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Review article

An overview of targeted cancer therapy

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ABSTRACT

Cancer is a multifactorial disease and is one of the leading causes of death worldwide. The contributing factors include specific genetic background, chronic exposure to various environmental stresses and improper diet. All these risk factors lead to the accumulation of molecular changes or mutations in some important proteins in cells which contributes to the initiation of carcinogenesis. Chemotherapy is an effective treatment against cancer but undesirable chemotherapy reactions and the development of resistance to drugs which results in multi-drug resistance (MDR) are the major obstacles in cancer chemotherapy. Strategies which are in practice with limited success include alternative formulations e.g., liposomes, resistance modulation e.g., PSC833, antidotes/toxicity modifiers e.g., ICRF-187 and gene therapy. Targeted therapy is gaining importance due to its specificity towards cancer cells while sparing toxicity to off-target cells. The scope of this review involves the various strategies involved in targeted therapy like-monoclonal antibodies, prodrug, small molecule inhibitors and nano-particulate antibody conjugates.

1. Introduction

Cancer is the second leading cause of deaths all over the world. Globally 7.6 million deaths are caused by cancer which represents 13% of all global deaths [1]. Surgery, chemotherapy, and irradiation are the mainstream therapeutic approaches for cancer, chemotherapy being an important component of treatment for cancer patients. However, its success is limited due to lack of selectivity for tumor cells over normal cells resulting in insufficient drug concentrations in tumors, systemic toxicity and the appearance of drug-resistant tumor cells [2]. Several strategies have been proposed which include alternative formulations e.g., liposomes [3], resistance modulation e.g., PSC833 [4], antidotes/toxicity modifiers e.g., ICRF-187 [5] and gene therapy. Recently targeted therapy is gaining importance due to its specificity towards cancer cells while sparing toxicity to off-target cells.

Targeted therapy aims at delivering drugs to particular genes or proteins that are specific to cancer cells or the tissue environment that promotes cancer growth. Effectiveness of the therapy lies in targeted release of therapeutics at the disease site while minimizing the off-target side effects caused to normal tissues. It is often used in conjunction with chemotherapy and other cancer treatments. Targeted therapy involves developing drugs that block cancer cell proliferation, promote cell cycle regulation or induce apoptosis or autophagy and targeted delivery of toxic substances specifically to cancer cells to destroy them. Targeted therapy involves the use of monoclonal antibodies or oral small drugs [6].

Monoclonal antibodies are the focus of intense research in the field of cancer therapeutics since mid 1970s when the customized monoclonal antibody production was reported. Monoclonal antibody production, antibody engineering with display and screening innovations such as phage display meant the binding of antibody to a wide range of targeted antigens with exceptional specificity. Cancer immunotherapy involves the use of gemtuzumab (Mylotarg®; Wyeth, CT, USA), a CD-33 specific monoclonal antibody conjugated to a calicheamicin used for the treatment of acute myeloid leukemia [7]. On a similar note, radioisotope conjugated targeting antibodies have been developed for imaging (immunoscintigraphy) and radioimmunotherapy strategies. ^{90}Y metal isotope based anti-CD20 ibritumomabtiuxetan (Zevalin®; Spectrum Pharmaceuticals, CA, USA) has been developed for use in clinical therapy [8, 9].

Moreover, apart from being used as therapeutic agents antibodies also serve as targeting agents. They are used in targeted therapy for the delivery of active therapeutics [10], prodrug activation enzymes [11, 12] and chemotherapy toxins [13-15]. Monoclonal antibodies block a specific target on the outside of cancer cells or in the tissue surrounding it. Monoclonal antibodies are used to deliver chemotherapeutic drugs and radioactive substances, directly to cancer cells. Being large compounds these drugs are usually given intravenously.

Prodrug cancer therapy involves selective activation of prodrug(s) in tumor tissues by exogenous enzyme(s) which can be accomplished by several methods which include: gene-directed enzyme prodrug therapy (GDEPT), virus-directed enzyme prod-

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rugtherapy (VDEPT), and antibody-directed enzyme prodrug therapy (ADEPT). The important aspect of prodrug cancer therapy is to deliver drug-activating enzyme or gene or functional protein to tumor tissues, followed by systemic administration of a prodrug [2].

Prodrug cancer therapy is a two step process, the first step involves, targeting the drug-activating enzyme and its expression in tumors followed by the systemic administration of the nontoxic prodrug, which is the substrate for the exogenous enzyme that is targeted and expressed in tumors [16-18]. This in turn helps in localization of activated anticancer drug (toxic drug) in high concentration in tumors. The success of the prodrug therapy requires that both enzymes and prodrugs should meet certain criteria: the enzyme should be of nonhuman origin or a human protein either absent or has low expression levels in normal tissues [19, 20] but should find sufficient expression in tumors with high catalytic activity [21]. The prodrug should not be activated by the endogenous enzymes in non-tumor tissues but must be a good substrate for the expressed enzyme in tumors. The prodrug should be highly diffusible and be activated in the tumor cell with high cytotoxic potential. Further, it must exhibit 'bystander' killing effect by being actively taken up by the nonexpressing neighboring cancer cells. The half life of the prodrug should be long enough to exhibit bystander effect but should not permit drug leakage to systemic ciruculation [22]. The targeting strategies for enzyme/prodrug can be divided into two major classes: (a) delivery of genes that encode prodrug-activating enzymes to tumor tissues (GDEPT, VDEPT, GPAT etc.); and (b) delivery of active enzymes onto tumor tissues (ADEPT).

Gene directed enzyme prodrug therapy, is a technique that involves delivery of a gene that encodes a foreign enzyme to tumor cells where it finds expression and activates a systemically administered nontoxic prodrug [16, 23, 24]. The enzyme/prodrug systems applied in GDEPT include: HSV-TK/GCV, *Escherichia coli* CD/5-FC and *E. coli* NTR/CB1954 which act intracellularly by converting prodrugsinto active drugs within cancer. Cell-cell contact is essential for this mode of action for effective killing. An extra-cellular cytotoxic effector system includes the conversion of an inactive glucuronidated derivative of doxorubicin (HMR 1826) to the cytotoxic doxorubicin in the tumor cells by the secreted form of lysosomal human glucuronidase. In the extracellular system the hydrophilic prodrug gets converted into a lipophilic, cell-permeable cytotoxic drug outside cells and hence targets both transduced and nontransduced cells. It exhibits enhanced cytotoxic potential as cell-cell contact is not required for a bystander effect [16].

Virus directed enzyme prodrug therapy (VDEPT) uses viral vectors to deliver a gene that encodes an enzyme that can convert a systemically administrated nontoxic prodrug into a cytotoxic agent within tumor cells. The NTR/CB1954 combination is used against colorectal and pancreatic cancer cells to sensitize them to CB1954 after retro-viral transduction and expression of the *E. coli* NTR gene [25, 26].

The viruses used for VDEPT include: retroviruses, adenoviruses, HSV [27], adeno-associated virus [28-30], lentivirus and EBV [31]. Over the years, many drug-activating enzyme gene/prodrug combinations have been delivered into tumors *in vitro* or *in vivo* by VDEPT, the majority using CD/5-FC or HSV-TK/GCV with the involvement of retroviral and adenoviral vectors [32].

Genetic prodrug activation therapy (GPAT) induces the selective expression of a drug-metabolizing enzyme for activation of prodrug into a toxic moiety using the known transcriptional differences between normal and tumor cells [33, 34]. Several tumor-specific Transription responsive elements (TREs) have been used, which include genes that are either tumor specific or tumor associated antigens, such as CEA for colorectal cancer or N-myc for neuroblastoma [2].

Antibody directed enzyme prodrug therapy (ADEPT) uses a conjugate which consists of tumor specific antibody linked to a drug-activating enzyme which when administered systemically targets tumor tissues. This targeted enzyme which is localized on the tumor surface, converts the systemically administered nontoxic prodrug into a toxic drug resulting in cytotoxic effects in tumor cells [12, 35-40]. The ideal drugs for ADEPT include diffusible small molecules, which can diffuse in to both antigen-positive and antigen-negative tumor cells, and cause a bystander effect [35-37]. The interval between enzyme and prodrug administrations should be optimized to enhance the conjugate accumulation in tumors and avoid their leakage to blood and normal tissues, to avoid systemic toxicity.

The important criteria for ADEPT include: the target antigen should be accessible, therefore it should preferably be a membrane bound antigen associated with the tumor cell membrane or secreted into the extracellular matrix of the tumor [41], and the antibody should be a monoclonal antibody with high affinity [35]. The enzyme should have optimal activity at a pH close to that of the tumor extracellular fluid.

The interval between enzyme and prodrug administrations is important for ADEPT, studies carried out in animals regarding the optimal interval showed that with the enzyme CPG2 linked to the anti-CEA antibody A5B7, the prodrug CMDA can be safely given 48 h or 72 h after antibody-enzyme administration [36]. In human subjects, the prodrug can be administered safely after 7 days to avoid systemic toxicity due to the activation of prodrug in plasma, as it takes 7 days for the adequate clearance of antibody-enzyme conjugate from the plasma [35]. The Phase I clinical trials carried out with CMDA/CPG2 prodrug/ enzyme system in colorectal carcinoma patients has revealed promising results. The bacterial enzyme CPG2 was conjugated to the F(ab)2 fragment of murine A5B7 monoclonal Ab, and a galactosylated second clearing Ab against CPG2 was also used to lower levels of conjugate in the circulation and other nontumor tissues. The plasma levels of the prodrug CMDA and active drug CJS11, a bifunctional alkylating agent, released from prodrug by the action of CPG2 localized in tumors were measured. The results showed that after applying the clearing agent, CPG2 activity was found in metastatic tumor biopsies, but not found in normal tissues. Further, a rapid appearance of the active drug with half-life of 36 ± 14 min in plasma was encouraging [42].

The limitations of ADEPT include: restricted delivery of the large conjugate in poorly vascularized tumors, therefore it is not possible to deliver antibody/enzyme conjugate to all of the tumor cells [43]. With low levels of the enzyme, adequate quantities of active drug to reach the cytotoxic concentration cannot be achieved. The antigen heterogeneity does not permit the binding of the conjugate to the cell surface. Other disadvantages of ADEPT include cost and availability of purified antibodies, immunogenicity of antibodies, accessibility of tumor to the enzyme/antibody conjugate, and the conversion of prodrugs in nontumor tissues [41]. The main problem being the immunogenicity of the antibody-enzyme conjugate, which limits multiple cycles of its application this can be overcome with the use of humanized proteins and concomitant administration of immunosuppression [35].

2. Small molecule inhibitors in cancer therapy

Small drugs constitute a pill that a patient takes orally. As they are smaller chemical components than monoclonal antibodies, the body absorbs them better. The small drugs usually are targeted against specific molecular targets which are important for cancer cell proliferation or metastasis or angiogenesis. The current state of cancer drug discovery and development focuses on small molecule inhibitors which act against new molecular targets that determine therapeutic outcome. The molecularly targeted cancer therapies have resulted in improving the lives of a large number of cancer patients. The successful treatment of patients with acute promyelocytic leukaemia harbouring translocations in the *RARα* retinoic acid receptor gene with all-*trans* retinoic acid [44] and chronic myeloid leukaemia in which the malignancy is driven by the *BCR-ABL* translocation with the ABL inhibitor imatinib [45, 46] serve as the proof of the concept of molecular targeting of pathogenetic driver abnormalities with a small molecule in the clinical setting. The other small molecule inhibitors of cancer targets include, e.g. the gefitinib - inhibitor of epidermal growth factor receptor (EGFR) kinase and erlotinib- the inhibitor of EGFR in non small cell lung cancer (NSCLC) patients; the lapatinib- inhibitor of EGFR/ERBB2 for ERBB2-positive breast cancer; and the sorafenib- inhibitor of vascular epidermal growth factor receptor (VEGFR) kinase, in renal cancer [47]. The recent addition to the list is, abiraterone- the CYP171A1 inhibitor which blocks androgen synthesis, approved for treatment of late stage, castration-resistant prostate cancer [48], crizotinib -inhibitor of the protein kinase ALK approved for the treatment of NSCLC patients with a pathogenic rearrangement of the *ALK* gene [49] and vemurafenib – inhibitor of *BRAF* kinase [50] for metastatic melanoma with the BRAF V600E mutation. The progress with small molecule drugs is mirrored by the successful introduction of protein-based therapeutics, particularly antibodies, as exemplified by the anti-ERBB2 monoclonal antibody trastuzumab in ERBB2-positive breast cancer [51, 52]. These examples provide ample evidence of the success in targeting the pathogenic drivers to which cancer cells are 'addicted' [53, 54].

Despite the considerable progress in cancer therapy with the advent of new molecularly targeted therapies, therapeutic options are still limited for many patients and the process of new drug development is frustratingly slow with high failure rates [52, 55], The reasons for slow progress is that, frequently, a patient with a particular anatomically and histologically defined solid tumor respond to the treatment with a particular class of kinase inhibitor that matches the predominant pathogenic driver mutation e.g., NSCLC patients with EGFR mutations respond to EGFR inhibitors while those with ALK translocations respond to ALK inhibitors [47, 56]. Which necessitates understanding the value of specific gene targeting and selection of patient specific companion biomarkers on cancer drug discovery.

Another important task is identification of specific molecular targets through the sequencing of various cancer genomes which revealed extraordinary complexity with several genetic alterations and considerable genetic heterogeneity, not only between different tumours but also within an individual cancer [57-59]. Further, the heterogeneous population of tumors also include drug-resistant stem [60] and other host cells which aid in tumour progression [47]. This heterogeneity leads to drug resistance and the need for combinatorial therapy.

After identification of a potential novel therapeutic target, the next challenge is that of selecting and validating the best targets. This requires establishing a causal linkage of the proposed target and target modulation to deliver a therapeutically meaningful biological effect in relevant experimental models. 'Druggability gap' is the main concern in the drug discovery for medicinal chemists using small molecules. Frequently, the promising targets are regarded as technically undruggable as they cannot be targeted with small molecules which is referred to as 'druggability gap' e.g., RAS proteins, c-MYC or hypoxia inducible factor (HIF) [61]. Unfortunately, sometimes, cancer cells develop resistance for drugs with therapeutic efficacy as proved by the successful completion of clinical trials as shown recently by crizotinib [62] and vemurafenib [63]. This could be due to the mutation of the target gene [64], or activation of feedback loops [65] or development of alternative oncogenic pathways [66, 67]. In such cases combinatorial regimen helps to overcome such problems [68].

3. Antibody-conjugated Nanoparticles for targeted Cancer Therapy

Recently research in nanoparticle based targeted therapy has gained momentum which saw the use of a full spectrum of nanoparticles in diagnostic and therapeutic applications of cancer [69]. Antibody-NP conjugates are being used for targeted delivery of chemotherapeutics and are considered as better therapeutic agents compared to NP conjugates due to their ability to circumvent some of the problems associated with direct conjugates, such as possible inactivation of the drug and the release of the drug in nonspecific areas once internalized into endosomal/lysosomal vesicles through pH labile or reducible linkers [70, 71]. Moreover, the limitation with respect to the amount of drug that can be delivered to targeted area with direct antibody conjugated drug can be overcome by the use of antibody-NP complexes, which maximize the concentration of drug that can be targeted to the disease site. Recent studies focused on the development of antibody-coated lipid and non-lipid based nanoparticulates for antitumor research. The nanoparticulate antibody-targeting research is focused on antitumor strategies, where the antibody is used to target cell-surface markers of disease which are frequently upregulated or are specifically expressed in tumor cells [70-72].

Thus, the cancer drug discovery involves genome wide sequencing, understanding molecular pathology through bioinformatics and systems biology approaches for understanding how cancer cells can be targeted through single agents or on several fronts through drug combinations [73-75]. Although targeted treatment is considered a breakthrough in cancer treatment, latest research findings show that tumor heterogeneity with respect to molecular targets cause failure in many cases. This lead to evolution of concept of matching a patient to treatment which in other words is known as personalized medicine. In order to find the most effective treatment, the patient will be screened for the genes, proteins, and other factors unique to your tumor. After identifying the appropriate molecular targets the best suitable treatment will be recommended. Personalized medicine is gaining importance which ensures that the right drug is given to the right patient at the right time whereby maximum therapeutic benefit to patients is achieved. Pharmacologists and basic researchers are working together towards the discovery of effective and safe clinical candidates against the new targets trying to bridge the gap frequently referred to as technically druggable.

REFERENCES

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Xu G, McLeod HL. Strategies for enzyme/prodrug cancer therapy. Clin Cancer Res 2001; 7: 3314-24.

[3] Krishna R, Mayer LD. Liposomal doxorubicin circumvents PSC 833-free drug interactions, resulting in effective therapy of multidrug-resistant solid tumors. Cancer Res 1997; 57: 5246-53.

[4] Fracasso PM, Westervelt P, Fears CL, Rosen DM, Zuhowski EG, Cazenave LA, *et al*. Phase I study of paclitaxel in combination with a multidrug resistance modulator, PSC 833 (Valspodar), in refractory malignancies. J Clin Oncol 2000; 18: 1124-34.

[5] Hasinoff BB, Chee GL, Thampatty P, Allan WP, Yalowich JC. The cardioprotective and DNA topoisomerase II inhibitory agent dexrazoxane (ICRF-187) antagonizes camptothecin-mediated growth inhibition of Chinese hamster ovary cells by inhibition of DNA synthesis. Anticancer Drugs 1999; 10: 47-54.

[6] Gerber DE. Targeted therapies: a new generation of cancer treatments. Am Fam Physician 2008; 77: 311-9.

[7] Sorokin P. Mylotarg approved for patients with CD33+ acute myeloid leukemia. Clin J Oncol Nurs 2000;4:279-80.

[8] Jacobs SA. Yttrium ibritumomab tiuxetan in the treatment of non-Hodgkin's lymphoma: current status and future prospects. Biologics 2007; 1: 215-27.

[9] Vitolo U, Ladetto M, Boccomini C, Evangelista A, Gamba E, Russo E, *et al*. Brief Chemoimmunotherapy R-FND with Rituximab Consolidation Followed by Randomization Between Rituximab Maintenance Vs. Observation As First Line Treatment in Elderly Patients with Advanced Follicular Lymphoma (FL): Final Results of a Prospective Randomized Trial by Italian Lymphoma Foundation (FIL). Blood 2011; 118: 352-53.

[10] Lode HN, Xiang R, Becker JC, Gillies SD, Reisfeld RA. Immunocytokines: A promising approach to cancer immunotherapy. Pharmacol Ther 1998; 80: 277-92.

[11] Kerr DE, Vrudhula VM, Svensson HP, Siemers NO, Senter PD. Comparison of recombinant and synthetically formed monoclonal antibody-beta-lactamase conjugates for anticancer prodrug activation. Bioconjug Chem 1999; 10: 1084-89.

[12] Wolfe LA, Mullin RJ, Laethem R, Blumenkopf TA, Cory M, Miller JF, *et al*. Antibody-directed enzyme prodrug therapy with the T268G mutant of human carboxypeptidase A1: *In vitro* and *in vivo* studies with prodrugs of methotrexate and the thymidylate synthase inhibitors GW1031 and GW1843. Bioconjug Chem 1999; 10: 38-48.

[13] Kawakami K, Nakajima O, Morishita R, Nagai R. Targeted anticancer immunotoxins and cytotoxic agents with direct killing moieties. Thescientific World Journal 2006; 6: 781-90.

[14] Hamann PR, Hinman LM, Hollander I, Beyer CF, Lindh D, Holcomb R, *et al*. Gemtuzumab ozogamicin, a potent and selective anti-CD33 antibody-calicheamicin conjugate for treatment of acute myeloid leukemia. Bioconjug Chem 2002; 13: 47-58.

[15] Henry MD, Wen S, Silva MD, Chandra S, Milton M, Worland PJ. A prostate-specific membrane antigen-targeted monoclonal antibody-chemotherapeutic conjugate designed for the treatment of prostate cancer. Cancer Res 2004; 64: 7995-8001.

[16] Weyel D, Sedlacek HH, Muller R, Brusselbach S. Secreted human beta-glucuronidase: a novel tool for gene-directed enzyme prodrug therapy. Gene Ther 2000; 7: 224-31.

[17] Hamstra DA, Rehemtulla A. Toward an enzyme/prodrug strategy for cancer gene therapy: endogenous activation of carboxypeptidase A mutants by the PACE/Furin family of propeptidases. Hum Gene Ther 1999; 10: 235-48.

[18] Springer CJ, Niculescu-Duvaz I. Prodrug-activating systems in suicide gene therapy. J Clin Investig 2000; 105: 1161-67.

[19] Riggs CD. Meiotin-1: the meiosis readiness factor? BioEssays 1997; 19: 925-31.

[20] Rainov NG, Dobberstein KU, Sena-Esteves M, Herrlinger U, Kramm CM, Philpot RM, *et al*. New prodrug activation gene therapy for cancer using cytochrome P450 4B1 and 2-aminoanthracene/4-ipomeanol. Human Gene Ther 1998; 9: 1261-73.

[21] Niculescu-Duvaz I, Spooner R, Marais R, Springer CJ. Gene-directed enzyme prodrug therapy. Bioconjug Chem 1998; 9: 4-22.

[22] Friedlos F, Court S, Ford M, Denny WA, Springer C. Gene-directed enzyme prodrug therapy: quantitative bystander cytotoxicity and DNA damage induced by CB1954 in cells expressing bacterial nitroreductase. Gene Ther1998; 5: 105-12.

[23] Clark AJ, Iwobi M, Cui W, Crompton M, Harold G, Hobbs S, *et al*. Selective cell ablation in transgenic mice expressing E-coli nitroreductase. Gene Ther 1997; 4: 101-10.

[24] Bridgewater JA, Knox RJ, Pitts JD, Collins MK, Springer CJ. The bystander effect of the nitroreductase CB 1954 enzyme prodrug system is due to a cell-permeable metabolite. Human Gene Ther 1997; 8: 709-17.

[25] McNeish IA, Green NK, Gilligan MG, Ford MJ, Mautner V, Young LS, *et al*. Virus directed enzyme prodrug therapy for ovarian and pancreatic cancer using retrovirally delivered *E-coli* nitroreductase and CB1954. Gene Ther 1998; 5: 1061-69.

[26] Grove JI, Searle PF, Weedon SJ, Green NK, McNeish IA, Kerr DJ. Virus-directed enzyme prodrug therapy using CB1954. Anti-Cancer Drug Des 1999; 14: 461-72.

[27] Nakamura H, Mullen JT, Chandrasekhar S, Pawlik TM, Yoon SS, Tanabe KK. Multimodality therapy with a replication-conditional herpes simplex virus 1 mutant that expresses yeast cytosine deaminase for intratumoral conversion of 5-fluorocytosine to 5-fluorouracil. Cancer Res 2001; 61: 5447-52.

[28] Warrington KH, Teschendorf C, Cao LG, Muzyczka N, Siemann DW. Developing VDEPT for DT-diaphorase (NQO1) using an AAV vector plasmid. Int J Radia Oncol Biol Phys 1998; 42: 909-12.

[29] Kanazawa T, Urabe M, Mizukami H, Okada T, Kume A, Nishino H, *et al*. gamma-Rays enhance rAAV-mediated transgene expression and cytocidal effect of AAV-HSVtk/ganciclovir on cancer cells. Cancer Gene Ther 2001; 8: 99-106.

[30] Fukui T, Hayashi Y, Kagami H, Yamamoto N, Fukuhara H, Tohnai I, *et al*. Suicide gene therapy for human oral squamous cell carcinoma cell lines with adeno-associated virus vector. Oral Oncol 2001; 37: 211-5.

[31] Westphal EM, Ge JQ, Catchpole JR, Ford M, Kenney SC. The nitroreductase/CB1954 combination in Epstein-Barr virus-positive B-

cell lines: Induction of bystander killing *in vitro* and *in vivo*. Cancer Gene Ther 2000; 7: 97-106.
[32] Curiel DT. The development of conditionally replicative adenoviruses for cancer therapy. Clin Cancer Res 2000; 6: 3395-99.
[33] Rigg A, Sikora K. Genetic prodrug activation therapy. Mol Med Today 1997; 3: 359-66.
[34] Pandha HS, Martin LA, Rigg A, Hurst HC, Stamp GWH, Sikora K, *et al*. Genetic prodrug activation therapy for breast cancer: A phase I clinical trial of erbB-2-directed suicide gene expression. J Clin Oncol 1999; 17: 2180-89.
[35] Syrigos KN, Epenetos AA. Antibody directed enzyme prodrug therapy (ADEPT): A review of the experimental and clinical considerations. Anticancer Res 1999; 19: 605-13.
[36] Stribbling SM, Martin J, Pedley RB, Boden JA, Sharma SK, Springer CJ. Biodistribution of an antibody-enzyme conjugate for antibody-directed enzyme prodrug therapy in nude mice bearing a human colon adenocarcinoma xenograft. Cancer Chemother Pharmacol 1997; 40: 277-84.
[37] Syrigos KN, Rowlinson-Busza G, Epenetos AA. *In vitro* cytotoxicity following specific activation of amygdalin by beta-glucosidase conjugated to a bladder cancer-associated monoclonal antibody. Int J Cancer 1998; 78: 712-19.
[38] Florent JC, Dong X, Gaudel G, Mitaku S, Monneret C, Gesson JP, *et al*. Prodrugs of anthracyclines for use in antibody-directed enzyme prodrug therapy. J Med Chem 1998; 41: 3572-81.
[39] Pedley RB, Sharma SK, Boxer GM, Boden R, Stribbling SM, Davies L, *et al*. Enhancement of antibody-directed enzyme prodrug therapy in colorectal xenografts by an antivascular agent. Cancer Res 1999; 59: 3998-4003.
[40] Haisma HJ, Sernee MF, Hooijberg E, Brakenhoff RH, van den Meulen-Mulleman IH, Pinedo HM, *et al*. Construction and characterization of a fusion protein of single-chain anti-CD20 antibody and human beta-glucuronidase for antibody-directed enzyme prodrug therapy. Blood 1998; 92: 184-90.
[41] Mason DW, Williams AF. The Kinetics of Antibody-Binding to Membrane-Antigens in Solution and at the Cell-Surface. Biochem J 1980; 187: 1-20.
[42] Martin J, Stribbling SM, Poon GK, Begent RHJ, Napier M, Sharma SK, *et al*. Antibody-directed enzyme prodrug therapy: Pharmacokinetics and plasma levels of prodrug and drug in a phase I clinical trial. Cancer Chemother Pharmacol 1997; 40: 189-201.
[43] Denny WA, Wilson WR. The design of selectively-activated anticancer prodrugs for use in antibody-directed and gene-directed enzyme-prodrug therapies. J Pharm Pharmacol 1998; 50: 387-94.
[44] Huang ME, Ye YC, Chen SR, Chai JR, Lu JX, Zhoa L, *et al*. Use of All-Trans Retinoic Acid in the Treatment of Acute Promyelocytic Leukemia. Blood 1988; 72: 567-72.
[45] O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, *et al*. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 2003; 348: 994-1004.
[46] Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, *et al*. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 2006; 355: 2408-17.
[47] Yap TA, Workman P. Exploiting the cancer genome: strategies for the discovery and clinical development of targeted molecular therapeutics. Annu Rev Pharmacol Toxicol 2012; 52: 549-73.
[48] de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, *et al*. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011; 364: 1995-2005.
[49] Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, *et al*. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010; 363: 1693-703.
[50] Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, *et al*. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011; 364: 2507-16.
[51] Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, *et al*. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344: 783-92.
[52] Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov 2004; 3:711-16.
[53] Weinstein IB. Cancer. Addiction to oncogenes--the Achilles heal of cancer. Science 2002; 297: 63-4.
[54] Weinstein IB, Joe A. Oncogene addiction. Cancer Res 2008; 68: 3077-80; discussion 80.
[55] DiMasi JA, Grabowski HG. Economics of new oncology drug development. J Clin Oncol 2007; 25: 209-16.
[56] Collins I, Workman P. New approaches to molecular cancer therapeutics. Nat Chem Biol 2006; 2: 689-700.
[57] McDermott U, Downing JR, Stratton MR. Genomics and the continuum of cancer care. N Engl J Med 2011; 364: 340-50.
[58] Sellers WR. A blueprint for advancing genetics-based cancer therapy. Cell 2011; 147: 26-31.
[59] De Palma M, Hanahan D. The biology of personalized cancer medicine: facing individual complexities underlying hallmark capabilities. Mol Oncol 2012; 6: 111-27.
[60] Jordan CT, Guzman ML, Noble M. Cancer stem cells. N Engl J Med 2006; 355: 1253-61.
[61] Verdine GL, Walensky LD. The challenge of drugging undruggable targets in cancer: lessons learned from targeting BCL-2 family members. Clin Cancer Res 2007; 13: 7264-70.
[62] Sasaki T, Koivunen J, Ogino A, Yanagita M, Nikiforow S, Zheng W, *et al*. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. Cancer Res 2011; 71: 6051-60.
[63] Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, *et al*. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). Nature 2011; 480: 387-90.
[64] Gibbons DL, Pricl S, Kantarjian H, Cortes J, Quintas-Cardama A. The rise and fall of gatekeeper mutations? The BCR-ABL1 T315I paradigm. Cancer 2012; 118: 293-9.
[65] Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, Poulikakos PI, Scaltriti M, Moskatel E, *et al*. mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. Cancer Discov 2011; 1: 248-59.
[66] Johannessen CM, Boehm JS, Kim SY, Thomas SR, Wardwell L, Johnson LA, *et al*. COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. Nature 2010; 468: 968-U370.
[67] Nazarian R, Shi HB, Wang Q, Kong XJ, Koya RC, Lee H, *et al*. Melanomas acquire resistance toB-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature 2010; 468: 973-U377.
[68] Whitehurst AW, Bodemann BO, Cardenas J, Ferguson D, Girard L, Peyton M, *et al*. Synthetic lethal screen identification of chemosensitizer loci in cancer cells. Nature 2007; 446: 815-19.

[69] Gleysteen JP, Newman JR, Chhieng D, Frost A, Zinn KR, Rosenthal EL. Fluorescent labeled anti-EGFR antibody for identification of regional and distant metastasis in a preclinical xenograft model. Head and Neck-Journal for the Sciences and Specialties of the Head and Neck 2008; 30: 782-89.

[70] Fay F, Scott CJ. Antibody-targeted nanoparticles for cancer therapy. Immunotherapy 2011; 3: 381-94.

[71] Vanniasinghe AS, Bender V, Manolios N. The Potential of Liposomal Drug Delivery for the Treatment of Inflammatory Arthritis. Semin Arthritis Rheum 2009; 39: 182-96.

[72] Cirstoiu-Hapca A, Buchegger F, Lange N, Bossy L, Gurny R, Delie F. Benefit of anti-HER2-coated paclitaxel-loaded immuno-nanoparticles in the treatment of disseminated ovarian cancer: Therapeutic efficacy and biodistribution in mice. J Control Release 2010; 144: 324-31.

[73] Stratton MR. Exploring the Genomes of Cancer Cells: Progress and Promise. Science 2011; 331: 1553-58.

[74] MacConaill LE, Garraway LA. Clinical Implications of the Cancer Genome. J Clin Oncol 2010; 28: 5219-27.

[75] Kitano H. Cancer robustness - Tumour tactics. Nature 2003; 426: 125-25.

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Review article

Assessing the risk: Scoring systems for outcome prediction in emergency laparotomies

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ABSTRACT

Emergency laparotomy is the commonest emergency surgical procedure in most hospitals and includes over 400 diverse surgical procedures. Despite the evolution of medicine and surgical practices, the mortality in patients needing emergency laparotomy remains abnormally high. Although surgical risk assessment first started with the ASA Physical Status score in 1941, efforts to find an ideal scoring system that accurately estimates the risk of mortality, continues till today. While many scoring systems have been developed, no single scoring system has been validated across multiple centers and geographical locations. While some scoring systems can predict the risk merely based upon preoperative findings and parameters, some rely on intra-operative assessment and histopathology reports to accurately stratify the risk of mortality. Although most scoring systems can potentially be used to compare risk-adjusted mortality across hospitals and amongst surgeons, only those which are based on preoperative findings can be used for risk prognostication and identify high-risk patients before surgery for an aggressive treatment. The recognition of the fact, that in the absence of outcome data in these patients, it would be impossible to evaluate the impact of quality improvement initiatives on risk-adjusted mortality, hospital groups and surgical societies have got together and started to pool data and analyze it. Appropriate scoring systems for emergency laparotomies would help in risk prognostication, risk-adjusted audit and assess the impact of quality improvement initiative in patient care across hospitals. Large multi-centric studies across varied geographic locations and surgical practices need to assess and validate the ideal and most apt scoring system for emergency laparotomies. While APACHE-II and P-POSSUM continue to be the most commonly used scoring system in emergency laparotomies,studies need to compare them in their ability to predict mortality and explore if either has a higher sensitivity and specificity than the other.

1. Introduction

Emergency laparotomy is most common emergency surgery and describes an exploratory procedure for which the clinical presentation, underlying pathology, anatomical site of surgery, and perioperative management vary considerably. The fact that over 400 different surgical procedures have been recorded during an emergency laparotomy, reflect the diverse nature of this surgical cohort [1]. The varied surgical pathology and the emergent nature of the procedure limits the time to optimize these patients [1]. Although there is scarcity of outcome data after emergency laparotomy, it is generally recognized to be poor [1]. Even after innumerable advances in surgical skills, antimicrobial agents and supportive care, the mortality of peritonitis remains high, and is presently reported to be between 14.9-19.5% [1, 2].

Early prognostic evaluation of these patients is desirable to select the high-risk patients for a more aggressive treatment [3]. The continuous monitoring and audit of clinical practice is an essential part of making improvements in medical science and enhancing patient care [4]. It is also essential to ensure that patients are well informed of risks and to improve quality of care in hospitals.

Knowing which patient is at risk of developing complications or dying contributes to the quality of surgical care and cost reduction [5]. Doctors are legally bound to inform their patients of the potential risks involved with a particular treatment [5-7]. It is therefore essential to identify and make appropriate decision on those patients who are at high-risk of developing serious complications or die [5, 7-9]. Categorizing patients into different risk groups would also help prognosticate the outcome, select patients for intensive care and determine operative risk, thereby helping to choose the nature of the operative procedure, e.g. damage control *vs.* definitive procedure.

An ideal scoring system is desirable, so that an accurate prediction of outcome could then be made, allowing the treating team to present a more informed choice to the patient on whether

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surgery or supportive care is the optimal management [10]. It should also allow analysis for risk-adjusted comparison between surgeons, hospitals and across geographical distributions [11].

2. The scoring systems

Various scoring systems are available to predict surgical outcomes. They range from the general scoring systems to surgery specific scoring systems. While most scoring systems compare post-operative mortality as an outcome parameter, some are also designed to predict morbidity. While American College of Surgeons recommend the Universal ACS NSQIP Surgical Risk Calculator [12] for mortality and morbidity risk assessment for informed consent and to facilitate decision making for patients and surgeons, P-POSSUM scoring system was used to assess improved outcomes in patients undergoing emergency laparotomy after the implementation of emergency laparotomy pathwayquality improvement care (ELPQuiC) bundle [13]. While those which can calculate the risk based on pre-operative parameters are most useful in prognostication and triage of patients, those that need intra-operative data are best utilized for retrospective quality audits. While many scoring systems have been used in emergency laparotomies, till date no specific scoring system has been developed for emergency laparotomies.

3. The general scoring systems

3.1. ASA

The oldest available scoring system [14], the American Society of Anesthesiologists' (ASA) physical status score is often used to subjectively estimate preoperative health status. While it was initially designed for "statistical data collection and reporting", it is today used to predict the perioperative risk [15]. This subjective scoring is associated with inter-observer variability [14] and has "no specific role" [15] in predicting the outcome in emergency laparotomy. The ASA Scores range from ASA I for a normal healthy patient, ASA II for a patient with mild systemic disease, ASA III for a patient with severe systemic disease, ASA IV for a patient with a severe systemic diseas that is a constant threat to life and ASA V for a moribund patient who is not expected to survive without the operation. An addition of E besides the ASA Grade denotes emergency surgery. A study on 10864 patients using receiver-operating characteristic (ROC) curve area, Sankar A *et al*. found that its ability to predict mortality (ROC Curve area 0.69) and cardiac complications (ROC Curve area 0.70) was only moderate [14]. However, the ASA Score is simple, able to predict mortality as well as morbidity in general surgical patients, and has also been able to predict mortality well in a particular subset of patients undergoing emergency laparotomy, those with peptic ulcer perforation [15].

3.2. Apgar score for surgery

It is a 10 point score considering the estimated amount of blood loss, lowest heart rate, and lowest mean arterial pressure. Scores of less than or equal to 4 was associated with significant higher mortality [16]. However it can only be calculated at the end of surgery. This scoring system can has been reported to predict death with a significant degree of accuracy (P = 0.0001 in univariate logistic regression, c-statistic 0.92) [16]. Scores of 9-10 was associated with 0% death, 7-8 with 0.3% mortality, 5-6 with 4.9% mortality and 0-4 was associated with a mortality of 13.8% [16]. This scoring system was also able to predict major complications with a only 4% complications scores with scores of 9-10 and a 50% risk of major complications with a score of 4 [16]. It has beentested mostly in general surgical and vascular surgeries, and not been validated to specifically assess the risk in emergency laparotomies [16].

3.3. Sickness assessment (SA)

It was essentially used to predict mortality in the geriatric population undergoing emergency surgery. It is a simple scoring system using only three parameters at the time of admission, hypotension, pre-existing severe chronic disease and whether or not the patient was functionally independent [17]. In patients above 65 years undergoing emergency laparotomy, an SA Score of 1 was associated with a 52% mortality, a score of 2 was associated with 60% mortality and a score of 3 was associated with 100% mortality [17]. The mortality was 15% in those with SA score as zero [17]. Although it is a simple scoring system to identify the high risk group, its utility in large multi-centric studies or across age-groups has not been evaluated till date. Kennedy RH et al found that SA Scores had significant predictive ability ($P < 0.001$) in predicting mortality and the APACHE II scoring system was not superiorto it [17]. No study till date has evaluated its correlation with peri-operative morbidity.

3.4. Calculation of post-operative risk in emergency surgery (CORES)

The Calculation of post-Operative Risk in Emergency Surgery (CORES) [18] was constructed based on a regression model and needs only 6 preoperative variables to predict the in-hospital mortality. The predicted mortality is calculated using an equation based on presence or absence of (1) Japan Coma Scale >30, (2) ASA Class3, (3) ASA Class 4, (4) Whie blood cell count of <2,500 cells/μL, (5) platelet count<150,000 or>300,000 cells/μL and (6) blood urea nitrogen >/=40 mg/dL [18]. After development, its accuracy was further assess on 1471 cases across six hospitals and found to be as discriminative as P-POSSUM [18]. In predicting in-hospital mortality, the area under the receiver operating characteristic curve (AUC) (95% CI) of the CORES model was high (0.85), the observed-to-estimated mortality ratio (OE ratio) was also high (0.70), and the calibration power was also good (chi-square = 19.9, degrees of freedom = 8, P = 0.81) [18]. Although this model was developed to specifically predict mortality, the CORES scores were also significantly correlated to other post-operative morbidity [18]. While this was the first, and possibly only specific model predicting the post-operative risk for emergency surgery, it is yet to be validated specifically on emergency laparotomies [18].

3.5. Estimation of physiologic ability and surgical stress (E-PASS)

The Estimation of Physiologic Ability and Surgical Stress (E-PASS) scoring system aims to quantify the patient's reserve and surgical stress was initially developed to predict morbidity and mortality in elective gastrointestinal surgery [19]. They have been subsequently been evaluated in emergency gastrointestinal

study and was "useful for assessing the risks of emergency abdominal surgery" [20].

This system uses a preoperative risk score (PRS) and a surgical stress score (SSS) to calculate a comprehensive risk score (CRS) [19]. PRS is calculated from a formula using age, presence or absence of severe heart-disease, severe pulmonary disease and diabetes mellitus, along with performance status index (based on the definition by Japanese Society for Cancer Therapy) and American Society of Anesthesiologists physiological status classification [19]. Surgical stress score (SSS) is calculated by an equation based on blood loss/body weight (mL/kg), operation time (in hours), and extent of skin incision [19].

With increase in CRS, there was a significant increase in incidence of postoperative morbidity and mortality ($P < 0.0001$) [19]. With CRS <0.1 the morbidity and mortality was 12.5% and 0%, respectively [19]. With CRS 0.5-0.75, the morbidity and mortality rates were 45.0% and 5.0%, respectively, and when the CRS > 1.0, the morbidity and mortality rates were 76.9% and 38.5%, respectively [19]. However it is yet to be validated in large multicentric trials.

3.6. ACS NSQIP surgical risk calculator

American College of Surgeons National Surgical Quality Improvement Program (ACS NSQIP) Surgical Risk Calculator [13] is based on 21 preoperative risk factors identified from 1,414,006 patients across 393 ACS NSQIP hospitals in the United States. It also allows a Surgeon Adjustment Score to reasonably the modify the score based on their clinical impression. This scoring system can be used for over 1500 procedures across surgical specialties. It can specifically predict the risk for 10 different Current Procedural Terminology (CPT) codes for laparotomies. CPT code is the descriptive terms for identifying and reporting medical services and procedures in the United States. Apart from mortality, it is able to predict the risk of serious or any other post-operative complication including pneumonia, cardiac complication, surgical site infection, urinary tract infection, venous thromboembolism, renal failure or return to the operating room. Although the discriminative ability has been found to be reasonable accurate as compared to other risk calculators [13], it is yet to be accurately evaluated specifically for emergency laparotomies. Besides, it is yet to be evaluated across geographical locations and the Surgeon Adjustment Score is based on subjective assessment with "no quantitative evidence that these adjusted risks are more accurate" [13].

Other surgical risk assessment tools like the Charlson Comorbidity Index validated in over 5500 studies since 1987 for its ability to predict mortality based on co-morbidity [21], the Fitness Score [10] in major abdominal surgery (including emergency surgery) and Reiss Index [10] used specifically in laparotomies in elderly patients have all been used to assess the surgical risk. However, none of the scoring system has been specifically been assessed in patients undergoing emergency laparotomies across all age groups.

4. The critical care and sepsis scoring systems

4.1. APACHE II

The original APACHE score based on physiology score for acute illness and chronic health status was developed in 1981 by Knaus WA *et al*. [22] and was subsequently simplified to create APACHE II in 1985 [23]. The score is calculated based on the patient's age, 12 routine physiological measurements and whether they are scheduled for routine or emergency surgery. An integer score from 0 to 71 is computed based on these values; higher scores are associated with an higher risk of death [23]. Till date, this remains the most widely used illness severity score worldwide [24]. It has been evaluated to predict mortality in patients undergoing in general surgical or patients undergoing laparotomy since 1990. Till date, at least 14 studies [25-38] (Table 1) have tried to correlate the APACHE II with the risk of mortality and re-exploration.

In the study by Oka Y *et al*. in patients with peritonitis undergoing laparotomy, APACHE II scores amongst survivors ranged from 0-21 (Mean 5.0) , with a mean of 5.0 [30]. Amongst those patients who died, the scores ranged from 15-38 (Mean 23.3) [30]. The difference between groups was significant ($P < 0.05$) [30]. The co-relation with morbidity was not reported in this study.

Another study by Scheien M *et al.* in patients with perforated peptic ulcer reported zero mortality in patients with scores below 11 points and 35% mortality rate in patients with APACHE II scores above 11 [25]. In other studies by Lee FY *et al.* also, APACHE II was found to predict both morbidity and mortality but AUC or relative risks were not reported in this study [36].

4.2. Simplified acute physiology score (SAPS), multiple organ dysfunction score (MODS), sepsis-related organ failure assessment (SOFA) score, sepsis score, multiple organ failure (MOF) score

While SAPS, MODS and SOFA Scores were able to predict mortality in peritonitis patients, they were unable to predict "ongoing infection needing a relaparotomy" [34]. The SAPS II score is calculated from 12 physiological and 3 disease-related variables. The SAPS II score ranges from 0 to 163 points. A MODS is calculated from 0-4 scoring for each of respiratory, hematologic, hepatic, cardiovascular, Glasgow Coma Scale and renal parameters. The scores range from 0-24.

The SOFA Score is also calculated from 0-4 scoring for each of respiratory, cardiovascular, nervous, hepatic, coagulation and renal systems with scores ranging from 0-24. The MOF Score is derived from 0-2 score (0: Normal function, 1: Organ dysfunction, 2: Organ failure) for each of respiratory, cardiovascular, renal, hepatic, hematologic, gastrointestinal and the central nervous systems with scores ranging from 0-14. Higher scores are associated with increased risk of morbidity and mortality.

The ability of the scoring system to predict mortality was assessed using the area under the receiver operating characteristic (ROC) curve (AUC) by Oddeke van Ruler et al. and they found it to be 0.8 for SAPS-II, 0.72 for SOFA (day 1) and 0.76 for MODS (day 1) [34]. In their study, the authors found the ability of this scoring system to be statistically significant in all the 3 scoring systems [34]. Other morbidity was not studied in this trial but this study failed to demonstrate the correlation of these scoring systems to predict relaparotomy [34]. Similarly, the Sepsis Score and Multiple Organ Failure (MOF) Score may also predict mortality, but has not been extensively studied in patients undergoing emergency laparotomy.

4.3. Mannheim peritonitis index (MPI)

The MPI based on retrospective analysis of 1253 patients with peritonitis has 8 proven risk factors based on their predictive

Table 1 − APACHE II scoring for outcome in emergency general surgery or laparotomy.

Year	Patient Category	Outcome
1990	Perforated peptic ulcers	APACHE II scoring system accurately stratified patients according to risk [25]
1997	Peritonitis and intra abdominal sepsis	Combination of the APACHE II and the MPI provides the best scoring system [26]
2007	Peritonitis due to hollow viscus perforation	APACHE-II scoring system can be used to assess group outcomes in patients with peritonitis due to hollow viscus perforation [27]
2007	General surgical patients	M-POSSUM is more accurate than POSSUM and APACHE II in predicting postoperative morbidity and mortality [28]
2007	Perforated peptic ulcer	Compared to the APACHE II & III & the simplified acute physiology score II, the mortality probability models (MPM) II predicted mortality at admission better [29]
2010	Patients with peritonitis	APACHE II is accurate in predicting mortality has definitive advantages and is therefore more useful [30]
2010	Generalized secondary peritonitis	Independent mortality predictors were APACHE II > or = 16 [31]
2011	obstructing colon cancer	APACHE II score ≥11 was a prognostic factor for poor outcome [32]
2011	Perforation peritonitis	APACHE II is superior in prediction of the outcome as compared to SAPS I, Sepsis score, MOF, TISS-28 and MPI [33]
2011	Abdominal sepsis that have ongoing infection and would need relaparotomy	All evaluated scoring systems (APACHE-II score, SAPS-II, Mannheim Peritonitis Index (MPI), MODS, SOFA score, and the acute part of the APACHE-II score) were predictive of mortality, none predicted need for laparotomy [34]
2012	Secondary peritonitis of colorectal origin to predict relaparotomies	APACHE II score might be helpful in predicting the need for relaparotomies [35]
2013	Perforated peptic ulcer	APACHE II has been shown to predict outcome well also for PPU patients [36]
2014	Patients of intra-abdominal sepsis and treated with planned relaparotomy	APACHE II scoring system is reliable for prediction of mortality [37]
2015	Gall bladder perforation	Both POSSUM and APACHE II scores were superior to ASA score in risk prediction [38]

power [39]. MPI scores of pre were associated with a mortality of 5%, scores of 21-29 had a mortality of 14% and scores ≥ had a mortality of 14% 50% [39]. MPI score of 25 had sensitivity and specificity of 72.09% and 71.43% respectively in predicting mortality, and 80.65% and 57.89% sensitivity and specificity respectivelyfor predicting morbidity [39]. Other studies also observed strong association between increasing MPI score and adverse outcomes in patients with secondary peritonitis [40].

5. The disease specific scoring systems

5.1. Perforated peptic ulcer scoring systems: boey score, Hacettepe score, Jabalpur score and the peptic ulcer perforation (PULP) score

The Boey score was the first developed scoring system to predict mortality in perforated peptic ulcer in 1982 [41]. Subsequent studies in 1987 validated the value of the three independent variables in this scoring system, severe medical illness, pre-existing shock and longstanding perforation in predicting mortality [42]. Risk score 0 was not associated with any mortality, risk score 1 was associated with a mortality of 10%, Risk score 2 was associated with a mortality of 45.5% and a Risk score 3 was associated with a mortality of 100%. The accuracy rate in predicting mortality was 93.9% and there were no false negative errors [42].

The Hacettepe score uses coexisting medical illness, acute renal failure, raised white cell count and male sex as the 4 variables for predicting mortality with mortality increasing with rising scores. When developed in 1992, "the sensitivity was 83%, the specificity 94%, and the overall predictive accuracy 93%." [43]. However it has not been found to be better than other scoring systems used to predict outcome in peptic ulcer perforation [36].

The Jabalpur score used multiple regression analysis and developed a scoring system based on six identified risk factors which included age, co-morbid illness, perforation to surgery time interval, preoperative shock, heart rate and serum creatinine levels to predict post-operative mortality [44]. Scores of 0-4 was associated with 14% morbidity and 0% mortality, scores of 5-9 was associated with 48% morbidity and 7% mortality, scores of 10-14 were associated with 71% morbidity and 38% mortality, while scores of 15-21 was associated with 100% morbidity and mortality [44]. There was good correlation of the Jabalpur score in predicting both morbidity and mortality with correlation coefficient being 0.67 ($P < 0.001$) and 0.81 ($P < 0.001$) for mortality and morbidity respectively [44].

The Peptic Ulcer Perforation (PULP) score is based on age, presence of comorbid diseases, concurrent use of steroids, shock on admission, serum creatinine levels, time from perforation to admission and ASA scores [45]. Scores of 0-7 was associated with low risk (<25%) and scores of 8-18 was associated with high risk (>25%) of mortality [45]. Its accuracy to predict mortality by the area under receiver operating characteristics curve (AUC) was better (AUC 0.83 for PULP) than the Boey score (AUC 0.70) and ASA score (AUC 0.78) [45]. This study did not analyze other post operative morbidity.

5.2. Patients with Liver Disease: Child-Turcotte-Pugh (CTP) classification&MELD (model for end stage liver disease) score

Nearly 10% of the patients undergoing surgery have some form of liver disease. The CTP classification is based on serum albumin and bilirubin levels, prothrombin time, and the degree of encephalopathy and ascites. In major abdominal surgery, the mortality in Child's class A was reported to be 10%, 30-31% in class B and 76-82% in class C [46]. It also correlated well with postoperative complications including "liver failure, worsening encephalopathy, bleeding, infection, renal failure, hypoxia and intractable ascites" [46].

Although the MELD was initially designed to predict mortality after Transjugular intrahepatic portosystemic shunt (TIPS), it is based on a linear regression model, assessing the risk from a score derived from the patient's creatinine level, serum bilirubin and International Normalized Ratio of the Prothrombin Time (PT/INR) [46]. It has been shown to prognosticate the risk of mortality in patients undergoing abdominal, orthopedic and cardiac surgery with 30-day mortality ranging from 5.7% for a MELD score </= 7, 10.3% for MELD Score of 8-11 and 25.4% for MELD score of 12-15 with emergency surgery being an independent predictor of the duration of hospital stay [47]. The risk of mortality increased linearly for MELD Scores above 8.

However, none of the scoring systems have been studied to stratify the risk of morbidity or mortality in patients undergoing emergency laparotomy. Possibly, this subset of patients with liver disease, depending on the preexisting hepatic insult, the hemodynamic instability and the nature of surgery, would be at a much higher risk as compared to those without pre-existing liver disease. The existing scoring system needs to be further validated in these patients.

5.3. Colorectal Surgery: AFC-index and Cleveland clinic colorectal cancer model

Although the APACHE, ASA or POSSUM or its modified form for colorectal surgery have been most commonly been used in these subset of patients in whom the survival has increased substantially over the last 25 years, the French Association for Surgery (Association Française de Chirurgie, AFC) identified independent factors leading to death by multivariate logistic regression analysis and developed the AFC-index [48]. The four independent preoperative risk factors,namely emergency surgery, loss of more than 10% of weight, neurological disease history and age > 70 years have been shown to predict mortality with the same sensitivity and specificity as P-POSSUM [48]. An AFC Score of 0 was associated with a mortalityof 0.5%, score of 1 with a mortality of 1.6%, score of 2 with a mortality of 7.2%, score of 3 with a mortality of 46.8% and a score of 4 was associated with a mortality of 70% [48]. Although the study by Arnaud Alves *et al*. did report a postoperative morbidity of 23% in 239 studied patients, the did not establish any correlationwith the AFC-index [48].

The Cleveland clinic colorectal cancer model was developed using a multilevel Bayesian logistic regression model and it identified age, ASA grade, TNM Staging, urgent need for surgery, cancer resection status and the hematocrit levels as independent risk factors for mortality [49]. The model offered excellent correlation between the observed and predicted mortality and an area under receiver operator characteristic (ROC) curve (AUC) of 0.801 [49].

6. Surgical audit scoring systems

6.1. Possum & its varients

Copeland *et al*. first described POSSUM (Physiological and Operative severity for the enumeration of mortality and morbidity) in 1991 as a scoring system for surgical audit [8]. They used logistic regression analysis to predict both morbidity and mortality. However, it was found to over predict death, especially amongst the low risk patients [50]. This led to the modification of the logistic regression and development of the Portsmouth POSSUM (P-POSSUM) [50]. P-POSSUM used the same physiological and operative scoring methods initially described by Copeland *et al.* and its predicted mortality matched with the observed mortality [51]. It uses 12 physiological and 6 operative parameters which were divided into 4 grades with exponentially increasing score (1, 2, 4, and 8) to calculate the risk of mortality. The minimum score is 12 and maximum score is 88, with higher scores predicting higher mortality.

POSSUM has subsequently been modified for application in various types of surgeries, O-POSSUM for orthopedic surgeries [52, 53], V-POSSUM for vascular surgeries [54] and Cr-POSSUM for colorectal surgeries [55].

P-POSSUM still remains the scoring system of choice for general surgeries and also for emergency laparotomies, especially in the United Kingdom. Numerous studies have validated POSSUM or one of its variants in general surgery, laparotomy or in high risk patients (Table 2 [56-67]).

P-POSSUM has emerged as the most dependable scoring system for audit purposes and for evaluating the impact of quality improvement initiatives across the United Kingdom in patients undergoing emergency laparotomy. In a recent multicentre study across four National Health Service (NHS) hospitals, ELPQuiC bundles (Table 3) brought about a significant reduction in P-POSSUM risk-adjusted 30-day mortality in patients undergoing [13].

Sreeharsha H *et al*. used linear analysis for comparing the observed and predicted mortality using POSSUM. The observed to predicted ratio (O: P) was 0.71 and there was "no statistically significant difference between the predicted and observed values" [67]. An O:P ratio of 1.19 suggested that there was no significant difference between the observed and predicted morbidity also. Chieng *et al*. observed P-POSSUM (O: P Ratio 0.721) to be a "better scoring system" compared to POSSUM (O: P Ratio 0.366) [11].

6.2. Surgical mortality probability model (S-MPM)

The S-MPM [68] is a 9-point 30-day mortality risk index. Patients were assigned points as per ASA Status (O for ASA I, 2 for ASA II, 4 for ASA III, 5 for ASA IV and 6 for ASA V), risk of surgery (1 for intermediate risk and 2 for high-risk procedures) and for emergency surgery (1 point). A total risk score <5 was associated with 0.5% mortality, score of 5-6 with 1.5-4% mortality and scores >6 with 10% mortality.

While this simple scoring system can be easily calculated at the bed-side and "used by surgeons andhospitals to internally audit their quality of care", it can also help risk-prognostication and prioritization of patients [68]. This scoring system is fairly accurate as compared to ACS NSQIP mortality model with "slightly worse discrimination and marginally better calibration" [68]. S-MPM

Table 2 − Use of POSSUM or one of its variants in general surgery, laparotomy or high risk surgical patients.

Year	Patient Category	Outcome
2004	Patients needing damage control laparotomy	Lower mortality than that predicted by P-POSSUM and POSSUM with Damage Control Surgery [56]
2004	Patients undergoing emergency laparotomy	POSSUM is a good predictor of morbidity and mortality. P-POSSUM predicts mortality equally well. Both can be used for risk-adjusted surgical audit [57]
2005	High risk patients undergoing surgery	p-POSSUM predicted mortality well but POSSUM over-predicted mortality [58]
2006	elective and emergency laparotomy	It is a useful predictor of morbidity and mortality [59]
2007	General surgery	M-POSSUM correlates better with postoperative complications and mortality than POSSUM [60]
2008	cases of ileal perforations	Significant correlation between POSSUM score and postoperative complications and deaths [61]
2009	Patients undergoing emergency laparotomy	P-POSSUM predicts mortality better than POSSUM. Exponential method is better than linear regression analysis [62]
2009	Unresectable pancreatic cancer during exploratory laparotomy	POSSUM scoring system is an independent predictor of survival in multivariate analysis [63]
2009	oncologic gastric surgery	Mortality lower than that predicted by POSSUM and higher than that predicted by P-POSSUM [64]
2010	patients undergoing emergency surgery	ASA grade and POSSUM scores were the better predictors of mortality than EWS, APACHE II, and age [65].
2010	general surgical laparotomy	P-POSSUM is a better overall predictor of mortality than POSSUM [11].
2011	General surgical patients	Both POSSUM and P-POSSUM are valid indices for risk prediction of morbidity and mortality [66]
2012	secondary peritonitis of colorectal origin	CR-POSSUM had the highest sensitivity and specificity to predict mortality as compared to MPI & APACHE-II [35]
2014	Emergency laparotomy	POSSUM is an accurate predictor of mortality and morbidity and can be used for surgical audit [67]

Table 3 − Evidence-based care bundle for patients undergoing emergency laparotomy [13].

Bundle	Element
1	Early warning score assessment for all emergency admissions with graded escalations
2	All patients with suspicion of peritoneal soiling or diagnosis of sepsis to receive early broad-spectrum antibiotics
3	Laparotomy within 6 hours of decision to operate
4	Goad directed resuscitation as soon as possible, or within 6 hours of admission
5	ICU admission for all patients in the immediate post-operative period

using only 3 predictors had an ability for discrimination with C-Statistic (which predicts that outcome is better than chance) of 0.90 as compared to the ACS NSQIP risk adjustment model using 35 variables which has a C-Statistic of 0.94. This model has only been applied on limited types of procedures and its validity specifically in emergency laparotomy remains to be ascertained.

7. Conclusion

Although numerous scoring systems for risk prognostication (Table 4) has been developed since the ASA Score was first introduced in 1941 [68], none have been able to comprehensively achieve the goals of being easy to calculate, fairly accurate in its prediction, reproducible across geographical locations, able to audit surgical outcomes across hospitals and assess the change brought about by any quality improvement initiatives.

Emergency laparotomies remain the commonest emergency surgery across most hospitals continue to be associated with significantly higher mortality as compared to most other major general surgical procedures or elective surgeries [1]. Therefore, a scoring system is not only necessary to predict mortality in this category of patients, initiative resulting in improved quality of care should also reflect on the surgical risk adjusted mortality. Emergency laparotomies involves "considerable cost" [69] to the healthcare providing agencies or the individual, either directly or through insurance. Similarly, quality improvement initiatives like availability of operation theatre space or consultant coverage round the clock would also involve financial implications. Therefore studies like those Huddart S. *et al.* (on behalf of the ELPQuiC Collaborator Group) [38] which showed that evidence based care bundles (Table 3) saved 8.11 lives per 100 patients treated, could also justify the efforts to enhance the quality of care and also its financial impact. Besides, it would also help the

Table 4 − Scoring systems for emergency laparotomy.

Scoring System		Preoperative risk evaluation possible
General Scoring Systems		
	ASA	Yes
	Apgar Score for Surgery	No
	Sickness Assessment (SA)	Yes
	Calculation of post-Operative Risk in Emergency Surgery (CORES)	Yes
	Estimation of Physiologic Ability and Surgical Stress (E-PASS)	No
	ACS NSQIP Surgical Risk Calculator	Yes
The Critical Care & Sepsis Scoring Systems		
	APACHE II	Yes
	Simplified Acute Physiology Score (SAPS)	Yes
	Multiple Organ Dysfunction Score (MODS)	Yes
	Sepsis-related Organ Failure Assessment (SOFA) score	Yes
	Sepsis Score	Yes
	Multiple Organ Failure (MOF) Score	Yes
	Mannheim peritonitis index (MPI)	No
The disease specific scoring systems		
Perforated Peptic Ulcer Scoring Systems	Hacettepe score	Yes
	Boey score	Yes
	Jabalpur score	Yes
	Peptic Ulcer Perforation (PULP) score	Yes
Patients with Liver Disease	Child-Turcotte-Pugh (CTP) classification	Yes
	MELD (model for end stage liver disease) score	Yes
Colorectal Surgery	AFC-index	Yes
	Cleveland clinic colorectal cancer model	No
Surgical Audit Scoring systems	POSSUM & its variants	No
	Surgical Mortality Probability Model (S-MPM)	No

medical fraternity in identifying those quality improvement initiatives which actually bring about a risk-adjusted benefit, as compared to those, which in absence of consensus, are being practiced merely based on individual perception [70].

Till very recently, ease of calculation, especially at the bedside, used to be an extremely essential criteria for any scoring system. However, the advent of smart phones and mobile applications have made the use of even intricate scoring systems like the APACHE-II, P-POSSUM and ACS NSQIP Surgical Risk Calculator, very simple. Today, hand-held devices like smartphones, personal digital assistants (PDAs) or tablets allows us to use complex formulas and various regression models to calculate the risk at the patient's bedside.

APACHE-II and P-POSSUM remain the most commonly used scoring system in emergency laparotomies (Table 1 & Table 2). Although P-POSSUM has been most frequently used for audit purposes in this cohort, it is associated with certain limitations. Operative variables such as estimated blood loss or peritoneal contamination may have significant inter-observer bias. A similar surgery by two different surgeons, one causing or estimating higher blood-loss than the other, will cause a change in the observed to expected (O/E) risk ratio. Besides, the delay in getting histopathology reports can also delay the risk assessment. APACHE-II scores can however be calculated preoperatively and has been shown to correlate well with postoperative mortality. However, unlike P-POSSUM, it does not consider etiology or degree of peritoneal contaminationand is purely based on the acute physiologic and chronic health status of the patient. While it does eliminate risk assessment based on subjective evaluation of certain risks in the P-POSSUM scoring system (example, peritoneal soiling or estimated blood loss), it does not consider the surgical procedure or the operative findings. However, it does factor-in emergency surgeries while calculating the risk.

Large multicentric studies across varied geographic locations and surgical practices need to assess and validate the ideal and most apt scoring system for emergency laparotomies. Studies need to compare APACHE-II and P-POSSUM in their ability to predict mortality and explore if either has a higher sensitivity and specificity than the other. Any impact on the risk-adjusted mortality can bring about significant reduction in mortality amongst patients undergoing one of the commonest emergency surgeries worldwide.

REFERENCES

[1] Saunders DI, Murray D, Pichel AC, Varley S, Peden CJ. UK Emergency Laparotomy Network. Variations in mortality after emergency laparotomy: the first report of the UK Emergency Laparotomy Network. Br J Anaesth. 2012 Sep; 109(3): 368-75. Epub 2012 Jun 22 [DOI:10.1093/bja/aes165]

[2] Vester-Andersen M, Lundstrøm LH, MøllerMH,Waldau T, Rosenberg J, Møller AM. Danish Anaesthesia Database. Mortality and postoperative care pathways after emergency gastrointestinal surgery in 2904 patients: a population-based cohort study. Br J Anaesth 2014; 112: 860-70.

[3] Ashish Ahuja, Ravinder Pal. Prognostic scoring indicator in evaluation of clinical outcome in intestinal perforations. J Clin Diagn Res 2013; 7: 1953-5.

[4] Mercer SJ, Guha A, Ramesh VJ. The P-POSSUM scoring systems for predicting the mortality of neurosurgical patients undergoing craniotomy: further validation of usefulness and application across healthcare systems. Indian J Anaesth 2013; 57: 587-91.

[5] Neary WD, Heather BP, Earnshaw JJ. The Physiological and Operative Severity Score for the enumeration of Mortality and morbidity. Br J Surg 2003; 90: 157-65.

[6] Knaus W, Wagner D, Draper E. The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalized adults. Chest 1991; 100: 1619-36.

[7] Chang R. Individual outcome prediction models for predictive care unit. Lancet 1989; 11: 143-6.

[8] Copeland GP, Jones D, Walters M. POSSUM: A scoring system for surgical audit. Br J Surg 1991; 78: 355-60.

[9] Bann SD. Comparative Audit: The trouble with POSSUM. JR Soc Med 2001; 94: 632-4.

[10] Rix TE, Bates T. Pre-operative risk scores for the prediction of outcome in elderly people who require emergency surgery. World J Emerg Surg 2007; 2: 16.

[11] Chieng TH, Roslan AC, Chuah JA. Risk-adjusted analysis of patients undergoing laparotomy using POSSUM and P-POSSUM score in Queen Elizabeth Hospital, Sabah. Med J Malaysia. 2010 Dec; 65: 286-90. [PMID: 21901947]

[12] Bilimoria KY, Liu Y, Paruch JL, Zhou L, Kmiecik TE, Ko CY, *et al*. Development and evaluation of the universal ACS NSQIP surgical risk calculator: a decision aid and informed consent tool for patients and surgeons. J Am Coll Surg 2013 Nov; 217(5): 833-42.e1-3. doi: 10.1016/j.jamcollsurg.2013.07.385. Epub 2013 Sep 18.PMID: 24055383

[13] Huddart S, Peden CJ, Swart M, McCormick B, Dickinson M, Mohammed MA, *et al*. ELPQuiC Collaborator Group; ELPQuiC Collaborator Group. Use of a pathway quality improvement care bundle to reduce mortality after emergency laparotomy. Br J Surg 2015 Jan; 102(1):57-66. doi: 10.1002/bjs.9658. Epub 2014 Nov 10.PMID: 25384994

[14] Sankar A, Johnson SR, Beattie WS, Tait G, Wijeysundera DN. Reliability of the American Society of Anesthesiologists physical status scale in clinical practice. Br J Anaesth 2014 Sep; 113(3): 424-32. doi: 10.1093/bja/aeu100. Epub 2014 Apr 11. PMID: 24727705

[15] Thorsen K, Søreide JA, Søreide K. Scoring systems for outcome prediction in patients with perforated peptic ulcer. Scand J Trauma ResuscEmerg Med 2013 Apr 10; 21: 25.

[16] Gawande AA, Kwaan MR, Regenbogen SE, Lipsitz SA, Zinner MJ. An Apgar score for surgery. J Am Coll Surg 2007 Feb; 204(2): 201-8. Epub 2006 Dec 27. PMID: 17254923

[17] Kennedy RH, al-Mufti RA, Brewster SF, Sherry EN, Magee TR, Irvin TT. The acute surgical admission: is mortality predictable in the elderly? Ann R CollSurg Engl 1994 Sep; 76(5): 342-5. PMID: 7979079

[18] Miyazaki N, Haga Y, Matsukawa H, Ishimura T, Fujita M, Ejima T, *et al*. The development and validation of the Calculation of post-Operative Risk in Emergency Surgery (CORES) model. Surg Today. 2014 Aug; 44(8): 1443-56. doi: 10.1007/s00595-013-0707-1. Epub 2013 Aug 31. PMID: 21969149

[19] Oka Y, Nishijima J, Oku K, Azuma T, Inada K, Miyazaki S, *et al*. Usefulness of an estimation of physiologic ability and surgical stress (E-PASS) scoring system to predict the incidence of postoperative complications in gastrointestinal surgery. World J Surg 2005; 29: 1029-33.

[20] Koushi K, Korenaga D, Kawanaka H, Okuyama T, Ikeda Y, Takenaka K. Using the E-PASS scoring system to estimate the risk of emergency abdominal surgery in patients with acute gastrointestinal disease. Surg Today. 2011 Nov; 41(11): 1481-5. doi: 10.1007/s00595-010-4538-z. Epub 2011 Oct 4. PMID: 21969149

[21] Quan H, Li B, Couris CM, Fushimi K, Graham P, Hider P, *et al*. Updating and validating the Charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. Am J Epidemiol 2011 Mar 15; 173(6): 676-82. doi: 10.1093/aje/kwq433. Epub 2011 Feb 17.

[22] Knaus WA, Zimmerman JE, Wagner DP, Draper EA, Lawrence DE. APACHE-acute physiology and chronic health evaluation: a physiologically based classification system. Crit Care Med 1981; 9: 591-7.

[23] Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med 1985 Oct; 13(10): 818-29 Knaus WA, Draper EA, Wagner DP, Zimmerman JE.APACHE II: a severity of disease classification system. Crit Care Med 1985 Oct; 13(10): 818-29.

[24] Vincent JL, Moreno R. Clinical review: scoring systems in the critically ill. Crit Care 2010; 14(2): 207. doi: 10.1186/cc8204. Epub 2010 Mar 26.

[25] Schein M, Gecelter G, Freinkel Z, Gerding H. APACHE II in emergency operations for perforated ulcers. Am J Surg 1990; 159: 309-13.

[26] Bosscha K, Reijnders K, Hulstaert PF, Algra A, van der Werken C. Prognostic scoring systems to predict outcome in peritonitis and intra-abdominal sepsis. Br J Surg 1997; 84: 1532-4.

[27] Kulkarni SV, Naik AS, Subramanian N Jr. APACHE-II scoring system in perforative peritonitis. Am J Surg 2007; 194: 549-52.

[28] Ding LA, Sun LQ, Chen SX, Qu LL, XieDF. Modified physiological and operative score for the enumeration of mortality and morbidity risk assessment model in general surgery. World J Gastroenterol 2007; 13: 5090-5.

[29] Koç M, Yoldaş O, Kiliç YA, Göçmen E, Ertan T, Dizen H, *et al*. Comparison and validation of scoring systems in a cohort of patients treated for perforated peptic ulcer. Langenbecks Arch Surg 2007; 392: 581-5.

[30] Malik AA, Wani KA, Dar LA, Wani MA, Wani RA, Parray FQ. Mannheim Peritonitis Index and APACHE II--prediction of outcome in patients with peritonitis. Ulus Travma Acil Cerrahi Derg 2010;

16: 27-32.
[31] Berreta J, Kociak D, Balducci A, De Feo F, Laplacette MV, Bellido F, *et al*. Generalized secondary peritonitis: predictors of in-hospital mortality and survival and mortality evolutive links. Acta Gastroenterol Latinoam 2010; 40: 105-16.
[32] Aslar AK, Ozdemir S, Mahmoudi H, Kuzu MA. Analysis of 230 cases of emergent surgery for obstructing colon cancer--lessons learned. J Gastrointest Surg 2011 Jan; 15(1):110-9. doi: 10.1007/s11605-010-1360-2. Epub 2010 Oct 26.
[33] Delibegovic S, Markovic D, Hodzic S. APACHE II scoring system is superior in the prediction of the outcome in critically ill patients with perforative peritonitis. Med Arh 2011; 65: 82-5.
[34] van Ruler O, Kiewiet JJ, Boer KR, Lamme B, Gouma DJ, Boermeester MA, *et al*. Failure of available scoring systems to predict ongoing infection in patients with abdominal sepsis after their initial emergency laparotomy. BMC Surg 2011 Dec 23; 11:38. doi: 10.1186/1471-2482-11-38.
[35] Viehl CT, Kraus R, Zürcher M, Ernst T, Oertli D, Kettelhack C. The Acute Physiology and Chronic Health Evaluation II score is helpful in predicting the need of relaparotomies in patients with secondary peritonitis of colorectal origin. Swiss Med Wkly 2012; 142: w13640.
[36] Thorsen K, Søreide JA, Søreide K. Scoring systems for outcome prediction in patients with perforated peptic ulcer. Scand J Trauma Resusc Emerg Med 2013; 21: 25.
[37] Das K, Ozdogan M, Karateke F, Uzun AS, Sozen S, Ozdas S. Comparison of APACHE II, P-POSSUM and SAPS II scoring systems in patients underwent planned laparotomies due to secondary peritonitis. Ann Ital Chir 2014; 85: 16-21.
[38] Ausania F, Guzman Suarez S, Alvarez Garcia H, Senra Del Rio P, CasalNuñez E. Gallbladder perforation: morbidity, mortality and preoperative risk prediction. Surg Endosc 2015 Apr; 29(4): 955-60. doi: 10.1007/s00464-014-3765-6. Epub 2014 Aug 27.
[39] V A M, C P M, S S, Srinivasarangan M. Efficacy of Mannheim Peritonitis Index (MPI) Score in Patients with Secondary Peritonitis. J Clin Diagn Res 2014 Dec; 8(12): NC01-3. doi: 10.7860/JCDR/2014/8609.5229. Epub 2014 Dec 5.
[40] Qureshi AM, Zafar A, Saeed K, Quddus A. Predictive power of Mannheim Peritonitis Index. J Coll Physicians Surg Pak. 2005; 15: 693-6.
[41] Boey J, Wong J, Ong GB. A prospective study of operative risk factors in perforated duodenal ulcers. Ann Surg 1982; 195: 265-9.
[42] Boey J, Choi SK, Poon A, Alagaratnam TT. Risk stratification in perforated duodenal ulcers. A prospective validation of predictive factors. Ann Surg 1987; 205: 22-6.
[43] Altaca G, Sayek I, Onat D, Cakmakçi M, Kamiloğlu S. Risk factors in perforated peptic ulcer disease: comparison of a new score system with the Mannheim Peritonitis Index. Eur J Surg. 1992; 158: 217-21.
[44] Mishra A, Sharma D, Raina VK. A simplified prognostic scoring system for peptic ulcer perforation in developing countries. Indian J Gastroenterol 2003; 22: 49-53.
[45] Møller MH, Engebjerg MC, Adamsen S, Bendix J, Thomsen RW. Acta Anaesthesiol Scand. The Peptic Ulcer Perforation (PULP) score: a predictor of mortality following peptic ulcer perforation. A cohort study. 2012 May; 56(5): 655-62. doi: 10.1111/j.1399-6576.2011.02609.x. Epub 2011 Dec 23.
[46] Lawrence S, Friedman MD. Surgery in the Patient with Liver Disease. Trans Am Clin Climatol Assoc. 2010; 121: 192-205. PMCID: PMC2917124
[47] Teh SH, Nagorney DM, Stevens SR, Offord KP, Therneau TM, Plevak DJ, *et al.* Risk factors for mortality after surgery in patients with cirrhosis. Gastroenterology. 2007 Apr; 132(4): 1261-9. Epub 2007 Jan 25.48.
[48] Alves A, Panis Y, Mantion G, Slim K, Kwiatkowski F, Vicaut E. The AFC score: validation of a 4-item predicting score of postoperative mortality after colorectal resection for cancer or diverticulitis: results of a prospective multicenter study in 1049 patients. Ann Surg 2007; 246: 91-6.
[49] Fazio VW, Tekkis PP, Remzi F, Lavery IC. Assessment of operative risk in colorectal cancer surgery: the cleveland clinic foundation colorectal cancer model. Dis Colon Rectum 2004; 47: 2015-24.
[50] Whiteley MS, Prytherch D, Higgins B, Weaver PC, Prout WG. An evaluation of the POSSUM surgical scoring system. Br J Surg 1996; 83: 812-15.
[51] Prytherch DR, Whiteley MS, Higgins B, Weaver PC, Prout WG, Powell SJ. POSSUM and Portsmouth POSSUM for predicting mortality. Physiological and operative severity score for the enumeration of mortality and morbidity. Br J Surg 1998; 85: 1217-20.
[52] Mohamed K, Copeland GP, Boot DA, Casserley HC, Shackleford IM, Sherry PG, *et al*. An assessment of the POSSUM system in orthopaedic surgery. Journal of Bone & Joint Surgery - British Volume 2002; 84: 735-9.
[53] Kurita M, Ichioka S, Tanaka Y, Umekawa K, Oshima Y, Ohura N, *et al*. Validity of the orthopedic POSSUM scoring system for the assessment of postoperative mortality in patients with pressure ulcers. Wound Repair Regen. 2009 May-Jun; 17(3):312-7. doi: 10.1111/j.1524-475X.2009.00486.x.
[54] Mosquera D, Chiang N, Gibberd R. Evaluation of surgical performance using V-POSSUM risk-adjusted mortality rates. ANZ J Surg 2008 Jul; 78(7): 535-9. doi: 10.1111/j.1445-2197.2008.04567.x.
[55] Ozkan O, Guner A, Kaya U, Kece C, Reis E, Kesici S. Evaluation of CR-POSSUM, original ACPGBI and new ACPGBI scoring systems for colorectal cancer surgery. Chirurgia (Bucur) 2014; 109: 800-5.
[56] Finlay IG, Edwards TJ, Lambert AW. Damage control laparotomy. Br J Surg 2004; 91: 83-5.
[57] Mohil RS, Bhatnagar D, Bahadur L, Rajneesh, Dev DK, Magan M. POSSUM and P-POSSUM for risk-adjusted audit of patients undergoing emergency laparotomy. Br J Surg 2004; 91: 500-3.
[58] Brooks MJ, Sutton R, Sarin S. Comparison of Surgical Risk Score, POSSUM and p-POSSUM in higher-risk surgical patients. Br J Surg 2005; 92: 1288-92.
[59] Campillo-Soto A, Flores-Pastor B, Soria-Aledo V, Candel-Arenas M, Andrés-García B, Martín-Lorenzo JG, *et al*. The POSSUM scoring system: an instrument for measuring quality in surgical patients. Cir Esp 2006; 80: 395-9.
[60] Ding LA, Sun LQ, Chen SX, Qu LL, XieDF. Modified physiological and operative score for the enumeration of mortality and morbidity risk assessment model in general surgery. World J Gastroenterol 2007; 13: 5090-5.
[61] Mohil RS, Singh T, Arya S, Bhatnagar D. Risk adjustment is crucial in comparing outcomes of various surgical modalities in patients with ileal perforation. Patient Saf Surg 2008; 2: 31. doi:

10.1186/1754-9493-2-31.

[62] Kumar P, Rodrigues GS. Comparison of POSSUM and P-POSSUM for risk-adjusted audit of patients undergoing emergency laparotomy. Ulus Travma Acil Cerrahi Derg 2009; 15: 19-22.

[63] de Castro SM, Houwert JT, Lagard SM, Busch OR, van Gulik TM, Gouma DJ. POSSUM predicts survival in patients with unresectable pancreatic cancer. Dig Surg 2009; 26: 75-9. doi: 10.1159/000194982. Epub 2009 Jan 23.

[64] Luna A, Rebasa P, Navarro S, Montmany S, Coroleu D, Cabrol J, *et al.* An evaluation of morbidity and mortality in oncologic gastric surgery with the application of POSSUM, P-POSSUM, and O-POSSUM. World J Surg. 2009 Sep;33(9):1889-94. doi: 10.1007/s00268-009-0118-z.

[65] Garcea G, Ganga R, Neal CP, Ong SL, Dennison AR, Berry DP. Preoperative early warning scores can predict in-hospital mortality and critical care admission following emergency surgery. J Surg Res. 2010 Apr; 159(2): 729-34. doi: 10.1016/j.jss.2008.08.013. Epub 2008 Nov 12.

[66] Yadav K, Singh M, Griwan M, Mishra Ts, Kumar N, Kumar H. Evaluation of POSSUM and P-POSSUM as a tool for prediction of surgical outcomes in the Indian population. Australas Med J. 2011; 4(7): 366-73. doi: 10.4066/AMJ.2011558. Epub 2011 Jul 31.

[67] Sreeharsha H, Sp R, Sreekar H, Reddy R. Efficacy of POSSUM score in predicting the outcome in patients undergoing emergency laparotomy. Pol Przegl Chir 2014 Apr; 86(4): 159-65. doi: 10.2478/pjs-2014-0029.

[68] Glance LG, Lustik SJ, Hannan EL, Osler TM, Mukamel DB, Qian F, *et al*. The Surgical Mortality Probability Model: derivation and validation of a simple risk prediction rule for noncardiac surgery. Ann Surg 2012 Apr; 255(4): 696-702. doi: 10.1097/SLA.0b013e31824b45af.

[69] Shapter SL, Paul MJ, White SM. Incidence and estimated annual cost of emergency laparotomy in England: is there a major funding shortfall?. Anaesthesia 2012 May; 67(5): 474-8. doi: 10.1111/j.1365-2044.2011.07046.x.

[70] Nightingale JJ, Burmeister L, Hopkins D. A national survey of the use of epidural analgesia in patients with sepsis undergoing laparotomy. Anaesthesia 2011 Apr; 66(4): 311-2. doi: 10.1111/j.1365-2044.2011.06672.x.

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Review article

Nutrition, psychoneuroimmunology and depression: the therapeutic implications of omega-3 fatty acids in interferon-α-induced depression

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ABSTRACT

The unmet need of current pharmacotherapy and the high occurrence of somatic symptoms and physical illness in depression imply that the 'monoamine hypothesis' is insufficient in approaching the aetiology of depression. Clinically, depressed patients manifest higher levels of inflammatory biomarkers, while pro-inflammatory cytokines induce neuropsychiatric symptoms (sickness behaviour) as well as major depressive episodes. Indeed, accumulating evidence suggests that inflammation dysregulation plays an important role in the pathophysiology of depression. Biological mechanisms that link inflammation to neuropsychiatric symptoms are vital in the understanding of the "mind-body" interface. IFN-α-induced depression is the most powerful support for the inflammation theory of depression. This clinical observation provides an excellent model for depression research. By comparing subjects with and without major depression induced by the cytokine treatment, statistical powers could be largely increased by reducing phenotypic variation (homogeneity in aetiological factors). In addition, the anti-inflammatory pathway has recently become an important topic in looking for new antidepressant therapies. For example, anti-inflammatory compounds, omega-3 polyunsaturated fatty acids (omega-3 PUFAs or n-3 PUFAs), have been found to be associated with the development and treatment for depression in human and animal models. Here I review recent epidemiological studies, cross-sectional and longitudinal case-controlled studies, interventional clinical trials, as well as basic animal and cellular studies to prove the linkage among omega-3 PUFAs, inflammation, and depression.

1. Introduction

Major depressive disorder (MDD) is a serious psychiatric illness with a high lifetime prevalence rate of up to one-tenth or possibly even one fifth [1]. Nevertheless, available treatments fail to meet the clinical needs of patients adequately, making this illness difficult to treat and burdensome to a patient's life, family, and career. The growing burden of major depressive disorder (MDD) is evidenced by the projection that depression will become a leading cause of disease or injury worldwide by 2020 [2].

Clinical features, biological markers, and treatment outcomes for MDD are heterogeneous. Therefore, using our current diagnostic schemas undoubtedly contributes to the difficulty in finding any reliable biological markers for the disease [3]. According to the diagnostic criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, 5th Edition (DSM-5), and the *International Statistical Classification of Diseases and Related Health Problems*, 10th Revision (ICD-10), individuals within the same diagnostic categories of MDD may have distinct clinical manifestations. Furthermore, the diagnostic classification does not provide reliable or predictive effects in treatment efficacy and/or the ability to predict the occurrence of adverse effects associated with specific antidepressants. Accordingly, with unsatisfactory outcomes for all the antidepressant treatments and the small-to-moderate effect sizes from all the biomarker studies and clinical trials, it is impossible to explain the whole picture of the aetiology of MDD with any single hypothesis.

The heterogeneity of depression could also be reflected by the current classification system with monoamine reuptake mechanisms for antidepressant agents (Figure 1). For example, the selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), which inhibit serotonin reuptakes, are the most commonly prescribed antidepressant agents. However, tianepine, which enhances serotonin reuptakes,

Abbreviation: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TNF, tumor necrotic factor; AA, arachidonic acid; PUFAs, polyunsaturated fatty acids; HCV, patients with chronic hepatitis C viral; IL, interleukin; IFN, interferon.

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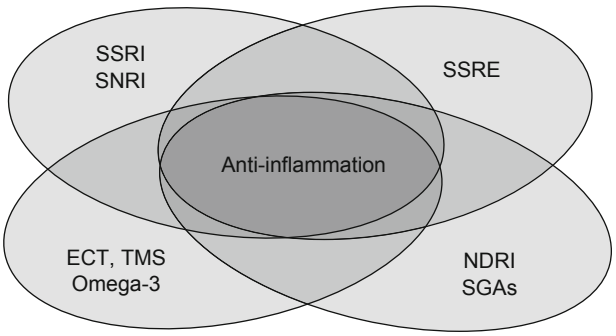

Fig. 1 - The current classification for antidepressant agents by monoamine reuptake mechanisms is insufficient to explain the aetiology of depression.
The heterogeneity of depression could be reflected by the limits of pharmacotherapy and pharmacological classification based on serotonin, norepinephrine, and dopamine. Controversially, the agents that inhibit (*i.e.*, SSRI & SNRI), enhance (*i.e.*, SSRE), or even neglect (*i.e.*, NDRI & SGAs) the serotonin reuptake action could all be approved to be antidepressant treatments, yet all of them seem to share the common mechanism of anti-inflammation. Interestingly, this common mechanisms is applicable not only for antidepressant agents from different categories but also for omega-3 PUFAs, electroconvulsive therapy (ECT) and repetitive transcranial magnetic stimulation (TMS).

is also approved as an antidepressant treatment. Furthermore, we have antidepressant agents that have nothing to do with serotonin reuptakes, such as norepinephrine-dopamine reuptake inhibitors (NDRIs) and second-generation antipsychotics (SDA). Indeed, the limits of pharmacotherapy and pharmacological classification based on serotonin, norepinephrine, and dopamine imply that the 'monoamine hypothesis' is woefully insufficient in approaching the aetiology of depression. Interestingly, all the antidepressant treatments seem to share the common mechanism of anti-inflammation.

Inflammation theory lights a promising path to resolve the dilemma of depression. Administration of therapeutic cytokine interferon-α (IFN-α) can lead to clinical depression [4-6]. In fact, looking for antidepressant therapies from anti-inflammatory pathways has become a hot topic in current medical research [7]. Chronic inflammation is linked with early childhood trauma, major psychiatric disorders, and several physical diseases [8, 9]; inflammation theory thus provides a clear window to investigate mind-body interface.

Omega-3 polyunsaturated fatty acids (omega-3 PUFAs or n-3 PUFAs) are anti-inflammatory and have been proposed to be associated with the neurobiology of depression. The human body holds two main serial types of PUFAs: omega-6 (n-6) derived from cis-linoleic acid (LA, 18:2) and omega-3 (n-3) derived from α-linolenic acid (ALA, 18:3). Omega-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and omega-6 PUFAs, arachidonic acid (AA), are important constituents of all cell membranes, essential for the survival of humans and other mammals [6, 10]. PUFAs appear active in biological functions; some of their functions require conversion to eicosanoids and products like prostaglandins (PGs), thromboxanes (TXs), and leukotrienes (LTs). A deficit of omega-3 PUFAs is reported to be associated with neurological, cardiovascular, cerebrovascular, autoimmune, and metabolic diseases, as well as bipolar disorder and depression [10]. This review summarizes current evidence about omega-3 PUFA biological mechanisms of and—inflammation in—depression.

2. Omega-3 fatty acids in depression

Societies with high consumption of fish in their diets appear to have a lower prevalence of MDD, mood disorders, coronary heart disease mortality, cardiovascular disease mortality, stroke mortality, and all-cause mortality [11], which implies a possibleprotective effect of omega-3 PUFAs in physical and psychiatric disorders. Consistent with epidemiological findings, patients with MDD show lower levels of omega-3 PUFAs in tissues of blood [12] and brain [13]. Deficits in omega-3 PUFA levels are reported in other populations with mood disorders: e.g., lower DHA and total omega-3 PUFAs in postpartum depression [14], lower DHA and EPA in social anxiety disorder [15], and lower DHA and AA in bipolar disorders [16].

Consistent with case-control studies of PUFA levels in human tissues, omega-3 PUFAs have been reported to be effective in treatment of MDD. Six meta-analytic reviews from four independent groups have reported on the antidepressant effect of PUFAs [17-22], yet three previous meta-analyses from the two of the four groups did not support these effects in heterogeneous populations (such as subclinical subjects in community samples) [23-26]. Negative findings must be interpreted with caution due to limitations: e.g., differing mood assessments, pooling heterogeneous populations, and implementing different intervention methods. For example, in a recent meta-analysis showing no benefits [24], their meta-analysis included clinical trials of enrolled individuals according to self-rating scales in settings like general practice surgery, a shopping mall and a university freshmen's fair [27]. It found no beneficial effects of omega-3 PUFAs and was weighted 31.7% of a pooled estimate among a total of 13 clinical trials. Similar results emerged from the meta-analytic review by Appleton *et al*. [26]. Take one clinical trial [28] included in Appleton's meta-analysis for example: Ness's study enrolled a relatively large number of patients, 452, yet it did not focus on treating depression or using appropriate tools for the diagnosis of—as well as the severity rating of—depression. Intervention with omega-3 PUFAs was defined as "advising" subjects with angina to "eat more fish." Treatment outcomes of omega-3 PUFAs in Ness's study were negative and contributed greatly to the pooled estimate in Appleton's meta-analysis.

Despite the uneven quality of published studies, recent meta-analytic evidence strongly supports the adjunctive use of omega-3 to treat bipolar depression [29]. However, studies regarding the effectiveness of omega-3 PUFAs in the acute manic phase of bipolar disorder are still lacking. To date, only one small double-blind placebo-controlled trial has been published, and it does not support omega-3 PUFAs' anti-manic effects [30, 31]. Omega-3 PUFAs offer promise in treating special populations with depression [32, 33]. Our 8-week double-blind, placebo-controlled study showed monotherapy with omega-3 PUFAs was associated with a significant improvement in depressive symptoms and a higher response rate in pregnant women with depression [34]. Most importantly, omega-3 PUFAs are safe for and well tolerated by depressed women during pregnancy and postpartum [35]. In addition, omega-3 PUFAs are proven effective and safe for children with depression [36], and supplementation with omega-3 PUFAs lowers the risk of suicide [37], alleviates MDD depressive symp-

toms associated with menopausal transition [38], and diminishes aggression in women with borderline personality disorder [39].

2.1. Safety and tolerability

Numerous clinical studies have shown omega-3 PUFAs are tolerated well by patients with chronic medical illnesses and mental disorders [6, 40-42]. Adverse reactions are rare; and if they occur at all, they usually involve belching, eructation or perhaps a fishy taste [43]. It is theorized that a potential anti-thrombotic effect of omega-3 PUFAs may increase the risk of bleeding. Clinical trials have shown that a high-dose consumption of omega-3 PUFAs is safe, even when concurrently administered with other agents that increase bleeding, such as aspirin and warfarin [40]. According to Harris's systematic review on 19 available clinical trials with n-3 PUFAs supplementation for patients with an already high risk of bleeding (n = 4397) [44], the risk of clinically significant bleeding was virtually nonexistent. Another potential safety concern is the susceptibility of omega-3 fatty acids to undergo oxidation, which may contribute to patient intolerance and potential toxicity. Yet it must be said that conclusions on this issue are quite inconsistent [42]. Adding an antioxidant like vitamin E to omega-3 PUFAs is a common way to reduce oxidation and rancidity, maintain freshness, and increase shelf life. The concurrent use of vitamin E with omega-3 PUFAs may also overcome the potential risk of oxidative stress,yet most published studies show either unchanged or decreased oxidation [42]. Given omega-3 PUFAs' antidepressant effects, another possible adverse effect is drug-induced mania. Yet until now, only one case report shows omega-3 PUFAs inducing hypomania [45]. A comprehensive assessment of manic symptoms in patients receiving omega-3 PUFAs is recommended for future clinical trials.

As depression is heterogeneous, any currently available antidepressant treatment only has modest effects. For example, the effect sizes of n-3 PUFAs in MDD treatment are only 0.17~0.23 [20, 22], which are similar to antidepressant drug treatments of 0.11 for mild to moderate, 0.17 for severe, and 0.47 for very severe MDD [46]. Therefore, it is of clinical interest to identify specific populations who might benefit from specific treatments.

3. Inflammation in depression

Accumulating evidence suggests that depression might be associated with activated inflammatory processes: e.g., depressed patients with elevated c-reactive protein (CRP), acute phase proteins, and pro-inflammatory cytokines [4, 7]. Depression is highly comorbid with chronic physical diseases [47]. In fact, children exposed to early-life adverse experiences display enduring low-grade systemic inflammation upon reaching adulthood [8], which is not only a risk factor for depression but also a feature of chronic physical diseases. Inflammation theory thus explains the high comorbidity of physical illness in depression and the potential "interface between mind and body [48]."

Systemic inflammatory challenges like lipopolysaccharide (LPS) or pro-inflammatory cytokine in experiments on animals cause behavioural changes induced by neuroinflammation that include anorexia, sleep abnormalities, reduction of locomotor activity and exploration, anhedonia, and cognitive disturbances, which share a strong similarity with the somatic symptoms of depression [4]. Sick individuals are often somewhat depressed and lethargic. The idea of a sickness's behaviour emanates from a series of observed symptoms related to infection and cytokine/prostaglandins administration in humans and animals. It offers a good model to study the effects of cytokine on the brain and behaviour [10, 49]. Excessive secretion of pro-inflammatory cytokines has been proposed to cause depression [50]. Microglia are the resident macrophages of the brain, and they act as the chief immune defense in the central nervous system (CNS) [51]. Upon activation, microglia up-regulate the expression of detrimental factors of reactive oxygen species such as nitric oxide *via* inducible nitric oxide synthase (iNOS) and induce oxidative stress, contributing to neuropsychiatric pathogenesis [52]. On the other hand, the expression of anti-oxidative enzymes like heme oxygenase-1 (HO-1) can reverse oxidative stress and may characterize antidepressant mechanisms [53, 54]. In addition, neuroinflammation reduces the survival of serotonergic neurons [55] and decreases neurogenesis [56], while antidepressants exert neuroprotection against microglia-mediated neurotoxicity [57].

3.1. IFN-α-induced depression

Substantiating evidence for the inflammation theory of depression is that interferon-alpha (IFN-α) induces clinical depression [5]. IFN-α is a standard cytokine therapy for chronic HCV infection, yet it associates with common and severe neuropsychiatric adverse effects. MDE during IFN-α therapy (IFN-α-induced depression) in patients with HCV is common, with incidence ranging from 23 to 45% [58]. Several biological mechanisms potentially play a role in this clinical phenomenon. For example, IFN-α-induced increases in IL-6 have been reported to predict development of depressive symptoms [59]. Cerebrospinal fluid (CSF) concentrations of 5-hydroxyindoleacetic acid (5-HIAA), but with no inflammatory markers, are associated with depressive symptoms induced by IFN-α [60]. Other studies are also mechanistically insightful by examining biomarkers such as plasma adrenocorticotropic hormone (ACTH), cortisol [61], serum tryptophan concentrations [62], and even brain function changes revealed in functional imagings [63]. Recent studies have identified genetic markers on serotonin transporters and interleukin-6 genes that seem to predict the development of IFN-α-induced depression [64].

4. Omega-3 fatty acids in interferon-α-induced depression

Chronic HCV infection is a major public health issue, and has a high rate of progression to liver cirrhosis and hepatocellular carcinoma [65, 66]. IFN-α is the standard therapy for chronic HCV infection, and will remain a cornerstone of therapy even with new combinations with ribavirin and protease inhibitors [67]. Because of the high rate of neuropsychiatric adverse effect like sickness behaviour and depression during IFN-α therapy, some clinicians consider adding prophylactic antidepressant use [68]. In patients with HCV infection, the prophylactic effects with SSRIs have been demonstrated by some [69-71], but not all [72-74] studies. Moreover, it has been associated with adverse events, including gastric discomfort, headache, dizziness, and an increased risk of rare but severe adverse effects, such as retinal haemorrhaging and cotton-wool spots [75, 76], bone marrow suppression, hepatotoxicity [74, 77], and manic episodes [78]. In addition, symptoms of IFN-α-induced sickness behaviour, once they develop, are only partially responsive to SSRIs [79]. As most patients receiving

IFN-α do *not* develop clinically significant depression with IFN-a therapy, the routine pre-treatment with antidepressant drugs might expose patients to unnecessary medications. It is thus important to find alternative strategies for the prevention of IFN-α-induced depression.

One advantage of nutritional medicine is its application in early intervention that can avoid unnecessary exposure to medication. Omega-3 PUFAs have been shown in numerous clinical studies to be tolerated well by patients with chronic medical illnesses, including liver diseases (Mori, 2004; Bays, 2006; Mozaffarian and Rimm, 2006; Bays, 2007; Lee *et al*., 2007). One of the hypothesized mechanisms underlying PUFAs' antidepressant effects is their anti-inflammatory action [10]. Moreover, omega-3 PUFAs have been found to have beneficial effects in cytokine-induced behavioural changes in animal models of depression [80, 81]. Of particular relevance, our previous study demonstrated that lower omega-3 PUFA levels in the peripheral blood are associated with an increased risk of developing IFN-α-induced depression over the following weeks [5]. Based on this and the other evidence discussed above, we further conducted a 2-week, double-blind, placebo-controlled trial, to test the differential effects of the omega-3 PUFAs, EPA and DHA, against a placebo, in the prevention of IFN-α-induced depression. We have specifically prescribed a short (2 weeks) intervention *before* IFN-α therapy, in order to potentially correct the lower omega-3 fatty acid levels that we had previously identified as a risk factor for the development of IFN-α-induced depression [5]. According to most studies, the active antidepressant component from omega-3 PUFAs is EPA [20, 22], but we also wanted to test DHA because, as mentioned above, we have found that lower levels of this omega-3 PUFA predispose patients to IFN-induced depression [5].

The results of that newly published clinical trial [41] support our previous findings, showing that omega-3 PUFAs play a role in the risk of IFN-α-induced depression. To summarize, the incident rates of IFN-α-induced depression were significantly lower in EPA-, but not in DHA-treated patients (rates: 10% and 28%, respectively, *vs*. 30% for placebo, $P = 0.037$), as compared with the placebo. Both EPA and DHA pre-treatment significantly delayed the onset of IFN-induced depression (average weeks of onset: 12.0 and 11.7, respectively, *vs*. 5.3 for placebo, $P = 0.002$). Previous clinical trials and meta-analyses have shown that the efficacy of omega-3 fatty acids as antidepressants might be dependent on the selection of the subject populations as well as the ratio of EPA and DHA, and have further suggested that EPA, rather than DHA, might be the most active component of omega-3 PUFAs' antidepressant effects [20, 22]. However, Mischoulon *et al*. found a dose-response effect supporting 1g/day as superior to 2 g/day or 4 g/day, though the latter study was limited by the lack of a placebo arm [82]. A recent meta-analysis has suggested that both EPA and DHA contribute to antidepressant effects, but that the effects of EPA are stronger [17]. Our current study, showing that EPA reduces the incidence of depression while DHA only delays the onset of depression, further supports this notion.

The anti-inflammatory action of omega-3 PUFAs is likely to be particularly important in the biological explanation for the antagonism of depressogenic effects of IFN-α. The model for IFN-α-induced depression reveals the increases of pro-inflammatory cytokines both in the periphery and in the brain of patients, with subsequent activation of the indoleamine 2,3-dioxygenase (IDO) pathway and the production of potentially depressogenic tryptophan metabolites, such as quinolinic acid [83]. EPA has numerous anti-inflammatory properties. Therefore, depressogenic mechanisms induced by proinflammatory cytokines and the IDO cascades are less likely to respond to standard antidepressants and more likely to respond to anti-inflammatory drugs [84-87]. In addition to this anti-inflammatory action, EPA and DHA may both exert their preventive effects also through neuroplasticity effects [88-90], which is a relevant molecular mechanism for antidepressant actions [91, 92].

5. Conclusions

The inflammation theory of depression draws support from several lines of evidence: e.g., increasing inflammatory biomarkers in clinical depression, and observed behavioral changes related to inflammatory activation. Interferon-α-induced depression in chronic HCV cases is the most notable clinical observation to support the inflammation theory of depression and an excellent model to probe the aetiology of depression in a prospective way. Anti-inflammatory omega-3 PUFAs prove beneficial in depression and several inflammation-related physical diseases. In addition, omega-3 PUFAs have been shown to have prophylactic effects in bipolar disorder [31, 42, 93, 94], psychotic transition in ultra-high risk individuals [95], and the development of post-traumatic stress disorder (PTSD) following accidental injury [96]. Furthermore, omega-3 PUFAs may particularly benefit children, pregnant women, and/or patients with comorbid cardiovascular or metabolic disorder, who all face greater risks of adverse effects from antidepressants, antipsychotics, and mood stabilizers. Therefore, our findings confirm and extend the notion that this nutritional intervention can have preventive effects in mental health populations, and they also corroborate the existing evidence that anti-inflammatory strategies may have antidepressant effects, especially in the context of depression associated with inflammation.

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REFERENCES

[1] Belmaker RH, Agam G. Major depressive disorder. N Engl J Med 2008; 358: 55-68.

[2] Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997; 349: 1498-504.

[3] Hasler G, Drevets WC, Manji HK, Charney DS. Discovering endophenotypes for major depression. Neuropsychopharmacology 2004; 29: 1765-81.

[4] Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol 2006; 27: 24-31.

[5] Su KP, Huang SY, Peng CY, Lai HC, Huang CL, Chen YC, *et al*. Phospholipase A2 and Cyclooxygenase 2 Genes Influence the Risk of Interferon-alpha-Induced Depression by Regulating Polyunsaturated Fatty Acids Levels. Biol Psychiatry 2010; 67: 550-7.

[6] Su KP. Inflammation in psychopathology of depression: Clinical, biological, and therapeutic implications. BioMedicine 2012; 2: 68-74.

[7] Maes M, Leonard B, Fernandez A, Kubera M, Nowak G, Veerhuis R, *et al*. (Neuro)inflammation and neuroprogression as new pathways and drug targets in depression: from antioxidants to kinase inhibitors. Prog Neuropsychopharmacol Biol Psychiatry 2011; 35: 659-63.

[8] Danese A, Moffitt TE, Pariante CM, Ambler A, Poulton R, Caspi A. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. Arch Gen Psychiatry 2008; 65: 409-15.

[9] Danese A, Pariante CM, Caspi A, Taylor A, Poulton R. Childhood maltreatment predicts adult inflammation in a life-course study. Proc Natl Acad Sci USA 2007; 104: 1319-24.

[10] Su KP. Biological Mechanism of Antidepressant Effect of Omega-3 Fatty Acids: How Does Fish Oil Act as a 'Mind-Body Interface'? Neurosignals 2009; 17: 144-52.

[11] Hibbeln JR, Nieminen LR, Blasbalg TL, Riggs JA, Lands WE. Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity. Am J Clin Nutr 2006; 83: 1483S-93S.

[12] Lin PY, Huang SY, Su KP. A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. Biol Psychiatry 2010; 68: 140-7.

[13] McNamara RK, Hahn CG, Jandacek R, Rider T, Tso P, Stanford KE, *et al*. Selective deficits in the omega-3 fatty acid docosahexaenoic acid in the postmortem orbitofrontal cortex of patients with major depressive disorder. Biol Psychiatry 2007; 62: 17-24.

[14] De Vriese SR, Christophe AB, Maes M. Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: further evidence that lowered n-PUFAs are related to major depression. Life Sci 2003; 73: 3181-7.

[15] Green P, Hermesh H, Monselise A, Marom S, Presburger G, Weizman A. Red cell membrane omega-3 fatty acids are decreased in nondepressed patients with social anxiety disorder. Eur Neuropsychopharmacol 2006; 16: 107-13.

[16] Chiu CC, Huang SY, Su KP, Lu ML, Huang MC, Chen CC, *et al*. Polyunsaturated fatty acid deficit in patients with bipolar mania. Eur Neuropsychopharmacol 2003; 13: 99-103.

[17] Sublette ME, Ellis SP, Geant AL, Mann JJ. Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression. J Clin Psychiatry 2011; 72: 1577-84.

[18] Freeman MP, Mischoulon D, Tedeschini E, Goodness T, Cohen LS, Fava M, *et al*. Complementary and alternative medicine for major depressive disorder: a meta-analysis of patient characteristics, placebo-response rates, and treatment outcomes relative to standard antidepressants. J Clin Psychiatry 2010; 71: 682-8.

[19] Freeman MP, Hibbeln JR, Wisner KL, Davis JM, Mischoulon D, Peet M, *et al*. Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. J Clin Psychiatry 2006; 67: 1954-67.

[20] Lin PY, Mischoulon D, Freeman MP, Matsuoka Y, Hibbeln J, Belmaker RH, *et al*. Are omega-3 fatty acids antidepressants or just mood-improving agents? The effect depends upon diagnosis, supplement preparation, and severity of depression. Mol Psychiatry 2012; 17: 1161-3.

[21] Lin PY, Su KP. A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids. J Clin Psychiatry 2007; 68: 1056-61.

[22] Martins JG, Bentsen H, Puri BK. Eicosapentaenoic acid appears to be the key omega-3 fatty acid component associated with efficacy in major depressive disorder: a critique of Bloch and Hannestad and updated meta-analysis. Mol Psychiatry 2012; 17: 1144-9.

[23] Appleton KM, Hayward RC, Gunnell D, Peters TJ, Rogers PJ, Kessler D, *et al*. Effects of n-3 long-chain polyunsaturated fatty acids on depressed mood: systematic review of published trials. Am J Clin Nutr 2006; 84: 1308-16.

[24] Bloch MH, Hannestad J. Omega-3 fatty acids for the treatment of depression: systematic review and meta-analysis. Mol Psychiatry 2012; 17: 1272-82.

[25] Appleton KM, Rogers PJ, Ness AR. Is there a role for n-3 long-chain polyunsaturated fatty acids in the regulation of mood and behaviour? A review of the evidence to date from epidemiological studies, clinical studies and intervention trials. Nutr Res Rev 2008; 21: 13-41.

[26] Appleton KM, Rogers PJ, Ness AR. Updated systematic review and meta-analysis of the effects of n-3 long-chain polyunsaturated fatty acids on depressed mood. Am J Clin Nutr 2010; 91: 757-70.

[27] Rogers PJ, Appleton KM, Kessler D, Peters TJ, Gunnell D, Hayward RC, *et al*. No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial. Br J Nutr 2008; 99: 421-31.

[28] Ness AR, Gallacher JE, Bennett PD, Gunnell DJ, Rogers PJ, Kessler D, *et al*. Advice to eat fish and mood: a randomised controlled trial in men with angina. Nutr Neurosci 2003; 6: 63-5.

[29] Sarris J, Mischoulon D, Schweitzer I. Omega-3 for bipolar disorder: meta-analyses of use in mania and bipolar depression. J Clin Psychiatry 2012; 73: 81-6.

[30] Chiu CC, Huang SY, Chen CC, Su KP. Omega-3 fatty acids are more beneficial in the depressive phase than in the manic phase in patients with bipolar I disorder. J Clin Psychiatry 2005; 66: 1613-4.

[31] Su KP, Shen WW, Huang SY. Are omega3 fatty acids beneficial in depression but not mania? Arch Gen Psychiatry 2000; 57: 716-7.

[32] Su KP, Shen WW, Huang SY. The use of omega-3 fatty acids for the management of depression and psychosis during pregnancy and breast-feeding. In: Peet M, Glen I, Horrobin DF, editors. Phospholipid spectrum disorder in psychiatry and neurology. 2 ed. Carnforth: Marius Press; 2003. pp. 391-9.

[33] Chiu CC, Huang SY, Shen WW, Su KP. Omega-3 fatty acids for depression in pregnancy. Am J Psychiatry 2003; 160: 385.

[34] Su KP, Huang SY, Chiu TH, Huang KC, Huang CL, Chang HC, *et al*. Omega-3 fatty acids for major depressive disorder during pregnancy: results from a randomized, double-blind, placebo-controlled trial. J Clin Psychiatry 2008; 69: 644-51.

[35] Freeman MP. Omega-3 fatty acids and perinatal depression: a review of the literature and recommendations for future research.

Prostaglandins Leukot Essent Fatty Acids 2006; 75: 291-7.
[36] Nemets H, Nemets B, Apter A, Bracha Z, Belmaker RH. Omega-3 treatment of childhood depression: a controlled, double-blind pilot study. Am J Psychiatry 2006; 163: 1098-100.
[37] Hallahan B, Hibbeln JR, Davis JM, Garland MR. Omega-3 fatty acid supplementation in patients with recurrent self-harm. Single-centre double-blind randomised controlled trial. Br J Psychiatry 2007; 190: 118-22.
[38] Freeman MP, Hibbeln JR, Silver M, Hirschberg AM, Wang B, Yule AM, *et al*. Omega-3 fatty acids for major depressive disorder associated with the menopausal transition: a preliminary open trial. Menopause 2011; 18: 279-84.
[39] Zanarini MC, Frankenburg FR. Omega-3 Fatty acid treatment of women with borderline personality disorder: a double-blind, placebo-controlled pilot study. Am J Psychiatry 2003; 160: 167-9.
[40] Bays HE. Safety considerations with omega-3 fatty acid therapy. Am J Cardiol 2007; 99: 35C-43C.
[41] Su KP, Lai HC, Yang HT, Su WP, Peng CY, Chang JP, *et al*. Omega-3 fatty acids in the prevention of interferon-alpha-induced depression: results from a randomized, controlled trial. Biol Psychiatry 2014; 76: 559-66.
[42] Su KP, Wang SM, Pae CU. Omega-3 polyunsaturated fatty acids for major depressive disorder. Expert Opin Investig Drugs 2013; 22: 1519-34.
[43] Bays H. Clinical overview of Omacor: a concentrated formulation of omega-3 polyunsaturated fatty acids. Am J Cardiol 2006; 98: 71i-6i.
[44] Harris WS. Expert opinion: omega-3 fatty acids and bleeding-cause for concern? Am J Cardiol 2007; 99: 44C-6C.
[45] Kinrys G. Hypomania associated with omega-3 fatty acids. Arch Gen Psychiatry 2000; 57: 715-6.
[46] Fournier JC, DeRubeis RJ, Hollon SD, Dimidjian S, Amsterdam JD, Shelton RC, *et al*. Antidepressant drug effects and depression severity: a patient-level meta-analysis. JAMA 2010; 303: 47-53.
[47] Katon W, Sullivan MD. Depression and chronic medical illness. J Clin Psychiatry 1990; 51 Suppl: 3-11.
[48] Su KP. Mind-body interface: the role of n-3 fatty acids in psychoneuroimmunology, somatic presentation, and medical illness comorbidity of depression. Asia Pac J Clin Nutr 2008; 17 Suppl 1: 151-7.
[49] Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci 2002; 25: 154-9.
[50] Smith RS. The macrophage theory of depression. Med Hypotheses 1991; 35: 298-306.
[51] Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 2007; 10: 1387-94.
[52] Lu DY, Leung YM, Su KP. Interferon-alpha induces nitric oxide synthase expression and haem oxygenase-1 down-regulation in microglia: implications of cellular mechanism of IFN-alpha-induced depression. Int J Neuropsychopharmacol 2013; 16: 433-44.
[53] Lu DY, Tsao YY, Leung YM, Su KP. Docosahexaenoic acid suppresses neuroinflammatory responses and induces heme oxygenase-1 expression in BV-2 microglia: implications of antidepressant effects for omega-3 fatty acids. Neuropsychopharmacology 2010; 35: 2238-48.
[54] Gozzelino R, Jeney V, Soares MP. Mechanisms of cell protection by heme oxygenase-1. Annu Rev Pharmacol Toxicol 2010; 50: 323-54.
[55] Hochstrasser T, Ullrich C, Sperner-Unterweger B, Humpel C. Inflammatory stimuli reduce survival of serotonergic neurons and induce neuronal expression of indoleamine 2,3-dioxygenase in rat dorsal raphe nucleus organotypic brain slices. Neuroscience 2011; 184: 128-38.
[56] Song C, Wang H. Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. Prog Neuropsychopharmacol Biol Psychiatry 2011; 35: 760-8.
[57] Zhang F, Zhou H, Wilson BC, Shi JS, Hong JS, Gao HM. Fluoxetine protects neurons against microglial activation-mediated neurotoxicity. Parkinsonism Relat Disord 2012; 18 Suppl 1: S213-S7.
[58] Asnis GM, De La GR. Interferon-induced depression in chronic hepatitis C: a review of its prevalence, risk factors, biology, and treatment approaches. J Clin Gastroenterol 2006; 40: 322-35.
[59] Bonaccorso S, Puzella A, Marino V, Pasquini M, Biondi M, Artini M, *et al*. Immunotherapy with interferon-alpha in patients affected by chronic hepatitis C induces an intercorrelated stimulation of the cytokine network and an increase in depressive and anxiety symptoms. Psychiatry Res 2001; 105: 45-55.
[60] Raison CL, Borisov AS, Majer M, Drake DF, Pagnoni G, Woolwine BJ, *et al*. Activation of central nervous system inflammatory pathways by interferon-alpha: relationship to monoamines and depression. Biol Psychiatry 2009; 65: 296-303.
[61] Capuron L, Raison CL, Musselman DL, Lawson DH, Nemeroff CB, Miller AH. Association of exaggerated HPA axis response to the initial injection of interferon-alpha with development of depression during interferon-alpha therapy. Am J Psychiatry 2003; 160: 1342-5.
[62] Capuron L, Neurauter G, Musselman DL, Lawson DH, Nemeroff CB, Fuchs D, *et al*. Interferon-alpha-induced changes in tryptophan metabolism. relationship to depression and paroxetine treatment. Biol Psychiatry 2003; 54: 906-14.
[63] Capuron L, Pagnoni G, Demetrashvili M, Woolwine BJ, Nemeroff CB, Berns GS, *et al*. Anterior cingulate activation and error processing during interferon-alpha treatment. Biol Psychiatry 2005; 58: 190-6.
[64] Bull SJ, Huezo-Diaz P, Binder EB, Cubells JF, Ranjith G, Maddock C, *et al*. Functional polymorphisms in the interleukin-6 and serotonin transporter genes, and depression and fatigue induced by interferon-alpha and ribavirin treatment. Mol Psychiatry 2009; 14: 1145.
[65] Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001; 345: 41-52.
[66] Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. Lancet 2003; 362: 2095-100.
[67] Schaefer M, Capuron L, Friebe A, ez-Quevedo C, Robaeys G, Neri S, *et al*. Hepatitis C infection, antiviral treatment and mental health: a European expert consensus statement. J Hepatol 2012; 57: 1379-90.
[68] Dieperink E, Ho SB, Thuras P, Willenbring ML. A prospective study of neuropsychiatric symptoms associated with interferon-alpha-2b and ribavirin therapy for patients with chronic hepatitis C. Psychosomatics 2003; 44: 104-12.
[69] Raison CL, Woolwine BJ, Demetrashvili MF, Borisov AS, Weinreib R, Staab JP, *et al*. Paroxetine for prevention of depressive symptoms induced by interferon-alpha and ribavirin for hepatitis C. Aliment Pharmacol Ther 2007; 25: 1163-74.

[70] de Knegt RJ, Bezemer G, Van Gool AR, Drenth JP, Hansen BE, Droogleever Fortuyn HA, *et al*. Randomised clinical trial: escitalopram for the prevention of psychiatric adverse events during treatment with peginterferon-alfa-2a and ribavirin for chronic hepatitis C. Aliment Pharmacol Ther 2011; 34: 1306-17.

[71] Schaefer M, Sarkar R, Knop V, Effenberger S, Friebe A, Heinze L, *et al*. Escitalopram for the Prevention of Peginterferon-alpha2a-Associated Depression in Hepatitis C Virus-Infected Patients Without Previous Psychiatric Disease: A Randomized Trial. Ann Intern Med 2012; 157: 94-103.

[72] Diez-Quevedo C, Masnou H, Planas R, Castellvi P, Gimenez D, Morillas RM, *et al*. Prophylactic treatment with escitalopram of pegylated interferon alfa-2a-induced depression in hepatitis C: a 12-week, randomized, double-blind, placebo-controlled trial. J Clin Psychiatry 2011; 72: 522-8.

[73] Morasco BJ, Loftis JM, Indest DW, Ruimy S, Davison JW, Felker B, *et al*. Prophylactic antidepressant treatment in patients with hepatitis C on antiviral therapy: a double-blind, placebo-controlled trial. Psychosomatics 2010; 51: 401-8.

[74] Morasco BJ, Rifai MA, Loftis JM, Indest DW, Moles JK, Hauser P. A randomized trial of paroxetine to prevent interferon-alpha-induced depression in patients with hepatitis C. J Affect Disord 2007; 103: 83-90.

[75] Hejny C, Sternberg P, Lawson DH, Greiner K, Aaberg TM, Jr. Retinopathy associated with high-dose interferon alfa-2b therapy. Am J Ophthalmol 2001; 131: 782-7.

[76] Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, *et al*. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med 2001; 344: 961-6.

[77] Loftis JM, Hauser P. Safety of the treatment of interferon-alpha-induced depression. Psychosomatics 2003; 44: 524-6.

[78] Wu PL, Liao KF, Peng CY, Pariante CM, Su KP. Manic episode associated with citalopram therapy for interferon-induced depression in a patient with chronic hepatitis C infection. Gen Hosp Psychiatry 2007; 29: 374-6.

[79] Capuron L, Gumnick JF, Musselman DL, Lawson DH, Reemsnyder A, Nemeroff CB, *et al*. Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. Neuropsychopharmacology 2002; 26: 643-52.

[80] Song C, Phillips AG, Leonard BE, Horrobin DF. Ethyl-eicosapentaenoic acid ingestion prevents corticosterone-mediated memory impairment induced by central administration of interleukin-1beta in rats. Mol Psychiatry 2004; 9: 630-8.

[81] Song C, Leonard BE, Horrobin DF. Dietary ethyl-eicosapentaenoic acid but not soybean oil reverses central interleukin-1-induced changes in behavior, corticosterone and immune response in rats. Stress 2004; 7: 43-54.

[82] Mischoulon D, Best-Popescu C, Laposata M, Merens W, Murakami JL, Wu SL, *et al*. A double-blind dose-finding pilot study of docosahexaenoic acid (DHA) for major depressive disorder. Eur Neuropsychopharmacol 2008; 18: 639-45.

[83] Raison CL, Dantzer R, Kelley KW, Lawson MA, Woolwine BJ, Vogt G, *et al*. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. Mol Psychiatry 2010; 15: 393-403.

[84] Cattaneo A, Gennarelli M, Uher R, Breen G, Farmer A, Aitchison KJ, *et al*. Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline 'predictors' and longitudinal 'targets'. Neuropsychopharmacology 2013; 38: 377-85.

[85] Carvalho LA, Torre JP, Papadopoulos AS, Poon L, Juruena MF, Markopoulou K, *et al*. Lack of clinical therapeutic benefit of antidepressants is associated overall activation of the inflammatory system. J Affect Disord 2013; 148: 136-40.

[86] Zunszain PA, Anacker C, Cattaneo A, Choudhury S, Musaelyan K, Myint AM, *et al*. Interleukin-1beta: a new regulator of the kynurenine pathway affecting human hippocampal neurogenesis. Neuropsychopharmacology 2012; 37: 939-49.

[87] Raison CL, Rutherford RE, Woolwine BJ, Shuo C, Schettler P, Drake DF, *et al*. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. JAMA Psychiatry 2013; 70: 31-41.

[88] Bazinet RP, Laye S. Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci 2014; 15: 771-85.

[89] Rao JS, Ertley RN, Lee HJ, DeMar JC, Jr., Arnold JT, Rapoport SI, *et al*. n-3 polyunsaturated fatty acid deprivation in rats decreases frontal cortex BDNF via a p38 MAPK-dependent mechanism. Mol Psychiatry 2007; 12: 36-46.

[90] Beltz BS, Tlusty MF, Benton JL, Sandeman DC. Omega-3 fatty acids upregulate adult neurogenesis. NeurosciLett 2007; 415: 154-8.

[91] Castren E, Hen R. Neuronal plasticity and antidepressant actions. Trends Neurosci 2013.

[92] Eisch AJ, Petrik D. Depression and hippocampal neurogenesis: a road to remission? Science 2012; 338: 72-5.

[93] Stoll AL, Severus WE, Freeman MP, Rueter S, Zboyan HA, Diamond E, *et al*. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. Arch Gen Psychiatry 1999; 56: 407-12.

[94] Su KP, Balanzá-Martínez V. Role of omega-3 fatty acids in mood disorders. In: McNamara RK, editor. The omega-3 fatty acid deficiency syndrome: opportunities for disease prevention. NY, USA: Nova Science Pub Inc.; 2013. pp. 315-36.

[95] Amminger GP, Schafer MR, Papageorgiou K, Klier CM, Cotton SM, Harrigan SM, *et al*. Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. Arch Gen Psychiatry 2010; 67: 146-54.

[96] Matsuoka Y, Nishi D, Yonemoto N, Hamazaki K, Hashimoto K, Hamazaki T. Omega-3 fatty acids for secondary prevention of posttraumatic stress disorder after accidental injury: an open-label pilot study. J Clin Psychopharmacol 2010; 30: 217-9.

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Review article

Wound dressings – a review

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ABSTRACT

Wound healing is a dynamic and complex process which requires suitable environment to promote healing process. With the advancement in technology, more than 3000 products have been developed to treat different types of wounds by targeting various aspects of healing process. The present review traces the history of dressings from its earliest inception to the current status and also discusses the advantage and limitations of the dressing materials.

1. Introduction

A wound is defined as a disruption in the continuity of the epithelial lining of the skin or mucosa resulting from physical or thermal damage. According to the duration and nature of healing process, the wound is categorized as acute and chronic [1, 2]. An acute wound is an injury to the skin that occurs suddenly due to accident or surgical injury. It heals at a predictable and expected time frame usually within 8-12 weeks depending on the size, depth and the extent of damage in the epidermis and dermis layer of the skin [3, 4]. Chronic wounds on the other hand fail to progress through the normal stages of healing and cannot be repaired in an orderly and timely manner [5, 6]. Chronic wounds generally results from decubitis ulcer, leg ulcer and burns. Wound healing is a dynamic and complex process of tissue regeneration and growth progress through four different phases (i) the coagulation and haemostasis phase (immediately after injury); (ii) the inflammatory phase, (shortly after injury to tissue) during which swelling takes place; (iii) the proliferation period, where new tissues and blood vessels are formed and (iv) the maturation phase, in which remodeling of new tissues takes place [7-12]. These phases occur in an ordered manner overlapping with each other in a well-connected cascade [13, 14]. Promotion of these phases are largely depends on the wound type [15], and its associated pathological conditions and the type of dressing material. With the advancement in technology, currently, different types of wound dressing materials are available for all types of wounds. But the selection of a material for a particular wound is important to achieve faster healing. In this review, an attempt has been made to consolidate the different types of wound dressing materials and their function on healing process.

2. Factors affecting wound healing process

Wound healing is the result of interactions among cytokines, growth factors, blood and the extracellular matrix. The cytokines promote healing by various pathways such as stimulating the production of components of the basement membrane, preventing dehydration, increasing inflammation and the formation of granulation tissue. These pathways are affected by various local and systemic factors [16]. Local factors which includes hypothermia, pain, infection, radiation and tissue oxygen tension directly influence the characteristics of the wound where as systemic factors are the overall health or disease state of the individual that affect individual's ability to heal [17]. In addition to these factors, poor nutrition, age and protein, vitamins and mineral deficiency can also prolongs healing times.

2.1. Syndromes associated with abnormal healing

Ehlers-Danlos syndrome (EDS) is a genetic connective tissue disorder characterized by defects of the major structural protein Collagen. Autosomal dominant and autosomal recessive forms of EDS equally affect males and females. Since the collagen is a major structural protein and provide elasticity to body cells and tissues, its damage results in articular hyper mobility leading to partial or complete dislocation of joints and elastic skin. Based on the defects and inheritance mode, EDS is categorized into six major subtypes and they are distinct in affecting individuals [18].

Cutis Laxa is characterized by (Lysyl oxidase) enzyme deficiency resulting in abnormality of copper metabolism leads to abnormal loose skin, muscular organ and skeletal abnormality. Wrinkled skin, particularly on the neck and mild mental retarda-

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tion also characterized by this disorder. X-linked cutis laxa also called as (OHS) occipital horn syndrome, a rare disorder that was formely classified as a subtype of EDS. Cutis laxa is further classified into four genetic forms based on their pattern of inheritance. These includes sex-linked defective on X chromosome, autosomal dominant defective on autosomal chromosome and two types of autosomal recessive inheritance defective on chromosome 5 Among these types, autosomal recessive forms are more severe than other forms [19].

3. Characteristics of an ideal wound dressing

Based on the wound type, suitable dressing material must be used. Dressing selection should be based on its ability to a) provide or maintain moist environment b) enhance epidermal migration c) promote angiogenesis and connective tissue synthesis d) allow gas exchange between wounded tissue and environment e) maintain appropriate tissue temperature to improve the blood flow to the wound bed and enhances epidermal migration f) provide protection against bacterial infection and g) should be non-adherent to the wound and easy to remove after healing h) must provide debridement action to enhance leucocytes migration and support the accumulation of enzyme and i) must be sterile, non-toxic and non-allergic.

4. Wound Dressings

Wound, whether it is a minor cut or a major incision, it is important to care for it properly, part of this process includes wound dressing. Dressing is designed to be in contact with the wound, which is different from a bandage that holds the dressing in place. Historically, wet-to-dry dressings have been used extensively for wounds requiring debridement. In 1600 BC, Linen strips soaked in oil or grease covered with plasters was used to occlude wounds. Clay tablets were used for the treatment of wounds by Mesopotamian origin from about 2500 BCE. They cleaned wounds with water or milk prior to dressing with honey or resin. Wine or vinegar usage for cleaning the wounds with honey, oil and wine as further treatment was followed by Hippocrates of ancient Greece in 460-370 BCE. They used wool boiled in water or wine as a bandage [20]. There was a major breakthrough in the antiseptic technique during the 19th century, antibiotics were introduced to control infections and decrease mortality. Modern wound dressing arrival was in 20th century [21].

When the wound is closed with dressing they are continuously exposed to proteinases, chemotactic, complement & growth factors, which is lost in the wound exposed. So during late 20th century, production of occlusive dressing began to protect and provide moist environment to wound. These dressings helps in faster re-epithelialization, collagen synthesis, promotes angiogenesis by creating hypoxia to the wound bed and decreases wound bed pH which leads to decrease in the wound infection [22]. Woven absorbent cotton gauze was used in 1891. Until the mid 1900's, it was firmly believed that wounds healed more quickly if kept dry and uncovered whereas 'closed wounds heal more quickly than open wound' written in an Egyptian medical text -Edwin smith surgical papyrus in 1615 BC. Oscar Gilje in 1948 describes moist chamber effect for healing ulcers. In the mid 1980's, the first modern wound dressing were introduced which delivered important characteristics providing moisture and absorbing fluids (e.g. polyurethane foams, hydrocolloids, iodine-containing gels). During the mid 1990's, synthetic wound dressings expanded into various group of products which includes hydrogels, hydrocolloids, alginates, synthetic foam dressing, silicone meshes, tissue adhesives, vapor-permeable adhesive films and silver/collagen containing dressing.

4.1. Traditional wound dressing

Traditional wound dressing products including gauze, lint, plasters, bandages (natural or synthetic) and cotton wool are dry and used as primary or secondary dressings for protecting the wound from contaminations [30]. Gauze dressings made out of woven and non woven fibres of cotton, rayon, polyesters afford some sort of protection against bacterial infection. Some sterile gauze pads are used for absorbing exudates and fluid in an open wound with the help of fibres in these dressings. These dressings require frequent changing to protect from maceration of healthy tissues. Gauze dressings are less cost effective. Due to excessive wound drainage, dressings become moistened and tend to become adherent to the wound making it painful when removing. Bandages made out of natural cotton wool and cellulose or synthetic bandages made out of polyamide materials perform different functions. For instance, cotton bandages are used for retention of light dressings, high compression bandages and short stretch compression bandages provide sustained compression in case of venous ulcers. Xeroform™ (non-occlusive dressing) is petrolatum gauze with 3% of Bismuth tribromophenate used for non-exudating to slight exudating wounds. Tulle dressings such as Bactigras, Jelonet, Paratulle are some examples of tulle dressings commercially available as impregnated dressings with paraffin and suitable for superficial clean wound. Generally traditional dressings are indicated for the clean and dry wounds with mild exudate levels or used as secondary dressings. Since traditional dressings fail to provide moist environment to the wound they have been replaced by modern dressings with more advanced formulations [30].

4.2. Modern wound dressing

Modern wound dressing have been developed to facilitate the function of the wound rather than just to cover it. These dressings are focused to keep the wound from dehydration and promote healing. Based on the cause and type of wound, numerous products are available in the market, making the selection a very difficult task. Modern wound dressings are usually based on synthetic polymers and are classified as passive, interactive and bioactive products. Passive products are non-occlusive, such as gauze and tulle dressings, used to cover the wound to restore its function underneath. Interactive dressings are semi-occlusive or occlusive, available in the forms of films, foam, hydrogel and hydrocolloids. These dressings act as a barrier against penetration of bacteria to the wound environment [11-14].

4.2.1. Semi-permeable film dressings

These dressings are composed of transparent and adherent polyurethane which permits transmission of water vapor, O_2 and CO_2 from the wound and it also provides autolytic debridement of eschar and impermeable to bacteria [23]. Initially, films were made from nylon derivatives with an adhesive polyethylene frames as the support which made them occlusive. Originally nylon derived film dressings were not used for highly exudating wounds due

to their limited absorption capacity and caused maceration of the wound and the healthy tissues around the wound [24]. But, these dressings are highly elastic and flexible, and can conform to any shape and do not require additional tapping. Inspection of wound closure is also possible without removal of wound dressing because of transparent films. Hence these dressings are recommended for epithelializing wound, superficial wound and shallow wound with low exudates, e.g. Opsite™, Tegaderm™, Biooclusive™. Commercially available film dressings differ in terms of their vapour permeability, adhesive characteristics, conformability and extensibility [25].

4.2.2. Semi-permeable foam dressings

Foam dressings are made up of hydrophobic and hydrophilic foam with adhesive borders sometimes [26]. The hydrophobic properties of outer layer protect from the liquid but allow gaseous exchange and water vapor. Silicone-based rubber foam (silastic) molds and contours to wound shape. Foam has capability of absorbing varying quantities of wound drainage depending upon the wound thickness. Adhesive and non adhesive foam dressings are available. Foam dressings are suitable for lower leg ulcers and moderate to highly exudating wounds, also indicated for granulating wounds. They are generally used as primary dressings for absorption and secondary dressings are not required due to their high absorbancy and moisture vapour permeability [27, 28]. Disadvantage of foam dressing is requiring frequent dressing and is not suitable for low exudating wounds, dry wounds and dry scars as they depend on exudates for its healing [28] e.g. Lyofoam™, Allevyn™ and Tielle™.

4.2.3. Hydrogels dressing

Hydrogels are insoluble hydrophilic materials made from synthetic polymers such as poly (methacrylates) and polyvinyl pyrrolidine. The high water content of hydrogels (70-90 %) helps granulation tissues and epithelium in a moist environment. Soft elastic property of hydrogels provides easy application and removal after wound is healed without any damage. Temperature of cutaneous wounds is decreased by hydrogels providing soothing and cooling effect. Hydrogels are used for dry chronic wounds, necrotic wounds, pressure ulcers and burn wounds. Morgan [27] has reported that except infected and heavy drainage wounds, hydrogel dressings are suitable for all four stages of wound healing. Hydrogel dressings are non irritant, non reactive with biological tissue and permeable to metabolites. Many researchers have reported that hydrogel dressings are used to treat chronic leg ulcers. Difficulties of hydrogel dressings are exudate accumulation leads to maceration and bacterial proliferation that produces foul smell in wounds. Besides, low mechanical strength of hydrogels making it difficult to handle [29]. Some examples of hydrogels are Intrasite™, Nu-gel™, Aquaform™ polymers, sheet dressings, impregnated gauze and water-based gels.

4.2.4. Hydrocolloid dressing

Hydrocolloid dressings are among the most widely used interactive dressings and are consist of two layers, inner colloidal layer and outer water- impermeable layer. These dressings are made up of the combination of gel forming agents (carboxymethylcellulose, gelatin and pectin) with other materials such as elastomers and adhesives [30]. Hydrocolloids are permeable to water vapor but impermeable to bacteria and also have the properties of debridement and absorb wound exudates [31]. They are used on light to moderately exudating wounds such as pressure sores, minor burn wounds and traumatic wounds. These dressings are also recommended for paediatric wound care management, as they do not cause pain on removal [32]. When this hydrocolloids contact with the wound exudate they form gels and provide moist environment that helps in protection of granulation tissue by absorbing and retaining exudates. Granuflex™, Comfeel™, Tegasorb™ are available in the form of sheets or thin films. Disadvantage of hydrocolloids are they are not indicated for neuropathic ulcers or highly exudating wounds, also they are mostly used as a secondary dressings [30].

4.2.5. Alginate dressing

Alginate dressings are made from the sodium and calcium salts comprising mannuronic and guluronic acid units. Absorbent and biodegradable alginates are derived from seaweed. Absorption capability is achieved by strong hydrophilic gel formation, which limits wound exudates and minimizes bacterial contamination. Even though some studies have reported that alginate inhibits keratinocytes migration, Thomas *et al.*, [33] have reported that alginates accelerate healing process by activating macrophages to produce TNF-α which initiates inflammatory signals. Once alginate dressings are applied to the wound, ions present in the alginate are exchanged with blood to form a protective film. Alginate dressings are suitable for moderate to heavy drainage wounds and not suggested for dry wound, third degree burn wound and severe wounds with exposed bone. Also these dressings require secondary dressings because it could dehydrate the wound which delay healing. Sorbsan™, Kaltostat™, Algisite™ are some alginate dressings commercially available [30].

4.3. Bioactive wound dressings

The last type of modern wound dressing is bioactive dressings and is produced from biomaterials which play an important role in healing process. These dressings are known for their biocompatibility, biodegradability and non-toxic nature and are derived generally from natural tissues or artificial sources [34] such as collagen [35], hyaluronic acid [36], chitosan [37], alginate and elastin. Polymers of these materials are used alone or in combination depending on the nature and type of wound. Biological dressings are sometimes incorporated with growth factors and antimicrobials to enhance wound healing process.

Collagen, a major structural protein has been discussed by many researchers for their active role in natural healing process [35, 38, 39]. Collagen initiates fibroblast formation and accelerates endothelial migration upon contact with wound tissue [40]. Hyaluronic acid (HA) is a glycoaminoglycan component of extra cellular matrix (ECM) with unique biological and physicochemical features. Similar to collagen, HA also biocompatible, biodegradable and lack immunogenicity naturally [41]. Chitosan promotes the formation of granulation tissue during the proliferative stage of wound healing [42]. When compared to other dressings, biological dressings are reported to be more superior to other types of dressings.

4.4. Tissue engineered skin substitutes

Human skin or dermal equivalent (HSE) has two types of tissue-

engineered substitutes available, one mimics the layer of skin composed of Keratinocytes and fibroblast on collagen matrix (Cell containing matrix). Second contains only the dermal elements with fibroblast on collagen matrix (Acellular matrix). Major mechanism of HSE is to secrete and stimulate wound growth factor by which epithelialization is achieved. Bioengineered are capable of adapting to their environment so that they are able to release growth factors and cytokines incorporated in dressings. Bioengineered dressings are suitable for Diabetic foot ulcer and venous leg ulcer. Apligraf is a FDA approved skin equivalent substitute consists of keratinocytes and fibroblast-seeded collagen for venous ulcers. Some skin substitutes commercially available include, Alloderm™ composed of normal human fibroblasts with all cellular materials removed and Integra™ artificial skin consists of collagen/ chondroitin 6 sulphate matrix overlaid with a thin silicone sheet. Other few substitutes are Laserskin™, Biobrane™, Bioseed™, and Hyalograft3-DTM.

4.5. Medicated dressings

Medicated dressings incorporated drugs plays an important role in the healing process directly or indirectly by removal of necrotic tissues. This has been achieved by cleaning or debriding agents for necrotic tissue, antimicrobials which prevents infection and promotes tissue regeneration. Some commonly incorporated compounds include antimicrobial agents, growth factors and enzymes. Commercially available antimicrobial dressings include Cutisorb™. Silver impregnated dressings available are Fibrous hydrocolloid, Polyurethane foam film and silicone gels. Antiseptic Iodine dressing acts on bacterial cells via oxidative degradation of cell components by interrupting the function of protein, which is widely effective against pathogen. Prolong usage of iodine leads to skin irritation and staining [43]. The purpose of antimicrobials is mainly to prevent or combat infections especially for diabetic foot ulcers.

Normal tissue repair process in the body is controlled by cellular activities caused by growth factors that are naturally present in our body. In case of chronic wounds, growth factors and cells are arrested in the wound bed within the clots that affects the healing process. So exogenous application of growth factors benefits the wound healing process and this was proved by numerous studies. Among the different growth factors, platelet derived growth factor (PDGF) is the most commonly used growth factor which promotes chemotactic recruitment and proliferation of cells and increasing angiogenesis. Besides, PDGF, fibroblast growth factor (FGF), epidermal growth factor (EGF), and autologous platelet thrombin are also studied extensively for their application in healing process. Among which, PDGF and EGF are approved by FDA for human application.

Enzymatic debridement of necrotic tissues without harming healthy tissue is also a crucial part to promote normal healing process. Papain and collagenase based ointments are currently used to digest necrotic tissue. Collagenase acts on the collagen by attacking native collagen and gentle on viable collagen by gradual breakdown of tissue whereas papain attacks cystein residue and associated with inflammatory response. DebridaceTM is a commercially available dressing which increases proteolytic action.

4.6. Composite dressing

Composite dressings are versatile and convenient for both partial and full thickness wounds. A composite or combination dressings has multiple layers and each layer is physiologically distinct. Most of the composite dressings possess three layers. Composite dressings may also include an adhesive border of non-woven fabric tape or transparent film. They can function as either a primary or a secondary dressing on a wide variety of wounds and may be used with topical medications. Outer most layer protect the wound from infection, middle layer usually composed of absorptive material which maintains moisture environment and assist autolytic debridement, bottom layer composed of non adherent material which prevents from sticking to young granulating tissues. Composite dressings have less flexibility and they are more expensive [44].

5. Conclusion

Currently more than 3000 types of dressings are available in the market making the physician to address all aspects of wound care. But still there is no superior product that heals chronic wounds like venous leg ulcers, diabetic wound and pressure ulcers which often fail to achieve complete healing. Hence developing a dressing material that addresses the major interfering factors of normal healing process will help patients and wound care practitioners largely.

REFERENCES

[1] Robson MC, Steed DL, Franz MG. Wound healing: biological features and approaches to maximize healing trajectories. Curr Prob Surg 2001; 38: 77-89.

[2] Szycher M and Lee SJ. Modern wound dressings: a systemic approach to wound healing. J Biomater Appl 1992; 7: 142-213.

[3] Schreml S, Szeimies RM, Prantl L, Karrer S, Landthaler M, Babilas P. Oxygen in acute and chronic wound healing. Br J Dermatol 2010; 163: 257-68.

[4] Rajendran S, Anand SC. Hi-tech textiles for interactive wound therapies: Handbook of Medical Textiles; 2011.

[5] Lazurus GS, Cooper DM, Knighton DR, Margolis DJ, Pecoraro RE, Rodeheaver G, *et al*. Definitions and guidelines for assessment of wounds and evaluation of healing. Arch Dermatol 1994; 130: 489-93.

[6] Bischoff M, Kinzl L, Schmelz A. The complicated wound. Unfallchirurg 1999; 102: 797-804. [In German, English abstract]

[7] Tarnuzzer RW, Schultz GS. Biochemical analysis of acute and chronic wound environments. Wound Repair Regen 1996; 4: 321-5.

[8] Clark RAF. Wound repair Overview and general considerations. The molecular and cellular biology of wound repair 2nd edition. Plenum: New York; 1996. P 3-5.

[9] Dowsett C, Newton H. Wound bed preparation: TIME in practice. WOUNDS UK 2005; 1: 58-70.

[10] Vanwijck R. Surgical biology of wound healing. Bulletin et mem-

oires de l'Academie royale de medecine de Belgique 2000; 156: 175-84. [in French, English abstract]
[11] Degreef HJ. How to heal a wound fast. Dermatol Clin 1998; 16: 365-75.
[12] Hunt TK, Hopf H, Hussain Z. Physiology of wound healing. Adv Skin Wound Care 2000; 13: 6-11.
[13] Rivera AE, Spencer JM. Clinical aspects of full-thickness wound healing. Clin Dermatol 2007; 25: 39-48.
[14] Strecker-McGraw MK, Jones TR, Baer DG. Soft tissue wounds and principles of healing. Emerg Med Clin North Am 2007; 25: 1-22.
[15] Baxter E. Complete crime scene investigation handbook: CRC press. 2015; p 313.
[16] Finn G, Kirsner R, Meaume S, Munter C, Sibbald G. Clinical wound assessment a pocket guide, Coloplast 2006; p 6.
[17] Guo S, DiPietro L. Factors affecting wound healing. Journal of Dent Res 2010; 89: 219-29.
[18] Baranoski S, Ayello EA. Wound dressing: an evolving art and science. Adv Skin Wound Care: the J Preven Healing 2012; 25: 87-92.
[19] Fernandes NF, Schwartz RA. A hyperextensive review of Ehlers-Danlos syndrome: Pubfacts 2008; 82: 242-8.
[20] Daunton C, Kothari S, Smith L, Steele D. A history of materials and practices for wound management. Wound Pract Res 2012; 20: 174-86.
[21] Jayesh BS. The history of wound care. The journal of the American college of certified wound specialists 2011; 3: 65-6.
[22] Sujatha S. Recent advances in topical wound care. Indian J Plast Surg 2012; 45: 379-87.
[23] Moshakis V, Fordyce MJ, Griffiths J D, McKinna JA. Tegaderm versus gauze dressing in breast surgery. Br J Clin Pract 1984; 38: 149-52.
[24] Debra JB, Cheri O. Wound healing: Technological innovations and market overview. 1998; 2: 1-185.
[25] Thomas S, Loveless P. Comparative review of the properties of six semipermeable film dressings. Pharm J 1988; 240: 785-7.
[26] Morgan DA. Wounds- What should a dressing formulary include? Hosp Pharmacist 2002; 9: 261-6.
[27] Thomson T. Foam Composite. US Patent 7048966. 2006.
[28] Marcia RES, Castro MCR. New dressings, including tissue engineered living skin. Clin Dermatol 2002; 20: 715-23.
[29] Martin L, Wilson CG, Koosha F, Tetley L, Gray AI, Senel S, *et al*. The release of model macromolecules may be controlled by the hydrophobicity of palmitoyl glycol chitosan hydrogels. J Control Release 2002; 80: 87-100.
[30] Boateng JS, Matthews KH, Stevens HNE, Eccleston GM. Wound Healing Dressings and Drug Delivery Systems: A Review. Indian J Pharml Sci 2008; 97: 2892-923.
[31] Thomas S, Loveless PA. A comparative study of twelve hydrocolloid dressings. World Wide Wounds 1997; 1: 1-12.
[32] Thomas S. Hydrocolloids. J Wound Care 1992; 1: 27-30.
[33] Thomas A, Harding KG, Moore K. Alginates from wound dressings activate human macrophages to secrete tumour necrosis factor-a. Biomaterials 2000; 21: 1797-802.
[34] Barlett RH. Skin substitutes. J Trauma 1981; 21: S731.
[35] Ramshaw JAM, Werkmeister JA, Glatteur V. Collagen based biomaterials. Biotechnol Rev 1995; 13: 336-82.
[36] Doillon CJ, Silver FH. Collagen-based wound dressing: Effect of hyaluronic acid and fibronectin on wound healing. Biomaterials 1986; 7: 3-8.
[37] Ishihara M, Nakanishi K, Ono K, Sato M, Kikuchi M, Saito Y, *et al*. Photo crosslinkable chitosan as a dressing for wound occlusion and accelerator in healing process. Biomaterials 2002; 23: 833-40.
[38] Liu SH, Yang RS, Al-Shaikh R, Lane JM. Collagen in tendon, ligament and bone healing. Clin Orthop Res 1995; 318: 265-78.
[39] Rao KP. Recent developments of collagen based materials for medical applications and drug delivery. J Biomater Sci Polym Ed 1995; 7: 623-45.
[40] Mian M, Beghe F, Mian E. Collagen as a pharmacological approach in wound healing. Int J Tissue React 1992; 14: 1-9.
[41] Supp DM, Boyce ST. Engineered skin substitutes: Practices and potentials. Clin Dermatol 2005; 23: 403-12.
[42] Ueno H, Mori T, Fujinaga T. Topical formulations and wound healing applications of chitosan. Adv Drug Deliv Rev 2001; 52: 105-15.
[43] Liesenfeld B, Moore D, Mikhaylova A, Vella J, Carr R, Schultz G, *et al*. Antimicrobial wound dressings- mechanism and function. In: Symposium on advanced wound care; 2009.
[44] Laurie S. Wound dressing selection: types and usage 2011; Available from http://www.woundsource.com/blog/wound-dressing-selection-types-and-usage.

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Review article

A promising "TRAIL" of tanshinones for cancer therapy

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ABSTRACT

An ideal cancer therapy specifically targets cancer cells while sparing normal tissues. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) elicits apoptosis by engaging its cognate death receptors (DRs)—namely, DR4 and DR5. The cancer cell-selective proapoptotic action of TRAIL is highly attractive for cancer therapy, but clinical application of TRAIL is rather limited due to tumors' inherent or acquired TRAIL resistance. Combining TRAIL with agents that reverse resistance to it has proved promising in the sensitization of TRAIL-induced apoptosis. Noteworthy, natural compounds have already been validated as potential resources for TRAIL sensitizers. In this review, we focus on the recently identified TRAIL-sensitizing effect of tanshinones, the anticancer ingredients of the medicinal plant *Salvia miltiorrhiza* (Danshen in Chinese). Research from our laboratories and others have revealed the synergy of a tanshinones-TRAIL combination in diverse types of cancer cells through up-regulation of DR5 and/or down-regulation of anti-apoptotic proteins such as survivin. Thus, in addition to their anticancer mechanisms, tanshinones as TRAIL sensitizers hold great potential to be translated to TRAIL-based therapeutic modalities for combatting cancer.

1. Introduction

Cancer remains the leading cause of mortality globally. Despite advances in developing new therapeutic modalities for cancer, chemotherapy is still the fundamental tool for cancer treatment primarily through induction of apoptosis in cancer cells. Natural compounds isolated from medicinal plants have been seen as promising resources for novel chemotherapeutic drug discovery [1-3]. In this review, we summarize the anticancer potential of tanshinones, the bioactive components isolated from the dried root of the medicinal plant *Salvia miltiorrhiza* Bunge (Lamiaceae) (a.k.a. Danshen) (Figure 1) that has been frequently used in traditional Chinese medicine for over a thousand years to prevent or treat various conditions including menstrual disorders, hepatitis, and cardiovascular diseases [4, 5]. In particular, we focus on the recently discovered role of tanshinones as sensitizing agents of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), which has an attractive anticancer potential due to its cancer cell-selective proapoptotic action but is often limited by the development of TRAIL-resistance in many human tumors. The mechanisms whereby tanshinones overcome TRAIL resistance and the potential translation of tanshinones to TRAIL-based cancer remedies are also discussed herein.

2. Tanshinones

2.1. Tanshinones are the anticancer components of Danshen

In general, the bioactive components of Danshen can be categorized into two groups, namely, the lipophilic diterpene quinones and the water-soluble phenolic acids like salvianolic acids [6]. The lipophilic group, composed of more than 50 diterpenoid tanshinones, shows prominent anticancer potential in addition to showing anti-inflammatory and antioxidant activities [7]. Cryptotanshinone, tanshinone I, and tanshinone IIA are the three major elements of the lipophilic group (Figure 2), and numerous *in vitro* and *in vivo* studies have revealed the anticancer actions as well as the underlying mechanisms of these main tanshinones (Figure 3).

2.2. Anticancer modes of action of tanshinones

2.2.1. Induction of cell cycle arrest

Tanshinones induce the arrest of cancer cell cycle progression at the G1, S, or G2/M phases in a cell type-dependent manner, leading to the inhibition of cell proliferation [8-12]. Mechanistically,

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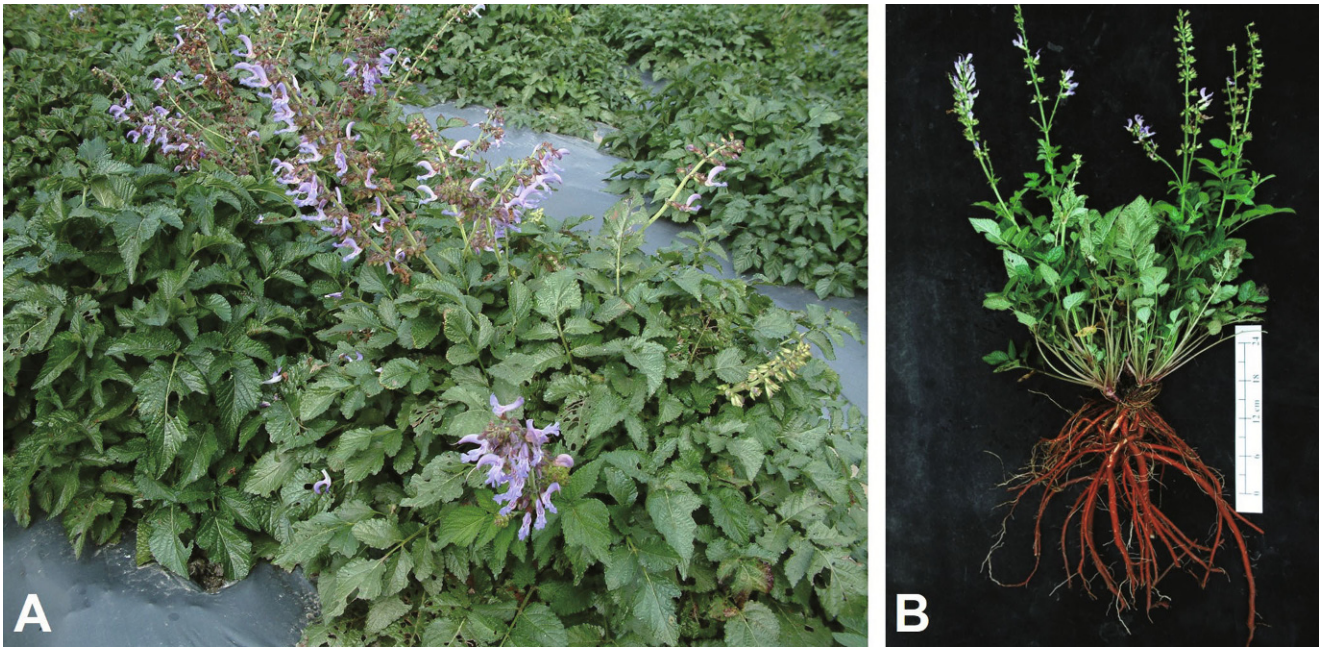

Fig. 1 - Photographs of *Salvia miltiorrhiza* Bunge (Lamiaceae). (A) Propagated plants of *Salvia miltiorrhiza*; (B) The aerial and root parts of harvested *Salvia miltiorrhiza*.

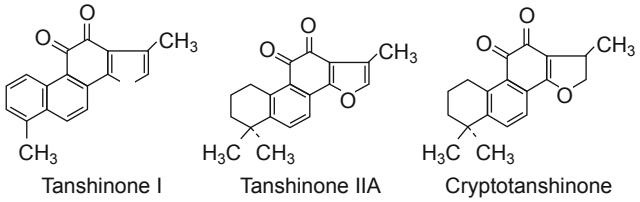

Fig. 2 - The chemical structure of the main tanshinones of Danshen. Tanshinone I (left); Tanshinone IIA (center); and Crytotanshinone (right).

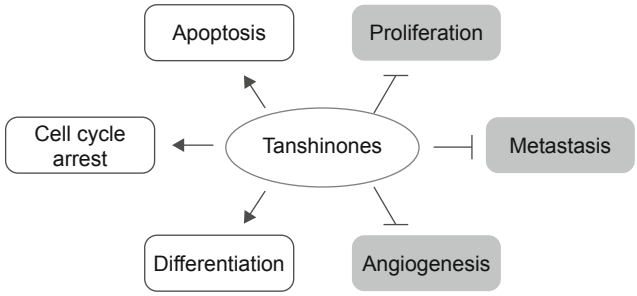

Fig. 3 - Anticancer mechanisms of action of tanshinones. Reported anticancer actions of tanshinones include: (1) inhibition of proliferation through arresting cell cycle progression, (2) induction of cancer cell apoptotic death, (3) anti-metastasis, (4) anti-angiogenesis, and (5) induction of cancer cell differentiation. Please refer to text for details.

tanshinone I has been shown to induce G1 arrest in lung cancer cells through the activation of the p53/p21/p27 pathway [13]. Cryptotanshinone and its synthetic derivatives as well as tanshinone IIA have all been observed to markedly repress prostate cancer cell growth *in vitro* and *in vivo* and to trigger G1 arrest by blocking the actions of the androgen receptor [11, 14-16].

2.2.2. Induction of cell death

The proapoptotic effects of all of the main tanshinones have been tested and validated in a broad range of cancer cell lines, primarily through engaging the mitochondrial apoptosis pathway. Of note, all three main tanshinones have suppressed the activation of pro-survival STAT3 to provoke apoptotic cell death [17-20]. Furthermore, dependent on the type of tanshinones, additional prosurvival mechanisms have been found to be targeted for suppression, including PI3K/AKT [21, 22], survivin [23], Erb-2 [24], Aurora A [25], MCL-1, and c-IAP2 [26]. In contrast, activation of JNK [27], p53 [11], and endoplasmic reticulum stress have been reported to mediate tanshinones' proapoptotic action [28, 29]. Intriguingly, the induction of autophagic cell death is something that contributes to the anti-leukemia effect of tanshinone IIA [30].

2.2.3. Anti-metastasis

The anti-metastasis effect of tanshinone I has been clearly validated in xenograft models of the breast cancer cell line MDA-MB-231 [31] and the lung adenocarcinoma cell line CL1-5 [32], and has also been established in a transgenic lung cancer model driven by overexpression of the human vascular endothelial growth factor (VEGF)-A_{165} variant [13]. Additionally, tanshinone IIA inhibited the metastasis of xenografted hepatocellular carcinoma cell line HepG2, likely through the inhibition of the activities of matrix metallopeptidases 2 and 9 [33].

2.2.4. Anti-angiogenesis

All of the main tanshinones demonstrate an anti-angiogenic effect at the *in vitro* and *in vivo* levels, as evidenced by reduced migration/

proliferation/tube formation of vascular endothelial cells and neovascularization of the chick chorioallantoic membrane, respectively [18, 34, 35]. Tanshinone IIA has also been shown to repress angiogenesis in mice xenografted with MDA-MB-231 cells [36]. It appears that tanshinones elicit anti-angiogenesis mainly through the down-regulation of hypoxia-induced factor 1α (HIFα) and the consequent reduction in VEGF using distinct mechanisms. Tanshinone I lowered HIFα levels by promoting the proteasomal degradation of HIFα [18], whereas tanshinone IIA attenuated HIFα translation by suppressing the mTOR-p70S6K-4E-BP1 signaling pathway [36].

2.2.5. Induction of cancer cell differentiation

All-*trans* retinoic acid (ATRA) is an effective chemotherapeutic for acute promyelocytic leukemia (APL) that works by inducing APL cell differentiation, but resistance to ATRA does eventually develop. Notably, tanshinone IIA has effectively induced APL cell differentiation in both ATRA-sensitive and –resistant cell lines, likely through inducing CCAAT/enhancer-binding protein β (C/EBPβ)-mediated differentiation [37].

3. TRAIL

3.1. TRAIL-induced apoptosis

TRAIL is a type II membrane protein belonging to the TNF death ligand superfamily, which also includes TNFα and Fas ligand (FasL/CD95L) [38]. TRAIL is unique in its ability to induce p53-independent apoptosis selectively in cancer cells while sparing normal cells, thus avoiding the adverse side effects frequently associated with current chemotherapeutic agents. TRAIL induces apoptosis primarily through the death receptors (DRs)-mediated apoptotic pathway (Figure 4). Four membrane-bound TRAIL receptors, including DR4 (TRAIL-R1), DR5 (TRAIL-R2), decoy receptor 1 (DcR1/TRAIL-R3) and DcR2 (TRAIL-R4), and one soluble receptor osteoprotegerin (OPG) share highly homologous extracellular TRAIL-binding domain. Both DR4 and DR5 are functional TRAIL receptors that carry the cytoplasmic death domain (DD) to transduce TRAIL-initiated apoptotic signals, whereas DcR1, DcR2 and OPG lack the cytoplasmic DD and therefore antagonize TRAIL's proapoptotic action. The binding of TRAIL to DR4 or DR5 induces receptor trimerization and consequently clusters the cytoplasmic DDs to recruit Fas-associated death domain (FADD) and pro-caspases 8 and 10 for the assembly of death-inducing signaling complex (DISC), leading to self-cleavage and thus activation of pro-caspases 8/10. Activated caspases 8/10 in turn initiate downstream caspase cascade to execute apoptosis program and, in certain types of cells, also evoke the mitochondrial apoptosis pathway through the truncation of the BH3-only protein BID (tBID) for the efficient induction of apoptosis [39].

3.2. TRAIL-based cancer therapy

The ability to induce cancer cell-selective apoptosis makes TRAIL an attractive choice for cancer therapy. Indeed, preclinical trials using soluble forms of recombinant TRAIL have shown a promising tumoricidal effect and, unlike TNFα and CD95L, barely caused systematic toxicity [39]. Clinical trials for TRAIL-based cancer therapies using either recombinant forms of the

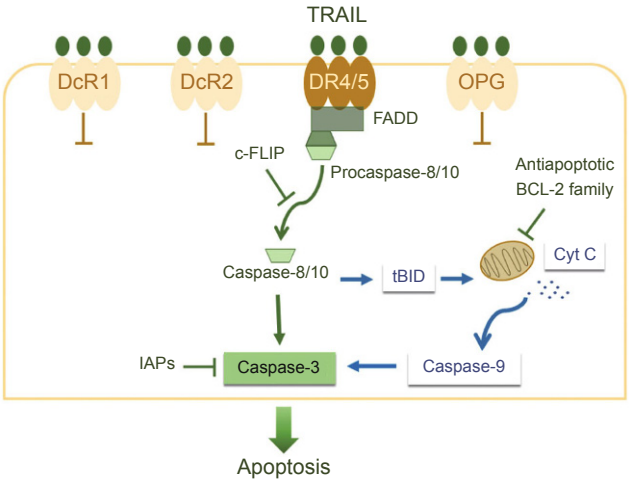

Fig. 4 - TRAIL-induced apoptosis signaling pathway. TRAIL initiates apoptosis through binding to DR4 and/or DR5. TRAIL binding induces receptor trimerization to promote the assembly of DISC (composed of FADD and procaspases 8/10) to induce self-cleavage and thus activation of caspases 8/10, which in turn trigger downstream caspase cascade to execute apoptosis program. In certain cell types, activated caspase 8 cleaves BID to generate truncated BID (tBID), which in turn triggers the mitochondrial apoptosis pathway. c-FLIP competes with procaspase 8 for recruitment to DISC, thereby suppressing activation of procaspase 8 and thus dampening TRAIL-initiated apoptosis stimuli. Antiapoptotic BCL-2 family members BCL-2, BCL-xL, and MCL-1 suppress the activation of the mitochondrial apoptosis pathway to blunt TRAIL-induced apoptosis. IAP proteins survivin and XIAP induce TRAIL resistance *via* the blockade of the activity of executioner caspases.

human TRAIL extracellular domain (dulanermin) or agonistic antibodies specifically targeting DR4 (mapatumumab) or DR5 (e.g. conatumumab) also revealed the safety and tolerability of these therapeutics. However, clinical trials of TRAIL-based therapies have failed to produce significant therapeutic responses in patients [38, 40, 41]. One of the key reasons for this limited therapeutic activity is TRAIL resistance, either inherent or acquired after repeated TRAIL administration.

3.3. TRAIL resistance mechanism

Our knowledge regarding the mechanisms of TRAIL resistance in tumor cells has advanced greatly in recent years. In general, the deregulation of the molecules involved in TRAIL-initiated apoptotic pathway is closely linked to the development of TRAIL resistance [41, 42]. Briefly, three fundamental mechanisms are commonly found in tumors with inherent or acquired TRAIL resistance. One, low levels of cell-surface DR4/DR5 and/or overexpression of DcR1/DcR2 effectively blunt TRAIL to turn on apoptosis. Two, TRAIL resistance can be caused by the up-regulation of c-FLIP, a caspase 8 homolog without caspase activity; increased c-FLIP levels compete with procaspase 8 for recruitment to DISC, thus impairing the activation of caspase 8 to mediate TRAIL's proapoptotic action. And three, overexpression of prosurvival proteins, including antiapoptotic BCL-2, BCL-xL and MCL-1 as well as inhibitors of apoptosis proteins (IAPs) such

as survivin and XIAP, contribute to TRAIL resistance by blocking caspase activities.

3.4. Strategies to overcome TRAIL resistance

Given an acquired resistance to TRAIL commonly develops in most human tumors, current clinical trials for TRAIL-based therapies employ combination strategies using agents that overcome TRAIL resistance, thus restoring sensitivity to TRAIL-induced apoptosis [39-45]. Intensive studies in recent years have identified a number of potent TRAIL sensitizers in the context of diverse cancer cell lines. These include conventional chemotherapeutic drugs (e.g. cisplatin) [46], proteasome inhibitors (e.g. bortezomib) [47], Hsp90 inhibitors (e.g. 17-AAG) [48], ER stress inducers (e.g. tunicamycin) [49], and autophagy inhibitors [45]. BH3 mimetics and Smac mimetics, which selectively target anti-apoptotic BCL-2 proteins and IAPs, respectively, synergize with TRAIL as well [50-53]. It is also noteworthy that some natural compounds have been validated as rich sources of TRAIL sensitizers [43, 44, 54, 55].

Reversing the mechanisms of TRAIL resistance forms the functional basis of TRAIL-sensitizing agents. Indeed, the majority of TRAIL sensitizers reported to date synergize with TRAIL by inducing up-regulation of DRs (particularly DR5), thus highlighting DRs' expression levels as the primary point of control for TRAIL-induced apoptosis. In this context, DR5 is up-regulated through distinct mechanisms of action [43]. Most natural TRAIL sensitizers induce transcriptional up-regulation of DR5 in p53-dependent or -independent manners, the latter of which often involves the ROS-(JNK)-CHOP pathway, whereas the proteasome inhibitor bortezomib up-regulates DR5 by facilitating DR5 protein stabilization. Alternatively, caspase 8 activation caused by the down-regulation of c-FLIP at the transcriptional or post-translational levels underlies the mode of action of some TRAIL sensitizers [45]. Likewise, small molecules that bind and stabilize the caspase 8 homodimers can function as TRAIL stabilizers by promoting caspase 8 activation upon TRAIL stimulation [56]. Chemotherapeutic drugs synergize with TRAIL mainly through lowering the apoptotic threshold by up-regulating proapoptotic BH3-only proteins while down-regulating antiapoptotic BCL-2 proteins and/or IAPs [39].

4. Tanshinones reverse TRAIL resistance

4.1. Tanshinones are a new class of natural TRAIL sensitizers

Using the TRAIL-resistant human ovarian cancer cell lines TOV-21G and SKOV3 as cellular models, in early 2013 we published the first report demonstrating the TRAIL-sensitizing effect of crytotanshinones, tanshinone I, and tanshinone IIA [57]. Later on, Tse *et al*. identified crytotanshinone as a TRAIL sensitizer in the human melanoma cell line A375 and the lung adenocarcinoma cell line A549, both refractory to TRAIL [58]. Likewise, Shin *et al*. recently reported that tanshinone I restores the sensitivity of the TRAIL-resistant human prostate cancer cell lines PC-3 and DU145 to TRAIL-induced apoptosis [59]. These studies altogether establish tanshinones as effective TRAIL sensitizers. Noteworthy, up-regulation of DR5 appears as the fundamental basis of TRAIL sensitization by tanshinones. Detailed mechanisms whereby tanshinones overcome TRAIL resistance are summarized in the following sections.

4.2. Tanshinone IIA synergizes with TRAIL to induce apoptosis by engaging the ROS-JNK-CHOP signaling axis to up-regulate DR5 while activating p38 MAPK to down-regulate survivin

We made the pioneering discovery that crytotanshione, tanshinone I, and tanshinone IIA all exert TRAIL-enhancing action on the TRAIL-resistant human epithelial ovarian cancer (EOC) cell lines TOV-21G and SKOV3, with tanshinone IIA showing the best potency [57]. Subsequent analyses validated the synergy of this tanshinone IIA-TRAIL combination in apoptotic killing of these EOC cell lines, as well as the transcriptional up-regulation of *DR5* along with increased cell-surface DR5 expression following tanshinone IIA stimulation [60].

The levels of DRs on the cell surface is essential for TRAIL to induce effective apoptotic signaling, whereas low levels of cell-surface DRs confer TRAIL resistance. Along this line, the functional blockade of DR5 by the DR5/Fc chimer protein abolished tanshinone IIA's action to sensitize TRAIL, indicating that DR5 up-regulation primarily determines tanshinone IIA as a TRAIL sensitizer in the context of EOC cells. Mechanistic studies on how tanshinone IIA up-regulates DR5 uncovered that tanshinone IIA triggers reactive oxygen species (ROS) production to induce JNK activation, leading to the transcriptional up-regulation of CAAT/enhancer-binding protein homologous protein (CHOP), a well-established transcriptional activator of the *DR5* promoter. The functional significance of the ROS-JNK-CHOP signaling axis in tanshinone IIA-mediated DR5 up-regulation is clearly supported by the failure of tanshinone IIA to induce DR5 when CHOP is depleted by CHOP shRNA, JNK-specific inhibitor SP600125, or ROS scavenger N-acetylcysteine (NAC) [60].

Down-regulation of IAPs such as survivin or XIAP represents an alternative approach to overcome TRAIL resistance aside from DR5 up-regulation [42]. Survivin is recognized as an attractive drug target owing to its selective expression in malignant cells [61]. Notably, high levels of survivin have been associated with TRAIL resistance [62]. Along this line, we have revealed that tanshinone IIA induces p38 MAPK-dependent transcriptional repression of survivin in TRAIL-resistant but not TRAIL-sensitive human EOC cell lines [23]. Of note, ectopic survivin expression to counteract tanshinone IIA-induced survivin reduction markedly attenuates the synergistic cytotoxicity of the tanshinone IIA-TRAIL combination, indicating survivin down-regulation as an important mode of action for transhinone IIA to overcome TRAIL resistance. Collectively, these findings have delineated that multiple mechanisms of action, including DR5 induction and survivin repression, are involved in the tanshinone IIA-mediated sensitization of TRAIL-resistant human EOC cells to TRAIL-induced apoptosis (Figure 5).

4.3. Tanshinone I restores TRAIL sensitivity through microRNA 135a-3p-mediated up-regulation of DR5

Using the human prostate cancer cell lines PC-3 and DU-145 as cellular models, Shin *et al*. demonstrated that Tanshinone I synergistically sensitizes TRAIL-induced apoptosis in these TRAIL-resistant cells [59]. Furthermore, the tanshinone I-TRAIL combination up-regulated the mRNA and protein levels of DR5 and activated the *DR5* promoter, and this was accompanied by an increase in cell-surface DR5 levels. Not surprisingly, the depletion of DR5 severely lowered the potency of tanshinone I to enhance TRAIL-induced apoptosis, confirming the essential role of DR5 up-regulation in tanshinone I-mediated TRAIL sensitization.

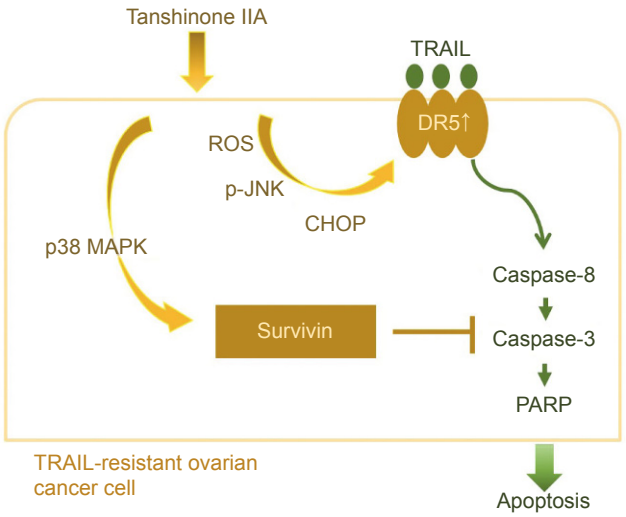

Fig. 5 - The mechanisms underlying tanshinone IIA-TRAIL synergy. Tanshinone IIA sensitizes TRAIL-resistant epithelial ovarian cancer (EOC) cells to TRAIL-induced apoptosis through at least two mechanisms. First, tanshinone IIA evokes ROS-dependent activation of JNK to increase CHOP expression, which in turn activate *DR5* transcription to up-regulate cell-surface DR5 levels for the potentiation of TRAIL-induced apoptosis stimuli. Second, tanshinone IIA engages the p38 MAPK-mediated pathway to induce transcriptional down-regulation of survivin, consequently promoting caspase activities to execute apoptosis.

Furthermore, Shin *et al.* made a unique, noteworthy discovery that microRNA 135a-3p (miR135-3p) elicited by the tanshinone I-TRAIL combination accounts for the up-regulation of DR5, whereas the miR135-3p inhibitor attenuated the apoptosis induced by the tanshinone I-TRAIL combination. However, how miR135-3p up-regulates DR5 was not demonstrated in the report. Likewise, although tanshinone I evoked ROS production in these cell lines, the roles of ROS in miR135-3p induction and TRAIL sensitization by tanshinone I were not addressed either.

4.4. Cryptanshinone facilitates TRAIL sensitization by activating the ROS-CHOP-DR5 pathway

To employ DR5 up-regulation as the strategy of to overcome TRAIL resistance, Tse *et al*. identified cryptotanshinone, among tanshinone I, dihydrotanshione I, and tanshinone IIA, as the most potent tanshinones to induce DR5 expression in the TRAIL-resistant human melanoma A375 cells [58]. Similar to tanshinone IIA, cryptotanshinone was revealed to induce transcriptional up-regulation of DR5 along with increased levels of cell-surface DR5 expression. DR5 up-regulation is essential for cryptotanshinone to overcome TRAIL resistance in the A375 cells, as DR5 depletion markedly abrogated cryptotanshinone-mediated sensitization to TRAIL-induced apoptosis. Similar to tanshinone IIA, cryptotanshinone elicited the ROS-dependent transcriptional induction of CHOP, leading to increased *DR5* transcription for enhancing TRAIL's proapoptotic action. It is also noteworthy that p53 is not required for cryptotanshinone to up-regulate DR5, as evidenced by the comparable induction of DR5 in the HCT116 cell lines with or without p53 expression. As a matter of fact, the p53-independent nature of DR5 up-regulation by cryptotanshinone is advantageous, considering that the majority of human cancer cells are deficient in p53-mediated apoptosis.

5. Conclusions and perspectives

TRAIL, which benefits from its malignant cell-selective proapoptotic action, is an ideal cancer therapeutic agent, but its potential is ironically sabotaged by either intrinsic or acquired resistance commonly developed by tumor cells. A combination of TRAIL with agents that overcome TRAIL resistance mechanisms has been validated in preclinical and clinical trials as a promising strategy to boost TRAIL's efficacy. In this regard, tanshinones as effective TRAIL sensitizers hold great potential to be included in TRAIL-based cancer therapeutic regimens. As for the TRAIL-sensitizing mechanisms of tanshinones, it appears that ROS-mediated DR5 up-regulation is the primary modes of action (except for transhinone IIA, which also targets survivin). It would be informative to decipher additional targets of TRAIL resistance mechanisms likely modulated by tanshinones in the context of various types of tumor cells, as a detailed molecular understanding of tanshinones-elicited TRAIL sensitization would allow for a rational design for more effective tanshinones-TRAIL synergy. Furthermore, although only cryptotanshinone, tanshinone I, and tanshinone IIA are validated as TRAIL sensitizers to date, additional members of tanshinones that are more effective to circumvent TRAIL resistance are likely present and remain to be discovered. It is also worth noting that the action of tanshinones as TRAIL sensitizers have been demonstrated only at the *in vitro* level so far. Therefore, preclinical validation of tanshinones-TRAIL synergy in xenograft or orthograft mouse cancer models is necessary for subsequent clinical trial design, which will hopefully lead to the future translation of tanshinones to TRAIL-based cancer therapy.

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Declaration of interest

The authors declare no conflicts of interest for this work.

REFERENCES

[1] Efferth T, Li PC, Konkimalla VS, Kaina B. From traditional Chinese medicine to rational cancer therapy. Trends Mol Med 2007; 13: 353-61.

[2] Chen CY, Adams JD2, Hou T3, Litscher G. When modern technology meets ancient traditional chinese medicine. Evid Based Complement Alternat Med 2015; 2015: 156581.

[3] Wang X, Feng Y, Wang N, Cheung F, Tan HY, Zhong S, *et al*. Chinese medicines induce cell death: the molecular and cellular mechanisms for cancer therapy. Biomed Res Int 2014; 2014: 530342.

[4] Zhou L, Zuo Z, Chow MS. Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol 2005; 45: 1345-59.

[5] Cheng TO. Danshen: what every cardiologist should know about this Chinese herbal drug. Int J Cardiol 2006; 110: 411-2.

[6] Li MH, Chen JM, Peng Y, Wu Q, Xiao PG. Investigation of Danshen and related medicinal plants in China. J Ethnopharmacol 2008; 120: 419-26.

[7] Zhang Y1, Jiang P, Ye M, Kim SH, Jiang C, Lü J. Tanshinones: sources, pharmacokinetics and anti-cancer activities. Int J Mol Sci 2012; 13: 13621-66.

[8] Chen L, Zheng SZ, Sun ZG, Wang AY, Huang CH, Punchard NA, *et al*. Cryptotanshinone has diverse effects on cell cycle events in melanoma cell lines with different metastatic capacity. Cancer Chemother Pharmacol 2011; 68: 17-27.

[9] Su CC, Chen GW, Lin JG. Growth inhibition and apoptosis induction by tanshinone I in human colon cancer Colo 205 cells. Int J Mol Med 2008; 22: 613-8.

[10] Wang L, Wu J, Lu J, Ma R, Sun D, Tang J. Regulation of the cell cycle and PI3K/Akt/mTOR signaling pathway by tanshinone I in human breast cancer cell lines. Mol Med Rep 2015; 11: 931-9.

[11] Won SH, Lee HJ, Jeong SJ, Lü J, Kim SH. Activation of p53 signaling and inhibition of androgen receptor mediate tanshinone IIA induced G1 arrest in LNCaP prostate cancer cells. Phytother Res 2012; 26: 669-74.

[12] Wang JF, Feng JG, Han J, Zhang BB, Mao WM. The molecular mechanisms of Tanshinone IIA on the apoptosis and arrest of human esophageal carcinoma cells. Biomed Res Int 2014; 2014: 582730.

[13] Tung YT, Chen HL, Lee CY, Chou YC, Lee PY, Tsai HC, *et al*. Active component of Danshen (Salvia miltiorrhiza Bunge), tanshinone I, attenuates lung tumorigenesis *via* inhibitions of VEGF, cyclin a, and cyclin b expressions. Evid Based Complement Alternat Med 2013; 2013: 319247.

[14] Xu D, Lin TH, Li S, Da J, Wen XQ, Ding J, *et al*. Cryptotanshinone suppresses androgen receptor-mediated growth in androgen dependent and castration resistant prostate cancer cells. Cancer Lett 2012; 316: 11-22.

[15] Xu D, Lin TH, Zhang C, Tsai YC, Li S, Zhang J, *et al*. The selective inhibitory effect of a synthetic tanshinone derivative on prostate cancer cells. Prostate 2012; 72: 803-16.

[16] Wu CY, Hsieh CY, Huang KE, Chang C, Kang HY. Cryptotanshinone down-regulates androgen receptor signaling by modulating lysine-specific demethylase 1 function. Int J Cancer 2012; 131: 1423-34.

[17] Shin DS, Kim HN, Shin KD, Yoon YJ, Kim SJ, Han DC, *et al*. Cryptotanshinone inhibits constitutive signal transducer and activator of transcription 3 function through blocking the dimerization in DU145 prostate cancer cells. Cancer Res 2009; 69: 193-202.

[18] Wang Y, Li JX, Wang YQ, Miao ZH. Tanshinone I inhibits tumor angiogenesis by reducing Stat3 phosphorylation at Tyr705 and hypoxia-induced HIF-1α accumulation in both endothelial and tumor cells. Oncotarget 2015; 6: 16031-42.

[19] Tang C, Xue HL, Huang HB, Wang XG. Tanshinone IIA inhibits constitutive STAT3 activation, suppresses proliferation, and induces apoptosis in rat C6 glioma cells. Neurosci Lett 2010; 470: 126-9.

[20] Lin C, Wang L, Wang H, Yang L, Guo H, Wang X. Tanshinone IIA inhibits breast cancer stem cells growth *in vitro* and *in vivo* through attenuation of IL-6/STAT3/NF-kB signaling pathways. J Cell Biochem 2013; 114: 2061-70.

[21] Liu JJ, Liu WD, Yang HZ, Zhang Y, Fang ZG, Liu PQ, *et al*. Inactivation of PI3k/Akt signaling pathway and activation of caspase-3 are involved in tanshinone I-induced apoptosis in myeloid leukemia cells *in vitro*. Ann Hematol 2010; 89: 1089-97.

[22] Won SH, Lee HJ, Jeong SJ, Lee HJ, Lee EO, Jung DB, *et al*. Tanshinone IIA induces mitochondria dependent apoptosis in prostate cancer cells in association with an inhibition of phosphoinositide 3-kinase/AKT pathway. Biol Pharm Bull 2010; 33: 1828-34.

[23] Lin JY, Ke YM, Lai JS, Ho TF. Tanshinone IIA enhances the effects of TRAIL by downregulating survivin in human ovarian carcinoma cells. Phytomedicine 2015; 22: 929-38.

[24] Su CC, Lin YH. Tanshinone IIA down-regulates the protein expression of ErbB-2 and up-regulates TNF-alpha in colon cancer cells *in vitro* and *in vivo*. Int J Mol Med 2008; 22: 847-51.

[25] Li Y, Gong Y, Li L, Abdolmaleky HM, Zhou JR. Bioactive tanshinone I inhibits the growth of lung cancer in part *via* downregulation of Aurora A function. Mol Carcinog 2013; 52: 535-43.

[26] Xu L, Feng JM, Li JX, Zhu JM, Song SS, Tong LJ, *et al*. Tanshinone-1 induces tumor cell killing, enhanced by inhibition of secondary activation of signaling networks. Cell Death Dis 2013; 4: e905.

[27] Zhang J, Wang J, Jiang JY, Liu SD, Fu K, Liu HY. Tanshinone IIA induces cytochrome c-mediated caspase cascade apoptosis in A549 human lung cancer cells *via* the JNK pathway. Int J Oncol 2014; 45: 683-90.

[28] Chiu SC, Huang SY, Chen SP, Su CC, Chiu TL, Pang CY. Tanshinone IIA inhibits human prostate cancer cells growth by induction of endoplasmic reticulum stress *in vitro* and *in vivo*. Prostate Cancer Prostatic Dis 2013; 16: 315-22.

[29] Pan TL, Wang PW, Hung YC, Huang CH, Rau KM. Proteomic analysis reveals tanshinone IIA enhances apoptosis of advanced cervix carcinoma CaSki cells through mitochondria intrinsic and endoplasmic reticulum stress pathways. Proteomics 2013; 13: 3411-23.

[30] Yun SM, Jung JH, Jeong SJ, Sohn EJ, Kim B, Kim SH. Tanshinone IIA induces autophagic cell death *via* activation of AMPK and ERK and inhibition of mTOR and p70 S6K in KBM-5 leukemia cells. Phytother Res 2014; 28: 458-64.

[31] Nizamutdinova IT, Lee GW, Lee JS, Cho MK, Son KH, Jeon SJ, *et al*. Tanshinone I suppresses growth and invasion of human breast cancer cells, MDA-MB-231, through regulation of adhesion molecules. Carcinogenesis 2008; 29: 1885-92.

[32] Lee CY, Sher HF, Chen HW, Liu CC, Chen CH, Lin CS, *et al*. Anticancer effects of tanshinone I in human non-small cell lung cancer. Mol Cancer Ther 2008; 7: 3527-38.

[33] Yuxian X, Feng T, Ren L, Zhengcai L. Tanshinone II-A inhibits invasion and metastasis of human hepatocellular carcinoma cells *in vitro* and *in vivo*. Tumori 2009; 95: 789-95.

[34] Chen W, Lu Y, Chen G, Huang S. Molecular evidence of cryptotanshinone for treatment and prevention of human cancer. Anticancer Agents Med Chem 2013; 13: 979-87.

[35] Tsai MY, Yang RC, Wu HT, Pang JH, Huang ST. Anti-angiogenic effect of Tanshinone IIA involves inhibition of matrix invasion and modification of MMP-2/TIMP-2 secretion in vascular endothelial cells. Cancer Lett 2011; 310: 198-206.

[36] Li G, Shan C, Liu L, Zhou T, Zhou J, Hu X, *et al*. Tanshinone IIA inhibits HIF-1α and VEGF expression in breast cancer cells *via* mTOR/p70S6K/RPS6/4E-BP1 signaling pathway. PLoS One 2015; 10: e0117440.

[37] Zhang K, Li J, Meng W, Xing H, Yang Y. C/EBPβ and CHOP participate in tanshinone IIA-induced differentiation and apoptosis of acute promyelocytic leukemia cells *in vitro*. Int J Hematol 2010; 92: 571-8.

[38] Micheau O, Shirley S, Dufour F. Death receptors as targets in cancer. Br J Pharmacol 2013; 169: 1723-44.

[39] Hellwig CT, Rehm M. TRAIL signaling and synergy mechanisms used in TRAIL-based combination therapies. Mol Cancer Ther 2012; 11: 3-13.

[40] Stuckey DW, Shah K. TRAIL on trial: preclinical advances in cancer therapy. Trends Mol Med 2013; 19: 685-94.

[41] Lemke J, von Karstedt S, Zinngrebe J, Walczak H. Getting TRAIL back on track for cancer therapy. Cell Death Differ 2014; 21: 1350-64.

[42] Dimberg LY, Anderson CK, Camidge R, Behbakht K, Thorburn A, Ford HL. On the TRAIL to successful cancer therapy? Predicting and counteracting resistance against TRAIL-based therapeutics. Oncogene 2013; 32: 1341-50.

[43] Prasad S, Kim JH, Gupta SC, Aggarwal BB. Targeting death receptors for TRAIL by agents designed by Mother Nature. Trends Pharmacol Sci 2014; 35: 520-36.

[44] Dai X, Zhang J, Arfuso F, Chinnathambi A, Zayed ME, Alharbi SA, *et al*. Targeting TNF-related apoptosis-inducing ligand (TRAIL) receptor by natural products as a potential therapeutic approach for cancer therapy. Exp Biol Med (Maywood) 2015; 240: 760-73.

[45] Trivedi R, Mishra DP. Trailing TRAIL resistance: novel targets for TRAIL sensitization in cancer cells. Front Oncol 2015; 5: 69.

[46] Newsom-Davis T, Prieske S, Walczak H. Is TRAIL the holy grail of cancer therapy? Apoptosis 2009; 14: 607-23.

[47] de Wilt LH, Kroon J, Jansen G, de Jong S, Peters GJ, Kruyt FA. Bortezomib and TRAIL: a perfect match for apoptotic elimination of tumour cells? Crit Rev Oncol Hematol 2013; 85:363-72.

[48] Siegelin MD, Habel A, Gaiser T. 17-AAG sensitized malignant glioma cells to death-receptor mediated apoptosis. Neurobiol Dis 2009; 33: 243-9.

[49] Jiang CC, Chen LH, Gillespie S, Kiejda KA, Mhaidat N, Wang YF, *et al*. Tunicamycin sensitizes human melanoma cells to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by up-regulation of TRAIL-R2 *via* the unfolded protein response. Cancer Res 2007; 67: 5880-8.

[50] Fulda S, Wick W, Weller M, Debatin KM. Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma *in vivo*. Nat Med 2002; 8: 808-15.

[51] Li L, Thomas RM, Suzuki H, De Brabander JK, Wang X, Harran PG. A small molecule Smac mimic potentiates TRAIL- and TNFalpha-mediated cell death. Science 2004; 305: 1471-74.

[52] Fakler M, Loeder S, Vogler M, Schneider K, Jeremias I, Debatin KM *et al*. Small molecule XIAP inhibitors cooperate with TRAIL to induce apoptosis in childhood acute leukemia cells and overcome Bcl-2-mediated resistance. Blood 2009; 113: 1710-22.

[53] Lecis D, Drago C, Manzoni L, Seneci P, Scolastico C, Mastrangelo E *et al*. Novel SMAC-mimetics synergistically stimulate melanoma cell death in combination with TRAIL and Bortezomib. Br J Cancer 2010; 102: 1707-16.

[54] Henrich CJ, Brooks AD, Erickson KL, Thomas CL, Bokesch HR, Tewary P, *et al*. Withanolide E sensitizes renal carcinoma cells to TRAIL-induced apoptosis by increasing cFLIP degradation. Cell Death Dis 2015; 6: e1666.

[55] Kumazaki M, Shinohara H, Taniguchi K, Ueda H, Nishi M, Ryo A, *et al*. Understanding of tolerance in TRAIL-induced apoptosis and cancelation of its machinery by α-mangostin, a xanthone derivative. Oncotarget 2015; 6: 25828-42.

[56] Bucur O, Gaidos G, Yatawara A, Pennarun B, Rupasinghe C, Roux J, *et al*. A novel caspase 8 selective small molecule potentiates TRAIL-induced cell death. Sci Rep 2015; 5: 9893.

[57] Chang CC, Lai JS, Tsai CS, Ma SW, Lin JY, Huang LR, *et al*. Proapoptotic and TRAIL-sensitizing constituents isolated from *Salvia militiorrhiza* (Danshen). J Biosci Bioeng 2013; 116: 516-23.

[58] Tse AK, Chow KY, Cao HH, Cheng CY, Kwan HY, Yu H, *et al*. The herbal compound cryptotanshinone restores sensitivity in cancer cells that are resistant to the tumor necrosis factor-related apoptosis-inducing ligand. J Biol Chem 2013; 288: 29923-33.

[59] Shin EA, Sohn EJ, Won G, Choi JU, Jeong M, Kim B, *et al*. Up-regulation of microRNA135a-3p and death receptor 5 plays a critical role in Tanshinone I sensitized prostate cancer cells to TRAIL induced apoptosis. Oncotarget. 2014; 5: 5624-36.

[60] Chang CC, Kuan CP, Lin JY, Lai JS, Ho TF. Tanshinone IIA facilitates TRAIL sensitization by up-regulating DR5 through the ROS-JNK-CHOP signaling axis in human ovarian carcinoma cell lines. Chem Res Toxicol. 2015; 28: 1574-83.

[61] Athanasoula KCh, Gogas H, Polonifi K, Vaiopoulos AG, Polyzos A, Mantzourani M. Survivin beyond physiology: orchestration of multistep carcinogenesis and therapeutic potentials. Cancer Lett 2014; 347: 175-82.

[62] Ryu BJ, Hwang MK, Park M, Lee K, Kim SH. Thiourea compound AW00178 sensitizes human H1299 lung carcinoma cells to TRAIL-mediated apoptosis. Bioorg Med Chem Lett 2012; 22: 3862-5.

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Case report

Influencing and moderating factors analyzed in the group art therapy of two schizophrenic inpatients

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ABSTRACT

Art therapy has been considered a guideline treatment for schizophrenia. Due to difficulty in the outcome measurement, the research is difficult and controversial. Here, we presented two schizophrenic patients receiving the regular art group therapy. We compared their characteristics and different outcome. Art therapy is difficult to quantify. However, we could qualify the improvement from the individual case. Further study might be focus on how to make appropriate qualification of art therapy and individualized difference instead of enrollment of huge data bank.

1. Introduction

Although art therapy has been considered a guideline treatment for schizophrenia [1, 2], the reviewed article shows an inconsistent result [3].

Here, we have presented two schizophrenic patients that received semi-structured art therapy by one trained psychiatrist. We have analyzed their clinical symptoms, psychosocial problems, and the images during the therapy.

All images were made with informed consent for publication and in anonymity.

2. Case reports

A 19-year-old, patient A was diagnosed with schizophrenia 4 years ago, with the presentation the disease being auditory hallucinations with voices commenting, referential delusions, and delusion of thought. She originally studied in the special education department of a university but stopped due to mental illness. Due to the aggravation of her psychotic symptoms and violent attempts toward her family, she was admitted to our acute psychiatric ward. Under a daily treatment of 15 mg of aripiprazole, she became relatively stable and began to take part in a semi-structured art therapy group weekly. She initially drew a girl with wonder in a white skirt on a green lawn. With the progression of time, her drawings became more abundant and integrated. She also began to become involved in the story telling of her creations. The themes started from the imprisoned long-hair princess in the high tower of a castle and the collage work she did by herself. After receiving the pharmacologic and art therapy treatment for around one month, the patient was relatively stable and was able to reestablish a good relationship with her family. Her Positive and Negative Syndrome Scale (PANSS) [4] was improved from 90 to 65 and Scale for the Assessment of Negative Symptoms (SANS) [5] went from 69 to 45.

Patient B is a 37-year-old housewife suffering from schizophrenia for the past 10 years with the manifestations of the disease being disorganized behavior, disorganized speech, auditory hallucinations, and olfactory hallucinations. She was compulsorily admitted to our acute psychiatric ward according to the Mental Health Act of Taiwan due to aggravation of disturbing and violent behavior. She received a risperidone consta depot injection because she refused treatment. What's interesting is that she did express interest in participating in the semi-structured art therapy group. She enjoyed using crayons to freely associate the theme denoted by the therapist. Her pictures were all of poorly understood symbols and religious subjects. Her PANSS improved from 114 to 92 and her SANS went from 94 to 69.

The semi-structured art therapy was performed by the trained psychiatrist, with a frequency of four times every month. Each session's guiding sentence for what the patients should draw were similar and used the photo card collection that was published by the Taiwan Art Therapy Association (TATA). The therapist used cards full of images, and the patients drew their favorite images. After completion of their works, the therapist invited them to re-

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Table 1 − A comparison of the two patients.

Variables	Patient A	Patient B
Age	19	37
Gender	Female	Female
Education	Senior high school	Junior high school
Occupation	Student	Housewife
Illness duration (years)	4	10
Schizophrenia subtype (DSM-IV)	paranoid	disorganized
PANSS improvement	90→65	114→92
SANS improvement	69→45	94→69
Medication	Oral atypical antipsychotics	Injection depot
Favorite theme(s)	Fairy tales	Religion, Symbols
Favorite tool(s)	Colorful pencils	Crayons
Frequency of art therapy (times/month)	4	4
Key family person	Parents	Husband
Psychosocial stressor	Sibling conflicts	Marital problems

Image 1 from the patient A, with the title of "The Dancer on the Earth"

Image 2 from the patient A with the title of "The Long-Haired Princess Imprisoned in the Castle"

Image 3 from the patient B with the tile of "Candy Time Machine"

late the themes of their pictures.

3. Discussion

From the images created by patient A and B, individual differences among their age, education, occupation, clinical symptoms, and even schizophrenia subtypes as defined by the DSM-IV [6] can be discerned. In summary, we outlined the comparison and difference from these two patients in the Table 1.

The literature reviews are consistent with our results that the better outcome as measured from the PANSS in clinical symptoms seem to be associated with a patient's younger age, higher level of functioning before the mental illness, paranoia type, and a lower SANS [7, 8]. In schizophrenic patients, improvement in negative symptoms predicted changes in Neuroticism and (inversely) in Extraversion and Openness [9].

Eligibility criteria in the previous MATISSE study were broad. For the small effect observed when group art therapy was offered to all patients with a diagnosis of schizophrenia, and the

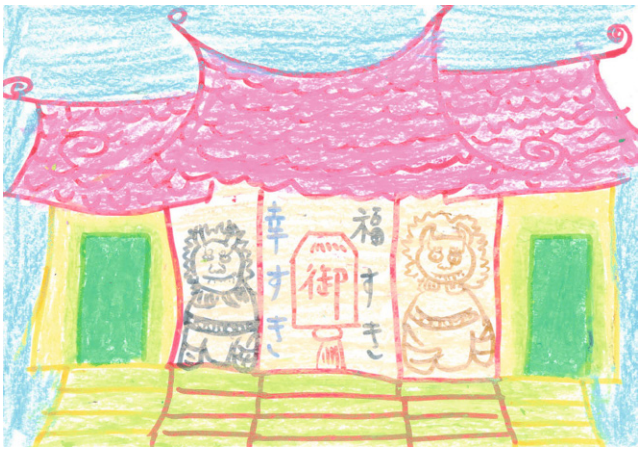

Image 4 from from the patient B with the tile of "One Buddha Temple"

resources involved in delivering such an intervention, it is important to identify patients who appear the most likely to benefit from such therapy [10].

From the two patients presented here, we might see that motivation plays an important role in improving the clinical symptoms of schizophrenics. Another interesting and novel finding in these two patients was that the younger patient tended to be involved in story telling style in the group art therapy while the older patient appeared to be more involved in her own fantastic and strange world.

Due to both the variety of art therapy and patient subgroups, further study might be needed to fully elucidate the benefits of group art therapy in patients suffering from schizophrenia.

REFERENCES

[1] Patterson S, Debate J, Anju S, Waller D, Crawford MJ. Provision and practice of art therapy for people with schizophrenia: results of a national survey. J Ment Health 2011; 20: 328.

[2] Montag C, Haase L, Seidel D, Bayerl M, Gallinat J, Herrmann U, *et al*. A pilot RCT of psychodynamic group art therapy for patients in acute psychotic episodes: feasibility, impact on symptoms and mentalising capacity. PloS One 2014; 9: e112348.

[3] Leurent B, Killaspy H, Osborn DP, Crawford MJ, Hoadley A, Waller D, *et al*. Moderating factors for the effectiveness of group art therapy for schizophrenia: secondary analysis of data from the MATISSE randomised controlled trial. Soc Psychiatry Psychiatr Epidemiol 2014; 49: 1703.

[4] Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987; 13: 261.

[5] Andereasen NC. Scale for Assessment of Negative Symptoms (SANS). Iowa City: The University of Iowa; 1981.

[6] Association AP. Diagnostic and Statistical Manual of Mental Disorders. 4 ed: American Psychiatric Association, Washington, D.C.; 1994.

[7] Jaaskelainen E, Haapea M, Rautio N, Juola P, Penttila M, Nordstrom T, *et al*. Twenty Years of Schizophrenia Research in the Northern Finland Birth Cohort 1966: A Systematic Review. Schizophr Res Treatment 2015; 2015: 524875.

[8] Castagnini AC, Munk-Jorgensen P, Bertelsen A. Short-term course and outcome of acute and transient psychotic disorders: Differences from other types of psychosis with acute onset. Int J Soc Psychiatry 2015.

[9] Boyette LL, Nederlof J, Meijer C, de Boer F, de Haan L, for G. Three year stability of Five-Factor Model personality traits in relation to changes in symptom levels in patients with schizophrenia or related disorders. Psychiatry Res 2015.

[10] Patterson S BR, Waller DE. Considering referral to art therapy: responses to referral and experiences of participants in a randomised controlled trial. Int J Art Ther 2013; 18: 8.