CLINICAL SPOTLIGHT
49 Craniofacial dysmorphism, what is your diagnosis?

REVIEW ARTICLES
51 Acupuncture as treatment for nervous system diseases
58 Stem cell therapy in amyotrophic lateral sclerosis
64 Spinal pelvic-urethra reflex potentiation
68 Inflammation in psychopathology of depression: Clinical, biological, and therapeutic implications
75 Glutamate theory in developing novel pharmacotherapies for obsessive compulsive disorder: Focusing on N-methyl-D-aspartate signaling

CASE REPORT
80 Nonketotic hyperglycinemia: A case report and brief review
BioMedicine

EDITORIAL BOARD

Consultant Editor

Noboru Mizushima  Professor, Department of Physiology and Cell Biology, Tokyo Medical and Dental University Graduate School and Faculty of Medicine, Japan

Editor-in-Chief

Fuu-Jen Tsai  Professor of Pediatrics and Dean, Office of Research and Development, China Medical University, Taiwan; Director, Genetic Center, China Medical University Hospital, Taiwan

Editorial Board

Jan-Gowth Chang  Vice Superintendent, Kaohsiung Medical University Hospital; Professor, School of Medicine, Kaohsiung Medical University, Taiwan

Chien-Jen Chen  Academician and Distinguished Research Fellow, Genomic Research Center, Academia Sinica; Professor, Graduate Institute of Epidemiology and Preventive Medicine, National Taiwan University College of Public Health, Taiwan

Chih-Ping Chen  Professor, Mackay Medical College; Professor, Department of Obstetrics and Gynecology, School of Medicine, National Yang-Ming University, Taiwan

Yuan-Tsong Chen  Academician and Distinguished Research Fellow, Academia Sinica, Taiwan

Jing-Gung Chung  Professor, Department of Biological Science and Technology, China Medical University, Taiwan

Chih-Yang Huang  Director and Professor, Graduate Institute of Basic Medical Science, and Deputy-Director, R&D Institution, China Medical University, Taiwan

Mien-Chie Hung  Vice President for Basic Research, and Distinguished Teaching Professor and Chair, Department of Molecular and Cellular Oncology, The University of Texas M. D. Anderson Cancer Center, USA

Kuo-Hsiung Lee  Kenan Distinguished Professor of Medicinal Chemistry, and Director, Natural Products Research Laboratories, University of North Carolina–Chapel Hill, USA

Chong-Kuei Lii  Professor, Department of Nutrition, China Medical University, Taiwan

Ming-Chei Maa  Professor, Graduate Institute of Molecular Systems Biomedicine, China Medical University, Taiwan

Catherine Fang-Yeu Poh  Associate Professor, Oral Biological and Medical Sciences, Faculty of Dentistry, University of British Columbia; Clinician Scientist, Integrative Oncology, BC Cancer Agency Research Centre, Canada

W. Gibson Wood  Professor, Department of Pharmacology, School of Medicine, University of Minnesota; Geriatric Research, Education and Clinical Center, VA Medical Center, USA

Mei-Chin Yin  Professor, Department of Nutrition, China Medical University, Taiwan
BioMedicine is a peer-reviewed journal that aims to publish high quality scientific research in the field of translational and personalized medicine, with the goal of promoting and disseminating medical science knowledge to improve global health. It is published quarterly by Elsevier.

Articles on clinical, laboratory and social research in translational and personalized medicine, rare diseases and other related fields that are of interest to the medical profession are eligible for consideration. Review articles, original articles, case reports, short communications, and letters to the editor are accepted.

Editorial Office
BioMedicine
No. 91, Hsueh-Shih Road, Taichung 40402, Taiwan
Tel: (+886) 4-22070672; Fax: (+886) 4-22070813
E-mail: biomed1958@gmail.com

Subscription Information
Delivery will be made only upon receipt of payment. All orders and subscription-related communication, including notification of change of address for delivery, payments for subscription and inquiries about membership, should be directed to the Editorial Office.

Individual
• Within Taiwan: NT$3000/year by surface mail.
• Outside Taiwan: US$200/year by surface mail.

Institutional
• US$350/year (4 issues) by surface mail.

Copyright Information
Submission of a manuscript implies:
• that the work described has not been previously published (except in the form of an abstract);
• that it is not under consideration for publication elsewhere;
• that it has been approved by all co-authors, if any, as well as by the responsible authorities at the institute where the work was carried out;
• that, if and when the manuscript is accepted for publication, the authors agree to automatic transfer of copyright to China Medical University;
• that the manuscript will not be published elsewhere in any language without consent from China Medical University;
• that written permission has been obtained by the authors from the copyright holders of material used from other copyrighted sources.

All articles published in BioMedicine are protected by copyright, which covers the exclusive rights to reproduce and distribute the article, as well as translation rights. No part of this publication may be reproduced, stored in any retrieval system, or transmitted in any form or by any means, electronic, mechanical, by photocopying, recording, or otherwise, without prior written permission from China Medical University.

Advertisements
Requests for information and orders should be addressed to the Editorial Office. Advertisements are reviewed in light of appropriate ethical considerations before being accepted for publication. The publication of advertisements relies on the responsibility of the advertiser to comply with all legal requirements relating to the marketing and sale of the products or services advertised. The publication of an advertisement neither constitutes nor implies a guarantee or endorsement, by China Medical University and Elsevier, of the product or service advertised, or the claims made for it by the advertiser. BioMedicine reserves the right to discontinue any advertisement it so wishes.

Disclaimer
While the advice and information in this journal are believed to be true and accurate at the date of it going to press, the authors, China Medical University and Elsevier, cannot accept any legal responsibility for any errors or omissions that may be made. They make no warranty, express or implied, with respect to material contained herein. To the extent permissible under applicable laws, no responsibility is assumed by China Medical University or Elsevier for any injury and/or damage to persons or property as a result of any actual or alleged libelous statements, infringement of intellectual property or privacy rights, or products liability, whether resulting from negligence or otherwise, or from any use or operation of any ideas, instructions, procedures, products or methods contained in the material herein. The opinions expressed in this journal belong to the authors and do not necessarily reflect the opinions of China Medical University and Elsevier.

Publisher
ELSEVIER
http://www.elsevier.com
Journal Manager: Janet Amali Joseph
TABLE OF CONTENTS

June 2012  Volume 2  Number 2

Clinical Spotlight

49  Craniofacial dysmorphism, what is your diagnosis?
Chung-Hsing Wang, Wei-De Lin, Fuu-Jen Tsai

Review Articles

51  Acupuncture as treatment for nervous system diseases
Ching-Liang Hsieh

58  Stem cell therapy in amyotrophic lateral sclerosis
Kuo-Wei Hsueh, An-Chang Hsieh, Horng-Jyh Harn, Shinn-Zong Lin

64  Spinal pelvic-urethra reflex potentiation
Hsien-Yu Peng, Tzer-Bin Lin

68  Inflammation in psychopathology of depression: Clinical, biological, and therapeutic implications
Kuan-Pin Su

75  Glutamate theory in developing novel pharmacotherapies for obsessive compulsive disorder: Focusing on N-methyl-D-aspartate signaling
Po-Lun Wu, Hsien-Yuan Lane, Hwa-Sheng Tang, Guochuan E. Tsai

Case Report

80  Nonketotic hyperglycinemia: A case report and brief review
Yu-Tzu Chang, Wei-De Lin, Zheng-Nan Chin, Chung-Shing Wang, I.-Ching Chou, Huang-Tsung Kuo, Fuu-Jen Tsai
A 12-year-old girl, presented to the Outpatient Department with congenital non-progressive cranial-facial-digital abnormalities and normal developmental milestones, had a past medical history of congenital heart disease (atrial septal defect, tricuspid valve regurgitation) under long-term follow-up. Clinically, specific phenotype was characterized by widely separated eyes (hypertelorism), broad nasal root, bifid nasal tip, clinodactyly over left 5th finger and slim fingers Fig. 1 (Panel A,B and C). Computed tomography of her head revealed no skull asymmetry or brachycephaly (Panel D). Her mother showed similar characteristics but with facial asymmetry (Panel E) due to isolated right coronal suture craniosynostosis that had received surgical correction. Although craniofrontonasal dysplasia was our first impression of diagnosis according to the index case’s clinical manifestations, mode of inheritance (most likely autosomal dominant or X-linked dominant disorder) and a hint of isolated craniosynostosis from her mother, several disorders having similar symptoms to those of craniofrontonasal dysplasia, including Aarskog syndrome (widely spaced eyes and broad nose; but lack of low-set ear, short, broad hands with stubby hands, genital malformations and mental retardation), Frontonasal dysplasia (for her hypertelorism, broad nose, vertical groove down the tip of the nose; fall short of nose split into two, brachycephaly, cleft lip and/or palate, microphthalmia and mental retardation; sporadic occurrence), Frontofacialnasal dysplasia (wide space between the eyes, no cleft lip and/or palate, telecanthus, brachycephaly, mid-face hypoplasia; autosomal recessive inheritance), Greig cephalopolysyndactyly syndrome (widely separated eyes; but no prominent forehead, polydactyly and/or syndactyly) and a variety of craniosynosostosis (not seen in this index patient, but her mother had prematurely closed right coronal skull suture) syndromes, should be on the list of differential diagnoses. Craniofrontonasal dysplasia has an X-linked dominant inheritance mode. Direct sequencing of all exons and exon-intron boundaries of the $EFNB1$ gene subsequently revealed C-to-G transversion at nucleotide 354 in exon 2 [$EFNB1$ (Xq12), exon2: c.354C>G, p.S118R] (Panel F). This disease-causing novel mutation was inherited from her mother with intrafamilial phenotypic variability. Craniofrontonasal dysplasia is inherited as an X-linked dominant inheritance mode. Direct sequencing of all exons and exon-intron boundaries of the $EFNB1$ gene subsequently revealed C-to-G transversion at nucleotide 354 in exon 2 [$EFNB1$ (Xq12), exon2: c.354C>G, p.S118R] (Panel F). This disease-causing novel mutation was inherited from her mother with intrafamilial phenotypic variability. Craniofrontonasal dysplasia is inherited as an X-linked dominant pattern. A female inherits one X chromosome from each parent, while a male gets an X chromosome from the mother and a Y from the father. The male
thus displays X-linked trait from the mother, while a female may have X-linked traits from either parent. Because of dominantly inherited pattern, only one copy of defective gene EFNB1 is necessary for the disease to appear. No definite therapy for craniofrontonasal dysplasia is available so far, except for plastic surgery.

Fig. 1 – Panel A,B,C - peculiar phenotypes of the index patient; Panel D - neuroimage of index case; Panel E - index patient’s mother on right side of the picture; Panel F - mutated nucleotide of EFNB1 gene depicted. (Note: Informed consent to publish patient photographs was obtained.)
Acupunture as treatment for nervous system diseases

Ching-Liang Hsieh a,b,c,*

a Graduate Institute of Acupuncture Science, College of Chinese Medicine, China Medical University, Taichung, Taiwan
b Acupuncture Research Center, China Medical University, Taichung, Taiwan
c Department of Chinese Medicine, China Medical University Hospital, Taichung, Taiwan

ABSTRACT

Acupuncture and moxibustion have been used for at least 2000 years to treat a wide range of diseases. In recognition of the increasing worldwide interest in the subject, the World Health Organization conducted a symposium on acupuncture in 1979 and put forth a list of 40 suitable diseases that can be treated with this approach. In Taiwan, acupuncture is widely used as a tool to treat diseases and disorders of the nervous system such as stroke, dementia, Parkinson’s disease, and carpal tunnel syndrome. Although numerous studies on the effectiveness of acupuncture have been conducted, the efficacy of acupuncture as treatment for nervous system diseases or disorders has been questioned mainly because only a limited number of controlled clinical trials have been published. The aim of this review is to determine whether there is enough evidence in previously published trials to support the beneficial effects of acupuncture on diseases of the nervous system.

Copyright © 2012, China Medical University. Published by Elsevier Taiwan LLC. All rights reserved.

1. Introduction

Acupuncture and moxibustion have been used to treat diseases in China for over 2000 years. The meridian theory of acupuncture was first recorded in detail in The Yellow Emperor’s Classic of Internal Medicine [1,2], although The Great Compendium of Acupuncture and Moxibustion, published in the Ming Dynasty, forms the basis of modern acupuncture theory and practice [2]. In recognition of the increasing worldwide interest in the subject, the World Health Organization conducted a symposium on acupuncture in 1979 and put forth a list of 40 suitable diseases that can be treated with this approach [3]. In Taiwan, acupuncture is commonly used to treat diseases and disorders of the nervous system, namely stroke, dementia, Parkinson’s disease, epilepsy, Bell’s palsy, carpal tunnel syndrome, and headache. Although numerous studies on the effectiveness of acupuncture have been conducted, the efficacy of acupuncture as treatment for nervous system diseases or disorders has been questioned mainly because only a limited number of controlled clinical trials have been published. The aim of this review is to determine whether there is enough evidence in previously published trials to support the beneficial effects of acupuncture on diseases of the nervous system.

2. Acupuncture in stroke

Stroke is one of the most common diseases in Taiwan, and was the third leading cause of death in that country in 2011 [4].
Hu et al. conducted a randomized, controlled study to evaluate the effect of acupuncture on acute stroke symptoms. A total of 30 patients with onset of symptoms within 36 hours were randomly assigned to receive acupuncture in combination with conventional supportive treatment or to receive supportive treatment only. Acupuncture was applied three times per week for 4 weeks and patients were then followed-up for 3 months. They found that neurologic outcome was significantly better in the acupuncture group on day 28 and on day 90, and that improvement in neurologic status was greatest in patients with a poor baseline neurologic score [5]. In a randomized controlled study on the effects of electro-acupuncture (EA) in patients with first-ever ischemic stroke, Hsieh et al. reported that patients who received eight courses of EA with stimulation pulses alternating between 3 Hz and 5 Hz over a 1-month period showed significantly better improvement in motor function than patients who received conventional rehabilitation treatment only [6]. Johansson et al. studied whether sensory stimulation can improve functional outcome in stroke patients. A total of 78 patients with severe hemiplegia were randomized within 10 days after stroke onset to receive either daily physiotherapy alone (40 patients) or in combination with EA (2–5 Hz) for 30 minutes twice a week for 10 weeks (38 patients). They found that patients who received EA recovered faster than controls and showed greater improvement in balance, activities of daily living (ADL), and quality of life [7]. In a multicenter controlled trial by the same group, 150 patients with moderate to severe hemiparesis were randomized 5–10 days after stroke onset to receive EA, transcutaneous electrical nerve stimulation (TENS), or subliminal electrostimulation (control group). A total of 20 treatment sessions were performed over a 10-week period and outcome variables were assessed at the 3-month and 1-year follow-ups. Interestingly, there were no significant differences among the three groups in improvement in motor function, walking ability or ADL [8], indicating that acupuncture did not produce a beneficial effect on functional outcome in stroke patients. Similar results were reported by Gosman-Hedström et al. in their randomized study on the effects of acupuncture treatment on daily life activities and quality of life in 104 patients with acute stroke [9], and by Sze et al. who found that there was no significant difference in outcome between stroke patients with moderate or severe functional impairment who received acupuncture at 10 acupoints for 10 weeks and stroke patients who received standard post-stroke motor rehabilitation training [10]. Lack of a beneficial effect of acupuncture as treatment for stroke patients has been reported in other studies as well [11,12].

A number of studies, however, have shown that acupuncture is an efficacious post-stroke therapy. For example, Naeser et al. reported that patients with right-sided hemiplegia due to left hemispheric ischemic infarction who received 20 acupuncture sessions over a period of 1 month beginning 1–3 months after stroke onset showed a significantly better response than patients who received sham acupuncture [13]. In addition, Kjendahl et al. found that acupuncture applied for 30 minutes three or four times per week for 6 weeks resulted in a positive long-term effect on motor function, daily life quality, and social interaction in patients with subacute stage (mean, 40 days) stroke 1 year after hospital discharge [14].

Recently, Liu et al. conducted a randomized, crossover pilot study on the effects of EA on motor recovery in chronic stroke survivors. A total of 10 stroke patients who had suffered a stroke more than 2 years prior to enrolment were randomized to receive either EA (1–2 Hz) plus strength training treatment twice per week for 6 weeks or a 6-week session of strength training treatment only. They found that patients who received 2 Hz EA plus strength training treatment had a marked reduction in muscle spasticity of the wrist and a marked increase in active wrist extension range of motion and Fugl-Meyer upper-limb upper-limb scores. These effects were not noted in patients who only received strength training treatment. The findings indicate that EA reduces muscle spasticity and enhances performance of motor tasks [15]. Results from a similar study also revealed that EA (twice per week) combined with muscle training exercises for 6 weeks reduced the degree of muscle spasticity in chronic stroke patients [16].

In addition, a recent study showed that acupuncture treatment accompanied by manual twisting of needles at the Baihui (GV20) acupoint and at the spirit acupoints for 20 minutes in patients with first-ever ischemic stroke resulted in significantly greater reduction in displacement area than acupuncture without twisting of needles, indicating that acupuncture with twisting of needles can improve balance function [17].

Rorsman and Johansson investigated whether EA or TENS influences cognitive and emotional outcome after stroke. In their study, 54 stroke patients with moderate or severe functional impairment were randomized to receive acupuncture including EA, TENS, or subliminal TENS (control group). Acupuncture started from 5–10 days after stroke onset, and was performed for 30 minutes, two times per week for 10 weeks. They found that there were no significant differences in changes among the three groups in emotional status or cognitive function at 3 or 12 months after treatment [18]. In contrast, Chou et al. showed that 1 EA (1 Hz) applied to the PC6 and HT7 acupoints for 20 minutes, twice per week for 8 weeks, had a positive effect on cognition and life quality in stroke patients with cognitive impairment [19].

The effectiveness of acupuncture on motor function, ADL, and cognitive function in patients with acute stroke remains controversial. More rigorous randomized controlled studies comprising larger patient populations are needed to definitively determine whether acupuncture is a valuable treatment for stroke patients.

3. Acupuncture as treatment for degenerative disorders

3.1. Alzheimer's disease and vascular dementia

Alzheimer’s disease is a chronic progressive degenerative disease. Several systematic review articles have revealed that acupuncture is not effective in patients with Alzheimer’s disease [20] or vascular dementia [21,22]. However, Zhou and Jin showed that acupuncture had a beneficial effect in patients with Alzheimer’s disease. The researchers used functional magnetic resonance imaging to evaluate brain changes in 26
patients with Alzheimer’s disease who underwent EA. Electro-
stimulation was applied to the Shenmen (HT7) acupoint as an anode and the Zusanli (ST36) acupoint as a cathode as well as to the Fenlong (ST40) acupoint as an anode and the Taixi (K13) acupoint as a cathode. They found that electro-
stimulation at these acupoints resulted in increased activity in the hippocampal gyrus and insula in the right hemisphere, the temporal lobe, and parietal lobe of the left hemisphere. These regions are associated with cognitive function such as memory and language, suggesting that acupuncture at those acupoints produces a beneficial effect in patients with Alz-
heimer’s disease [23]. In addition, Yang et al. demonstrated that acupressure could reduce the degree of agitation behavior in patients with dementia. The researchers applied acupres-
sure to the Fengchi (GB20), Baihui (GV20), Shenmen (HT7), Neiguan (PC6), and Sanyinjiao (SP6) acupoints for 2 minutes after a 5-minute warm-up activity, twice daily, 5 days a week for 4 weeks in 20 patients with dementia who demonstrated agitated behavior. They found that acupressure dramatically reduced the degree of agitation in their patients [24]. Addi-
tional randomized, controlled, double-blinded trials comprising larger patient populations are needed to determine whether acupuncture induces a beneficial effect in patients with Alzheimer’s disease or vascular dementia.

3.2. Parkinson’s disease

Parkinson’s disease is a chronic progressive disease charac-
terized by slow movements, tremors, and walking impair-
ment due to loss of midbrain nigrostriatal neurons and depletion of striatal dopamine. About 63% of patients with Parkinson’s disease in Korea [25] and 25% of patients with the disease in Singapore [26] use acupuncture as a complemen-
tary therapy; however, a number of large-scale studies have shown that acupuncture is not effective at alleviating the symptoms of Parkinson’s disease [27–30]. The results from small-scale studies are similar. For example, Eng et al. used acupuncture in combination with Yin Tui Na massage on a weekly basis as treatment for patients with Parkinson’s disease for 6 months and found that scores on the Unified Parkinson’s Disease Rating Scale (UPDRS) were significantly higher than baseline scores 6 months after treatment [31]. In addition, Shulman et al. found that acupuncture applied to body or scalp acupoints for 1 hour twice per week for 5–8 weeks in patients with Parkinson’s disease resulted in improvements in sleep and rest but did not improve other symptoms of the disease [32]. Functional magnetic resonance imaging studies have shown that acupuncture applied to the Yanglingquan (GB34) acupoint results in the activation of portions of the putamen and the primary motor cortex, resulting in improved motor function [33]. A positron emis-
sion tomography study showed that scalp acupuncture and Madopa therapy for 5 weeks resulted in an increase in glucose metabolism in five patients with Parkinson’s disease [34]. In another study, although EA administered to the scalp in addition to administration of levodopa for 5 weeks increased hemispheric regional blood flow, administration of those treatments did not result in changes in striatal dopamine transporter density in the basal ganglia [35]. Taken together, although acupuncture including EA does not seem to improve motor function or ADL in patients with Parkinson’s disease, acupuncture does seem to improve blood flow and glucose metabolism in brain tissue in patients with the disease, sug-
gesting that acupuncture does play a beneficial role in delay-
ing intellectual decline in patients with Parkinson’s disease. Further large-scale studies are warranted.

4. Acupuncture as treatment for headache

4.1. Migraine

Studies have shown that non-specific psychological interac-
tions play a major role in the improvement of many patients with headache, which might explain why some studies comparing the effects of acupuncture with those of placebo have demonstrated that there are no differences in outcome between the two treatments [36]. Nevertheless, a number of studies have shown that acupuncture is an effective treatment for certain types of headache. For example, studies have demonstrated that acupuncture is both a clinically efficacious and a cost-effective treatment for migraine, especially when applied to the Fenchi (GB20) and Taiyang (EX-HN5) acupoints for 30 minutes twice a week for at least 10 weeks [37–39]. Also, Allais et al. used the needle contact test to detect the most efficacious points on the ear for reducing pain during a migraine episode and found that the insertion of semi-permanent need-
dles into the anterior-internal part of the antitragus ipsilateral to the side of pain resulted in pain relief within 30 minutes and that it persisted for up to 24 hours [40,41].

The results of a multicenter, single-blinded randomized controlled trial comprising 480 patients with migraine revealed that patients who received acupuncture including electrostimulation had significantly fewer days of migraine pain than patients who received sham acupuncture during weeks 13–16 after treatment, indicating that acupuncture produces a clinically minor prophylactic effect [42]. Other studies have shown that acupuncture treatment is more effective than prophylactic drug treatment for migraine headaches [43–45]. In addition, Yang et al. demonstrated that acupuncture applied to the Cuanzhu (BL2), Taiyang (EX-HN5), Yintang (Ex-HN-3), and Fengchi (GB20) acupoints resulted in fewer episodes of migraine and led to fewer adverse events than topiramate treatment in patients with chronic migraine [46]. Studies have also demonstrated that acupuncture is more effective than fluornizine treatment at reducing the number of days of migraine pain [47]. Based on the abovementioned findings, acupuncture treatment appears to be beneficial for reducing the degree of pain associated with migraine head-
aches, particularly when applied as prophylactic treatment.

4.2. Tension-type headache

A Cochrane review of 11 clinical trials comprising 2317 patients with tension-type headache provided evidence that acupuncture treatment is valuable for patients who suffer frequent episodes of that type of headache. A number of other studies have also demonstrated that acupuncture results in a greater reduction in intensity and frequency of tension-type headache than sham acupuncture [48,49]. Acupuncture,
therefore, appears to be beneficial for reducing the frequency and severity of episodic tension-type headaches.

5. Acupuncture in epilepsy

Epilepsy is a chronic neurological condition characterized by excessive synchronization of neuronal networks, behavioral changes, and recurrent seizures. Most studies have demonstrated that acupuncture is not an effective treatment for epilepsy. For example, the authors of a Cochrane review concluded that there is not enough evidence to support the effectiveness of acupuncture as treatment for epilepsy, mainly because most of the studies were of poor methodological quality [50]. In addition, Kloster et al. showed that there was no difference in the frequency of seizure between patients with chronic intractable epilepsy who received acupuncture and patients who received sham acupuncture at bilateral Taichon (LR3), Hegu (LI4) and Baihui (GV20) acupoints for 30 minutes, three times per week for 7.5 weeks, indicating that acupuncture is not beneficial in patients with chronic intractable epilepsy [51]. A similar study on patients with intractable epilepsy [52].

---

**Fig. 1** — Acupuncture as treatment for nervous system disorders.
epilepsy showed that acupuncture treatment did not result in improved health-related quality of life [52]. Therefore, the results of many randomized, controlled trials indicate that acupuncture is not an effective treatment for epilepsy.

Vagal nerve stimulation and EA are promising neuroprotective therapies for patients with intractable epilepsy. Studies have shown that stimulation of acupoints stimulates the vagus nerve, and it is possible that vagal nerve stimulation and EA target the same center of the brain. The nucleus of the solitary tract is also the site of afferent signals produced by vagal nerve stimulation or EA when applied to scalp, face and auricular sites, and the neuroprotective effects of EA in epileptic patients may be due to the anti-inflammatory and neurotrophic actions produced through the nucleus of the solitary tract by stimulation of the vagus nerve [53, 54]. We found that 2 Hz and 100 Hz EA applied to bilateral Zusanli (ST36) acupoints resulted in reduced pulse rate, indicating that EA at Zusanli (ST36) induces parasympathetic activity [55]. He et al. hypothesized that auricular acupuncture may suppress epileptic seizures by activating parasympathetic activity. This increase in parasympathetic tone is believed to activate the nucleus of the solitary tract, which might result in the suppression of structures related to the pathogenesis of epilepsy or in the activation of the cholinergic anti-inflammatory pathway [56]. Further studies are needed to determine whether EA stimulation at specific acupoints has an antiepileptic effect as a result of EA-induced parasympathetic activity.

6. Acupuncture in peripheral nerve disease

6.1. Bell’s palsy

Bell’s palsy is defined as an idiopathic facial nerve paralysis and is the most common unilateral peripheral facial neuropathy. The cause of Bell’s palsy is unknown, but mounting evidence suggests that reactivated herpes virus infection plays a key role in its development [57, 58]. Although a Cochrane review concluded that there is inadequate evidence to support the effectiveness of acupuncture for Bell’s palsy [59], a number of studies have provided evidence that acupuncture is beneficial. For example, Li et al., in their multi-center, single-blinded, randomized controlled trial comprising 480 patients with Bell’s palsy, found that acupuncture applied to the Dicang (ST4), Jiache (ST6), Hegu (LI4), Yangbai (GB14), Xiaguan (ST7), and Yifeng (TE17) acupoints for 30 minutes followed by moxibustion for 5 minutes, five times per week for 4 weeks resulted in significant improvement in facial nerve function [60]. In addition, acupuncture treatment was shown to be safe and effective in improving functional and cosmetic outcome in a patient with a 7-year history of Bell’s palsy and in a pregnant patient with Bell’s palsy [61, 62]. More rigorous clinical studies are needed to determine whether acupuncture is an appropriate and effective treatment for patients with Bell’s palsy.

6.2. Carpal tunnel syndrome

Carpal tunnel syndrome (CTS) is a nerve disorder of the hand caused by compression of the median nerve. Surgical decompression is considered to be definitive treatment, although conservative treatment such as steroid injection (e.g., with prednisolone) and splinting is effective in some cases [63]. Acupuncture is often used as adjunct therapy, especially in Asian countries. Sim et al. reviewed 11 studies including 6 randomized controlled trials of the use of acupuncture as conservative therapy for CTS and found that there is not enough evidence to support its efficacy [64]. Yao et al., in their double-blinded, randomized controlled study of 34 patients with CTS, found that verum acupuncture was not superior to placebo acupuncture [65]. However, Napadow et al. used functional magnetic resonance imaging to evaluate differences in somatosensory cortical plasticity after acupuncture treatment between 13 adult patients with CTS and 12 age- and sex-matched healthy adults and found that acupuncture resulted in changes in digital cortical representation in CTS patients 5 weeks after acupuncture treatment. These changes, however, were not noted in the healthy adults [66]. They also found that verum acupuncture activated regions in the hypothalamus and deactivated regions in the amygdala in patients with CTS but not in healthy adults, indicating that acupuncture might modulate the limbic-paralimbic network [67]. In addition, studies have demonstrated that the effects of acupuncture applied to the Daing (PC7) and Neiguan (PC6) acupoints twice per week for 4 weeks on reducing symptoms of CTS were similar to those elicited by the administration of prednisolone [68], and that the effects of acupuncture treatment lasted longer than the effects obtained with that corticosteroid [69]. Although additional studies are needed to prove the efficacy of acupuncture for CTS, it appears that acupuncture is moderately beneficial as conservative treatment in patients with CTS.

7. Conclusion

Acupuncture treatment is beneficial in patients with subacute and chronic stroke, appears to be effective for migraine when used prophylactically, is an effective treatment for tension-type headache, and shows promise as an adjunct therapy for Bell’s palsy and carpal tunnel syndrome. More rigorous studies are needed to evaluate whether acupuncture is an efficacious treatment modality for Alzheimer’s disease, vascular dementia, Parkinson’s disease, and epilepsy (Fig. 1).

Acknowledgments

The author is grateful to Mr. Jeffrey Conrad for editorial assistance.

References


Amyotrophic lateral sclerosis (ALS) is a rare and lethal neurodegenerative disease for which there is no effective medical treatment. Although riluzole, an N-methyl-D-aspartate receptor antagonist, has been shown to be reasonably safe for patients with ALS, the drug has been demonstrated to prolong median survival by only 2–5 months. There is mounting evidence that stem cell-based gene therapy is a promising treatment modality for patients with ALS. In this review, we focus on the types, sources, and doses of stem cells that have been shown to be effective for ALS patients, the differences in cytokines or chemokines secreted from these various stem cells, and the immune-modulation activity of stem cells as treatment for ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an incurable, degenerative neurological disease. The clinical characteristics of ALS include muscle weakness and atrophy, spasticity, and eventual paralysis due to the progressive loss of spinal and brainstem motor neurons. Death typically occurs 3–5 years after symptoms begin [1,2]. The disease currently affects an estimated 350,000 people worldwide, and there are no effective treatments. Although riluzole, an N-methyl-D-aspartate receptor (NMDA) receptor antagonist, is the only drug approved by the US Food and Drug Administration for ALS, it has been shown to offer only a modest improvement in symptoms and to prolong median survival by a maximum of 3–5 months. Therefore, an effective treatment for ALS is urgently needed. This review article focuses on the emergence of stem cell-based gene therapy in ALS.

2. Molecular mechanisms mediating the development of ALS

It is still not fully understood why specific neuronal populations are selectively vulnerable in ALS. Mutations in several genes have been shown to be related to the development of the disease, including mutations in the SOD1, TARDBP...
(TDP-43), FUS/TLS, FIG4, and chromosome 9 open reading frame 72 (C9orf72) genes. A hexanucleotide repeat expansion of the C9orf72 gene has been identified as the underlying genetic cause of chromosome 9p21-linked frontotemporal lobar degeneration and ALS [3–5].

In addition, about 20% of cases of inherited ALS are caused by mutations in the superoxide dismutase-1 (SOD1) gene, particularly mutations that cause misfolding of the protein product [6]. Studies have shown that mutant SOD1 transgenic mice with loss of SOD1 function show phenotypic characteristics of motor neuron disease, including progressive deterioration of the brainstem and a functional loss of spinal motor neurons, resulting in weakness, loss of muscle function, and premature death. Interestingly, studies have shown that epigenetic factors, such as aging, are possible causes of ALS in more than 90% of patients with the disease [7].

3. Superoxide dismutases

In vivo, superoxide dismutases are responsible for peroxidation reactions in cells. These enzymes are divided into several species based on their intracellular locations. SOD1 (Cu/ZnSOD) is located in the mitochondrial intermembrane space and cytosol, while SOD2 (MnSOD) is found in the mitochondrial matrix. Although these dismutases are located in different intramitochondrial locations, these enzymes have the same catalytic functions.

4. Other molecular mechanisms of ALS

Excitotoxicity of motor neurons has also been implicated in the pathogenesis of ALS. Most patients with sporadic ALS express reduced levels of synaptosomal high-affinity glutamate uptake and glutamate transporters such as excitatory amino acid transporter 2 (EAAT2 or GLT1) in the motor cortex and spinal cord, resulting in apoptosis of motor neurons due to elevated extracellular glutamate concentrations [8,9]. The NMDA receptor antagonist riluzole effectively minimizes the overexcitation of motor neurons caused by elevated levels of extracellular glutamate and has been shown to have a good safety profile in patients with ALS; however, the drug only extends the lifespan of ALS patients by several months [10–14].

5. Pathology of mutant SOD1 transgenic mice

In 1994, Gurney et al established strains of transgenic mice that express mutant human SOD1 (mSOD1) in order to study the impact of overproduction of mutant SOD1 protein and its accumulation on motor neuron function in ALS [15,16]. Of these mSOD1 transgenic mice, a strain of hemizygous mice harboring human SOD1 with the G93A mutation in high copy number has been shown to be an appropriate model for studying ALS in mice with a short lifespan because these mice become completely paralyzed and die within 16–18 weeks of age. On the other hand, G93A-mSOD1 mice with a low transgene copy number are used to study ALS in mice with longer lifespans. These mice demonstrate much slower disease progression and die within 8–9 months of age [16]. The results of pathological studies of these mutant SOD1 mice have revealed accumulation of mutant SOD1 in the brainstem and spinal motor neurons, marked inflammation around the dying neurons, and overexpression of cytokines such as tumor necrosis factor alpha and interferon gamma in spinal lesions [17,18].

6. Stem cell therapy as treatment for ALS

Stem cells have the ability to continuously divide and differentiate into a number of different types of cell. Stem cells also secrete various cytokines, chemokines, and trophic factors that are known to modulate inflammation, attract other stem cells to sites of injury, enhance cell survival, and participate in angiogenesis and neurogenesis [19,20]. Tables 1 and 2 provide a review of clinical trials in ALS patients and animals.

<table>
<thead>
<tr>
<th>Humans</th>
<th>Stem cell source</th>
<th>Conditioning regimen</th>
<th>Delivery method</th>
<th>Dose</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS patients</td>
<td>CD34+ HSCs</td>
<td>Total body irradiation (450 cGy); tacrolimus (0.3 mg/kg/d IV) and methotrexate (5 mg/m² IV)</td>
<td>IV injection</td>
<td>Absolute neutrophil count &gt;5 x 10⁹/L</td>
<td>No clinical benefit</td>
</tr>
<tr>
<td>ALS patients</td>
<td>Autologous MSCs</td>
<td>None reported</td>
<td>Multiple intraspinal thoracic subcutaneous injections</td>
<td>Approximately 5.7 x 10⁶ cells total</td>
<td>Decelerated linear decline of forced vital capacity</td>
</tr>
<tr>
<td>ALS patients</td>
<td>Autologous CD133+ cells</td>
<td>None reported</td>
<td>Bilateral injection into frontal motor cortex</td>
<td>2.5–7.5 x 10⁶ cells/site</td>
<td>Survived more than 47 months</td>
</tr>
</tbody>
</table>

IV = intravenous; HSC = hematopoietic stem cells; MSC = mesenchymal stem cells; sALS = sporadic amyotrophic lateral sclerosis.
7. Hematopoietic stem cell therapy in ALS

CD34+ hematopoietic stem cells (HSTCs) were first used to treat patients with leukemia because these cells are easily isolated from bone marrow and peripheral blood. In 2008, however, Appel et al reported that CD34+ stem cell transplantation therapy could only be performed in patients who received peripheral blood HSTC from an identically matched human leukocyte antigen donor [21]. Transplantation by intravenous injection of HSTCs to ALS patients with an absolute neutrophil count > 0.5 × 10⁹/L revealed that inflammatory cells (macrophages and monocytes) proliferated in the spinal cord. The authors then used immunohistochemical staining to show that HSTCs accumulated in the spinal cord and released chemokines. Although the results showed that administration of HSTCs induced a strong immune response, the authors found that the use of CD34+ stem cells did not lead to any marked improvement in symptoms of ALS [21] (Table 3).

8. Effectiveness of mononuclear cells from umbilical cord blood in ALS

Recently, Garbuzova-Davis et al treated pre-symptomatic G93A-mSOD1 mice with an intravenous injection of a single low dose (10 × 10⁶ cells), a moderate dose (25 × 10⁶ cells), or a high dose (50 × 10⁶ cells) of umbilical cord blood-derived mononuclear cells (MNC-hUCBs) and found that the moderate dose (25 × 10⁶ cells) significantly increased lifespan by 20–25% and delayed disease progression by 15% [22]. The most beneficial effect on decreasing proinflammatory cytokines in the brain and spinal cord was found in mice that received moderate-dose therapy. In addition, results of hematological assays showed that the number of lymphocytes was significantly higher and the number of neutrophils significantly lower in the peripheral blood of mice that received a dose of 25 × 10⁶ cells than in the peripheral blood of mice that received low-dose or high-dose therapy. Moderate-dose therapy was also shown to result in a marked reduction in microglial density in the cervical and the lumbar spinal cord, indicating that MNC-hUCB cells transplanted via intravenous injection can move into cervical and lumbar tissue. The findings demonstrate that transplantation via intravenous injection of a moderate dose (25 × 10⁶ cells) of MNC-hUCB cells may provide a neuroprotective effect for motor neurons and prolong the survival rate of mice with ALS [20].

9. Mesenchymal stem cell therapy in ALS

Bone marrow-derived mesenchymal stem cells are widely used for the treatment of many human diseases [23]. Mazzini et al found that bone marrow-derived mesenchymal stem cells that had been cultured for two or three generations and then transplanted via multiple intraspinal thoracic injections at a dose of 57 × 10⁶ cells did not produce a strong

<table>
<thead>
<tr>
<th>Trial institutions</th>
<th>Stem cell source</th>
<th>Delivery method</th>
<th>Dose</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuralstem Inc.</td>
<td>Human spinal cord-derived neural stem cells</td>
<td>Surgical implantation</td>
<td>None reported</td>
<td>Phase I Safe. Delay in symptoms of disease</td>
</tr>
<tr>
<td>Fundacion para a Formacion e Investigacion Sanitarias de la Region de Murcia Corestem, Inc</td>
<td>Autologous bone marrow-derived stem cells</td>
<td>Intraspinal transplantation and intrathecal infusion of autologous bone marrow stem cells</td>
<td>None reported</td>
<td>Phase II</td>
</tr>
<tr>
<td>Mayo Clinic</td>
<td>Mesenchymal stem cells</td>
<td>Single intrathecal lumbar puncture</td>
<td>10 × 6 cells</td>
<td>Phase I</td>
</tr>
<tr>
<td>TCA Cellular Therapy</td>
<td>Autologous bone marrow-derived stem cells</td>
<td>Infusion of autologous bone marrow-derived stem cells</td>
<td>None reported</td>
<td>Phase I</td>
</tr>
<tr>
<td>Hadassah Medical Organization</td>
<td>Autologous cultured mesenchymal bone marrow stromal cells secreting neurotrophic factors</td>
<td>IM in patients with early ALS</td>
<td>IM: patients were injected at 24 sites with a total of 24 million cells IT: intrathecally via a standard lumbar puncture, with a total of 60 million cells</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

HYNR-CS = autologous bone marrow-derived stem cells; IM = intramuscular; IT = spinal cord injection. muscle injection.

Use of cell therapy for the treatment of amyotrophic lateral sclerosis in animal models.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Stem cell source</th>
<th>Conditioning regimen</th>
<th>Delivery method</th>
<th>Dose</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD1&lt;sup&gt;G93A&lt;/sup&gt; rats</td>
<td>Human NPCs</td>
<td>FK-506 (1 mg/kg daily)</td>
<td>Bilateral lumbar SC injections</td>
<td>2 × 10&lt;sup&gt;6&lt;/sup&gt; cells/site, eight sites</td>
<td>No NMJs with host muscle</td>
</tr>
<tr>
<td>SOD1&lt;sup&gt;G93A&lt;/sup&gt; rats</td>
<td>Rat GRPs</td>
<td>Ciclosporin A (10 mg/kg daily)</td>
<td>Bilateral cervical SC injections</td>
<td>1 × 10&lt;sup&gt;5&lt;/sup&gt; cells/site, six sites</td>
<td>Prevented MN loss; increased lifespan</td>
</tr>
<tr>
<td>SOD1&lt;sup&gt;G93A&lt;/sup&gt; rats</td>
<td>Human NPCs</td>
<td>Ciclosporin A (10 mg/kg daily)</td>
<td>Unilateral lumbar SC injections</td>
<td>1.2–1.8 × 10&lt;sup&gt;5&lt;/sup&gt; cells/site, four sites</td>
<td>Prevented MN loss; did not innervate muscle end plates</td>
</tr>
<tr>
<td>SOD1&lt;sup&gt;G93A&lt;/sup&gt; rats</td>
<td>Human MSCs</td>
<td>Ciclosporin A (10 mg/kg daily), focal muscular injury with bupivacaine hydrochloride (0.35 mg)</td>
<td>Bilateral muscle injections</td>
<td>1.2 × 10&lt;sup&gt;5&lt;/sup&gt; cells/site</td>
<td>Increased number of NMJs and MN cell bodies; prevented loss of proximal MNs</td>
</tr>
<tr>
<td>SOD1&lt;sup&gt;G93A&lt;/sup&gt; mice</td>
<td>Human MSCs</td>
<td>None</td>
<td>Unilateral lumbar SC injection</td>
<td>1 × 10&lt;sup&gt;6&lt;/sup&gt; cells/site</td>
<td>Delayed MN loss; improved motor performance</td>
</tr>
<tr>
<td>SOD1&lt;sup&gt;G93A&lt;/sup&gt; mice</td>
<td>Human umbilical cord blood cells</td>
<td>Ciclosporin A (10 mg/kg daily)</td>
<td>IV injection</td>
<td>10 × 10&lt;sup&gt;6&lt;/sup&gt; cells, 25 × 10&lt;sup&gt;6&lt;/sup&gt; cells, or 50 × 10&lt;sup&gt;6&lt;/sup&gt; cells per mouse</td>
<td>25 × 10&lt;sup&gt;6&lt;/sup&gt; cells was the most effective dose; increased lifespan (20–25%) and delayed disease progression (15%)</td>
</tr>
<tr>
<td>SOD1&lt;sup&gt;G93A&lt;/sup&gt;/PU.1, SOD1&lt;sup&gt;G93A&lt;/sup&gt;/RAG2 mice</td>
<td>Mouse BM</td>
<td>Gamma-irradiation (400 rads)</td>
<td>IP injection, SOD1&lt;sup&gt;G93A&lt;/sup&gt;/PU.1/mice; IV injection, SOD1&lt;sup&gt;G93A&lt;/sup&gt;/RAG2/mice</td>
<td>1 × 10&lt;sup&gt;7&lt;/sup&gt; cells per SOD1&lt;sup&gt;G93A&lt;/sup&gt;/PU.1 mouse; 3 × 10&lt;sup&gt;7&lt;/sup&gt; cells per SOD1&lt;sup&gt;G93A&lt;/sup&gt;/RAG2 mouse</td>
<td>Prolonged survival</td>
</tr>
</tbody>
</table>

BM; GRP; IP = intraperitoneal; IV = intravenous; MN = motor neuron; MSC = mesenchymal stem cells; NMJ = neuromuscular junction; NPC; SC = spinal cord.

There is mounting evidence that progression of ALS is related to inflammatory and immune responses. A recent study by Rentzos et al revealed that the levels of CD8 cytotoxic T-cells and natural killer T-cells were significantly higher and that the number of regulatory T-cells was significantly lower in the peripheral blood of ALS patients than in blood from normal controls [25]. Therefore, mesenchymal stem cell therapy might be able to modulate the host immune inflammatory response and extend the survival of ALS patients.

**10. Neuroprogenitor cell therapy in ALS**

CD133<sup>+</sup> stem cells have the ability to differentiate into multiple neural lineages. Recently, Martinez et al studied the effects of CD133<sup>+</sup> progenitor cells on survival in ALS patients. A total of 20 patients with ALS were randomized to a treatment group or a control group. The treatment group received a subcutaneous injection of 300 µg of filgrastim for 3 days to stimulate the overproduction of stem cells in bone marrow. CD133<sup>+</sup> stem cells were then separated from peripheral blood using magnetic bead separation. Patients then received 2.5–7.5 × 10<sup>5</sup> cells per 300 µL cerebrospinal fluid (CSF) by bilateral injection into the frontal motor cortex. They found that transplantation of CD133<sup>+</sup> progenitor cells resulted in a delay in disease progression and increased survival [26].

**11. Stem cell therapy in ALS at the China Medical University**

Mesenchymal stem cells can be derived from a number of tissue types, including adipose tissue, dental pulp, and umbilical cord blood. Our group is currently studying the effects of different types of stem cells in animal models of ALS to determine the type that is most effective at suppressing the immune and inflammatory responses and which can therefore be developed as an appropriate vector therapy.

Our preliminary data show that the effects of stem cells as treatment for neurodegenerative diseases may be due to their ability to secrete chemokines or their ability to regulate the immune response. We have found that transplantation of stem cells by cortical spinal tract injection prolongs the lifespan of mice with ALS by about 150 days. The results of immunohistochemical staining have revealed the presence of inflammatory response and did not result in abnormal cell proliferation in the spinal cord in ALS patients during 4 years of post-transplant follow-up. In addition, 50% of patients showed evidence of a significant slowing down of the linear decline of forced vital capacity [24]. Cell transplantation by spinal injection is a high-risk procedure, and the long-term safety profiles of administering stem cells via that approach need to be established in clinical trials involving larger numbers of patients.

...
several types of stem cell in the lumbar spinal cord and evidence of increased levels of chemokines and trophic factors, such as stromal cell-derived factor-1 (SDF-1), Brain-derived neurotrophic factor (BDNF), and C-X-C chemokine receptor type 4 (CXCR-4). The findings support our hypothesis that transplanted stem cells are attracted to sites of injury by inflammatory signaling molecules (Fig. 1).

In the future, we plan to conduct preclinical trials to study the effects of stem cells that have been transfected with wildtype SOD1 as well as the effects of other stem cell-based gene therapies on disease progression and survival in animal models of amyotrophic lateral sclerosis.

Acknowledgments

This work was supported by grants NSC 100-2314-B-039-006-MY3 from the National Science Council, Taiwan, and this study was supported in part by the Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH101-TD-B-111-004).

Fig. 1 – Hypothetical mechanisms governing the effectiveness of stem cell therapy as treatment for amyotrophic lateral sclerosis. Transplantation of stem cells results in increased levels of chemokines and trophic factors ( SDF-1, BDNF, CXCR-4), resulting in delayed progression of motor neuron disease. IFN = interferon; TNF = tumor necrosis factor; mSOD = mutant human superoxide dismutase; Treg = regulatory T-cell.

References


Spinal pelvic-urethra reflex potentiation

Hsien-Yu Peng a,b, Tzer-Bin Lin a,b,c,d,*

a Department of Urology, China Medical University Hospital, Taichung, Taiwan
b Department of Physiology, College of Medicine, China Medical University, Taichung, Taiwan
c Department of Medical Education, Saint Paul’s Hospital, Taoyuan, Taiwan
d Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei, Taiwan

Abstract

Spinal reflex potentiation (SRP) in the pelvic-urethra reflex activity is a form of activity-dependent neural plasticity, presumed to be essential for urethra contraction resulting in continence under physiological conditions and also to underlie the pelvic pain caused by pathology in the pelvic cavity. Studies have demonstrated that SRP could be induced by electrical shocks, bladder distension and activation of the lumbosacral (L6-S1) spinal glutamatergic NMDA and AMPA receptors. Conversely, blockage of glutamatergic receptors using selective antagonists either attenuated or abolished the established SRP. Electrical shocks on and nicotine microinjection into the pontine tegmentum area facilitated SRP induction, but intrathecal serotonin antagonists abolished electrical stimulation- and nicotine-induced facilitation on SRP. Finally, the induction of SRP is highly estrogen-dependent, because surgical ablation of menstruation diminished the SRP which is prevented by estradiol supplements, and SRP is more significant in proestrus (high estrogen but low progesterone) than in metestrus (both estrogen and progesterone are low) of the menstrual cycle. We propose that SRP is relevant to urine continence under physiological conditions, and pathological facilitation of SRP could result in pelvic pain.

1. Introduction

In the central nervous system (CNS), repetitive activation of synaptic connections could lead to strengthening of synaptic efficacy in a variety of brain structures [1–4]. In the CA1 area of the hippocampus, long-term potentiation (LTP) [5,6], a tetanization-induced enhancement in synaptic efficacy, has been investigated extensively in the last three decades, because it is considered a fundamental mechanism of learning and memory formation [4,7]. In addition, “windup”, a pain-related synaptic plasticity, characterized by a progressive increase in evoked activity in the wide dynamic dorsal horn neurons, is presumed to underlie the development of allodynia and/or hyperalgesia [8,9].

2. Pelvic-urethra reflex potentiation

The pelvic-urethral reflex (PUR), in which sensory impulses induced by bladder distension transmit centripetally onto the
dorsal horn neurons through the pelvic afferent fibers [10] and, after integrating within the spinal cord, motor impulses emerge via the pudendal efferent fibers, and therefore, cause external urethra sphincter contractions [11,12], has been shown to be essential for the urethra to develop sufficient resistance to maintain continence during the micturition cycle [13]. Spinal reflex potentiation (SRP) of the pelvic-urethra reflex was first reported in 2003, by demonstrating the firing of pudendal efferent nerves (PENs) and external urethra sphincter electromyogram (EUSE) evoked by pelvic afferent nerve (PAN) repetitive stimulation (RS, 1 stimulation/1 second), increased progressively following stimulation onset, then reached a plateau which was maintained until stimulation ceased. In contrast, PAN test stimulation (TS, 1 stimulation/30 seconds) evoked relatively constant baseline reflex activity with a single action potential [14]. Additionally, in parallel to inducing SRP, RS on PAN elongated the contraction wave of the urethra, implying the SRP of the pelvic-urethra reflex potentiation is physiologically relevant to urethra closure [14–16]. Moreover, stepwise saline distension of the urinary bladder from 0 to 4, 8, 12 and 16 mmHg dose-dependently potentiated TS-evoked baseline pelvic-urethra reflex activity, accompanied by elongation of the urethra contraction wave [17], suggesting physiological challenges, such as bladder distension, could induce SRP. Finally, during the early storage stage of a voiding cycle, negligible increments in intravesical pressure did not induce background spontaneous firing in EUSE, whereas, off-line analysis demonstrated that TS-evoked pelvic-urethra reflex activities were potentiated in parallel with intravesical pressure increases during this stage [17], suggesting that the strength of the pelvic-urethra reflex fluctuates following the micturition cycle. Together, these results imply that SRP could be a physiological phenomenon which occurs under physiological conditions.

3. Involved neurotransmission

Investigations using spinal administration of test agents demonstrated that SRP caused by repetitive electric shocks [18–25], bladder saline distension [17], rhythmic voiding cycle [22] and noxious visceral irritation [23–31] is blocked by intrathecal application of 2-amino-5-phosphono-valerate (APV), a glutamatergic NMDA receptor antagonist, and diminished 
administration of serotonin antagonist and high level spinal cord transaction at T1 level both abolished the facilitation on SRP caused by DPT stimulation [18,32]. In another study, microinjection of nicotinic agonist to the DPT, exhibited similar facilitatory effects on SRP as synchronized electric shocks did, and conversely, pharmacological blockage of the nicotinic cholinergic receptors (nACh) in this area abolished the modulation exhibited by the nicotinic agonist [33,34]. Additionally, the nicotinic agonist-induced facilitation on SRP was also blocked by intrathecal serotonin antagonist and T1 level spinal transaction [35]. Together these data suggest that activating nACh receptors at the DPT may modulate SRP induction via descending serotonergic neurotransmission.

4. Descending control

Researchers investigating the possible areas exhibiting descending control on the SRP, have revealed that at the dorsal pontine tegmentum (DPT), synchronized electrical shocks to PAN stimulation facilitated RS-induced SRP. Spinal administration of serotonin antagonist and high level spinal cord transaction at T1 level both abolished the facilitation on SRP caused by DPT stimulation [18,32]. In another study, microinjection of nicotinic agonist to the DPT, exhibited similar facilitatory effects on SRP as synchronized electric shocks did, and conversely, pharmacological blockage of the nicotinic cholinergic receptors (nACh) in this area abolished the modulation exhibited by the nicotinic agonist [33,34]. Additionally, the nicotinic agonist-induced facilitation on SRP was also blocked by intrathecal serotonin antagonist and T1 level spinal transaction [35]. Together these data suggest that activating nACh receptors at the DPT may modulate SRP induction via descending serotonergic neurotransmission.

5. Impacts of female gonadal hormones

Whether or not levels of circulatory estrogen affect lower urinary function through effects on SRP was first investigated using rats which received a sham operation (Sham), ovariectomy (OVX), or ovariectomy followed by estrogen supplementation (OVX+E). The magnitude of the RS-induced SRP and associated urethra contraction wave elongation decreased significantly in the OVX group, which was partially reversed by supplemental estrogen [15], indicating that estrogen impacts lower urinary function through modulating SRP. Moreover, by recording the evoked reflex activity in rats in different estrus stages of the female cycle, studies have shown noxious visceral stimulation induced SRP in both the proestrus and metestrus stages. However, the degree of reflex potentiation was significantly higher in the proestrus rats than the metestrus ones [31], implying that the strength of the SRP fluctuates in response to estrogen levels across different estrus stages. In addition to genomic actions mediated by nucleus receptors, administration of 17β-estradiol (5 μg/kg) was demonstrated to acutely facilitate noxious visceral stimulation-induced SRP which was reversed by intrathecal pre-treatment with ICI 182780, a non-selective membrane estrogen receptor antagonist [28], indicating a role of membrane estrogen receptor on the estrogen-dependent facilitation of SRP.

In addition to estrogen, a regimen of daily progesterone for 4 days attenuated RS-induced SRP and simultaneously regulated the expression of GABA_A receptor alpha 2, alpha 3, alpha 4 and delta subunits in ovariectomized rats. Finasteride, an antagonist of neurosteroid synthesis from progesterone, but not RU486, a progesterone receptor antagonist, reversed the progesterone-dependent inhibition of SRP. Moreover, SRP was attenuated after a short intrathecal treatment with the neurosteroids, allopregnanolone and 3α,5α-tetrahydrodexoxy-corticosterone (THDOC). Acute intrathecal administration of the GABA_A receptor antagonist biccuculline reversed the inhibition produced by progesterone, THDOC and allopregnanolone. These results imply that, through its metabolic neurosteroid, progesterone inhibits SRP by exerting effects on spinal GABA_A receptor expression [27]. This proposal is further supported by a study that showed progesterone, as well as two of its 3α,5α-derivatives, allopregnanolone and THDOC, is capable of producing acute GABA_A receptor-dependent inhibition of SRP.
6. Possible role in urethral spasms

To investigate the rationale that anal stretch could relieve the high urethral resistance in neurogenic bladder or detrusor-sphincter dysynergia, the impact of acute anal stretch on PUR potentiation was examined in urethane-anesthetized rats. Acute anal stretch using a mosquito clamp with a distance of 4 mm exhibited no effect, whereas distances of 8 mm attenuated and 12 mm abolished the repetitive stimulation-induced SRP. Intrathecal pretreatment with bicuculline, a GABA_A receptor antagonist, but not hydroxyaslofen, a GABA_A receptor antagonist, counteracted the anal stretch-dependent abolition of PUR potentiation [36]. These results suggested that GABAergic neurotransmission is an important spinal mechanism involved in inhibition of reflexive external urethra sphincter activity. Moreover, acute manual anal stretch, a maneuver that activates the spinal GABAergic system, may be used as an adjunct to assist voiding dysfunction in patients with overactive urethral sphincters.

7. Conclusion

The induction of spinal reflex potentiation in the pelvic-urethra reflex activity seems to be physiologically relevant to urethra closure, and pathologically relevant to pelvic pain. Therefore, its underlying mechanisms could provide information for developing a therapeutic strategy for neurogenic pelvic pain.

REFERENCES


Inflammation in psychopathology of depression: Clinical, biological, and therapeutic implications

Kuan-Pin Su a,b,*

a Graduate Institute of Neural and Cognitive Sciences, School of Medicine, China Medical University, Taichung, Taiwan
b Department of Psychiatry and Mind-Body Research Center (MBI-Lab), China Medical University Hospital, Taichung, Taiwan

Article history:
Received 13 March 2012
Received in revised form 14 March 2012
Accepted 21 March 2012
Available online 1 May 2012

Keywords:
antidepressant
anti-inflammatory
cytokines
depression
docosahexaenoic acid (DHA)
eicosapentaenoic acid (EPA)
inflammation
interleukin (IL)
interferon-α (IFN-α)
omega-3 (n-3) polyunsaturated fatty acids (PUFAs)

Abstract
Increasing evidence suggests that inflammation responses play an important role in the pathophysiology of depression. Clinically depressed patients manifest higher levels of inflammatory biomarkers, while proinflammatory cytokines induce neuropsychiatric symptoms (sickness behavior) as well as major depressive episode. Mechanisms that might be responsible for inflammation-mediated neuropsychiatric and depressive symptoms are vital in understanding "mind–body" interface; these have been studied in clinical and animal models (e.g., interferon-α-induced depression in patients with chronic hepatitis C, one of the most notable clinical models for testing inflammation theory of depression and an excellent approach to investigate development of depression in a prospective manner). Furthermore, the anti-inflammatory pathway has become a hot topic in looking for new antidepressant therapies. Recently, omega-3 polyunsaturated fatty acids (omega-3 PUFAs or n-3 PUFAs) have gained more attention as a promising treatment for depression. n-3 Polyunsaturated fatty acids, both natural anti-inflammatory and antidepressant agents. Here, we 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
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 [1]. MDD is a serious psychiatric illness with a high lifetime prevalence rate up to one-tenth or one-fifth [2]. In general medical practice, at least one in 10 outpatients has this condition; most cases go unrecognized or are inappropriately treated, leading to loss of productivity, functional decline, and higher mortality [2]. Nevertheless, currently available treatments fail to address many crucial needs of patients adequately, making this illness difficult to treat and burdensome to the patients life, family, and career.
Clinical features, biological markers, and treatment outcomes are heterogeneous. According to the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV), and/or the International Statistical Classification of Diseases and Related Health Problems, 10th Revision, individuals within diagnostic categories of MDD have distinct clinical manifestations. Use of the current diagnostic schemas thus undoubtedly contributes to difficulties in finding any single biological or genetic marker [3]. Treatment efficacy and occurrence of adverse effects associated with specific antidepressants vary widely among patients. Accordingly, with the unsatisfactory outcome of pharmacotherapy and small-to-moderate effect sizes from most biomarker studies and clinical trials, it is impossible to explain the whole picture of etiology of MDD with any single hypothesis. The inflammation theory lights a promising path to resolve the dilemma of depression. Clinical patients exhibit higher levels of inflammatory biomarkers [4]. Administration of therapeutic cytokine interferon-α (IFN-α) can lead to clinical depression [5]. In fact, it has become a hot topic in medical research to look for antidepressant therapies from anti-inflammatory pathways [4]. Chronic inflammation is linked with early childhood trauma, major psychiatric disorders, and several physical diseases; the inflammation theory provides a window to investigate the mind–body interface.

Nowadays, omega-3 polyunsaturated fatty acids (omega-3 PUFAs) or n-3 PUFAs) provide a promising path to understand 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 like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and omega-6 PUFAs like arachidonic acid (AA) are important constituents of all cell membranes, which are essential for survival of humans and other mammals. They cannot be synthesized in the body but must be obtained from our diet and are thus called essential fatty acids [6]. PUFAs themselves appear active in biological function; some of their functions require conversion to eicosanoids and products like prostaglandins, thromboxanes (TXs), and leukotrienes (LTs). 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 [6]. This review summarizes current evidence about omega-3 PUFAs biological mechanisms of and inflammation in depression.

2. Inflammation theory of depression

Increasing evidence suggests inflammation response playing an important role in pathophysiology of depression: for example, patients with elevated C-reactive protein, acute phase proteins, and proinflammatory cytokines [4]. The latter include tumor necrotic factor (TNF-α), interleukin (IL-1β, IL-6, soluble TNF-R2, soluble IL6-R), and interferon (IFN-γ and IFN-α), all found to interact with many pathophysiological domains that characterize depression: neurotransmitter metabolism, neuroendocrine function, synaptic plasticity, and behavior [5]. Systemic inflammatory challenges like lipopolysaccharide or proinflammatory cytokine not only cause a systemic inflammation, but also induce central neuroinflammation for sustained periods [7]. A series of behavioral changes induced by neuroinflammation in experiment animals include anorexia, sleep abnormalities, reduction of locomotor activity and exploration, anhedonia, and cognitive disturbances, which share a strong similarity with somatic symptoms of depression. Sick individuals are somewhat depressed and lethargic. The idea of sickness behavior emanates from a series of observed symptoms related to infection and cytokine/prostaglandins administration in humans and animals. It offers us a good model to study the effects of cytokine on the brain and behavior [6,8].

Excessive secretion of proinflammatory cytokines is proposed to cause depression [9]. Microglia are resident macrophages of the brain, acting as chief immune defense in the central nervous system [10]. Neuroinflammatory processes are proposed as contributing to neuropsychiatric disorders like Alzheimer’s or Parkinson’s disease, as well as depression, via microglial activation [10]. Engagement of immune-to-brain communication pathways by proinflammatory cytokines (e.g., IFN-α, IFN-γ, IL-1) ultimately leads to microglial activation and triggers inflammatory signaling pathways [10,11]. Upon activation, microglia up-regulate the expression of detrimental factors of reactive oxygen species such as nitric oxide via inducible nitric oxide synthase and induce oxidative stress [12], contributing to neuropsychiatric pathogenesis [10,13]. On the other hand, expression of antioxidative enzymes like heme oxygenase-1 can reverse oxidative stress and may characterize antidepressant mechanisms [12,14]. In addition, neuroinflammation reduces the survival of serotonergic neurons [15] and decreases neurogenesis [16], while antidepressants exert neuroprotection against microglia-mediated neurotoxicity [17].

Early-life adverse experiences are not only risk factors for psychiatric disorders but also physical diseases for adulthood. Children exposed to adverse psychosocial stressors display enduring low-grade systemic inflammation [18], which is not only a risk factor for depression but also a feature of chronic physical diseases: metabolic syndrome, type 2 diabetes, cardiovascular disease, coronary artery disease, cancer, and dementia. Interestingly, these physical diseases are all commonly comorbid in patients with depression [19]. The inflammation theory thus explains the high comorbidity of physical illness in depression and potential “interface between mind and body” [20].

2.1. IFN-α-induced neuropsychiatric symptoms: sickness behavior and depression

Most notably, this theory gains support from prospective clinical observations like major depressive episode (MDE) induced by cytokine therapy. IFN-α is the standard cytokine therapy for chronic HCV infection, yet it is associated with common and severe neuropsychiatric adverse effects. After an initial injection of IFN-α, almost all patients experience acute cytokine-induced sickness behavior: malaise, myalgias, arthralgias, anorexia, fatigue, apathy, poor concentration and attention, nonspecific painful symptoms, and acute flu-like symptoms [21,22] (fever, cough, dyspnea, pharyngitis, rhinorrhea, anorexia, rash) that generally subside in 1–2 weeks.
Yet fatigue, malaise, apathy, and cognitive and behavioral changes persist for weeks during treatment; “sickness behavior” induced by IFN-α corresponds to the effects of cytokine administration to animals [5,8,23]. These resemble somatic or vegetative symptoms in major depressive disorder [6,24].

MDE during IFN-α therapy (IFN-α-induced depression) in patients with HCV is common; incidence ranges from 23 to 45% [25]. Onset of symptoms usually occurs within 3 months of therapy initiation [25]. In fact, depression results in poor compliance and is the leading cause of discontinuation of IFN-α therapy [21]. Despite its clinical significance, it is still unsatisfactory to apply specific clinical features when predicting IFN-α-induced depression pathogenesis. Notably, a history of psychiatric disorder before starting IFN-α therapy does not unequivocally predict the occurrence of such symptoms [26]. Other potential clinical predictors include presence of mood and anxiety symptoms before treatment [22], history of major depression, female gender, higher IFN-α dosage, and longer treatment duration [22]. Biological predictors for IFN-α-induced depression are clinically important and can help define the molecular mechanisms of inflammation-associated depression. IFN-α-induced increases in IL-6 have been reported to predict the development of depressive symptoms, rather than MDE [27]. Cerebrospinal fluid concentrations of 5-hydroxyindoleacetic acid, but no inflammatory markers, are predictors of depressive symptoms [28]. Other studies have examined biomarkers such as plasma adrenocorticotropic hormone, cortisol [29], serum tryptophan concentrations [30], even brain function [31]; these found depression predicted by changes in biomarkers during IFN-α therapy, rather than by baseline (pretreatment) biomarker levels.

Recent studies identify genetic markers on serotonin transporter and interleukin-6 genes that seem to predict the development of IFN-α-induced depression [32]. Our study in a Han Chinese sample, however, did not support those findings. Instead, we found variations on PUFA metabolite genes associated with risk of IFN-α-induced depression [24]. A recent preliminary report cites inflammatory predictors for depression at baseline: for example, low DHA level [24] and increased soluble interleukin-2 receptor, interleukin-6, and interleukin-10 concentrations [33].

Chronic HCV infection is a major public health issue in Taiwan [34] and has a high rate of progression to liver cirrhosis and hepatocellular carcinoma. Because of the high rate of neuropsychiatric adverse effect like sickness behavior and depression during IFN-α therapy, some clinicians consider prophylactic antidepressant use. The selective serotonin reuptake inhibitor (SSRI) antidepressants are reported to decrease the occurrence of IFN-α-induced depression in HCV patients [35]. However, it has been associated with adverse events, including gastric discomfort, headache, dizziness, and increased risk of retinal hemorrhaging, cotton-wool spots [35], and manic episodes [36]. In addition, symptoms of IFN-α-induced sickness behavior, once they develop, are only partially responsive to SSRIs [37]. The limited efficacy and possible adverse effects of antidepressant medication make it critical to find alternative treatment and prevention measures in patients receiving IFN-α.

2.2. Anti-inflammatory effect as a common mechanism of antidepressant treatment

If activated inflammatory response is involved in depression etiology, one would expect antidepressive treatments to have anti-inflammatory effects. Interestingly, current antidepressant agents like tricyclic antidepressants, SSRIs, serotonin reuptake enhancer tianeptine, noradrenaline–dopamine reuptake inhibitor bupropion, reversible inhibitors of MAO-A moclobemide, might be diverse in their actions on neurotransmitters, but they all exert anti-inflammatory effects [4,12]. In animal models of depression, antidepressants increase antioxidant levels, normalize oxidative and nitrosative stress damages [4], and suppress IL-1beta and TNF-α [38,39] production. They decrease inflammation-induced peripheral and brain cytokine production and reverse depressive-like symptoms [40]. In cell cultures, tricyclic antidepressants and SSRIs significantly suppress IL-1beta, IL-6, and TNF-α production [39]. Antidepressants also attenuate microglial activation and nitric oxide metabolism in the brain [12,41]. In addition, anti-inflammatory effects are associated with mechanisms of antidepressant effects not only in traditional antidepressants but also in off-label pharmacological or nonpharmacological treatment for depression: for example, lithium [42], valproate [43], omega-3 PUFAs [12], atypical antipsychotics [44,45], electroconvulsive shock [46], exercise [47], and even psychosocial intervention [48].

Considering the broad evidence supporting the inflammation theory of depression, the anti-inflammatory pathways loom as a hot topic in the search for new antidepressant therapies [49]. Cytokine antagonists might be associated with significant side effects but block one only specific cytokine [50], while cytokine networks are broadly and only mildly activated [4]. The COX-2 inhibitor celecoxib has been shown to be effective [51] but can also cause mild to severe side effects (e.g., cardiovascular events [4]). Non-steroidal anti-inflammatory drugs cause gastrointestinal adverse effects and increase gut permeability, which may drive peripheral inflammation via bacterial translocation [52], and might contribute to pathogenesis of depression [53]. Above all, omega-3 polyunsaturated fatty acids (omega-3 PUFAs or n-3 PUFAs) might be one of the most promising treatments that are safe, health-promoting, and well accepted.

3. Role of omega-3 PUFAs in psychoneuroimmunology of depression

3.1. Clinical evidence

It has been observed that societies with high consumption of fish in diet appear to have lower prevalence of MDD, mood disorders, coronary heart disease mortality, cardiovascular disease mortality, stroke mortality, and all-cause mortality [54], which implies the protective 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 [55] and brain [56]. Deficits in omega-3 PUFA levels are reported in other populations with
mood disorders: for example, lower DHA and total omega-3 PUFAs in postpartum depression [57], lower DHA and EPA in social anxiety disorder [58], and lower DHA and AA in bipolar disorders [59].

3.2. Clinical applications

Consistent with case-control studies of PUFA levels in human tissues, omega-3 PUFAs are reported to be effective in treatment of MDD. Four meta-analytic reviews from three independent groups have reported the antidepressant effect of PUFAs [60–63], yet two previous meta-analyses from the same group did not support these effects in heterogeneous populations (such as subclinical individuals in community samples) [64,65]. Negative findings must be interpreted with caution due to limitations: for example, differing mood assessments, pooling heterogeneous populations, and implementing different intervention methods.

Omega-3 fatty acids might be “antidepressive” on patients with DSM-defined MDD but not “mood-improving” on symptomatic individuals if the diagnosis was not clinically confirmed. Recent meta-analysis by Bloch and Hannestad [66] found no benefits for depression; their review included clinical trials enrolling individuals according to self-rating scales in settings like general practice surgery, shopping mall, and university freshmen’s fair [65], which found no beneficial effect 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 [67]. Take one clinical trial [68] included in Appleton et al’s meta-analysis, for example. While Ness et al’s study enrolled a relatively large number of 452 patients, it did not focus on treating depression or using appropriate tools for diagnosis and severity rating of depression. Intervention with omega-3 PUFA was defined to “advise” patients with angina to “eat more fish.” The treatment outcome of omega-3 PUFAs in Ness’s study was negative and contributed greatly to the pooled estimate in Appleton et al’s meta-analysis.

Omega-3 PUFAs might only prevent depression but not mania in patients with bipolar disorder [69]. Despite the uneven quality of published studies, recent meta-analytic evidence strongly supports the adjunctive use of omega-3 to treat bipolar depression [70]. However, studies regarding the effectiveness of omega-3 PUFAs in the acute manic phase of bipolar disorder are still lacking. To date, one small double-blind placebo-controlled trial has been published and does not support omega-3 PUFAs’ anti-manic effects [71]. Future large-scale, double-blind, placebo-controlled trials are needed.

Omega-3 PUFAs offer promise in treating special populations with depression. We first reported a successful treatment with omega-3 PUFAs in a pregnant woman with major depression [72]. Our 8-week, double-blind, placebo-controlled study showed that monotherapy with omega-3 PUFAs was associated with significant improvement of depressive symptoms and higher response rate in pregnant women with depression [73]. Most importantly, omega-3 PUFAs are safe for and well tolerated by depressed women during pregnancy and postpartum [74]. Omega-3 PUFAs are proven effective and safe for children with depression [75]; supplementation lowers risk of suicide [76], alleviates MDD depressive symptoms associated with menopausal transition [77], and diminish aggression in women with borderline personality disorder [78].

3.3. Preclinical evidence

Preclinical studies further support the omega-3 PUFA hypothesis. Omega-3 PUFAs have antidepressant effects in the animal model of depression in rats [79,80]. Likewise, the level of brain DHA negatively correlates with immobility time and positively correlates with swimming time [80]. Interestingly, rats fed with lithium chloride, valproate, or carbamazepine showed reduced AA turnover within brain phospholipids, which may give rise to the hypothesis that lithium and anti-manic anticonvulsants act by targeting parts of “arachidonic acid cascade” that may be functionally hyperactive in mania [81]. Empirical evidence supports this “arachidonic acid cascade” hypothesis identified as a mechanism of mood stabilization: for example, higher ratio of AA [59,82] along with hyperactivity of its major metabolic enzyme phospholipase A2 in mood disorders [83], inhibitory effect on phospholipase A2 activity of mood stabilizers [84], and therapeutic effect of omega-3 PUFAs in mood disorders [61]. Another cellular mechanism underlying the antidepressant effects of omega-3 PUFAs is the biological regulation of neurotransmitters and signal transduction. Changes in omega-3 PUFA concentration in the brain, induced by chronic deficiency in dietary omega-3 PUFAs, could increase serotonin 2 (5-HT2) and decrease dopamine 2 receptor density in the frontal cortex [85]. Finally, EPA might be improving the hypothalamic–pituitary–adrenal axis dysfunction through the action of p-glycoprotein and multidrug resistance receptors [86].

3.4. Safety and tolerability

Numerous clinical studies have shown that omega-3 PUFAs are well tolerated by patients with chronic medical illnesses and mental disorders [60,87]. Adverse reactions are rare; if they occur, they usually involve belching, eructation, or perhaps fishy taste [88]. It is theorized that the potential antithrombotic effect of omega-3 PUFAs may increase the risk of bleeding. Clinical trials show high-dose omega-3 PUFAs consumption as safe, even when concurrently administered with other agents that increase bleeding, such as aspirin and warfarin [87]. According to Harris’s [89] systematic review of 19 available clinical trials with n-3 PUFAs supplementation for patients with high risk of bleeding (n = 4397), the risk of clinically significant bleeding is 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 conclusions are quite inconsistent [90]. Adding antioxidant 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 [90]. Given omega-3 PUFAs’ antidepressant effects, another possible
adverse effect is drug-induced mania. Until now, only one case report has shown omega-3 PUFAs inducing hypomania [91]; a comprehensive assessment of manic symptoms in patients receiving omega-3 PUFAs is recommended for future clinical trials.

4. Conclusions

The inflammation theory of depression draws support from several lines of evidence: for example, 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 etiology of depression in a prospective manner. Chronic low-grade inflammation links not only with psychiatric disorders but also certain physical diseases. The inflammation theory might thereby provide an interface between mind and body, along with a promising path for developing new treatments. Anti-inflammatory omega-3 PUFAs prove beneficial in depression and several inflammation-related physical diseases. Omega-3 PUFAs may particularly benefit children, pregnant women, and/or patients with comorbid cardiovascular or metabolic disorder, who face greater risks of adverse effects from antidepressants, antipsychotics, and mood stabilizers. The cost of omega-3 PUFAs is relatively modest as compared to many psychiatric treatments and other over-the-counter natural products. Given the potential benefits and safety, omega-3 PUFAs deserve greater attention and wider application.

Acknowledgments

Work included in this review was supported by Grants NSC-99-29111-1-039-002, NSC-98(99&100)-2627-B-039-003 and NSC 98-2628-B-039-020-MY3 from the National Science Council in Taiwan; NHRI-EX101-10144NI from the National Health Research Institute in Taiwan; and DMR-101-081, DMR99-114 and CMU97-336 from China Medical University in Taiwan.

References


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.


Rapoport SI, Bosetti F. Do lithium and anticonvulsants target the brain arachidonic acid cascade in bipolar disorder? Arch Gen Psychiatry 2002;59:592–6.


Bays HE. Safety considerations with omega-3 fatty acid therapy. Am J Cardiol 2007;99:35C–43C.


Review article

Glutamate theory in developing novel pharmacotherapies for obsessive compulsive disorder: Focusing on N-methyl-D-aspartate signaling

Po-Lun Wu, Hsien-Yuan Lane, Hwa-Sheng Tang, Guochuan E. Tsai

Department of Psychiatry, China Medical University Hospital, Taichung, Taiwan
Institute of Clinical Medical Sciences, China Medical University, Taichung, Taiwan
Department of Psychiatry, Taipei City Psychiatric Center, Taipei City Hospital, Taipei, Taiwan
Department of Psychology, National Chengchi University, Taipei, Taiwan
Department of Psychiatry, Harbor Medical Center, University of California, Los Angeles, California, United States

Article info

Article history:
Received 10 April 2012
Received in revised form 18 April 2012
Accepted 19 April 2012
Available online 29 May 2012

Keywords:
glutamate
N-methyl-D-aspartate
obsessive compulsive disorder

Abstract

Obsessive compulsive disorder (OCD) is a prevalent and debilitating illness that often follows a chronic course. Up to 40% of OCD patients received little or no benefit from currently available pharmacotherapy or exposure-based behavior psychotherapy. Thus, there is an urgent need to develop new strategies for the treatment of OCD. Although the neurobiology and etiology of OCD are not completely understood, growing clinical and preclinical evidence appears to support the abnormalities of glutamatergic neurotransmission, including N-methyl-D-aspartate subtype receptor (NMDAR) function, in the pathophysiology and treatment of OCD. This review summarizes the findings from neuro imaging, candidate genes, animal models, and treatment studies in the context of glutamatergic dysregulation, with particular emphasis on the synaptic NMDAR function. The converging evidence indicates the potential of glutamate-modulating agents in the development of novel treatment for OCD.

Copyright © 2012, China Medical University. Published by Elsevier Taiwan LLC. All rights reserved.

1. Introduction

Obsessive compulsive disorder (OCD) is characterized by intrusive thoughts or images (obsessions) that increase anxiety and by ritualistic behaviors (compulsions) that can temporarily relieve such anxiety. OCD is a debilitating psychiatric disorder estimated to affect 2%–3% of the world population [1]. Unfortunately, only 40%–60% of patients with OCD responded to currently available pharmacotherapy and exposure-based psychotherapy, and a great proportion of treatment responders remained markedly ill [2]. There is an urgent need to develop novel strategies for OCD patients who are inadequately responsive to currently available therapies.

Although clinical experience and research of OCD have mostly focused on the serotonergic and dopaminergic systems (see refs [3–5]), convergent lines of evidence support...
an important role of the glutamatergic system in the pathophysiology and treatment of OCD (see refs [6,7]). In this review, we will focus on recent finding on the N-methyl-D-aspartate (NMDA) subtype receptor (NMDAR) function in OCD.

2. Clinical studies with glutamate-modulating agents on the treatment of OCD

Preliminary case reports and small open trials showed that resistant OC symptoms might benefit from adjunctive nonselective glutamate antagonists such as riluzole [8–10], topiramate [11], and lamotrigine [12,13]. In three case reports and one small open-label trial, memantine, a weak uncompetitive NMDAR antagonist, proved to be efficacious as add-on treatment to resistant OCD [14–17]. Extracellular glycine is an obligatory coagonist with glutamate on the activation of NMDAR [18]. Greenberg and colleagues [19] conducted the first randomized trial using glycine as adjunctive treatment for refractory OCD. There seemed to be a trend favoring glycine treatment.

Following a new paradigm using D-cycloserine (DCS), a partial agonist acting on NMDA glycine site, as “cognitive enhancer” [20] to facilitate exposure therapy for anxiety disorders [21,22], two trials on OCD found advantage of adjunctive DCS over placebo [23,24], but another one did not [25]. But the administration of DCS is intermittent, immediately before each exposure session.

3. Genetic, neuroimaging, and animal studies on OCD

Genetic association studies of OCD have identified two susceptibility genes, which are vital for glutamatergic neurotransmission: a glutamate transporter gene, SLC1A1 [26–29] and the N-methyl-D-aspartate receptor (NMDAR) subunit 2B gene, GRIN2B [30]. Functional neuroimaging studies for OCD demonstrated metabolic disturbance in the frontal-subcortical circuit (FSC) [31], where glutamatergic neurotransmission play the role as the principal input [32]. Two recent transgenic animal models demonstrated phenotypical compulsive behavior: SAPAP3 knockout mouse which demonstrated striatum-specific alteration of NMDAR subunit composition [33] and G72/G30, a presumed D-amino acid oxidase (DAO) activator [34], transgene mouse [35]; while DAAO is the main degrading enzyme of D-serine, an allosteric coactivator of NMDARs. Both of them were associated with NMDA functional alteration.

It is suggested that OCD is a prefrontal cortex hyper-glutamatergic condition [36]. Glutamate levels estimated by magnetic resonant spectroscopy are significantly elevated in the caudate of treatment-naïve pediatric OCD patients [37], but significantly reduced in anterior cingulated cortex (ACC) in drug-naïve pediatric OCD patients [38]. Cerebral spinal fluid (CSF) glutamate levels are greatly elevated in OCD patients than in normal controls [39]. NMDAR antagonists such as (2R)-amino-5-phosphonovaleric acid, ketamine, and phencyclidine caused pathologically increased glutamate efflux in the hippocampus, prefrontal cortex, and possibly the striatum [40–42], which was reversible with the use of either NMDAR agonists such as glycine or nonspecific glutamate inhibitors such as lamotrigine, in both animal and human preclinical studies [43,44].

A NMDAR antagonist MK-801 exacerbated the repetitive climbing and leaping behavior in a transgenic D1CT-7 mouse model of comorbid Tourette syndrome (TS) and OCD [45]. Therefore, potentiation of NMDA function may correct the OCD-like behaviors. However, memantine, amantadine, and MK-801, but not α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) antagonist and riluzole, significantly inhibited murine marble-burying behavior, a potential animal model for OCD [46], suggesting both agonist and partial antagonist of NMDA receptors can improve the symptoms of OCD.

4. Focusing on modulation of NMDAR functioning

Taken together, insufficient clinical data is available to explain why both nonspecific glutamate inhibitors (or a weak uncompetitive NMDAR antagonist) and NMDA agonist/partial agonist as add-on treatment may benefit patients with OCD. A nonspecific glutamate inhibitors (or a weak uncompetitive NMDAR antagonist) and NMDA agonists may share a common mechanism of reducing glutamatergic neurotransmission in frontal regions and provide therapeutic efficacy for the patients with OCD [19,45]. We postulate that enhancing NMDA neurotransmission in FSC may be beneficial for OCD. Glycine transporter-1 (GlyT-1) regulates and maintains subsaturating concentrations of glycine at the glycine site of NMDARs [47,48]. Blockade of glycine uptake by glial GlyT-1 could increase the availability of synaptic glycine near NMDARs [47,48] and potentiate NMDA excitatory postsynaptic potential (EPSCs) [48].

N-methylglycine (sarcosine) is a potent endogenous antagonist of GlyT-1 [47,49]. In an open-label [50], flexible-dosing study to investigate the potential efficacy and safety of sarcosine therapy in patients with OCD, we found that (a) sarcosine treatment for 10 weeks significantly reduced the Yale-Brown obsessive compulsive scores in patients with OCD, especially those who were drug naïve, (b) five of the eight final responders met criteria of response within 2 to 4 weeks of sarcosine treatment, which is quicker than the onset of therapeutic response with serotonin reuptake inhibitors, and (c) the therapeutic effect occurs with doses lower than the dose for the patients with schizophrenia at 2 g/day [51–53]. The study is limited in its open-label design, a relatively small sample, and concurrent treatment with psychotropic medications in the add-on group. Despite these limitations, the low dropout rate, significant improvement in Yale-Brown Obsessive Compulsive scale (Y-BOCS) scores, particularly the naïve group, and overall favorable tolerability suggest that sarcosine may be of clinical benefit to the patients with OCD. The efficacy of sarcosine adds to the literature implicating the NMDA neurotransmission in the pathophysiology of OCD, while GlyT-1 may be a novel therapeutic target for OCD treatment.

As yet, we do not know how GlyT-1 inhibitors or glycine exert their therapeutic effects in OCD. What have we known from clinical samples and animal studies were: The
polymorphism of NR2B subunit gene GRIN2B has been involved with OCD [30], and the SAPAP3 gene-deleted mouse has the striatum NR2A/NR2B ratio decreased, the field EPSCs significantly reduced, and exhibited OCD-related phenotype [33].

In preclinical studies, distinct NMDA modulating agents may have pharmacologically, regionally and temporally differential effects in the FSC relevant to OCD, given that:

1. The developmental and physiological properties of NMDARs: NMDARs are composed of different subunits (NR1, NR2A–D, and, in some cases, NR3A or NR3B) and differentially expressed both regionally in the brain [54–56] and temporally during development [57]. Alternative composition of the NMDAR channel results in functional diversity of the channel [55,56,58].

2. Differences in neuronal NMDAR properties are largely attributed to the NR2 subunits. NMDARs containing the NR2A subunit have the highest affinity for competitive antagonists [59], while NMDARs containing the NR2B subunit have greater affinity for agonists such as glycine and D-serine [54,56,59,60]. It is the presence of the NR2A subunit that allows increased glycine concentrations to potentiate the NMDAR response [61].

3. From an anatomical perspective, synaptic processing of excitatory input is different in the ventromedial and dorsolateral striatum; either glycine or D-serine increased the peak current of NMDAR mediated excitatory postsynaptic currents selectively in dorsolateral striatum [59]. Interestingly, electrophysiological experiments demonstrated that glycine or another GlyT-1 inhibitor CP-802,079 exerted an inverted-U dose-response profile for the synthetically evoked NMDA currents in prefrontal cortex slices [48], while ketamine has a dose-associated biphasic influence on the outflow of glutamate in the prefrontal cortex [40].

Given the molecular, anatomical and physiology complexities of NMDA function, the “direct” and “indirect” pathways unbalanced hypothesis [38,62] may partially explain the clinical and preclinical reports that both NMDA agonists and some uncompetitive NMDA antagonists are efficacious for OCD.

5. The relationship between serotonergic and NMDA signaling

The cortico-raphe glutamatergic and raphe-cortical serotonergic projections may form an excitatory-inhibitory loop by which excitatory input signals are converted into inhibitory output projecting back to cerebral cortex [64,65]. It is possible that the therapeutic effect of SRIs or sarcosine for OCD may converge on diminishing ventromedial basal ganglia activity relative to that in the dorsolateral system, or reducing glutamatergic hyperactivities in the frontal cortex. Either SRIs or NMDA agents alone may reach a therapeutic ceiling and combination treatment can bring less improvement in the drug-exposed group than the drug-naïve group. This can be understood provided that chronic administration of SRIs leads to altered levels of mRNA encoding NMDAR subunits and region-specific change of NMDAR function in CNS [65,66]. Besides, the effects of SRIs were different on lateral PFC versus ventral frontal paralimbic serotonin regulation [67], while serotonin may exert dual actions by stimulating 5-hydroxytryptophan (5-HT) 2A receptors on γ-aminobutyric acid (GABA)ergic interneurons and 5-HT1A receptors on glutamatergic neurons in the prefrontal cortex [63,64], thus indirectly inhibiting the primary glutamatergic output to the ventral striatum.

6. Conclusion and future perspectives

In recent years, converging lines of evidence implicate glutamatergic neurotransmission in the pathophysiology and treatment of OCD. Glutamatergic signaling through NMDAR had showed controversial effects in clinical and preclinical studies. This may attribute to that the distinct NMDA modulating agents may have pharmacologically, regionally, and temporally differential effects in the frontal-striatal circuitry relevant to OCD. The use of animal models for screening NMDAR modulating agents, and combining genetic and neuroimaging studies in clinical patients may expand our understanding of the neurobiology as well as novel treatments for OCD.

Acknowledgment

This work was supported by the National Science Council, Taiwan (NSC-97-2314-B-039-006-MY3, NSC-98-2627-B-039-001), National Health Research Institutes, Taiwan (NHRI-EX101-9904NI), Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH101-TD-B-111-004), and the China Medical University Hospital in Taiwan (DMR99-IRB115 and DMR99-IRB117).

REFERENCES


of ketamine with lamotrigine: support for
hyperglutamatergic effects of N-methyl-D-aspartate
[44] Moghaddam B. Glutamatergic animal models of
Glutamatergic drugs exacerbate symptomatic behavior in
a transgenic model of comorbid Tourette’s syndrome and
[46] Egashira N, Okuno R, Harada S, Matsushita M, Mishima K,
Iwasaki K, et al. Effects of glutamate-related drugs on
marble-burying behavior in mice: implications for obsessive-
[47] Bergeron R, Meyer TM, Coyle JT, Greene RW. Modulation of
N-methyl-D-aspartate receptor function by glycine
[48] Chen L, Muhlhauser M, Yang CR. Glycine tranporter-1
blockade potentiates NMDA-mediated responses in rat
prefrontal cortical neurons in vitro and in vivo. J
requirements for activation of the glycine coagonist site of
N-methyl-D-aspartate receptors expressed in Xenopus
[50] Wu PL, Tang HS, Lane HY, Tsai CA, Tsai GE. Sarcosine
therapy for obsessive compulsive disorder: a prospective,
[51] Tsai G, Lane HY, Yang P, Chong MY, Lange N. Glycine
transporter I inhibitor, N-methylglycine (sarcosine), added to
antipsychotics for the treatment of schizophrenia. Biol
Glycine transporter I inhibitor, N-methylglycine (sarcosine),
added to clozapine for the treatment of schizophrenia. Biol
[53] Lane HY, Lin CH, Huang YJ, Liao CH, Chang YC, Tsai GE. A
randomized, double-blind, placebo-controlled comparison
study of sarcosine (N-methylglycine) and D-serine add-on
therapy for schizophrenia. Int J Neuropsychopharmacol
[54] Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K,
Kushiya E, Araki K, et al. Molecular diversity of the NMDA
[55] Aghajanian GK, Marek GJ. Serotonin model of schizophrenia:
emerging role of glutamate mechanisms. Brain Res Brain Res
[56] Skolnick P. Antidepressants for the new millennium. Eur J
[57] Pittaluga A, Raiteri L, Longordo F, Luccini E, Barbiero VS,
Racagni G, et al. Antidepressant treatments and function of
glutamate ionotropic receptors mediating amine release in
[58] el Mansari M, Bouchard C, Blier P. Alteration of serotonin
release in the guinea pig orbito-frontal cortex by selective
serotonin reuptake inhibitors. Relevance to treatment of
obsessive-compulsive disorder. Neuropsychopharmacology
Case report

Nonketotic hyperglycinemia: A case report and brief review

Yu-Tzu Chang, Wei-De Lin, Zheng-Nan Chin, Chung-Shing Wang, I.-Ching Chou, Huang-Tsung Kuo, Fuu-Jen Tsai

Departments of Pediatrics, Children’s Medical Center, China Medical University Hospital, Taichung, Taiwan
Department of Medical Research, China Medical University and Hospital, Taichung, Taiwan
School of Post Baccalaureate Chinese Medicine, China Medical University, Taichung, Taiwan
Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan

Abstract

In encephalopathic infants, cerebrospinal fluid hyperglycinemia and elevated cerebrospinal fluid to plasma glycine ratio are considered pathognomonic of nonketotic hyperglycinemia (NKH). We present a case of NKH complicated by neonatal intractable seizures. Increased ratio of cerebrospinal fluid to plasma glycine concentrations of 0.28 was seen as a strong diagnostic indicator of nonketotic hyperglycinemia. Evaluating sick neonates with hypotonia, encephalopathy, and/or seizures is a diagnostic challenge. NKH should be considered; elevated cerebrospinal fluid/plasma glycine ratio will allow correct identification and treatment more often in the future.

1. Introduction

Glycine encephalopathy, also known as nonketotic hyperglycinemia (NKH), is an inborn error of glycine metabolism caused by deficiency in the glycine cleavage system (GCS) and characterized by large quantities of glycine accumulated in all body tissues, especially in serum and cerebrospinal fluids. Most glycine encephalopathy cases occur during the neonatal period. The neonatal form manifests in the first hours to days of life with progressive lethargy, hypotonia, myoclonic jerks, hiccups, and apnea, which often lead to coma or death. Outcome is usually poor, with mortality up to 50% during the first week of life. Surviving infants have profound intellectual disability and intractable seizures. Atypical forms include milder disease, with onset from late infancy to adulthood, which presents various neurological symptoms: seizure, motor and/or cognitive impairments, aggressive behavior, and impaired work or school performance. A rare transient form has been described in which newborns have elevated cerebrospinal fluid and plasma glycine, which is biochemically and clinically indistinguishable from the classic form. In the rare form, glycine levels normalize over time without pharmacologic intervention and often have few or no neurologic sequelae. We present a case of NKH neonatal intractable seizures. Evaluating a sick neonate who presents with hypotonia, encephalopathy, and/or seizures is a diagnostic challenge; a high index of suspicion for timely diagnosis and treatment could prevent severe complications.
2. Case report

A two month-old girl was transferred to our hospital for evaluation and management of seizure. She was a full-term baby, her parents not consanguineous, pregnancy uneventful, and labor and vaginal delivery uncomplicated. Apgar scores at birth were 9 at 1 minute and 10 at 5 minutes birth body weight 3116 g (50th–75th percentile), birth height 52 cm (75th–90th percentile), head circumference 33.5 cm (50th–75th percentile). Physical and neurologic examinations were normal except for mild hypertonic muscle tone. Sudden onset of general convulsions accompanied by increased heart rate and oxygen desaturation appeared 3 hours after birth. Symptoms lasted about 3 to 4 minutes. One day after birth, general convulsions were noted every hour. Seizure frequency increased even under antiepileptic drug (AED) treatment. Laboratory examination showed that blood cell count, electrolyte, glucose, ammonia, and lactate were all within the normal range. Brain echography showed negative findings. Urine organic acid examination and blood liquid chromatography-mass spectrometry were unremarkable. The infant was transferred to the ward due to poor seizure control. After admission to our intensive care unit, she showed frequently paroxysmal general tonic postures, associated with oxygen desaturation and increased heart rate. Seizure was aggravated by touching. Phenytoin and phenobarbital were given, but in vain. Sleep EEG revealed paroxysmal sharp waves over both central areas. Video on long-term EEG monitor proved seizure associated with epileptic form discharges, particularly in the frontal region. Ictal recording showed general tonic seizure with tachycardia, associated with epileptic form spikes. Brain magnetic resonance imaging (MRI) showed no remarkable findings. Frequency and intensity of seizures did not improve after pyridoxine challenge and prescription of multiple AEDs. Under the impression of suspect NKH due to intractable seizures, cerebrospinal fluid examination was performed. Amino acid analyses (MRM mode) of glycine were as follows: CSF = 51.9 μM; plasma = 183.0 μM; and CSF/plasma ratio = 0.28 (Fig. 1). Diagnosis of NKH was strongly suspected. Regimen of frequent feedings, together with AEDs, including phenobarbital and clonazepam, sodium benzoate and dextromethorphan were prescribed. Episodes of seizures and apnea decreased, and the patient was followed-up at the outpatient clinic. Unfortunately, the patient expired at home when she was 5-month-old due to sudden onset of cardiac arrest and apnea.

3. Discussion

In neonatal seizures, issues to consider include hypoxia-ischemia insult, hemorrhage or intracranial infarction, trauma, infection, cerebral malformations, neurocutaneous syndromes, drugs or toxic agents, and metabolic and idiopathic disorders. In our case, the birth course was smooth. Brain imaging study revealed no abnormal brain malformation or hemorrhage, yet refractory epilepsy was noted, which led us to survey for possible inborn errors. In many metabolic disorders, epilepsy may even dominate the clinical picture, especially in newborns and infants. One must always consider the possibility of inborn metabolic errors in neonates with unexplained and refractory epilepsy [1,2]. This patient's metabolic study showed unremarkable urine organic acid examination and blood liquid chromatography-mass spectrometry. Blood tests including electrolytes, glucose, ammonia, and lactate were within the normal range. Pyridoxine treatment was eventually ineffective, suggesting that pyridoxine-dependent epilepsy was unlikely. Therefore, further study of NKH was enrolled.

NKH is an autosomal recessive metabolic disorder characterized by glycine accumulating in the brain due to defective GCS. Incidence of glycine encephalopathy in British Columbia is 1:63,000 live births and 1:55,000 newborns in Finland [5]. Classically, NKH is associated with normal pregnancy and birth, then neonatal apnea, lethargy, hypotonia, and seizures occur, followed by severe psychomotor retardation in those who survive. While the present case manifested mild hypertonia, it may arise from severe encephalopathy. In the laboratory, NKH is characterized by elevated glycine concentrations in plasma, CSF, and brain with a CSF glycine to plasma glycine ratio greater than 0.08, which is diagnostic. A ratio of >0.04 is suggestive but require further confirmation through enzymatic analysis of liver or mutation detection. In our case, glycine CSF/plasma ratio is 0.28. Elevated cerebrospinal fluid glycine is reported in infants with encephalopathy: e.g., hypoxic ischemic encephalopathy, congenital stroke, central nervous system infection[3]. No perinatal insult or infection appeared in this case.

Glycine is an inhibiting neurotransmitter in the spinal cord and excitation modulator of N-methyl D-aspartate (NMDA) receptors in the telecephalon and cerebellum [6]. The GCS consists of four distinct (P, T, H, L) proteins. Over 80% of NKH patients show defects in the P-protein, as high as 15% human T-protein defect, with H-protein deficiencies being rare [7–9]. NKH can manifest one of four forms: classic neonatal, transient, infantile, and late; differences lie in time of onset, severity of
clinical presentation, and outcome [10,11]. The typical neonatal form presents in the first few days after birth with progressive lethargy, hypotonia, hiccup, and seizures, progresses to central apnea, and often death. Surviving infants often have profound developmental delay and intractable seizures. The infantile form occurs in the first few months of life and is also characterized by hypotonia, developmental delay, and seizures. Increased CSF glycine level (typically 20–30 times normal) along with elevated CSF/plasma glycine ratio, suggests diagnosis. Gold standard for diagnosis is still liver biopsy, but this is not feasible in many cases. A noninvasive [13] C-glycine breath test and screening system for genomic deletion with GLDC also help confirm diagnosis [12,13]. Genes known to associate with NKH are GLDC (encoding P-protein component of the GCS complex), AMT (encoding T-protein component), and GCSD (encoding H-protein component) [12]. Molecular genetic testing of all three genes is available on a clinical basis. Mutations associated with residual enzyme activity seem associated with a milder outcome and infantile presentation; two mutations with no residual enzyme activity seem linked with severe outcome and neonatal onset [14,15]. Initial EEG typically shows a burst-suppression pattern that evolves into hypsarrhythmia.

MRI can show normal, agenesis of the corpus callosum, delay in myelination, vacuolation, gliosis, or, less frequently, retro-cerebellar cysts with subsequent hydrocephalus [16–18]. Prior reports revealed that hydrocephalus appears to predict poor outcome [2,17]. A glycine peak on magnetic resonance spectroscopy (MRS) result is seen in the most severely affected infants, carrying poor prognosis. MRS techniques may play a key role in assessing adverse outcome in NKH. Identification of NAA/Ins-Gly as an indicator of severity could be ultimately applied to monitor and predict evolution of NKH and fine-tune existing and future treatments [18].

To date, no effective treatment exists for NKH. The standard treatment strategies for NKH include sodium benzoate (to reduce plasma concentration of glycine) and NMDA receptor antagonists (ketamine, dextromethorphan, felbamate, and topiramate) [19]. Both sodium benzoate and dextromethorphan may improve alertness and decrease seizure frequency if prescribed during the newborn period [2]. Others focus on seizure control with AEDs. Among AEDs, valproate should be avoided in glycine encephalopathy, since it raises blood and CSF glycine concentrations by further inhibiting the GCS and may increase seizure frequency [20]. Higher glycine concentration would result in severe lethargy, seizures, chorea (especially in mildly affected patients), and coma [21]. Other management includes gastrostomy tube that are used for feeding problems and physical therapy can prevent several complications. Surveillance developmental assessment is vital throughout the first years of life; molecular genetic and/or biochemical tests of at-risk symptomatic siblings are recommended to promote early diagnosis and treatment.

Acknowledgments

This study was supported in part by the China Medical University Hospital (grant number DMR-100-058).

References

BioMedicine aims to publish high quality scientific research in the field of translational and personalized medicine, with the goal of promoting and disseminating medical science knowledge to improve global health.

Articles on clinical, laboratory and social research in translational and personalized medicine and related fields that are of interest to the medical profession are eligible for consideration. Review articles, original articles, case reports, short communications, and letters to the editor are accepted. The journal is published quarterly, with a total of four issues a year.

The Editorial Board requires authors to be in compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (URMs); current URMs are available at http://www.icmje.org.

1. Manuscript Submission

Manuscripts should be submitted online through Elsevier's Editorial System (EES). This system can be accessed at http://ees.elsevier.com/biomed. This site will guide authors stepwise through the submission process. If assistance is required, please refer to the tutorials and/or customer support that are available on the website, or you may contact the Editorial Office.

Editorial Office
BioMedicine
No. 91, Hsueh-Shih Road, Taichung 40402, Taiwan.
Tel: (+886) 4-22070672; Fax: (+886) 4-22070813
E-mail: biomed1958@gmail.com

1.1. Important Information

- Articles submitted should be in Microsoft Word document format and prepared in the simplest form possible. We will add in the correct font, font size, margins and so on according to the journal’s style.
- You may use automatic page numbering, but do NOT use other kinds of automatic formatting such as footnotes, headers and footers.
- Put text, references, and table/figure legends in one file.
- Figures must be submitted separately as picture files, at the correct resolution. The files should be named according to the figure number, e.g., “Article1_Fig1”, “Article1_Fig2”. Also see Section 9.7. below.

1.2. Supporting Documents

The following documents must be included (refer also to the Checklist that follows these author instructions):

(1) Cover Letter. This must include the name, address, telephone and fax numbers, and e-mail address of the corresponding author.

(2) Authorship Statement. You may use the form that follows these author instructions. ALL the authors’ signatures must be included.

(3) Conflict of Interest Statement. You may use the form that follows these author instructions. Also see Section 2 below.

(4) Copyright Transfer Agreement. You may use the form that follows these author instructions.

(5) Ethics Statement. Articles covering human or animal experiments must be accompanied by a letter of approval from the relevant review committee or authorities. Also see Section 3 below.

(6) Consolidated Standards of Reporting Trials (CONSORT) flow chart for randomized controlled trials submitted for publication. Also see Section 4 below.

(7) Articles where human subjects can be identified in descriptions, photographs or pedigrees must be accompanied by a signed statement of informed consent to publish (in print and online) the descriptions, photographs and pedigrees from each subject who can be identified. Also see Section 5 below.

(8) Where material has been reproduced from other copyrighted sources, the letter(s) of permission from the copyright holder(s) to use the copyrighted sources must be supplied.

2. Disclosure of Conflicts of Interest

All authors are required to sign and submit a financial disclosure statement at the time of manuscript submission, for example:

I certify that all my affiliations with or financial involvement in, within the past 5 years and foreseeable future, any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript are completely disclosed (e.g., employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, royalties).

Authors who have no relevant financial interests should provide a statement indicating that they have no financial interests related to the material in the manuscript. Any non-financial conflicts of interest should also be explicitly declared in your own words.

3. Ethical Approval of Studies and Informed Consent

For human or animal experimental investigations, appropriate institutional review board or ethics committee approval is required, and such approval should be stated in the methods section of the manuscript. For those investigators who do not have formal ethics review committees, the principles outlined in the Declaration of Helsinki should be

For investigations in humans, state explicitly in the methods section of the manuscript that informed consent was obtained from all participating adults and from parents or legal guardians for minors or incapacitated adults, together with the manner in which informed consent was obtained (ex. oral or written). For work involving experimental animals, the guidelines for their care and use should be in accordance with European Commission Directive 86/609/EEC for animal experiments (available at http:// ec.europa.eu/environment/chemicals/lab_animals/ legislation_en.htm); this should be stated in the methods section of the manuscript.

4. Reporting Clinical Trials

All randomized controlled trials submitted for publication should include a completed Consolidated Standards of Reporting Trials (CONSORT) flow chart (available at http://www.consort-statement. org). This Journal has adopted the proposal from the International Committee of Medical Journal Editors (ICMJE) that require, as a condition of consideration for publication of clinical trials, registration in a public trials registry. Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) do not require registration. Further information can be found at http://www.icmje.org.

5. Identification of Patients in Descriptions, Photographs and Pedigrees

A signed statement of informed consent to publish (in print and online) patient descriptions, photographs and pedigrees should be obtained from all subjects (parents or legal guardians for minors who can be identified [including by the subjects themselves] in such written descriptions, photographs or pedigrees. Such persons should be shown the manuscript before its submission. Omitting data or making data less specific to de-identify patients is acceptable, but changing any such data is not acceptable.

6. Previous Publication or Duplicate Submission

Submitted manuscripts are considered with the understanding that they have not been published previously in print or electronic format (except in abstract or poster form) and are not under consideration in totality or in part by another publication or electronic medium.

7. Basic Criteria

Articles should be written in English (using American English spelling) and meet the following basic criteria: the material is original, the information is important, the writing is clear and concise, the study methods are appropriate, the data are valid, and the conclusions are reasonable and supported by the data.

8. Article Categories

8.1. Review Articles

These should aim to provide the reader with a balanced overview of an important and topical subject in the field, and should be systematic and critical assessments of literature and data sources. They should cover aspects of a topic in which scientific consensus exists as well as aspects that remain controversial and are the subject of ongoing scientific research. All articles and data sources reviewed should include information about the specific type of study or analysis, population, intervention, exposure, and tests or outcomes. All articles or data sources should be selected systematically for inclusion in the review and critically evaluated.

By invitation only. The format for review articles will be jointly decided by the Editors and the contributing author. Typical length: no more than 4000 words, 50–100 references.

8.2. Original Articles

These may be randomized trials, intervention studies, studies of screening and diagnostic tests, laboratory and animal studies, cohort studies, cost-effectiveness analyses, case-control studies, and surveys with high response rates, which represent new and significant contributions to the field.

Section headings should be: Abstract, Introduction, Methods, Results, Discussion, Acknowledgments (if applicable), Conflicts of Interest (if any), and References.

The Introduction should provide a brief background to the subject of the paper, explain the importance of the study, and state a precise study question or purpose.

The Methods section should describe the study design and methods (including the study setting and dates, patients/participants with inclusion and exclusion criteria, or data sources and how these were selected for the study, patient samples or animal specimens used, explain the laboratory methods followed), and state the statistical procedures employed in the research.

The Results section should comprise the study results presented in a logical sequence, supplemented by tables and/or figures. Take care that the text does not repeat data that are presented in tables and/or figures. Only emphasize and summarize the essential features of any interventions, the main outcome measures, and the main results.
8.3. Case Reports
These are short discussions of a case or case series with unique features not previously described that make an important teaching point or scientific observation. They may describe novel techniques, novel use of equipment, or new information on diseases of importance. Section headings should be: Abstract, Introduction, Case Report, Discussion, Acknowledgments (if applicable), Conflicts of Interest (if any), and References.

The introduction should describe the purpose of the report, the significance of the disease and its specificity, and briefly review the relevant literature.

The Case Report should include the general data of the case, medical history, family history, chief complaint, present illness, clinical manifestation, methods of diagnosis and treatment, and outcome.

The Discussion should compare, analyze and discuss the similarities and differences between the reported case and similar previously reported cases. The importance or specificity of the case should be restated when discussing the differential diagnoses. Suggest the prognosis of the disease and possibility of prevention. Typical length: no more than 1500 words, 20–40 references.

8.4. Short Communications
These should be concise presentations of clinical or preliminary experimental results. Section headings should be: Abstract, Introduction, Methods, Results, Discussion, Acknowledgments (if applicable), Conflicts of Interest (if any), and References.

Typical length: no more than 1000 words, 20–40 references, with no more than four figures or tables. The Editors reserve the right to decide what constitutes a Short Communication.

8.5. Letters to the Editor
Letters are welcome in response to previously published articles, and may also include interesting cases that do not meet the requirement of being truly exceptional, as well as other communications of general interest. Letters should have a title and include appropriate references, and include the corresponding author's mailing and e-mail addresses. Letters are edited, sometimes extensively, to sharpen their focus.

They may be sent for peer review at the discretion of the Editors. Letters are selected based on clarity, significance, and space. Typical length: no more than 600 words, 5–10 references; 1 table and/or 1 figure may be included.

8.6. Editorials
Editorials are invited articles or comments concerning a specific paper in the Journal or a topical issue in the field. While normally invited, unsolicited editorials may be submitted. Typical length: no more than 1500 words, 15–30 references.

9. Manuscript Preparation
Text should be typed double-spaced on one side of white A4 (297 × 210 mm) paper, with outer margins of 2.5 cm. A manuscript should include a title page, abstract, text, acknowledgments (if any), conflicts of interest statement (if any), references, and figures and tables as appropriate. Each section of the manuscript should begin on a new page. Pages should be numbered consecutively, beginning with the title page.

9.1. Title Page
The title page should contain the following information (in order, from the top to bottom of the page):

- category of paper
- article title
- names (spelled out in full)* of all the authors, and the institutions with which they are affiliated; indicate all affiliations with a superscripted lowercase letter after the author's name and in front of the appropriate affiliation
- corresponding author details (name, e-mail, mailing address, telephone and fax numbers)

*The name of each author should be written with the family name last, e.g., Jing-Lin Chang. Authorship is restricted only to direct participants who have contributed significantly to the work.

9.2. Abstract and Keywords
Abstracts should be no more than 300 words in length. Abstracts for Original Articles should be structured, with the section headings: Background/Introduction, Purpose(s)/Aim(s), Methods, Results, Conclusion. Abstracts for Case Reports are unstructured, but should include the significance and purpose of the case presentation, the diagnostic methods of the case, the key data, and brief comments and suggestions with regard to the case. Abstracts for Review Articles and Short Communications should also be unstructured. No abstract is required for Letters to the Editor and Editorials. For the article categories that require an abstract, 3–5 relevant keywords should also be provided in alphabetical order.

9.3. Main Text
The text for Original Articles should be organized into the following sections: Background/Introduction, Purpose(s)/Aim(s), Methods, Results and Discussion. Sections for Case Reports are: Introduction, Case Report, and Discussion. Each section should begin on a new page.
9.3.1. Abbreviations
Where a term/definition will be continually referred to, it must be written in full when it first appears in the text, followed by the subsequent abbreviation in parentheses. Thereafter, the abbreviation may be used. An abbreviation should not be first defined in any section heading; if an abbreviation has previously been defined in the text, then the abbreviation may be used in a subsequent section heading. Restrict the number of abbreviations to those that are absolutely necessary.

9.3.2. Units
Système International (SI) units must be used, with the exception of blood pressure values which are to be reported in mmHg. Please use the metric system for the expression of length, area, mass, and volume. Temperatures are to be given in degrees Celsius.

9.3.3. Names of drugs, devices and other products
Use the Recommended International Non-proprietary Name for medicinal substances, unless the specific trade name of a drug is directly relevant to the discussion. For devices and other products, the generic term should be used, unless the specific trade name is directly relevant to the discussion. If the trade name is given, then the manufacturer name and the city, state and country location of the manufacturer must be provided the first time it is mentioned in the text, for example, “...SPSS version 11 was used (SPSS Inc., Chicago, IL, USA).”

9.3.4. Statistical requirements
Statistical analysis is essential for all research papers except case reports. Use correct nomenclature of statistical methods (e.g., two sample t test, not unpaired t test). Descriptive statistics should follow the scales used in data description. Inferential statistics are important for interpreting results and should be described in detail.

All p values should be expressed to 2 digits to the right of the decimal point, unless p < 0.01, in which case the p value should be expressed to 3 digits to the right of the decimal point. The smallest p value that should be expressed is p < 0.001, since additional zeros do not convey useful information; the largest p value that should be expressed is p > 0.99.

9.3.5. Personal communications and unpublished data
These sources cannot be included in the references list but may be described in the text. The author(s) must give the full name and highest academic degree of the person, the date of the communication, and indicate whether it was in oral or written (letter, fax, e-mail) form. A signed statement of permission should be included from each person identified as a source of information in a personal communication or as a source for unpublished data.

9.4. Acknowledgments and Conflicts of Interest Statement
General acknowledgments for consultations, statistical analysis, etc., should be listed concisely at the end of the text, including the names of the individuals who were directly involved. Consent should be obtained from those individuals before their names are listed in this section. All financial and material support for the research and work from internal or external agencies, including commercial companies, should be clearly and completely identified. Ensure that any conflicts of interest (financial and/or non-financial) are explicitly declared.

9.5. Abbreviation list
A term that appears more than three times in a paper should be abbreviated. Spell out the term on first mention, followed by the abbreviated form in parentheses. Thereafter, please use the abbreviated form. Supply a list of nonstandard abbreviations used in the paper at the end of the main text, in alphabetical order, giving each abbreviation followed by its spelled-out version.

9.6. References
9.6.1. In the main text, tables, figure legends
- References should be indicated by numbers in square brackets in line with the text, and numbered consecutively in order of appearance in the text.
- References cited in tables or figure legends should be included in sequence at the point where the table or figure is first mentioned in the main text.
- Do not cite uncompleted work or work that has not yet been accepted for publication (i.e., “unpublished observation”, “personal communication”) as references. Also see Section 9.3.5. above.
- Do not cite abstracts unless they are the only available reference to an important concept.

9.6.2. In the references section
- References should be limited to those cited in the text and listed in numerical order, NOT alphabetical order.
- References should include, in order, author surnames and initials, article title, abbreviated journal name, year, volume and inclusive page numbers. The last names and initials of all the authors up to 6 should be included, but when authors number 7 or more, list the first 6 authors only followed by “et al”. Abbreviations for journal names should conform to those used in MEDLINE.
- If citing a website, provide the author information, article title, website address and the date you accessed the information.
• Reference to an article that is in press must state the journal name and, if possible, the year and volume.

Authors are responsible for the accuracy and completeness of their references and for correct text citation.

Examples are given below.

Standard journal article

Journal supplement

Journal article not in English but with English abstract

Book

Book chapter in book with editor and edition

Bulletin

Company/manufacturer publication/pamphlet

Electronic publications


Items presented at a meeting but not yet published


Item presented at a meeting and published

Material accepted for publication but not yet published


Theses and dissertations


Website

9.7. Tables
Tables should supplement, not duplicate, the text. They should have a concise table heading, be self-explanatory, and numbered consecutively in the order of their citation in the text. Information requiring explanatory footnotes should be denoted using superscripted lowercase letters in alphabetical order (a, b, c, etc.). Asterisks (*, **) are
used only to indicate the probability level of tests of significance. Abbreviations used in the table must be defined and placed after the footnotes. If you include a block of data or table from another source, whether published or unpublished, you must acknowledge the original source.

9.8. Figures

9.8.1. General guidelines
The number of figures should be restricted to the minimum necessary to support the textual material. They should have an informative figure legend and be numbered in the order of their citation in the text. All symbols and abbreviations should be defined in the legend. Patient identification should be obscured. All lettering should be done professionally and should be in proportion to the drawing, graph or photograph. Photomicrographs must include an internal scale marker, and the legend should state the type of specimen, original magnification and stain.

Figures must be submitted as separate picture files at the correct resolution (see Section 9.7.2. below). The files should be named according to the figure number, e.g., “Article1_Fig1”, “Article1_Fig2”.

9.8.2. Formats
Regardless of the application used, when your electronic artwork is finalized, please “save as” or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

- EPS: Vector drawings. Embed the font or save the text as “graphics”.
- TIFF: Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
- TIFF: Bitmapped line drawings: use a minimum of 1000 dpi.
- TIFF: Combination of bitmapped line/half-tone (color or grayscale): a minimum of 600 dpi is required.
- DOC, XLS or PPT: If your electronic artwork is created in any of these Microsoft Office applications, please supply “as is”.

Please do not:
- Supply files that are optimized for screen use (like GIF, BMP, PICT, WPG); the resolution is too low;
- Supply files that are too low in resolution;
- Submit graphics that are disproportionately large for the content.


10. The Editorial and Peer Review Process

As a general rule, the receipt of a manuscript will be acknowledged within 1 week of submission, and authors will be provided with a manuscript reference number for future correspondence.

If such an acknowledgment is not received in a reasonable period of time, the author should contact the Editorial Office.

Submissions are reviewed by the Editorial Office to ensure that it contains all parts. The Editorial Office will not accept a submission if the author has not supplied all the material and documents as outlined in these author instructions.

Manuscripts are then forwarded to the Editor-in-Chief, who makes an initial assessment of it. If the manuscript does not appear to be of sufficient merit or is not appropriate for the Journal, then the manuscript will be rejected without review.

Manuscripts that appear meritorious and appropriate for the Journal are reviewed by at least two Editorial Board members or expert consultants assigned by the Editor-in-Chief. Authors will usually be notified within 6 weeks of whether the submitted article is accepted for publication, rejected, or subject to revision before acceptance. However, do note that delays are sometimes unavoidable.

11. Preparation for Publication

Once a manuscript has been accepted for publication, the authors should submit the final version of the manuscript in MS Word format, with all tables/figures as applicable, to the Editorial Office.

Accepted manuscripts are copyedited according to the Journal’s style and PDF page proofs are e-mailed by the Publisher to the corresponding author for final approval. Authors are responsible for all statements made in their work, including changes made by the copy editor.

12. Publication Charges and Reprints

Authors receive 10 stapled offprints of their articles free of charge, which will be sent by the Editorial Office to the corresponding author. Professional reprints (which include a cover page for the article) may be ordered from the Publisher at prices based on the cost of production. A reprint order form can be downloaded from the journal website at www.e-biomedicine.com.

13. Copyright

BioMedicine is the official peer-reviewed publication of China Medical University (the Proprietor), Taichung, Taiwan. Published manuscripts become the permanent property of the Proprietor. All articles published in the Journal are protected by copyright, which covers the exclusive rights to reproduce and distribute the article, as well as translation rights. No part of this publication may be reproduced, stored in any retrieval system, or transmitted in any form or by any means, electronic, mechanical, by photocopying, recording, or otherwise, without prior written permission from the Proprietor.
CHECKLIST

Only complete manuscript submissions will be considered for publication. Complete submission must include:

☐ Cover letter for manuscript submission
☐ Authorship statement signed by all authors
☐ Signed conflicts of interest disclosure statement
☐ Signed copyright transfer agreement
☐ Manuscript in MS Word format

AND, where applicable

☐ Letter of approval from review committee for use of human samples in research and human experiments
☐ Letter of approval from relevant authority for use of animals in experiments
☐ CONSORT flow chart for randomized controlled trial
☐ Signed consent to publish (in print and online) from human subjects who can be identified in your manuscript
☐ Letter(s) of permission from copyright holder(s) to use copyrighted sources in your manuscript

In the actual article, ensure that the following information is provided:

☐ Title page
  ☐ Article category
  ☐ Article title
  ☐ Name(s) and affiliation(s) of author(s)
  ☐ Corresponding author details (name, e-mail, mailing address, telephone and fax numbers)
☐ Abstract: structured for Original Article; unstructured for Review Article, Case Report, Short Communication (none required for Editorial, Letter to the Editor)
☐ 3–5 relevant keywords in alphabetical order: required for Review Article, Original Article, Case Report, Short Communication (MeSH terms are recommended; see http://www.ncbi.nlm.nih.gov/mesh?term)
☐ Main text
☐ References in the correct format, cited in numerical order, and all references in the List are cited in the Text/Tables/Figures, and vice versa

AND, where applicable

☐ Acknowledgments
☐ Conflicts of interest statement
☐ Table headings and tables, each on a new page
☐ Figure legends, on a new page
☐ Electronic picture files of all figures; resolution of 300 dpi for halftone images, 600 dpi for combination art (halftone + line art), and 1000 dpi for line art

Further considerations:
☐ Manuscript has been spell-checked and grammar-checked
☐ Color figures are clearly marked as being intended for: (I) color reproduction on the Web (free of charge) and in print; or (II) color reproduction on the Web (free of charge) and in grayscale in print (free of charge). If option (II), then grayscale versions of the figures are also supplied for printing purposes.
AUTHORSHIP STATEMENT

Article title: ____________________________________________________________
..................................................................................................................
..................................................................................................................
..................................................................................................................

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in BioMedicine.

Authorship contributions
Please indicate the specific contributions made by each author (list the authors’ initials followed by their surnames, e.g., Y.L. Chang). The name of each author must appear at least once in each of the three categories below.

Category 1
Conception and design of study: ______________, ________________, _______________, __________________;

acquisition of data: ___________________, ___________________, ___________________, ___________________

analysis and/or interpretation of data: ______________, _____________, ______________, ________________.

Category 2
Drafting the manuscript: _________________, _________________, _________________, ____________________;

revising the manuscript critically for important intellectual content: _________________, _________________,

_________________, _________________.

Category 3
Approval of the version of the manuscript to be published (the names of all authors must be listed):

________________, ________________, _________________, ________________, ________________, _______________,

________________, ________________, ________________, ________________.

Acknowledgments
All persons who have made substantial contributions to the work reported in the manuscript (e.g., technical help, writing and editing assistance, general support), but who do not meet the criteria for authorship, are named in the Acknowledgments and have given us their written permission to be named. If we have not included an Acknowledgments, then that indicates that we have not received substantial contributions from non-authors.
This statement is signed by all the authors *(a photocopy of this form may be used if there are more than 10 authors)*:

<table>
<thead>
<tr>
<th>Author's name (typed)</th>
<th>Author's signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CONFLICTS OF INTEREST STATEMENT

Manuscript title: ________________________________________________________________
____________________________________________________________________________
____________________________________________________________________________
The authors whose names are listed immediately below certify that they have NO affiliations
with or involvement in any organization or entity with any financial interest (such as hono-
raria; educational grants; participation in speakers’ bureaus; membership, employment, con-
sultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing
arrangements), or non-financial interest (such as personal or professional relationships, affili-
atations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Author names:

The authors whose names are listed immediately below report the following details of affilia-
tion or involvement in an organization or entity with a financial or non-financial interest in
the subject matter or materials discussed in this manuscript. Please specify the nature of the
conflict on a separate sheet of paper if the space below is inadequate.

Author names:
This statement is signed by all the authors to indicate agreement that the above information is true and correct (a photocopy of this form may be used if there are more than 10 authors):

<table>
<thead>
<tr>
<th>Author's name (typed)</th>
<th>Author's signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>