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Treating metastatic castration-resistant prostate cancer with novel polyamine-free oral nutritional supplementation: Phase I study

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ABSTRACT

Background: Polyamine (PA) dietary deprivation may be of clinical interest in castration-resistant prostate cancer (CRPC).
Purpose: We assessed tolerance and side effects of PA-free oral nutritional supplement (ONS) combined with partial intermittent intestinal decontamination (PIID) in a Phase I trial.
Methods: Ten volunteers of mean age 68 ± 12 years and with symptomatic, metastatic CRPC were enrolled. PA-free ONS was given as the only food source three times daily during the first 2 weeks; twice daily with one PA-reduced meal for 3 weeks; and then once daily with two PA-reduced meals for 7 weeks. Oral neomycin was administered at 0.75 g/day as PIID every other week. Toxicity, performance, and pain status were rated on World Health Organization and European Organisation for Research and Treatment of Cancer scales. Prostate-specific antigen, blood counts, ionograms, and hepatic transaminases were regularly assessed. Bone and computed tomography scans were performed at weeks 0, 5 and 12.
Results: One patient disliked the taste and stopped on Day 4. Nine patients experienced transient Grade I diarrhea. Performance status and pain score were significantly improved in five patients and maintained in three patients. No significant differences in body weight, hemoglobin, serum proteins, and ionograms were noted. Four patients had 20–40% prostate-specific antigen baseline decline during the first 5 weeks of the trial. Five patients had bone and computed tomography scan stabilization.
Conclusion: This PA-free ONS was safe and well tolerated with PIDD. It seemed to benefit quality of life and control pain. The effects were dose dependent, with maximum improvement observed during the first 5 weeks when PA depletion was maximal.

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1. Introduction

Polyamines (PAs) belong to a wide range of biogenic amines involved in many physiological functions [1]. These ubiquitous chemical entities have roles in cellular proliferation and differentiation. In mammals, cellular PAs such as putrescine, spermidine, and spermine are derived from endogenous biosynthesis as well as from the diet and intestinal microorganisms. Putrescine is synthesized from ornithine by a reaction catalyzed by ornithine decarboxylase (ODC), a limiting enzyme in polyamine synthesis. Cadaverine, a PA chemically similar to putrescine, is produced by microorganisms and is generated by the decarboxylation of lysine by lysine decarboxylase. Two other mammalian PAs are derived from putrescine by successive attachment of two propylamine groups by aminopropyl transferases: spermidine synthase and spermine synthase. The propylamine group donor is S-adenosyl-S-methyl-homocysteamine, derived from S-adenosyl-methionine by the action of S-adenosyl-methionine decarboxylase. Abnormalities in homeostatic control of PA metabolism are implicated in several pathological processes, including cancer. PA metabolism is a potential target in prostate cancer treatment, because ODC is overexpressed in prostate cancer [2]. Additionally, high PA levels are present in circulating red blood cells and are correlated with tumor stage and aggressiveness [3,4].

In vitro, difluoromethylornithine (DFMO), an ODC inhibitor, effectively inhibits malignant cell proliferation. However, its effectiveness *in vivo* is reduced because tumor cells can take up PAs released into the circulation by normal and cancer cells, as well as from gut microflora or dietary sources. Tumor-bearing animals fed with a PA-deficient diet and treated with DFMO and neomycin, which partially decontaminates the gastrointestinal tract, exhibit significant inhibition of tumor progression and metastatic spread [5]. In these animals, anticancer immunity is stimulated [6], and deleterious secondary effects are not observed. Moreover, we have previously observed 40% inhibition in tumor progression and metastatic spread in tumor-grafted animals fed only with a PA-deficient diet and receiving neomycin in the drinking water, without DFMO treatment [5]. Single-agent treatment with neomycin or DFMO did not slow progression.

A preliminary clinical trial performed in 13 metastatic castrate resistant prostate cancer (CRPC) patients previously showed the feasibility of nutrition therapy, based on a diet with reduced PAs, combined with intermittent oral neomycin treatment for 6 months [7]. This trial demonstrated that combining reduction of PA dietary intake with partial intestinal decontamination is adhered to well and well tolerated. Likewise, this regimen seemed to benefit quality of life and control pain. Both performance status and pain were improved during the regimen but deteriorated 3 months after cessation.

We have previously published PA contents of 233 food and drink items. This food list and PA content can serve as the basis for establishing a PA-reduced diet (PRD). Quality of life was maintained for 33 prostate cancer patients treated with a PRD; 30 of whom had metastatic HRPC disease. PRD tolerance and adherence were also good [8]. To reduce PA dietary intake significantly, we designed an industrially processed, PA-free oral nutritional supplement (ONS) containing 100 pmol/mL PA, which covered all human nutritional needs. In hormone-resistant Dunning Mat LyLu prostatic and Lewis L lung carcinoma tumor-bearing animals, PA-free ONS monotherapy reduced tumor growth and pulmonary metastatic spread by 40% without weight loss or toxicity (unpublished results).

Here, we tested the feasibility, adherence, tolerance, and toxicity of PA-free ONS combined with oral neomycin PIID administered to symptomatic metastatic HRPC patients in a palliative setting.

1.1. *Patients and methods*

Ten volunteers with symptomatic metastatic CRPC of mean age 68 ± 12 years (range: 46–81 years) were enrolled in a clinical trial assessing PA-free ONS and PIID. All eligible patients had CRPC, as defined by prior treatment with androgen-blocking hormonal manipulations, castrate testosterone serum levels, and rising prostate-specific antigen (PSA) levels. Mean time from palliative hormonal treatment to hormonal escape was 35 ± 18 months, and mean time from hormonal escape to trial was 18 ± 8 months. All cases were nonresponsive to prior chemotherapy with either estramustine phosphate or oral low-dose cyclophosphamide (100 mg/day). During this trial, chemotherapy was stopped, but hormone therapy continued as usual. Bone scans assessing metastatic bone spread showed one patient with ≤5 hotspots (Grade I), six with 6–20 hotspots (Grade II), and three with >21 hotspots (Grade III). As such, most patients had extensive metastatic disease.

PA-free ONS covers all human nutritional needs, with an energy content of 1800 kcal. PRD, as previously described [8], was proposed in combination with ONS during the trial. We designed the trial to mimic a Phase I trial, with three different phases of PA dietary depletion, starting with 14 days of the least possible dietary intake and gradually increasing dietary PAs with time.

The following polyamine-free ONS was proposed. (1) Three times daily (three cans) as sole food during the first 2 weeks. Hungry patients could eat some bread and butter, which have low PA content. Patients were not limited for consumption of water, tea, or coffee. (2) Twice daily (2 cans) with one PRD meal for 3 weeks. (3) Once daily (1 can) with two PRD meals for the last 7 weeks. (4) Patients then continued with a PRD.

The primary objective was to assess feasibility, tolerance, and adherence of these three consecutive periods of PA dietary reduction and of combining PA-free ONS and PIID. Secondary objectives were to assess biological and clinical responses. Performance and pain status were assessed according to World Health Organization (Table 1) and European Organisation for Research and Treatment of Cancer (Table 2) scales. Body weight, PSA, alkaline phosphatases (APs), blood counts, serum and urine ionograms, serum urea, creatinine, proteins, cholesterol, hepatic transaminases, and bilirubin were also regularly monitored. Bone and computed tomography (CT) scans were performed on Days 0, 35 and 85.

1.2. *Polyamine-free ONS*

Polyamine-free ONS is presently manufactured and marketed under the tradename Castase by Nutrialys Medical Nutrition

Table 1 – World Health Organization performance status scale.

Grade 0: Patient able to carry out all normal activity without restriction
Grade 1: Patient restricted in physically strenuous activity but ambulatory and able to carry out light work
Grade 2: Patient ambulatory and capable of all self-care but unable to carry out any work, up and about for >50% of waking hours
Grade 3: Patient capable of only limited self-care, confined to bed or chair for >50% of waking hours
Grade 4: Patient completely disabled, cannot carry out any self-care, totally confined to bed or chair

(Saint Grégoire, France). PA content is 100 pmol/mL. At the time of the study, three 500-mL cans supplied 1800 kcal/day and covered all adult nutritional needs, including proteins, fatty acids, sugars, vitamins, iron, calcium, and magnesium.

1.3. *PIID*

Patients were prescribed with daily doses of 0.75 g oral neomycin, starting with antibiotics at Day 0 and continuing treatment daily for 1 week. Treatment stopped during the second week, resumed during the third, stopped during the fourth, and continued in that manner until the end of the trial. This intermittent prescription was proposed to reduce side effects of long-term intestinal decontamination. Results are expressed as mean ± standard deviation or median values, and statistics were calculated by nonparametric Wilcoxon test using BI@LOGINSERM software. The local ethics committee approved the trial.

2. Results

2.1. *Tolerance and adherence*

One patient disliked the taste of the ONS and experienced intestinal discomfort. He stopped participating in the trial on Day 4 but continued with PRD and is still alive. No other voluntary dropouts were recorded.

2.2. *Toxicity*

Nine patients had mild transient Grade I diarrhea, defined by an increase in stools, but fewer than four over baseline daily, for a mean 2.2 ± 1.7 days (range: 0–4 days) during the first 2 weeks. No other significant toxicity was reported. Table 3 reports body weight and main biological parameters assessed.

Table 2 – European Organization for Research and Treatment of Cancer pain scale.

Grade 0: No analgesics
Grade 1: Occasional non-morphine analgesics
Grade 2: Regular non-morphine analgesics
Grade 3: Occasional morphine analgesics
Grade 4: Regular morphine analgesics

2.3. *Quality of life*

Performance status and pain scores were improved in five patients, stabilized in three patients, and deteriorated in one patient. Their mean values were significantly reduced during the first 5 weeks of the trial (Fig. 1).

2.4. *Treatment response*

Five patients had bone and CT scan stabilization, and four patients showed disease progression. Four patients had a 10%, 27%, 28% or 42% decline in PSA from baseline during the first 5 weeks. One patients showed PSA stabilization, and four others showed PSA progression. At the end of the trial, five patients had secondary PSA decline of 55–70% over an average of 90 days, following reintroduction of estramustine phosphate or low-dose cyclophosphamide therapy. Median levels of APs fell significantly at Days 18 and 35 (Table 3 and Fig. 2). From Day 4 onwards, total lymphocyte counts significantly improved (Table 3 and Fig. 3).

2.5. *Patient survival*

Two patients died in the final stages of the trial. One patient aged 81 years on Day 70 died of a previously recorded bone marrow insufficiency, which was unaffected by diet. Another patient died of terminal cancer on Day 77. Both had advanced disease with Grade III bone scans. One patient died of an unrelated cause (endocarditis) at 10 months. Cancer-specific survival after hormonal escape and initiation of trial were 30 ± 9 months (range: 16–42 months) and 9 ± 5 months (range: 3–17 months), respectively.

3. Discussion

Prostate cancer is the second leading cause of male cancer mortality in western countries. Managed in its early stages, cures are possible most of the time. However, when radical treatment fails, including radical prostatectomy, external beam radiation or brachytherapy, treatment becomes palliative, as resistance to androgen ablation inevitably occurs [9]. Currently, docetaxel chemotherapy is standard for management of symptomatic CRPC and was the first chemotherapy regimen to increase cancer-specific survival significantly. Yet, this improvement is only marginal and does not exceed 2 months, compared to mitoxantrone chemotherapy or prednisolone alone [9–11]; HRPC management generally focuses on quality of life and pain control.

Here, we report the results of the first PA-free ONS given in combination with intermittent oral neomycin as a palliative treatment for CRPC patients and as an adjunct to continuous androgen deprivation. We estimate that this nutrient, given as sole food source food during the first 14 days of the trial, enables a 2500-fold reduction of daily dietary PA intake. One patient disliked the taste of the product, which is now produced in vanilla, strawberry, chocolate, coffee, biscuit and vegetable flavors. Adherence was excellent in other patients. Side effects were moderate and included transient Grade 1 diarrhea; whether this was related to PA-free ONS, to neomycin, or to

Table 3 – Body weight and main biological parameters assessed during trial.

	D0	D4	D7	D18	D35	D50	D85
Body weight (kg)	74 ± 12	74 ± 10	73 ± 10	73 ± 11	74 ± 10	74 ± 10	74 ± 10
Hb (g/L)	10.85	11.05	11.15	10.55	9.5	10.6	11.4
WBC (/mL)	7700	6200	7150	6850	7300	6400	7200
Lymphocytes (/mL)	631	994*	798*	902*	679*	861*	756*
Platelets (/mL)	219,500	231,500	262,000	210,500	212,000	236,000	247,000
Proteins (g/L)	63	63	67	65	61	61	66
Albumin (g/L)	33	33	34	36	34	35	38
Urea (g/L)	0.34	0.33	0.46	0.58	0,37	0.36	0.48
Creatinine (g/L)	8	8.5	9.5	10,5	8	8	11
Glycemia (g/L)	1.02	0.9	1.15	1.15	1	0.9	1
Calcium (mg/L)	84	88,5	90,5	89	88	87	91
Cholesterol (g/L)	1.88	1.94	1.99	1.92	1.78	1.89	1.9
Iron (g/L)	68	59,5	70	44	49	72	66
Bilirubin	5	5	5	5	5	5	5
Transaminases	22	26	29	31	30	25	25
GGT	12	12	51	59	57	65	42
AP	161	153	145	120*	126*	161	162
PSA (ng/ml)	782	765	667	668	594	692	859

*Significant difference ($p < 0.05$) compared to D0 values expressed as median values, except for body weight and PSA, which are expressed as mean ± standard deviation due to large variations between patients.
AP = alkaline phosphatase; D = day; GGT = γ-glutamyl transferase; Hb = hemoglobin; PSA = prostate-specific antigen; WBC = white blood cells.

their combination is unclear, although it was mainly observed with neomycin. Body weight was maintained and no vital biological parameters were significantly impaired, particularly during the first two phases (5 weeks) of minimal PA dietary intake. By contrast, blood lymphocyte counts significantly improved. Two deaths recorded at the end of trial were not attributed to treatment itself but to the terminal state of the disease.

Marked improvements in pain control and performance status were observed. Rapid pain improvement, measured by stark reduction of analgesic consumption, was observed on Day 4. Performance status improvement was slightly delayed and observed on Days 7–14. These improvements occurred during the first two phases of the trial, with maximal PA dietary reduction. During the third phase, pain and performance status scores increased but remained below initial values. Rapid pain control was most likely not due to tumor control, because progressive cases also exhibited pain improvement. It possibly relates to the fact that PAs are known to regulate N-methyl-D-aspartate (NMDA) receptors and, in particular, their NR2B subunit, which is involved in pain perception [12–15]. This NMDA receptor is widely expressed in the nervous system and has been implicated in a variety of synaptic signaling events that also modulate other functions: for example, learning, memory processing, and feeding. Recent research supports the involvement of this receptor in several human disorders, such as seizures, schizophrenia, Parkinson's disease, and drug abuse [12].

PA deprivation has potential direct analgesic effects. Experimentally, PA deprivation provokes analgesic effects in animals submitted to painful stimuli [16,17]. Studies have shown that spermine is directly implicated in nociception, and exogenous intrathecal administration of spermine produces pain-related behavior in mice, an effect attributed to activation of NMDA receptors [18]. PA-deficient diets suppress

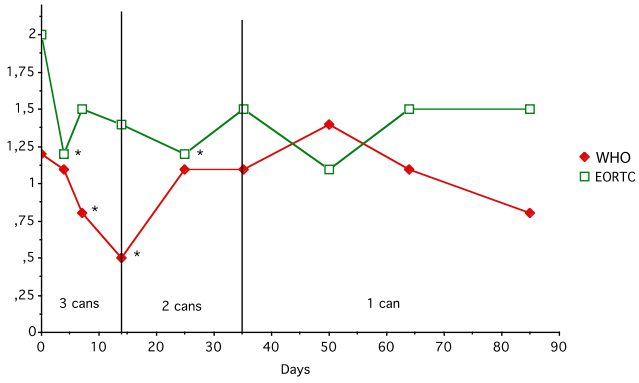

Fig. 1 – Mean performance status values (World Health Organization) and pain scores (European Organization for Research and Treatment of Cancer). Patients received three cans daily during Phase 1, two cans daily and one polyamine-reduced diet (PRD) meal daily during Phase 2, and one can daily and two PRD meals during Phase 3.
*** $p \leq 0.05$.**

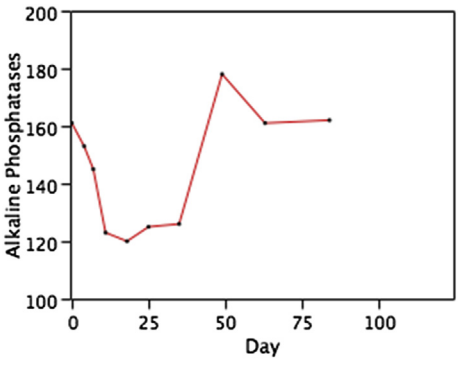

Fig. 2 – Median alkaline phosphatase values significantly declined during Days 11–35 ($p < 0.05$).

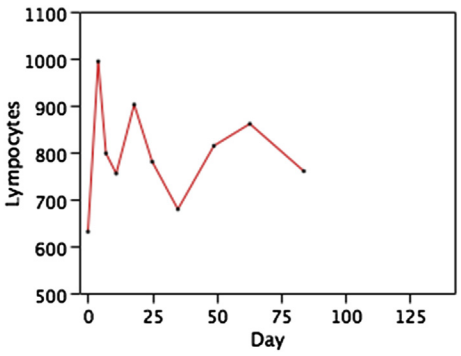

Fig. 3 – Median total circulating blood lymphocyte values significantly increased during Days 4–85.

morphine-induced hyperalgesia and allodynia in rats [19]. Reducing exogenous polyamine pools could thus be useful as a pain management strategy. Furthermore, PRD is simple and cost-effective.

Objective CT and bone scan stabilization were observed in five of nine evaluable patients. AP levels significantly dropped on Days 18–35. As bilirubin values were normal at treatment initiation and not modified during the trial, this AP reduction was not of hepatic origin but may have arisen from positive metastatic bone response. Four cases had a 10–42% decline in PSA, and one showed stabilization of PSA. Although these observed PSA declines did not reach 50%, which represented a positive response, they remained outstanding for nutrition therapy alone with neomycin, especially in symptomatic patients showing aggressive disease with extensive metastases. These responses were observed during the first weeks of the trial, when PA reduction was maximal. Furthermore, after the trial, some patients who remained on a natural PRD showed a secondary 55–73% decline in PSA in response to prior ineffective therapies, such as low-dose cyclophosphamide or estramustine phosphate.

Circulating lymphocyte levels significantly increased during the trial. Lymphocytes are involved in antitumor immunity. In a preclinical *in vivo* setting with the androgen-independent prostate carcinoma Mat LyLu model, PA deprivation prevented tumor-induced immunosuppression in tumor-bearing animals [6]. Although specific lymphocyte measurements were not performed in the present trial, we may speculate that global lymphocyte count improvement could reflect positive antitumor immune response. These results suggest that in clinical settings, dietary PAs can have similar potential implications in tumor control, immune response and chemosensitization, as highlighted previously by the Dunning Mat LyLu rodent prostatic adenocarcinoma model [6,20]. Dietary PAs appear to be inversely correlated with dosage, because maximum depletion yields maximum beneficial clinical effects.

In conclusion, as a novel therapeutic nutritional approach to CRPC, this PA-free nutrient combined with intermittent gut decontamination is safe, adhered to well, and well tolerated with no major toxicity. Treatment proved beneficial to quality of life and pain control, with objective stabilization achieved in half of the patients. PSA decline was observed during the trial, then secondarily after reintroduction of low-dose chemotherapy. The effect of PA dietary depletion seems to be dose-dependent. Results merit further investigation. Phase II trials combining PA dietary depletion by means of a PA-free ONS and docetaxel chemotherapy are currently in progress.

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