Biomedicine & Pharmacotherapy 105 (2018) 558–565

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Metal- and redox homeostasis in prostate cancer with vitamin D_3 supplementation

Krisztina Süle[a,b,*], Klára Szentmihályi[a], Gergő Szabó[b], Dénes Kleiner[b], Imre Varga[c], Anna Egresi[b], Zoltán May[a], Péter Nyirády[d], Miklós Mohai Jr.[e], Anna Blázovics[b]

[a] Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences of the Hungarian Academy of Sciences, Magyar Tudósok körútja 2, H-1117 Budapest, Hungary
[b] Institute of Farmacognosy Semmelweis University, Üllői út 26, H-1086 Budapest, Hungary
[c] Medical Center of Dunakeszi, Fő út 75-86, H-2120 Dunakeszi, Hungary
[d] Department of Urology and Urooncological Centre, Semmelweis University, Üllői út 78/b, H-1082 Budapest, Hungary
[e] Budapest University of Technology and Economics, Műegyetem rkp. 3, H-.1111 Budapest, Hungary

ARTICLE INFO

Keywords:
Micro-macro elements
prostate cancer
vitamin D_3
ICP-OES

ABSTRACT

Vitamin D_3 supplementation has a beneficial effect on cancerous patients, although it can influence the redox- and metal homeostasis. The aim of our investigation was to demonstrate the effect of vitamin D_3 consumption on the redox- and metal homeostasis in prostate cancer, because of the recommended daily dose increased from 200 IU to 2000 IU in recent years in Hungary. Forty-three volunteers were involved in the study. The grouping was applied according to the clinical routine laboratory parameters (vitamin D_3) and the tumor markers (PSA, *f*PFA). Patients were divided into 5 groups: (A) patient control (N = 8), (B) patient control with vitamin D_3 treatment (N = 9), (C) high-risk prostate cancer group (N = 6), (D) high-risk prostate cancer group with vitamin D_3 treatment (N = 8) and (E) vitamin D_3 treated cancerous group with androgen deprivation therapy (N = 11). The element concentrations were determined with ICP-OES. Among the redox parameters, free radical scavenging capacity and H-donating ability were determined with luminometry and spectrometry. Vitamin D_3 treatment caused differences in the metal- and redox homeostasis in either patient control and cancerous groups. The concentration of Fe, Cr, and Pb significantly increased in the erythrocytes of prostate cancer patients. According to the higher scavenging capacity by vitamin D_3 treatment, it seems that vitamin D_3 helps to equilibrate the redox homeostasis that could affect the outcome of cancer positively. However, the tendency in the metal element status does not give a clear explanation of cancer's outcome, but the accumulation of Pb by vitamin D_3 supplementation needs to be taken into more serious consideration in set terms of occupational diseases.

Abbreviations: Al, aluminium; ALT, alanine transaminase; AP-1, activated protein–1; AR, androgen receptor; AST, aspartate transaminase; B, boron; Ba, barium; BMI, body mass index; Ca, calcium; CDK4, cyclin dependent kinase; CEA, carcynoembrionic antigene; Chol, cholesterol; CHR, reticulocyte haemoglobin content; CKI, cyclin dependent kinase inhibitor; Co, cobalt; Cr, chromium; CREA, creatinine; CRP, c-reactive protein; Cu, copper; DNA, deoxyribonucleic acid; DV, declared value; EGF, epidermal growth factor; Fe, iron; fPSA, free prostate specific antigen; G1, gap 1 phase; G2, gap 2 phase; GADD45α, growth arrest and DNA-damage-inducible protein alpha; GGT, gamma-glutamyl transferase; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; GSK-3, glycogen synthase kinase; H_2O_2, hydrogen peroxide; HCHO, formaldehyde; HCl, hydrogen chloride; HGB, haemoglobin; HIF-1, hypoxia induced factor; HNO_3, nitric acid; ICP-OES, inductively coupled plasma optical emission spectrophotometry; IKEB, Intézeti Kutatási Etikai Bizottság = Instructional Research Ethics Committee; IL-2, -6, -10, interleukin-2, -6, -10; INK4, inhibitors of CDK4; IU, international unit; LDH, lactate dehydrogenase; Li, lithium; LMWCr, low molecular weight chromium; M, metaphase checkpoint; MCV, mean corpuscular volume; Mg, magnesium; Mn, manganese; MV, measured values; NADH, nicotinamide adenine dinucleotide; NFAT, nuclear factor of activated cells family; NF-κB, nuclear factor-kappaB; Ni, nickel; P, phosphorus; p21waf1, cyclin-dependent kinase inhibitor protein 1; p27kip1, multifunctional cyclin–dependent kinase inhibitor; p53, tumor suppressor p53; Pb, lead; PDGF, platelet-derived growth factor; PSA, prostate specific antigen; R, recovery; RLU, as relative light unit; RNA, ribonucleic acid; RXR, retinoid X receptor; S, sulfur; Sr, strontium; Ti, titanium; TNF-α, tumour necrosis factor alpha; TSC, total scavenger capacity; TUKEB, Tudományos és Kutatásetikai Bizottság = Scientific and Research Ethics Committee; UA, uric acid; V, vanadium; VDR, vitamin D_3 receptor; VEGF, vascular endothelial growth factor; WBC, white blood cell; Zn, zinc

* Corresponding author at: Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences of the Hungarian Academy of Sciences, Magyar Tudósok körútja 2, H-1117 Budapest, Hungary.

E-mail addresses: sule.krisztina@ttk.mta.hu (K. Süle), szentmihalyi.klara@ttk.mta.hu (K. Szentmihályi), szabo2gergo@gmail.com (G. Szabó), kleiner.denes@pharma.semmelweis-univ.hu (D. Kleiner), dkszakrend@gmail.com (I. Varga), egresi.anna@pharma.semmelweis-univ.hu (A. Egresi), may.zoltan@ttk.mta.hu (Z. May), nyirady.peter@med.semmelweis_univ.hu (P. Nyirády), mohaiv29@gmail.com (M. Mohai), blazovics.anna@pharma.semmelweis-univ.hu (A. Blázovics).

https://doi.org/10.1016/j.biopha.2018.05.090
Received 30 December 2017; Received in revised form 18 May 2018; Accepted 20 May 2018

1. Introduction

In the literature, there are a few investigations about the effect of vitamin D_3 against oxidative stress, but they show positive results, because vitamin D_3 increases the SOD activity, reverses the alteration in the oxidative and nitrosative stress parameters [1]. Epidemiological studies indicate that vitamin D_3 ($1\alpha,25(OH)_2D_3$) deficiency is an important factor in various human cancer type [2–4]. In Europe, prostate cancer is one of the most commonly diagnosed malignant diseases among men after non-melanoma skin cancer, colorectal and lung cancer whose mortality increases with age. Besides genetic diversity and adverse environmental aspects, vitamin D_3 deficiency plays an important role in the incidence of prostate cancer. Clinical researches and molecular studies have already established that vitamin D_3 inhibits cell proliferation on androgen receptor (AR)-positive and AR-negative in the prostate tumor cells. Antiproliferative effect of vitamin D_3 is mediated by the vitamin D receptor (VDR), as calcitriol ($1\alpha,25(OH)_2D$) binds to VDR, and it interacts with the retinoid X receptor (RXR) [5,6]. Several forms of cholecalciferol could be bound to the vitamin D_3 receptor, but 1,25-dihydroxycholecalciferol is biologically more potent due to its 1000 times higher affinity to VDR than that of to 25-hydroxycholecalciferol [7]. Nevertheless, a hormonally active form of vitamin D_3 (25-dihydroxycholecalciferol) activates the apoptotic pathways through the expression of p53 tumor suppressor protein, modulates cell cycle by inhibition of the G_1–S transition and inhibits cell differentiation in tumors through the signal transduction processes [8–10]. In vitro studies on human prostate cancer cell lines and mouse models have demonstrated that vitamin D_3 and its derivatives can inhibit the growth of cancer cells, by inducing apoptosis and preventing cell proliferation [11–14]. Also, vitamin D secosteroids can activate cyclin-dependent kinase inhibitors, e.g. INK4 proteins, p21waf1, and p27kip1 [15].

With these findings, it is highly considered that the treatment of vitamin D_3 and its analogs could be used as alternative therapy in prostate cancer.

There are still not enough indicators available to diagnose prostate cancer at an early stage and differentiate the patients who require prostatectomy or oncological treatment for healing. Even the level of prostate-specific antigen (PSA), whose height is the most common sign of the disease occurrence, can stay within the normal range or give a false negative result, especially at the early stage. Several studies were carried out to find a specific biomarker of the redox system to determine the disease severity. Some studies in human tumor cell lines refer to the increase of interleukins (IL-6, IL-10), tumor necrosis factor alpha (TNF-α), VEGF signal protein, and the decrease of IL-2 in metastasis [16,17]. In one of our previous studies, the levels of cytokines and growth factors were lower at the early stage prostate cancer patients than the one in the controls. Furthermore, remarkable differences were found in the results of bound formaldehyde (HCHO), Zn-protoporphyrin and free protoporphyrin in erythrocytes of taxane-treated metastatic, histologically negative and positive patients compared to the healthy controls [18]. These findings are not only important in the process of DNA hypomethylation because it could lead to high mutation rate in most cases [19], but they are also in strong connection with the redox status [20].

On the other hand, it was also demonstrated that not only the metal elements could assist the absorption of vitamin D_3 but conversely, the uptake of micro and macronutrients (e.g., Ca, Mg, Cu, Zn, Fe, Se), and certain transition metals might be facilitated by vitamin D_3 in the gastrointestinal tract in healthy individuals [21]. Even the absorption of heavy metals can be assisted by vitamin D_3. For that reason, despite the beneficial effects of vitamin D_3, heavy metal accumulation needs to be taken into account, since the absorption of non-essential metal elements could be affected by the vitamin D_3 intake in human [22]. It was also demonstrated that the metal element status alters in the case of prostate cancer with special emphasis on the Pb level [23], but there is no study for investigating the metal element status in the function of vitamin D_3 supplementation in prostate cancer.

Vitamin D_3 deficiency could be a risk factor for prostate cancer in the Hungarian man population since the vitamin D_3 is lower than the optimal level in 50% of the Hungarian population even in the summer period [24]. There is no information available in the literature about that how vitamin D_3 treatment effects on the metal element and redox homeostasis in prostate cancer patients. Therefore, in the current study our aim was to perform a comprehensive investigation on how vitamin D_3 supplementation in patient control and prostate cancer patients influences the metal element- and redox homeostasis after a three-year treatment.

2. Materials and methods

2.1. Materials

Vitamin D_3 was a general consumer product on sale in the pharmacy. Each gelatine capsule contained 3000–3300 IU (75–82.5 μg) cholecalciferol (vitamin D_3). Standard solutions, nitric acid, and hydrogen chloride were purchased from Reanal (Budapest). 1,1-diphenyl-2-picrylhydrazyl, stable radical, hydrogen peroxide, luminol, microperoxidase were obtained from Sigma (St. Luis). Spectro multi-element standard solutions were used for ICP (CPAchem; Stara Zagora, Bulgaria). The CHR hemoglobin reagent solution was purchased from Reagents Ltd., Hungary.

2.2. Patients

42 volunteers with a mean age of 62.1 ± 15.9 years were examined, of which 25 were the outpatients of the Medical Centre of Dunakeszi and 17 from the Department of Urology and Urooncological Centre at the Semmelweis University, Budapest Hungary. The grouping was executed by the treating physician according to cancer's progressivity (PSA, digital rectal examination, biopsy) and the vitamin D_3 treatment. Harmful habits and other comorbidities of some patients (smoking, alcohol consumption, diabetes mellitus, another type of cancers) were considered. Conforming to the latter, two patients had to be excluded from the investigation. Patients with vitamin D_3 treated groups received vitamin D_3 treatment for three years continuously under strict physician control.

The diet of the patients didn't alter from the conventional Hungarian dietary, the intake of vitamin D_3 was 2.5 μg/day in men and 1.9 μg/day in women of 35–64 years old, while Ca was 759 mg/day in men and 690 mg/day in women; Cu was 1.18 mg/day in men and 0.94 mg/day in women; and Zn was 9.65 mg/day in men and 7.31 mg/day in women [24]. According to their state, they were following a normal diet without taking any food supplements. The patients didn't work under extreme conditions and they weren't exposed to any metal toxicity (foundries, battery recycling, etc.). Blood samples were collected in spring (March-April) after the three-year treatment. The patients were divided into 5 groups according to the Table 1:

Table 1
PSA level (μg/L) in different patients' groups after three-year treatment.

Patients	PSA (μg/L)
Patient control group (N = 8)	1.11 ± 0.45
Patient control group with vitamin D_3 treatment (N = 9)	0.75 ± 0.40
High-risk prostate cancer group (N = 6)	13.0 ± 5.4*
High-risk prostate cancer group with vitamin D_3 (N = 8)	5.68 ± 1.11*,**
Vitamin D_3 treated cancerous group with androgen deprivation therapy (N = 11)	1.40 ± 2.44**

* significant difference to the patient control $p < 0.01$.

** significant difference to the high-risk prostate cancer without vitamin D_3 treatment $p < 0.01$.