Journal of Steroid Biochemistry & Molecular Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

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

Review

Vitamin D supplementation and antibacterial immune responses in adolescents and young adults with HIV/AIDS☆

Rene F. Chun [a], Nancy Q. Liu [a], T. Lee [a], Joan I. Schall [c], Michelle R. Denburg [b,e], Richard M. Rutstein [d,e], John S. Adams [a], Babette S. Zemel [c,e], Virginia A. Stallings [c,e], Martin Hewison [a,*]

[a] UCLA Orthopaedic Hospital, Department of Orthopaedic Surgery, Orthopedic Hospital, University of California, Los Angeles, CA 90095, USA
[b] Division of Nephrology, USA
[c] Gastroenterology, Hepatology and Nutrition, USA
[d] General Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA
[e] Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, 19104

ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form 26 July 2014
Accepted 31 July 2014
Available online xxx

Keywords:
HIV
Monocytes
Antibacterial
Cathelicidin
Vitamin D-supplementation

ABSTRACT

Human monocytes activated by toll-like receptor 2/1 ligand (TLR2/1L) show enhanced expression of the vitamin D receptor (VDR) and the vitamin D-activating enzyme 1α-hydroxylase (CYP27B1). The resulting intracrine conversion of precursor 25-hydroxyvitamin D_3 (25OHD) to active 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) can stimulate expression of antibacterial cathelicidin (CAMP). To determine whether this response is functional in HIV-infected subjects (HIV+), serum from HIV+ subjects pre- and post-vitamin D supplementation was utilized in monocyte cultures with or without TLR2/1L. Expression of *CYP27B1* and *VDR* was enhanced following treatment with TLR2/1L, although this effect was lower in HIV+ vs HIV− serum ($p < 0.05$). *CAMP* was also lower in TLR2/1L-treated monocytes cultured in HIV+ serum ($p < 0.01$). In a dose study, supplementation of HIV+ subjects with 4000 IU or 7000 IU vitamin D/day increased serum 25OHD from 17.3 ± 8.0 and 20.6 ± 6.2 ng/ml (43 nM and 51 nM) at baseline to 41.1 ± 12.0 and 51.9 ± 23.1 ng/ml (103 nM and 130 nM) after 12 weeks (both $p < 0.001$). Greater percent change from baseline 25OHD was significantly associated with enhanced TLR2/1L-induced monocyte *CAMP* adjusted for baseline expression ($p = 0.009$). In a randomized placebo-controlled trial, 7000 IU vitamin D/day increased serum 25OHD from 18.0 ± 8.6 to 32.7 ± 13.8 ng/ml (45 nM and 82 nM) after 12 weeks. Expression of *CAMP* increased significantly from baseline after 52 weeks of vitamin D-supplementation. At this time point, TLR2/1L-induced *CAMP* was positively associated with percent change from baseline in 25OHD ($p = 0.029$ overall and 0.002 within vitamin D-supplemented only). These data indicate that vitamin D supplementation in HIV-infected subjects can promote improved antibacterial immunity, but also suggest that longer periods of supplementation are required to achieve this.

Contents

☆ This work was supported by the NIH/National Center for Complementary and Alternative Medicine, Grant R01AT005531, the National Center for Research Resources, Grant UL1RR024134, and is now at the National Center for Advancing Translational Sciences, Grant UL1TR000003. Ex vivo analyses were supported by NIH/NIAMS grant AR063910.

* Corresponding author at: UCLA Orthopaedic Hospital Department of Orthopaedic Surgery, 615 Charles E. Young Drive South, Room 410D, Los Angeles, CA 90095-7358, USA. Tel.: +1 310 206 1625; fax: +1 310 825 5409.
E-mail address: mhewison@mednet.ucla.edu (M. Hewison).

http://dx.doi.org/10.1016/j.jsbmb.2014.07.013
0960-0760/

1. Introduction

Vitamin D promotes a range of extra-skeletal responses that may impact on diverse aspects of human physiology [1]. Prominent amongst these are the immunomodulatory effects of active 1,25-dihydroxyvitamin D (1,25(OH)$_2$D), that can influence both innate and adaptive immunity [2,3]. Responses to 1,25(OH)$_2$D are mediated via the nuclear vitamin D receptor (VDR) which is expressed by many immune cells [4]. However, monocytes are particularly important targets for vitamin D because they express the enzyme 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) that catalyzes conversion of pro-hormone 25-hydroxyvitamin D (25OHD) to active 1,25(OH)$_2$D [5,6]. The resulting intracrine mechanism plays a pivotal role in innate antibacterial responses, where binding of pathogen-associated molecular patterns (PAMPs) to monocyte pattern-recognition receptors (PRR) stimulates expression of both VDR and CYP27B1 [7]. Intracrine conversion of 25OHD to 1,25(OH)$_2$D by monocytes promotes several antibacterial responses, including enhanced expression of antibacterial proteins [7,8], induction of autophagy [9,10], and regulation of intracellular iron homeostasis [11]. These effects appear to be dependent on the availability of substrate 25OHD for intracrine conversion to 1,25(OH)$_2$D. As 25OHD is the main circulating form of vitamin D, variations in serum 25OHD status have the potential to influence monocyte antibacterial responses, with vitamin D-deficiency compromising, and vitamin D-supplementation enhancing, antibacterial responses [7,12].

Vitamin D-induced antimicrobial responses have been reported for several different cell types and may occur in response to a variety of pathogens [3]. In particular, vitamin D-induced antibacterial responses in monocytes have been closely linked to mycobacterial infections such as tuberculosis [13]. The tuberculosis pathogen *Mycobacterium tuberculosis* (*M. tb*) is phagocytosed by monocytes and macrophages, but *M. tb* PAMPs also promote innate immune responses when recognized by PRR such as the toll-like receptor (TLR) 2/1 heterodimer [14,15]. TLR2/1 ligands (TLR2/1L) associated with *M. tb* have been shown to stimulate monocyte expression of *CYP27B1* and *VDR*, with the resulting intracrine induction of the antibacterial protein cathelicidin (CAMP) acting to promote intracellular killing of *M. tb* [7].

These observations provide a mechanistic rationale for the historical link between vitamin D and the treatment of tuberculosis, in which ultraviolet light (the primary mode of vitamin D generation in normal physiology) and cod liver oil (a rich source of dietary vitamin D) were at one time used as a treatment for tuberculosis [16,17]. More recently, epidemiology has shown that vitamin D-insufficiency is associated with increased incidence of tuberculosis [18–21], and several clinical trials of vitamin supplementation and tuberculosis have also been reported with varying degrees of success [21–24]. Vitamin D-deficiency is also prevalent in HIV+ subjects where there is increased risk of infection by pathogens such as *M. tb* [25,26]. Supplemental vitamin D may help to promote antibacterial responses in HIV+ subjects by utilizing intracrine vitamin D pathways [27]. However, vitamin D may also stimulate anti-retroviral responses in the setting of HIV-infection, with a recent study showing that 1,25(OH)$_2$D-induced autophagy in macrophages not only stimulates killing of *M. tb*, but also inhibits replication of HIV [28]. To investigate the possible importance of vitamin D for antibacterial responses in HIV+ subjects, we carried out two vitamin D supplementation trials and used serum from the trial participants to assess monocyte antibacterial activity. Furthermore, we investigated potential differences in anti-bacterial responses in HIV+ subjects compared to healthy control subjects (HIV−) of similar age and serum vitamin D status.

2. Materials and methods

2.1. Human subjects

Subjects with HIV were recruited from Philadelphia regional urban centers for two vitamin D supplementation trials. Both protocols were approved by Institutional Review Board at CHOP. Written informed consent was obtained from subjects ages 18.0 to 24.9 years, emancipated minors presenting for care alone and parents/legal guardians of subjects <18.0 years. Healthy control subjects were drawn from two studies, one investigating vitamin D status in subjects with sickle cell disease and one in subjects with renal insufficiency. HIV− control subjects were similar in age and sex to HIV+ subjects. The baseline characteristics of HIV+ subjects from the Dose Study and the RCT compared to the HIV− subjects used in the current analyses are summarized in Supplemental Table 1.

Supplementry material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.jsbmb.2014.07.013.

2.2. Vitamin D dose study

Forty-four subjects were recruited from three regional centers and randomized to receive supplemental vitamin D$_3$ at 4000 IU/d (Nutraceutical Science Institute, Lexington, NC) or 7000 IU/day

Please cite this article in press as: R.F. Chun, et al., Vitamin D supplementation and antibacterial immune responses in adolescents and young adults with HIV/AIDS, J. Steroid Biochem. Mol. Biol. (2014), http://dx.doi.org/10.1016/j.jsbmb.2014.07.013