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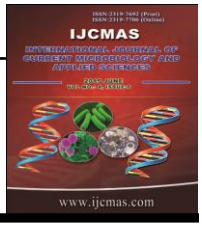

Short Communication

Effect of Vitamin D Ingestion on iNOS Activity and Antimicrobial Property in Murine Peritoneal Macrophages

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A B S T R A C T

Macrophages are a part of innate immune response that rapidly recognizes microbial pathogens and serve as a first line of defense by mounting an antimicrobial response primarily by metabolizing arginine to nitric oxide with the help of nitric oxide synthase (NOS) activity. Vitamin-D has recently been shown to play important roles in innate and adaptive immunity. Here is an attempt to find out if in-vivo Vitamin –D treatment in mice can help to enhance the iNOS (inducible nitric oxide synthase) activity level in peritoneal macrophages leading to a greater NO production and increased phagocytosis of foreign substances by those macrophages.

Introduction

The immune response to microbial pathogens relies on both innate and adaptive components. Innate immunity is the first line of defense against microbial infection. It is mediated primarily by white blood cells, such as macrophages, neutrophils and dendritic cells (Aderem A et al,1999). Macrophages are found in all body tissues, where they serve as sentinels in wait for pathogens, the invaders shed a variety of chemotactic agents that alerts the macrophages to the infection site. The cells bind the pathogen via phagocytic receptors that initiate the cytoskeletal rearrangements and membrane trafficking that is required for phagocytosis (Aderem A et al,1999; Underhill DM et al,2002;Greenberg S et all,2002;).Once the pathogen is internalized the phagosome matures to become a phagolysosome where most of the pathogens are killed by a wide variety of microbicidal mechanism including reactive oxygen species and toxic peptides(Aderem A et al,2003).

Research has also demonstrated that immunologic activation of mouse macrophages induces the activity of nitric oxide synthase (NOS), which oxidizes a guanine nitrogen of L-arginine, yielding citrulline and the reactive radical, nitric oxide (Cardelli J et al,2005).A review of biochemical and immunological

regulation of this pathway in macrophages provides a backdrop to evaluate its effector functions. Many reports suggest that synthesis of nitric oxide mediates much of antimicrobial activity of mouse macrophages against some fungal, helminthic, protozoal and bacterial pathogens (Nathan CF et al,1991).

Evidence exists that Vitamin-D has a potential antimicrobial activity and its deficiency has deleterious effects on general well-beings and longevity (Dima A. Youssef et al,2011).It may reduce the risk of infection through multiple mechanisms; one of which is boosting of innate immunity by modulation in the production of anti-microbial peptides and cytokine response (Dima A. Youssef et al,2011).Moreover, Vitamin-D helps in boosting the activity of monocytes and macrophages thereby contributing to a potent systemic anti-microbial effect. A vitamin- D replete state appears to benefit most infections (Dima A. Youssef et al,2011). In innate immune responses, activation of Toll-like receptors (TLRs) triggers direct antimicrobial activity against intracellular bacteria, which in murine monocytes and macrophages is mediated principally by nitric oxide (Philip T et al,2006). It has been reported that TLR activation of human macrophages up-regulated activity level of the Vitamin D receptor and the vitamin D-1 hydroxylase genes, leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular pathogens (Philip T et al,2006).

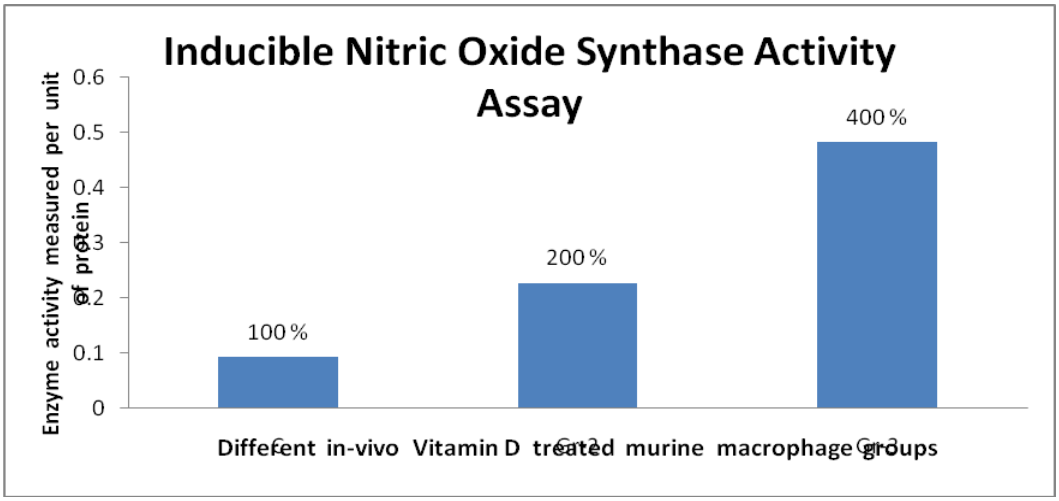
Materials and Methods

In order to carry out the experiments 15 BALB/c mice housed into three different groups [Group-I for control and was given normal diet, Group-II was oral Vitamin-D (1,25 OH_2D_3) treated mice at dose of 0.048µM/day and Group-III was oral Vitamin-D (1,25 OH_2D_3) treated mice at dose of 0.48µM/day]. The oral supplementation was continued for 7 days. The mice were given starch treatment 4 days before sacrifice; and peritoneal macrophages were isolated by following standard protocol (Xia Zhang et al,2008). A heamocytometer count of this population was taken. *Salmonella typhi* were used as stress factor by exposing them to isolated macrophages from each group (Tatsou et al,1993). The stressed macrophages were cultured in DMEM, (supplemented with 10% FBS, $NaHCO_3$ and antibiotics Pen-Strep); incubated at 37^0C in 5% CO_2 atmosphere for 16 hours. The cultured cells were harvested and the phagocytic activity was checked under phase contrast microscope using a 20 minutes yeast phagocytosis assay (I Szabo;1993). The cell suspension of macrophages were utilized to quantify the iNOS activity at 420mµ (Nins et al,1996).

Results and Discussion

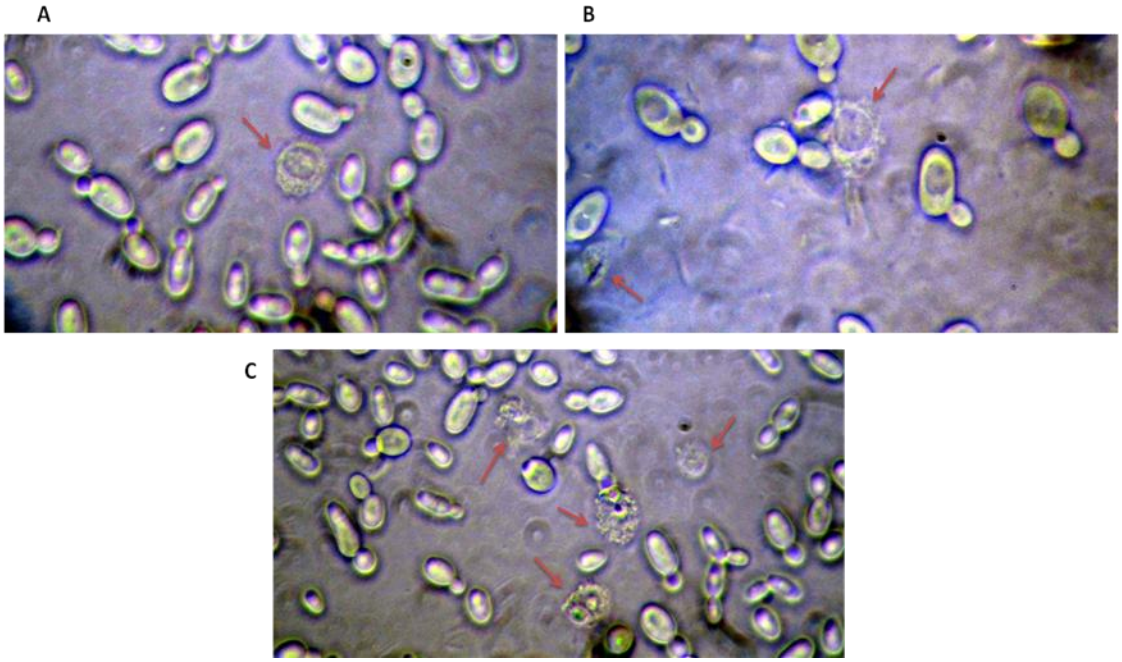
During this study we observed that oral administration of vitamin-D supplementation has increased the inducible Nitric Oxide Synthase(iNOS) activity of peritoneal macrophages on exposure to bacterial stress (Figure-1). The higher the dose of vitamin-D in vivo, higher is the level of iNOS in the macrophages as obtained by standard spectrophotometric assays (Nins et al,1996).When macrophages from the three groups were cultured after giving bacterial stress, and observed under phase contrast for phagocytosis with yeast particles, it was seen that the number of yeast particles phagocytosed in case of the higher dose of Vitamin-D supplemented group was more in comparison to the lower dose treated group or control group[Fig-2].

Figure.1 Inducible nitric oxide synthase assay



Macrophage cell lysate from three different group of mice (Contol-C), [0.048µM/day in-vivo Vitamin D treated – Gr-2], [0.48µM/day in-vivo Vitamin D treated – Gr-3] were assayed for NOS enzyme using a reaction system (0.5 mM arginine as the substrate, Nins et al,1996) and observed spectrophotometrically at 420 mµ.

Figure.2 Cultured murine macrophage phagocytosis assay with yeast under phase contrast microscope



Macrophages that suffered bacterial stress were cultured and assayed for phagocytosis using yeast particles. **A)** represents macrophages from control group that was not supplemented with oral Vitamin D dose. **B)** represents macrophages from the lower oral dose of vitamin D treated group(0.048µM/day). **C**) represents macrophages from the higher oral dose of Vitamin D supplemented group(0.48µM/day)

The present study also show a field here where the number of macrophages were also more in the higher dose treated group than the other two groups. Since the higher number of ingested foreign particle relates to higher macrophage activity, and more

number of macrophages relates to a greater immune response against invading microbes, we can predict from the results that the higher oral dose of vitamin-D has brought about an increase in both these macrophage properties. Thus Vitamin D has an antibacterial effects which creates a prominent outcome on helping the macrophages to work on pathogens with even better efficiency.

The above data from the experiments can help us to say that there lies a basic correlation among iNOS activity level and macrophage activity, and Vitamin-D upon in-vivo oral administration enhances the activity level of iNOS, producing greater amount of NO that again further stimulates the macrophages to act better against the invading pathogens. Further work needs to be carried out to reveal the detailed mechanism underlying this outcome. These preliminary observations can now help to explain some of the speculations about the role of Vitamin-D in different infections. Consequently, consideration might be given to clinical trials of inexpensive vitamin-D supplementation at appropriate doses to enhance innate immunity to microbial infections.

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References

Aderem A, Underhill DM. 1999. Mechanisms of phagocytosis in macrophages. *Annu Rev. Immunol.* 17: 593-623

Aderem A. 2003. Phagocytosis and inflammatory response. *The Journal of Infectious Diseases*.187(Suppl 2) S340-5

Cardelli J. 2001. Phagocytosis and macropinocytosis in dictyostelium : phosphoinositide-based processes. *Biochemically Distinct Traffic*. 2; 311-20

Dima A. Youssef, 2011 ;Antimicrobial implications of vitamin D Dermato-Endocrinology 3:4, 220-229;

Szabo, I M Rajavin,J L Bussiere,T K Eisenstein,M W Adler and T J Rogers;1993,Suppression of peritoneal macrophage phagocytosis of Candida albicans by Opioids,267;703-706;

Greenberg S , Grinstein S 2002. Phagocytosis and innate immunity. *Curr Opin Immunol*. 14: 136-45

Nathan CF, Hibbs JB.Jr. 1991. Role of nitric oxide synthesis in macrophage antimicrobial activity. *Curr Opin Immunol*. Feb 3(1): 65-70

Philip T. Liu et al. 2006. Toll-Like receptor triggering of a Vitamin D–mediated human antimicrobial response. *Science.* 311,1770

Umderhill DM, Ozinsky A. 2002. Phagocytosis of microbes complex in action. *Annu Rev Immunol.* 20: 825-52

Xia Zhang, Ricardo Gonclaves and David M Mosser, November 2008,The isolation and characterization of murine macrophages, Curr Protoc Immunol Chapter Unit-14.1.