Vitamin D acts as independently from glucocorticoid receptor in animal model of asthma

Ashok Agrawal1,*, Anita Mehta1

1Dept. of Pharmacology, L M College of Pharmacy, Ahmedabad, Gujarat, India

ABSTRACT

Now a day most of the patients on long term therapy of glucocorticoid become insensitive to corticosteroids and addition of vitamin D to such patients may enhance to response to corticosteroids. Therefore, we investigated anti-inflammatory activity of vitamin D in presence of glucocorticoids receptor antagonist; mifepristone, to assess whether, vitamin D acts as dependent or independently from glucocorticoids receptor. Wistar albino rats were sensitized with ovalbumin (OVA) and, upon OVA challenge, developed airway eosinophilia and neutrophilia. Administration of dexamethasone, vitamin D and combination of dexamethasone+vitamin D, and dexamethasone+vitamin D+mifepristone significantly (P<0.05) attenuated OVA-induced eosinophils and neutrophils but fail to attenuate in animals pretreated with mifepristone and dexamethasone +mifepristone. Moreover, we did not find any effects on monocytes, lymphocytes and total cells counts. The results of present study indicated that vitamin D acts as independently from glucocorticoids receptor and play significant role in glucocorticoids insensitive patients.

© This is an open access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

1. Introduction

Asthma is one among the foremost common chronic disease and is ranked within the top 10 prevalent conditions. Approximately 300 million people of worldwide are affected by asthma and is projected to extend to 400 million by 2025.1 In 2010 the casualty from this disease was quite 300 thousands.2 Mast cells, infiltration of eosinophils and increased activated T helper 2 (Th2) lymphocytes3,4 plays a significant role in chronic asthma. Now a days, inhaled corticosteroid has become first-line treatment,5 however, about 5–10% of asthma patients becomes steroid-insensitive or difficult to regulate asthma.6

Recently many studies has suggested that vitamin D play a significant role in the regulation of immune system and vitamin D receptor (VDR) are present on activated B, T cells, monocytes, and dendritic cells.5–10 Moreover, vitamin D inhibits Th1 (11), Th211 and Th1712 associated cytokines while some evidence shows that vitamin D may enhance (14) Th2 responses. Regulatory T-cells (Tregs) modulates the immune system by inhibiting transcription of IL-2, expressing IL-10, and potentially converting effector T cells to hyporesponsive or regulatory forms.7 Vitamin D causes induction as well as proliferation of Tregs7 by increasing IL-10-secretion and toll-like receptor (TLR)-9 expression.13 Both in vivo and in vitro studies also have shown that vitamin D enhances the production of anti-inflammatory cytokine IL-10 by human T cells.14 In a pilot study, administration of vitamin D to steroid resistant asthmatic patients results in enhanced response to dexamethasone which suggests that vitamin D could potentially increase the therapeutic response of glucocorticoids in steroid-resistant patients.15 Therefore, present study was undertaken to investigate the role of vitamin D in presence of glucocorticoids receptor antagonist.
2. Materials and Methods

2.1. Animal husbandry and feeds

Wistar albino rats (200-250 g) of either sex were housed in a room maintained at 22 ± 1°C with a relative humidity of 55 ± 5% and a 12 h light-dark cycle. Animals had given free access to standard pellet animal diet (Amrut brand rodent feed, Pranav Agro Industries, Pune, India) and filtered tap water. All experiments were conducted as per protocol (LMCP/Cology/12/12) approved by the Institutional Animal Ethics Committee (IAEC) and as per Indian norms laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi.

2.2. Sensitization and challenge with OVA and Treatment

Animals were divided into nine groups (n=6/group) as mentioned follow.

- **Group I:** non-sensitized controls, received distilled water (2.5 ml/kg).
- **Group II:** ovalbumin sensitized and received distilled water (2.5 ml/kg).
- **Group III:** reference standard group- ovalbumin sensitized and received Dexa (2.5 mg/kg).
- **Group IV:** ovalbumin sensitized and received vitamin D (50 IU/kg).
- **Group IV:** ovalbumin sensitized and received combination of vitamin D (50 IU/kg) and Dexa (2.5 mg/kg).
- **Group VI:** ovalbumin sensitized and received mifepristone (5 mg/kg).
- **Group VII:** ovalbumin sensitized and received combination of mifepristone (5 mg/kg) and vitamin D (50 IU/kg).
- **Group VIII:** ovalbumin sensitized and received combination of mifepristone (5 mg/kg) and Dexa (2.5 mg/kg).
- **Group IX:** ovalbumin sensitized and received combination of mifepristone (5 mg/kg), Dexa (2.5 mg/kg) and vitamin D (50 IU/kg).

All rats were sensitized with 100 µg ovalbumin adsorbed in 100 mg/ml of aluminum hydroxide in saline by i.p. injection on days 0 and 14 (general sensitization) in all rats. Rats were anesthetized before being challenged with 100 µg of OVA in 100 µl of saline; intranasally for 3 days. On days 21–23, for group II to IX daily doses of drug or vehicle by i.p. injection 1 h prior to OVA administration.16

3. Results

3.1. Effect of treatments on Eosinophils

The eosinophils count in blood samples recovered from the model control animals were markedly increased (p<0.05) compared to the non-sensitized controls. However, the numbers of circulating eosinophils (p<0.05) in the blood were significantly decreased in dexamethasone, vitamin D, combination of vitamin D-dexamethasone, combination of mifepristone-vitamin D, and combination of mifepristone-vitamin D-dexamethasone treated animals, respectively, compared to those numbers seen in the ovalbumin sensitized rats (Figure 1).

![Fig. 1: Effect of treatments on eosinophils. Values shown are the mean±S.E.M. (n=6). @ p< 0.05 compared to the non-sensitized control. * p<0.05, compared to the OVA (ovalbumin)-sensitized vehicle-treated model control, # p<0.05 compared to the dexamethasone treated, $ p<0.05 compared to the mifepristone. S=Ovalbumin sensitized, VitD=Vitamin D, Dexa=Dexamethasone, M=Mifepristone.](image)

3.2. Effect of treatments on neutrophils

The neutrophils count in blood samples recovered from the model control animals were markedly increased (p<0.05) compared to the non-sensitized controls. However, the numbers of circulating eosinophils (p<0.05) in the blood were significantly decreased in dexamethasone, vitamin D, combination of vitamin D-dexamethasone, combination of mifepristone-vitamin D, and combination of mifepristone-vitamin D-dexamethasone treated animals, respectively, compared to those numbers seen in the ovalbumin sensitized rats (Figure 2).

3.3. Effect of treatments on Monocytes

We did not find any significant changes in the neutrophils count in blood samples recovered from the model control animals and treatment groups (Figure 3).
3.4. Effect of treatments on Lymphocytes

We did not find any significant changes in the lymphocytes count in blood samples recovered from the model control animals and treatment groups (Figure 4).

3.5. Effect of treatments on total cell

We did not find any significant changes in the total cell count in blood samples recovered from the model control animals and treatment groups (Figure 5).

4. Discussion

Steroid insensitivity is major concern in severe asthmatic patients was associated with considerably poorer quality of life (QOL). Mifepristone is well known synthetic steroid with antiglucocorticoid activity that binds so strongly to the glucocorticoid receptors with binding affinity approximately three times greater than dexamethasone. In the present study we observed that animals pretreated with vitamin D, combination of vitamin D and dexamethasone decreased the production of the inflammatory cells; eosinophils and neutrophils. However, animals pretreated with mifepristone and received dexamethasone are unable to suppress inflammatory cells like eosinophils and neutrophils but those animal who were pretreated with mifepristone and received vitamin D are able to suppress inflammatory cells like eosinophils and neutrophils. It is clear that vitamin D may act by other mechanism and does not involved glucocorticoids receptor. Therefore; vitamin D may be useful in that case who are steroid insensitive. In this experiment we could not find any effects of treatments on
Fig. 5: Effect of treatments on total cell. Values shown are the mean±S.E.M. S= Ovalbumin sensitized, VitD= Vitamin D, Dexa= Dexamethasone, M= Mifepristone.

inflammatory cells like lymphocytes and monocytes. This may be due to short study period of experiments or may be biological or environmental variations in animals.

5. Conclusion
Vitamin D does not involved glucocorticoids receptor and may be played a significant role in glucocorticoids insensitive patients.

6. Source of Funding
None.

7. Conflict of Interest
The authors declare that there is no conflict of interest.

References
1. Masoli M, Fabian D, Holt S, and RB. The global burden of asthma: executive summary of the GINA Dissemination Committee Report. *Allergy*. 2004;59(5):469–78.
2. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study. *Lancet*. 2010;380(9859):2095–128.
3. Barnes PJ. Inhaled glucocorticoids for asthma. *N Engl J Med*. 1995;332(13):868–75.
4. Barnes PJ, Adcock I, Spedding M, Vanhoutte PM. Anti-inflammatory actions of steroids: molecular mechanisms. *Trends Pharmacol Sci*. 1993;14(12):436–41.
5. Barnes PJ, Adcock IM. Glucocorticoid resistance in inflammatory diseases. *Lancet*. 2009;373(9678):1905–17.
6. Morishima Y, Ano S, Ishii Y, Ohtsuka S, Matsuyama M, Kawaguchi M, et al. Th17-Associated Cytokines as a Therapeutic Target for Steroid-Insensitive Asthma. *Clin Dev Immunol*. 2013;2013:1–9.
7. Chambers ES, Hawrylowicz CM. The Impact of Vitamin D on Regulatory T Cells. *Curr Allergy Asthma Rep*. 2011;11(1):29–36.
8. Bouillon R, Garg M, Versypt A, Segae S, Casteels K, Mathieu C. Paracrine role for calcitriol in the immune system and skin creates new therapeutic possibilities for vitamin D analogs. *Eur J Endocrinol*. 1995;133(1):7–16.
9. Griffin MD, Xing N, Kumar R. Vitamin D and its analogs as regulators of immune activation and antigen presentation. *Ann Rev Nutr*. 2003;23:117–45.
10. Dong X, Craig T, Xing N, Bachman LA, Paya CV, Weih F. Direct transcripational regulation of RelB by 1alpha,25-dihydroxyvitamin D3 and its analogs: physiologic and therapeutic implications for dendritic cell function. *J Biol Chem*. 2003;278:49378–85.
11. Topilski I, Flaishon L, Naveh Y, Harmelin A, Levo Y, Shachar I. The anti-inflammatory effects of 1,25-dihydroxyvitamin D3 on Th2 cells in vivo are due in part to the control of integrin-mediated T lymphocyte homing. *Eur J Immunol*. 2004;34(4):1068–76.
12. Tang J, Zhou R, Luger D, Zhu W, Silver PB, Grajewski RS, et al. Calciotriol Supresses Antiretroviral Autoimmunity through Inhibitory Effects on the Th17 Effector Response. *J Immunol*. 2009;182(8):4624–32.
13. Urzy Z, Xystrakis E, Richards DF, McDonald J, Sattar Z, Cousins DJ, et al. Ligation of TLR9 induced on human IL-10–secreting Tregs by 1α,25-dihydroxyvitamin D3 abrogates regulatory function. *J Clin. Investig*. 2009;119:387–98.
14. Provvedini DM, Tsoukas CD, Deftos LJ. I alpha, 25-Dihydroxyvitamin D3-binding macromolecules in human B lymphocytes: effects on immunoglobulin production. *J Immunol*. 1986;136:2734.
15. Xystrakis E. Reversing the defective induction of IL-10-producing regulatory T cells in glucocorticoid-resistant asthma patients. *J Clin Investig*. 2005;116(1):146–55.
16. Sitruk-Ware R, Spitz IM. Pharmacological properties of mifepristone: mechanisms of action. *Gynecol. Investig*. 2003;5:68(6):409–20.
17. Wamboldt FS, Spahn JD, Klinnert MD, Wamboldt MZ, Gavin LA, Szefler SJ, et al. Clinical outcomes of steroid-insensitive asthma. *J Allergy Asthma Immunol*. 1999;83(1):55–60.

Author biography
Ashok Agrawal, PhD Scholar [https://orcid.org/0000-0002-8023-8355](https://orcid.org/0000-0002-8023-8355)

Anita Mehta, Professor and HOD

Cite this article: Agrawal A, Mehta A. Vitamin D acts as independently from glucocorticoid receptor in animal model of asthma. *Indian J Pharm Pharmacol*. 2021;8(1):74–77.