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AN ANTIHISTAMINIC ACTIVITY OF VITAMIN D IN EXPERIMENTAL MODELS

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ABSTRACT

Background: The inverse correlation exists between plasma vitamin D level and development of asthma whereas the direct correlation exists between occurrence of asthma and histamine level. Therefore, we aimed to evaluate anti-histaminic activity of vitamin D in experimental models. Materials and Methods: The histaminic actions modulating role of vitamin D was assessed using histamine induced bronchospasm in guinea pigs, clonidine induced rat mast cell degranulation and haloperidol induced catalepsy in mice. Results: The vitamin D treatment showed significant ($p<0.001$) increased in histamine induced proconvulsive time in guinea pigs, inhibited clonidine induced rat mast cell degranulation and attenuated haloperidol-induced catalepsy in mice. Conclusion: The current study revealed that vitamin D possesses antihistaminic activity by inhibiting H1 receptor action and histamine release from the cells.

KEYWORDS: Vitamin D, histamine, asthma.

INTRODUCTION

Histamine is a key spasmogen that causes bronchoconstriction, airway hyper-responsiveness and airway inflammation.[1,2] It is released during early and late phase of allergic reaction.[3] It induces secretion of mucus that leads to airways oedema and contraction of airway smooth muscle.[4] Histamine stimulates macrophages recruitment, epithelial and smooth muscle hyperplasia in the alveolar area that causes airway inflammation.[5] The currently available

anti-histaminics acts by inhibiting histamine release and blocking histamine binding to the receptor. However, the uses of such anti-histaminics are limited in controlling allergic asthma.[6] Vitamin D is a steroid in nature that binds with nuclear receptor for regulating immune system. In asthmatic patient, the decrease in serum vitamin D levels have been associated with severity of asthma, increased airway hyper-responsiveness, and increased the risk of glucocorticoid resistance.[7-9] Vitamin D inhibits expression of pro-inflammatory genes and induces expression of anti-inflammatory genes.[10-12] It showed increase in the production and release of IL-10, an anti-inflammatory cytokine, from T-cells in the *in vitro* and *in vivo* experiments.[13] These inverse relation between asthma and vitamin D[14-16] level, direct relation between histamine and asthma directed us to study the anti-histaminic potential of vitamin D.

MATERIALS AND METHODS

Animals

Male Dunkin–Hartley guinea pigs (300– 500 g), Male Wistar rats (150– 1800 g), and Male Swiss albino mice (20-25 g) were housed in a climate-controlled room (temperature 22±1°C; relative humidity 55±5%) on a 12-h light–dark cycle. Animals had access to standard pellet diet (certified Amrut brand rodent feed, Pranav Agro Industries, Pune, India) and filtered tap water *ad libitum*. All experiments were carried out after approval of Institutional Animal Ethics Committee (IAEC) with protocol no. LMCP/cology/12/12 and in accordance to ethical guidelines laid by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Drugs and chemicals

The drugs were given as a gift sample; vitamin D (Cadila Pharmaceutical, India), (Sigma-Aldrich, USA), haloperidol (Sunpharma, India) and chlorpheniramine maleate (Cadila Healthcare, India). The histamine and ketotifen fumarate (Sigma-Aldrich, USA) were purchased. Vitamin D was dissolved in 96% (v/v) ethanol and freshly diluted in saline solution before administration.

Histamine induced bronchospasm in guinea pigs[17]

Guinea pigs were placed in aerosol chamber (24×14×24 cm perplex glass chamber) for 15 days (30 minutes/day) to acclimatize. They were divided into five groups (n=8); control (saline, 1 mL/kg, i.p.), vitamin D (50, 100 and 200 IU/kg, i.p.) treated and chlorpheniramine (2 mg/kg, i.p.) treated guinea pigs. At 0, 1, 4 and 24 hr of respective treatment, guinea pigs

were place in aerosol chamber and exposed to 0.25% histamine aerosol that was produced by an ultra-sound nebulizer. The preconvulsive time (PCT) i.e. the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion, was noted for each animals. As the preconvulsive dyspnoea was noted the pigs were removed from the chamber and placed in fresh air to recover. The data of 0hr were considered as a basal value that was used to calculate % protection.

% protection = [1-T1/T2] x 100
Where, T1 = the mean of PCT at 0 hr, and
T2 = the mean of PCT at 1 hr, 4 hr and 24 hr.

Clonidine-induced rat mast cell degranulation[18]

The male albino Wistar rats were selected and anesthetized using ketamine (50mg/kg, ip). The heparinized (5 IU/ml) saline was injected in the peritoneal cavity of anaesthetized rats. After a gentle abdominal message for 3 times, the peritoneal fluid containing mast cells was collected in centrifuge tubes and placed over ice. They were centrifuged at 2,000 rpm for 5 min and supernatants were discarded. Mast cells were washed twice with saline and suspended in 1 ml of saline. The 0.1 ml of the peritoneal cell suspension was transferred to 4 test tubes and was treated as follows.

Test tube no. 1 – 0.1 ml Saline
Test tube no. 2 – 0.1 ml Saline
Test tube no. 3 - 0.1 ml of (100nM, ~10IU/ml) vitamin D
Test tube no. 4 - 0.1 ml of (10µg/ml) ketotifen fumarate

Each test tube was incubated for 15 min at 37°C, later clonidine (0.1 ml, 100µg/ml) was added to each test tube except test tube no. 1 (0.1 ml saline was added in test tube no. 1) and again incubated for 15 min at 37°C. After this, the cells were stained with 0.1% toluidine blue solution made in distilled water and examined under the high power of light microscope. The % mast cell degranulation were calculated by mean of six times counted number of degranulated (blue in appearance) mast cells from total of 100 mast cells counted (blue + pink in appearance).

Haloperidol-induced catalepsy in mice[19]

Swiss albino mice were divided into five groups (n=8); divided into five groups (n=8); control (saline, 1 mL/kg, i.p.), vitamin D (50, 100 and 200 IU/kg, i.p.) treated and chlorpheniramine (2 mg/kg, i.p.) treated rats. Rats were received haloperidol (1 mg/kg, i.p.) at 1 h after respective treatment. The forepaws of mice were placed on a horizontal bar (1 cm in diameter, 3 cm above the table) and the time taken to remove paws from the bar (duration of catalepsy) was noted for each animal. The cut of time was five min. The duration of catalepsy was repeatedly measured at 0, 30, 60, 90, 120, 150 and 180 min after administration of haloperidol. The control and chlorpheniramine treated rats were considered as a negative and positive control respectively.

STATISTICAL ANALYSIS

Data were presented as mean±S.E.M. Statistical analyses were done using a one-way analysis of variance (ANOVA) followed by Dunnett's test. The $p<0.05$ was considered as a statistically significant. All statistical analyses were performed using the Graph Pad software (San Diego, CA, USA).

RESULTS AND DISCUSSION

The vitamin D (at 50IU/kg, 10IU/ml and 50IU/kg) showed anti-histaminic activity in the histamine induced bronchospasm, clonidine induced mast cell degranulation and haloperidol induced catalepsy, respectively.

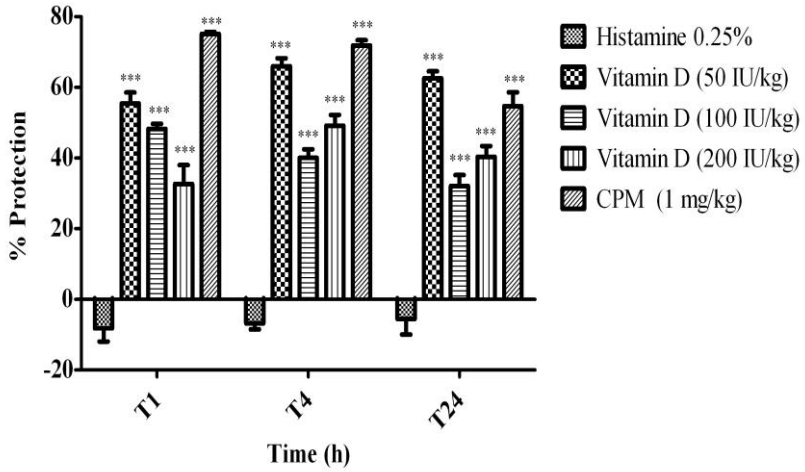

Fig. 1: Effect of vitamin D on histamine induced bronchospasm in guinea pigs. Values are expressed as a mean±S.E.M. (n=8). $^{***}p< 0.001$ as compared to the control group (only histamine aerosolized group). CPM= chlorpheniramine maleate.

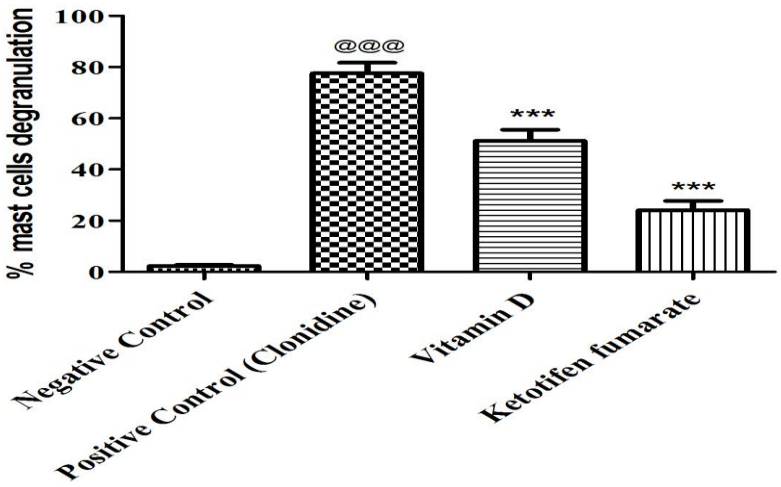

Fig. 2: **Effect of vitamin D on clonidine induced rat mast cell degranulation. Values are expressed as a mean±S.E.M. (n=8). $^{@@@}$p< 0.001 as compared to negative control group and ***p< 0.001 as compared to positive control group.**

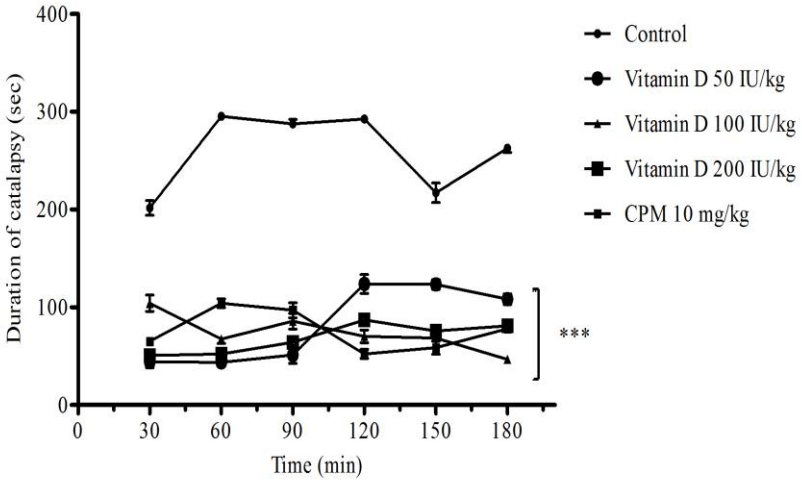

Fig. 3: **Effect of vitamin D on haloperidol-induced catalepsy. Values are expressed as a mean±S.E.M. (n=8). ***p< 0.001 as compared to the control group. CPM= chlorpheniramine maleate**

Effect of vitamin D on histamine induced bronchospasm

Guinea pigs are extremely sensitive to histamine induced bronchoconstriction and also react to histamine in a manner similar to humans.[20] When guinea pigs exposed to histamine; it causes hypoxia by bronchoconstriction and leads to convulsion. Apart from this, Histamine causes profound hypotension, and capillary dilation in guinea pigs. The second-generation antihistamines ebastine, cetirizine and loratadine inhibited the histamine induced

preconvulsive action in guinea pigs by blocking H1 receptor mediated airway smooth muscle contraction. Moreover, the effects of ebastine, loratadine and cetirizine were prolonged up to 21, 19 and 15 h, respectively.[21] In present study, A significant ($p<0.001$) increase in preconvulsion time was observed due to pretreatment with vitamin D (50/100/200 IU/kg) when the guinea pigs were exposed to histamine aerosol. The increase in preconvulsion time by vitamin D (50IU/kg at 24 h) was comparable to chlorpheniramine maleate (standard drug) 1mg/kg (Fig. 1). This suggests the H1 receptor inhibitory action of vitamin D.

Effect of vitamin D on clonidine induced mast cell degranulation

Mast cell degranulation is an important process in the initiation of immediate responses following exposure to allergens. Degranulated cells liberate mediators of inflammation such as histamine, leukotrienes, platelet activating factors and chemotactic factors for eosinophils, neutrophils etc. from mast cells. They play a significant role in airway inflammatory response such as airway eosinophilia, late asthmatic response and airway hyperresponsiveness as well as in immediate hypersensitivity reaction like bronchial contraction.[6] Uvans has studied mast cell degranulation and its correlation with the release of histamine after administration of a mast cell degranulating agent such as compound 48/80.[22] Lakdawala et al. have shown that clonidine release histamine from mast cells in a manner similar to a compound 48/80.[23] Histamine secretion is mediated by calcium release from intracellular store of mast cell.[24] Clonidine produced significant ($p<0.001$) disruption of mast cells which was significantly ($p<0.001$) inhibited by pretreatment with the vitamin D (10IU/ml) and ketotifen fumarate (10μg/ml, Fig. 2). Other than this, previous reports indicated that vitamin D inhibited the maturation of mast cells via inducing apoptosis in mast cell precursors and also inhibited mast cell differentiation at various stages through triggering vitamin D receptor (VDR) in mast cells.[25] *In vivo* studies suggested that the absence of VDR signaling in mice led to accelerated maturation of mast cells and increased number of mast cells.[26] Vitamin D inhibited mast cell activity by down-regulating mast cell development and differentiation.[27] Vitamin D also inhibited calcium ionophore-induced histamine release from rat peritoneal mast cell.[28] Therefore, the mast cell degranulation inhibitory action of vitamin D in the current in vitro condition may be related to direct calcium depletion in the mast cells.

Effect of vitamin D on haloperidol-induced catalepsy

Several drugs are known to induce catalepsy in animals and different stages of catalepsy appear to be directly correlated with brain histamine content.[29] Haloperidol induces

catalepsy by inhibiting dopamine D2 receptor and dopamine secretion. Dopamine is an agonist for adrenaline and adrenaline is a physiological antagonist of histamine. Therefore, the decrease in dopamine is directly correlated with high level of histaminergic action in brain.[30] Puchacz et al. reported that vitamin D increased availability of dopamine, noradrenaline and adrenaline in rat's brain.[31] The intracerebroventricular injection of histamine in conscious mice induced catalepsy, which was inhibited by H1-receptor antagonist but not by H2- receptor antagonist.[32] The vitamin D was significantly ($p<0.001$) inhibited haloperidol-induced catalepsy. The inhibition of catalepsy was comparable to standard drug, chlorpheniramine maleate in current study (Fig. 3). This suggests the anti-histaminic actions of vitamin D might be related to inhibition of other histaminergic stimuli and stimulation of anti-histaminic stimuli in the body.

CONCLUSION

Ultimately, vitamin D has a significant antihistaminic activity by inhibiting H1 receptor action and histamine release from the cells.

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