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RESEARCH ARTICLE

Biocompatibility of newer formulation of Vitamin-D3 with Azelastine and Fluticasone nasal spray preparation- Invitro analysis

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Abstract: Intranasal steroids are effective in treating allergic rhinitis patients. Emerging evidence suggests a potential link between vitamin D deficiency and allergic rhinitis severity. Vitamin-D plays a role in immune regulation and inflammation modulation potentially impacting on Allergic rhinitis, which includes its effects on T cells, cytokine production and regulatory pathways. There is a strong need of alternative routes of administration of Vitamin-D to overcome difficulties related to oral administration and advantages of high rate of absorption and patient compliance. The aim of this study was to evaluate the cytotoxicity and permeability in vitro and to assess the drug's relevance for incorporating in drug delivery systems for nasal administration of newer combined formulation of nasal sprays containing vitamin-D with steroid component. Mouse fibroblast cell model was used to evaluate drug permeability in vitro. The cytotoxicity of the spray solution was evaluated using 3T3L1 fibroblast cells at different concentrations for 24h. After 24h, the cell viability was assessed by MTT assay. The morphology of the cells also complimented the cell viability results with no significant changes in the cellular structure.

Keywords: Vitamin-D, Azelastine & Fluticasone (Azeflo) nasal spray, Mouse fibroblast cell, Allergic rhinitis, cytotoxicity.

INTRODUCTION

Allergic rhinitis (AR) is a rapidly increasing global health issue, marked by an IgE-mediated type 1 hypersensitivity reaction to allergens such as pollen, dust mites, animal dander, and mold. This condition often manifests with nasal symptoms like sneezing, rhinorrhea, nasal congestion, and itching, accompanied by associated issues such as watery, itchy eyes, throat or palate itching, postnasal drip, and chronic cough [2]. It affects 20% of the global population and mainly affects the children of age 5-12 yrs and adolescents of > 20 yrs of age . Vitamin D is a fat-soluble vitamin that plays a vital role in calcium and phosphate homeostasis, bone health, and, importantly, immunoregulation. Its immunomodulatory effects are mediated through vitamin D receptors (VDRs), which are expressed on various immune cells, including T-cells, B-cells, dendritic cells, and macrophages [13].Whereas vitamin-D deficiency is more common in children and adults, now a days vit-D is fortified with food products for children and for adults it is given a single dose per week or single dose per month for adults. The pathophysiology of Vitamin-D in Allergic Rhinitis plays a role in Immunoregulatory effects, Reduction of IgE levels, Anti-inflammatory action, Epithelial barrier integrity.

Immunoregulatory Effects of Vitamin D mitigates the hyperactive Th2 response in allergic rhinitis by rebalancing Th1/Th2 cytokines. It fosters T-regulatory cell activity, reducing inflammation and hypersensitivity to allergens[5]. By inhibiting IgE production in B-cells, vitamin D limits allergen-triggered immune responses. This results in reduced AR symptoms and a lower frequency of exacerbations [14]. Vitamin D curtails mast cell and basophil activation, minimizing histamine release and this alleviates itching, sneezing, and rhinorrhea in AR patients by inducing anti-inflammatory action. Vitamin D strengthens the nasal epithelial barrier by stimulating antimicrobial peptide production. Hence prevents allergen entry and subsequent immune activation and this shows Epithelial barrier integrity[15]. Thus, Vitamin-D plays a crucial role in regulating the immune system by influencing both innate and adaptive immune responses. There is poor patient compliance as patient may forget to take the medications. Hence, Combining two beneficial compounds into one product can enhance convenience
for users.

CYTOTOXICITY EVALUATION BY MTT ASSAY:

Mouse fibroblast cells (3T3L1, NCCS, Pune) were grown in 25 cm^2 vented cell culture flasks in a humidified incubator at 37°C with 5% CO_2. The cells were maintained in Dulbecco's modified Eagle medium (DMEM) (Invitrogen Life Technologies, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, USA) and 1% penicillin–streptomycin antibiotics (Life Technologies, Auckland, NZ, USA)[10]. When the 3T3L1 cells reached 70–80% confluence, they were seeded directly onto a 96-well plate (1×10^4 cells) and incubated for 24–48 hours to obtain a confluent culture. 100µL solution of nasal spray

containing Vitamin D and Azelastine with fluticasone was prepared (w/v), with 100µL corresponding to 100mg. Untreated group was used as the control. Once the cells reached 70–80% confluence, they were exposed to different concentrations of the Vitamin D + Azelastine with fluticasone solution (10, 20, 40, 80, and 100µg/mL) for 24 hours. After this, 50µL of MTT dye (Sigma-Aldrich) (5mg/mL) was added to each well and incubated at 37°C for 2 hours. Following the experimental period, 150µL of DMSO was added to solubilize the formazan crystals and the absorbance was measured at 490nm using a TECAN multiplate reader. Cytotoxicity in fibroblasts, exposed to the Vitamin D +

Azelastine with fluticasone spray solution, was assessed by observing cell morphology under a phase contrast microscope. Abnormal changes in fibroblast morphology were captured through images taken with the microscope.

DISCUSSION:

Allergic rhinitis (AR) is a chronic inflammatory condition affecting the nasal lining, triggered by an overreaction of the immune system to airborne allergens. It is mainly mediated employing IgE.The prevalence rate is 10% to 40% worldwide. Allergic rhinitis affects the day-to-day activities of individuals [11].

The primary source of vitamin D for the human body is sunlight, as ultraviolet B (UVB) rays stimulate its production in the skin. This natural synthesis accounts for most of the daily vitamin D requirement, making sunlight an essential factor in maintaining optimal levels. Dietary sources and supplements, while helpful, typically contribute only a smaller fraction[2]. The production of vitamin D in the body is influenced by various factors, including the intensity of sunlight, the extent of skin exposed, and the duration of exposure. Additionally, the sun's zenith angle, skin thickness, and pigmentation play significant roles in determining individual variations in vitamin D synthesis. These factors collectively explain why sunlight's effectiveness in promoting vitamin D production differs from person to person[2,3].When antigens are inhaled through the nasal mucosa, they bind to immunoglobulin E (IgE) antibodies attached to mast cells distributed throughout the nasal mucosa. This antigen- antibody interaction triggers the release of various chemical mediators, primarily histamine and peptide leukotrienes, from the mast cells. These mediators stimulate nerve endings and blood vessels, resulting in symptoms such as sneezing, rhinorrhea, and nasal congestion. Approximately 6 to 10 hours after antigen exposure, the production of cytokines, chemokines, and activated eosinophils leads to late-phase reactions, contributing to prolonged inflammation and symptoms [2].

Vitamin D plays a crucial role in regulating various biological processes in the body, and its deficiency increases the risk of developing conditions such as allergic rhinitis (AR) and asthma [12]. Vitamin D alleviates AR symptoms via reducing IgE and cytokines. vitamin D has immunoregulatory effects on T cells, B cells, dendritic cells, monocytes, and macrophages [4]. Inhalational steroids causes vitamin-D deficiency.1,25-Dihydroxyvitamin

D inhibits T cell proliferation, promotes a shift from Th1 to Th2 responses, and enhances the synthesis of T regulatory cells, which are vital in regulating immune activity during Allergic Rhinitis[5-7]. Additionally, vitamin

D induces apoptosis in activated B cells, suppresses plasma cell differentiation, and reduces immunoglobulin secretion, including IgE, thereby mitigating allergic inflammation [8-9].The supplementation of vitamin-D significantly reduces the serum interleukin IL-4, IL-5, and IFN-γ, which indicates that vitamin D was inversely associated with blood eosinophils in patients with persistent Allergic Rhinitis . hence there are strong effects of vitamin D on AR and the mechanism of it.

Statistical Analysis:

Statistical analysis was performed using GraphPad Prism 8 (San Diego, USA). Experimental data are presented as the Mean ± standard error of the mean (SEM). One-way ANOVA followed by Post Hoc Tukey test was conducted to assess statistically significant differences between groups with a 95% confidence interval ($p<0.05$).

RESULTS:

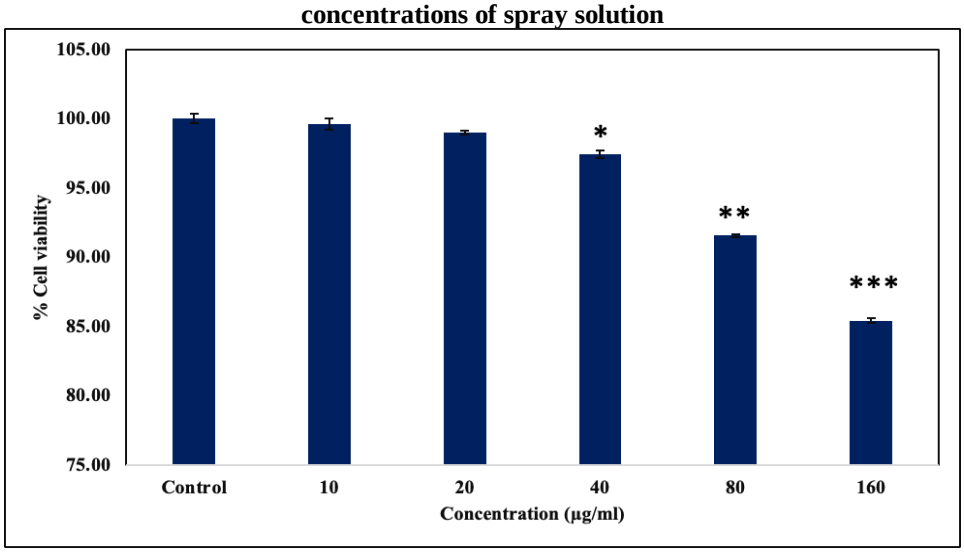
The cytotoxicity of the spray solution was evaluated using 3T3L1 fibroblast cells at different concentrations for 24h. After 24h, the cell viability was assessed by MTT. The results showed (Table 1) that the spray solution did not affect the viability of the cells indicating that the spray solution is non-toxic to cells. The control group showed almost 100% viable cells and the spray solution from 10-80 g/ml showed cell viability of more than 90%. The 160 g/ml treated group showed cell viability of 85% as in (Figure 1). The morphology of the cells also complimented the cell viability results with no significant changes in the cellular structure (Figure 2).

Table 1: showing the percentage cell viability of 3T3L1 fibroblast cells treated with different concentrations of spray solution

Concentration (µg/ml)	% cell viability
Control	100.02±0.35
10	99.60±0.40
20	99.03±0.13
40	97.44±0.30
80	91.57±0.11
160	85.43±0.20

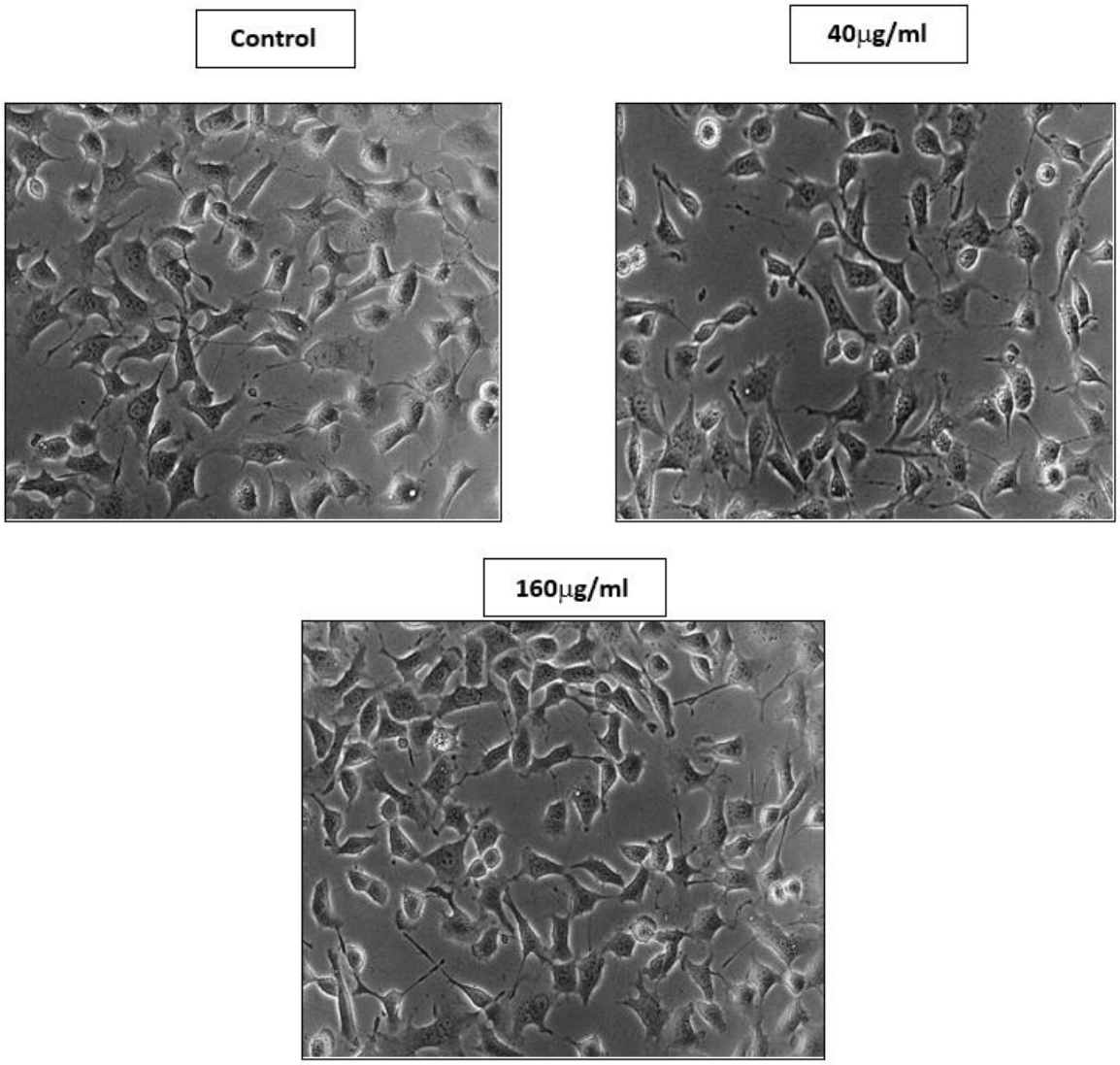
Data expressed in terms of Mean□SEM (n=3)

Figure 1: Bar graph showing the percentage cell viability of 3T3L1 fibroblast cells treated with different concentrations of spray solution



Data expressed in terms of Mean□SEM (n=3)

Figure 2: Representative microscopic images showing morphological changes in the 3T3L1 fibroblast treated with spray solution



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