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Mometasone furoate and fluticasone furoate are equally effective in restoring nasal epithelial barrier dysfunction in allergic rhinitis

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ABSTRACT

Tight junction defects (TJ) have been associated with a defective epithelial barrier function in allergic rhinitis (AR). Intranasal corticosteroids are potent drugs frequently used to treat AR and are shown to restore epithelial integrity by acting on TJs and by reducing type 2 cytokine production. However, the effect of different classes of intranasal corticosteroids on the epithelial barrier has not been studied. Therefore, we compared the effect of 2 intranasal corticosteroids, ie, fluticasone furoate (FF) and mometasone furoate (MF) on epithelial barrier function. Both FF and MF similarly increased trans-epithelial electrical resistance of primary nasal epithelial cell cultures from AR patients. In a house dust mite-induced allergic asthma mouse model, FF and MF had similar beneficial effects on fluorescein isothiocyanate–dextran 4 kDa mucosal permeability, eosinophilic infiltration and IL-13 levels. Both molecules increased mRNA expression of the TJ proteins occludin and zonula occludens-1, thereby restoring epithelial barrier function. Lastly, we showed that long-term FF treatment also increased expression of occludin in AR patients compared to controls. In conclusion, both FF and MF effectively restore epithelial barrier function by increasing expression of TJ proteins in AR patients.

TO THE EDITOR

The airway epithelium acts as the first barrier, separating the antigenic airway lumen from the external environment. Maintaining proper integrity of this epithelial barrier is of pivotal importance for tissue homeostasis and disease prevention. Tight junctions are essential cell-to-cell adhesion proteins involved in the formation of a physical barrier.[1] A defective epithelial barrier has been described in the pathogenesis of allergic rhinitis (AR) and chronic rhinosinusitis (CRS), due to structural and functional abnormalities of tight junctions.[2,3] Intranasal corticosteroids (INS) represent a first-line treatment in various forms of

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airway diseases, such as AR and CRS.[4] Corticosteroids act on allergic inflammation by regulating the production and secretion of cytokines from immune cells and have the ability to promote the formation of the airway epithelial barrier by acting on tight junctions.[5] Different classes of INS are available to treat AR.[6] Two frequently used INS are fluticasone furoate (FF) and mometasone furoate (MF). Both FF and MF are effective single-modality treatment for AR. However, to our knowledge, there has been no head-to-head study comparing the effect of FF and MF on epithelial barrier function in patients with AR *in vitro* and *ex vivo,* and in a mouse model of house dust mite (HDM)-induced allergic asthma. Hence, this study was performed to compare the efficacy between FF and MF to promote epithelial integrity.

Firstly, we tested the effect of different concentrations of FF and MF on primary nasal epithelial cells of non-allergic controls and patients with HDM-induced AR, grown at air-liquid interface for 21 days on Transwell inserts as described previously.[3] Both FF and MF dose-dependently increased trans-epithelial electrical resistance (TEER) of diseased epithelial cells (Fig. 1A). Interestingly, no effect of FF or MF on TEER was observed in nasal epithelial cell cultures of non-allergic controls (Supplementary Fig. 1). Next, we investigated the effect of FF and MF on mucosal integrity in a mouse model of HDM-induced allergic asthma (Fig. 1B).[3] One hour before each HDM challenge (50 μl), mice received endonasally either 50 μl sham, FF (0.3 mg/kg bodyweight) or MF (0.3 mg/kg body weight). Twenty-four hours after the last HDM challenge, 20 μl FITC-dextran 4 kDa (FD4) was applied in the nose to evaluate mucosal permeability. FD4 mucosal permeability was significantly increased in HDM-challenged mice, which was significantly reduced to levels found in saline control mice after treatment with FF and MF (Fig. 1C). Additionally, FF and MF significantly attenuated the levels of

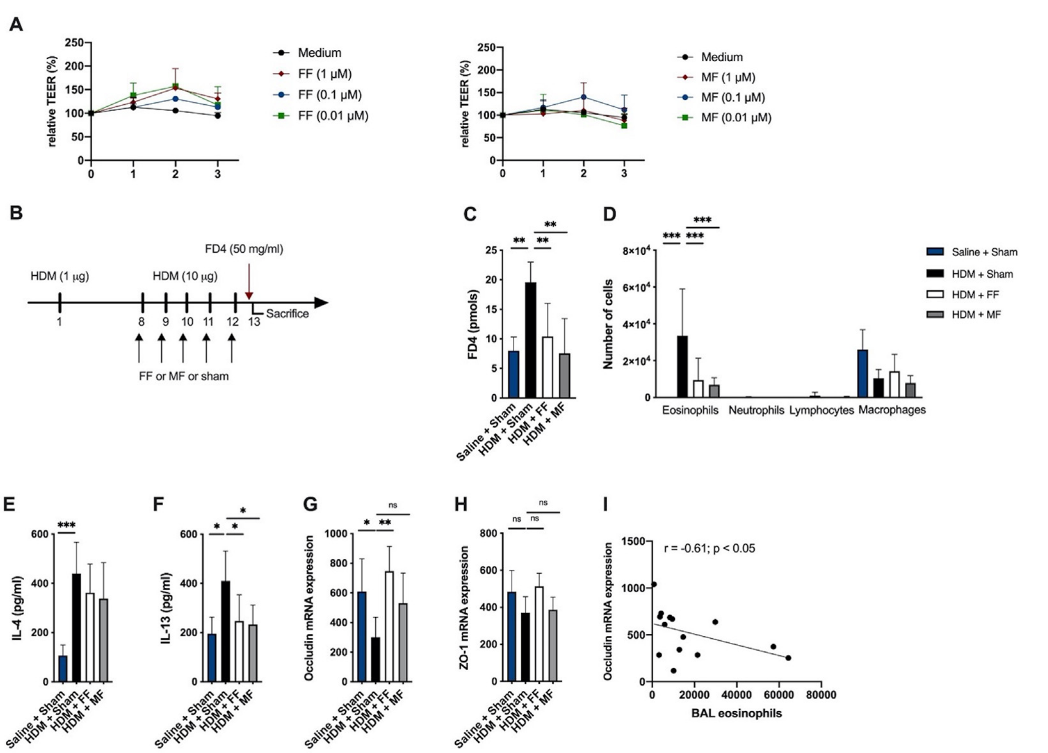

Fig. 1 Effect of fluticasone furoate and mometasone furoate on nasal epithelial integrity *in vitro* and *in vivo*. A. Effect of fluticasone furoate (FF) and mometasone furoate (MF) on *trans*-epithelial electrical resistance of primary nasal epithelial cells of patients with allergic rhinitis at air-liquid interface (n = 4 donors). **B.** Graphical representation of house dust mite (HDM)-induced allergic asthma mouse model and the therapeutic interventions. N = 5 mice/group. **C.** Effect of FF and MF on FD4 mucosal permeability. **D.** Effect of FP and MF on differential cell count in bronchoalveolar lavage fluid. **E.-F.** IL-4 and IL-13 levels measured in bronchoalveolar lavage fluid. **G.-H.** mRNA expression of occludin and ZO-1 in the nasal mucosa. Expression is relative to housekeeping gene B2M. **I.** Correlation between mRNA expression of occludin and number of eosinophils in bronchoalveolar lavage fluid of all house dust mite-challenged mice. One-Way ANOVA with Holm-Sidak's multiple comparisons test. Data presented as mean ± SD. *p < 0.05; **p < 0.01 and ***p < 0.001

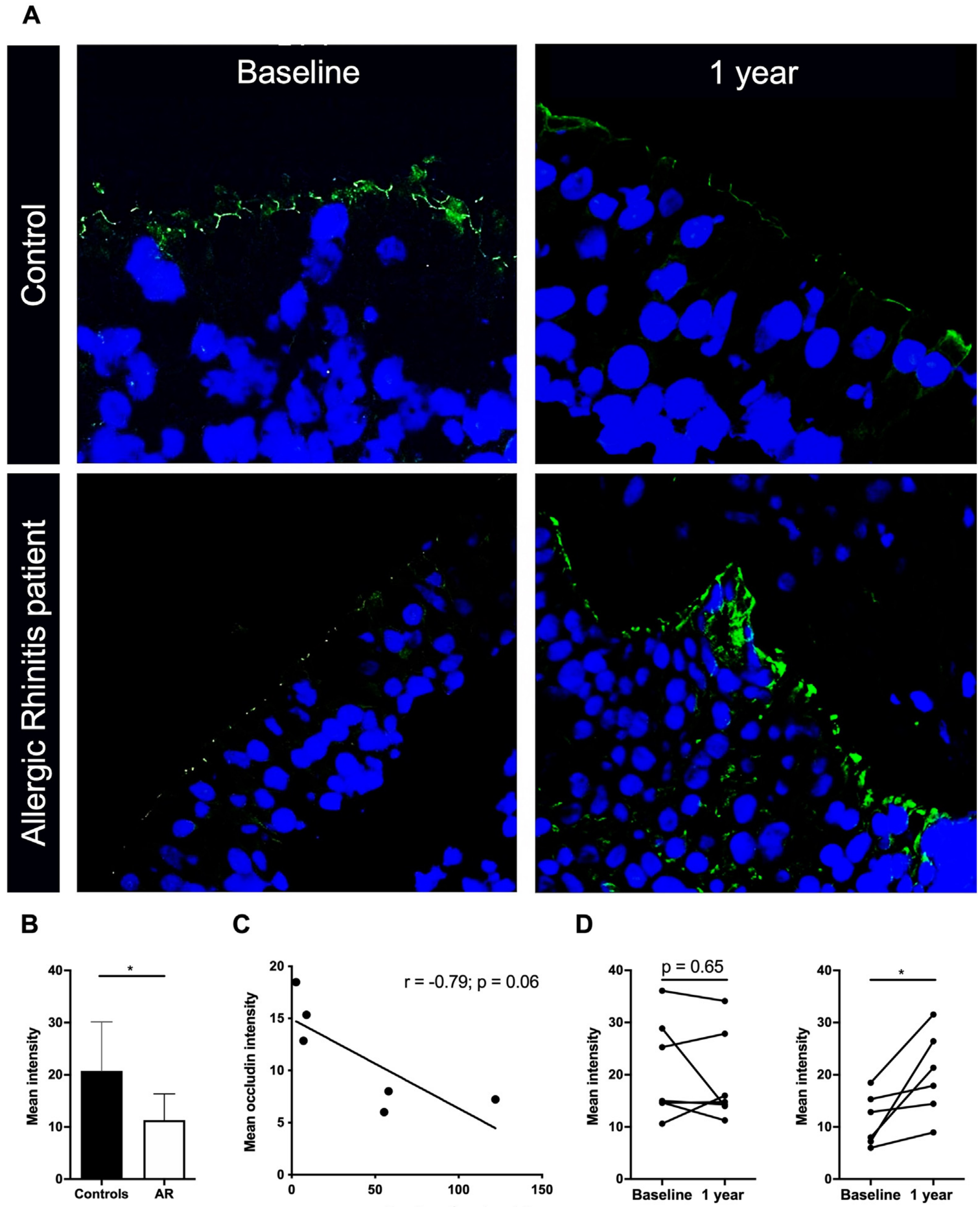

Fig. 2 Effect of year-long treatment with fluticasone furoate on occludin expression in nasal mucosa of controls and patients with allergic rhinitis. A. Representative immunofluorescence staining for occludin in the nasal mucosa of controls and patients with allergic rhinitis at baseline and after 1 year. N = 6 patients. **B.** Mean fluorescence intensity of occludin at baseline in controls and patients. Image J was used to measure this. **C.** Correlation between mean fluorescence intensity of occludin and the number of eosinophils in the lamina propria at baseline in patients with allergic rhinitis. **D.** Effect of year-long treatment with fluticasone furoate on the mean fluorescence intensity of occludin in controls and patients. Student's *t*-test (Fig. 2B) and paired *t*-test (Fig. 2D), data presented as mean ± SD.*$p < 0.05$

eosinophils in bronchoalveolar lavage fluid (Fig. 1D). Both INS reduced IL-13, but not IL-4 levels in bronchoalveolar lavage fluid (Fig. 1E–F). Next, we investigated the effect of both INS on occludin and ZO-1 mRNA expression in the nasal mucosa and found that HDM-challenged mice showed reduced levels of both occludin and ZO-1 (Fig. 1G–H). Treatment with FF, but not MF, increased mRNA expression. Levels of eosinophils in bronchoalveolar lavage fluid correlated inversely with occludin mRNA expression (Fig. 1I). Similar effects on inflammation and epithelial barrier function were observed when FF and MF were applied in the nose of mice in a therapeutic setting (Supplementary Fig. 2).

As both INS molecules are capable of reducing inflammation and restoring mucosal barrier function, we wondered what the long-term effect of INS on mucosal barrier function was. Therefore, 5 µm thick serial sections of snap-frozen nasal mucosal biopsy specimens from a previously performed study were used to evaluate the expression of occludin in controls and patients with AR at baseline and after one year on daily FF treatment.[7] We found that occludin expression was severely disrupted at baseline in patients compared to controls, which recovered after one year of daily treatment (Fig. 2A). Mean occludin fluorescence intensity was significantly decreased in patients compared to controls, which inversely correlated—though without reaching significance—with levels of eosinophils found in the lamina propria (Fig. 2B–C). Lastly, after one year of treatment with FF, mean occludin fluorescence intensity significantly increased. No effect was found in controls (Fig. 2D).

Epithelial barriers play a pivotal role in maintaining mucosal immune homeostasis. In AR, however, the homeostasis balance is characterized by reduced junctional integrity, and overwhelming mucosal inflammation.[1] Disruption of epithelial tight junctions might increase the susceptibility to allergen sensitization, as well as decrease the threshold of allergen exposure to drive local antigen-driven inflammation. Indeed, we previously reported that allergen sensitization was facilitated in the presence of epithelial barrier defects and that preventing epithelial injury hampered allergen sensitization.[8]

The ability of INS to restore barrier function has been demonstrated in a number of *in vitro* and *in vivo* models.[3,9] However, the effect of different classes of INS on epithelial integrity has not been extensively studied. Here, we show that both FF and MF significantly restore epithelial barrier integrity, and similarly reduce mucosal permeability and eosinophilic inflammation *in vivo*. Both INS directly restore the epithelial barrier by increasing mRNA and protein levels of tight junctions. Clinically, both FF and MF have been proven effective in the treatment of AR.[10,11] Here, we demonstrate that long-term INS treatment in patients with AR promotes the expression of occludin, illustrating the beneficial effect of the treatment on epithelial integrity. Furthermore, we show that both INS have a similar effect on inflammation *in vivo*. Corticosteroids have been associated with reduced type 2 inflammation.[12] Type 2 cytokines, including IL-4 and IL-13, are capable of reducing tight junction function and integrity.[13] From our results, we cannot exclude the contribution of INS in reducing inflammation indirectly by suppression of type 2 cytokines. Similarly, INS might also have an effect on the epithelial cytokines IL-25, IL-33 and thymic stromal lymphopoietin, which are also contributing to type 2 inflammation and epithelial disruption.[14]

Based on our results, we propose that both INS may prevent epithelial defects by enhancing the ability of the epithelium to withstand environmental triggers, thereby reducing mucosal permeability and inflammation. A year-long treatment with INS reconstitutes a defective epithelial barrier in patients with AR by promoting tight junction expression and function.

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Data availability

All data is available upon reasonable request.

Author's contribution

MD, KM, MV and BS performed experiments. MD, TW and BS wrote the manuscript. WF, EP, RF and PWH critically revised the manuscript.

Ethical approval

All experiments were approved by the Medical Ethical Committee of the University Hospitals Leuven (S59865), and the Ethical Committee for Animal Research at the KU Leuven (P150/2017).

Consent for publication

All co-authors have evaluated the document and provided written approval for publication.

Declaration of competing interest

The authors declare no competing interest in relation to this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2021.100585.

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