Pharmacological characterization of a novel and potent $\alpha_4\beta_1$ integrin antagonist effective in experimental allergic conjunctivitis

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Allergy is a common hypersensitivity disorder that affects 15% to 20% of the population and its prevalence is increasing worldwide. Signs include conjunctival hyperaemia, chemosis, eyelid swelling and sometimes a mild papillary reaction on the palpebral conjunctiva. The central mechanism in the pathogenesis of this disease is the IgE-mediated mast cell degranulation and infiltration and activation of T lymphocytes, basophils, eosinophils, and other cells in the conjunctiva, with the involvement of conjunctival epithelial cells. Its severity correlates with the degree of eosinophil infiltration into the conjunctiva, which is mediated by chemokines that stimulate the production of adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on the endothelial cell surface. The $\alpha_4\beta_1$and $\alpha_4\beta_7$ integrins are expressed in eosinophils and contribute to their activation and infiltration in AC through the binding to VCAM-1 or fibronectin, expressed on vascular endothelial cells. The $\alpha_4\beta_1$, also known as very late antigen-4 (VLA-4), and $\alpha_4\beta_7$ integrins mediate cell adhesion to VCAM-1 and fibronectin and function as both adhesion and signaling molecules; $\alpha_4\beta_7$ interacts also with mucosa addressin cell adhesion molecule (MadCAM).

The mechanisms regulating eosinophil adhesion to the ocular surface during allergic inflammation are still largely unknown but the $\alpha_4\beta_1$ integrin/VCAM-1 pathway is crucial for the firm adhesion and transmigration of eosinophils into the conjunctiva through vascular endothelial cells. The inhibition of conjunctival eosinophil infiltration by an α_4 integrin monoclonal antibody has been reported in a guinea pig model of AC. These findings suggest that the blockade of α_4 integrins might be a therapeutical achievement in allergic eye diseases.

Several reports have confirmed that small-molecule α_4 antagonists decrease airway inflammation in experimental animal models. These compounds are considered potential alternatives to therapeutic antibodies because of their increased selectivity for specific α_4-containing integrin complexes. In addition, their short half-lives compared with those of antibodies permits a relatively fast elimination from the body.

Firstly, we performed a screening of several small molecules derived from a library of putative α_4 antagonists through an in vitro cell adhesion assay and we identify the most effective one, DS 70, that show an IC_{50} in the nanomolar range against $\alpha_4\beta_1$ integrin in Jurkat cells and in the eosinophilic cell line EOL-1. This compound was able to prevent cell adhesion to VCAM-1 and FN in vitro. In a scintillation proximity assay DS70 displaced ^{125}I-FN binding to human $\alpha_4\beta_1$ integrin and, in flow cytometry analysis, it antagonized the binding of a primary antibody to $\alpha_4\beta_1$ integrin expressed on the Jurkat cells surface as well. Furthermore, we analysed also its effects on integrin $\alpha_4\beta_1$ signalling: the results showed that VCAM-1 or FN mediated phosphorylation of ERK 1/2 and focal adhesion kinase (FAK) was significantly reduced by DS70.

Finally, in an vivo model of allergic conjunctivitis, topical DS70 reduced the clinical aspects of EPR (early phase reaction) and LPR (late phase reaction), by reducing clinical score, eosinophil accumulation, mRNA levels of cytochines and chemochines pro-inflammatory and the conjunctival expression of α_4 integrin.

In conclusion, DS70 seems a novel antiallergic ocular agent that has significant effects on both early and late phases of ocular allergy.