CONTRIBUTION OF $\alpha_4\beta_1$ INTEGRIN TO THE ANTIALLERGIC EFFECT OF LEVOCABASTINE IN EXPERIMENTAL CONJUNCTIVITIS

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Levocabastine is an ophtalmic antiallergic agent acting as histamine H1-receptor antagonist. It has been proposed that, in allergic conjunctivitis (AC), levocabastine may also interfere with up-regulation of the adhesion molecule ICAM-1 expressed on epithelial conjunctival cells; whereas little is known on its effects on eosinophils, which are important effector cells in AC. The adhesion molecule $\alpha_4\beta_1$ integrin and its ligand vascular cell adhesion molecule 1 (VCAM-1), are expressed in eosinophils and are known to play important roles in their infiltration and activation observed in allergic reactions. In this study we investigated if levocabastine may act as $\alpha_4\beta_1$ integrin antagonist and may affect conjunctival expression of this receptor in a model of AC. A scintillation proximity assay (SPA) has been developed to measure levocabastine binding to integrin $\alpha_4\beta_1$, that was purified by affinity chromatography from HEK-293 cells stably transfected with the human gene of this receptor. This SPA assay allowed measurement of specific ^{125}I-fibronectin binding as defined by displacement of the fibronectin (FN) CS-1 fragment (1978-1985). IC_{50} value for displacement of ^{125}I-FN binding by levocabastine was 585 μM. Furthermore, cell-based adhesion assays to study the effect of potential integrin antagonists were adopted. The adhesion of Jurkat cells and of the eosinophilic cell line EoL-1, which express $\alpha_4\beta_1$ integrin, to plates coated with the counter receptor VCAM-1 was blocked by levocabastine (in Jurkat cells, IC_{50} = 395,6 μM; in EoL-1 cells, IC_{50} = 453,9 μM) or by the purported antagonist BIO1211 (in Jurkat cells, IC_{50} = 84,1 pM). In order to confirm if levocabastine antagonizes integrin receptor binding, a flow cytometry assay was also adopted. In Dunkin-Hartley guinea pigs, sensitised by intra-peritoneal injection of ovalbumin (OVA), a significant increase of conjunctival integrin $\alpha_4\beta_1$ expression (detected by western blotting) was observed after antigen topic challenge. Levocabastine eye drops (500 μg/eye), applied 60 and 30 min before the antigen challenge, produced a noteworthy protection from AC, and prevented the conjunctival elevation of integrin $\alpha_4\beta_1$ levels as well as conjunctival eosinophil infiltration. Our findings indicate that levocabastine, acting as an integrin $\alpha_4\beta_1$ antagonist, may interfere with conjunctival eosinophil infiltration observed in late phase of AC.