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Effect of GLC756, a novel mixed dopamine D1 receptor antagonist and dopamine D2 receptor agonist, on TNF-alpha release in vitro from activated rat mast cells

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Abstract

Tumor necrosis factor-α (TNF-α) is released from activated mast cells via an IgE-dependent mechanisms, and plays a crucial role in ocular allergic inflammation. This study examined the influence of three antiglaucoma drugs differing in their chemical structure and pharmacological profile (i.e. latanoprost, timolol, GLC756) on TNF-α release from activated rat mast cells. A rat basophilic leukemia mast cell line (RBL-2H3) was activated via IgE/anti-IgE. Rat mast cells were incubated with latanoprost, timolol, GLC756 or betamethasone (positive control) at concentrations of 0.1, 1, 10 and 30 μM. TNF-α concentration in supernatant was measured by ELISA 5 h post-activation. Compared to controls, the prostaglandin derivative latanoprost and the beta-blocker timolol in the concentration range 0.1–30 μM, had no significant effect on TNF-α release from rat mast cells measured 5 h after activation. By contrast, the dopaminergic drug GLC756 compared to controls in the concentration range 1–30 μM significantly inhibited TNF-α release from activated rat mast cells in a concentration-dependent manner. The positive control betamethasone inhibited TNF-α release almost completely at all concentrations tested. In conclusion, the results of this study suggest that latanoprost and timolol do not reduce inflammation triggered by activated mast cells. By contrast, the dopaminergic drug GLC756 inhibited TNF-α release from activated mast cells, suggesting an palliative potential of dopaminergic compounds on allergic conjunctivitis in topical glaucoma medication.

Keywords: dopamine D1 receptor antagonist; dopamine D2 receptor agonist; GLC756; in vitro model; latanoprost; mast cells; timolol; TNF-α; topical glaucoma medication

1. Introduction

Allergic eye disease is a common problem among individuals suffering from allergies (Bielory, 2000a,b, Part 1 and 2). Ocular allergic conjunctivitis is typically associated with IgE-mediated mast-cell activation in conjunctival tissue (Stahl et al., 2002). The binding of IgE to the high affinity receptor (FcεRI) on the surface of mast cells (MC) and basophils and its subsequent crosslinking with an allergen is the first step in a cascade of processes leading to the release of pro-inflammatory mediators (Shaikh et al., 1997). In addition to its crucial role in MC activation, IgE has been shown to regulate the FcεRI expression on the surface of MC (Welker et al., 1997; MacGlashan, 2005; Yamaguchi et al., 1997, 1999) and basophils (Lantz et al., 1997; MacGlashan et al., 1997). Therefore, increased IgE concentration induces a higher FcεRI density which in turn leads to an increased release of pro-inflammatory cytokines such as TNF-α (Brazis et al., 2002). Among the MC-derived pro-inflammatory mediators, TNF-α plays a prominent role. Therefore, compounds modifying TNF-α release from

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MC in response to allergen challenge may be of potential value for therapeutic intervention (Cook et al., 2000).

To investigate the potential of antiglaucoma medication to improve or possibly aggravate ocular allergic inflammation three topically active antiglaucoma drugs i.e. latanaprost, timolol and GLC756 which differ in their chemical structure and pharmacological profile were selected to examine their influence on TNF-α release from activated rat mast cells. Latanoprost, a prostaglandin F2α (PGF2α) analogue, is very effective in lowering elevated intra ocular pressure (IOP) by increasing uveoscleral outflow (Ziai et al., 1993; Alm and Stjernschantz, 1995). GLC756 a benzoquinoline derivative exhibits the profile of a mixed dopamine D1-receptor antagonist and dopamine D2 receptor agonist. It lowered IOP in man and experimental animals by an yet unknown mechanism (Pruente and Flammer, 1995) and in addition improved perfusion of the optic nerve in experimental animals (Prünte et al., 1995; Chiou and Chen, 1992; Markstein et al., 1996; Prunte et al., 1996). GLC 756 was assessed in this experiment since in a previous study it was found to decrease TNF-α levels in the serum of Lewis rats after intravenous injection of 160 μg lipopolysaccharide from Salmonella typhimurium (Laengle et al., 2006). Finally, timolol, a non-selective beta-receptor blocking agent which lowers IOP by decreasing aqueous humor inflow is a widely used antiglaucoma drug (Konstas et al., 2003). As positive control for the inhibition of TNF-α release the corticosteroid betamethasone, a potent anti-inflammatory drug (Xu et al., 2005), was used. In the present study, the effect of the test compounds was assessed in-vitro using a basophilic leukemia RBL-2H3 mast cell line. RBL-2H3 cells have been utilized by a number of laboratories as a model to study signal transduction mechanisms through the IgE pathway (Barsumian et al., 1981).

2. Methods

2.1. Drug preparation

Latanoprost was commercially available from Cayman Chemical (Ann Arbor, MI, USA) as a solution in methyl acetate, purity >98%. The compound was used as a solution in ethanol after evaporation of the methyl acetate under a stream of nitrogen. GLC756, as a powder, received from Novartis Pharma AG (Switzerland) was reported to be 100% pure by certificate of analysis. The drug was dissolved in DMSO (Sigma, Switzerland). Timolol was available commercially as a powder (100% pure) from Sigma, Switzerland. The powder was dissolved in a 2.5% glycerol solution. Betamethasone was available commercially as a powder (99.7% pure) from Sigma, Switzerland. The powder was dissolved in a 40% PEG300 solution.

2.2. RBL-2H3 cell culture, activation and treatment

The 2H3 subclone of the rat basophilic leukaemia (RBL) cells are considered homologous to rat mucosal mast cells (Seldin et al., 1985). They were purchased from the American Type Culture Collection (ATCC, France) and maintained as monolayer culture in 75 cm^2 tissue flasks (T-75, Costar, Switzerland) in Eagles minimum essential media (MEM, ATCC, France) supplemented with 15% of heat inactivated fetal calf serum (FCS, Gibco, Switzerland) and 100 units/mL penicillin and 100 μg/mL streptomycin (Gibco, Switzerland) and incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. For secretion studies, cells were dislodged from the flasks with trypsin (0.05%) and EDTA (0.02%) solution (Gibco, Switzerland), rinsed in phosphate buffered saline (PBS) then plated in 24-well plates at 2×10^5 cells/well overnight. After removal of the medium and any unattached cells, the cells were sensitized by pre-exposure to purified rat IgE (0.5 μg/mL, 1 mL/well, Zymed, Switzerland) for a period of 24 h after which the plates were thoroughly rinsed with PBS by centrifugation at $300 \times g$, 5 min, at 4 °C. The test substances (500 μL/well), latanoprost, timolol, bethametasone and GLC756 were then added to the cells at 0.1, 1, 10 and 30 μM for 20 min. The cells were then triggered by the addition of purified rabbit anti-rat IgE (1 μg/mL, 5 μL/well, Zymed, CH). An equal volume of carrier solution was added to the control wells. The cells were incubated for a period of 5 h after which the plates were centrifuged at $300 \times g$ and the medium was carefully removed and aliquoted in Eppendorf tubes which were stored at −70 °C until further use.

The same cell line with the same number of passages under identical experimental conditions was used in 3 independent assays. The control group received culture medium or culture medium plus corresponding vehicle DMSO or acetic methyl acetate or H_2O at 0.1%.

2.3. Determination of TNF-α secretion from RBL-2H3 cells

Prior to analysis of antigen-induced secretion of TNF-α from treated RBL-2H3 cells, the samples were thawed and centrifuged at $800 \times g$ for 4 min. TNF-α was measured using a commercially available ELISA kit (BioSource International, Inc, Switzerland) according to the manufacturer's instructions. Briefly, 100 μl samples diluted at ½ were transferred to microtiter strips pre-coated with a monoclonal antibody specific for rat TNF-α and incubated 3 h at room temperature. The wells were washed six times and incubated with 100 μl of biotinylated anti-TNF-α solution for 45 min at room temperature. After 6 washing steps, 100 μl of Streptavidin-HRP was added to each well for 45 min at room temperature. After washing, 100 μl of stabilized chromogen was added to the wells and incubated for 20 min at room temperature in the dark and followed by the addition of 100 μl stop solution. The enzyme reaction product was measured spectrophotometrically at 450 nm. Standard curves (range = 0–150 pg/ml) were obtained from freshly prepared solutions of TNF-α in a buffered solution and the optical density of the samples was calibrated for TNF-α content against the standard curve.

2.4. Data analysis

All measurements and calculation were automatically processed by the ELISA reader (VERSAmax, Molecular devices,