Anti-inflammatory effects of glucocorticoids on Endotoxin-Induced Uveitis (EIU) in rats: impact of the mode of administration.

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Background

The model of Endotoxin-Induced Uveitis (EIU) in the rat is a useful animal model for human anterior uveitis (1). The systemic administration of lipopolysaccharides (LPS) results in an acute inflammatory response in the anterior and posterior segments of the eye with a breakdown of blood-ocular barrier and inflammatory cell infiltration. Clinical signs of EIU, including protein flare and cell in the aqueous humor, miosis and posterior synechiae, fibrin clots and hypopyon, reflect the changes seen in the human disease.

Measurement of Infiltrating Cells and Protein Concentration in Aqueous Humor

At 24 hours after LPS injection, the rats were euthanized and 10µl of the aqueous humor (AH) was collected immediately and thereafter diluted 10-fold in PBS. The total protein concentration in the aqueous humor samples was measured with the Bradford method and expressed in milligrams per milliliter.

Levels of IL-1β, IL-6, IL-12 in Aqueous Humor

The aqueous humor samples were stored at -80°C until testing. The levels of cytokines were determined by the Luminex xMAP detection protocol. The results were expressed in picograms per milliliter.

Clinical Evaluation

24h after LPS injection, untreated animals displayed clinical signs of uveitis, in contrast to the glucocorticoid treated groups, which showed a similar diminution of the clinical signs, regardless of the route of administration. Intraocular dexamethasone phosphate administration reduced the total clinical score of uveitis from 3.0 (+/-0.3) for untreated group down to 0.3 (+/-0.1), 0.4 (+/-0.2) and 0.6 (+/-0.2). Control rats without any induction had no clinical signs of uveitis.

Results

Clinical score (0-7) for multiple instillations of 0.1% dexamethasone.

Material and method

Animals, EIU Induction and Treatment

EIU was induced in male Lewis rats, weighing 180 to 200 g by footpad injection of 200 µg of LPS that had been diluted in 0.1 ml of sterile water. Animals were then randomized in four groups. The first two groups received either a single intravenous dose of 2.5 mg/kg dexamethasone phosphate (immediately after LPS injection) or multiple instillations of 0.1% dexamethasone (1h before and 1h, 2h and 3h after induction). The third group received a single subconjunctival dose of 1 µg methylprednisolone. A non-treated group was used as control of induction.

Clinical Evaluation of EIU

Animals were examined with a slit lamp biomicroscope 24 hours later, corresponding to the peak severity for EIU. Clinical ocular inflammation was scored in each eye using a scale from 0 to 7 as follows: iris hyperemia and cell in the anterior chamber 0-2, (0 = no sign; 1 = mild; 2 = severe) and flare, miosis and hypopyon were scored 0 for no sign or 1 for presence. The maximum possible score was 7 (sum of the 5 parameter scores).

Inflammatory Cells and Protein Concentration in Aqueous Humor

24 hours after LPS injection, inflammatory cells were found in the anterior segment. In the LPS group, the mean of inflammatory cells infiltrated into the aqueous humor was 264 cells/µl (+/-281). Rats treated with glucocorticoids showed a significant reduced count of inflammatory cells, 54 cells/µl (+/-12) were found with intraocular dexamethasone phosphate, 797 cells/µl (+/-147) with subconjunctival administration of methylprednisolone and 79 cells/µl (+/-13) with multiple instillations of 0.1% dexamethasone. In the control group, no infiltrating cells were detected in the aqueous humor.

The cytokine levels were measured with the Bradford method and expressed in milligrams per milliliter.

Levels of IL-1β, IL-6, IL-12 in Aqueous Humor

In the naive group, protein concentration in the aqueous humor was 43 mg/ml (+/-1.2) and was increased to 31.4 mg/ml (+/-3.4) with LPS injection. In the rats treated with glucocorticoids, protein concentration was reduced by 98% for intravenous dexamethasone phosphate, by 70% subconjunctival administration of methylprednisolone, by 97% for multiple instillations of 0.1% dexamethasone.

Conclusion

Both intravenous and topical administrations of dexamethasone markedly decreased clinical signs of EIU, inflammatory cell counts, protein concentration, and levels of IL-1ß, IL-6 and IL-12 in aqueous humor. Subconjunctival administration of methylprednisolone also decreased the symptoms of EIU but to a lesser extent than the other two treatments.

Topical administration of dexamethasone allows for a therapeutic effect on the anterior segment of the eye in the rat EIU model and may provide a viable alternative to systemic administration of glucocorticoids.

References


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