Effect of Triamcinolone Acetonide on Proliferation of Retinal Pigment Epithelium Cells

Jost B Jonas, Ulrich HM Spandau

Medical Faculty, Department of Ophthalmology, Mannheim of the Ruprecht-Karls-University of Heidelberg, Heidelberg, Germany

Correspondence: Jost B Jonas, Universitäts-Augenklinik, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Heidelberg, Germany
Phone: 49-621-383-2242, Fax: 49-621-383-3803, e-mail: jost.jonas@umm.de

ABSTRACT

Purpose: To evaluate the influence of triamcinolone acetonide on the proliferation of retinal pigment epithelium cells in an experimental cell culture study.

Methods: Pooled fresh porcine retinal pigment epithelium cell cultures were exposed to triamcinolone acetonide in increasing concentrations after the solvent agent had been removed. The proliferation rate was measured using the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay (MTS assay).

Results: The number of proliferating retinal pigment epithelium cells decreased significantly (p < 0.01) with increasing concentrations of triamcinolone acetonide up to 1 mg/mL.

Conclusions: In porcine retinal pigment epithelium cell cultures, low concentrations of triamcinolone acetonide lead to a dosage dependent reduction in the cell proliferation rate. It may be of importance for the safety and the therapeutic effect of intravitreal use of triamcinolone acetonide for treatment of intraocular edematous and proliferative diseases, such as proliferative vitreoretinopathy.

Keywords: Intravitreal triamcinolone, Retinal pigment epithelium, Macular diseases, Age-related macular degeneration, Proliferative vitreoretinopathy.

INTRODUCTION

In an increasing number of clinical studies, triamcinolone acetonide as an intermediate-acting corticosteroid suspension has intravitreally been applied for treatment of intraocular edematous, neovascular and proliferative diseases, such as diabetic macular edema, proliferative diabetic retinopathy and proliferative vitreoretinopathy. It has remained unclear, so far, whether triamcinolone acetonide influences the proliferation rate of retinal pigment epithelium cells in vivo. It may be of importance for the safety of the intravitreal use of triamcinolone acetonide, and for the therapeutic effect of intravitreal triamcinolone acetonide in diseases, such as proliferative vitreoretinopathy.

METHODS

Pooled porcine retinal pigment epithelium cells, freshly obtained from slaughterhouse pigs and frozen at – 80°C, were thawed and put into a cell culture consisting of Dulbecco's modified Eagle's medium, containing 2 mM L-glutamine supplemented with 10% fetal bovine serum and an antibiotic mixture of 100 U/mL penicillin G and 100 µg/mL streptomycin sulfate (Sigma-Aldrich Co, D-82024 Taufkirchen, Germany). Cell suspensions with a cell volume of 1000 cells/0.1 mL were seeded onto 96-well tissue culture plates. Incubation at 37°C and in an environment containing 95% O₂ and 5% CO₂ was performed for three days. Cells were adherent at the bottom of the well. The medium was exchanged against fresh Dulbecco's modified Eagle's medium, and crystalline triamcinolone acetonide was added in increasing concentrations (0 mg/mL to 1.0 mg/mL). The solvent agent of triamcinolone acetonide was removed by centrifuging the original drug [Volon AR (Bristol-Myers Squibb, Munich, Germany)] three times, removing the supernatant and keeping the triamcinolone acetonide crystals. For each of the six concentrations of triamcinolone acetonide, a series of six wells was prepared and measured on day 1, 2, 3, 4, 5 and 8. The whole test series was repeated once so that altogether 72 wells were prepared and analyzed. The proliferation rate of the retinal pigment epithelium cells was measured using the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay (MTS assay; Promega Co., Madison, WI, USA). In this assay, the MTS tetrazolium compound was bioreduced by viable cells into a colored formazan product and its concentration was measured by recording the absorbance at 490 nm.

RESULTS

From the third day onwards after adding triamcinolone acetonide to the cell culture, increasing concentrations of triamcinolone acetonide caused a significant (p < 0.01) reduction in the absorbance in the MTS assay as equivalent of the number of viable retinal pigment epithelium cells (Fig. 1).
DISCUSSION

The present investigation showed that triamcinolone acetonide caused a significant decrease in retinal pigment epithelium cell numbers across the whole range of concentrations (0.01-1 mg/mL) as long as the cells had been exposed to triamcinolone acetonide for more than two days in the cell culture. The results agree with recent studies in which human retinal pigment epithelial cell lines were used, in contrast to the freshly obtained porcine cells used in the present study. It may partially be in contrast to a previous investigation in which triamcinolone acetonide produced an inhibition of rabbit dermal and conjunctival fibroblast proliferation in cell cultures at a concentration of 150 mg/L, but paradoxically increased proliferation almost two-fold at concentrations ranging from 1 to 30 mg/L under identical culture conditions. It has remained unclear whether and how the results of the present cell culture study may be transferred into clinical application. Preceding clinical studies did not show a detected toxicity of intravitreal triamcinolone acetonide at doses normally used in clinical practice. A recent experimental cell-culture study by Narayanan et al suggested that triamcinolone acetonide was toxic to proliferating cells of retinal origin in vitro at doses normally used in clinical practice. In another experimental study by Yeung et al the cytotoxic effect of triamcinolone on human retinal pigment epithelium (ARPE19) and human glial cells over a range of concentrations and durations of exposure was examined. The authors reported that triamcinolone was cytotoxic to both glial cells and ARPE19 cells with a higher efficacy on the glial cells. Triamcinolone caused an activation of the caspase-3 pathway more readily than the cell-protective c-fos and c-jun pathways in the glial cells, making those cells more vulnerable than the ARPE19 cells. The authors concluded that triamcinolone toxicity in one cell type may not reliably indicate its toxicity in other cells. Different cells within the retina may react to triamcinolone differently, or triamcinolone may cause changes in the gene expressions differentially with different concentrations of the same stimulus.

In view of the widespread use of triamcinolone acetonide for treatment of diseases associated with macular edema, the results of our study, Narayanan’s investigation and Yeung’s study may warrant additional investigations for the effect of intravitreal triamcinolone acetonide in vivo, since the results of these in vitro study cannot be directly extrapolated to clinical practice. The results may be interesting for the discussion of a medical adjunctive treatment of proliferative vitreoretinopathy.

REFERENCES


Fig. 1: The absorbance in the MTS test as equivalent of the number of viable porcine retinal pigment epithelium cells in cell culture with triamcinolone acetonide added in various concentrations (0-1.0 mg/mL)