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The effects of triamcinolone crystals on retinal function in a model of isolated perfused vertebrate retina

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ABSTRACT

A good clinical experience of intravitreal triamcinolone acetonide (TA) has been reported in several studies, but there are growing indications that epiretinal crystals of TA exhibit retinal toxicity. To investigate the effects of TA on retinal function we used a model of an electrophysiological in vitro technique for testing retinal toxicity. Isolated bovine retinas were perfused with an oxygen saturated nutrient solution. The electroretinogram (ERG) was recorded as a transretinal potential using Ag/AgCl electrodes. After reaching stable ERG-amplitudes TA at the maximum solubility equilibrium (36 μg/ml) was either applied to the nutrient solution for 45 min or TA was administered epiretinally at concentrations (1 mg/ml, 4 mg/ml, 8 mg/ml, 20 mg/ml and 40 mg/ml) above the maximum solubility equilibrium to assure direct contact of the TA crystals with the isolated perfused retinas. After that the retinas were reperfused for 75 min with the standard nutrient solution. The percentage of a- and b-wave reduction directly after the application and at the washout was calculated. To assess the effects of TA at the level of the ganglion cell layer a Viability/Cytotoxicity Kit for mammalian cells was used. No changes of the ERG-amplitudes were detected during the exposure to 36 μg/ml TA for 45 min (b-wave: 9.6 μV ± 2.1 vs. 8 μV ± 2.1 ($p = 0.135$); a-wave: −11 μV ± 2.7 vs. −10.6 μV ± 2.3 ($p = 0.889$)) and at the washout (b-wave: 8 μV ± 2.1 vs. 8.3 μV ± 2.4 ($p = 0.18$); a-wave: −10.6 μV ± 2.3 vs. −12 μV ± 2.6 ($p = 0.225$)). At concentrations higher than 1 mg/ml TA induced a decrease of the a- and b-wave in a concentration dependent manner. These changes were reversible for concentrations of TA up to 20 mg/ml (b-wave: 9 μV ± 2.4 vs. 6.6 μV ± 2.5 ($p = 0.08$); a-wave: −11.4 μV ± 2.0 vs. −11.2 μV ± 2.2 ($p = 0.37$)), but irreversible at 40 mg/ml even at the end of the washout (b-wave: 9.8 μV ± 1.9 vs. 3 μV ± 1.7 ($p = 0.009$); a-wave: −9.8 μV ± 2.1 vs. −2.6 μV ± 2.1 ($p = 0.001$)). Histological examination of the preparations revealed a dramatic ganglion cell death, in which an application of 20 mg/ml and 40 mg/ml TA led to a 60.53% ($p = 0.013$) and 82.35% ($p = 0.002$) ganglion cell death, respectively. The epiretinal application of 4 mg/ml TA and higher resulted in distinct effects on the ERG of the isolated perfused retinas. Ganglion cell death was induced at a concentration of 20mg/ml and higher. TA shows an asymmetric and partly high concentrated distribution after intravitreal application. Therefore, we consider concentrations of 4 mg/ml and higher might be toxic and should be avoided in clinical use.

1. Introduction

The intravitreal use of triamcinolone acetonide (TA) was found to be effective in the treatment of cystoid macular edema caused by diabetic retinopathy and retinal vein occlusion unresponsive to laser photocoagulation. The treatment with TA can improve visual acuity associated with a decrease of the macular edema (Chen et al., 2006; Jonas and Söfker, 2001; Martidis et al., 2002; Jonas et al., 2003). A major advantage of TA is the sustained release of minute amounts from its crystalline form. Kim et al. found that the vitreous half-life of TA is associated with the amount of intravitreally applied TA (Kim et al., 2006). In addition, the vitreous half-life of TA is also dependent on the anatomical structure of the injected eye and vitrectomized patients have a distinct lower TA half-life than non-vitrectomized patients (Beer et al., 2003; Chin et al., 2005).

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Side effects of TA therapy can occur and comprise elevation of intraocular pressure and cataractogenesis like topical ocular corticosteroid. However, cytotoxicity to retinal or pigment epithelial cells is currently under discussion. In vitro studies have shown that 200 μg/ml TA and lower could be toxic to retinal pigment epithelial cells, glial cells and retinal cells, respectively (Yeung et al., 2003; Narayanan et al., 2006). This is in contrast to the good clinical experience of TA. Recently, we could clarify this discrepancy between in vitro and in vivo retinal toxicity of TA. We demonstrated that TA exhibits only moderate toxicity if the crystals are not in direct contact to the cell surface. In contrast, direct exposure to even minute crystalline deposits caused a rapid progressive and irreversible cell death being significant far below clinically used concentrations (Szurman et al., 2006, 2007). TA was originally developed and approved for labelled use only as a therapeutic agent in arthritis. The evaluation of an appropriate safe therapeutic range for intravitreal injection is important. Therefore, the aim of our study was to assess safe concentrations of TA, using an electrophysiological model for the evaluation of retinal toxicity, which can simulate the situation in vivo more appropriate than cell culture methods testing the functional integrity of the retina after TA application.

2. Materials and methods

Aspartate, glucose and other chemicals were obtained from Merck (Merck Pharma GmbH, Darmstadt, Germany) at pro analysis grade. TA (Volon-A 40, Bristol-Myers-Squibb, Munich, Germany) containing 40 mg crystalline suspension was purified according to a standard protocol for human use with reduced benzyl alcohol (Hernaez-Ortega and Soto-Pedre, 2004). Finally, suspensions in concentrations of 1 mg/ml, 4 mg/ml, 8 mg/ml, 20 mg/ml and 40 mg/ml were prepared for the study. In addition, 40 mg TA was dissolved in 1.1 l of the standard nutrient solution leading to a final concentration of 36 μg/ml that corresponds to the maximum solubility equilibrium of TA.

Bovine eyes were obtained directly post mortem and transported in darkness in a serum-free standard medium containing 120 mM NaCl, 2 mM KCl, 0.1 mM $MgCl_2$, 0.15 mM $CaCl_2$, 1.5 mM NaH_2PO_4, 13.5 mM Na_2HPO_4 und 5 mM glucose. The preparation was performed as described recently (Lüke et al., 1997, 2005; Sickel, 1965; Walter et al., 1999). The electroretinogram (ERG) was recorded in the surrounding serum free standard medium *via* two silver/silver-chloride electrodes (scientific workshop, Tuebingen, Germany) on either side of the retina. The recording chamber containing a piece of retina was placed in an electrically and optically insulated box (scientific workshop). The perfusion velocity was controlled by a roller pump and set to 1 ml/min and the temperature was kept constant at 30 °C. The perfusing medium was preincubated and saturated with oxygen. The electroretinogram from the dark-adapted retinas was elicited at intervals of 5 min using a 1-Hz single white xenon flash (xenon tube; Grass Technologies, West Warwick, RI) for stimulation. The flash intensity was set to 6.3 mlx at the retinal surface using calibrated neutral density filters (Wratten Filter; Kodak, Stuttgart, Germany).

The duration of light stimulation was 10 μs controlled by a timer (Photopic Stimulator PS33 Plus; Grass Technologies). The ERG was filtered and amplified (100-Hz high pass filter, 50-Hz notch filter, 100,000× amplification) using a Grass RPS312RM Amplifier (Grass Technologies). The data were processed and converted with an analog-to-digital data acquisition board (PCI-MIO-16XE-50; National Instruments, Austin, TX) in a desktop computer (PC compatible).

The retina was perfused with the serum-free nutrient solution and stimulated repeatedly until stable b-wave amplitudes were recorded. Thereafter, the nutrient solution with TA at a concentration of 36 μg/ml ($n = 6$) was applied and responses were recorded for 45 min. To test the effects of the crystalline suspensions, TA in concentrations of 1 mg/ml, 4 mg/ml, 8 mg/ml, 20 mg/ml and 40 mg/ml ($n = 5$, for each concentration) were directly applied onto the vitreoretinal surface of the isolated perfused retinas through a thin cannula. For all test series, the retinas were reperfused for 75 min with normal nutrient solution after exposure and the changes of the b-wave amplitude were recorded. The b-wave amplitude was measured from the trough of the a-wave to the peak of the b-wave (Fig. 1A).

To investigate the effects of TA on the photoreceptors under scotopic conditions (6.3 mlx flash light intensity), the b-wave was suppressed by adding 1 mM aspartate to the nutrient solution. Under these conditions, the influence of TA on the photoreceptor potential P III was analyzed. Aspartate is an inhibitor of synaptic transmission at the level of the first retinal synapse and enables the recording of unmasked photoreceptor potential P III by abolishing the b-wave (Fig. 1B) (Hanawa and Tateishi, 1970). We recorded a stable photoreceptor potential P III for 30 min. Thereafter, in an analogous manner TA was applied at the same concentration

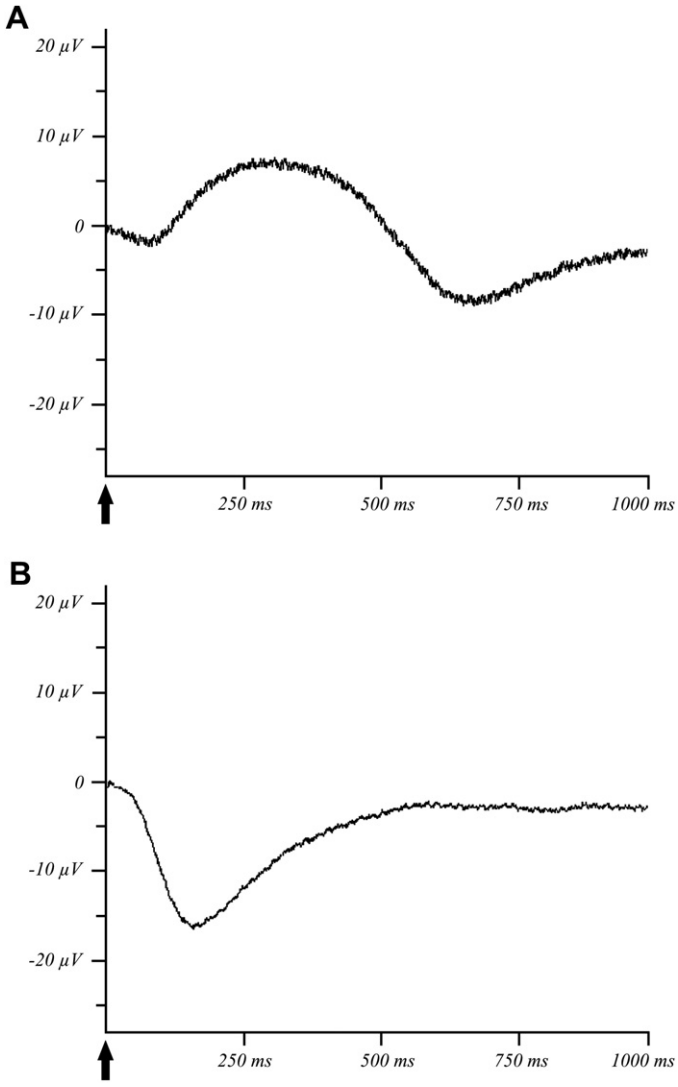

Fig. 1. The ERG from the isolated perfused bovine retina. **A**: The b-wave is dominant in the ERG of the isolated perfused bovine retina under scotopic light conditions. It results from a 10 μs light stimulus at a light intensity of 6.3 mlx at scotopic lighting conditions. **B**: The a-wave is dominant in the ERG of the isolated perfused bovine retina after blocking the b-wave by 1 mM aspartate to the nutrient solution. The a-wave was generated by using a 10 μs light stimulus of 6.3 mlx at scotopic lighting conditions.