Graefes Arch Clin Exp Ophthalmol (2008) 246:1069–1070
DOI 10.1007/s00417-008-0814-7

LETTER TO THE EDITOR

Effect of intravitreal triamcinolone acetonide on retinal apoptosis in experimental retinal neovascularization

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Received: 7 January 2008 / Revised: 27 February 2008 / Accepted: 3 March 2008 / Published online: 17 April 2008

Dear Editor,

Within the last 3 years, triamcinolone acetonide has increasingly been applied intravitreally as a treatment option for various intraocular neovascular and edematous proliferative disorders [1]. Despite its worldwide use, with an estimated number of more than 1 million injections performed so far, few studies addressed safety issues of the treatment [2–5]. It was, therefore, the purpose of the present study to examine the effect of intravitreal triamcinolone on the rate of retinal cell apoptosis. The results of such a study may indicate specific caveats for the discussion of the safety of the treatment, as well as on the possible neuroprotective effect of intravitreal triamcinolone [6]. In order to demonstrate an in vivo effect of triamcinolone on retinal apoptosis, an established model of retinopathy of prematurity was employed [7].

The experimental study included 12 newborn C57BL/J6 mice which were exposed to 75% oxygen from postnatal day 7 to postnatal day 12 with their nursing mothers. On the 12th day, the mice were returned to room air. Using a Hamilton syringe, 0.04 mg of crystalline triamcinolone acetonide in 1 μl (40 mg/ml; Volon A^R; Bristol-Myers-Squibb, Anagni, Italy) was injected into the vitreous cavity of one eye at the corneoscleral junction at the 6 o'clock position. The dosage of 0.04 mg triamcinolone in a mouse eye is equivalent to a dosage of 4 mg triamcinolone in the eye of an adult human. The contralateral eyes of the mice in the study underwent no intervention [6]. At day 15 (n=7) and day 17 (n=5), the mice were sacrificed. Additionally, three age-matched mice without treatment or oxygen exposure served as control group and were sacrificed at postnatal day 17. The enucleated eyes were fixed in 4% formalin and histologically examined using a TdT-dUTP terminal-nick-end labeling (TUNEL) assay for cell apoptosis (Promega, Madison, WI, USA). An Axiophot microscope equipped for immunofluorescence (Zeiss Co., Oberkochen, Germany) was used. The fluorescein 12-dUTP labeled deoxyribonucleic acid (DNA) was directly visualized by fluorescence microscopy. Positive cells were counted with an ocular square grid at randomly selected areas of the central and peripheral region of the retina. All experiments were performed in accordance with the guidelines for the design and conduct of animal experiments of the Association for Research in Vision and Ophthalmology, and were approved by local animal care authorities.

In the study group with concentrated oxygen exposure, the eyes treated with triamcinolone compared with the contralateral untreated eyes did not vary significantly in the count of apoptotic retinal cells in the inner nuclear layer in the central region [2.3±2.6 cells versus 3.5±3.0 cells; P=0.30; 95% confidence interval (CI): −1.19, 3.65] and in the peripheral region (9.4±12.2 cells versus 14.0±12.5 cells; P=0.37; 95% CI: −5.88, 15.05) nor in the outer nuclear layer in the center (13.2±15.8 cells versus 12.2±9.8 cells; P=0.85; 95% CI:

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−12.3, 10.3) and the peripheral region (27.6±17.5 cells versus 38.4±21.3 cells; P=0.19; 95% CI: −5.67, 23.35). In the control group, the count of apoptotic cells was significantly ($P < 0.05$) lower than in the study group [outer nuclear layer, central (3.7±2.6 cells) and peripheral (9.3±3.0 cells)].

Confirming a previous study [8], the results suggest that the experimental model of retinopathy of prematurity is associated with an increased amount of retinal apoptosis which was not markedly influenced by intravitreal triamcinolone. There are limitations of the study. One of them is that the vitreous of a young mouse is very well formed, and it is possible that there is less distribution of an intravitreally applied drug close to the retina than there might be in the more liquid vitreous of a diabetic patient or an elderly patient with macular degeneration. It may lead to a lower retinal drug toxicity in a young mouse than in patients with a more liquid vitreous. Another limitation of the study is that the retinopathy of the prematurity model has a high level of apoptosis. Therefore, possible minute changes in apoptotic effect through intravitreal triamcinolone may remain undetected. Since the rate of apoptosis was not markedly elevated in the triamcinolone group compared with the contralateral eyes, the data may serve as a hint for the safety of intravitreal triamcinolone. Since the rate of apoptosis was not markedly reduced, the data do not suggest a marked neuroprotective effect of intravitreal triamcinolone in this retinopathy of the prematurity model.

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