Quantification of latanoprost acid after subtenon application of a latanoprost-loaded drug depot

Kopp F[1], Eickner T[2], Falke K[1], Grabow N[2], Guthoff RF[1]

[1] University Medical Center Rostock, Department of Ophthalmology, Rostock, Germany

[2] University Medical Center Rostock, Institute for Biomedical Engineering, Rostock, Germany

Purpose

The efficiency of topical glaucoma treatment is impaired by poor patient adherence and low bioavailability. Local drug delivery systems, providing sustained release of pharmaceuticals over a prolonged period of time, are a promising new therapeutic approach. They can circumvent the need for patient adherence and potentially provide higher bioavailability. In this animal study, the drug release kinetics of a subtenon, *in situ* polymerizing, biodegradable, two-component delivery system, loaded with the hypotensive prodrug latanoprost (LP), were investigated.

Methods

Five female New Zealand White rabbits received subtenon injections of an LP-loaded polymer depot in the right eye under general anaesthesia (Fig. 1A, B). The polymer is a two-component system consisting of hyaluronic acid and an isocyanate functionalized ethylene glycol bis dilactic acid derivate, which were administered manually in a 1/1 (v/v) ratio (Fig. 1C). To assess drug release from the depot, ocular and systemic concentrations of latanoprost acid (LA) and latanoprost (LP) were quantified using liquid chromatography mass spectrometry (LCMS). For this purpose, aqueous humor was aspirated using a 30 G needle, tear fluid was collected with Whatman filter paper, and blood was drawn from the *arteria auricularis*, every three weeks. Intraocular pressure (IOP) was monitored for 6 weeks prior to and during the duration of the study. Measurements were conducted once a week using the Icare TAO1 (Finland) rebound tonometer. Upon sacrificing the animals, the tissue surrounding the injection site was collected and residual drug concentration measured with LCMS.

Results

The acid of latanoprost was detected 24 hours after polymer injection in aqueous humor of the treated eye (4.90 ± 4.49 ng/ml), but not the contralateral eye. In tear fluid, LA was quantifiable in both eyes for 6 weeks (0.09 – 0.64 ng/mg), and could be detected below the quantification limit up to 4 months post injection. Latanoprost was detected in aqueous humor (AH) of the treated and the contralateral eye 30 minutes after injection, 0.55 ± 0.24 ng/ml and 1.33 ± 0.82 ng/ml, respectively. Serum levels of LP after 30 minutes were 1.92 ± 2.00 ng/ml. Similar concentrations were also found after 24 hours in AH of both eyes as well as in serum. After 6 months, LP concentrations in AH and serum were below 0.1 ng/ml. LP was quantifiable in tear fluid of both eyes up to 10 weeks after injection (treated: 0.98 ± 1.14 ng/mg; contralateral: 1.95 ± 1.77 ng/mg) (Fig. 2). After the study duration of 38 weeks no residual latanoprost or latanoprost acid was detectable in a *post mortem* analysis of scleral and conjunctival tissue surrounding the injection site. The IOP did not change significantly after depot application.

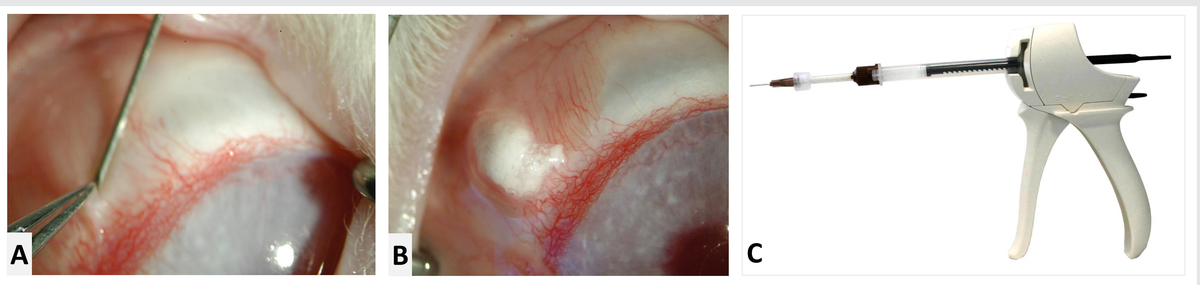

Fig. 1: (**A**) Injection in close proximity to *musculus rectus superior bulbi*. (**B**) Drug depot immediately after application. (**C**) Injection device consisting of a double chamber syringe with an extruding applicator and a 27 G needle placed in a gun-type dispenser.

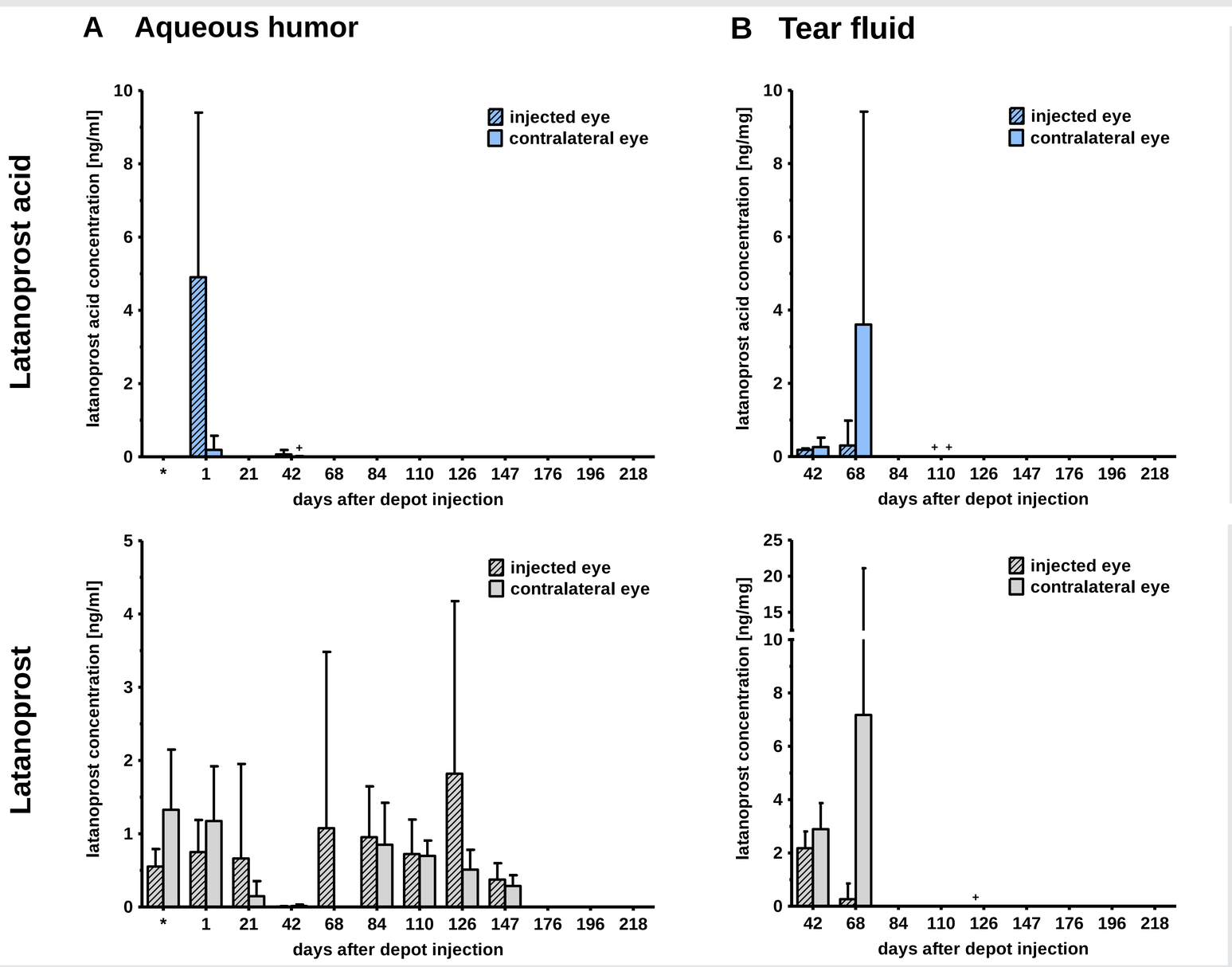

Fig. 2: Concentrations of latanoprost acid (LA; blue bars) and latanoprost (LP; grey bars) as determined with LCMS in the injected eye (hatched bars) and the contralateral eye (solid bars). (**A**) LA was detected in aqueous humor up to 6 weeks, while LP was present in aqueous humor of both eyes over the course of 21 weeks. (**B**) LA and LP were quantifiable in tear fluid of both eyes up to 10 weeks. Due to experimental difficulties sampling of tear fluid started on day 42 (**C**) LA was detectable in serum only up to 24 hours after injection, whereas LP was present in serum throughout day 147. Graphs depict mean ± SD for 5 animals, except for tear fluid on day 42 (n = 3; injected; n = 4 contralateral). * represents the time point 30 minutes after depot injection.
+ represents detection of LP or LA below the lower limit of quantification (LP: 0.01 ng/ml; LA: 0.1 ng/ml).

Conclusions

Detection of the biologically active compound latanoprost acid in aqueous humor and tear fluid proves that a subtenon, *in situ* polymerizing delivery system is a suitable drug delivery vehicle for the treatment of primary open angle glaucoma. The polymer depot provided continuous release of latanoprost in the ng/ml range over several months. After this proof-of-concept study further investigations will be conducted to adjust drug release kinetics to treatment requirements.

Kontakt: franziska.kopp@uni-rostock.de

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Potenzielle Interessenkonflikte:
1. nein, 2. nein, 3. nein, 4. nein, 5. nein