Pharmacokinetic and Pharmacodynamic Aspects of an Ophthalmic Pilocarpine Nanoparticle-Delivery-System

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The regional pharmacokinetics as well as the pharmacodynamics of pilocarpine-loaded nanoparticles for the treatment of glaucoma were investigated and compared to a solution of this drug. Polybutylcyanoacrylate nanoparticles were prepared by an emulsion polymerization process. Formulations with different drug concentrations (2–6%) as well as different particle concentrations were investigated and analyzed for size and drug loading. Drug binding to the particles was achieved at a level of 10–18% of the total drug content. The colloidal nanoparticles were sufficiently small (diameter: 100–300 nm) for a non-irritating application to the eye. All preparations were applied to the eyes of New Zealand white rabbits which were treated with betamethasone before to create an elevated intraocular pressure (IOP). Pilocarpine concentrations, assayed from aqueous humor using gaschromatography, increased by 23% (AUC) for nanoparticle suspensions compared to aqueous reference solutions. Additionally, tmax was prolonged and the elimination coefficient was significantly decreased. Pharmacodynamic effects such as miosis and IOP reduction were investigated. tmax values of aqueous humor concentration were observed to be in a similar time range as miosis tmax readings. It was found that at lower drug contents a more pronounced prolongation of miosis was achieved with nanoparticles versus a standard solution. The IOP-reduction was significantly prolonged with nanoparticles preparations; whereas maximum reduction was obtained with a reference solution after 1–2 hours, it was reached with nanoparticles at about 2–3 hours. Differences between nanoparticles and aqueous solutions were most pronounced at lower drug concentrations.

KEY WORDS: pilocarpine; glaucoma; nanoparticles; betamethasone; miosis; intraocular pressure.

INTRODUCTION

Human glaucoma is one of the most common ophthalmic diseases occurring in 2% of the population older than 40 years (1). Pilocarpine is still used by most ophthalmologists to initiate glaucoma therapy. New drugs with the same pharmacological profile, such as carbachol and aceclidine are also employed for medication (2). In addition, other classes of drugs were introduced in glaucoma therapy. Alpha-receptor mediation of IOP is achieved with dipivalylepinephrine, adrenaline and clonidine. The β-agonist isoproterenol was first shown to lower IOP almost 40 years ago (3). L-Ti-molol, a non-selective β-antagonist, was introduced clinically in 1978. The same pharmacological class of drug is also represented by carteolol and metipranol. Carbonic anhydrase inhibitors, especially acetazolamide and methazolamide have been given orally to lower IOP in man for 40 years.

Despite these available therapeutics, the treatment of glaucoma needs to be improved since topical aqueous ophthalmic preparations like pilocarpine eyedrops only achieve a bioavailability between 1 and 3% and must be applied frequently (4). Unfortunately in the case of pilocarpine the major part of the drug does not penetrate into the eye, but it is lost by physiological drainage. This effect is generally described as non-productive loss (5). This loss and, subsequently, a nasal or oral absorption of the drug becomes responsible for systemic side effects like salivation, lacrimation, sweating, and nausea setting the limits for ophthalmic treatments.

Recent investigations have shown the potential of polybutylcyanoacrylate (PBCA)-nanoparticles as ophthalmic drug delivery system (6) with improved drug action. Applications were demonstrated for antiglaucoma drugs e.g. pilocarpine (7,8). Despite the fact that a prolonged drug action and an antiglaucomatos effect was observed in different glaucoma models (9), the relation between the pharmacokinetic and the pharmacodynamic response provoked by pilocarpine administered with nanoparticle preparations remained unknown.

Therefore the goal of this study was to evaluate in animals with an artificially induced glaucoma using the betamethasone model (10): I. ophthalmic aqueous humor pharmacokinetics of pilocarpine nitrate delivered by nanoparticles at different drug and nanoparticle concentrations, II. pharmacodynamic responses such as miosis and IOP-reduction, and III. possible relations between pharmacokinetic and pharmacodynamic effects.

MATERIALS AND METHODS

Nanoparticle Preparation

The particles containing pilocarpine nitrate were prepared as described earlier (9,11). Briefly, an anionic emulsion-polymerization was performed in an aqueous solution of 50 ml 0.01 M HNO3 (preparations see Table I). Pilocarpine nitrate and poloxamer 188 were dissolved completely. The solution was stirred with a magnetic stirrer at 300–500 rpm while 2-butyl-cyanoacrylate (BCA) was added slowly. Stirring was continued for 4 h, and the resulting nanoparticle suspension subsequently was buffered with 0.1 M NaOH to pH 6. In order to complete the reaction, stirring was maintained for 30 min. The suspension was purified by filtration through a G1 glass filter (Schott, Mainz, Germany), freeze dried (LyoVac GT 2, Leybold, Germany) and stored at 4°C in a refrigerator until use. Prior to application, the nanoparti-
icles (PBCA-nanoparticles) were resuspended in a aliquot of Michaelis-acetate-buffer, pH 5.45, by sonification (Bandelin RK106, Berlin, Germany) for 30 min.

**Particle Size**

The size of PBCA-nanoparticles was determined by photon correlation spectroscopy (PCS) (BI-90, Brookhaven Instruments Corporation, Holtsville, USA; Laser: 5 mW, HeNe). Samples were prepared as follows: the nanoparticle suspension was diluted with distilled water (HPLC grade, particle free filtered with a 0.2 μm filter, Millipore, Eschborn, Germany) between 1:100 and 1:1000 depending on the optimum count rate of the instrument. The samples were sonicated to minimize aggregation before taking the measurements (Bandelin RK106, Berlin, Germany).

**Drug Loading**

After preparation the nanoparticle suspensions were filtered through an Anotop 10, 0.02 μm, disposable syringe filter (Merck, Darmstadt, Germany). The filtrate was diluted with distilled water 1:100. The pilocarpine content in the particle-free filtrate was determined by UV-spectroscopy (Beckman DU-7, Fullerton, CA, USA). The measurements were carried out relative to a blank sample prepared by the same procedure without pilocarpine nitrate. The absorption at 215 nm was used to calculate the drug content. For quantification of the drug which was bound to nanoparticles the data were compared to measurements with reference solutions containing 2, 4 and 6% pilocarpine nitrate.

**Betamethasone Model**

For all in vivo experiments New Zealand white rabbits, age approximately 1/2 to 1 year, weight 3–4 kg (Hoechst AG, Frankfurt a.M., Zentrale Toxikologie Kastengrund, Germany) were used. The animals were placed in restrainer boxes and a local anaesthetising pretreatment followed by dosing 50 μl of a proxymetacain- HCl solution (5,5 mg/ml) (Ophthamik, Pharm-Allergan GmbH, Karlsruhe, Germany) into the left cul-de-sac. After 5 min, 0.4 to 0.8 ml of a betamethasone : disodium-betamethasone-21-dihydrogenphosphate = 4 : 1.32 mg/ml crystal suspension (Betnesol-Kristall suspension, Glaxo GmbH, Bad Oldesloe, Germany) were injected under the left conjunctiva (subconjunctivally) of the elevated lower lid. A visible bubble of the suspension remained forming a depot. The treatments were repeated weekly over a period of three weeks until an elevated IOP was achieved (10). Animals which did not respond to the treatments were not used in further experiments. In some cases, according to the physical conditions of the rabbits, the injections were continued for an additional week to stabilize the IOP.

**Miosis Measurements**

New Zealand white rabbits as specified above were used for the miosis measurements. The pupillary diameter was measured by using a video system (Panasonic NV-M7, VHS-HQ, Matsushita, Japan) under standard lighting conditions (15–20 lux), while the animals were placed in restrainer boxes (Techni Plast, Italy). The focus of the camera was adjusted to the iris and a metric measure tape (1 cm) was mounted under the eye in the same optical section.

Measurements were performed with 8 to 10 animals. 25 μl of the nanoparticle preparations as well as the reference solutions were applied with a pipette into the everted conjunctival sack of the left eye and both lids were gently pressed together in order to minimize loss of the dosage form for a few seconds.

Pupil pictures were recorded after baseline readings were employed prior each series of measurements. The pupil size was measured using the still picture frame function of a video tape recorder (AKAI, VS-767, AKAI, Egelsbach, Germany) and was calculated in relation to the measure tape.

**Aqueous Humor Sampling Procedure**

Dosage forms were applied as outlined for the miosis measurements. For each preparation 4 to 6 animals were used. The animals were rapidly sacrificed by injection of 1 ml T6l (Hoechst AG, Frankfurt, a.M., Germany) into the left ear vein. The anterior chamber of the eye was punctured with a cannula (26G × 12mm Nr. 18, Terumo Europ-N.V., Belgium) and the aqueous humor was withdrawn with a syringe. In general, 200–300 μl liquid was obtained and the samples were stored in a freezer at −25°C until further analytics were performed.

**Pilocarpine Determination**

Due to sensitivity and selectivity, capillary gaschromatography (Hewlett Packard 5890 series II, Integrator: HP 3396A, HP-1 Methyl Silicone, 5 m × 0.53 × 2.65 mm film thickness, Hewlett Packard, Böblingen, Germany) equipped with a nitrogen selective detector (NPD) was used for the pilocarpine assay.

The aqueous humor samples were processed as follows: the frozen samples were thawed and divided in two aliquots each containing 100 μl aqueous humor. Each aliquot was filled into a 3 ml extraction vial (Alltech, München, Germany). To one aliquot 50 μl of an isopilocarpine solution (1 μg/ml isopilocarpine) was added as internal standard. 600 μl sodium hydrogen carbonate solution (30 mg/ml sodium hydrogen carbonate) and 1 ml dichloromethane were finally added. Analogously, to the second aliquot 50 μl distilled water was added instead of the 50 μl isopilocarpine solution. Both samples were extracted for 1 min by shaking and the organic phases were transferred each into a clean vial. Dichloromethane was evaporated under nitrogen at room temperature until approx. 10 μl remained. From each vial 3 μl of the residual organic phase was injected splitless.

The specified GC method was developed for the pilocarpine assay (He carrier 3.5 ml/min, splitless inj., temp. 75–250°C, rate 18°C/min). As described above each sample was divided into two parts in order to subtract the aqueous humor isopilocarpine content from the amount that was added as internal standard.

**IOP Measurements**

Readings were performed using a pneuma tonometer as commonly used for human ophthalmic examinations (Digilab Modular One, Pneuma Tonometer, Bio-Rad Ophthalmic De-