Aqueous humor and serum penetration of tacrolimus after topical and oral administration in rats: an absorption study

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Purpose: To investigate the penetration of tacrolimus to the aqueous and serum after topical and oral administration.

Methods: Thirty Wistar rats were divided into three groups. In the first group (n = 12), a single dose of 0.3 % isotonic tacrolimus solution was applied topically to the rat eyes. The second group (n = 12), received 0.1 mg/kg/day tacrolimus for three days. In control group (n = 6), the rats were administered a single drop of 0.3% salin solution topically. Following collection of samples, the amounts of tacrolimus in aqueous and serum samples were compared with each other.

Results: In group I, the mean concentration of tacrolimus was 35.16 ng/ml in aqueous and 2.22 ng/ml in serum. In group II, the mean concentration of tacrolimus was 13.08 ng/ml in aqueous and 13.45 ng/ml in serum. There was no significant difference in the serum concentration levels of tacrolimus between group I and control group. However there was a significant difference of the drug concentrations in aqueous between the groups (p < 0.001). The mean aqueous and serum concentrations of tacrolimus in group II, differed significantly from the mean concentrations in group I (p < 0.001) and the control group (p < 0.001).

Conclusion: Tacrolimus may be a promising treatment modality in intraocular inflammation by topical application besides systemic administration.

Keywords: FK506, humor aqueous, tacrolimus, penetration into the eye, systemic application, topical application

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Introduction

Tacrolimus (FK506) is a macrolide lactone isolated from the culture broth of *Streptomyces tsukubaensis* (Kino, Hatanaka, Hashimoto, et al 1987). It has been shown in vitro and in vivo to be a potent immunosuppressive agent. It is 100 times more powerful than cyclosporin in vitro in inhibiting T-cell proliferative responses, including mixed lymphocyte reactivity and cytotoxic T-cell generation (Kino, Hatanaka, Miyata, et al 1987; Sawada et al 1987). Preclinical studies of FK506 showed prolonged corneal allograft survival in various animal models (Koyabashi et al 1989; Nishi et al 1993; Mills et al 1995; Okada et al 1996; Hikita et al 1997; Reis et al 1998). FK506 has shown promise in the management of corneal and limbal grafts (Dua and Azuara-Bianco 1999; Sloper et al 2001). It suppresses endotoxin-induced uveitis and autoimmune uveitis in experimental uveitis models (Hikita et al 1995; Kawashima et al 1998; Whitcup et al 1998). FK506 was effective in the treatment of human noninfectious uveitis as well (Mochizuki et al 1991; Ishioka et al 1994; Kilmartin et al 1998; Sloper et al 1999).

The efficacy of FK506 for uveitis led us to investigate if the drug can reach therapeutic levels in the eye following topical and systemic application. The first animal studies on the penetration of topical FK506 have been published by using liposomal and oil formulations of FK506 (Pleyer et al 1993).

The purpose of this experiment was to determine the penetration of tacrolimus into the rat aqueous humor and serum after topical and oral administration. For topical administration, FK506 solution which was dissolved in castor oil and ethanol was used.

Methods

Thirty adult male Wistar rats were used in this study. All animals were obtained from Ankara University Faculty of Pharmacy (Ankara, Turkey) and housed in environmentally controlled rooms with a 12-h light and dark cycle. All procedures were performed according to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. Food and water were given ad libitum. The rats were randomly assigned to one of 3 groups. The first group (n = 12 rats), received only one drop of 0.3% FK506 in one eye chosen at random. Drops were applied using a micropipette with each drop containing 7 μl (Hikita et al 1997). 0.3% FK506 drops (Hikita et al 1997) were prepared from commercially available tacrolimus concentrate for infusion containing 5 mg tacrolimus, castor oil, and ethanol. The solution was preserved in a sterile dark colored bottle and below 25 °C. The second group (n = 12 rats), received 0.1 mg/kg/day tacrolimus for three days. The drug was given via oral gavage twice daily (bid). The control group (n = 6 rats), was given 7 μl drop of 0.3% saline solution in one eye chosen randomly.

Intraperitoneal injection of ketamine was used to kill the animals ten minutes after topical application of the drops and two hours after oral administration (the mean peak concentration (t_{max}) time of tacrolimus is approximately 1–2 h). The eyes which received topical drops were carefully rinsed with normal saline and dried with filter paper to remove remaining drug before obtaining the samples. Aqueous humor samples were obtained by inserting a 27-gauge needle on a tuberculine syringe into the anterior chamber from the eyes. A blood sample was obtained by intracardiac puncture. Serum was collected by centrifugation of the blood sample. Tacrolimus concentrations in all samples were measured by microparticle enzyme immunoassay (MEIA).

Differences between groups were evaluated using the Kruskal-Wallis test for the global comparison and multiple comparison test for paired comparison.

Results

Tacrolimus concentrations were determined in aqueous humor and serum at 10 min after topical and 2 h after systemic administrations. In all experiments no ocular side effects were observed in animals receiving topical drops. In group I, (topical tacrolimus applied eyes), the mean concentration of tacrolimus was 35.16 ± 4.26 ng/ml (31–42 ng/ml) in aqueous humor. The serum levels ranged between 1.3 to 7.6 ng/ml with the mean being 2.22 ± 3.14 ng/ml in this group. In the second group, (oral tacrolimus-given rats), the mean concentration of tacrolimus was 13.08 ± 1.08 ng/ml (10–17 ng/ml) in aqueous humor and 13.45 ± 5.5 ng/ml (9.3–20.8 ng/ml) in serum.

Negative controls were performed on serum and aqueous humor from animals receiving topical saline solution. No measurable FK506 drug concentrations were detected in aqueous humor and serum in this group.

The mean drug concentrations in aqueous humor differed significantly between all groups ($p < 0.001$). However, there was no significant difference in the serum concentrations between group I and the control group. The mean aqueous humor and serum concentrations of tacrolimus in group II differed significantly from the mean concentrations in group I ($p < 0.001$) and the control group ($p < 0.001$).

Discussion

The results of this study indicate that topically applied tacrolimus resulted in high concentrations of the drug in aqueous humor, but low levels of tacrolimus in serum in rats. An additional finding is that significantly higher drug concentrations were obtained in aqueous humor and serum after oral administration.

Animal studies indicate tacrolimus is widely distributed into most tissues including the lungs, spleen, heart, kidney, pancreas, brain, muscle, and liver. It is present in breast milk at similar levels to those reported in the plasma (Scott et al 2003). Since the greater the lipid solubility coefficient of a substance, the greater its ability to penetrate the blood-aqueous barrier, tacrolimus is expected to pass the blood-aqueous barrier easily. In our study, the aqueous humor concentrations of tacrolimus were high and close to serum concentrations in the oral tacrolimus group.

FK506 has been shown to be a potent immunosuppressive agent in several models of organ transplantation and in clinical practice. The concentrations required to control immune-mediated diseases of the eye have not been established. However, organ transplantation studies show serum concentrations of tacrolimus for immunosuppressive effect should be in the range of 5–15 ng/ml (Pirsch et al 1997) which can be considered the effective target organ concentration to control immune response. In our study, the serum concentration was 2.22 ± 3.14 ng/ml after topical and 13.45 ± 5.5 ng/ml after oral administration.

Tacrolimus is poorly absorbed from the gastrointestinal tract (Honbo et al 1987). However, a solid dispersible formulation of tacrolimus in hydroxypropyl methylcellulose was developed for clinical use which has rapid oral absorption and good stability (Honbo et al 1987). We used 0.1 mg/kg/day tacrolimus which has this formulation by oral route and obtained therapeutic concentrations in aqueous humor and serum.

FK 506 is a 23-membered macrolide lactone with a molecular weight of 822 g/mol. This hydrophobic compound has low solubility in aqueous solutions but it dissolves readily in alcohols and some oils (Kino, Hatanaka, Hashimoto, et al 1987). In our experiment, tacrolimus solution which was dissolved in castor oil and ethanol was used for topical application.

Pleyer and colleagues (1993) investigated the systemic and ocular absorption of topically applied FK506 in rabbits. They used liposomal and oil formulations of FK506. They detected low intraocular drug concentrations after the topical application of FK506 dissolved in olive oil but obtained higher drug concentrations with liposome-bound FK506 in aqueous humor and vitreus. Liposomes increase corneal drug absorption and have tendency to accumulate in the conjunctival folds (Fitzgerald et al 1987). Conjunctival and scleral penetration is important in delivering poorly absorbed drugs to intraocular tissues.

The penetration of topical and systemic tacrolimus into aqueos humor reached a high concentration in our study. This result is different from the findings with the topical application of FK506 dissolved in olive oil (Pleyer et al 1993). This difference may be due to combination of castor oil and ethanol in formulation we used in our study. This particular drug formulation penetrated well into the eye compared with only oil-based formulations. Since tacrolimus is very soluble in alcohols (Kino, Hatanaka, Hashimoto, et al 1987; Tanaka et al 1987), the addition of ethanol to castor oil increased its solubility.

Side effects of the drug include kidney damage, tremors, high serum pressure, diabetes, high serum potassium, headache, insomnia, confusion, and seizures. Cosmetic complications such as gum hyperplasia and hirsutism are less commonly associated with tacrolimus than cyclosporin (Reyes et al 2000). Local ocular immunosuppression provides adequate therapeutic level of local immunosuppressive activity while avoiding the risk of generalized immunosuppression and systemic adverse reactions. In addition, serum levels of tacrolimus have to be measured regularly in systemic use because of variable absorption after oral administration.

From the data observed in the present study, we conclude that high aqueous humor and serum levels of tacrolimus were obtained after systemic, and high aqueous levels were also obtained after topical administration. The drug concentrations are between the minimal effective concentration levels (5–15 ng/ml) to control the immune response in immune-mediated eye diseases.

This experiment was designed as an absorption study not a pharmacokinetic study. Drug levels at different time courses were not studied. Further studies to obtain a time course of the drug levels following administration would be useful how long were these drug levels maintained.

Acknowledgments

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