Ocular Penetration of Cyclosporin A

The Rabbit Eye

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In the rabbit, after oral ingestion of 20 mg/kg/day of cyclosporin A (CsA) a high level of the drug is found in the blood. This level increases steadily during the first 3 days, leveling off by days 5–7. Although the blood level of CsA was within the “therapeutic window” of 400–600 ng/ml, the drug was not detected within the ocular tissues. Local application using 2% CsA in olive oil induced a high concentration of the drug in the cornea and conjunctiva but no detectable levels within the intraocular structures. When a significant intraocular inflammation is induced in one of the rabbit eyes, CsA ingested orally reaches detectable levels in nearly all tissues of the inflamed eyes. Highest concentrations of the drug were observed within the chorioretinal complex in these eyes. In the contralateral (noninflamed) eyes, however, no detectable CsA was found either intraocularly or extraocularly. In the significantly inflamed eyes, local application of the drug induced a high CsA level within the anterior segment only, without any detectable levels within the choroid and retina. Invest Ophthalmol Vis Sci 31:1362–1366, 1990

Unlike most immunosuppressive drugs, cyclosporin A (CsA), in pharmacologic concentrations, has no cytotoxic effect on the lymphocytes. It appears that its mode of action is based on the ability to inhibit the synthesis of Interleukin-2 (IL-2) mRNA by the T-helper lymphocytes. Thus, the T-helper functions and the generation of cytotoxic T cells are aborted in the presence of CsA without any significant influence on the other activities of the immune system. As the result of the specific mode of action of CsA, the B cell, T-suppressor cell, and macrophage functions remain largely unaffected. Therefore, CsA induces a state of immunomodulation rather than immunosuppression. Because of these characteristics, CsA rapidly became the drug of choice for the inhibition of graft rejection. Nussenblatt et al first reported on the inhibition of experimental autoimmune uveitis (EAU) in rats. These observations paved the way for investigations regarding the potential therapeutic effects of CsA in patients with endogenous uveitis and Behcet’s disease refractive to conventional therapy.

Soon, however, it became evident that systemic treatment with CsA is not without side effects, the most worrisome being nephrotoxicity. Therefore, studies regarding the possible use of CsA eye drops or ointment for ocular diseases were initiated. Instillation of CsA eye drops inhibited the rejection of corneal grafts in poor-risk patients and the drops were very beneficial in the treatment of severe vernal keratoconjunctivitis. We and others (Nussenblatt RB: Personal communication, 1987), however, did not find any effect of CsA local drops on uveitis. These observations raised crucial questions concerning the ocular penetration of CsA. Conflicting results regarding the intraocular levels of CsA after local instillation of the drug have been published.

We report herein our observations regarding the CsA levels attained in the rabbit eye after various routes of drug administration.

Materials and Methods

Mongrel male and female rabbits weighing 3–4 kg were used. The ARVO Resolution on the Use of Animals in Research was followed.

Routes of Cyclosporin A Administration

Oral administration: For oral administration, 20 mg/kg/day from a 10% solution (100 mg/ml) in olive oil was given with a syringe with an esophageal cannula.

Local instillation: One drop from a 2% solution (20 mg/ml) in olive oil was introduced in the lower cul-de-sac three times daily.

Assessment of CsA tissue levels: Unless otherwise stated, all samplings for assessments of CsA levels...
were made after at least 1 week of continuous daily treatment. For the rabbits taking oral ingestion, sampling was performed 12 hr after the last dose. For those under local treatment, tests were performed 2 hr after the last scheduled drop of CsA. Blood samples were obtained from the marginal ear vein. Tapping of the anterior chamber was performed with a 30-G needle after thorough washing of the eyes with isotonic saline solution and local anesthesia with 2% benoxinate HCl (Novesine).

For evaluation of the various ocular tissues, the rabbits were killed by intravenous injection of 1.0 ml/kg of a 6% solution of sodium Pentothal® (Abbott Hospital Products, North Chicago, IL). Five minutes after the injection, the eyes were washed three times with isotonic saline. Blood and aqueous humor samples were immediately obtained and the eyes enucleated.

After enucleation, the globes were immersed three times into a solution of isotonic saline. Rapid and careful dissection of the various ocular components followed. Attempts to remove whole tissue from each enucleated eye were made. The separated tissues were thoroughly rinsed in buffered saline. The cornea and sclera were initially minced with scissors, and all tissues were incubated overnight in 2 ml buffered saline 0.25% Triton X-100® (Packard Instrument Company, Meriden, CT). After incubation, the tissues were homogenized in two steps using first a hand homogenizer and finally an electric microhomogenizer (Sorvall, Newton, Connecticut). The homogenates were centrifuged and the supernatants collected.

In all experiments, radioimmunoassay (RIA) kits with nonspecific polyclonal antibodies or RIA kits with monoclonal antibodies (Sandoz, Basle) were used for the assessment of CsA levels. However, because the purpose of this study was to determine the concentration of CsA (including any fraction of it that had been metabolized), only the data obtained with the nonspecific polyclonal RIA kits detecting both the parent drug and most of its derived metabolites are illustrated and discussed.

To enhance accuracy of CsA levels evaluation, we used separate standard curves for each of the tissue extracts, whole blood, aqueous humor, and vitreous. These curves were obtained by diluting a standard concentration of CsA in the corresponding tissue extract supernatants or fluids obtained from nontreated control animals. Determinations of CsA levels within the experimental ocular tissues and fluids were made by reference to the appropriate standard curves for each tissue extract or fluid.

To evaluate the ocular penetration of CsA in inflammatory conditions, 100 ng or 200 µg of lipopolysaccharide (LPS from Escherichia coli) was injected into the vitreous of the right eye of rabbits, inducing a mild (100 ng) or severe (200 µg) uveitis. In these cases, the CsA levels in inflamed (right) eyes and in the noninflamed (left) eyes were compared. The severely inflamed rabbit eyes also showed corneal changes, including edema and epithelial damage.

For each evaluation, three rabbits were used and the whole experiment was repeated four times. Twice, the levels of CsA were evaluated openly, and twice the evaluations were performed in a masked manner. There were no significant differences in the levels observed with the use of the open or masked evaluations. Therefore, all results are represented as the mean and standard error of all evaluations of the same experiment.

Results

In the normal rabbits, significant CsA blood levels were detected 3 hr after the rabbits ingested an oral dose of 20 mg/kg. The blood levels were slightly lower during the first day, increasing thereafter. During the same period, no CsA was measured in the aqueous humor of these rabbit eyes (Fig. 1). After a thrice daily instillation of 2% CsA eye drops, no detectable blood or aqueous humor levels of CsA were observed during the 7 days of this experiment (Fig. 1).

After 1 week of oral treatment, the aqueous humor, cornea, conjunctiva, sclera, iris, lens, vitreous, choroid, and retina were devoid of measurable CsA levels (Fig. 2). After instillation of eye drops, significant levels of CsA were detected only in the cornea and conjunctiva. In the sclera, low levels of 40–60 ng/ml were measured in most eyes. The other intraocular tissues had no detectable levels of CsA (Fig. 3).

Induction of mild uveitis (100 ng of LPS) did not change the pattern of CsA distribution in the various
ocular tissues when the drug was administered locally or after oral treatment.

After the intravitreal injection of 200 μg of LPS in the right eyes and the induction of significant intraocular inflammation, a different pattern of CsA distribution within the ocular tissues was observed. Figure 4 illustrates the results obtained in rabbits that ingested the drug orally. In these cases, the blood levels were similar to those observed in normal rabbits. In the ocular tissues, however, there was a significant difference between the right (inflamed) eyes and the left (noninflamed) eyes. In the eyes with inflammatory reaction, high levels of CsA were detected in the aqueous humor and chorioretinal tissues. Detectable levels of CsA were also found in the cornea, conjunctiva, iris, and vitreous of the inflamed eyes. In tissues obtained from the noninflamed (left) eyes, no detectable levels of CsA were found (Fig. 4).

When treatment with CsA was given locally, significant CsA levels were found in the cornea, conjunctiva, aqueous humor, and iris of the inflamed eyes (Fig. 5). In the noninflamed eyes, high CsA levels were observed in the cornea and conjunctiva only.

The levels of CsA in the sclera were lower, with similar levels observed in inflamed and noninflamed eyes of those rabbits treated locally.

The CsA blood and aqueous humor levels in normal rabbit eyes and in eyes with mild or severe intraocular inflammation are illustrated in Figures 6 and 7. One can see that oral ingestion induced high blood CsA levels in all rabbits, whereas aqueous humor levels were observed only in significantly inflamed eyes (Fig. 6).

After local instillation of the CsA, no detectable blood levels could be observed in any of the rabbits. Detectable levels of CsA in aqueous humor were observed only in eyes with significant intraocular inflammation. No CsA was found in the aqueous humor of normal eyes, mildly inflamed eyes and their contralateral eyes, or the left eyes of rabbits with severe inflammation of the right eyes (Fig. 7).

Discussion

After the rabbits ingested 20 mg CsA/kg/day orally, the drug was not detected in ocular tissues of healthy
rabbit eyes. Although the lack of CsA within the avascular ocular tissues in these rabbits is readily understandable, because of the high CsA concentration in the blood its lack within the vascular tissues and especially within the choroid and retina is puzzling. These findings may be reconciled if one assumes that because of the very low blood content within the extracts of these ocular tissues the CsA concentration remains below the level of sensitivity of the RIA method (25–30 ng/ml). This latter postulation is reinforced by our recent observations using radioactive CsA and testing its penetration within the rat eye. In these experiments, radioactive CsA is detected within the choroid and retinal complexes. Topical application of 2% CsA in olive oil solution to normal rabbit eyes, while failing to induce any detectable levels of the drug within the eye, showed significant concentration in the cornea and conjunctiva and lower levels in the sclera. These data appear contradictory to the previously reported results using either a 10% CsA ointment or 1% solution in olive oil and are in line with the findings of Wiederholt et al regarding the intraocular penetration of CsA. Of course, it can be argued that our method of CsA evaluation using the RIA method was inadequate and unable to detect the relatively low levels of CsA that do penetrate the eye. Our findings of detectable CsA levels in the significantly inflamed rabbit eyes and the corroborating results regarding the lack of intraocular penetration of topically applied CsA in normal rat eyes with the use of a radiolabeled drug would dispute this argument.

Because of these apparent discrepancies, we have carefully repeated our studies four times. In all experiments, no detectable levels (concentrations higher than 25–30 ng/ml) of CsA could be observed within the normal rabbit eye when the drug was administered orally for 7–14 days at a dose of 20 mg/kg/day. Topical application of CsA in our experiments failed to show any significant penetration within the eye beyond the cornea, conjunctiva, and sclera. The discrepancies between our findings and those of others may be explained by the lack of appropriate standard curves for each tested tissue used in the study by Moesteller et al and the possible radioactive contamination of the ocular tissues in the results reported by Kaswan.

Induction of mild intraocular inflammation had no influence on the CsA ocular distribution after local application of the drug. After oral ingestion, detectable levels of CsA were found in the aqueous humor of only one of the mildly inflamed eyes and in none of the noninflamed contralateral eyes. However, after induction of significant intraocular inflammation, CsA has been detected in most of the extraocular and intraocular tissues of all inflamed eyes of the rabbits treated orally. Highest concentrations of CsA have been detected in the chorioretinal complex and in the aqueous humor. Measurable low levels of the drug have also been found in every intraocular tissue of the inflamed eyes. The high levels of CsA in the blood achieved by the oral ingestion of 20 mg/kg/day and the "leaky" nature of the vessels within the inflamed eyes (after the breakdown of the blood retinal barrier) are probably responsible for the high ocular concentration of CsA in these cases. It is interesting that the contralateral (noninflamed) eyes of these rabbits demonstrated a pattern similar to that observed in normal rabbit eyes. At least in our experiments, the "recirculation effect" observed by others did not take place even when significant intraocular inflammation (uveitis) was induced in one of the eyes.

After local application of CsA, the inflamed eyes showed high CsA levels in aqueous humor and iris but no detectable levels within the vitreous or chorio-
retinal complex. The finding of high levels in the aqueous humor of the significantly inflamed eyes is understandable in view of the fact that in these eyes the corneas were edematous and the epithelial surface was damaged. However, the lack of detectable CsA within the posterior segment is unexpected. Maybe the increased systemic reabsorption into the leaky vascular system of the anterior segment of these inflamed eyes and the aqueous humor flow and the lens barrier are responsible for the poor diffusion of CsA to the posterior segment. Similar observations of high CsA levels within the anterior segment along with the lack of detectable levels in the posterior segment have been made in our laboratory with the use of radiolabeled CsA and the rat eye as a model. 19

From the data obtained in this study, it is clear that the presently available formula of 2% CsA in olive oil solution cannot be used for the routine treatment of posterior uveitis even in severely inflamed eyes. An appropriate hydrophilic vehicle (adequate liposomal preparation) or changes within the CsA molecule may, in the future, enhance the ocular penetration of the present lipophilic formula. Experiments on this line are being designed.

Key words: cyclosporin A, uveitis, ocular, inflammation, aqueous humor

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References