Ocular Aspirin Distribution: A Comparison of Intravenous, Topical, and Coulomb-Controlled Iontophoresis Administration

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Purpose. To evaluate the pharmacokinetics and safety of aspirin delivered by a single, noninvasive, transscleral, coulomb-controlled iontophoresis (CCI) treatment; topical application; or by one intravenous (IV) injection.

Methods. Forty-one adult New Zealand White rabbits received either a single IV injection, topical, or CCI administration of aspirin at a concentration of 10 mg/mL. Histologic evaluation was performed in four CCI-treated eyes to assess safety. Pharmacokinetic distribution in all ocular tissues and fluids was studied at 0.5, 1, 2, 4, 6, and 8 hours after the treatments. Immediately after death, the eyes were dissected and salicylic acid (SA) concentration was determined by HPLC analysis. Blood was sampled at 0.5, 1, 2, 4, 6, and 8 hours, and plasma SA levels for systemic distribution were measured by HPLC analysis.

Results. No tissue damage was observed clinically or histologically. SA was found in all tissues and fluids throughout the study period of 8 hours. The highest concentrations of SA were observed with CCI immediately after treatment for all tissues and were the highest SA tissue peaks obtained by the studied delivery methods. IV administration demonstrated a delayed tissue peak of salicylate at 2 hours after administration. At 8 hours, ocular SA concentrations were in the same range for CCI and IV administration. IV injection resulted in blood plasma levels up to 28 times higher than CCI and remained significantly elevated until 8 hours after the treatments.

Conclusions. CCI is a safe and effective method of administering aspirin to the eye while avoiding the systemic side effects associated with IV injection. (*Invest Ophthalmol Vis Sci.* 2002; 43:3299–3306)

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs in medicine. Their action of inhibiting prostaglandin (PG) synthesis by blocking the key enzyme cyclooxygenase has been demonstrated in numerous studies and is the basis for their use as antipyretic and analgesic drugs.[1–2] Multiple in vitro and in vivo studies have demonstrated that PGs, the biologically active metabolites derived from the arachidonic acid cascade, are produced in such ocular tissues as the cornea,[3–4] iris,[5] retina,[6–7] and optic nerve.[8] PGs have been found to act as chemical mediators in a variety of pathologic conditions of the retina, including retinal detachment,[9] diabetic retinopathy,[10] and retinopathy of prematurity.[11] PGE_2 has been implicated in directly inducing angiogenesis[12–14] and through induction of VEGF and bFGF in Müller cells.[15] In addition, vitreous levels of PGs have been detected in cystoid macular edema.[16–18]

Although PGs are implicated in the pathophysiology of various ocular disease processes, the usage of NSAIDs is relatively limited in ophthalmology. It is possible that the restricted utility of NSAIDs in ophthalmology is due to the inability of current delivery methods to transfer drug into the appropriate ocular tissues efficiently. Pharmacokinetic studies focusing on different methods for administration of NSAIDs to the eye, such as topical, subconjunctival, or systemic application demonstrate high drug concentrations in the anterior chamber, but low or insignificant amounts in the posterior segment.[19–21] NSAIDs are the established treatment for many clinical disorders, such as cystoid macular edema,[22] allergic conjunctivitis, scleritis, and episcleritis. Topical drug administration, although the most common method of administration, results in subtherapeutic levels for the treatment of posterior segment diseases.[23–24] Attempts to solve this problem have been focused on developing different administration techniques, including drug carrier systems such as nanoparticles, nanocapsules, or NSAID silicon oil emulsions.[25–26]

Of special interest to our laboratory was the development of a system of drug administration capable of obtaining therapeutic drug concentrations in the entire eye while avoiding systemic exposure. Therefore, we investigated topical noninvasive coulomb-controlled iontophoresis (CCI) drug delivery,[27] because it offers a defined and controlled administration of NSAIDs to the anterior and posterior segments of the eye.

Iontophoresis, the transfer of charged drug molecules across tissues through an electric field, appears to be a useful means of application of NSAIDs. Numerous studies have already documented the enhanced delivery of various drugs into the anterior and posterior segments of the eye by using iontophoresis.[28] Water-soluble low-molecular-weight drugs, which are in their ionized form at physiological pH, are preferable for iontophoretic administration in ophthalmic therapy.

From an electrochemical standpoint (low molecular weight [180.16] and a negative logarithm of acid ionization [pK_a] of 3.5) aspirin was an ideal candidate for this pharmacokinetic study. Clinically, aspirin is of interest as a potential ocular

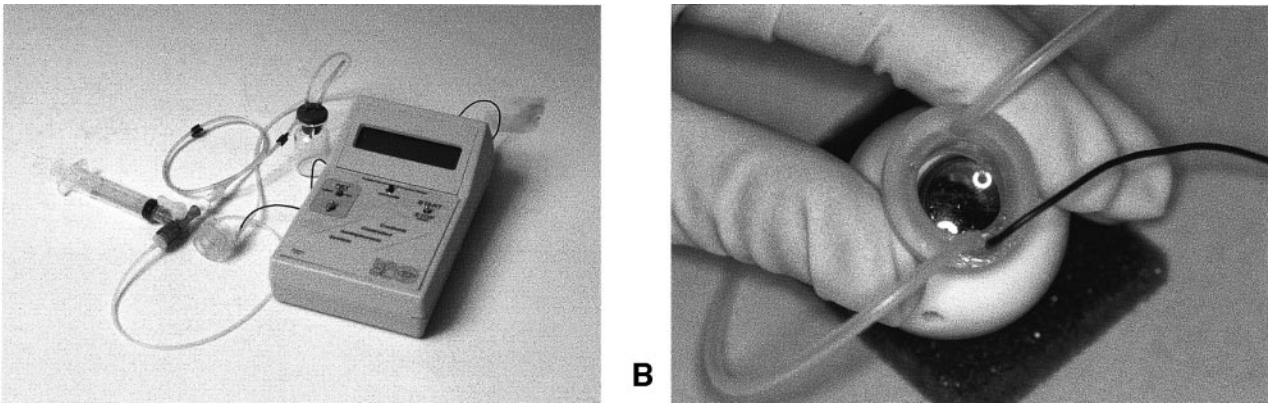

FIGURE 1. CCI system. **(A)** Battery-operated programmable control unit connected to the ocular applicator with fluidic lines connected to a syringe and the drug reservoir. **(B)** Autoclavable silicone rubber CCI ocular applicator shown on a plastic model eye.

therapeutic agent, as suggested by Kahler et al.,[29] who studied acetylsalicylic acid's inhibition of fibroblast growth-promoting activity of intraocular fluids in patients with proliferative vitreoretinopathy. They demonstrated that the growth-promoting activity of intraocular fluid in proliferative vitreoretinopathy was significantly antagonized by inhibition of cyclooxygenase with acetylsalicylic acid (ID_{50} = 0.9 μg/mL).

The ability of a drug delivery system to administer therapeutic ocular acetylsalicylic acid concentrations is of crucial importance to its usage as a treatment modality. Therefore, the determination of the in vivo efficacy and safety of ocular aspirin delivery by CCI in comparison with topical administration and systemic intravenous (IV) injection was the purpose of the present study.

MATERIALS AND METHODS

The study protocol was approved by the University of Miami School of Medicine Animal Care and Use Review Board. All experiments in this study were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the institutional guidelines regarding animal experimentation in ophthalmic and vision research.

New Zealand White rabbits of an average weight of 3.37 ± 0.33 kg were used. Before each treatment and before death the animals were anesthetized with intramuscular injection of ketamine (14 mg/kg) and xylazine (7 mg/kg). Animals were killed with an IV injection of 390 mg pentobarbital sodium.

Aspirin (acetylsalicylic acid, $C_9H_8O_4$; Sigma–Aldrich Chemie GmBH, Steinheim, Germany) at a concentration of 10 mg/mL (pH 7.3, dissolved in balanced saline solution [BSS]) was used in the transscleral CCI applicator and for IV injection (15 mg/kg of body weight of aspirin solution administered in the right ear vein).

The battery operated, microprocessor programmable CCI instrument (Fig. 1, left) produces a constant current (in milliamps) and uniform electrical field (in volts per square centimeter) for the treatment duration selected by the surgeon.[27] Because aspirin is charged negatively at pH 7.3, the transscleral applicator was made the cathode. Fabricated of silicone elastomer (MED 6033; Nusil, Inc., CA), the custom-made conical transscleral probe for rabbit has an annular surface of 0.5 cm^2 and an outer diameter of 17 mm, assuring its location between pars plana and limbus, with a clear opening of 13 mm to avoid contact with the cornea (Fig. 1, right). Before treatment, the eye was proptosed, and CCI was applied for 10 minutes at a current density of 5 mA/cm^2. A peristaltic pump induced circulation under a maximum suction pressure of 25 mm Hg to ensure constant drug flow. A low-impedance, 2-cm^2 custom-made rectal probe served as the anodal return electrode, because it avoids the erratic impedance problems associated with dermal patches or subcutaneous needles in rabbits. An audiovisual alarm indicated poor contact or accidental disruption of the circuit, and because the instrument continuously recorded the total coulomb delivered, a controlled and calibrated delivery of the drug was ensured. The transscleral ocular probe was sterilized by autoclaving, and the drug solutions were prepared under an aseptic laminar flow hood and filtered through a 0.22-μm filter.

Safety Study

Two rabbits received a single 10-minute 2.5-mA transscleral CCI application of drug or vehicle in each eye. The right eyes were treated with aspirin and the left with BSS (control treatment). Eight hours after treatment, the rabbits were put under general anesthesia and the anterior segment, vitreous cavity, and fundus were examined by slit lamp biomicroscopy and indirect ophthalmoscopy. Immediately after death, the eyes were enucleated and immersed in 10% formalin, serially sectioned, and stained with hematoxylin and eosin. Light microscopic examination was performed in a masked fashion.

Aspirin Blood Plasma Concentration

At 0.5, 1, 2, 4, 6, and 8 hours after CCI, topical, and IV treatments, blood was taken out of the ear artery in every living animal to analyze the systemic distribution of aspirin. The last blood samples were taken immediately before death.

Transscleral CCI

Forty eyes received transscleral aspirin CCI (2.5 mA, 10 minutes). These rabbits were killed and the eyes enucleated at 0.5 (n = 4), 1 (n = 6), 2 (n = 6), 4 (n = 6), 6 (n = 12), and 8 (n = 6) hours after treatment and the eyes dissected under a surgical microscope.

Topical Application

Three animals received a 10-minute application of aspirin in both eyes through the transscleral applicator, with no current applied. One hour after treatment, the rabbits were put under general anesthesia, and the anterior segment, vitreous cavity, and fundus were examined by slit lamp biomicroscopy and indirect ophthalmoscopy. Thereafter, the animals were killed and the eyes (n = 6) enucleated and immediately dissected.

Intravenous Injection

Sixteen animals were used. After death, the eyes were enucleated at 1 (n = 6), 2 (n = 6), 4 (n = 4), 6 (n = 6), and 8 (n = 10) and dissected.

Tissue Dissection and HPLC Analysis

After enucleation, the aqueous humor and vitreous were aspirated with a 1-mL syringe and a 25-gauge needle, and the eyes were dissected under the surgical microscope. To analyze the ocular distribution of aspirin, the conjunctiva, muscle, orbital fat tissue, anterior sclera, cornea, anterior uvea (iris and ciliary body), lens, retina, choroid, posterior sclera, and optic nerve were harvested in vials (Eppendorf, Fremont, CA) and kept frozen at −80°C until further processing. All ocular tissues were dissected and used in their whole. The anterior sclera was defined as the area of the location of the transscleral eye electrode between the limbus and pars plana. This area was approximately 2 mm in annular length. To ensure the reproducibility of the dissection, the same person always performed the process.

Before extraction, wet and dry weights of all tissue samples were measured (reproducibility, >0.1 mg). Defined volumes of distilled water (Millipore, Bedford, MA.) were added (100–600 μL related to dry tissue weight) to the lyophilized tissues and kept at room temperature overnight. After centrifugation for 25 minutes at 15,000*g*, supernatant was extracted and methanol was added (ratio 1:2 by volume; HPLC grade, Sigma-Aldrich). Samples were vortexed for 1 minute and centrifuged at 15,000*g* for 25 minutes for protein precipitation and, if necessary, filtered. Liquid samples were diluted in methanol (1:2) and processed as described earlier.

Although administered as acetylsalicylic acid, concentrations of salicylate were measured in ocular tissues and fluids, because it is the active and stable metabolite of aspirin. SA levels were measured by an HPLC system, consisting of an isocratic pump (model LC 250), an autosampler (model LC ISS 2000), and a variable spectrophotometer (model LC 95 UV/visible; all from Perkin Elmer, Wellsley, MA). The column selected was a reversed-phase column (pore size 8 nm, particle size 5 μm, 25 cm × 4.6 mm; model OD5-C18; Baxter International, Deerfield, IL) fitted with a guard column (5 μm, 10 × 4.6 mm, Spherisorb ODS-2; Sigma-Aldrich) and equilibrated at room temperature with a mobile phase of a mixture of distilled water-acetonitrile-phosphoric acid (76:24:0.5, , HPLC-grade, pH 1.85; Sigma-Aldrich) with a flow rate of 1 mL/min. The detection limit was on the range of 0.01 μg/mL at UV detection at 295 nm.[30–31]

To calibrate the HPLC system, salicylic acid (SA) standard solutions were prepared in acetonitrile in five concentrations spanning the range of samples tested. The method was found to be linear over the concentration range of SA examined. Calibration was repeated several times during the study to ensure reliability.

Statistical Analysis

Data are expressed as the mean ± SD. Statistical evaluation was performed on a logarithmic scale. Two tailed *t*-test and ANOVA with the Duncan multiple range and the least-significant-difference post hoc tests were used to examine the relation between concentrations, time course, treatment. $P < 0.05$ was considered significant.

Results

Safety

Slight conjunctival injection was noted for a few eyes immediately after removal of the applicator. Similar observations were made in animals that received topical aspirin administration through the same applicator without current. The injection disappeared within 8 hours. No retinal detachment or other intraocular complication was observed ophthalmoscopically in CCI- and topically treated eyes. Histologically, no signs of inflammation and no tissue damage were found in the anterior and posterior segments. Topical and transscleral CCI applications of aspirin appear to be safe (Fig. 2).

CCI Treatments

Salicylic acid (SA) levels in the anterior and posterior segments of the eye were measurable at all time points evaluated. The obtained tissue levels, including those of intraocular fluids, significantly exceeded the in vitro evaluated ID_{50} of 0.9 μg/mL as described by Kahler et al.[29] The highest concentrations of SA were achieved immediately after treatment in all eye tissues, with a preference for the anterior segment due to direct application above the pars plana region (Tables 1, 2). Although a decrease in the concentration of SA with time was observed in the whole eye, two different distribution trends appeared. A steady decline of salicylate was observed in the anterior chamber. In the posterior segment, including the vitreous, concentrations showed a slower decrease, with a plateau at 2 hours, which remained stable until 8 hours (Table 2; Figs. 3, 4).

To evaluate systemic distribution of SA, blood plasma levels were measured at 0.5, 1, 2, 4, 6, and 8 hours after application by CCI. For the ocular drug distribution study at the 0.5-hour time point, only the right eyes ($n = 4$) of four different animals were treated. The blood plasma levels measured at this time point were 4.9 ± 1.5 μg/mL. Furthermore, the blood plasma salicylate levels measured at 1 hour after CCI treatment on both eyes were 4.3 ± 1.5 μg/mL, which is in the same range as the treatment on one eye. We decided to apply CCI on both eyes, because the effect of systemic distribution by blood circulation seemed negligible.

Although the right and the left eyes were treated by CCI, the measured blood plasma levels stayed remarkably low (4.3 ± 1.5 to 2.4 ± 0.4 μg/mL). This result suggests that this means of administration is a method involving nearly negligible systemic drug exposure. The achieved ocular tissue levels cannot be explained by systemic uptake and rediffusion of SA, because the blood plasma concentrations measured (4.9 ± 1.5 to 2.4 ± 0.4 μg/mL) are insufficient to produce the amounts of SA obtained in intraocular tissues (Table 2; Fig. 4).

Topical Application

Tissue samples of the control group demonstrated significantly lower concentrations of SA in the posterior segment of the eye than animals treated by CCI or IV injection. Levels for CCI were 8 times higher in the retina ($P < 0.001$), 5 times higher in the choroid ($P < 0.001$), and 11 times higher in the optic nerve ($P < 0.001$) than in control eyes (Table 3). Levels with IV injection were 5 times higher in the retina and choroid ($P < 0.001$) and up to 17 times higher in the optic nerve ($P < 0.001$) than with topical application (Table 3).

For the anterior segment and external tissues, such as conjunctiva and muscle, topical application produced concentrations lower than or similar to CCI and IV (Table 3). These findings suggest that with a 10-minute topical application of aspirin, passive diffusion occurs. However, by using an eye cup, we avoided tear film and eyelid motion effects. Because dilution of the drug by the tears and evacuation of the drug through the lacrimal canal does not occur, SA applied by topical drops is likely to result in even lower tissue concentrations.

Eye tissue concentrations of topically applied SA were significantly lower than with CCI- and IV treatments overall, but especially in the posterior segment. Blood levels after CCI and topical application were in the same range, but blood levels of intravenously treated animals were 28 times ($P < 0.001$) higher. These data prove that high tissue levels reached by CCI are an effect of current-enforced migration of drug molecules into the eye and are not of systemic distribution and blood transport as in IV application.

Treatment by IV Injection

SA levels were measured in all tissues of the eye until 8 hours after administration. The highest amounts of SA were observed 2 hours after treatment, suggesting a systemic distribution

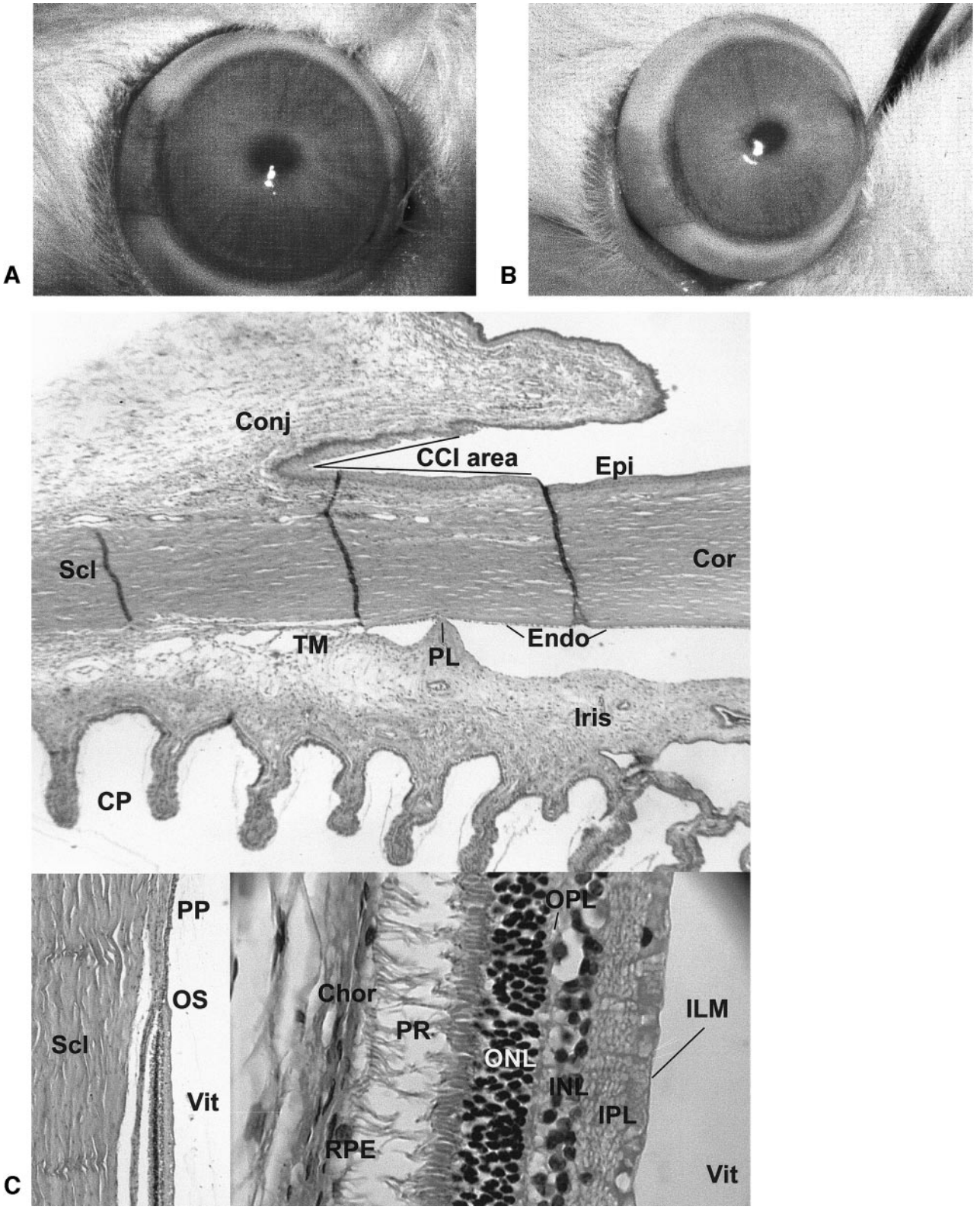

FIGURE 2. Clinical appearance of a New Zealand white rabbit eye immediately after transscleral CCI (**A**) and after 8 hours (**B**). Histomicrograph composite (**C**) of an eye 8 hours after transscleral CCI, showing the conjunctival surface in contact with the CCI's annular applicator (CCI area), the immediate surrounding and underlying tissues, and the peripheral retina closest to the probe. No histopathologic alterations are present. Chor, choroid; Conj, conjunctiva; Cor, cornea; CP, ciliary processes; Epi, epithelium; ILM, inner limited membrane; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, ora serrata; PL, pectum ligament; PP, pars plana; PR, photograph receptors; RPE, retinal pigment epithelium; Scl, sclera; TM, trabecular meshwork; Vit, vitreous. Staining, H&E.

TABLE 1. SA Distribution in Anterior Segment Tissues after CCI and IV Injection of Aspirin

Tissue	0.5 hour CCI (n = 4)	2 hours CCI (n = 6) IV (n = 6)	4 hours CCI (n = 6) IV (n = 4)	6 hours CCI (n = 12) IV (n = 6)	8 hours CCI (n = 6) IV (n = 10)
Conjunctiva	452.3 ± 177.4	65.3 ± 15.5	38.6 ± 11.8	36.0 ± 13.1	35.2 ± 14.5
		292.4 ± 91.2	97.0 ± 31.0	38.3 ± 5.8	41.2 ± 14.3
Muscle	126.4 ± 10.4	25.5 ± 4.8	11.7 ± 1.9	13.1 ± 6.5	15.7 ± 5.1
		93.6 ± 23.7	63.1 ± 23.7	43.9 ± 9.4	20.3 ± 7.3
Orbit	97.4 ± 23.7	8.9 ± 1.8	5.2 ± 1.7	12.6 ± 4.3	10.9 ± 4.0
		28.1 ± 5.4	25.9 ± 9.0	27.8 ± 9.1	12.2 ± 3.5
Cornea	933.8 ± 236.7	110.7 ± 31.9	135.0 ± 44.8	81.6 ± 21.6	38.3 ± 12.6
		132.7 ± 37.6	88.1 ± 30.1	43.6 ± 5.7	40.5 ± 18.4
Anterior sclera	515.1 ± 73.3	83.8 ± 11.9	97.3 ± 23.6	42.9 ± 10.5	26.9 ± 4.6
		151.5 ± 57.1	121.6 ± 49.6	32.8 ± 6.3	36.9 ± 11.7
Anterior uvea	1614.2 ± 280.7	139.8 ± 29.3	159.4 ± 50.4	99.7 ± 29.5	62.0 ± 13.5
		278.4 ± 90.1	148.3 ± 74.9	99.6 ± 28.1	79.1 ± 19.8
Lens	27.8 ± 1.7	5.4 ± 2.7	4.0 ± 1.0	6.3 ± 3.0	5.1 ± 1.2
		4.8 ± 1.2	4.6 ± 1.0	2.9 ± 0.3	3.5 ± 0.4

Concentrations are mean nanograms per milligram dry tissue weight ± SD.

through peripheral tissue diffusion of the drug and its metabolites. At all time points, the highest level was found in the anterior uvea, conjunctiva, and choroid. The concentration of SA increased between 1 and 2 hours for all tissues followed by a steady decrease to 8 hours, at which point approximately 30% of the highest peaks remained (Tables 1, 2; Figs. 3, 4, 5). In the aqueous and vitreous humors, the trend was similar (Fig. 4).

CCI Versus IV Injection

Peak concentrations obtained were higher with CCI treatment than those reached by IV injection. However, CCI and IV application showed different SA distribution patterns in ocular tissues. With CCI, the highest levels were measured 0.5 hour after treatment, whereas with systemic application SA peaks showed a delay of 2 hours (Figs. 3, 4, 5), at which time IV levels exceeded those with CCI in all ocular tissues and fluids except the aqueous humor (Tables 1, 2). This difference was not significant in retina ($P = 0.17$), choroid ($P = 0.13$), cornea ($P = 0.33$), and lens ($P = 0.77$). SA concentrations were in the same range with both treatment modalities at 4, 6, and 8 hours, at which point no significant differences were found in the retina ($P = 0.48$) and vitreous ($P = 0.8$). However, IV administration of aspirin resulted in remarkably high SA blood concentrations, from 76 ± 38.1 μg/mL at 1 hour, to more than 103 ± 29.7 μg/mL at 2 hours, and 11 ± 4.3 μg/mL at 8 hours, compared with the average of 3 ± 1 μg/mL levels produced by CCI ($P < 0.001$ for all time points; Fig. 4).

DISCUSSION

This study compared three different application methods, systemic IV injection, short-duration topical application and transscleral CCI. In this study the amount of aspirin per body weight in IV application resembled a plasma profile obtained by an administration of approximately 1.2 g in humans.[32]

We found that a single transscleral CCI application of aspirin achieved higher initial intraocular concentrations than IV application. At 2 hours after treatments, tissue levels of CCI and systemic drug administration were similar. In contrast, throughout the measured time, CCI application of aspirin produced significantly lower blood levels ($P < 0.001$) than IV administration. Comparing the maximal tissue concentrations achieved by CCI and IV injection, significantly higher levels were reached by CCI in the anterior uvea ($P < 0.001$), aqueous ($P < 0.001$), retina ($P < 0.001$), and choroid ($P < 0.001$). The difference in vitreous ($P = 0.54$) and optic nerve ($P = 0.88$) levels were not significant.

TABLE 2. SA Distribution in Posterior Segment Tissues and Fluids after CCI and IV Injection of Aspirin

Tissue	0.5 hour CCI (n = 4)	2 hours CCI (n = 6) IV (n = 6)	4 hours CCI (n = 6) IV (n = 4)	6 hours CCI (n = 12) IV (n = 6)	8 hours CCI (n = 6) IV (n = 10)
Retina	443.0 ± 91.6	102.6 ± 21.5	89.9 ± 10.0	80.7 ± 17.8	65.4 ± 19.3
		131.6 ± 38.9	147.7 ± 27.2	61.2 ± 5.3	58.7 ± 19.7
Choroid	1276.0 ± 277.7	150.5 ± 48.5	135.7 ± 21.6	124.1 ± 28.9	120.6 ± 35.7
		222.9 ± 61.9	218.1 ± 50.8	112.8 ± 30.9	78.0 ± 26.2
Posterior sclera	208.8 ± 38.8	113.4 ± 33.0	41.0 ± 6.8	40.3 ± 15.6	14.5 ± 1.5
		206.9 ± 31.5	104.1 ± 48.6	41.6 ± 16.2	24.9 ± 6.7
Optic nerve	163.6 ± 44.6	54.1 ± 12.7	55.8 ± 14.8	52.8 ± 7.5	54.4 ± 8.7
		157.9 ± 37.2	102.7 ± 33.8	43.2 ± 6.1	42.9 ± 11.3
Aqueous	495.9 ± 214.6	27.1 ± 12.1	27.1 ± 6.7	9.5 ± 5.6	5.7 ± 1.5
		12.5 ± 2.8	8.2 ± 3.1	5.9 ± 2.4	4.1 ± 0.9
Vitreous	9.1 ± 1.6	4.3 ± 1.2	4.3 ± 0.9	3.9 ± 1.1	3.9 ± 0.7
		8.4 ± 3.2	6.6 ± 2.1	3.9 ± 1.0	3.8 ± 0.8
Blood	4.9 ± 1.5	3.5 ± 0.9	3.5 ± 0.8	2.9 ± 0.7	2.4 ± 0.4
		103.1 ± 29.7	41.1 ± 20.9	*	11.3 ± 4.3

Concentrations are expressed as mean nanograms per milligram dry tissue or mean micrograms per milliliter fluid ± SD.
* No data available.

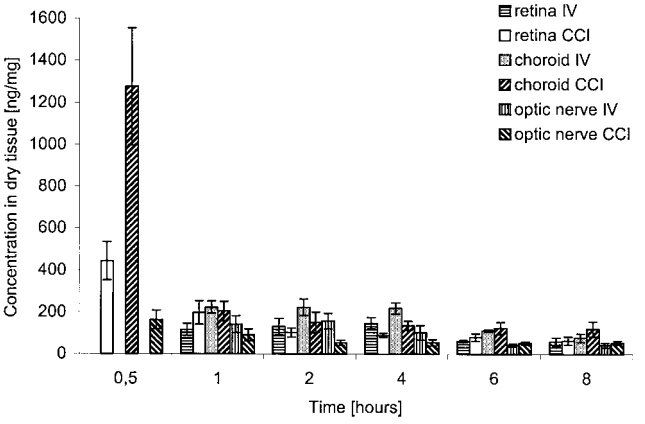

FIGURE 3. Distribution of SA in the posterior segment after aspirin treatment.

TABLE 3. SA Distribution 1 Hour after Treatment with Aspirin

Tissue	CCI (n = 6)	Control (n = 6)	IV (n = 6)
Conjunctiva	259.9 ± 77.6	105.7 ± 30.5	181.8 ± 40.9
Muscle	30.3 ± 7.3	25.5 ± 9.1	61.8 ± 17.1
Orbit	14.0 ± 2.3	14.5 ± 3.4	27.2 ± 6.7
Cornea	1080.3 ± 256.1	277.9 ± 135.6	51.8 ± 9.9
Anterior sclera	335.2 ± 155.0	146.9 ± 45.6	183.3 ± 72.6
Anterior uvea	534.3 ± 223.9	301.3 ± 157.2	152.8 ± 48.3
Lens	8.0 ± 1.9	3.6 ± 0.6	2.4 ± 0.3
Retina	197.4 ± 55.8	24.8 ± 7.7	115.8 ± 29.0
Choroid	204.2 ± 46.1	41.2 ± 18.6	222.6 ± 47.7
Posterior sclera	117.0 ± 38.4	86.5 ± 38.4	109.2 ± 19.9
Optic nerve	91.1 ± 28.5	8.2 ± 3.7	141.9 ± 40.2
Aqueous	81.5 ± 40.7	26.5 ± 11.5	7.4 ± 0.9
Vitreous	4.9 ± 1.0	2.8 ± 0.6	7.0 ± 1.6
Blood	4.3 ± 1.5	2.7 ± 1.3	76.3 ± 38.1

Concentrations are expressed as in Table 2.

Transscleral CCI resulted in a high initial drug concentration in the anterior segment tissues, producing a depot effect, and prolonged diffusion through the choroid and retina. It appears as though the migration of drug molecules from the anterior chamber to the posterior segment does not using the direct pathway from the iris-ciliary body through the vitreous to the posterior pole. A direct distribution by the vitreous diffusion route would probably result in higher vitreous SA levels than those measured in our experiment. It seems much more likely that the transscleral CCI-induced drug molecule migration of SA may go from the anterior to the posterior pole by using the directly affected tissues such as the sclera, retina, and choroid. Therefore, the insignificant salicylate concentration differences in the vitreous between the CCI and IV injection may be the result of distribution of the drug molecules from the posterior segment tissues into the liquid of the vitreous by simple passive diffusion. Elsewhere (e.g., the retina and choroid), the drug distribution pattern appears to be more affected by such factors as the blood flow for systemic distribution or, in the case of transscleral CCI, on the area where the electrode is placed. Topical application resulted in the lowest concentrations, demonstrating that the electrical field induced the penetration of aspirin into the eye.

Maurice and Hughes[33] detailed the main factors influencing iontophoretic drug delivery as current density, duration of treatment, drug concentration, pH, and the permeability capacity of the tissue for the drug molecules. Transcorneal and transscleral iontophoresis of antifungal, antibacterial, and antimitotic agents have been reported on animals with varying results.[34–41] Furthermore, studies on iontophoresis of oligonucleotides and peptides have revealed the potential of this application method for future drug delivery.[42–44] However, as demonstrated by several toxicity and safety studies, this treatment modality is not without risk of complications.[27,45–47] Transscleral iontophoresis may lead to retinal and choroidal cell damage, resulting in thinning and disorganization of retinal layers. When tissues are being damaged by heat, changes in hydration level, and/or mechanical disorganization during treatment, their impedance changes with time, resulting in variable electrical fields (volts per square centimeter), which affects the iontophoretic drug transfer characteristics. This can easily occur at the epithelial surface (e.g., conjunctiva) when high current densities are applied. To avoid these problems, a CCI system was developed[27] that produces and maintains a constant electrical field across the conjunctival epithelium barrier, a constant drug flow, and minute negative pressure that secures and maintains continuity of the tissue to drug solution interface. The system adapts to tissue impedance changes that mainly occur at the site of the return electrode and automatically maintains a constant current, thereby assuring a constant defined drug supply.

The threshold for avoiding ocular toxicity due to transscleral iontophoresis has been determined to be a current density of 500 mA/cm^2 at a duration of 5 minutes.[28] In our experiment, we administered a current density of 5 mA/cm^2, which is 100 times less than the recommended safety param-

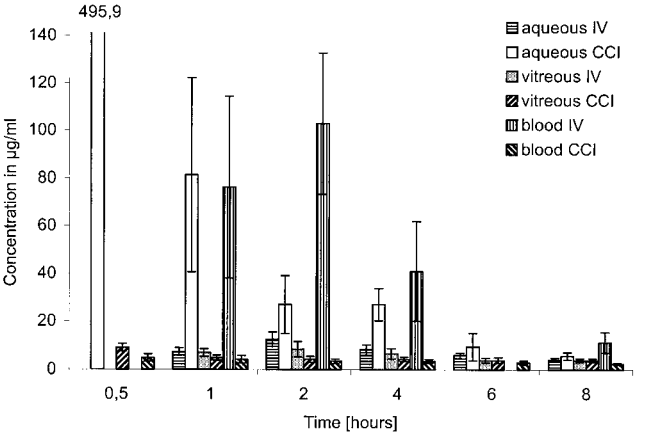

FIGURE 4. Distribution of SA in aqueous and vitreous humors and blood after treatment.

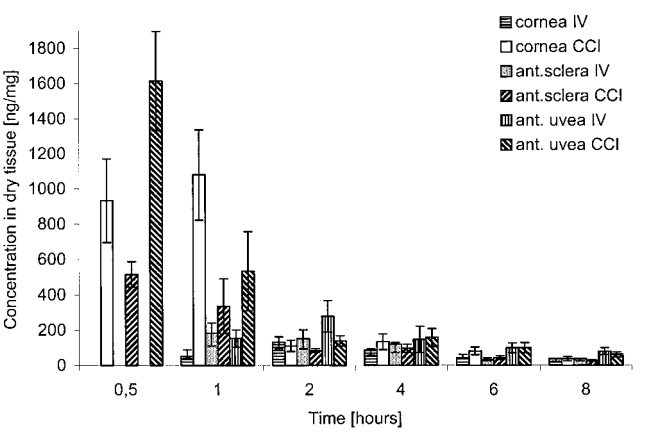

FIGURE 5. Distribution of SA in the anterior segment after aspirin treatment.

eter. Although we applied CCI for 10 minutes instead of 5 minutes, by keeping the applied current density at such a low level, we were able to avoid retinal toxicity. Furthermore, the electrode lies over the less visually critical pars plana. Because the electrode covers an annular area between the limbus and pars plana with a clear corneal window, corneal exposure is also avoided.

In conclusion, the treatment duration of 10 minutes at a current density of 5 mA/cm^2 was shown to be safe[48] and, as we demonstrated in this study, efficacious for intraocular drug delivery. Additional animal studies are needed to assess pharmacokinetics beyond 8 hours and the safety and efficiency of repetitive CCI treatments.[49] Transscleral CCI was shown in animal studies to be painless and easy to apply and the treatment time relatively brief. The application of the CCI electrode does not require any surgical skill. In pilot human trials a similar ocular electrode was used and the return electrode, a 3-M patch, was placed on the skin of the forehead. The participating patients did not demonstrate or report any subjective signs of discomfort or pain.[50]

Our study confirms the safety of administering aspirin by CCI. No damage was observed in the anterior or posterior segment of the eye, except for slight conjunctival injection that disappeared 8 hours after treatment. We also demonstrated transscleral application of aspirin by CCI to be an effective noninvasive drug delivery system capable of achieving higher initial SA levels in ocular tissues than IV administration while avoiding systemic exposure. The low plasma levels that resulted from CCI may avoid the undesirable systemic effects associated with aspirin, such as platelet dysfunction, drug interactions, gastrointestinal bleeding, aspirin-induced asthma, hepatotoxicity, and plasma acid-base alterations.

To demonstrate and study the potential therapeutic utility and efficacy of transscleral administration of aspirin or other NSAIDs by CCI several pathogenic pathways may be of investigational interest. These include direct cyclooxygenase inhibition, indirect mediation of the expression of VEGF and bFGF,[15] inhibition of T-lymphocyte[56–57] activity, and PGE_2 inhibition for the management of epinephrine-induced cystoid macular edema,[51] neovascularization,[52] retinopathy of prematurity,[53] proliferative vitreoretinopathy,[54–55] and experimental uveitis.[39–40]

CCI delivery of NSAIDS may be an alternative treatment modality for a wide range of ophthalmic complications. It is a safe, topical, noninvasive drug administration system that has been shown to achieve high intraocular drug concentrations while minimizing systemic exposure. The ease of administration and the potential for repetitive application of drug by transscleral CCI make this treatment modality of special interest in chronic and long-term intraocular diseases.

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