Noncross-Linked Collagen Discs and Cross-Linked Collagen Shields in the Delivery of Gentamicin to Rabbits Eyes

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Using fluorescent polarization immunoassay, in vitro absorption and elution of gentamicin by noncross-linked collagen discs was measured. This technique was compared with that of cross-linked collagen shields and topical drops to provide adequate gentamicin levels in the cornea and aqueous humor of the rabbit eye. In vitro results showed that the noncross-linked collagen discs absorbed increased gentamicin with prolonged soaking time. All 2-hr presoaked discs completely dissolved within 6 min after being placed in the lower fornix of the rabbit eye. The presoaked discs released most of their gentamicin load within 0.5 hr of elution. Gentamicin levels in the cornea and aqueous humor, using 2-hr presoaked discs, were similar to those obtained with a single drop (P > 0.05) of topical solution and significantly lower than those obtained by applying a collagen shield at all intervals (P < 0.01) and by hourly drops measured at 4- and 6-hr intervals (P < 0.01). These results suggest that, in their current formulation, the presoaked collagen discs may not be an effective alternative to collagen shields or topical drops for gentamicin delivery because of their rapid dissolution in the eye. Invest Ophthalmol Vis Sci 33:2194–2198, 1992

One of the significant problems with the delivery of ophthalmic drops is that drug administration is pulsed, with an initial period of overdosing followed by a longer period of relative underdosing. To overcome this disadvantage, various drug delivery devices have been used, such as therapeutic soft contact lenses,1–3 drug-impregnated inserts,4,5 and liposomal systems.6 Recently, investigators became interested in using soluble porcine and bovine cross-linked collagen shields as a drug-delivery vehicle.7–12 The collagen shield acts as a drug reservoir, lengthening the contact time between the cornea and the drug, and enhancing drug delivery to the cornea and aqueous humor. Collagen discs, sometimes referred to as minishields, with no cross-linkage, are a new design that can provide lubrication and relief from discomfort after surgery. They may be used in conjunction with artificial-tear solutions in the treatment of dry eyes. We investigated the in vitro absorption and elution of gentamicin by collagen discs and compared the usefulness of this technique with collagen shields and topical drops of antibiotic solution to provide adequate gentamicin levels in the cornea and aqueous humor of the rabbit eye.

Materials and Methods

Collagen discs and shields were provided by Bausch & Lomb (Rochester, NY). The disc is a clear pliable thin film that is fabricated from porcine scleral tissue, without cross-linking, and is 0.013–0.071 mm thick in an oval shape measuring 8.3 × 5.0 mm. Because of its size and shape, the disc can be inserted easily into the lower fornix. After insertion, it absorbs tears, softens, and conforms to the shape of the eye; then, it gradually dissolves. The collagen shield is composed of cross-linked porcine collagen and has a base curve of 9.0 mm and a diameter of 14.5 mm. It dissolves on the corneal surface over a period of 24 hr.

Absorption of Gentamicin by Collagen Discs and Shields in Vitro

The discs were immersed in 1 ml of gentamicin solution (15 mg/ml; Strong Memorial Hospital, Uni-
versity of Rochester Medical Center, Rochester, NY) for 5, 15, 30, 60, and 120 min at room temperature. They were removed, rinsed briefly in 5 ml of phosphate-buffered saline (PBS, 0.02 mol/l, pH 7.2) and individually placed on a microscope slide before mincing with a razor blade. The fine pieces were placed in a small vial containing 6 ml of PBS. The vials were agitated on a stirrer overnight at room temperature. Five discs were used at each time point, and the mean of the absorbed amounts of gentamicin was calculated. From this, the amount of gentamicin in the collagen disc was determined. Three collagen shields were soaked in the gentamicin solution for 120 min. The gentamicin elution from the shields was measured identically.

Release of Gentamicin by the Collagen Discs in Vitro

The discs were soaked in 1 ml of gentamicin solution (15 mg/ml) for 2 hr, then removed, rinsed briefly, and placed serially into small vials containing 1 ml of PBS for elutions of 0.5, 1.5, 2, and another 2 hr at room temperature without a change of medium. A total of five discs were used in this experiment. The mean of released amounts of gentamicin at each time was calculated.

Delivery of Gentamicin to Rabbit Eyes

Female New Zealand white rabbits (n = 40) weighing 2.1–2.7 kg were used in accordance with ARVO Resolution on the Use of Animals in Research. The animals were anesthetized by injecting sodium pentobarbital (30 mg/kg body weight; Butler, Columbus, OH) through the marginal ear vein with reinforcements of topical proparacaine hydrochloride 0.5%. Under a surgical microscope, the central cornea was marked with a 5-mm diameter trephine, and the epithelium on both eyes was removed with surgical cotton sponges. The epithelial defect was made to simulate the clinical situation of microbial keratitis requiring intensive antibiotic treatment and to enhance antibiotic penetration into the cornea. The rabbits were divided randomly into four groups to receive the presoaked discs and shields and single-drop and hourly drop treatments. There were ten rabbits in each group, except for the collagen-shield group consisting of eight rabbits. The discs and shields were soaked in 1 ml of gentamicin solution (15 mg/ml) for 2 hr before application. After epithelial debridement, the presoaked disc was placed into the lower fornix of each rabbit eye. The presoaked shield was placed on the cornea. Only one drop (approximately 50 μl) of gentamicin solution (15 mg/ml) was applied to the central cornea of each eye throughout the treatment period in the single-drop group; we administered one drop every hour in hourly drop group. The last drop was applied 1 hour before the animals were killed except for the 0.5-hr interval. The lower eyelid was held away from the eye during administration to avoid immediate overflow.

The animals were killed by injecting 1.5 ml of Suc comb (Butler) into their marginal ear veins. The shields were removed, and the eye surface was irrigated with PBS. The aqueous humor was aspirated with a 25-gauge needle attached to a tuberculin syringe. The corneas were excised with an 11-mm diameter trephine, and the corneal buttons were rinsed and kept in a freezer (−80°C) until prepared for assay. Each corneal button was placed on a clean glass microscopic slide and minced into fine pieces using a razor blade, and then it was placed into a preweighed microcentrifuge tube to determine its weight. We added 0.8 ml of PBS to each tube. The tubes were incubated in a 37°C agitated water bath overnight and then centrifuged for 5 min at 2000 rpm. The supernatant was used for the gentamicin assay. The concentration of gentamicin in the cornea was derived as follows: Ca = (W + V) Cb/W, where Ca indicates micrograms of gentamicin per gram of the corneal tissue; Cb, micrograms of gentamicin per milliliter of elution; W, weight of the corneal tissue in grams; and V, the original volume of elution in milliliters.

All samples of corneal supernatant, aqueous humor, and in vitro elution were measured for their gentamicin levels using fluorescence polarization immunoassay (TDx System Analyzer; Abbott, Irving, TX) in the Clinical Microbiology Laboratory, University of Rochester Medical Center. The application of this technique to assays of gentamicin and other antibiotics previously was described in detail. All samples were stored in a 4°C refrigerator before assay.

The gentamicin levels in cornea and aqueous humor produced by the presoaked discs were compared with those achieved using the presoaked shields and hourly drop and single-drop regimens. A Wilcoxon rank-sum test was used for comparison; the accepted level of significance was P < 0.05.

Two rabbits treated with presoaked discs and shields soaked in 0.02 mol/l PBS were used as control animals.

Results

The collagen discs gradually thickened and absorbed increased amounts of gentamicin with prolonged soaking times (mean, 199.4, 390.6, 442.4, 575.0, and 822.0 μg at 5, 15, 30, 60, and 120 min, respectively). There were significant differences be-
between the absorbed amounts of any two intervals \( (P < 0.05, \text{by Wilcoxon's rank-sum test}) \) except between the 15- and 30-min intervals \( (P > 0.05) \). The 2-hr presoaked shield absorbed a mean of 575.4 \( \mu \)g of gentamicin, markedly less than 822.0 \( \mu \)g absorbed by the 2-hr presoaked disc \( (P < 0.01, \text{by Wilcoxon rank-sum test}) \).

The in vitro release determinations showed that the presoaked disc released a mean total of 729.85 \( \mu \)g of gentamicin over 6 hr, with 513.5 \( \mu \)g (70.36\%) of total drug released during the first 0.5 hr. The rest was released into the 2-, 4-, and 6-hr elution.

Surprisingly, we found that all 2-hr presoaked discs rapidly dissolved, even at the 0.5-hr time point, and all shields were intact at each interval. Therefore, we did an additional experiment to determine the dissolution time of the discs in rabbit eyes. A total of nine discs were presoaked in the gentamicin solution (15 mg/ml) for 5, 15, and 120 min, respectively (three discs at each time). Then, each disc was placed in the lower fornix of a normal rabbit eye and observed every 2 min until it dissolved completely. We found that both 5- and 15-min presoaked discs dissolved approximately 15 min after insertion; the 2-hr presoaked discs dissolved in approximately 6 min.

Our in vivo results showed that peak gentamicin levels in the cornea and aqueous humor were attained at 0.5-hr for all groups and then declined in each. There were no statistically significant differences in gentamicin concentrations between disc and single-drop treatments at any tested time \( (P > 0.05; \text{Figs. 1, 2}) \). The disc treatment showed significantly lower gentamicin levels in the cornea and aqueous humor at all times compared with shield treatment \( (P < 0.01) \) and at 4- and 6-hr intervals compared with hourly drop treatment \( (P < 0.01; \text{Figs. 1, 2}) \). The levels obtained by shield treatment were significantly higher than those obtained by single-drop therapy at 0.5, 2, and 4 hr \( (P < 0.01) \) and at 6 hr \( (P < 0.05; \text{Figs. 1, 2}) \). In comparison with the hourly drop treatment, shield therapy provided significantly higher gentamicin levels in the cornea and aqueous humor at 0.5 and 2 hr \( (P < 0.01) \), no significant difference at 4 hr \( (P > 0.05) \), and lower levels at 6 hr \( (P < 0.01; \text{Figs. 1, 2}) \). The control corneas and aqueous humor samples showed no detectable levels of gentamicin \( (< 0.3 \mu \text{g/ml}) \).

**Discussion**

The noncross-linked collagen discs swelled gradually and absorbed increased gentamicin with prolonged soaking time in gentamicin solution. This characteristic seems to be different from that of the cross-linked collagen shields; there was no significant change in the absorbed amount of drug between 5 or 15 min and longer soaking times.8,9-10 This might be related to the absence of cross-linking in the collagen discs.

The in vitro gentamicin release by the discs was rapid, with most of the drug liberated within 30 min; this was similar to the results obtained with the collagen shield in vitro.8 A rapid in vitro release rate also was observed by others using collagen shields soaked with trifluridine,9 5-fluorouracil,11 and amphotericin B.12 This appears to indicate that the absence or presence of cross-linking has no effect on the in vitro release rate. Although the mechanism of the absorption and release of the drug by the collagen is not clear, it seems to be independent of cross-linking. Methods
that prolong the release of these drugs from the disc or shield could be beneficial for therapeutic applications of these devices.

Although the 2-hr presoaked disc absorbed more gentamicin than the 2-hr presoaked shield, the rapid dissolution of the disc in the rabbit eye resulted in an abrupt complete release of gentamicin. Therefore, it was not surprising that the gentamicin levels in ocular tissues using the presoaked discs were similar to those obtained by instilling a single drop of gentamicin in the lower fornix. However, the 5- and 15-min pre-soaked discs also showed a relatively rapid dissolution (approximately 15 min); however, the 2-hr presoaked collagen shields were still intact 6 hr after placement. This suggests that the cross-linking is an important process that prolongs the degradation time of the collagen in the eye. The immediate dissolution of the discs curtails their contact time with the ocular surface and precludes the possibility of using them as a drug reservoir. A 5-min presoaked disc was placed in the inferior fornix of a normal human eye; it dissolved in 90 min (unpublished observation). This suggests that the dynamics of disc dissolution differ between these two species. We should be cautious in extrapolating our results to a human clinical setting. The dissolution of these inserts probably depends on host factors, such as tear volume, tear enzyme concentration, degree of inflammation, and blink rate.14 However, if cross-linked, the discs might become an effective delivery vehicle for antibiotics and other medications. Others4 used 6-hr dissolving succinylated collagen inserts impregnated with gentamicin in rabbit eyes and obtained higher tear and corneal concentrations of gentamicin compared with a single drop, ointment application, or subconjunctival injection. When discs were punched out from the cross-linked collagen shields to treat patients with dry eyes, statistically significant symptomatic relief and reduced frequency of artificial tear administration was reported.14

The levels of gentamicin in the cornea and aqueous humor achieved at 4- and 6-hr intervals by hourly drop administration were slightly lower than those obtained at 0.5- and 2-hr intervals. However, gentamicin levels were unchanged between 4- and 6-hr intervals. We speculate that this may be attributed to the waning of anesthesia related to the epithelial defect resulting in consistent tearing by the animals, which diluted and washed away the instilled gentamicin in the lower fornix. During anesthesia, tear turnover is decreased; this may increase the amount of drug available for corneal penetration.9 The fluorescence polarization immunoassay has been shown to be a fast effective accurate technique for assaying gentamicin, with a low sensitivity limit of 0.3 μg/ml.8

Although the collagen disc absorbed more gentamicin, in its current form, its rapid dissolution rate limits its therapeutic potential. Thus, cross-linking should be incorporated to enhance drug delivery.

Our experiment also showed that gentamicin levels in the cornea and aqueous humor obtained with collagen shields were significantly higher than those obtained by applying discs at all time intervals ($P < 0.01$). They also were higher than those found using hourly drops at 0.5- and 2-hr intervals ($P < 0.01$) but were equivalent at 4 hr. These results suggest that the collagen shield-assisted delivery of gentamicin to the cornea and aqueous humor is comparable with or superior to topical drop administration during short-term application. In addition, the shields decrease the required frequency of drop application around the clock, are potentially more comfortable, and require no fitting because they rapidly conform to the shape of the cornea after placement.

Although gentamicin, as a charged drug, is not well absorbed across the cornea and is not a typical drug either in its uptake into collagen or release from it, nonetheless, it is one of the antibiotics most often used clinically to treat bacterial corneal infections and provide postoperative prophylaxis of ocular surgery. It is known that the intact corneal epithelium is a major barrier to impede the penetration of hydrophilic drugs, such as gentamicin, through the cornea. Deep-epithelialization enhances the penetration of hydrophilic drugs into the cornea.15

Key words: collagen disc, collagen shield, cross-linkage, gentamicin, drug delivery

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References


