In Vitro and in Vivo Evaluation in Rabbits of a Controlled Release 5-fluorouracil Subconjunctival Implant Based on Poly(D,L-lactide-co-glycolide)

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Purpose. To design a controlled release 5-fluorouracil (5-FU) implant to provide prolonged antifibroblast concentrations of 5-FU in the subconjunctival tissues but low concentrations of 5-FU in other ocular tissues.

Method. Implants (5 mg; 2.5 mm diameter × 1.2 mm thickness) of 5-FU or 9:1, 8:2, 7:3 5-FU to polymer mass ratios were made by compression. Poly(D,L-lactide-co-glycolide) polymers with 50:50 and 75:25 lactide to glycolide ratios were used. In vitro release characteristics of four types of implants were studied: 5-FU alone (CT), 5-FU/polymer matrices (MT), coated 5-FU/polymer matrices with a central hole drilled through the matrix and coating (CM1), and with a central hole in the coating (CM2). MT and CM1 (9:1 drug/polymer) were selected for subconjunctival implantation in rabbits. ^{14}C-5-FU levels in various ocular tissues and retrieved pellets were monitored.

Results. First-order release was observed from CT, MT and CM1 implants. Zero-order release profiles were observed from CM2 implants. Drug release was retarded by formulating 5-FU in a matrix comprising poly(D,L-lactide-co-glycolide) which in turn could be modified by the lactide/glycolide ratio of the polymer, the drug to polymer ratio, coating, and hole dimensions. First-order release kinetics were observed for MT and CM1 implants in the in vivo study in rabbits. A correlation between in vitro and in vivo release was established which allowed in vivo release to be predicted from in vitro release data. For CM1, therapeutic tissue concentrations of 35.2μg/g (conjunctiva) and 17.7μg/g (sclera) were found at the implantation site up to 200 hours post-implantation. Tracer levels were undetectable in other ocular tissues.

Conclusions. The CM1 implant maintained steady antifibroblast levels in target tissues and minimized levels in nontarget tissues.

KEY WORDS: 5-fluorouracil; ocular; implants; poly(D,L-lactide-co-glycolide); subconjunctiva.

INTRODUCTION

Recent studies have suggested that postoperative subconjunctival 5-fluorouracil (5-FU) injections have considerably increased the success of glaucoma filtering surgery in eyes that are at high risk of failure (1–3). However, 5-FU subconjunctival injection has been associated with side effects such as recurrent corneal epithelial erosions and thin conjunctival blebs that may leak, resulting in hypotony and complications such as choroided effusions and macular oedema. These thin blebs may also increase the risk of subsequent endophthalmitis (4). In ocular clinical practice, there are various treatment regimens, but most surgeons give daily injections for up to 14 days in a subconjunctival site close to the fistula and the bleb because of the short ocular half-life of 5-FU (5). This requires daily visits for the patient, daily discomfort, risk of conjunctival haemorrhage, infection, conjunctival scarring, ocular perforation and risk of multiple puncture wounds in the conjunctiva that may lead to deflation of the drainage bleb, additional postoperative inflammation and toxicity to both corneal and conjunctival epithelium (6).

The peak concentration of 5-FU has been implicated as the cause of ocular toxicity (7) and the toxic concentration of 5-FU on the retina has been shown to be 100 μg/ml (8). Khaw et al. (9) reported that high dose 5-FU may result in gradual fibroblast and corneal epithelial cell death. In tissue culture, exposure to 5-FU concentration of 1000 μg/ml for a day or more, or 100 μg/ml for 12 days, caused gradual loss of viability of the fibroblast population.

In our previous pharmacokinetic study in rabbits (unpublished data), high concentrations of 5-FU were detected in the cornea, sclera and conjunctiva following subconjunctival injection. A reduction in these toxic levels in both target and nontarget tissues could result in improved clinical success if the ocular 5-FU retention time was increased.

In this paper, we describe 5-FU implants prepared from poly(D,L-lactide-co-glycolide) and the effect of lactide/glycolide ratio in the polymer, drug loading, coating, hole anatomy and hole dimensions on the release of 5-FU in an in vitro study. In addition, the in vivo release characteristics were investigated and tissue levels of 5-FU from the most promising formulations were determined.

MATERIALS AND METHODS

Materials

5-Fluorouracil was purchased from Sigma (St. Louis, U.S.A.). A carbon-14 labelled 5-FU, [6-C^{14}] (Amersham, Australia, 55 mCi/mmol) was used as received. Tissue solubilizer and scintillation fluids (organic counting scintillant (OCS) and aqueous counting scintillant (ACS)) were from Amersham (Oakville, U.S.A.). 75/25 and 50/50 poly(D,L-lactide-co-glycolide) were a gift from Birmingham Polymers Inc. (Birmingham, U.S.A.). They have nominal molecular weights of 100,000 and 60,000 respectively (10). Dichloromethane was analytical grade and sodium azide medical grade. Other chemicals were either analytical or spectroscopic grade. All solutions were prepared with distilled water. Rabbits, approximately 2–6 months of age and of either sex and weighing 1.5–2.5 kg were used. The research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23 revised 1985).

Preparation of Implants

Implants (CT; Fig. 1) containing only 5mg of 5-FU were made by direct compression using flat punches (2.5 mm diame-

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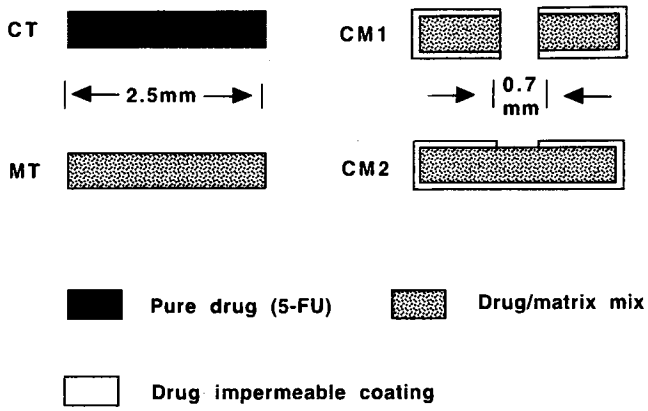

Fig. 1. Schematic representation of pure, matrix and coated implants. CT: pure drug only implant; MT: matrix implant; CM1: coated matrix implant with a hole drilled through the implant and coating; CM2: coated matrix implant with a hole drilled through the coating on one side.

ter). Matrix implants (MT; Fig. 1) were prepared by mixing either 50/50 or 75/25 poly(D,L-lactide-co-glycolide) /dichloromethane solution (0.5 g/ml), with 5-FU powder to produce a wet mass which was dried in a vacuum oven at 50 ± 1°C for 12h. The resultant dry solid was ground, sieved (16 mesh) and compressed as described above. Various drug to polymer mass ratios (90:10; 80:20; 70:30) were used.

Carbon-14 labelled 5-FU was added to the implant by mixing labelled 5-FU (250 μCi) in ethanol (15 ml) with 5 g of unlabelled 5-FU while the ethanol was evaporated under nitrogen. The content of labelled 5-FU in implants prepared from this 5-FU was measured by dissolving the implant in 100 ml buffer prior to scintillation counting.

Coated Matrix Implants

Some matrix implants were dip-coated with the same polymer as used in the manufacture of the matrix implant by immersing in a polymer solution containing 0.5 g polymer in 3 ml dichloromethane. A hole (0.7 mm) was then drilled by hand using a dental drill through the implant and the polymer coating (CM1; Fig. 1). In other cases a 0.7mm (unless specified otherwise) diameter hole was drilled through the coating only (CM2; Fig. 1).

The implants were 2.5 mm in diameter and about 1.5 mm thick; each implant contained 5 ± 0.2 mg of drug and varied in thickness depending on the manufacturing procedure (type, drug loading or coating). However, within each formulation the thickness varied by no more than 0.15 mm. Each implant weighed between 6–7 mg depending upon the percentage of polymer used in the implant. The coating weight of the polymer-coated implants was approximately 10–15% of the total implant weight.

In Vitro Release Study

Each implant was weighed and placed in 10ml of isotonic phosphate buffer (pH 7.4, 0.02% sodium azide, 37°C) shaken at 100 cycles per min. Samples (2 ml) were taken with replacement, acidified to about pH4.6 and analysed for 5-FU using a validated spectrophotometric assay (Shimadzu UV-240, Kyoto, Japan) at 266 nm. Percentage drug released was calculated from the original mass of 5-FU determined from the mass of the implant before coating. Dissolution tests were done in pentuplicate.

In Vivo Release Study

MT and CM1 implants containing a 9:1 mass ratio of 5-FU to polymer (75/25) were investigated in the in vivo study. In our previous pharmacokinetic study in rabbits (unpublished data), the results showed that no labelled 5-FU could be detected in the right eye after a single subconjunctival injection of 5-FU into left eye. Thus both eyes were used in this study. Each rabbit was anaesthetised with a mixture of oxygen and halothane (2.5% halothane in oxygen) at a rate of 1.0 L min^{-1} for about 15 mins. The eye was opened using an ocular spring, the conjunctiva cut and a weighed implant inserted beneath the conjunctiva 0.5 cm from the limbus. The incision was self-closing and the implants remained stable over time with no migration. At 24, 48, 72, 96, 144, or 192 hours postimplantation, a rabbit was re-anaesthetised and the implants removed and gently dried with a tissue. The concentration of 5-FU in a recovered implant was measured by UV spectrophotometry for matrix implants containing only 5-FU and by scintillation counting for the coated matrix implants containing labelled 5-FU.

Tissue Drug Measurement

After the carbon-14 labelled 5-FU implants were removed, approximately 0.3 ml of blood was withdrawn from the temporal area of the eyeball and another 2ml was collected by cardiac puncture. Rabbits were then sacrificed by injecting a lethal dose of pentobarbitone sodium solution (Nembutal®, Auckland, New Zealand) into the marginal ear vein. Tissue extraction procedures were then performed as described by Chiang and Schoenwald (11) with slight modification.

For conjunctiva, cornea, iris/ciliary body, lens, and sclera, 0.5ml of tissue solubilizer was added and the samples digested at 50°C overnight, then neutralised with 50 μl of glacial acetic acid to quench chemiluminescence and 10 ml of scintillation fluid (OCS) added. ACS scintillation fluid (10 ml) was directly added to vials containing serum, aqueous humour and vitreous humour. All samples were vortexed and dark-adapted overnight before counting. Disintegrations per minute (DPM) (Beckman scintillation counter, Model LS 3801) were corrected for background and tissue concentrations (μg/g) were calculated according to the ratio of labelled 5-FU and cold 5-FU. The counting efficiency was more than 40%.

Analysis of Results

Release data were fitted to either first-order (Eq. 1 and 2) or zero-order (Eq. 3) equations similar to those employed by Wagner (12) and Gibaldi and Feldman (13).

$$D_t = D_m - D_m \exp(-K_s t) \quad (1)$$

$$D_t = D_m - D_m \exp[-K_s(t - t_o)] \quad (2)$$

$$D_t = A + K_o t \quad (3)$$

D_m is the maximum cumulative percentage released and D_t is the cumulative percentage released at time t, K_s is the first-

order rate constant and K_o is zero-order rate constant. t_o is the lag time. The estimates of the parameters D_m, K_s, t_o and K_o were obtained by nonlinear regression analysis using the program Minim 2.0β (14) on a Macintosh computer (Model LC, U.S.A.). The largest value of R^2 and minimum AIC (15) were used to select the most appropriate model.

RESULTS

In Vitro Release

In vitro, 5-FU dissolved rapidly from CT and MT implants, whereas CM1 and CM2 (Fig. 2) released slowly. The MT, CM1 and CM2 were still intact at the end of the release study. In all cases release data were described by first order or zero order equations with R^2 values greater than 0.992. The effects of different polymers and 5-FU loads on the rate constants are shown in Table I.

Effect of Hole Diameter on the Drug Release

In order to examine the effect of hole size on drug release, a CM2 implant with 9:1 ratio of drug to 75/25 polymer was produced with a single hole of varying diameters in the coating. Increasing the hole diameter increased the release rate (Fig. 3).

In Vivo Release

In vivo release data were determined by measuring the drug remaining in recovered implants which had been implanted subconjunctivally for various times. Matrix implants released rapidly whereas the CM1 implants gave a prolonged release over 200 hours (Fig. 4).

Correlation between in Vitro and in Vivo Data

Since both in vitro and in vivo release profiles for CM1 were described by a first-order model, a correlation of in vitro and in vivo release data was established using times for 10, 20, 30, 40, 50 and 60% release of the incorporated dose (Fig. 5). The error bars are the inverse 95% confidence limits at the various percentages released obtained using the linearized forms of Eq. 1 and 2.

Table I. Release Rate Constants for 5-FU from the Various Implants (n = 5)

Formulations	Polymer Composition lactide/ glycolide	Drug Loading % drug	Drug Loading % polymer	Rate Constants (± SE)
CT		100	0	1.3662 (0.0186)[a]
MT	50/50	70	30	0.4469 (0.0163)[a]
MT	50/50	80	20	0.5633 (0.0156)[a]
MT	50/50	90	10	0.6093 (0.0202)[a]
MT	75/25	70	30	0.6337 (0.0365)[a]
MT	75/25	80	20	0.6215 (0.0231)[a]
MT	75/25	90	10	0.8355 (0.0398)[a]
CM1	50/50	70	30	0.0873 (0.0036)[a]
CM1	50/50	80	20	0.0998 (0.0046)[a]
CM1	50/50	90	10	0.1036 (0.0076)[a]
CM2	50/50	70	30	0.1610 (0.0076)[b]
CM2	50/50	80	20	0.1529 (0.0049)[b]
CM2	50/50	90	10	0.2181 (0.0099)[b]
CM1	75/25	70	30	0.0680 (0.0015)[a]
CM1	75/25	80	20	0.0832 (0.0056)[a]
CM1	75/25	90	10	0.0882 (0.0080)[a]
CM2	75/25	70	30	0.1464 (0.0077)[b]
CM2	75/25	80	20	0.1481 (0.0078)[b]
CM2	75/25	90	10	0.1384 (0.0053)[b]

[a] First order rate constant (h^{-1}).
[b] Zero order rate constant (mg/h).

Drug Concentration in Ocular Tissues

For CM1 only levels in sclera and conjunctiva near the implantation site were measurable (Fig. 6). Radioactivity levels

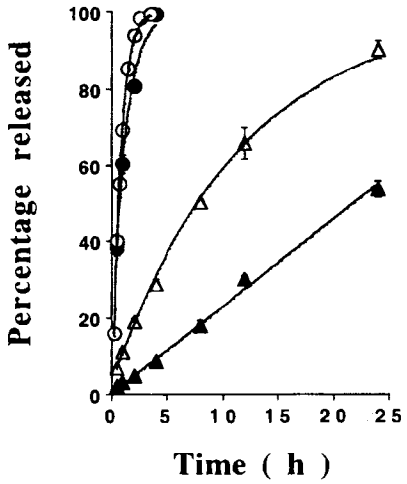

Fig. 2. Typical release profiles for the various implants CT (○), MT (●), CM1 (Δ) and CM2 (▲). Each matrix implant contained a 9:1 mass ratio of 5-FU to polymer (75/25). CM1 and CM2 had 0.7 mm holes. (mean ± SE, n = 5).

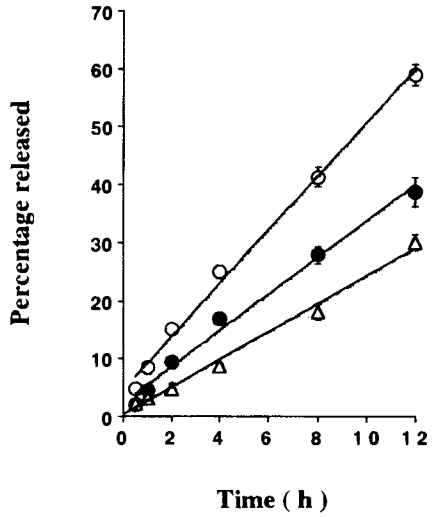

Fig. 3. Effect of hole diameter on the zero-order release profile of 5-FU from CM2 implants. Hole diameter: 0.7mm (Δ), 1.0 mm (●) and 1.4 mm (○). Lines are zero order fits over the first 12h. Each matrix implant contained a 9:1 mass ratio of 5-FU to polymer (75/25). (mean ± SE, n = 5).

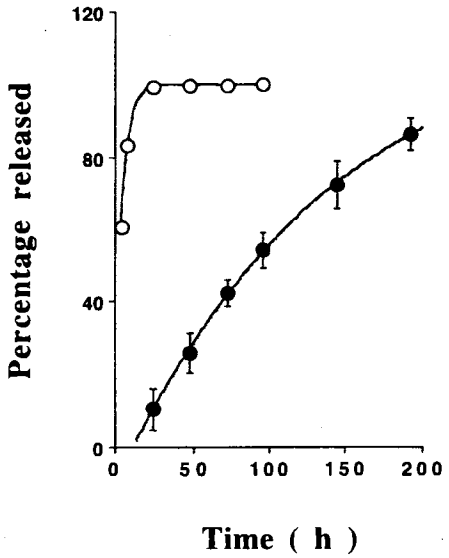

Fig. 4. In vivo release for 5-FU from MT (○) (n = 2) and CM1 (●) (mean ± SE, n = 4) implants following subconjunctival implantation in rabbits. Each matrix implant contained a 9:1 mass ratio of 5-FU to polymer (75/25) and CM1 a 0.7 mm diameter hole.

in aqueous humor, cornea, iris/ciliary body, lens, vitreous humor, sclera, serum in eye and serum in body were below twice the background level.

DISCUSSION

Studies have suggested that the success rate of filtering surgery is improved with postoperative subconjunctival injections of 5-FU; however local toxicity and administration procedures remain a problem (5,16,17). In the past 10 years several investigators have explored the use of controlled drug delivery systems for 5-FU. Liposomes (18), collagen shield implants (19), membranes (20) and intravitreal implants (21) of 5-FU have been reported in the literature. However, these approaches have not completely overcome the problems of delivering 5-FU over prolonged periods to the conjunctiva and sclera while maintaining low concentrations in non-target tissues.

In this study, 5-FU dissolved rapidly from the CT implant formulation under the experimental conditions (Fig. 2). Greater than 98% of the incorporated drug dissolved within 2 hours suggesting this formulation would not be suitable for ocular use. Copolymer type [50/50 and 75/25 poly(D,L-lactide-co-glycolide)] affected release in that decreasing the proportion of lactide in the polymer mix resulted in a decrease in release rate of 5-FU (Table I). The effect is unlikely to be due to different degradation rates of the polymers because after a 24 hour period little polymer degradation would have occurred. Typical degradation half-lives for these polymers are 15 days in pH 6.8 phosphate buffer at 37°C (10). Thus, the results may be due to the differences in the physical properties of the copolymers or the adsorption of the drug onto the copolymer.

For the matrix implant (MT) the release rate increased with the drug to polymer ratio (Table I) there being a significant difference between the 7/3 and the 9/1 loading ratio ($p < 0.05$). Presumably, this occurs because an increase in drug loading results in the formation of a more porous matrix structure within the implant (23). Observation by eye of the intact implants at the end of the release study revealed that a network of pores had formed throughout the implant. However, the delay in release using a matrix was not great so the matrix formulation (MT) was considered unsuitable for prolonged release.

To overcome this problem the matrix implant was coated to retard the rate of release of 5-FU. Preliminary experiments showed that for coating weights in the range of 10–15% of implant weight, the coat was impermeable to 5-FU as no release was detected over a 12 hour dissolution trial. In order to promote

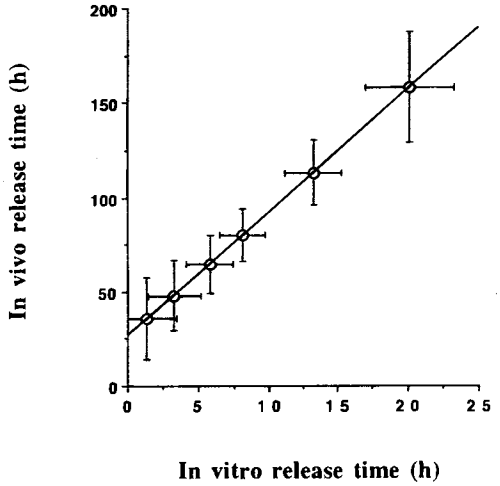

Fig. 5. Correlation between times taken for a given percentage of incorporated 5-FU to be released from CM1 implants following in vitro assessment (x axis) and subconjunctival implantation in rabbit eyes (y axis). Error bars represent inverse 95 % confidence limits. Each CM1 matrix implant contained a 9:1 mass ratio of 5-FU to polymer (75/25) and a 0.7 mm diameter hole.

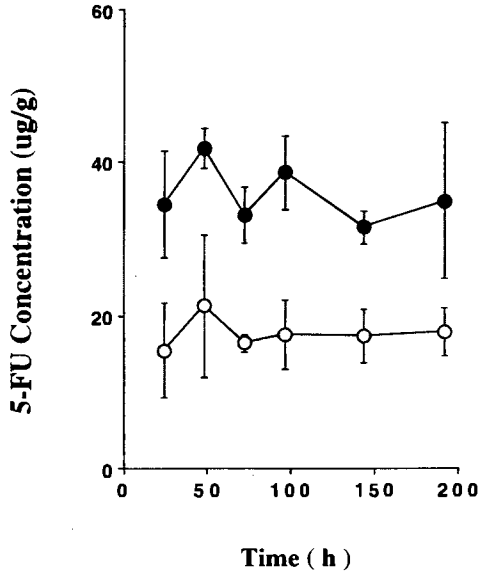

Fig. 6. Concentration of 5-FU in sclera (○) and conjunctiva (●) at implantation site following subconjunctival implantation of CM1 implants in rabbit eyes. Each matrix implant contained a 9:1 mass ratio of 5-FU to polymer (75/25) and a 0.7 mm diameter hole. (mean ± SE, n = 4).

release, a hole was formed (Fig. 1) in either one (CM2) or both surfaces (CM1). The first-order model gave a better fit for the CM1 implant and the zero-order model a better fit for the CM2 implant (Fig. 2) based upon the R^2 and AIC values. Previous authors have reported zero order release from coated devices with a single hole (24–26). In these devices the geometry was such that the diffusional release area increased with time compensating for the increase in diffussional distance of drug transport. Whether this occurs for CM2 has not been investigated. In contrast, for CM1 implants the release rate decreased with time possibly because of the increased diffusional distances of drug transport following release of the 5-FU near the surface.

Zero order release profiles for implants with hole diameters of 0.7, 1.0, and 1.4 mm were observed over the first 12h for 1.0 and 1.4 mm implants and 24h for 0.7 mm implant. Linear regression analyses showed that the zero-order release rate constant was linearly related to the diameter of hole according to:

$$k_o = -0.0062 + 0.201\ D \quad (4)$$

where k_o is the zero-order rate constant (mg/h) and D is diameter (mm) of the hole. The intercept ± SE (0.0062 ± 0.017) was not significantly different from zero. However when k_o was regressed on area of the hole, the relationship was linear but the intercept (0.092 ± 0.009) significantly greater than zero. Given that a linear relation between rate constant and area might be expected the reason for the positive intercept requires investigation.

The in vivo release rate of 5-FU from the MT implant was fast (Fig. 4), 80% of drug dose being released within 12 hours. This release rate was faster than that required for a sustained release preparation delivering over a period of about 14 days. The in vitro studies demonstrated that decreasing the drug to polymer ratio resulted in a decrease in 5-FU release rate. So the release in vivo might be slowed by reducing the drug to polymer ratio. However, a low drug to polymer ratio is undesirable in vivo because too much polymer would remain in the conjunctiva after the drug was exhausted. Incorporation of 5mg 5-FU would also result in too large an implant for subconjunctival implantation. From a clinical practice point of view, the ratio of drug to polymer is better kept as high as possible, i.e. 9:1. Coating such implants may prolong in vivo release of 5-FU for about 10 days. Although CM2 implants gave zero order release profiles in vitro, they were not chosen for in vivo assessment since it is desirable to achieve therapeutic levels of 5-FU rapidly. CM1 implants with rapid initial release were chosen for in vivo evaluation. The first-order release rate was about one tenth that observed in the in vitro study and a lag time of about 13 hours was observed from the in vivo release profiles. This lag time suggests that a loading dose would be required in the final formulation for use in clinical practice, in order to achieve an immediate effective concentration in the conjunctiva.

Since the in vivo release of a drug is not easily assessed, an approach is to choose an in vitro test which closely mirrors the in vivo conditions. In this study since the first-order release of 5-FU was observed in both in vivo and in vitro studies, a correlation between release data was developed as:

$$T_{in\ vivo} = 15.26 + 9.21\ T_{in\ vitro} \quad (5)$$

where $T_{in\ vivo}$ and $T_{in\ vitro}$ refer to the times taken for a selected percentage of the incorporated dose to be released from the implant in the in vivo and in vitro studies, respectively. 15.26 is the lag time in hours. Eq. 5 could be used to estimate and adjust in vivo release rate profiles following a relatively simple in vitro release study for this implant.

Steady-state concentrations of 5-FU were reached in the conjunctiva and sclera at the implantation site 24h after implantation and continued for 200h. In all the other tissue examined (aqueous humour, cornea, iris/ciliary body, lens, vitreous humour, sclera and conjunctiva (non-implantation site), serum in eye and serum in body) there was no detectable drug.

At steady state, the mean 5-FU concentrations in the conjunctiva (35.2 μg/g) and sclera (17.7 μg/g) at the implantation site were higher than the desired effective concentration, which is about 10 μg/ml (EC_{90}), but lower than the toxic concentration (100 μg/ml) (8). The mean 5-FU concentration in the conjunctiva at the implantation site is much lower than that of a previously investigated liposomal delivery system (18) and higher than that of an intravitreal implant (21).

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