Intravitreous Delivery of Dexamethasone Sodium m-Sulfobenzoate from Poly(DL-Lactic Acid) Implants

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Biodegradable intravitreal rod-shaped implants containing dexamethasone sodium m-sulfobenzoate (DMSB) were prepared from blends of poly(1-lactic acid) (PLA) with number-average molecular weight 2000 (PLA2000) and 4000 (PLA4000). The effect of the fraction of PLA2000 on the release of DMSB from the implant was investigated after implantation in the vitreous body of rabbit eyes. After the initial burst, the drug was released slowly from the blended PLA implants with a PLA2000 fraction of below 30 wt% in normal eyes within a period of 28 d. For the implants with a higher PLA2000 fraction of over 50 wt%, the drug was released following approximately first order kinetics. In the vitrectomized eyes, the release of DMSB from the PLA2000/PLA4000 (5/5) implant was 2.5 times more rapid than in normal eyes, and the clearance of drug was also appreciably accelerated as compared with that in normal eyes.

Key words dexamethasone sodium m-sulfobenzoate; poly(1-lactic acid); implant; vitreous; vitrectomy; rabbit

Several drug delivery approaches have been reported for treating vitreoretinal diseases to improve the poor penetration of topical instilled drugs into the posterior segment of the eye. These include intravitreal injection,1,2 iontophoresis,3 and implant,4,5 but the polymeric delivery systems have particularly been of interest to improve drug delivery to the site of action around the vitreous body.5 Poly(1-lactic acid) (PLA) has been widely investigated since its degradation product, lactic acid, is metabolized in the body. The drug release pattern from PLA can be varied with the molecular weight and the amount of drug loading.6,7

We report here the preparation of rod-shaped ocular implants consisting of blends of PLA with number-average molecular weight 2000 (PLA2000) and 4000 (PLA4000). We evaluated the effect of the fraction of PLA2000 in the blended PLA implant on the release of dexamethasone sodium m-sulfobenzoate (DMSB), water soluble dexamethasone derivative, and the vitreous concentration of DMSB after implantation in the vitreous body of normal rabbit eyes. We also measured the drug release rate and the drug level in the vitreous after implantation in the vitrectomized eyes for comparison with those in the normal eyes.

MATERIALS AND METHODS

Materials PLA with number-average molecular weight of 2000 (PLA2000) and 4000 (PLA4000) was supplied by Japan Synthetic Rubber Co., Ltd. (Tokyo, Japan). DMSB was obtained from Nippon Uclaf Co., Ltd. (Tokyo). All other chemicals were of reagent grade.

Preparation of Ocular Implants DMSB (1 g) and PLA (1 g) with a desired fraction of PLA2000 were dispersed homogeneously by melt-treating at 50 °C. The drug–polymer mixture was then cooled to −20 °C for 1 h, and crushed in a mortar with a pestle. About 4 mg of the drug–polymer mixture was charged into a Teflon tube with an inner diameter of 0.8 mm and then compressed into a rod-shaped implant by a steel rod at 50 °C. The amount of DMSB loaded was 50% by weight and the implant was 5 mm in length (average drug loading level of 2 mg/implant).

Animal Experiments Japanese albino rabbits weighing 2 to 2.5 kg were used. Vitrectomy was performed by a gas-mediated vitreous compression method.9 The implant was inserted into the vitreous cavity of normal rabbit eyes or vitrectomized rabbit eyes 3 mm posterior to the corneoscleral limbus with an 18 gauge needle under local anesthesia. At predetermined time intervals, rabbits were sacrificed with an overdose of intravenous sodium pentobarbital. The implant and the vitreous gel were then removed from the eyes. The amount of DMSB remaining in the implant and the mean concentration of DMSB in the vitreous body were determined by HPLC.

In Vitro Release Experiments The implants were incubated in 100 ml of an aqueous solution (pH 7.4) containing 0.796% sodium chloride, 0.036% potassium chloride, 0.018% calcium chloride, 0.03% magnesium sulfate, 0.1% sodium citrate, and 0.06% sodium acetate at 37 °C. At predetermined time intervals, the amount of DMSB remaining in the implant was determined by HPLC.

Determination of DMSB Concentration The implant was dissolved in a mixture of acetonitrile and distilled water (1:1) and DMSB in the solution was assayed by HPLC. The concentration of DMSB in the vitreous body was determined after extraction with ethanol. Chromatography was performed with an octadecysilane column (TSKgel ODS-80TM, Tosoh Corporation, Tokyo) and a mobile phase of a mixture of 45 ml of acetonitrile and 55 ml of 10 mM sodium dihydrogenophosphate aqueous solution containing 5 mM tetra-n-butylammonium chloride. The detection wavelength was 235 nm. The quantitation limit was 20 ng/ml and the coefficient of variation was 0.26%.

RESULTS AND DISCUSSION

Effect of PLA2000/PLA4000 Ratio on Release of DMSB The release profiles of DMSB from the PLA2000/PLA4000 implants in the vitreous body of normal rabbit eyes are shown in Fig. 1. About 90% of DMSB was released from the pure PLA2000 implant 10 d after implantation and only 18% was released from the pure PLA4000 implant after

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Fig. 1. Effect of PLA2000 Fraction on Release of DMSB from Blended PLA Implants in the Vitreous Body

○, PLA4000; △, PLA2000/PLA4000 (3/7); □, PLA2000/PLA4000 (5/5); ▽, PLA2000/PLA4000 (7/3); ●, PLA2000. Data are represented as the mean±S.D. (n=4).

Fig. 2. Concentration–Time Profiles of DMSB in the Vitreous Body after Implantation of the Blended PLA Implants

○, PLA4000; △, PLA2000/PLA4000 (3/7); □, PLA2000/PLA4000 (5/5); ▽, PLA2000/PLA4000 (7/3); ●, PLA2000. Data are represented as the mean±S.D. (n=4).

28 d. For the PLA2000/PLA4000 blend implants, the drug release was controlled by varying the fraction of PLA2000 in the implant. The amount of DMSB remaining after the first 3 d was 59±1.8% for the PLA2000/PLA4000 (7/3), 61±3.3% for the PLA2000/PLA4000 (5/5), and 65±6.2% for the PLA2000/PLA4000 (3/7). The fraction of PLA2000 may be inclined to increase during drug release, a tendency probably due to increase in the hydrophilic region in the implant, although none of the values was statistically significant. From the PLA2000/PLA4000 (3/7), the drug was released slowly after the initial burst of ca. 35%, while it was released mono-exponentially from the blended PLA implants with the PLA2000 fraction of more than 50 wt%. This was probably due to the difference in the rate of bulk erosion after hydration between the PLA2000 and PLA4000. 7

Figure 2 shows the concentration of DMSB in the vitreous body after implantation of the devices in normal eyes. The PLA2000/PLA4000 (5/5) implant maintained more than 10 µg/g-tissue level for 14 d and approximately 2.5 µg/g-tissue level during days 21 to 28. Cheng et al. reported that the dexamethasone device which maintained the drug level of 2.5±1.2 µg/ml in the vitreous could effectively control ocular inflammation in a rabbit model of uveitis. 8 The DMSB concentration after implantation of the PLA2000/PLA4000 (3/7) implant was 17 µg/g-tissue at 3 d after implantation and remained from 3 to 7 µg/g-tissue during 7 to 28 d. For an injection of DMSB in the vitreous body of normal rabbit eyes, on the other hand, the concentration of DMSB decreased monoenexponentially with the elimination rate constant of 0.258 h⁻¹ and the elimination half-life of 2.7 h. 9 It is obvious from these findings that the blended PLA implants maintained an effective therapeutic drug level in the vitreous body for one month.

Release Behavior of DMSB in Vitrectomized Eye

Vitreous surgical techniques include vitrectomy, scleral buckling, and dissection of preretal membrane. Therefore, it is necessary to evaluate the drug release behavior from the implant and clearance of the drug in vitrectomized eyes.

Figure 3 compares the release profiles of DMSB from the PLA2000/PLA4000 (5/5) implant in normal eyes and vitrectomized eyes. The release of DMSB from the implant followed approximately first order kinetics in both: (release rate, −0.083 d⁻¹; r²=0.967) and (release rate, −0.083 d⁻¹; r²=0.966), respectively. The half-life for the amount of drug remaining in the implant was 8.3 and 3.4 d, respectively. The vitreous in normal eye is a relatively high viscous gel-like mass consisting of collagen fibrils and hyaluronic acid. Vitrectomy, that is, removal of the vitreous gel produces a low viscosity fluid in the vitreous cavity and facilitates the turnover of water in the vitreous cavity. The difference in the rate of DMSB release is mainly attributable to the difference in the dissolution process of DMSB such as water penetration into the implant, drug dissolution, and leaching of drug in the vitreous humor between the two types of eyes.

The concentration–time profiles in the normal eyes and the vitrectomized eyes are shown in Fig. 4, where the profile for the injection of DMSB (2 mg) in the vitrectomized eyes is...
also plotted for comparison. For the injection in the vitrectomized eyes, the mean drug concentration was 345 μg/g-tissue at 1 d and rapidly declined to 4 μg/g-tissue at 3 d. The level in vitrectomized eyes was 56 μg/g-tissue at 3 d after implantation which was approximately 3 times higher than that in normal eyes, but it decreased more rapidly than that in normal eyes. The higher drug concentration in vitrectomized eyes at 3 d was presumably due to the greater amount of drug released in them. The rate of drug release in these eyes was 2.5 times higher than that in normal eyes. The behavior of drug molecules following intravitreal delivery may involve distribution throughout the vitreous by diffusion, then diffusion through the annular gap between the lens and the ciliary body into the posterior chamber, where drug elimination may proceed through the turnover of aqueous humor, and transport across the retina, where absorption into capillary blood will occur.11) Ohtori and Tojo reported that the diffusion coefficient of DMSB through the vitreous gel of rabbits, 5.1×10⁻⁶ cm²/s, was the same order of the magnitude as in aqueous solution in the in vitro experiment.12) Therefore, the rapid drug clearance in vitrectomized eyes probably reflects the loss of barrier function of the anterior vitreous/hyaloid membrane with consequent enhanced aqueous drainage, and the retina with facilitated transport into the subretina space. Indeed, the rapid clearance of 5-fluorouracil13) and triamcinolone acetonide14) in vitrectomized eyes following intravitreal injection have been also reported.

**Correlation between in Vivo and in Vitro Release**

Figure 5 compares the in vivo and in vitro release from the PLA2000/PLA4000 (5/5) implant. A good correlation between the two releases was observed in vitrectomized eyes (r²=0.991), but not in normal eyes.

In conclusion, the release rate of DMSB from the blended PLA implant was controlled by varying the fraction of PLA2000 in the implant. The drug release from the implant and the clearance of drug in vitrectomized eyes were appreciably faster than in normal eyes. The present intravitreal implant maintained an effective therapeutic level of DMSB in the vitreous for a longer period than the intravitreal injection. Further studies are required to precisely control the drug level in vitrectomized eyes.

**REFERENCES**