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Use of Fibrin Film as a Carrier for Drug Delivery: A Long-acting Delivery System for Pilocarpine into the Eye[1)]

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Fibrin film was evaluated as a new carrier for a long-acting delivery system for pilocarpine. The release characteristics of the pilocarpine-fibrin film system were investigated in *in vitro* and *in vivo* test models. The results indicated that the use of the pilocarpine-fibrin film system is more effective than that of the conventional liquid dosage form for prolonging the duration of a desirable pupillary response. Its good biocompatibility, flexibility, and heat processability suggest the material to be a good candidate system for pilocarpine delivery into the eye.

Keywords——fibrin film; pilocarpine; drug delivery; drug release; matrix; pupillary response; rabbit

The possible use of fibrin film as a new biodegradable carrier for drug delivery has been examined.[2)] Fibrin film is a bioplastic prepared from human plasma and is a flexible, nontoxic, sterilizable, and absorbable material.[3)] The safety and biocompatibility of fibrin film are reflected in its use as a dural substitute and in the prevention of meningocerebral adhesions.[4)] In addition, fibrin film is now available for use as an artificial skin,[5)] and an absorbable surgical implant material[3)] in the fields of gynecology[6)] and ophthalmology.[7)] Fibrin film shows good biocompatibility and should be useful as a carrier for implanted, surface-applied or inserted devices.

Many techniques have been utilized to modify the response to drugs which are delivered topically to the eye. In the development of a controlled drug delivery system for opthalmological applications, the use of the hydrophilic contact lens[8)] and the Ocusert system[9)] to prolong the action of pilocarpine on the eye has been reported. However, disadvantages included problems of insertion or retention, leakage, and discomfort.[10)] These vehicles are also more expensive than eyedrops. In order to circumvent some of these problems, the application of telopeptide-poor, reconstituted collagen[11)] and chitin derivatives[12)] as biodegrad able carriers for drug delivery was studied. However, the effectiveness of prolongation of drug action seems questionable. It is still desirable to develop less costly, more convenient systems.

In the present study, the potential use of fibrin film as a new carrier for a long-acting delivery system for pilocarpine has been examined.

Experimental

Materials——Wet fibrin film, with a stated thickness of 0.15 ± 0.05 mm and a fibrin content of 50% or more, was obtained from Green Cross Co., Osaka. Pilocarpine hydrochloride was of J.P. X grade. Water content in the film, determined from the weight loss, was about 58%.

Preparation of Pilocarpine-Fibrin Films——The fibrin film was rinsed with distilled water to remove surface contaminants and then immersed in a large volume of water and allowed to equilibrate at 37°C for 24 h before use. Strips, 1.8×1.8 cm, were cut from the fibrin film and soaked in 5 ml of 5% or 10% pilocarpine hydrochloride in water for 24 h at 37°C. The wet fibrin film was then dried by placing it in a desiccator (silica gel). The dried fibrin film prepared as above was crushed in a mortar and the particles obtained were melt-pressed (Tester Sangyo Co., Tokyo) at 160—170°C under 600 kg/cm^2 pressure for 2 min to produce films

of uniform thickness. The final circular fibrin film was 7.48±0.23 mm (mean±S.D., $n=15$) in diameter and 0.73±0.04 mm in thickness. The pilocarpine-fibrin film prepared was used for *in vitro* and *in vivo* studies within one month.

***In Vitro* Study**——The drug-fibrin film was placed separately in 20 ml vials containing 6 ml of distilled water. The drug release was followed with shaking at a rate of 60 strokes/min on a laboratory shaker at 37°C. Each film was successively transferred to fresh vials containing 6 ml of water. Analysis of the drug release into each 6 ml fraction was carried out by the U.S.P. colorimetric method.[13] Release studies were done at least in triplicate and the average values were plotted.

***In Vivo* Study**——Miotic studies were conducted using albino, 1.8—2.1 kg, male rabbits. The rabbits were kept in restraining boxes and allowed 1 h to acclimate to laboratory conditions. A solution (53% w/v, 0.01 ml) of pilocarpine hydrochloride in 0.9% NaCl was delivered from a microsyringe into the lower cul-de-sac of the left eye of four rabbits. The fibrin films were placed in the lower sac after being soaked for 30 s in 0.9% NaCl to allow the film to assume a semiplastic consistency and reduce the degree of initial contact irritation. Only one eye of each animal was used and the other eye served as a control.

The size of each pupil was measured with a micrometer held always at the same distance from the eye, by the same operator. Lighting and temperature in the test room were constant throughout the study. Separate formulations were tested on the same eye of the same rabbits at least one week apart.

Results and Discussion

Initially, the amount of pilocarpine which can be released from the drug-fibrin film into water was determined. Figure 1 shows plots of the data, expressed as the cumulative amount of the drug released, *versus* time. It is apparent that the release of pilocarpine proceeds more rapidly during the first 60 min. In this period, about 87 to 97% of the total release of the drug occurred. When the dried films of fibrin are placed in an aqueous environment, the fibrin rapidly hydrates and the drug is equally rapidly leached from the matrices.

The amount of the drug release from these matrix systems was found to be dependent upon the amount of the drug incorporated. The amount of the drug contained in the fibrin film was determined by measuring the drug concentration in the release medium after 24 h. Fibrin films, prepared by the above method after being presoaked with aqueous 5% and 10% pilocarpine, contained about 3.0±0.2 (mean±S.E., $n=4$) mg and 5.3±0.2 ($n=3$) mg of the drug, respectively.

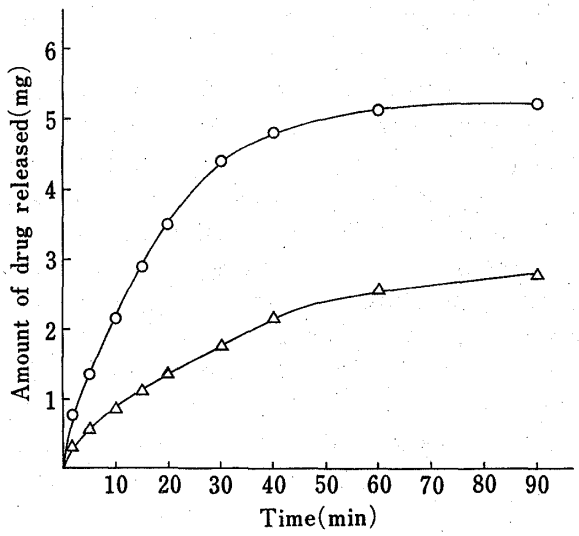

Fig. 1. *In Vitro* Release of Pilocarpine from Fibrin Film prepared after being presoaked with 5% (△) and 10% (○) Aqueous Drug Solutions at 37°C

This preliminary *in vitro* study indicated that fibrin film readily takes up and releases pilocarpine.

Figure 2 shows typical data obtained in the rabbit *in vivo* system. Pupillary responses show that duration of pupil size reduction was greatly increased by delivery of pilocarpine *via* the fibrin films as compared with that of the liquid dosage system. Restoration of normal pupillary diameter with the ophthalmic solution was observed to occur at between 4 and 5 h in contrast to more than 8 h for the drug-fibrin film systems. The results also indicate that an increase in drug content from 3.0 to 5.3 mg does not result in an appreciable increase in the pupil size reduction effect.[9] No inflammation was seen in the treated eyes and no pupillary constriction was noted in the control eyes.

Pilocarpine-fibrin film systems as tested *in vitro* did not release the drug in a manner consistent with the observed prolonged biological activity *in vivo*.[14] There is probably a

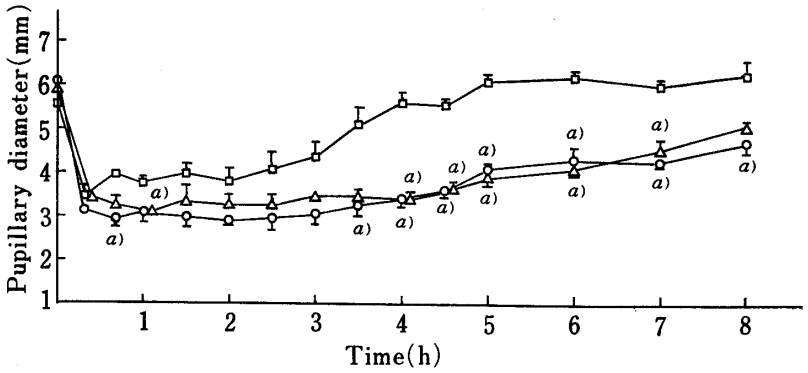

Fig. 2. Miotic Response Curve for Aqueous Pilocarpine (□), and Fibrin Films Containing 3.0 mg (△) and 5.3 mg (○) Pilocarpine

Each point represents the mean of 4 determinations and is shown with the standard error.
a) Denotes significant difference at $p<0.01$ from the liquid dosage system (t-test).

fairly rapid release *in vivo*, but the small amount of the drug still present after the initial stage of release does seem to continue to make a pharmacologic contribution. It is also suggested that the retention of the drug in the eye can be increased by the use of hydrophilic fibrin films. These factors contribute to the increased duration of drug action. Further studies are necessary on the mechanism involved. However, these preliminary results indicate that the use of fibrin film is more effective than that of conventional liquid dosage forms for prolonging the duration of a desirable pupillary response. Its good biocompatibility, flexibility, and heat processability suggest the material to be a good candidate system for pilocarpine delivery into the eye. In addition, fibrin film offers the possibility of controlling the rate of drug release in terms of the rate of absorption in the body because of its biodegradable property. The rate of absorption can be modified by suitable treatment of the fibrin.[15-17]

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