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Collagen ophthalmic inserts for pilocarpine drug delivery system

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Summary

Pepsin-treated telopeptide-poor foetal calf skin collagen has been employed as a carrier for a controlled release of pilocarpine nitrate. Three types of collagen–pilocarpine nitrate drug delivery systems were developed. In vitro release of pilocarpine nitrate from these systems was studied. The release studies indicated that after an initial boost release, pilocarpine was released at a constant rate following zero-order kinetics. The release of the drug can be manipulated based on the type of modification made on the collagen carrier. The release rate of pilocarpine nitrate could be regulated from 5 to 15 days depending on the modification made on collagen carrier. Collagen film, because of its biological inertness, structural stability and good biocompatibility, proved to be the most promising carrier for ophthalmic drug delivery systems.

Introduction

New ocular drug delivery systems have received much attention (Kim et al., 1980). This is partly because of the expected emergence of new drugs with short biological half-lives whose usefulness may depend on a more continuous drug supply than eye drops can provide, and also in part because of the potential of some drug delivery systems to reduce the side effects of potent new drugs (Capozza et al., 1978; Dohlman et al., 1972; Gurny, 1981).

Many techniques have been utilized to modify the response to drugs which are delivered topically to the eye. Some ocular delivery systems extend the duration of drug action by enhancement of corneal absorption (Gurny, 1981); these include soluble gels and emulsions (Bamba et al., 1979; March et al., 1982; Goldberg et al., 1979), hydrophilic ocular inserts (Coury et al., 1984), ion-pair associations (Davis et al., 1978), prodrugs and liposomes (Knight, 1981; Schaeffer et al., 1982; Smolin et al., 1981; Stratford et al., 1983). Although pilocarpine is widely used as a topical miotic for controlling the elevated intraocular pressure associated with glaucoma, the drug presents significant delivery problems. Its ocular bioavailability is very low and its duration of action is very short. The frequent administration of large amounts of pilocarpine is associated with transient peaks of high drug concentration in the eye, which in turn results in undesirable side effects such as myopia and miosis (Brown et al., 1976; Mazor, 1979).

The shortcomings of pilocarpine may probably be overcome by the development of a controlled

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release drug delivery system (Bensinger et al., 1976; Saettone et al., 1982; Ticho et al., 1979). By using delivery systems that release pilocarpine at a slow constant rate, the unnecessary high peak concentrations of the drug can be avoided in the eye and in the general circulation as well. Even though hydrophilic polymer matrices were used instead of eyedrops for controlled delivery of pilocarpine, it rapidly diffused from hydrophilic matrices due to its high water solubility. The diffusional release of pilocarpine from water soluble polymer matrices can be retarded, e.g. by increasing the molecular weight or the cross-linking density of the polymer. Even though the water solubility of the polymer decreases, a ghost matrix without drug remains in the conjunctival fornix for a long time after the drug has been released. Matrices from which drug and polymer dissolve at slow constant rates would seem to be potentially promising bioerodible systems of drug delivery for ophthalmic use.

In order to circumvent some of these problems, the possible use of collagen film as a vehicle for pilocarpine delivery has been examined by Rubin et al. (1973). However, the details are lacking and more systematic studies are needed.

In the present study, the potential use of collagen film as a new biodegradable carrier for a long-acting delivery system for pilocarpine has been examined. These studies were aimed at developing collagen delivery systems which are cheap, convenient and comfortable. Three drug delivery systems employing collagen in its native, cross-linked and chemically modified forms were developed and characterized. The in vitro release of pilocarpine from these systems has been studied.

Materials and Methods

Materials

Drug. Pilocarpine nitrate was obtained from C.H. Boehringer Sohn Ingelheim Am Rhein, F.R.G.

Collagen. Fresh calf foetus were obtained from a local slaughterhouse and preserved in a freezer until the removal of the skin from the foetus for further processing. All other reagents were of reagent grade and were used as obtained.

Methods

Preparation of soluble collagen. Soluble collagen was prepared by a standard method modified as described below. Foetal calf skin was used for the preparation of collagen as it proved to be a richer source of getting pure collagen than tendon and other sources. The calf skin was scraped on its outer and inner surfaces to remove hair and subcutaneous fat. The extraction procedure is as follows:

The skin was cut into small pieces (at + 4 °C). The small cut pieces were treated with acetone for 6 h using 3 vols. of the solvent. The acetone was decanted and the tissues were washed with 3 vols. of cold distilled water (4 times). The acetone-free tissue obtained was then treated with 10% sodium chloride for 24 h using 3 vols. of the solution. The sodium chloride solution was decanted and the tissues were washed again as described above. The salt-free tissues were soaked in citrate buffer of pH 4.3 for 48 h. The swollen tissue obtained was homogenised in ice-cold water, using a Kinematica polytron homogeniser (below 25 °C). The homogenised solution of collagen obtained was fibronated in ethanol for 24 h using vol. of ethanol per 5 vols. of collagen solution. The fibronated collagen solution was finally lyophilised.

Preparation of telopeptide-poor collagen. A solution containing 1% of the lyophilized collagen was prepared by dissolving 1 g of collagen in 100 ml of 0.001 M hydrochloric acid. The collagen was then made telopeptide-poor by treatment with pepsin (approximate ratio of enzyme to collagen is 1 : 400) at 20 °C with intermittent stirring for 5 days. Viscous solubilized collagen was filtered through cheese cloth and its pH was adjusted to 10.0 by sodium hydroxide solution and allowed to stand for 24 h at 4 °C to inactivate the pepsin. The pH of the collagen was subsequently adjusted to 7.0 and the collagen was precipitated. The precipitate was collected by centrifugation and washed with water 3 times. The enzyme solubilised telopeptide-poor collagen was then lyophilized. The purity of the collagen was evaluated by estimating the hydroxyproline using the method of Neuman and Logan (1950).

General procedure for the preparation of collagen films. A known volume of collagen solution (1%)

prepared by dissolving lyophilized, telopeptide-poor collagen in 0.001 M hydrochloric acid was poured on a teflon-laminated glass plate and allowed to evaporate at 20–24° C. The film thus formed was cut into circles with a circular die of diameter 1.5 cm. These circular films were stored in a closed bottle and kept in a refrigerator until they were used. Thicker films were also prepared to study the effect of film thickness on the amount of drug released.

Pilocarpine nitrate entrapment in collagen film. Telopeptide-poor unmodified collagen solution (1%) prepared by dissolving lyophilized collagen in 0.001 M hydrochloric acid, was transferred to conical flasks and a required amount of pilocarpine nitrate was added. The solution was thoroughly stirred to dissolve the drug and poured to spread uniformly on a teflon-laminated glass plate to get a film of uniform thickness as described earlier.

Pilocarpine nitrate entrapment in a cross-linked collagen. The entrapment of the drug was carried out exactly as described above, except the cross-linking of the film. The cross-linking of dried collagen film was carried out in 1% glutaraldehyde solution at pH 7.4 using phosphate buffer medium for 2 min.

Coupling of pilocarpine nitrate to collagen hydrazide. Collagen films were prepared as described above in the general procedure. The coupling reaction of pilocarpine nitrate onto modified collagen films was carried out by the following three steps: (i) methylation of collagen, (ii) preparation of collagen hydrazide and (iii) coupling of the drug to the collagen hydrazide.

Methylation of collagen films. Collagen films (circles) were methylated in dehydrated methanol (400 ml/g of collagen) containing 0.1 M hydrochloric acid for 7 days at 27° C in a tightly sealed vessel. Dehydration of methanol containing hydrochloric acid prior to addition of collagen was carried out by intermittent stirring with excess anhydrous sodium sulfate. The methylated collagen films (circles) were then dried quickly in the air.

Preparation of collagen hydrazide. The methylated collagen films (circles) were treated with 2% hydrazine hydrate solution in methanol (5 ml/circle) for 12–15 h at 20° C. The films were then washed with methanol and dried at 25–27° C.

Coupling of pilocarpine nitrate to collagen hydrazide. Required quantity of pilocarpine nitrate was dissolved in acetate buffer of pH 6. The films (circles) containing collagen hydrazide were then treated with the drug solution at 27° C at pH 6. An aliquot of the solution was withdrawn everyday and the amount of pilocarpine nitrate coupled to the collagen hydrazide was estimated as described below. The films (circles) were left in the acetate buffer solution till the coupling reaction was complete.

Estimation of pilocarpine nitrate. Pilocarpine nitrate was estimated by a standard method (U.S. Pharmacopeia, 18th revision, 1970). A standard curve was first obtained using pilocarpine nitrate as standard. A stock solution of pilocarpine nitrate containing 400 μg/ml of the drug was prepared. Known volumes of this solution ranging from 0.1 ml to 0.5 ml were taken in test tubes and made up to 5 ml with distilled water. One ml each of hydroxylamine hydrochloride solution (7%) and sodium hydroxide solution (14%) were added to these test tubes. The tubes were shaken well and left as such for 16 min. 1 ml of hydrochloric acid (6 N) and 1 ml of ferric chloride (5% solution in 0.6 N hydrochloric acid) were added and shaken well. Colour was developed and the absorbance of these solutions were noted at 500 nm. A standard curve of absorbance versus concentration of pilocarpine nitrate was drawn.

The pilocarpine nitrate concentration from unknown solutions was measured in the same way as described for standard pilocarpine.

'In vitro' release studies. The collagen films (circles) with pilocarpine nitrate entrapped in them were taken in culture bottles in phosphate buffer (6 ml for each circle) at pH 7.4. The culture bottles were shaken in a thermostatic water bath at 37° C. The amount of drug released was monitored colorimetrically every hour on the first day and every 6 h on subsequent days as described above.

Thermogravimetric analysis. Thermogravimetric analysis of plain collagen, methylated collagen

and collagen hydrazide were carried out with a DuPont 990 Thermogravimetric Analyser at a heating rate of 20 °C/min.

Micro shrinkage temperature measurements. Micro shrinkage temperature measurements of plain, cross-linked and methylated collagens and collagen hydrazide were carried out using a micro-shrinkage tester at a heating rate of 2 °C/min.

Intrinsic viscosity measurement. These studies were carried out with an Ubblehode viscometer. Viscosity was estimated by working at different concentrations of collagen in 0.05 M acetic acid at 20 °C. The flow time for water at 20 °C in the viscometer was 60 s. Intrinsic viscosity (η) was measured by extrapolating the reduced viscosity ($\eta_{sp/C}$) to zero concentration.

Results and Discussion

A number of solid-state drug carriers have been utilised for ophthalmic medication, for example the water soluble polymers polyvinyl alcohol and hydroxypropyl cellulose. Ocular inserts made of these polymers have been used to deliver anti-inflammatory, anti-infective and anti-glaucoma drugs (Benedetto et al., 1975; Bloomfield et al., 1977; Maichuk and Tishina, 1971). However, very scanty literature is available using collagen as a carrier for the delivery of the ophthalmic drugs. Delivery of gentamicin by an ocular insert made of succinylated collagen has been described by Bloomfield et al. (1978) and this approach appears promising for the treatment of ocular infection. Collagen-pilocarpine films in the treatment of glaucoma was employed by Rubin et al. (1973). 'In vivo' studies in rabbits indicated prolonged physiological activity. We have carried out a systematic study in developing ophthalmic ocular systems. Collagen films were chosen as a matrix for simple entrapment of pilocarpine nitrate on plain and cross-linked collagens. Collagen was also modified chemically by preparing their hydrazides and this material was used for covalent coupling of pilocarpine nitrate.

Pure telopeptide-poor collagen from foetal calf skin has been prepared as detailed in Materials and Methods. The purity of the collagen was measured by hydroxyproline estimation and intrinsic viscosity measurements. Intrinsic viscosity of the soluble collagen was found to be around 10.9 which is in agreement with the published data and which indicated the native structure of collagen.

Collagen films prepared as described earlier were used for the incorporation of pilocarpine. Pilocarpine nitrate was incorporated into circular collagen films of definite size (diameter 1.5 cm) containing 6 mg of collagen per circle of collagen film. The circular films were cut from the sheet of collagen film by making a punch using a stainless steel die. One set of films were cross-linked with glutaraldehyde after the incorporation of pilocarpine nitrate. The amount of drug in the film was estimated using the standard modified colorimetric method.

Collagen films of different thicknesses were prepared maintaining the collagen to drug ratio constant, for assessing the effect of thickness on the amount of the drug released. All the films after the drug incorporation were washed for 1 h for removing the adsorbed and loosely bound drug. These films were taken for in vitro release study. In all the graphs, initially μg/h release followed by μg/day release data is given for better presentation and understanding of the release data of the pilocarpine.

'In vitro' release of pilocarpine nitrate from collagen (plain and cross-linked) films

'In vitro' release studies of pilocarpine nitrate using both plain and cross-linked collagen films in physiological phosphate buffer of pH 7.4 at 37 °C have been carried out. Figs. 1 and 2 show the 'in vitro' release data of pilocarpine nitrate from the plain and cross-linked collagen films.

In Fig. 1, the 'in vitro' release data indicated that there was a boost release of 350 μg of the drug initially in the first 4 h. The release decreased to 140 μg the next day. This rate was maintained for another 5 days indicating the zero order kinetics (6 μg/h).

In the case of collagen films cross-linked with glutaraldehyde, the boost release was 265 μg in the first 4 h (Fig. 2). The release levels off to 120

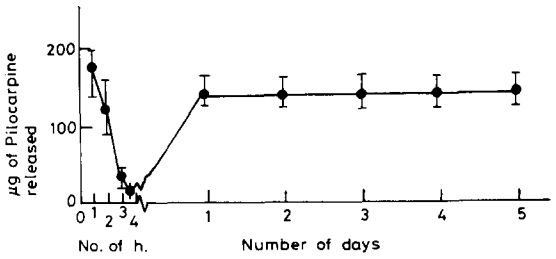

Fig. 1. Pilocarpine nitrate in vitro release by plain collagen film made from pepsin-digested foetal calf skin collagen. Error bars, ± S.E.M. of mean values.

µg/day which maintains for 7 days. These results clearly indicated the zero order kinetics in the cross-linked film too, except the boost release in the first few hours.

There is no marked difference in the release pattern between the plain and cross-linked films except that the release was extended for two more days for cross-linked film. Nevertheless it was expected that by cross-linking, the release rate could be extended for longer duration. The insignificant increase in the release pattern in the present case may be due to lower cross-linking of collagen matrix as compared to the plain film.

The 'in vitro' release data for pilocarpine nitrate using films of different thicknesses (Fig. 3) followed the same pattern as in the case of collagen film of 0.02 mm thickness. However, it is clearly indicated in these curves that a greater amount of the drug can be incorporated in the films of higher thicknesses and release of the drug (µg/day) is correspondingly higher than that from the films of lower thicknesses. From these data, it was clear

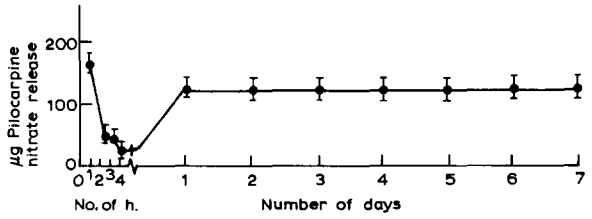

Fig. 2. Pilocarpine nitrate in vitro release by cross-linked collagen film made from pepsin-digested foetal calf skin collagen. Error bars, ± S.E.M. of mean values.

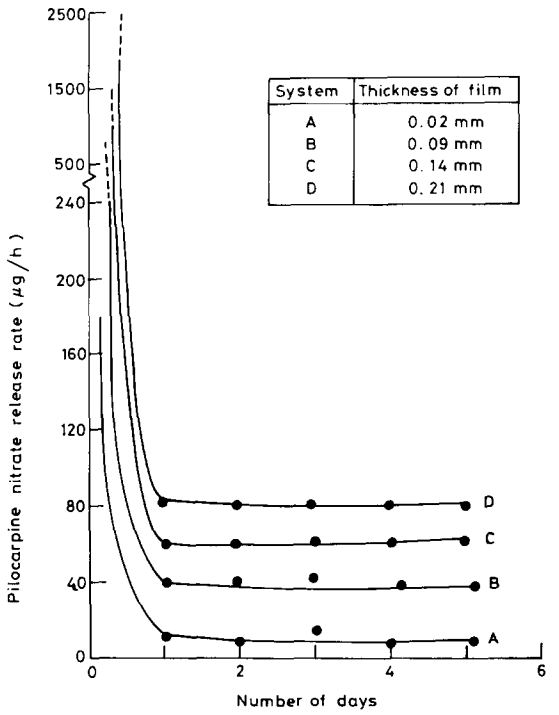

Fig. 3. Pilocarpine nitrate in vitro release from plain collagen film made from pepsin-digested foetal calf skin collagen. Effect of thickness on drug release.

that the rate of release of the drug can be manipulated based on the collagen film thickness. By gradual increase of film thickness from 0.02 to 0.21 mm (0.02 mm < 0.09 mm < 0.14 mm < 0.21 mm) a corresponding increase in release of the drug was observed from 8 µg/h to 82 µg/h. Furthermore, the rate of release was also found to be dependent on the nature of the collagen film, whether collagen was cross-linked or plain, or covalently linked to the drug.

Pilocarpine nitrate uptake by collagen hydrazide films

Collagen hydrazide was prepared as reported in Materials and Methods. Pilocarpine nitrate uptake by collagen hydrazide was estimated at different time intervals. It was found that the drug uptake was 80% after 5 days. It appears to be a slow reaction.

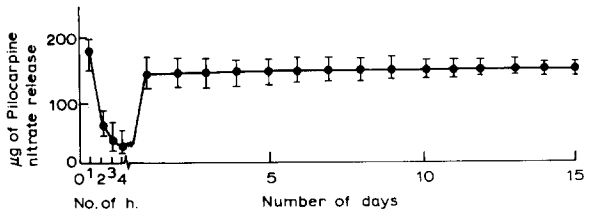

Fig. 4. Pilocarpine nitrate in vitro release by chemically modified collagen film made from pepsin-digested foetal calf skin collagen. Error bars, ±S.E.M. of mean values.

'In vitro' release study of pilocarpine nitrate from collagen hydrazide films

Fig. 4 shows 'in vitro' release curves for pilocarpine nitrate coupled to collagen hydrazide. It was clearly indicated that there was a boost release of about 310 µg out of 2400 µg in the first 4 h and then onwards the release rate followed the zero order pattern, at the rate of 140 µg day. The release lasted for 15 days as compared with 5 days for pilocarpine nitrate release from plain collagen and 7 days for cross-linked collagen films. This is expected since pilocarpine nitrate was covalently coupled through its keto groups to the collagen hydrazide by forming collagen hydrazone of pilocarpine nitrate. The reaction scheme is given in Fig. 5.

In the present investigation, shrinkage temperature of foetal collagen film without any modification gave a value of 48°C whereas methylated collagen film gave a value of 52°C. Collagen hydrazide gave a shrinkage temperature of 103°C as compared to 88–90°C in the case of glutaraldehyde cross-linked film. These results are

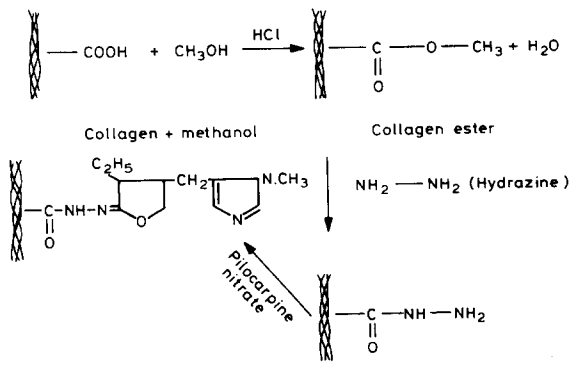

Fig. 5. Formation of collagen hydrazone of pilocarpine nitrate.

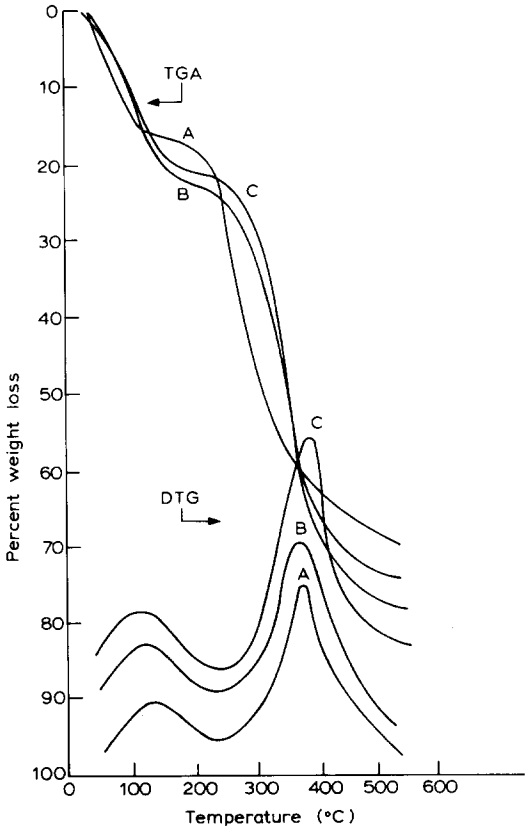

Fig. 6. Thermogram of (A) telopeptide-poor foetal calf skin collagen; (B) methylated collagen of the above; (C) modified collagen.

in agreement with the data obtained for the TGA characterisation of modified collagens. It was observed that the stability of collagens has been increased by the hydrazide treatment.

Thermogravimetric analysis of control and methylated collagens as well as collagen hydrazide was undertaken to study the stability of the modified collagens as compared to control collagen. Typical weight loss curves are shown in Fig. 6. In the present investigation, two peaks were observed, the first and second peaks correspond to water and protein respectively. The first peak, observed from 100°C to 200°C, corresponds to weight loss of residual or absorbed moisture in all 3 curves. The second stage starts at about 250°C and extends to 550°C with a maximum weight loss. The second stage reaches a maximum ratio of weight loss at about 375°C. In the case of methyl-

ated collagen, the peak at 375 °C corresponds to the peak at 375 °C for control collagen without any change in its stability. However, in the case of collagen hydrazide derivative, the peak was shifted by 5 °C. Both the TGA and shrinkage temperature data clearly indicated the increase in thermal stability of collagen by the hydrazide treatment. This may be due to some type of cross-linking in collagen. However, we have not tried to establish the type of cross-linking.

Sterilisation of collagen membrane for the control release of pilocarpine nitrate was not carried out in the present investigation. However, for 'in vivo' ophthalmic application, collagen film which carries the drug has to be sterilised. Collagen films may be sterilised by exposure to ultraviolet radiation at 120 °C for 2 h. This procedure has been followed (Dunn et al., 1967) for corneal transplantation of collagen derived membrane.

In conclusion, we have tried to prepare ocular inserts made of plain collagen, cross-linked collagen and collagen hydrazide derivative for controlled release of pilocarpine nitrate. This approach appears to be very promising from the 'in vitro' release data for the treatment of glaucoma. Investigations are in progress to evaluate the efficacy of these systems by 'in vivo' studies using rabbit model. These studies will be reported elsewhere.

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