APPLICATION OF ATELOCOLLAGEN SOLUTION FOR LACRIMAL DUCT OCCLUSION

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1. INTRODUCTION

Dry eye is a condition of dry, irritated, burning or gritty feeling in the eyes. It is mainly caused by ocular surface diseases, immunomodulation or injuries that affect tear secretion or composition.[1,2] The diagnosis and treatment of dry eyes have improved dramatically during recent years. Application of artificial tears is usually carried out as a method of treatment, but it requires frequent application and provides only temporary effect. In addition, it leads to epithelial cell toxicity, changes in epithelial membrane permeability and increased chances of eye infections.[3,4] Punctal occlusion prevents the discharge of natural tears from the lacrimal punctum.[5,6] Punctal occlusion prolongs the duration of tears on the ocular surface of the eye and improves the symptoms of dye eye significantly. The conventional method of punctal occlusion is the application of solid-type punctal plugs such as collagen-rod, silicone or plastic plugs. Such plugs often cause an unpleasant foreign-body sensation, corneal epithelial cell damage, granulation and accidental dropout.[7,8] To overcome such problems, we developed 3% atelocollagen solution, which forms fibrils at 37°C and neutral pH, for lacrimal duct occlusion as a new method for the treatment of dry eye. Atelocollagen is capable of forming fibrils (gel) at

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body temperature.[9] The gel formed from 3% atelocollagen solution is smooth and solid, so that when it is injected into the lacrimal duct, it forms fibrils and occludes the lacrimal canal. In the present investigation, we have studied the effect of atelocollagen solution for lacrimal duct occlusion using beagle dogs as an animal model.

2. METHODS

Atelocollagen was extracted from calf skin with pepsin digestion.[10] The extracted collagen was purified by isoelectric precipitation and filtration. The purified collagen was sterilized by micro-filtration. The atelocollagen solution was adjusted to 3% in 0.1 M phosphate buffer, pH 7.4.

Six beagle dogs (12 eyes) were used to evaluate the effect of atelocollagen on lacrimal duct occlusion. The dogs basal tear secretion, residual tear volume and tear turnover rate were studied on days 0–7 as control values. The third eyelid, which secretes 30–60% of basal lacrimal secretion,[11] was removed on day 8 under general anesthesia to reduce the basal tear volume. On day 56, after anesthetizing the animals with sodium pentobarbital, atelocollagen solution was injected into the lower lacrimal punctum until it overflowed through the upper punctum. The atelocollagen solution was applied in both eyes of all six dogs.

Schirmer tear test (STT)[12] was used to assess the basal tear secretion level before and after removal of the third eyelid. STT paper was obtained from Showa Pharmaceuticals (Tokyo, Japan). To avoid over-sensitization, 1 drop of Benoxil (Santen Pharmaceuticals, Osaka, Japan) was added to eye, and after 5 min the tear-testing paper was held to the lower eyelid for about 1 min and extent of the wet portion was measured.

Residual tear volume was determined before and after application of atelocollagen by phenol red thread tear (PRT, Showa Pharmaceuticals, Tokyo, Japan) test.[13] The cotton thread was held for 15 sec in the lower eyelid and length of the red color portion, which is directly proportional to the residual tear volume, was measured.

The tear turnover rate (TTR) was measured as described by Shimizu et al.[14]. To measure TTR, 5 μl FITC-labeled dextran (MW = 4400, Sigma, St. Louis, MO, USA) was applied in the eye and fluorescence intensity on the corneal surface was measured at 4, 6, 8, 10 and 12 min using an anterior fluorometer (Kowa, Tokyo, Japan). The values were converted to fluorescein concentration and the natural logarithm of the fluorescein concentration was plotted versus time. TTR was calculated from the regression curve.

The mean and standard error were calculated from the data. The results were evaluated using one-way analysis of variance (ANOVA). The mean values in each group were compared using contrast analysis method. The value of $P < 0.05$ was considered statistically significant.

3. RESULTS

The value of STT decreased significantly ($P < 0.05$) from day 19 onward after removal of the third eyelid, and remained at a reduced level after injection of atelocollagen (Fig. 1). The residual tear volume decreased significantly ($P < 0.001$) after removal of the third eyelid (Fig. 2). However, after injection of atelocollagen, the residual tear volume increased considerably ($P < 0.01$).

TTR decreased significantly ($P < 0.05$) after removal of the third eyelid (Fig. 3). Punctal occlusion with atelocollagen resulted in a further decrease of TTR values ($P < 0.001$).

4. DISCUSSION

The purpose of this investigation was to evaluate the effect of atelocollagen solution as a novel method for occlusion of the lacrimal duct. Immunochemical studies of collagen revealed that telopeptides are the major immunologic sites in native tropocollagen.[15] Atelocollagen obtained by pepsin digestion is a poor antigen[16] and is best suitable for occlusion of the lacrimal duct because of its poor antigenicity and excellent tissue compatibility.

In the present study we selected beagle dogs as an animal model because dogs are a good mammalian model for ocular studies and easy to maintain under laboratory conditions. It was necessary to remove the third eyelid of the dogs, which secretes 30–60% of basal tear secretion,[11] to create the environment of dry eye. STT proved the basal tear secretion decreased considerably after removal of the third eyelid. In this study we confirmed occlusion of the lacrimal duct by PRT and TTR test.

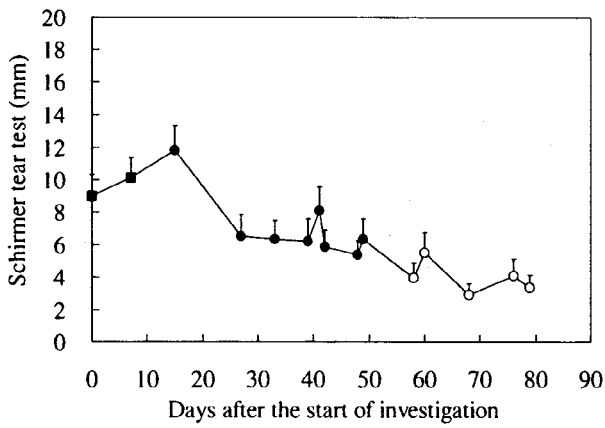

Figure 1. Basal tear secretion level before (■) and after (●) removal of the third eyelid and after application of 3% atelocollagen solution into the lacrimal duct (○) (Mean ± Standard error).

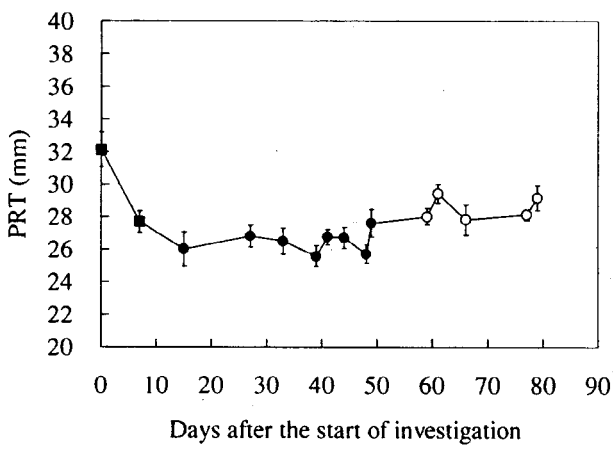

Figure 2. Residual tear volume before (■) and after (●) removal of the third eyelid and after application of 3% atelocollagen solution into the lacrimal duct (○) (Mean ± Standard error).

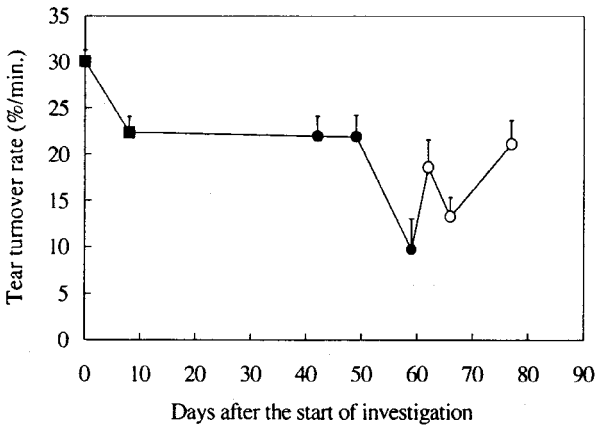

Figure. 3. TTR before (■) and after (●) removal of the third eyelid and after application of 3% atelocollagen solution into the lacrimal duct (○) (Mean ± Standard error).

Occlusion of the lacrimal duct by 3% atelocollagen solution is effective up to 3 weeks. When 3% atelocollagen solution is administered into the lacrimal duct of rabbits, occlusion is effective up to 7 weeks.[17] Importantly, the rabbit has a considerably bigger lacrimal sac compared to that of the dogs.

Our results suggest occlusion of the lacrimal duct by 3% atelocollagen solution is an effective method for treatment of dry eye. The atelocollagen solution can be injected into the lacrimal duct, regardless of its size, to form the soft fibrils without foreign-body sensation or corneal cell injury.

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