Effects of Bromhexine on the Secretions of Saliva and Tears

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Abstract—Effects of bromhexine and pilocarpine on the secretions of submaxillary saliva in dogs and of tears in rabbits were investigated including their effects on lysozyme activity in an attempt to elucidate the efficacy of bromhexine on Sjögren’s syndrome. Pilocarpine (0.3 mg/kg, p.o.) significantly increased spontaneous salivary flow rate, but bromhexine (20 and 40 mg/kg, p.o.) had almost no influence on spontaneous salivary flow rate. Pilocarpine increased total protein, saccharide, lysozyme and IgA secretions in saliva under electrical stimulation of the chorda tympani. Bromhexine did more markedly increase total lysozyme and IgA secretions in saliva, with minor increases in total protein and saccharide secretions. Pilocarpine (0.4 mg/kg, i.v.) had almost no influence on lysozyme concentration in tears, whereas it markedly increased tear secretion volume leading to an increase in total lysozyme secretion. On the other hand, bromhexine (4 and 8 mg/kg, i.v.) significantly increased both lysozyme concentration and total lysozyme secretion in tears from 50 min after injection, without influencing tear secretion volume. From these findings, it is suggested that bromhexine may work effectively on Sjögren’s syndrome by acting to accelerate the secretions of lysozyme and IgA in saliva and tears, which are known to have antiinflammatory and bacteriocidal effects.

Sjögren’s syndrome (SjS) is an autoimmune disease, characterized by the triad of keratoconjunctivitis (KCS), xerostomia and a connective tissue disorder (1). In SjS patients are observed the following symptoms: 1. a decrease in salivary secretion (2-4), 2. changes in composition of saliva such as high sodium and IgA concentrations and, especially, a marked low lysozyme activity (2-5), 3. a decrease in tear secretion (6), 4. a low lysozyme activity in tear fluid (6-8).

Lysozyme, which was discovered by Flemming (9), is mainly present in saliva and tear fluid, and it plays an important role in the protection of the oral cavity and eyes from infection. Scharf et al. (10) have reported that lysozyme activity is the most sensitive test for diagnosis of KCS, and, furthermore, can be used to evaluate the efficacy of treatment.

On the other hand, there has been no effective drug for SjS until recently except for steroids. Isager et al. (11) and Frost-Larsen et al. (12) recently reported that bromhexine, which is a mucolytic agent widely used in chronic obstructive lung diseases (13), has a beneficial effect on SjS. Since then, many investigators have ascertained that bromhexine is clinically effective in the treatment of SjS (14-16). However, there is no definite result concerning the effects of bromhexine on salivary and tear secretions and on the components in them, including lysozyme, in humans (17, 18). Moreover, the effect of bromhexine on secretions of saliva and tear fluid has not been studied in animal experiments.

The present study was thus undertaken in animals to determine the effects of bromhexine on the volumes of saliva and tear fluid and, furthermore, to determine its effect on the lysozyme activities in these fluids.

Materials and Methods
Saliva collection and determination of salivary components: The experiments were
performed on male mongrel dogs, weighing 8–15 kg, anesthetized with sodium pentobarbital (30 mg/kg, i.v.). They were deprived of food for 24 hr before the experiment, but had free access to water. A polyethylene tube (O.D., 1.0 mm; I.D., 0.5 mm) was inserted into the duct of a submaxillary gland for collecting saliva. The rate of salivary flow was calculated from the volume of saliva secreted during 30 min. In one series of experiments, the effects of drugs on the spontaneous salivary flow rate were investigated. In another series of experiments, the salivary secretion from the submaxillary gland was provoked by stimulation of the chorda tympani to determine the effects of drugs on the enhanced salivary flow rate and especially on the composition of saliva. The stimulation was given with square-wave electrical pulses (4 Hz, 2 msec, 3 V) 0.5 hr and immediately prior to and at 0.5, 1, 2, 3, 4, 5 and 6 hr after drug administration. Each stimulation was applied for 4 min because exhaustion of the salivary gland was caused if a continuous electrical stimulation was applied. Only the saliva that was secreted in a 2-min period between 2 and 4 min after the stimulation was collected in a plastic test tube with the capacity of 10 cc submerged in crushed ice and was weighed to calculate salivary flow rate. The chemical components of saliva samples were analyzed for protein, saccharide, lysozyme and IgA. Protein was determined by the method of Lowry et al. (19), and saccharide was determined by the method of Trevelyan and Harrison (20). Lysozyme and IgA were assayed according to the lysoplate method (21) and single radial immunodiffusion method (22), respectively. Each component in the fluids was evaluated as the total amount secreted during one min.

Drugs used were bromhexine hydrochloride (Nippon Boehringer Ingelheim Co.) and pilocarpine hydrochloride (Sanko). Pilocarpine was dissolved in and bromhexine was suspended in distilled water. The doses were represented as the respective bases. Either drug was administered into the stomach by the use of a catheter in a volume of 2 ml/kg of body weight.

Tear collection and tear lysozyme assay:

The experiments were conducted on male New Zealand white rabbits weighing 2.5–3.0 kg. The animals were anesthetized with i.p. injection of a mixture (50:1) of urethane (1000–1250 mg/kg) and sodium pentobarbital (20–25 mg/kg) and were given supplementary doses (urethane 250–500 mg/kg and sodium pentobarbital 5–10 mg/kg), if necessary. When no righting reflex, pain response and blink appeared at all, the animals were kept in rabbit fixed cages (Nippon Rabbit Co. 801) during the experiment. Tear secretion volume was measured according to Schirmer’s test (23) with a slight modification. A notch was bent at 5 mm from one end of a Schirmer tear test strip (Alcon, 5 mm×40 mm), and the strip was applied to a rabbit’s eye. The notch (5 mm in length) was within the conjunctival sac, and the remaining 35 mm was downwards outside the eye. At 90 sec after placing a part of the test strip inside the eye, the strip was removed and the notch was cut away at the bent part at 5 mm from one end of the strip. After a time lapse of another 60 sec, during which tear fluid was still descending along the strip, the length of the moistened area was measured using a millimeter scale (slide caliper) and expressed in terms of μl of tear volume per 90 sec from the relationship between tear volume (μl) and length (mm) of the wet portion of the strip. The procedure was repeated every 10 min with new strips for one side of the eyes. When the tear secretion volume obtained every time had been stable over 30 min, saline (control group), bromhexine or pilocarpine was injected i.v. in a volume of 2 ml/kg of body weight via the marginal vein of an ear.

Bisolvon Injection solution (Nippon Boehringer Ingelheim Co.) was used as bromhexine hydrochloride, and pilocarpine hydrochloride (Sanko) was dissolved in saline.

Lysozyme concentration in the collected tear fluid on the Schirmer’s test strip was assayed with the Schirmer lysoplate method (24). Lysozyme secretory activity was expressed as nanogram of total lysozyme output per 90 sec. These experiments were carried out in an air-conditioned room (20±1°C, 55–65% humidity).
Statistical analysis was performed using Student’s t-test.

Results

Effects of bromhexine on salivary secretion and components: As shown in Fig. 1, oral administration of bromhexine in doses of 20 and 40 mg/kg had almost no effect on spontaneous salivary flow rate, while pilocarpine, a cholinomimetic drug, in a dose of 0.3 mg/kg, p.o., caused a significant increase in spontaneous salivary flow rate in dogs (P<0.01), the effect lasting over 6 hr.

Next, the effect of bromhexine on the salivary secretion provoked by the electrical stimulation of the chorda tympani, as described in Materials and Methods, was studied. The basal salivary flow under the stimulation was 0.50±0.04 ml/min (N=6). Bromhexine (20 and 40 mg/kg, p.o.) and even pilocarpine (0.3 mg/kg, p.o.) had, in this case, no effect on the pre-existing enhanced salivary flow rate.

The results on salivary components shown below (Figs. 2 to 5) are those obtained in the experiments done under the stimulation of the chorda tympani. Figure 2 shows the time-response relationship of the changes in total protein secretion in saliva (for one min) after drug administration. In the control group, protein secretion decreased with time, while 0.3 mg/kg, p.o., of pilocarpine increased the secretion 0.5 to 4 hr after administration, a significant increase (P<0.01) being observed at 1 hr, as compared with the control group. Bromhexine in a dose of 40 mg/kg, p.o., also increased total protein secretion 1 hr after administration in comparison with the control group (P<0.01), but 20 mg/kg, p.o., of the drug showed no significant change. The time-response relationship of the changes in total saccharide secretion in saliva was almost identical with that in protein secretion (Fig. 3). Total saccharide secretion was significantly increased 1 hr after the administrations of 40 mg/kg, p.o., of bromhexine and 0.3 mg/kg, p.o., of pilocarpine (P<0.05) as compared with the control group. Figure 4 shows the time-course of changes in total lysozyme secretion in saliva after drug administration. In spite of a decrease in lysozyme secretion in the control group, lysozyme secretion was markedly increased by the treatments with 20 and 40 mg/kg, p.o., of bromhexine and also 0.3 mg/kg, p.o., of pilocarpine. The increases were evident from 0.5 hr after each drug administration, and the significant increase in lysozyme secretion by bromhexine was in a dose-dependent manner.
Fig. 2. Effects of bromhexine and pilocarpine on total protein secretion for one min in canine submaxillary saliva provoked by electrical stimulation. Each value represents the mean with S.E. of 5 to 7 experiments. Significant at **P<0.01 compared to the control value.

Fig. 3. Effects of bromhexine and pilocarpine on total saccharide secretion for one min in canine submaxillary saliva provoked by electrical stimulation. Each value represents the mean with S.E. of 5 to 7 experiments. Significant at *P<0.05 compared to the control value.

Figure 5 shows the time-course of changes in total IgA secretion in saliva after drug administration. IgA secretion was significantly increased 2 hr after administration of 20 and 40 mg/kg, p.o., of bromhexine and 0.3 mg/kg, p.o., of pilocarpine.

Effects of bromhexine on tear secretion and tear lysozyme: As shown in Fig. 6, 4 and 8 mg/kg, i.v. of bromhexine had almost no influence on tear flow rate, while 0.4 mg/kg, i.v., of pilocarpine significantly increased the rate in rabbits, the peak being observed shortly after the injection. Figure 7 shows the time-response relationship of the changes in
lysozyme concentration in the tears after drug injection. Pilocarpine in a dose of 0.4 mg/kg, i.v., caused a slight increase in the concentration. However, the increase was not statistically significant. On the other hand, bromhexine in doses of 4 and 8 mg/kg, i.v., markedly increased lysozyme concentration especially at 50 and 60 min after the injection, and bromhexine in a dose of 8 mg/kg, i.v., also significantly increased total lysozyme secretion at 50 min after the injection (P<0.05) (Fig. 8).

Discussion

New Zealand white rabbits were used for the tear experiments in the present study.
because rabbit orbital glands are generally considered to resemble human orbital glands anatomically and functionally (25), and high lysozyme activity is detected in rabbit lacrimal glands (26). In our preliminary experiments, a constant tear outflow during several hours in rabbits was obtained under anesthesia with a mixture of urethane and pentobarbital-Na. Therefore, tear collection was done under this anesthesia. For measurement of tear secretion volume, Schirmer's test was used in this study. To avoid underestimation of tear volume due to evaporation of tear water from the test strip, we attempted to express the length (mm) of the wet portion of the strip as tear volume (μl); for making the

![Graph Fig. 6](image1)

**Fig. 6.** Effects of bromhexine and pilocarpine on tear flow rate in rabbits. Each value represents the mean with S.E. of 5 experiments. Significant at *P<0.05, **P<0.01 and ***P<0.001, compared to the control value.

![Graph Fig. 7](image2)

**Fig. 7.** Time course of changes in tear lysozyme concentration following treatments with bromhexine and pilocarpine in rabbits. Each value represents the mean with S.E. of 5 experiments. Significant at *P<0.05 and **P<0.01, compared to the control value.
calibration curve, saline was used instead of tears. For the saliva experiments, dogs were used because it was easy to get a large amount of saliva which allows the determination of its components.

In saliva, total protein, saccharide, lysozyme and IgA secretions in the control group decreased with time until 60 min. Since the salivary outflow was kept constant by stimulating the glands with electrical stimuli, the rate of synthesis may be reduced rather than the secretion volume from salivary glands.

Submaxillary glands of carnivores are known to be composed of both mucous and serous cells (27). Some organic components such as protein and saccharide in submaxillary saliva originate mainly from mucous cells (27). On the other hand, lysozyme and IgA may originate mainly from serous cells (28, 29). In the present study, pilocarpine significantly increased not only the spontaneous salivary flow rate, but also increased total protein, saccharide, lysozyme and IgA secretions in saliva. Similarly, pilocarpine significantly increased flow rate and total lysozyme secretion. These findings suggest that pilocarpine increases the secretory activity of serous and mucous cells without changing the concentration of these components in saliva or tears. On the other hand, bromhexine in a dose of 20 mg/kg, p.o., markedly increased total lysozyme and IgA secretions in saliva with no change in salivary flow rate and did not cause a significant change in total protein and saccharide secretions, suggesting that bromhexine may mainly increase the secretory activity of serous cells.

SjS patients usually suffer from feelings of dry eyes (burning, itching, foreign-body sensation and increased mucinous discharge from eyes) and dry mouth (increased dental caries, oral soreness, difficulty chewing and oral aphthae) (30). These symptoms are based on atrophy and destruction of the gland structure. The most dramatic changes in compositions of saliva and tears in SjS patients are in the concentrations of lysozyme and IgA. In these patients, it has been reported that lysozyme concentrations in both saliva and tears are decreased, and salivary IgA concentration is sometimes increased (2-8). In the present study, bromhexine had almost no effect on salivary and tear flow rates, but did significantly increase lysozyme secretion in both saliva and tears. Our results in animal experiments confirmed the clinical result of Scharf et al. (10) that bromhexine has little influence on tear secretion, but increases...
the level of lysozyme in SjS patients.

Pilocarpine is sometimes applied systemically for the treatment of radiation xerostomia (mouth dryness) (31). However, this drug often causes significant side effects characterized by exaggeration of various parasympathomimetic effects such as hypermotility and -secretion of the gastro-intestinal tract and miosis. Recently, Isager et al. (11) and Frost-Larsen et al. (12) found that bromhexine has a beneficial effect on SjS. Since then, bromhexine has been used to manage SjS symptoms. Ben-Aryeh et al. (32) and Nahir et al. (33) reported that the sensation of mouth dryness in SjS patients could be caused by compositional changes of saliva. Ichikawa et al. (30) reported that there was no correlation between the sensation of dryness and the volume of tear or salivary secretion. It is thus considered that the involvement of the sensation of dryness in SjS patients by bromhexine may be mainly due to changes of the chemical composition such as an increase in lysozyme rather than an increase in the volume of salivary or tear secretion.

Lysozyme is normally present in abundance in saliva and tears (9). The enzyme is capable of lysing bacteria and thus plays an important role in the protection from infection in the oral cavity and eyes (9, 34). Lysozyme may additionally act on the mucus and then reduce the high viscosity of mucus in KCS. In the present study, i.v. injection of bromhexine significantly increased the concentration of lysozyme and total lysozyme secretion in tears at 50 min and later after the injection. Furthermore, lysozyme secretion in saliva increased with time until 6 hr by intragastric treatment of bromhexine. These slow onsets of responses may be ascribed to the following reasons: Tear fluid collected according to Schirmer's test was not that which was collected directly from the lacrimal gland excretory duct, but was that accumulated in the lower conjunctival sac. This may be associated with the slow response. An electronmicroscopic study (35) showed that an increase in the number of granules in serous-secreting cells in bronchial glands of the dog accrued at 30 min after i.v. injection of bromhexine. Thus a possible reason for the delayed action is that bromhexine metabolites may also be involved in the increase in lysozyme secretion.

In the present study, bromhexine significantly increased the concentration of lgA and total lgA secretion in saliva. Kado (36) reported that bromhexine also increased lgA secretion in the respiratory tract fluid in guinea pigs and humans. lgA is the major immunoglobulin produced by salivary gland tissue and is demonstrated to have a bacteriocidal effect (37). Moreover, evidence is accumulating for a close functional relationship between lysozyme and lgA in bacteriolysis (28). Therefore, the protective function against inflammation and infection in the oral cavity may be promoted by the accelerated secretion of lysozyme and lgA by bromhexine.

In conclusion, the results of the present study suggest that bromhexine may work efficiently on Sjögren's syndrome through the action to accelerate the secretions of lysozyme and lgA in saliva and tears, which have antiinflammatory and bacteriocidal effects.

References

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