Original Article

Rebamipide and mosapride enhance pilocarpine-induced salivation

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Background: During esophageal acid clearance, salivation plays an important role in defending the esophageal mucosa. Mosapride, an agent used in chronic, long-term therapy of gastro-esophageal reflux disease (GERD) was regarded as mediating its efficacy through prokinetic properties. Rebamipide is also widely used as an anti-gastritis and anti-ulcer agent in GERD patients with chronic gastritis. The aim of this study is to investigate the effects of rebamipide, mosapride, and risperidone on the salivation induced by pilocarpine. **Materials and Methods:** The experiments were conducted on 4-week male SD rats (120-150g). The salivation was induced by intraperitoneally administrated pilocarpine and saliva was collected using preweighted small cotton balls inserted into the animal's mouth every 30 min for 180 min. Thirteen minutes before intraperitoneal administration of pilocarpine, rebamipide, mosapride, and risperidone were administered intraduodenally. Control rats were conducted by intraperitoneal administration of saline and intraduodenal administration of 0.5% methylcellulose solution. **Results:** The saliva weight at 0-30 min was significantly ($p<0.01$) increased after administration of pilocarpine, compared to control rats. An additional administration of mosapride and rebamipide increased the saliva weight at 0-30 min. The total volume of saliva for 150 min after administration of pilocarpine was the highest after preadministration of rebamipide, followed by mosapride, and risperidone. **Conclusions:** Increase in salivation produced by i.p. pilocarpine was enhanced by preadministration of rebamipide and mosapride. (Urita Y, Watanabe T, Maeda T, Sasaki Y, Hike K, Muto H, Sanaka M, Shimada N, Nakajima H, Sugimoto M. **North Am J Med Sci** 2009; 1: 121-124).

Keywords: pilocalpine- induced salivation; mosapride; rebamipide.

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Introduction

Since exposure of the distal esophagus to acid is implicated in elicitation of both symptoms and mucosal damage, the importance of esophageal clearance is generally recognized [1, 2]. During esophageal acid clearance, salivation plays an important role in defending the esophageal mucosa [3, 4] . It is considered that systemically administered pilocarpine induces the salivary secretion. Additionally, it has been reported that intracerebroventricular injection of pilocarpine also induces salivary secretion in anesthetized rats [5, 6]. Takakura et al [7] also demonstrated that the pretreatment with intracerebroventricular injection of atropine inhibited the salivation induced by intraperitoneally administrated pilocarpine, suggesting that the salivary secretion elicited by systemically administered pilocarpine is mediated through the central nervous system as well as through the salivary glands.

Many studies suggest that proton pump inhibitors (PPIs) are the most effective medical therapy to control gastro-esophageal reflux disease (GERD) symptoms and heal esophagitis [8, 9]. PPIs are the major acid-suppressing drugs used for the treatment of GERD and have better characteristics for the long-term treatment of GERD, because they have a long-lasting, strong effect of raising intragastric pH and have no tachyphylaxis/tolerance phenomena on repeated dosing. However, PPI failure has become more prevalent with the increasing use of PPI as the first-line agent in the treatment of GERD [10]. On the other hand, laryngopharyngeal reflux (LPR) is a major cause of laryngeal inflammation and presents with a constellation of symptoms different from classic gastroesophageal reflux disease. Although LPR is frequently treated with empiric PPIs, most patients require more aggressive and prolonged treatment to achieve regression of symptoms [11].

Mosapride, which has been known to have both a 5-HT4 receptor agonistic and a 5-HT3 antagonist action and to be an agent used in chronic, long-term therapy of GERD was regarded as mediating its efficacy through prokinetic properties. Rebamipide is also widely used as an anti-gastritis and anti-ulcer agent in GERD patients with chronic gastritis. However, these other effects of the study drugs would make these agents even more attractive in the treatment of patients with GERD. Therefore, in the present study, we investigated the effects of rebamipide, mosapride, and risperidone on the salivation induced by pilocarpine.

Material & Methods

The experiments were conducted on 4-week male SD rats (120-150g). They were maintained under standard animal-housing conditions and had access to water and laboratory pellets except during the experimental period. After a 24-h fast, under urethane anesthesia, a tracheal catheter was inserted after incising the trachea to secure the airway.

Laboratory diet pellets were removed one hour before the measurement of saliva ry secretion. The salivation was induced by intraperitoneally administrated pilocarpine (0.5 mg/kg of body w eight), and saliva was collected using preweighted small co tton balls inserted into the animal's mouth every 30 min for 180 min. On the day of the experiments, rats were sedated with urethane (1mg/g) intrapertoneally, and kept in lateral decubitus. The cotton ball, 0.5 cm in diameter, was prepared and weighed in an analytic electronic scale. The first cotton ball was inserted under the rat's tongue. The salivary excretion is determined through the difference in weight of the cotton ball before and after co llection. The procedure of saliv a collection with the cotton ball was do ne at 30 -min intervals after pil ocarpine was administered intraperitoneally. Thirteen minutes before intraperitoneal administration of pilocarpine, rebamipide (10mg/kg), mosapride (1mg/kg), and risperidone (1mg/kg) were administered intraduodenally using a metallic tube. Control rats were co nducted by intraperitoneal administration of saline (1 mL/kg) and intraduodenal administration of 0.5 % methylcellulose solution. Each group consisted of 15 rats.

Results

Figure 1 shows the time-course changes in salivary secretion in four stimulated groups and unstimulated control group. The salivary excretion was stimulated with intraperitoneal administration of pilocarpine alone (pilocarpine group), pilocarpine and risperidone (risperidone group), pilocarpine and mosapride (mosapride group), and pilocarpine and rebamipide (rebamipide group). Saliva secretion reached a p eak at 0-30 min and decreased gradually to the baseline at 150 min. The saliva weight at 0-30 min was si gnificantly ($p<0.01$) increased after administration of pilocarpine, compared to control rats. Contrary to the expectation, the saliva weight at 0-30 min was significan tly ($p<0.01$) lower aft er additional intraduodenal administration of risperidone than after intraperitoneal administration of pilocarpine alone. An additional administration of m osapride and rebamipide increased the saliva weight at 0-30 min, but the differences did not reach a statistical significance.

Figure 2 demonstrated changes in saliva weight percent of risperidone, mosapride, and rebamipide group versus pilocarpine group. The sal iva weight was higher in rebamipide group but lower in risperidone group at each time point than in pilocarpine group. Mosapride group exceeded rebamipide group in salivary secretion at 90-120 min and had the highest value at 120-150 min.

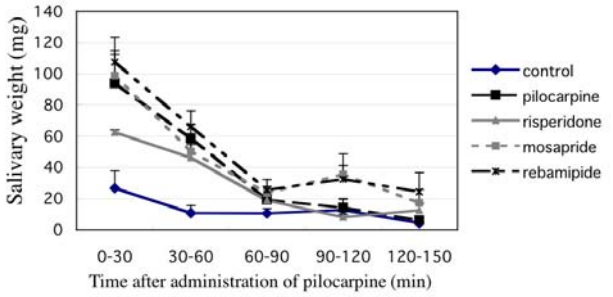

Fig. 1 The time-course changes in salivary secretion in four stimulated groups and unstimulated control group. Generally, saliva secretion reached a peak at 0-30 min and decreased gradually to the baseline at 150 min. The saliva weight at 0-30 min was sign ificantly lower in risperidone group than in pilocarpine alone.

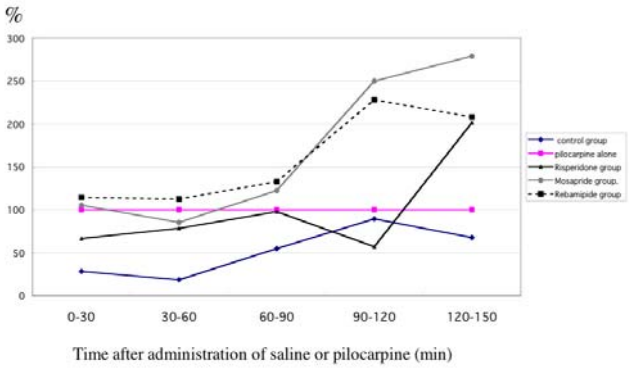

Fig. 2 Changes in m ean salivary wei ght percent of risperidone, mosapride, and rebamipide group versus pilocarline group. Mosapride group e xceeded rebamipide group in salivar y secretion at 90-120 min and had the highest value at 120-150 min.

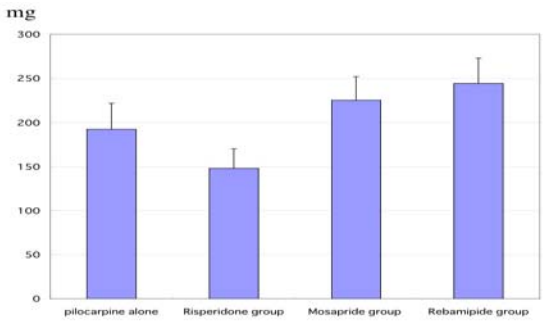

Fig. 3 Total volume of saliva fo r 150 m in each group. Rebamipide group had a maximum of total salivary secretion, followed by Mosapride group, Pilocarpine group, and Risperidone group.

Total volume of saliva for 150 min after administration of pilocarpine was 192.2 +/- 29.6 mg without premedication, 148 +/- 22 .2 mg with preadministration of risperidone, 225.3 +/- 26.7 mg with preadministration of mosapride, and 244.4 +/- 28.7 mg with preadministration of

rebamipide (Fig. 3). These differences (versus pilocarpine alone) did not reach the s tatistical significance. Fig.4 demonstrated the percent of saliva weight in ris peridone, mosapride, and rebamipide group in comparison with pilocarpine group at 0-60 min, 60-120 min, and 0-150 min. Increase in saliv a volume after preadministration of rebamipide and mosapride is the maximum at 60-120 min (176.6% and 173.4%, respectively). In contrast, saliva secretion was reduced after p readministration of risperidone.

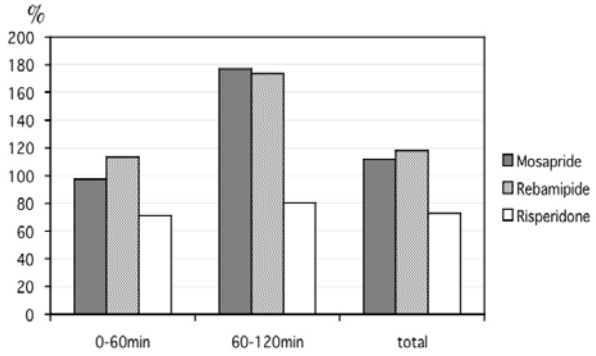

Fig. 4 The percent of saliva weight in risperidone, mosapride, and rebamipide group in comparison with pilocarpine group at 0-60 min, 60-120 min, and 0-150 min. The effects of mosapride and rebamipide on saliva secretion were almost equal.

Discussion

The major salivary glands produce 90% of the approximately 1.5L of saliva per d ay. In the basal state, 70% of saliva is sec reted by the submandibular and sublinguinal glands [12]. The various functions of sal iva include mechanical cleansing of the oral cavity , contributing to oral homeostasis and d ental health. The lubrication property of saliva depends on its con tents of mucins forming a gel that coats the food and makes it more easily moved about in the mouth. Various kinds of enzymes are present in saliva, i ncluding amylase, lysozyme, sialoperoxidase, linguinal lipase, ri bonuclease, deoxyribonuclease, and kallikreins. Amylase is th e major digestive enzyme and begins the digestion of starches. Lysozyme and sialoperoxidase provide important protective functions.

Xerostomia is the subjective feeling of a dry mouth, which is not necessarily linked with a sign ificant reduction in salivary flow. Saliva secretion is vital for maintaining oral health and function; thus, complications arising from hyposalivation such as dental caries, d ifficulty in swallowing, speaking and chewing, and an inc reased incidence of oral infection. The prevalence of xerostomia varies within a very wide range, from 10 to 80% [13]. This variation is what might be expected, as well as a l ogic consequence of how the question about the sensatio n of dry mouth is formulated. In a Swedish population sample of 4200 subjects, the positive answers to the question "Does your mouth usually feel dry?" corresponded to 21.3% and 27.3% for men and women, respectively [14]. There are a variety of ca uses but t he major ones are medication, especially tricyclic an tidepressants and sympathomimetic drugs, head and neck irradiation, and auto-immune inflammatory diseases such as Sjogren's syndrome, which targets exocrine glands in general [13].

Hyposalivation is not usually possible to distinguish from xerostomia because the etiology of hyposalivation is not very different from that of x erostomia. The di agnosis of hyposalivation is m ade by means of saliva flow rate measurement, and for chewing stimulated whole saliva, a cut-off value of 0.5 mL/min has been suggested to represent pathological secretion [15]. Esophageal acid clearance mainly depends on esophageal perstalsis and gravity leaving only a m inimal residue that sustains an acidic pH i n the esophageal mucosa until it is neutralized by swallowed saliva [16]. Salivary flow, volume, clearance, and alterations in the salivary electrolytic composition can influence the protective capacity of t he regional mucous membrane [17]. Thus, hyposalivation is asso ciated with various diseases.

The secretion of sal iva can be induced by several pharmacological drugs that mimic the natural neurotransmitters particularly those of the parasympathetic nervous system. Acetylcholine rapidly elicits a l arge volume of w atery saliva, particularly from the serous parotid glands. Pilocarpine, a presentative sialogogue, is well known for its largely parasympathetic stimulation and reduces depression of salivary secretion in human [18]. Despite the increased salivary secretion after administration of pilocarpine, thirst in the mouth is also induced via the ce ntral nervous system [19]. Higher dosage of pilocarpine can result in not only increased salivary secretion but also increased water intake. This may raise seri ous problems when pilocarpine is u sed as a medication for xerostomia. In order to attenuate the side effects of pilocarpine, the other drugs taken together have been used in clinical practice.

Nizatidine, a histamine H2 receptor antagonist, has bee n reported to inhibit acetylcholin e esterase, with a res ultant increase in acetylcholine, in the cholinergic system [20]. Adach et al [21] reported increased salivary secretion and bicarbonate output by nizatidine. Mosapride is a novel prokinetic agent which seems to exert its action via a high affinity and specificity for 5-HT4 receptors. 5-HT4-mediated actylcholine release from postganglionic neurons in the myenteric plexus has been suggested as an important mechanism behind the effects of prokinetic substances [22]. As expected, pilocarpine-induced salivation was enhanced by mosapride in the present study. On the other hand, contrary to our expectations, rebamipide also enhanced pilocarpine-induced salivation. Rebamipide is also widely used as an anti-g astritis and anti-ulcer agent in patients with chronic gastritis because it has oxygen radical scavenging effects and stimulates prostaglandin generation in the gastric mucosa [23]. Since the secretion of saliva is mainly in response to cholinergic nerve stimulation, we examined expression of dopamine 2 receptor (D2R) using immunohistochemistry in salivary

glands of rats. D2R was expressed more densely in rats with rebamipide than in those with mosapride and in controls (data not shown). It has been reported that D2Rs are localized to cholinergic nerve endings in the gastric myenteric plexus, and these presynaptic D2Rs mediate inhibition of acetylcholine release [24]. Therefore, antagonism of D2Rs results in an increase in acetylcholine release. Based on the fact that salivary secretion is regulated by parasympathetic nervous system and D2R are expressed in salivary glands of rats, D2R antagonists might be able to enhance the output of saliva.

Conclusions

Increase in salivation produced by intraperitoneal administration of pilocarpine was enhanced by preadministration of rebamipide and mosapride. The stimulatory impact of these drugs on salivary secretion would benefit GERD patients. We have to make a further study to confirm the stimulatory effect in human.

Conflict of Interest Disclosure

We hereby declare that there are not any potential conflicts of interest that are relevant to the manuscript.

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