− ORIGINAL ARTICLE −

Effect of 6-month administration of Cevimeline hydrochloride on salivary flow rate and s alivary components in primary Sjögren's syndrome patients

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一次性シェーグレン症候群患者の唾液分泌量と唾液成分に対する塩酸セビメリン投与後６ヶ月の効果

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Abstract : The aim of this study was to clarify pre- and post-cevimeline hydrochloride administrative changes of salivary flow rate and salivary components in primary Sjögren's syndrome (SS) patients. Twelve females who were diagnosed with primary SS were subjects in this study. Thirty milligrams of cevimeline hydrochloride was administered 3 times daily for 6 months. Whole stimulated saliva was collected from patients before and after administration. MMP(matrix metalloproteinase)-9 was measured by the sandwich-ELISA (enzyme-linked immunosorbent assay) method. The levels of Na^+, Cl^- and K^+ ions were measured by the ISE (ion-selective electrode) method. The level of Ca^{2+} ion was measured by the OCPC (ortho-cresolphthalein-complexone) method and amylase activity was determined by the enzyme coupling method. Whole stimulated saliva secretion was significantly higher ($p = 0.034$) after administration. Na^+,Cl^-,Ca^{2+} ions and MMP-9 levels were not different after administration. Amylase activity($p = 0.105$)and K^+ ion level($p = 0.105$)decreased but not significantly after administration. Therefore, Cevimeline hydrochloride at a dosage of 30 mg, 3 times daily may improve some symptoms of xerostomia in patients with primary SS, though there was no change of salivary components.

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抄録：この研究の目的は一次性シェーグレン症候群患者（pSS）の唾液分泌量と唾液成分に対する塩酸セビメリン投与前後の変化を調べることである。この研究の対象者は一次性ｐＳＳ患者12人であった。６ヶ月間１日３回30mgの塩酸セビメリンを投与した。試料としての全唾液は投与前後に集めた。Matrix Metalloproteinase-9（MMP－９）はsandwich-ELISA(enzyme-linked immunosorbent assay)方法で測定した。Na，Cl とＫイオンはOCPC(ortho-cresolphthalein-complexone)方法で測定し，アミラーゼ活性はEnzyme coupling法で測定した。その結果，全刺激唾液は投与後で有意に増加した（P = 0.034）。Na, Cl, Caイオンと MMP-9 レベルは投与後で変化がみられなかった。アミラーゼ活性（P = 0.105）とＫイオンレベル（P = 0.105）は投与後に減少傾向を示したが，有意差は認められなかった。それゆえ，塩酸セビメリン30mgの１日３回投与によって，一次性シェーグレン症候群患者（pSS）の唾液成分は変化しなかったが，口腔乾燥症状は改善する可能性が示された。

Introduction

Sjögren's syndrome(SS)is a systemic chronic autoimmune disease, characterized by symptoms of dry mouth and dry eyes, and can be primary or secondary to connective tissue diseases such as rheumatoid arthritis, systemic lupus erythematosus, polymyositis, and systemic sclerosis. Furthermore, it is said that SS is divided into primary and secondary classes according to symptoms and more prevalent in climacteric women. Pharmacotherapeutic approaches to xerostomia in SS include lubricating oral mucosa with saliva substitutes, treatment with immunosuppressive drugs and stimulating salivary gland activity with muscarine agents. Cevimeline hydrochloride(Saligren®, Evoxac®)is an acetylcholine analogue with high affinity for the muscarinic M3 receptor located on lacrimal and salivary gland epithelium and is an orally administered drug that relieves the symptoms in patients with SS[1]. In this study, we investigated the effect of cevimeline hydrochloride (Saligren®, Evoxac®) administration on salivary flow rate and salivary components in patients with primary SS.

Materials and Methods

1. Materials

Materials were as follows: Cevimeline hydrochloride (Saligren® Nippon Kayaku Inc. Japan; Evoxac® Daiichi-Sankyo Pharmaceutical Company Japan); MMP-9 enzyme-linked immunosorbent assay (ELISA) system kit (Amersham Bioscience, USA).

2. Subjects

The patients were diagnosed according to the preliminary classification criteria proposed by the Japanese Study Group on Diagnostic Criteria for primary SS[2]. Before administration of cevimeline hydrocloride, there were twelve female patients [average age 55.25±14.40 years (mean±S.D.)] with primary SS who had been referred to Niigata University, Medical and Dental Hospital. On the other hand, the control subjects were twenty one females [average age 54.00±9.76 years (mean±S.D.)]. All patients and control subjects gave informed consent and the Declaration of Helsinki (September 1989) was followed throughout the study. Statistical analysis was performed only on the patients in which enough saliva was available for measurements at all stages (before treatment, 1 month after treatment and 6 months after treatment).

3. Saliva sample

Patients were administered with 30 mg of Cevimeline hydrochloride 3 times daily for 6 months. Whole saliva was collected during stimulation by chewing paraffin for 10 min from patients between 9:00 am and 12:00 noon before administration as well as after administration at their first- and sixth-month visits.

The saliva of control subjects was collected in the same way as the patients. Immediately after the collection, the saliva was centrifuged at 12,000 rpm for 5 min at 4℃ to remove the precipitate. The supernatant was frozen quickly at -80℃ until use. The patients were asked not to eat on the day of examination. Except saliva flow rate measurement, the determination item numbers were not the same in all stages. The reason for this was the differences in the volume of saliva secretion among individuals. Therefore, for some patients, saliva volume was not enough to measure all items. The priority for measurement was MMP-9 ELISA assay.

4. One-step sandwich EIA system for MMP-9 ELISA assay

MMP-9 (Amersham Pharmacia Biotech Inc.) was

measured by the sandwich-ELISA method using an ELISA kit, as in our previous report[3].

5. Measurement s for Na^+, Cl^-, K^+, Ca^{2+} ions and amylase activity

The levels of Na^+, Cl^-, and K^+ ions were measured by the ISE (ion-selective-electrode) method[4]. The level of Ca^{2+} ion was measured by the OCPC (ortho-cresolphthalein-complexone) method[5]. Amylase activity was determined by the enzyme coupling method[6].

6. Statistical analysis

All values were expressed as the mean standard deviation (S.D.). All data were compared using the Friedman test. $P < 0.05$ was regarded as statistically significant.

Results

1. Salivary flow rate

The stimulated saliva secretion was significantly higher ($P = 0.034$) after administration (before ;2.6 ± 2.6ml/10 min, after 1 month ; 3.2 ± 2.6ml/10min, after 6 months ; 4.2 ± 3.3 ml/10min) as shown in Table 1. The salivary flow rate in control subjects could not be measured.

2. Na^+, Cl^- K^+, Ca^{2+} ion levels and amylase activity

Na^+, Cl^-, Ca^{2+} ion levels did not change after Cevimeline administration. On the other hand, amylase activity ($P = 0.105$) and K^+ ion level ($P = 0.105$) tended to decrease, but were not significantly different (Table 1).

3. MMP-9 level

For MMP-9, there was no difference, before and after cevimeline administration, as shown in Table 1.

Discussion and Conclusion

Secretion of saliva gradually increased over the administration period, suggesting the effectiveness of Cevimeline hydrochloride (Table 1). Especially, a long administration period (1 to 6 months) have shown improved effects in this study. Therefore, the patients have experienced relief from their symptoms following Cevimeline treatment, in agreement to other reports[1,7]. However, as placebo was not given in this study, a placebo effect cannot be ruled out.

On the other hand, control data were almost consistent with the patients with before treatment except Na^+ and Cl^- ions. These ions were lower compared to the patients with before treatment (data were not shown).

In our previous report, administration of Cevimeline hydrochloride improved mental issues, oral clinical examination (atrophy of lingual papilla) and subjective

Components		n	Mean	SD	Min	Max	probability*
saliva secretion (ml/10min)	before	12	2.66	2.67	0.0	8.0	0.034
	after 1 month	12	3.15	2.62	0.5	8.4	
	after 6 month	12	4.20	3.39	0.1	11.0	
Na(mmol/l)	before	4	39.75	28.00	16	78	0.549
	after 1 month	4	35.25	26.74	6	63	
	after 6 month	4	37.50	28.38	11	64	
Cl(mmol/l)	before	4	43.25	24.30	26	78	0.368
	after 1 month	4	35.50	20.09	14	58	
	after 6 month	4	35.75	22.47	16	59	
Amylase(IU/l)	before	4	5069.75	3741.84	2161	10220	0.105
	after 1 month	4	3206.25	1614.99	1819	5510	
	after 6 month	4	3044.00	1549.83	1675	4460	
K(mmol/l)	before	4	21.68	2.70	18.6	24.8	0.105
	after 1 month	4	18.28	3.37	14.6	21.7	
	after 6 month	4	18.73	2.23	16.6	21.2	
Ca(mg/dl)	before	4	2.33	1.14	1.4	3.9	0.549
	after 1 month	4	2.55	1.65	1.4	5.0	
	after 6 month	4	2.33	1.46	1.4	4.5	
MMP-9(ng/ml)	before	5	47.07	24.49	4.68	65.59	0.449
	after 1 month	5	45.96	22.38	13.87	68.11	
	after 6 month	5	50.34	20.28	16.79	67.32	

* : using Friedman test

Table I　Changes in salivary components before and after Cevimeline hydrochloride administration in primary Sjögren's syndrome patients

symptoms (the degree of talking with difficulty) in patients with primary SS[8]. Moreover, we previously reported that the MMP-9 level in saliva from patients with primary SS was significantly higher than that of healthy controls, suggesting that this contributes to destruction of the salivary gland[3]. In this study, administration of cevimeline hydrochloride did not affect a change of MMP-9 level, even though we had expected some differences after administration. This may be possibly because of the small number of patients.

The decreasing tendency of amylase activity may have resulted from dilution in saliva secretion, although not significantly ($p = 0.105$). Furthermore, it has been reported that secretion of amylase is closely associated with the sympathetic system and is increased in the presence of stress[9]. This is consistent with our previous report, which showed increased saliva secretion and reduction of patient stress with administration of Cevimeline hydrochloride[8]. In this study, the levels of Na^+, Cl^-, Ca^{2+} ions were not influenced by Cevimeline administration. Amylase activity and K^+ ion level tended to decrease, but were not significantly different compared to the levels before administration ($P = 0.105$). In cases of increase in rate of saliva, it was previously reported that Na^+, Cl^- ion levels were more increased and K + ion level had more tendency to decrease because of inability of reabsorption[10]. Our data were not consistent with this report except K^+ ion, maybe because of the small number of patients. Therefore, our findings have suggested that Cevimeline hydrochloride at a dosage of 30 mg, 3 times daily may improve some symptoms of xerostomia in patients with primary SS, though there was no change of salivary components. However, it is considered important to take steps to deal with side effects such as gradually increasing dosage, combination of use Cevimeline hydrochloride with trimebutine maleate (Cerekinon®) that act on smooth muscles directly and operate on opioid receptor, and is not a muscarinic agonist. Moreover, the introduction of rinse method has been recommended [11]. This study may suggest that these diagnostic approaches have been shown to be a valuable therapeutic option for the relief of dry mouth that accompanies SS.

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