

ORIGINAL ARTICLE

Effect of pilocarpine hydrochloride on unstimulated whole saliva flow rate and composition in patients with chronic graft-versus-host disease (cGVHD)

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A double-blind, placebo-controlled study was conducted to evaluate the effect of orally administered pilocarpine on unstimulated whole-saliva flow and composition in 28 patients with chronic graft-versus-host disease (cGVHD). Thirteen patients were treated with pilocarpine of 20 mg/day orally for 7 days, and 15 patients with placebo. Unstimulated whole saliva was collected in the morning on four occasions (30 min before pilocarpine or placebo intake, and 1 h, 1 day and 7 days after the first intake). Significantly, higher salivary flow rates, and sodium and total protein outputs were observed in the second samples of pilocarpine-treated patients compared with controls, whereas calcium and IgA outputs were not altered. Changes in these parameters were not significant in the third and fourth samples, although they were higher in the pilocarpine group. Patients who had received pilocarpine expressed satisfaction with their treatment. These data suggest that pilocarpine may improve salivary flow rate and the feeling of xerostomia in patients with cGVHD.

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Introduction

Bone marrow transplantation is being used as a therapy for hematological disease and other disorders.¹ Unfortunately, a number of patients who receive allogeneic BMT suffer from chronic graft-versus-host disease (cGVHD) following the procedure.^{2–6} Oral symptoms in extensive form are present in approximately 80% of patients.⁵ Usually,

atrophy of mucous membranes promotes the development of severe periodontitis and caries. Owing to the involvement of the salivary and lacrimal glands, the majority of patients with disseminated cGVHD develop sicca syndrome.⁷ In addition to the subjective complaints, patients with salivary gland dysfunction are susceptible to increased caries, oral pain, frequent infections and difficulties in speaking, chewing and swallowing. These difficulties may lead to inadequate nutrition and a decline in quality of life.⁸ This salivary flow reduction can be ameliorated by the administration of oral pilocarpine.³

The purpose of this study was to evaluate the unstimulated whole-salivary biochemical composition in cGVHD patients before and after the administration of oral pilocarpine.

Materials and methods

Patients and study design

Twenty-eight patients with cGVHD (as diagnosed by a physician), who were suffering from xerostomia with some remaining salivary function, were enrolled in a double-blind, placebo-controlled design study in the BMT Center of Sharyaty Hospital at Tehran University of Medical Sciences (TUMS) between August 2005 and August 2006.

Criteria for exclusion from the study were age, gender matching considerations and patients with cardiovascular diseases, uncontrolled asthma, angle closure glaucoma or hepatic impairment.

Thirteen patients were randomly assigned to case (eight men and five women, average age 33.5, range from 20 to 48 years) and 15 to control groups (10 men and 5 women, average age 31.6, range from 20 to 49 years). The case group was orally administered 5 mg of pilocarpine (Salagen, Novartis, UK), four times per day (20 mg/day) for 7 days. The control group received an identical-looking placebo.

The clinical physician and patients were unaware of the case and control drugs. The Ethics Committee of TUMS, Iran, approved the study protocol. Informed verbal and oral consent were obtained from each patient.

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Saliva collection

Saliva was collected in the morning from each participant; thereby controlling any possible effects that circadian rhythm may produce in saliva composition. Unstimulated whole saliva was performed by the spitting method. All subjects refrained from eating and drinking for 1 h before the sampling. Each individual was seated for approximately 5 min, before 3–5 ml of unstimulated saliva was collected into a pre-weighed plastic cup over a period of about 25 min. The saliva-filled cups were weighed and the weight of the cup subtracted. The flow rate was calculated in g/min, which is almost equivalent to ml/min.⁹

Unstimulated whole saliva was collected four times for each patient. The first samples were collected 30 min before pilocarpine or placebo intake, and subsequent samples collected 1 h, 1 day and 1 week after drug administration. The saliva specimens were immediately stored at -70°C for later determination of saliva composition.

Saliva analysis

Whole saliva was assessed colorimetrically by a spectrophotometer and using affiliated kits (Pars Azmoon, Tehran, Iran) for the analysis of whole-saliva calcium, total protein and IgA concentrations. Total protein concentration was measured by the Biurt method,¹⁰ using bovine serum albumin as a standard; calcium by the Arsenozo reaction method,¹¹ sodium concentration was measured by flame photometry and IgA by immunoturbidometry.¹⁰ Saliva outputs of these parameters were calculated by multiplying flow rate and each saliva composition. Changes for all saliva parameters were calibrated from the primary saliva (base line).

Statistical analysis

Results were tested for statistical significance in the differences between case and control groups by un-paired Student's *t*-test. Differences among means were considered statistically significant if $P < 0.05$. Data are expressed as mean \pm s.e.m.

Results

There was a significant difference in flow rate between case and control groups in the second samples, taken 1 h after drug administration (Table 1); mean saliva flow rate of case was higher than control group. However, the mean difference of saliva flow rate between case and control groups in the other samples was not significant. Saliva flow rate changes were significant in the second samples, but were not significant in the third and fourth samples (Figure 1).

No significant differences were observed in mean Na, Ca, IgA and total protein concentrations and their changes

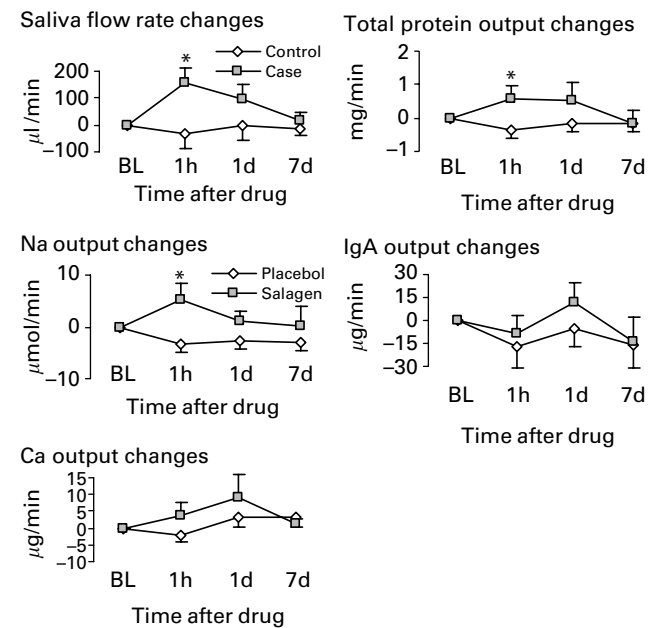


Figure 1 Unstimulated whole-saliva changes of Na, Ca, total protein and IgA outputs and flow rate following administration of placebo or pilocarpine, 20 mg/day orally (5 mg four times a day). Each line represents the mean and s.e.m. BL = base line (30 min before treatment); 1 h, 1 d and 7 d = 1 h, 1 day and 7 days elapsed following treatments, respectively. *Different from placebo, $P < 0.05$.

Table 1 Unstimulated whole-saliva flow rate and Na, Ca, total protein and IgA concentrations and their outputs following administration of placebo or pilocarpine, 20 mg/day orally (5 mg four times a day)

Time after drug	Base line		1 h		1 day		7 days	
	Placebo	Pilocarpine	Placebo	Pilocarpine	Placebo	Pilocarpine	Placebo	Pilocarpine
Flow rate (ml/min)	0.24 \pm 0.06	0.22 \pm 0.05	0.21 \pm 0.04	0.39 \pm 0.07*	0.24 \pm 0.05	0.33 \pm 0.06	0.23 \pm 0.06	0.24 \pm 0.05
Na (mmol/l)	52.5 \pm 8.7	61.0 \pm 11.1	45.40 \pm 8.2	56.3 \pm 12.3	44.2 \pm 8.4	50.8 \pm 11.6	45.8 \pm 8.7	54.3 \pm 13.2
Na output ($\mu\text{mol}/\text{min}$)	10.4 \pm 2.1	13.6 \pm 4.1	6.9 \pm 1.2	18.9 \pm 5.4*	7.9 \pm 1.8	15.2 \pm 6.2	7.8 \pm 1.6	15.5 \pm 6.7
Ca ($\mu\text{g}/\text{ml}$)	64.1 \pm 10.8	42.0 \pm 7.7	44.1 \pm 7.7	34.7 \pm 8.7	53.7 \pm 8.5	49.6 \pm 6.7	67.3 \pm 10.9	45.2 \pm 9.3
Ca output ($\mu\text{g}/\text{min}$)	9.8 \pm 1.4	8.3 \pm 1.9	7.8 \pm 1.9	11.8 \pm 4.2	13.1 \pm 3.5	17.6 \pm 6.3	12.2 \pm 2.7	10.2 \pm 2.4
Total protein (mg/ml)	8.3 \pm 1.1	7.4 \pm 1.2	7.4 \pm 1.2	6.0 \pm 1.2	6.9 \pm 1.1	5.7 \pm 1.0	7.1 \pm 1.2	6.0 \pm 0.8
Total protein output (mg/min)	15.3 \pm 2.3	14.7 \pm 3.3	12.0 \pm 2.4	20.6 \pm 4.0*	13.3 \pm 2.2	20.8 \pm 8.7	12.5 \pm 2.5	12.8 \pm 2.7
IgA ($\mu\text{g}/\text{ml}$)	271 \pm 52	219 \pm 53	207 \pm 35	134 \pm 39	214 \pm 37	165 \pm 39	184 \pm 31	172 \pm 35
IgA output ($\mu\text{g}/\text{min}$)	54.6 \pm 13.4	45.1 \pm 15.3	37.7 \pm 9.8	37.1 \pm 9.1	49.1 \pm 14.2	61.2 \pm 25.1	32.6 \pm 7.3	31.9 \pm 7.0

Base line = 30 min before treatment; 1 h, 1 day and 7 days are elapsed time following treatment. Data represent the mean \pm s.e.m.

*Different from placebo, $P < 0.05$.

between case and control groups at any sample point (Table 1 and Figure 2).

There was significant difference in mean salivary sodium and total protein outputs, and their output changes, between case and control groups in the second samples alone (Table 1 and Figure 1). Sodium and total protein outputs and their output changes in case group were higher than in control. However, no significant differences were found between case and control groups in Calcium and IgA outputs or their output changes at any sample point (Table 1 and Figure 1).

Patients who had received pilocarpine expressed satisfaction with their treatment. There was only one report of drug-related adverse effects – one patient claimed that his migraines had increased in intensity; in contrast, two patients claimed amelioration of their ocular dryness.

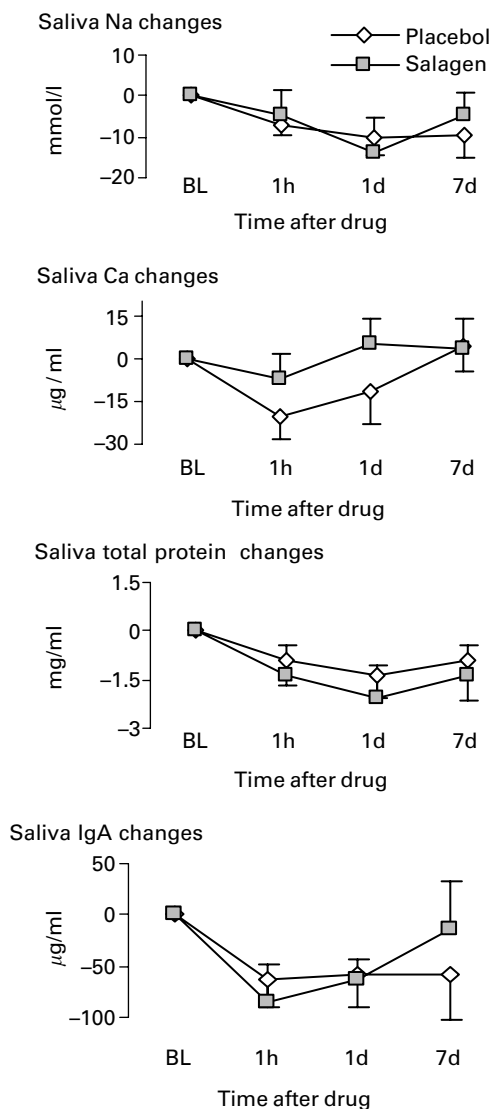


Figure 2 Unstimulated whole-saliva changes of Na, Ca, total protein and IgA concentrations following administration of placebo or pilocarpine, 20 mg/day orally (5 mg four times a day). BL = base line (30 min before treatment); 1 h, 1 d and 7 d = 1 h, 1 day and 7 days elapsed time following treatment, respectively. Each line represents the mean and s.e.m.

Discussion

Saliva is critical to the preservation and maintenance of oral health.^{8,12–17} Xerostomia is a major feature of cGVHD.^{6,18–20} Temporary symptomatic relief can be offered by moistening agents and saliva substitutes, and is the only option for patients without residual salivary function. The carboxymethylcellulose-based artificial saliva does not replace the many salivary macromolecules critical to protective and other functions of saliva. In patients with residual salivary function, oral administration of pilocarpine is effective in increasing salivary flow and improving the symptoms of xerostomia.^{8,21} Pilocarpine was found to be more effective than the artificial saliva.²² It has been shown that pilocarpine increases goblet cell numbers.²² Furthermore, more patients reported that it ameliorated their xerostomia, and more patients wanted to continue with it after the study.^{23,24} However, pilocarpine was found to be associated with more side effects than the artificial saliva. These side effects were usually reported as being mild.^{23,24} Some side effects, such as increasing lacrimal flow rate, are pleasant and can be considered as a benefit of pilocarpine in the treatment of xerostomia in cGVHD. In this study, there was only one drug-related adverse effect reported – one patient claimed an increase in migraines. In contrast, two patients had improvement in ocular dryness.

Our data indicated that there was no significant difference in flow rate of pilocarpine- and placebo-treated patients in the first, third and fourth samples, although flow rate of the case group in the third sample was higher than control group. But the flow rate and its changes in case group were significantly higher than control group in the second sample, 1 h after intake. In salivary gland hypofunction, the peak action of oral pilocarpine administration in Fox's report²⁵ was 30 min after intake, and afterward it decreased gradually. It seems that the second sampling was at the peak of pilocarpine action, because it had the most secretion, but the third and fourth samplings may not have been at the peak of drug action due to the variation in elapsed time after drug intake, when the samples were taken.

It has been shown that some saliva composition such as Na, IgA and total protein increased in cGVHD.¹⁹ Oral pilocarpine was able to reduce and normalize the elevated levels of Na, total protein and IgG salivary concentrations.¹⁹ In this study, no significant differences were observed in Na, Ca, IgA and total protein concentrations and their changes between case and control groups in any of the samples. There were also no significant differences in Ca and IgA outputs and their changes. However, a significant difference in Na and total protein outputs and their changes were observed in the second samples. The changes in Na, Ca, IgA and total protein outputs in the case group were higher than the control. It seems that pilocarpine may increase the output of saliva composition. Consistent with others^{3,4,19,26} our study may indicate that pilocarpine normalizes salivary flow and composition alteration in cGVHD patients.

A significant statistical increase was observed only in the second sample; nevertheless, this elicited comments of satisfaction from patients after treatment. Any increase in

salivation, no matter how small, which is accompanied by an increase in the production of beneficial constituents, may be a benefit to patients with xerostomia.^{3,4}

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