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Evaluations on Salivary Flow Induction and Dissolution Patterns in Saliva of Pilocarpine Chewing Tablet in Healthy Human Volunteers

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건강한 성인 지원자를 대상으로 한 필로칼핀 저작정의 타액분비 유도 및 타액중 용출패턴 평가

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Xerostomia is caused by organic or functional changes affecting the salivary system at different levels. Patients suffering from xerostomia may also complain of an oral burning sensation, ulceration or soreness, difficulty in swallowing, and poor denture retention. And pilocarpine is administered orally to induce salivary secretion. In Seoul National University Hospital(SNUH) pharmacy, the pilocarpine chewing tablets are prepared and supplied to patients of xerostomia in request of the dental hospital in SNUH. And we tested the salivary flow induction and the dissolution patterns of these products in saliva by a double-blind, sequential cross-over trials to eight healthy human volunteers with placebo. The pilocarpine chewing tablet contained 5 mg of pilocarpine, and placebo consisted of same materials as test drug, but didn't contain pilocarpine. *In vivo* experiment, all subjects were instructed to chew as 60-80 times/min. Mixed saliva was collected in the ranges of intervals such as 0-2, 2-5, 5-10, 10-15, 15-20, 20-30, 30-45 and 45-60 min after pilocarpine chewing tablet or placebo administration. Saliva volume was measured in each collecting time interval, and saliva pilocarpine concentrations were determined by reversed phase HPLC. The 82.5 percent (4.13±0.69 mg) of pilocarpine was extracted from chewing tablets during mastication of 60-80 times per minute for 60 minutes. Among these dissolved amounts, 90 percent was extracted within 20 minutes. The salivary flow rates were more increased in a group who administered pilocarpine chewing tablet at the interval of 5-10, 10-15, 20-30 and 45-60 min rather than a placebo-group, but only extracted amount of pilocarpine at 45-60 min interval is significanly different between two groups ($p<0.05$). But total amounts of saliva secreted for 1 hour in two group -pilocarpine and placebo treated- were 46.36±9.72 m*l* and 39.09±7.81 m*l*, respectively, and were not significantly different between two groups

Keywords—Xerostomia, Salivary flow induction, Dissolution, Pilocarpine chewing tablet, Human volunteers, HPLC, Double blind, Cross over trials

Pilocarpine is considered to a parasympathomimetic drug and has often been used to stimulate salivary secretion in patients with salivary gland dysfunction and dry mouth (xerostomia) or postradiation xerostomia.[1-3] The effects of insufficient salivary flow are not trivial. Significant salivary gland dysfunction impairs mastication, deglutition, and gustation while heightening susceptibility of the oral and soft tissues to a variety of destructive processes. To overcome xerostomia, pilocarpine hydrochoride 2.5 mg or 5 mg capsules are administered to these patients, but side effects occurring with the greatest frequency were transient sweating, a sensation of warmth

or flushing, and an increase in the urgency or frequency of urination.[4,5]

The dental hospital in SNUH designed a new dosage form, pilocarpine chewing tablet, to overcome the side effects following oral ingestion of pilocarpine capsules. And the purpose of our study was to examine the dissolution pattern of pilocarpine in saliva and the local effects of pilocarpine to xerostomia by spitting out saliva during mastication of pilocarpine chewing tablet.[6]

Experimental

Study Design

A double-blind, placebo-controlled, cross-over study was conducted to evaluate efficacy of pilocarpine chewing tablet in two groups (each group n=4), which was evaluated by the measuring the flow rate and amount of saliva secretion at each collecting time interval for 1 hr. And dissolusion patterns of pilocarpine in saliva were also checked during mastication. This experiment was carried out for 1 hour.

Human Volunteers

Eight healthy adult volunteers (male=2, female=6) were participated in this study after obtaining consent forms. All volunteers were healthy, and had no problems in physical examinations and clinical laboratory tests of blood chemistry and urine analysis. And all volunteers also had no imflammatory disease in the mouth. The average age of them was 25.3 years old, and the average body weight was also 52.3 Kg within in the ranges of 10 percent of ideal body weight.

Preparation of Pilocarpine Chewing Tablet

A 5 mg of pilocarpine chewing tablet was prepared at the department of pharmacy in SNUH. The prescription of pilocarpine chewing tablet was provided from dental hospital in SNUH, which was as follows (Table I). Pilocarpine hydrochloride (K.P.) was purchased from Kook-Jun Company located in Seoul, Korea. And other substances were supplied from Saehan Pharmaceutical Company. Placebo chewing tablet was prepared with same components which didn't contain pilocarpine hydrochloride.

Table I— *The Prescription of Pilocarpine Chewing Tablet which contains 5 mg pilocarine in 2.85 gram weight of chewing tablet*

Component	Amount
Tablet base	918.88 mg
Pilocarpine	5.00 mg
Lecithin	9.18 mg
Hydrogenated oil	30.60 mg
Liquid glucose	408.70 mg
D-Sorbital solution	63.70 mg
Dextrose	299.00 mg
Sucrose	050.00 mg
Aspartame	10.20 mg
TiO_2	8.14 mg
Peppermint powder	18.40 mg
Peppermint oil	32.90 mg
Colour red #3 (Al-lake)	1.10 mg

Experiment Procedures

All volunteers were divided randomly into two groups. Each group contained 4 subjects. And a double-blind, placeo-controlled, cross-over study design mentioned above was applied. All subjects in two groups received one tablet of pilocarpine chewing tablet or placebo at 09:00-10:00 AM, respectively. The mixed saliva samples (parotid+submandibular+sublingual saliva) were collected immediately as drug free saliva (baseline condition) before drug administration and then at time intervals of 0 - 2, 2 - 5, 5 - 10, 10 - 15, 15 - 20, 20 - 30, 30 - 45 and 45 - 60 min. No foods and drugs were permitted for 12 hours before experiments. The mastication rate of pilocarpine tablet was fixed 60-80 times per minute. Saliva was collected for fixed time intervals into preweighed vials added ice and NaF as preservative. All samples stored at -20°C until HPLC analysis.

Pilocarpine Assay in Saliva by HPLC Method

Saliva pilocarpine concentrations were quantitated by a modified HPLC method.[7-10] First of all, saliva samples stored in refrigerator thawed slowly in ice water and then added acetonitrile

by 1:3 ratio (saliva : acetonitrile, v/v %) to precipitate protein and cetrifuged at 4500 rpm. After then 30 μl of supernent was injected into HPLC. In a present assay method, internal standard was not used because direct protein precipitaion had simple precedure of experiment and good reproducibility.

The HPLC system (Model 322, Altex Scientific) was equipped with injection valve (Beckman Model 210A), a variable wavelength UV detector (Model 11-30, Hitachi Instrument) set 214 nm, and a pump (Model 110A, Altex Scientific). Chromatographic peak recording and peak-area integration were achieved with an SP 4290 integrator (Spectra Physics). The HPLC column was Bondex® ODS (10 μm, 300×3.9 mm, Phenomenex). All injections were made using a 50 μl Hamilton syringe into the 20 μl loop of the injection valve. The mobile phase composition was 7 mM potassium phosphate buffer (pH 4.0), acetonitrile and methanol (55:35:25, v/v/v %). The flow rate of mobile phase was maintained at 1.2 ml/min.

Data Analysis

Saliva volumes were measured according to fixed time interval, and cumulative amounts of pilocarpine also were determined from collected saliva volume and the concentration of pilocarpine in saliva. The mean flow rates of saliva in time-intervals were calucated by dividing the volume of collected saliva by the collecting time of sample. The absorbed amount through buccal mucosa was calculated from administed dose and cummulative amounts collected from saliva spitted out. Because it wasn't permitted to swallow saliva during mastication of pilocarpine chewing tablet for 1 hour, and it means there is no drug swallowed into GI-tract from mouth with saliva. The difference between administered pilocarpine dose and collected dose with saliva was considered to be absorbed amount through buccal mocosa, because it was found out that pilocarpine was extracted completely within 30 min during mastication.

Statistical analysis of data between two groups was conducted with Student t-test, and significance of difference was checked at $p<0.05$ value.

Results and Discussion

HPLC Chromagram of Pilocarpine

The HPLC chromatograms of pilocarpine from saliva sample were shown at Fig 1. It was noted that there was no interferring peak from the chromatograms of blank saliva, standard drug spiked saliva and human volunteer saliva sample collected during mastication of pilocarpine chewing tablet. The retention time of pilocarpine appeared at 8.3 min. Calibration curve was prepared between pilocarpine concentration in saliva and peak height of HPLC chlomatogram in the ranges of 25 to 300 mcg/ml in saliva, and had a good linear correlation, Y (peak height,cm) = 0.29992 + 0.13279 X (concen-

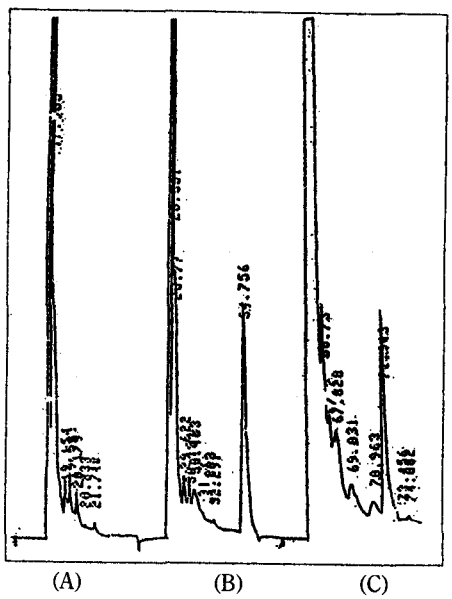

Figure 1—The HPLC chromatograms of pilocarpine in saliva. Key : A: Blank saliva, B: Standard spiked (50 mcg/ml), C: Sample of subject 2 at 20 min (41.35 mcg/ml). # Retention time of pilocarpine was 8.3 minute.

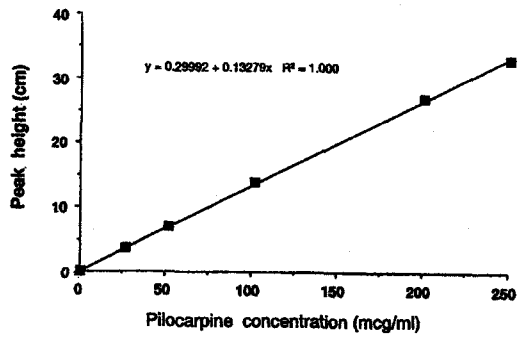

Figure 2—The calibration curve of pilocarpine in saliva

tration, mcg/m*l*), R^2=1.000 over the concentraion range 25-300 mcg/m*l*, and was shown at Fig. 2. Coefficients of variation were 5.6 and 2.3 % (n=6) at 25 and 300 mcg/m*l* and 6.7 and 4.5 %(n=6) at the same concentraions for intra- and inter-day determination, respectively.

Dissolution Patterns in Saliva of Pilocarpine during Mastication of Pilocarpine Chewing Tablet

Dissolved amounts of pilocarpine were determined from collected whole saliva volume and the concentration of pilocarpine in saliva at each time intervals. Cumulative dissolved amount and dissolved amounts of pilocarpine in each time interval were shown at Fig. 3. Total amount of pilocarpine dissolved from chewing tablet preparation to saliva within 20 min. was 4.127(±0.688)mg, 82.5% of administered dose, and 90% of total cumulative amount for 1 hour, and also it was found out that pilocarpine was extracted completely within 30 min during mastication chewing pilocarpine tablet. From the above results, it was noted that the different amount of about 17.5% of administered dose between administered dose and total collected dose for 1 hour, was considered to be absorbed through buccal mucosa, because in present study, it was not permitted to swallow saliva during mastication and gastro-intestinal (GI) absorption of pilocarpine could be ignored.

Whole Salivary Flow Rate

Whole saliva flow rates were calculated by dividing the secreted saliva volumes by collecting times of saliva during each collecting time interval. As shown Fig. 4, mean saliva flow rates were appeared to be different at the time intervals, such as 5-10, 10-15, 20-30 and 45-60 min between pilocarpine chewing tablet and placebo groups. But there was signifficant different ($p<0.05$, Student's t-test) only at 45-60 min inverval. At this interval, the saliva flow rates of pilocarpine chwing tablet and placebo groups were 0.767 and 0.523 m*l*/min, respectively.

Whole Saliva Excretion during Mastication of Pilocarpine Chewing Tablet

Total secreted volumes of saliva from pilocarpine chewing tablet and placebo groups were 46.36±9.72 m*l* and 39.09±7.81 m*l*, respectively. There was no signifficant difference between two groups ($p>$

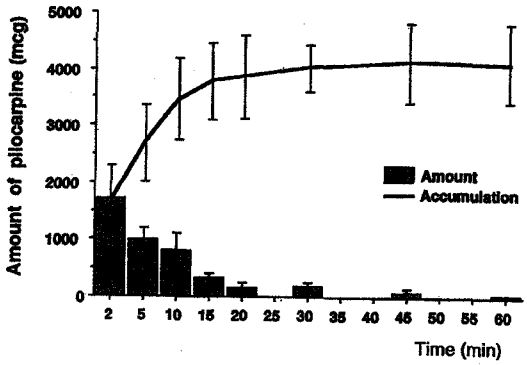

Figure 3—Released amount and accumulation of pilocapine at collecting time interval during mastication of pilocarpine chewing tablet at rate of 60-80 times per minute.

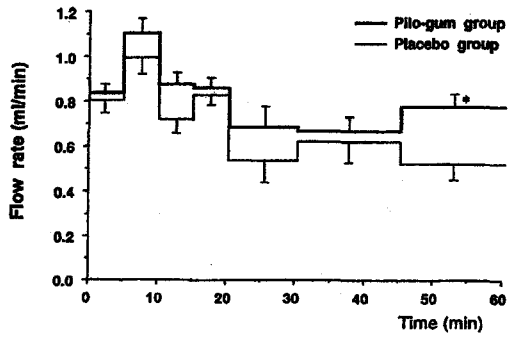

Figure 4—The comparisons of mean flow rates of pilocarpine at collecting time for 1 hr.
*Significantly different ($p<0.05$).

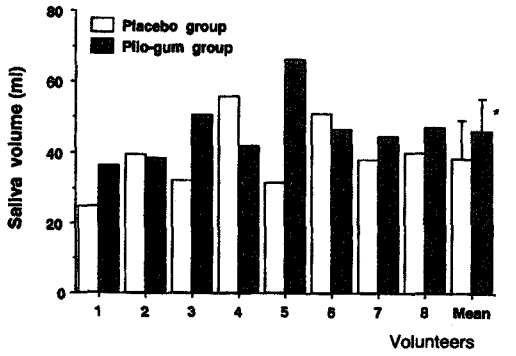

Figure 5—The comparisons of saliva volumes secreted during mastication of pilocarpine chewing tablet or placebo for 1 hr.
*Not significantly different ($p<0.05$)

0.05). The secreted saliva volume of each subject and mean value(±SD) were shown Fig. 5

Conclusions

From the this experiment, it was noted that the 82.5 percent (4.13±0.69 mg) of pilocarpine was extracted from pilocarpine chewing tablet during mastication of 60-80 times per minute for 1 hr. Among these dissolved amounts, 90 percent was extracted within 20 minutes. And total amounts of saliva secreted for 1 hour were not significantly different between two groups. It means that there is not dirrect stimulation of pilocarpine to saliva gland. In facts, when patients chew the pilocarpine tablet, they swallow saliva, and the extracted pilocarpine into saliva may be absorbed in GI-tracts. And more investigations of pilocarpine tablet is planed under above mentioned conditions.[11,12]

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