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Development of Controlled Release Oral Drug Delivery System by Membrane-Coating Method-II

—Correlation Between Acetaminophen Concentrations in Plasma and Saliva Samples of Man—

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피막법에 의한 경구투여용 제어방출 제제의 개발—II

—사람에 있어서 아세트아미노펜 혈장 중 농도와 타액 중 농도와의 상관성—

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Plasma and saliva concentrations of acetaminophen (AAP) were determined at various time points by HPLC after oral administration of AAP tablets (AAP 500 mg) to four healthy male Korean subjects. Saliva concentrations (S) of AAP were significantly correlated with plasma AAP concentrations (P). The S/P ratio of AAP was calculated to be 1.05 ($r=0.944$, $p<10^{-6}$) for all the data points from the subjects. It showed a little intersubject variation and ranged from 0.89 to 1.46 in each subject. Bioavailability parameters such as AUC, C_{max} and T_{max} which are usually obtained from the plasma concentration data will be predictable approximately by saliva concentration data. Saliva seems to be very convinient and useful samples for the preliminary studies of bioavailability and bioequivalence of AAP preparations, since it can be collected frequently without any painful venipuncture to the subjects, that is inevitable in plasma sampling. Evaluation of the bioavailability of a preparation by saliva samples will reduce the cost, time and safety risk greatly in developing a new drug delivery system for AAP.

Keywords—acetaminophen, saliva concentration, plasma concentration, correlation, S/P ratio, bioavailability, oral drug delivery system.

Monitoring blood or plasma level of a drug is usually essential in evaluating the boavailability of a drug in dosage forms. Blood sampling, however, is often avoided because it needs frequent venipuncture and gives pain to the subjects. It will be convinient and safe if bioavailability of a drug can be estimated without painful venipuncture. Some biological fluids such as saliva, tear and urine can be collected without pains. Among them, urine samples are usually used in place of plasma samples, however, they can not represent exactly the plasma concentration of a drug at the time point urine-

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collected since they are collected as a mixture of urine excreted during a relatively long period of time. For tear, there has been no evidence that lasma concentration of a drug can be predicted from it.

Saliva has been interested by many investigators. Many drugs[1] such as procainamide, pethidine, antipyrine, theophylline, phenobarbital, amobarbital, propranolol, phenytoin, chlorpropamide and tolbutamide were reported to be secreted into saliva with a respective saliva/plama (S/P) concentration ratio. Saliva was reported by us[2] to be very useful in evaluating the bioavailability of theophylline from the sustained-release tablets.

Acetaminophen (AAP) was also reported to be secreted into saliva[3-6]. Adithan and Thangam[4] reported a S/P ratio of 1.14, and Lowenthal *et al.*[5] reported 1.08. Kamali *et al.*[6] reported a little higher S/P ratio for the first 50 min after the drug dosing than that for the elimination phase.

In this study, correlation between S and P concentrations in Korean subjects was examined in order to confirm the usefulness of saliva samples in estimating bioavailability of the controlled release AAP tablets prepared by us previously[7].

EXPERIMENTAL

Materials

Tyrenol (AAP 500 mg) was provided from Korea Cilag Co. Acetaminophen was purchased from Hong Sung Sa and KP V grade. Sulfamerazine (Sigma), isopropanol (James Burrough, F.A.D.) and barium hydroxide (Mallinc krodt Chem. Works) were of analysis grade. Methanol (Merck) and acetonitrile (Merck) were of HPLC grade.

Subjects

Four healthy male volunteers, ranging in age from 20 to 23 years (mean 21.5), in weight from 53 to 70 kg (mean 60.8) participated in this study. One of the subjects was nonsmoker and the others were smokers. Informed consents were obtained from the subjects. All subjects had a normal history, physical examination and laboratory tests (complete blood count, creatinine, total protein, albumin, alkaline phosphatase, glucose, SGOT, SGPT, inorganic phosphate and calcium, cholesterol, blood urea notrogen (BUN) and total bilirubin).

Drug Administration

Acetaminophen (AAP) was given as a tablet (Tyrenol, AAP 500 mg, Korea Cilag) with 200 m*l* of water. Subjects were not allowed to administer any drugs at least during two days before the experiment. Food and water were permitted *ad libitum* until the morning of the experiment day. Drug was administered between 8~9 a.m. and no food or drink other than water was permitted until 4 h after dosing, and no sleeping was allowed during the experiment. Standard meal was allowed 4 h after drug administration.

Blood Sampling

Venous blood samples (5 m*l*) were withdrawn from a forearm on heparin (25-unit vacuum tube), immediately before and at 15, 30, 45, 60, 90, 120, 150, 225 and 270 min after dosing. After centrifugation, the plsma samples were transferred to sterilized vials and frozen at -20 ℃ until analysis.

Saliva Sampling

Saliva samples were collected at the same intervals as plsma sampling before and after the drug dosing. A small amount (20 mg) of citric acid, a salivary flow stimulant, was put on the tongue and held for 1 min. Then a 5m*l* sample of saliva was collected in the test tube and kept frozen until assay.

HPLC Assay of Acetaminophen in Plasma

The method of Colin and Sirois[8] was modified. To the 0.1 m*l* of plasma in a glass-stoppered test tube, 1 m*l* of acetonitrile:isopropanol (1:1) solution of sulfamerazine (internal standard; 3 μg/m*l*) was added. After vortexing (30 sec) and centrifugation (1000 × g, 10 min), 600 μl of the supernatant was transferred into a conical tube. It was evaporated to dryness using a water bath (40 ℃) and a stream of dry nitrogen. The residue was redissolved in 300 u*l* of filtered deionized water and a 20 μl aliquot was injected drectly onto the column (Lichrosorb, RP-18 10 μm, 4.0 × 250 mm) of a reversed-phase HPLC (Hitachi, model 638-50) with a UV detector

adjusted at 245 nm. The mobile phase was a 14:86 mixture of acetonitrile:water. The flow rate was 1.0 m*l*/min and the mean operating pressure was 44 bar. A standard curve was run with each set of determinations and prepared by the peak height ratio of AAP against the internal standard. Linearity of the standard calibration curves was found in the range from 0.5 to 20 μg/m*l*.

HPLC Assay of Acetaminophen in Saliva

To the 1.0 m*l* of centrifuged (600×g, 1 min) saliva in a glass-stoppered test tube, 1 m*l* of 0.3 N-barium hydroxide was added. After vortexing for 2 min, 1 m*l* of 5% (w/v) zinc sulfate was added. After vortexingfor 1 min and centrifuging for 10 min (6000×g), 20 u*l* of the supernatant was injected onto the HPLC column. HPLC was performed on the same condition to that of plasma samples as described above. The AAP concentration in the saliva was determined by the peak height of AAP in the sample using a standard calibration curve prepared by adding known amounts of AAP to saliva. Linearity of the calibration curves was found in the range from 0.5 to 20 μg/m*l*.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatograms of AAP in the plasma and saliva samples. The peak of AAP was clearly seperated from those of internal standard (sulfamerazine), plasma or saliva components.

Fig. 2 and 3 show the plsma and saliva concentration-time profiles of AAP in four subjects respectively. There was a great intersubject variation in boh profiles, however, the average plasma

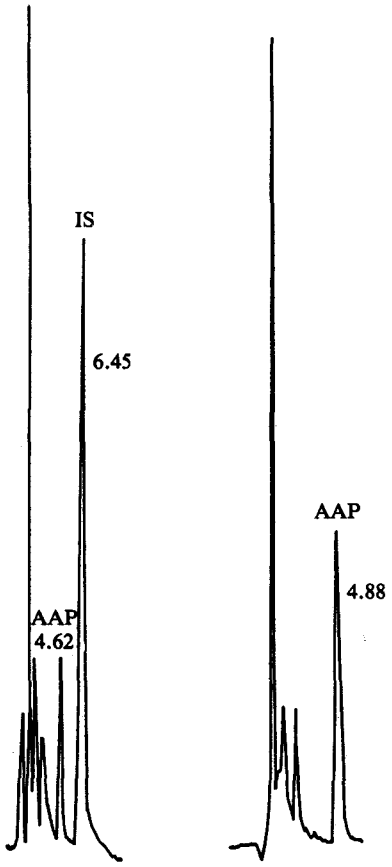

Figure 1—Chromatograms of AAP in plama(left) and saliva (right) samples.

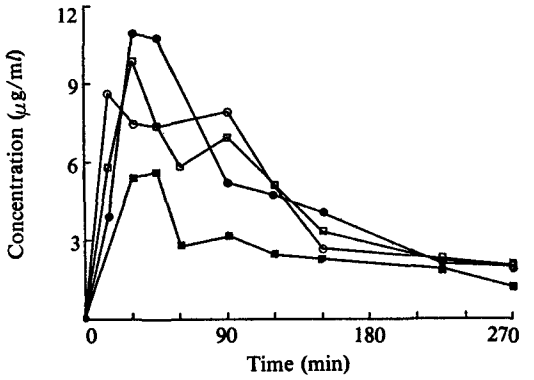

Figure 2—Plasma concentration-time curves of AAP in each subject.

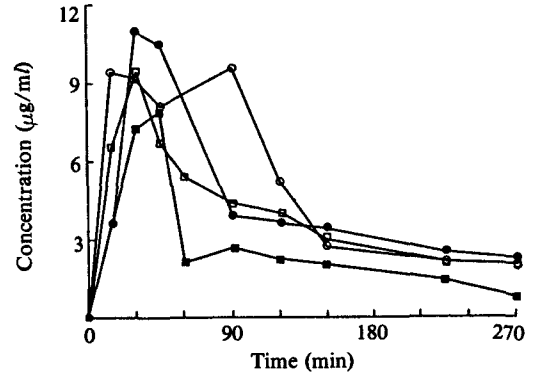

Figure 3—Saliva concentration-time curves of AAP in each subject.

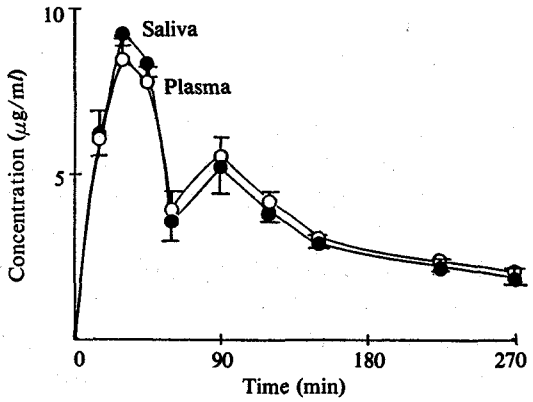

Figure 4—Average plsma and saliva concentration-time curves of AAP Each point represents mean ± SD of four subjects.

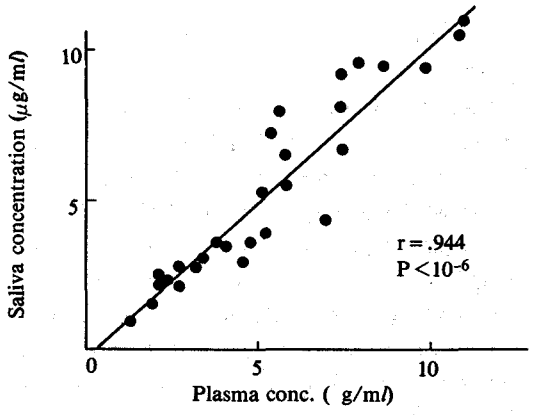

Figure 6—Correlation between plasma and saliva concentrations of AAP in all subjects.

and saliva concentration-time profiles were very similar in pattern and almost superimposable with each other (Fig. 4). There were two distinct peaks in most ofthe curves in Fig. 2~4, as reported earlier[9].

Fig. 5 shows the correlation betwen AAP concentrations of plasma and saliva. The S/P ratio in four subjects showed an intersubject variation ranging from 0.89 to 1.46, however, there were significant linear relationships between plasma and saliva concentrations of each subject.

Fig. 6 shows the correlation between plasma and saliva concentrations of AAP obtained for 34 pairs of data. It showed a significant linear relationship

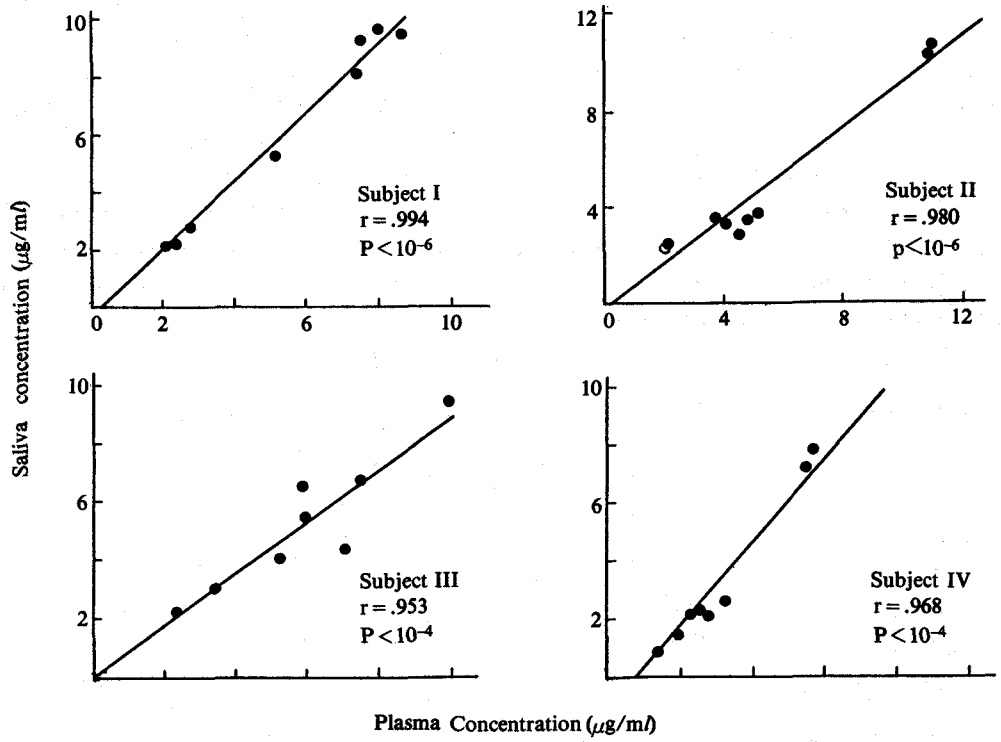

Figure 5—Correlation between plasma and saliva concentrations of AAP in each subjects.

($r=0.944$, $p<10^{-6}$) between S/P concentrations and the S/P ratio was 1.05, which is very close to the previously reported values[3~5].

Considering the intersubject variation in S/P ratio as examplifid in Fig. 5, plasma *AUC* of AAP predicted from saliva *AUC* and average S/P ratio may over- or underestimate bioavailability of the the drug in some cases. This problem can be solved if the bioavailability of a dosage form is calculated as a ratio of *AUC* to the reference dosage form (comparative bioavailability). Bioequivalence test is usually performed using plasma *AUC* data of the drug. Plasma *AUC* data, however, can be predicted from saliva *AUC* data if S/P ratios in all the subjects participated in the test are constant. Saliva seems to be very convinient and safe samples for the estimation of bioavailability and bioequivalence of AAP-containing preparations, comparing the difficulty of the saliva sampling with that of plasma sampling.

ACKNOWLEDGEMENT

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