EVALUATION OF A NEW SUSTAINED RELEASE THEOPHYLLINE FORMULATION BY THE MEASUREMENTS OF SALIVARY LEVELS OF THE DRUG IN HUMANS

MASAHIRO NAKANO,* YUHKO NAKAMURA, KAZUHIKO JUNI, AND TOSHIAKI TOMITSUKA

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan

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Dry agar beads containing theophylline were prepared. The release rate of theophylline from the agar beads in vitro was smaller than the dissolution rate of the drug from pure drug powders. The salivary levels of the drug following oral administration of the agar beads to three healthy volunteers were determined by reversed-phase high pressure liquid chromatography. Results of pharmacokinetic analyses indicated sustained absorption of the drug following the administration of the agar beads.

Keywords — theophylline; agar beads; hydrogel; sustained release; oral administration; salivary excretion; pharmacokinetic analysis; humans; high pressure liquid chromatography; reversed-phase liquid chromatography

Theophylline is widely used for the treatment of asthma and chronic obstructive lung diseases. It is one of the drugs that exhibit a relatively narrow range between therapeutic and toxic plasma concentrations. In general, the therapeutic range in plasma is considered to be 10–20 μg/ml and concentrations greater than 20 μg/ml tend to cause frequent incidences of gastrointestinal disturbances and other toxic effects.1-2 In addition, pharmacokinetic studies of theophylline have shown remarkable individual variations in the rate of elimination.3-6 These toxicological and pharmacokinetic properties of theophylline necessitate plasma level monitoring in order to set up an appropriate dosage regimen to achieve an effective and safe treatment.1

Recently, a number of investigators have examined the use of saliva to predict serum levels of the drug7 and some of them reported a relatively linear correlation between the theophylline concentration in plasma and that in saliva.8-11

Theophylline concentrations in biological fluids have been determined by spectrophotometric,12,13 gas chromatographic,14-17 and high pressure liquid chromatographic18-21 procedures. In this work, the usefulness of dry agar beads containing dispersed theophylline for the preparation of a sustained release dosage form was examined by reversed-phase high pressure liquid chromatography using the procedure developed for use with saliva samples. The results indicated that assay of salivary level may be useful for comparison of rate and extent of bioavailability of the drug following administrations of different preparations of theophylline.

MATERIALS AND METHODS

Materials — Powdered agar was of first grade, Japanese Industrial Standard, purchased from Wako Pure Chemical Industries, Osaka. Theophylline anhydrate and isopropanol (reagent grade) were obtained from Wako Pure Chemical Industries, and 8-chlorotheophylline was purchased from Tokyo Kasei Kogyo Co., Tokyo. Ethyl acetate, ethanol, acetonitrile, and chloroform were distilled prior to use. All other chemi-

* Present address: Department of Pharmacy, Kumamoto University Hospital, Honjo 1-chome, Kumamoto 860, Japan.
Salivary Level of Theophylline in Humans

...cals were of reagent grade from Wako Pure Chemical Industries and were used as received.

Preparation of Agar Beads — Dry agar beads containing 530 mg of theophylline per gram of the preparation were prepared by the method previously developed. The drug content was determined from the total amount of the drug released in the release study in vitro. The beads are spherical with the average diameter of 3.0 mm.

Release Studies — Ten beads containing theophylline were added to 80 ml of the release medium at 37°C in a 125 ml wide-mouth bottle in a constant temperature waterbath. The medium was Clark & Lubs HCl-KCl solution at pH 1.0 to simulate acidic environments which may be encountered after oral administration of the beads. The suspension was stirred by a magnetic stirring bar by means of a submersible magnetic stirrer (Acrobat Stirrer, MS Instruments, Osaka). At suitable time intervals, 1.0 ml portion of the medium was taken and diluted with 0.2 M acetate buffer, pH 5.0, and subjected to absorbance measurements at 272 nm using a digital spectrophotometer (model 200-20, Hitachi Manufacturing Co., Tokyo).

Drug Administration and Saliva Sampling — Three healthy volunteers between 22 and 42 years of age and weighing between 45 and 65 kg participated in the study. To eliminate any intake of theophylline from other sources and possible formation of theophylline in vivo following intake of caffeine, the subjects were told to abstain from coffee, tea, chocolate, and cola. Alcoholic beverages were also withheld because of the possible effects on disposition of theophylline. Blank saliva samples were collected a few minutes before the administration of the drug. A single dose of theophylline powder or the agar beads, 5 mg of theophylline/kg of body weight was administered wrapped in a sheet of wafer to eliminate contact of the drug with mucosa of the mouth, with 100 ml of water after an overnight fasting. Saliva was thereafter collected by the following method at appropriate intervals up to 24 hr. A small amount, about 10 mg, of citric acid, a salivary flow stimulant, was put on the tongue and held in the mouth for 1–2 min, then the saliva was spat into a test tube to collect a 2-ml sample. Saliva samples were frozen in a freezer until analysis. No food was taken for 4 hr postadministration. The crossover design was used and the minimum interval of one week was allowed between trials.

Analysis of Theophylline Levels in Saliva — Reversed-phase high pressure liquid chromatography for serum samples was modified for saliva samples as follows. Each saliva sample, after thawing, was centrifuged at 3500 rpm for 15 min to remove any sediment. One-milliliter of a saliva supernatant was pipetted into a 10 ml glass-stoppered centrifuge tube containing 400 mg of ammonium sulfate. The contents were mixed with a Vortex-type mixer. Six milliliters of isopropanol–chloroform (5/95 v/v) containing 1 μg/ml of 8-chlorotheophylline was added and the tube was shaken for 10 min prior to centrifugation at 3500 rpm for 10 min. Five milliliters of the organic layer was transferred to a test tube and evaporated under nitrogen in a waterbath at 60°C. The residue was dissolved in 250 μl of acetonitrile. A 10 μl supernatant was then injected into a chromatograph via the loop injection valve. A high pressure liquid chromatograph (TRI ROTAR, Japan Spectroscopic Co., Tokyo) with a variable wavelength UV spectrophotometer (UVDEC-100, Japan Spectroscopic Co.) and a recorder (model 056, Hitachi Manufacturing Co.) was employed. The column was of a reversed-phase type (Jasopak SS-100DS-B, 4.6 φ×250 mm, Japan Spectroscopic Co.). The detector was set at 273 nm. The mobile phase was 25% acetonitrile in 10 mM sodium acetate buffer at pH 4.0 and the flow rate was 1.5 ml/min.

Preparation of a Standard Curve — A 500 μg/ml stock theophylline solution was prepared in ethanol. Aliquots of 4, 8, 12, 16, and 20 μl were added to glass-stoppered centrifuge tubes. After ethanol was evaporated under a nitrogen stream, 1.0 ml of the blank saliva was added to each tube. Then theophylline in the tube was extracted and analyzed by the procedure described above.
RESULTS AND DISCUSSION

Release Patterns

In contrast with a rapid dissolution from the theophylline powders, sustained release was obtained in the release profile from the dry agar beads (Fig. 1). Agar beads are expected to absorb water to form a hydrogel. Sustained release of dibucaine from another hydrogel, konjac, has been reported earlier.

Assay of Salivary Levels

Among assay methods of theophylline, the UV spectrophotometric procedure, although a popular method, requires relatively large sample volumes and background absorption correction because of the possibility of interference by xanthines and other UV absorbing substances. Some GLC methods are sensitive and specific but require derivatization after extraction steps. After examining these methods for assay of theophylline in saliva and examining a few types of columns in high pressure liquid chromatography, reversed-phase liquid chromatographic determination of the drug was found to be satisfactory for the present purpose. In this procedure, theophylline was extracted into an organic sol-

FIG. 2. A Chromatogram of a Mixture of Theophylline (1), Caffeine (2), and 8-Chlorotheophylline (3, Internal Standard)

FIG. 1. A Release Profile of Theophylline from the Agar Beads (●) compared with a Dissolution Pattern from Pure Drug Powders (○) at pH 1.0 and 37°C

FIG. 3. Peak Height Ratios of Theophylline to Internal Standard Plotted against Concentrations of Theophylline
vent containing an internal standard to eliminate the need of a pre-column.

Several methods for the collection of saliva have been proposed. In the method used in this study, salivary flow stimulated with citric acid was rapid enough to collect saliva samples in a few minutes. Saliva obtained by this procedure was less viscous than that obtained in the absence of the stimulant and contained little insoluble matter.

Figure 2 shows a chromatogram for a mixture (about 20 μg/ml of each) of theophylline, caffeine, and 8-chlorotheophylline (internal standard). Theophylline was well separated from dietary caffeine and the internal standard. The standard curve was linear in the range studied (Fig. 3). **Salivary Excretion Patterns**

Although the use of salivary theophylline levels measured by UV spectrophotometric method for prediction of plasma theophylline levels was questioned because of rather large standard errors in the saliva/plasma concentration ratio, reliability of salivary theophylline levels measured by reversed-phase high pressure liquid chromatography to predict plasma theophylline levels has been demonstrated. Since theophylline was assayed by reversed-phase high pressure liquid chromatography in this study, saliva/plasma concentration ratios may be assumed to remain constant in each subject. Thus, changes in theophylline concentrations in saliva may be assumed to reflect changes in the drug concentrations in plasma.

The fate of theophylline following oral administration of pharmaceutical preparations is depicted in the following scheme (Fig. 4). Since the tissue and central compartments could not be separated pharmacokinetically following oral administration of the drug, they were treated as a single compartment.

According to the scheme the drug is absorbed into blood after release or dissolution from pharmaceutical preparations and is then excreted into saliva. The concentration of an unmetabolized drug in saliva, Cₘ, is assumed to be proportional to the plasma level, Cₚ, of the drug according to the following relationship,

\[ Cₘ = r Cₚ \]

where \( r \) = saliva to plasma concentration ratio.

Since the plasma level of the unmetabolized drug is expressed by the following equation,

\[ Cₚ = \frac{kₐ f D₀}{Vₐ (kₚ - kₐ)} (e^{-kₚ t} - e^{-kₐ t}) \]

where \( kₚ \) = absorption rate constant, \( f \) = fraction of dose absorbed, \( D₀ \) = dose, \( Vₐ \) = volume of distribution, and \( kₚ \) = elimination rate constant, the salivary level is expressed by the following equation,

\[ Cₘ = \frac{r kₐ f D₀}{Vₐ (kₚ - kₐ)} (e^{-kₚ t} - e^{-kₐ t}) \]

Figure 5 shows the salivary levels of the drug in three subjects plotted on a sheet of semilogarithmic graph paper and indicates that salivary levels of the drug following oral administration of the agar beads are sustained in comparison with those of the powders. Sustained salivary

![FIG. 4. Fate of a Drug following Administration of Pharmaceutical Preparations](image-url)
levels are reflection of the sustained plasma level profile due to slow absorption, which in turn is attributed to sustained release from the agar beads. Therefore, sustained levels in saliva are the results of sustained release in vivo.

In order to quantitatively compare the absorption and excretion patterns of the drug following administration of two preparations, pharmacokinetic analysis was made to obtain elimination and absorption rate constants.\(^{250}\) Apparent elimination rate constants were obtained from the elimination phase of log salivary level vs. time plots based on the following equation,

\[
\log C_{x, el} = \log \frac{r k_s f D_0}{V_d (k_s - k_{el})} - \frac{k_{el}}{2.303} t
\]

where \(C_{x, el}\) is salivary level in the elimination phase, whereas apparent absorption rate constants

![Graph showing the salivary excretion profiles of theophylline following administration of powders (○) or the agar beads (●) to three volunteers.](image)

**FIG. 5. Salivary Excretion Profiles of Theophylline following Administration of Powders (○) or the Agar Beads (●) to Three Volunteers**

**TABLE I. Apparent Absorption and Elimination Rate Constants of Theophylline Following Oral Administration of Powders or Agar Beads to Three Volunteers**

<table>
<thead>
<tr>
<th>Subject</th>
<th>(k_{sa}), hr(^{-1})</th>
<th>(k_{eb}), hr(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Powders</td>
<td>Agar beads</td>
</tr>
<tr>
<td>K.T.</td>
<td>Very large</td>
<td>0.59</td>
</tr>
<tr>
<td>Y.N.</td>
<td>1.44</td>
<td>0.61</td>
</tr>
<tr>
<td>M.N.</td>
<td>1.44</td>
<td>0.98</td>
</tr>
</tbody>
</table>
were obtained from the plots of residuals in log salivary level vs. time plots based on the following equation,

$$\log C_{s,\text{res}} = \log \frac{r_k f D_0}{V_d (k_a - k_{ci})} - \frac{k_a}{2.303} t$$

where $C_{s,\text{res}}$ is salivary level in the residual plots.

The elimination and absorption rate constants thus obtained are tabulated in Table I. Since the above procedures used for calculating rate constants are not strictly valid for calculation of the rate constants following the administration of sustained release preparations, it should be kept in mind that the absorption and elimination rate constants calculated for the agar beads are very cursory estimates and only approximate the true values.

Half-lives of salivary levels in the elimination phase following administration of theophylline powders ranged from 5.9 to 7.7 hr with the average value of 6.7 hr. These values are within the ranges of half-life values obtained in healthy volunteers reported earlier from measurements of drug levels in plasma.

Apparent half-lives of salivary levels in the elimination phase following administration of the agar beads were somewhat longer indicating possible sustained absorption of the drug due to sustained release in the gastrointestinal tract. Sustained absorption is indicated by smaller values for absorption rate constant following administration of the beads than those of the powders (Table I) as calculated from the slopes of the residual plots.

If the assumption made in calculation of rate constants is valid, measurements of salivary levels can be used to find differences in absorption rate following administration of different preparations. Thus, salivary level measurements may be used to evaluate rate and extent of bioavailability of theophylline preparations from the initial part of the salivary level curves and areas under the salivary level-time curves, respectively. Although the degree of sustained release from the agar beads containing theophylline was rather small in this study, such agar beads may be examined for the preparation of a sustained release dosage form of drugs with smaller water-solubility than theophylline since water-solubility has been shown to affect release rates of drugs from hydrogels.

In the present preparation, theophylline was completely released from the agar beads (Fig. 1) and absorbed as completely as the powder judging from almost equal areas under the curve following the administration of the powder and beads, whereas some of the sustained release preparations failed to release all theophylline (27, 28).

Since theophylline preparations have been used to treat asthma in children, the evaluation of rate and extent of bioavailability of each preparation as well as examination of compliance with dosage regimens by using a convenient, painless, and noninvasive sampling method as an alternative to the determination of theophylline levels in plasma may be useful for more individualized treatment of asthmatic children.

REFERENCES

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