Bioavailability of Theophylline and Thiamine Disulfide Incorporated into Mucoadhesive Microspheres Consisting of Dextran Derivatives and Cellulose Acetate Butyrate

Yasunori Miyazaki,*a,a Kanako Ogihara,a Shigeru Yakou,a Tsuneji Nagai,b and Kozo Takeyama,b

*a Pharmaceutical Department, Tokyo Women’s Medical University Daini Hospital; 2–1–10 Nishigou, Arakawa-ku, Tokyo 116–8567, Japan; and b Faculty of Pharmaceutical Sciences, Hoshi University; 2–4–41 Ebara, Shinagawa-ku, Tokyo 142–8501, Japan. Received July 29, 2003; accepted September 19, 2003

Mucoadhesive microspheres made of oppositely charged dextran derivatives and cellulose acetate butyrate (Ad-MS) were evaluated for their ability to improve the bioavailability of theophylline (TH) and thiamine disulfide (TDS). A drug suspension (or solution) and non-adhesive microspheres (MS) were used as references. In vitro drug release profiles from MS and Ad-MS were similar for each drug, whereas their gastrointestinal transit times differed. The plasma concentration after oral administration of drug suspension (or solution), MS and Ad-MS was investigated in rats. In the case of TH, sustained plasma level profiles were observed after MS or Ad-MS administration, with similar Cmax, Tmax and MRT∞ values. AUC∞ values of the suspension, MS and Ad-MS were statistically equivalence. These indicated that Ad-MS achieved a sustained plasma level profile without a decrease of AUC. In the case of TDS, MRT∞ and AUC∞ of Ad-MS were significantly larger than those of the solution and MS, indicating that the plasma level was sustained and the extent of bioavailability was increased. These results suggested that Ad-MS is a promising device for improvement of bioavailability of drugs those absorption windows are limited to upper part of the gastrointestinal tract.

Key words mucoadhesive microsphere; gastrointestinal transit; bioavailability; theophylline; thiamine disulfide; dextran derivative

The gastrointestinal (GI) absorption of orally administered drugs is determined not only by the permeability of the GI mucosa but also by the GI transit time of the preparation. The residence time in the GI tract is important since it may affect the amount of drug available for absorption.

Oral mucoadhesive drug delivery systems alter the GI transit rate. For example, mucoadhesive microspheres adhered to the GI mucosa, prolonging the GI transit time.1,2) Although localization and retention of the drug to the absorption site are known to improve the bioavailability, there are few reports dealing with bioavailability problems.3)

Recently, we have developed mucoadhesive microspheres (Ad-MS) made of oppositely charged dextran derivatives and cellulose acetate butyrate, used as mucoadhesive polymers4) and hydrophobic polymer, respectively. We confirmed that they adhered to the rat gastric mucosa by direct observation of the inner GI tract. Moreover, we evaluated GI transit patterns of microspheres after oral administration, and revealed that their gastric residence time was prolonged.2)

The objective of this study was to evaluate Ad-MS for their ability to improve the bioavailability of drugs by comparison of the pharmacokinetics in both Ad-MS and non-adhesive microspheres (MS). Theophylline (TH) and thiamine disulfide (TDS) were selected as model drugs, having different absorption sites. TH is absorbable from the entire GI tract, whereas the absorption site of TDS is limited to the upper part of the GI tract.6)

MATERIALS AND METHODS

Materials Dextran sulfate (DS, Mw 500000), [2-(diethylamino) ethyl] dextran (EA, Mw 500000) and theophylline (TH) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Lactose (Lac) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). These chemicals were used after sieving through a 200-mesh sieve (less than 75 μm). Cellulose acetate butyrate (CAB, Mw 30000) was obtained from Fluka Chemical Co. (Buchs, Switzerland). Thiamine disulfide (TDS) was purchased from ICN Biomedicals, Inc. (Aurora, OH, U.S.A.). Sugar ester DK F-10 used as a surfactant was kindly supplied by Daiichi Kougyou Seiyaku Co. Ltd. (Kyoto, Japan). 7-(2-Hydroxyethyl)-theophylline (HETH, Tokyo Kasei Kogyo, Tokyo, Japan) was used as an internal standard for TH assay. Acid phosphatase (EC 3.1.3.2, Nacalai Tesque, Inc., Kyoto, Japan) was used for hydrolysis of thiamine esters. All other chemicals and solvents were of reagent grade and used as received.

Preparation of Microspheres Microspheres were prepared by an emulsion solvent evaporation method, as follows. Drug (TH or TDS), DS and EA were dispersed in 15 ml of acetone containing 1 g of CAB, and then poured into 150 ml of liquid paraffin containing 1% w/v of sugar ester, at 20 °C under agitation (300 rpm). The mixture was mechanically stirred under atmospheric pressure to form a w/o emulsion. After 30 min, the solution was heated to 50 °C to evaporate acetone. Then the solution was gradually cooled at 20 °C and then decanted off. The removal of residual oil was performed by washing the microspheres with 50 ml of n-hexane 3 times. The microspheres were dried under vacuum at room temperature. The microspheres sieved at 425—600 μm were used for further experiments. In the case of MS, DS and EA were not added to acetone. In addition, MS for TH, Lac was added to acetone in order to modulate drug release rate.

Drug content of the microspheres was determined as follows. Ten milligrams of microspheres were dissolved (or dispersed) in 50 ml of methylene chloride. The solution was subjected to sonication at room temperature for 10 min. The resultant solution was filtered through a 0.2 μm membrane filter and analyzed spectrophotometrically at 274 nm for TH and 237 nm for TDS. All drug content determinations were
performed in triplicate.

**In Vitro Drug Release** In vitro release experiments were conducted at 37 ± 0.5 °C by a paddle method (60 rpm). Typically, 50 mg of microspheres were placed into a dissolution vessel filled with 900 ml of the JP XIV 1st fluid (pH 1.2, 0.07 m HCl and 0.0342 m NaCl) containing 0.1% (w/v) of polysorbate 80. Five milliliter samples were withdrawn at appropriate intervals and filtered with a membrane filter (pore size 0.45 μm). The filtrate was analyzed spectrophotometrically at 274 nm for TH content and 242 nm for TDS content. An equal volume of the same dissolution medium was added to maintain a constant volume. These dissolution medium contained a small amount of surfactant in order to improve the wettability of microspheres.

**Oral Administration to Rats** Male Sprague-Dawley rats weighing 300—390 g (Sankyo Labo, Co., Ltd.) were fasted for 24 h before the experiments. MS (or Ad-MS) containing 10 mg TH/kg or 3 mg TDS/kg were orally administered to conscious rats using a polyethylene tube attached to a gastric sonde with 0.2 ml of water. TH suspended in a 5% (w/v) arabic gum solution at a concentration of 20 mg/ml and 0.5% (w/v) TDS aqueous solution were used as references and also administered orally to each rat using a gastric sonde.

The blood samples were taken from the tail vein in heparinized micro-tubes at 0.5, 1, 2, 3, 4, 6, 8, 10 h for TH and at 0.5, 1, 2, 3, 4, 6 h for TDS. The rats were kept in the fasting state until 10 h. Plasma was obtained by centrifugation of the blood at 3000 rpm for 3 min and stored at −20 °C until assay.

**Determination of Plasma Concentrations of TH** Reverse-phase HPLC for TH in plasma was employed using HETH as an internal standard. A reversed phase HPLC column (Shim-pak ODS, 3 mm, 4.6 × 1.5 cm, Shimadzu Co., Ltd., Kyoto, Japan) was used at room temperature. The mobile phase was a 1 : 9 mixture of acetonitrile and 0.01 M acetate buffer (pH 4.0), flow at a rate of 2 ml/min. The wavelength for determination was 274 nm.

Prior to the analysis, the frozen samples were thawed out at room temperature and any precipitants removed. A 100 μl aliquot was placed in a centrifuge tube and spiked with a 10 μg/ml internal standard. Plasma 100 μl aliquot of the supernatant was introduced into HPLC.

**Analysis of Total Thiamine Levels in the Plasma** Total thiamine levels in the plasma were measured using a normal-phase HPLC technique after precolumn derivatization with mercuric chloride. Overnight enzymatic hydrolysis using acid phosphatase at pH 4.5 in acetate buffer converted any thiamine esters to their free form, and the thiamine was then oxidized to thiochrome which was then extracted into isobutanol. The isobutanol extracts were then injected directly onto a LiChrosorb NH2 column (5 μm, 4.6 × 250 mm) attached to a Shimadzu LC-10AS apparatus with a Shimadzu PF-10AXL detector set at 365 nm (excitation) and 440 nm (emission) at room temperature.

**Pharmacokinetic Analysis** The maximum plasma level (Cmax) and time to maximum plasma level (tmax) were determined according to the standard procedure. The area under the plasma level–time curves (AUC∞) was calculated by the linear trapezoidal method with a monoeponential extrapolation of the terminal phase. Mean residence time (MRT∞) was computed by moment analysis.8)

**Statistical Analysis** The in vivo parameters were subjected to statistical analysis of variance (ANOVA) followed by Tukey’s multiple range test. A difference was considered to be significant when the p value was less than 0.05.

**RESULTS AND DISCUSSION**

**Drug Release Behavior** The microspheres used for this study are listed in Table 1. The loading efficiencies of both drugs and both formulations were excellent. The drug content of the microspheres in the all cases showed good correlation with the theoretical drug loadings. Figure 1 shows the release profiles of TH and TDS from MS and Ad-MS sieved at 425—600 μm. MS and Ad-MS showed similar release profiles in both drugs. More than 90% of TH was released at 4 h and then completely released thereafter. About 85% of TDS was released within initial 2 h, showing a faster release than TH. This is a result of differences in their solubility in the dissolution medium, namely 8.2 mg/ml for TH9) and 37.7 mg/ml for TDS.10)

**Plasma Profiles of TH** After administration of a single dose of either arabic gum suspension, MS or Ad-MS to rats, the plasma TH levels were analyzed for a 10-hour period. Figure 2 shows the mean plasma concentration–time profiles after oral administration at the dose of 10 mg TH/kg. The TH suspension exhibited maximum concentration 1 h after administration. The TH release from MS and Ad-MS was suppressed at the initial phase, and prolonged later. The pharmacokinetic parameters are presented in Table 2. MS and Ad-MS showed small values in Cmax and large values in tmax and MRT∞ compared with the suspension, indicating an excellent sustained release performance. MS and Ad-MS, however, showed an equivalent AUC∞ value to that of the suspension. This demonstrated that the Ad-MS preparation provided excellent sustained release without showing a decrease in AUC.

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### Table 1. Formulation and Drug Content of Non-adhesive (MS) and Adhesive Microspheres (Ad-MS)

<table>
<thead>
<tr>
<th>Sample name</th>
<th>TH (g)</th>
<th>TDS (g)</th>
<th>CAB (g)</th>
<th>DS (g)</th>
<th>EA (g)</th>
<th>Lac (g)</th>
<th>Drug content [% (w/w)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH, MS</td>
<td>0.75</td>
<td>—</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>0.75</td>
<td>30.8 ± 1.5</td>
</tr>
<tr>
<td>TH, Ad-MS</td>
<td>0.5</td>
<td>—</td>
<td>1.0</td>
<td>0.25</td>
<td>0.75</td>
<td>—</td>
<td>23.9 ± 1.5</td>
</tr>
<tr>
<td>TDS, MS</td>
<td>—</td>
<td>1.5</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>63.8 ± 3.4</td>
</tr>
<tr>
<td>TDS, Ad-MS</td>
<td>—</td>
<td>0.5</td>
<td>1.0</td>
<td>0.25</td>
<td>0.75</td>
<td>—</td>
<td>23.3 ± 0.2</td>
</tr>
</tbody>
</table>

The results are expressed as the mean±S.D. (n=3).
In general, sustained-release preparations tend to show reduced AUC because they pass through the drug absorption site despite incomplete drug release. On the contrary, TH is well absorbed from the entire gastrointestinal including the colon. Therefore, there was no significant difference among AUC∞ values of these preparations.

Thiamine Levels in Plasma The total thiamine (thiamine plus its esters) in the plasma was examined by a thiochrome method after enzymatic hydrolysis and expressed as the amount of thiamine hydrochloride. Therefore, the total thiamine included thiamine and its metabolisms resulting from TDS and feed. In order to analyze the plasma level profiles caused by administration of TDS, the change in the total thiamine level was calculated by subtracting the thiamine concentration just before feeding as a baseline.

Figure 3 shows the change in the mean plasma levels of total thiamine after administration of solution, MS or Ad-MS containing 3 mg TDS/kg to rats. Zero of the vertical axis represents the baseline thiamine level and pharmacokinetic analysis was performed using the changes from the baseline. The pharmacokinetic parameters of thiamine are summarized in Table 3. There was no significant difference between the solution and MS in all parameters. As for Ad-MS, a significantly more sustained plasma level was observed. Moreover,
the AUC∞ data revealed that the bioavailability of TDS from Ad-MS was improved significantly compared with the other formulations.

According to a previous study,3) the bioavailability of both furosemide and riboflavin, the absorption of which was limited to the upper part of the GI tract, encapsulated in adhesive microspheres was improved compared with that of non-adhesive microspheres. It was considered that adhesive microspheres could, because of their adhesion to the gastric mucosa, reside for longer periods in the stomach or the upper small intestine, close to the absorption windows. In the present study, the pharmacokinetic analysis revealed that Ad-MS showed improved bioavailability of thiamine. Considering the strong adhesion to the gastric mucosa, the absorption of TDS, which has an absorption window in the small intestine, could be enhanced and prolonged by being released slowly.

CONCLUSION

The bioavailability of TH and TDS from Ad-MS in rats was investigated and compared with that from suspension (or solution) and MS. In the case of TH, the AUC∞ value of Ad-MS was equivalent to those of the suspension and MS. In the case of TDS, the AUC∞ value of Ad-MS was increased from those of the solution and MS. The Ad-MS performed well at sustaining the plasma level and improved the bioavailability of the encapsulated drugs whose absorption windows are limited to upper part of the gastrointestinal tract.

REFERENCES


Table 3. Pharmacokinetic Parameters for the TDS Solution, MS, and Ad-MS after Oral Administration at a Dose of 3 mg/kg in Rats

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>Cmax (ng/ml)</th>
<th>Tmax (h)</th>
<th>AUC∞ (ng·h/ml)</th>
<th>MRT∞ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution</td>
<td>122.4±42.6</td>
<td>1.15±0.25</td>
<td>316.7±103.8</td>
<td>2.22±0.56</td>
</tr>
<tr>
<td>MS</td>
<td>122.0±9.3</td>
<td>1.10±0.62</td>
<td>319.6±134.4</td>
<td>2.40±1.16</td>
</tr>
<tr>
<td>Ad-MS</td>
<td>111.3±37.2</td>
<td>1.60±0.48</td>
<td>683.6±269.1†</td>
<td>5.76±2.47†</td>
</tr>
</tbody>
</table>

The results are expressed as the mean±S.D. (n=4). *† represent significant differences: *versus solution, p<0.05; †versus MS, p<0.05.