Application of Curdlan to Controlled Drug Delivery. II.

In Vitro and in Vivo Drug Release Studies of Theophylline-Containing Curdlan Tablets

Motoko Kanke,* Hirokazu Katayama, and Masayo Nakamura

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Higashimura-cho, Fukuyama, Hiroshima
729-02 Japan. Received January 18, 1995; accepted April 8, 1995

Tablets (300 mg) having two different surface areas were prepared from spray-dried particles of curdlan (100 mg)/theophylline(200 mg). Drug release from the tablets was studied in vitro and in vivo. The in vitro drug release from a tablet with a larger surface area (Tab L) was faster than that with a smaller one (Tab S). The water uptake of Tab L was larger than that of Tab S, probably due to the difference in the tablets' surface areas. However, the water uptake was not a rate-determining step for the drug release from curdlan tablets containing a large amount of theophylline. A straight line was obtained when release % was plotted vs. time. The slope of each curve was calculated as 0.59 for Tab L and 0.58 for Tab S. This indicates that the release mechanism is non-Fickian diffusion controlled. In addition, the curdlan tablets or theophylline powder were administered orally to 5 healthy volunteers, and saliva concentrations of theophylline were determined. Each saliva concentration was converted to plasma concentration using the saliva to plasma ratio of the drug in each subject. The AUC of Tab L was nearly the same as that of powder, while the AUC of Tab S was smaller than that of powder. The mean residence times (MRTs) of theophylline powder, Tab S and Tab L were 11.1 ± 1.5, 25.4 ± 6.3 and 17.1 ± 1.5 h (N = 4–5, mean ± S.D.), respectively. The mean dissolution times (MDTs) of Tab L in vitro and Tab S in vitro were 5.0 ± 2.1 (N = 5, mean ± S.D.) and 13.9 ± 4.4 h (N = 4, mean ± S.D.), respectively. On the other hand, the MDTs of Tab L in vitro and Tab S in vitro were 4.8 and 11.2 h, respectively. In vivo drug release was very similar to in vitro drug release in both tablets. The lower bioavailability of Tab S suggested that the drug release had not been completed during the gastrointestinal transit period. Tab L would thus be a better controlled release form than Tab S.

Key words curdlan; tablet; controlled-release; bioavailability; theophylline; spray-drying

Curdlan (β-1,3-glucan), a thermogelable polysaccharide, was discovered by Harada et al. 1) This linear polymer is not water soluble but dissolves in alkaline solutions, formic acid and dimethylsulfoxide. Curdlan has been officially approved and used as a food additive, 2) but it has not yet been applied to pharmaceutical preparations. We previously reported the evaluation of sustained-release curdlan tablets containing theophylline prepared by compressing spray-dried particles of curdlan/theophylline. 3) The spray-drying technique was utilized for the preparation of sustained-release tablets. 4) The drug release from the curdlan tablets was controlled and was not affected by pH or various ions in the dissolution media in vitro. The release mechanism of the tablets was found to be diffusion-controlled.

In this study, 300 mg curdlan tablets containing 200 mg of theophylline were prepared with two different surface areas. Their drug release behaviors were first studied in vitro. Next, the tablets were administered orally to healthy volunteers in order to evaluate the applicability of curdlan to controlled release preparations.

MATERIALS AND METHODS

Materials Curdlan was purchased from Wako Pure Chemicals Co., Ltd. Theophylline and β-hydroxyethyl theophylline were obtained from Nacalai Tesque, Inc. and Sigma Chemical Co., respectively.

Spray-Drying Condition A spray-dryer, Pulvis MiniSpray, Model GA-31, Yamato Scientific Co., Ltd., was used. The drug (10 g) and curdlan (5 g) were dissolved in 5% ammonia solution (500 ml). The solution was kept at 70 ± 2°C and subsequently fed to the spray-dryer. The inlet temperature was set at 200°C, and the outlet temperature was 60–80°C. Air pressure was 1.0 kg/cm² and the flow rate of the solution was 7 ml/min.

Tablet Preparation Spray-dried particles (3 ± 1 μm) of curdlan or curdlan/theophylline (1:2) were directly compressed for 4 min at the force of 200 kg/cm² using a Shimadzu hand press for KBr tablets for IR spectroscopy. The 300 mg tablets with two different diameters (1.0 and 1.3 cm) and surface areas, named Tab S and Tab L, were prepared (Table 1).

In Vitro Release Studies The release of theophylline from the tablets was determined using a JP XII dissolution test apparatus with a paddle stirrer at 100 rpm. The dissolution medium used was 500 ml of pH 7.4 isotonic phosphate buffer (PB) or JP XII disintegration test medium No.1 (pH 1.2), and the medium was kept at 37 ± 0.5°C during measurements. Aliquots (0.2 ml) of sample solutions were withdrawn at appropriate time intervals and diluted with 3 ml of PB. The sample solutions were analyzed spectrophotometrically for theophylline at 270 nm using a Shimadzu UV 260 spectrophotometer. Tablet weight at time t (W) was determined after pat-drying the surrounding dissolution medium in order to estimate the hydration of the curdlan matrix in the tablet. "Water uptake" and "water uptake ratio" were calculated according to the following equations:

$$\text{water uptake} = \frac{(W_s - W_i) - W_o}{W_o} \times 100 \% \quad (1)$$

$$\text{water uptake ratio} = \frac{\text{water uptake}}{\text{water uptake at steady state}} \quad (2)$$

© 1995 Pharmaceutical Society of Japan
Table 1. Characteristics of Curdlan Tablets Used in This Study before and 48 h after Dissolution Test

<table>
<thead>
<tr>
<th></th>
<th>Diameter (cm)</th>
<th>Thickness (cm)</th>
<th>Weight (g)</th>
<th>Volume (cm³)</th>
<th>SA° (cm²)</th>
<th>SA°/volume (cm²/cm³)</th>
<th>Apparent density (g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tab S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before (A)</td>
<td>1.00</td>
<td>0.26</td>
<td>0.300</td>
<td>0.204</td>
<td>2.39</td>
<td>11.7</td>
<td>1.47</td>
</tr>
<tr>
<td>After (B)</td>
<td>1.23</td>
<td>0.32</td>
<td>0.498</td>
<td>0.380</td>
<td>3.62</td>
<td>9.5</td>
<td>1.31</td>
</tr>
<tr>
<td>(B)/(A)</td>
<td>1.20</td>
<td>0.32</td>
<td>1.66</td>
<td>1.86</td>
<td>1.51</td>
<td>0.81</td>
<td>0.89</td>
</tr>
<tr>
<td>Tab L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before (C)</td>
<td>1.30</td>
<td>0.16</td>
<td>0.300</td>
<td>0.212</td>
<td>3.30</td>
<td>15.6</td>
<td>1.42</td>
</tr>
<tr>
<td>After (D)</td>
<td>1.60</td>
<td>0.20</td>
<td>0.514</td>
<td>0.402</td>
<td>5.03</td>
<td>12.5</td>
<td>1.28</td>
</tr>
<tr>
<td>(D)/(C)</td>
<td>1.20</td>
<td>1.25</td>
<td>1.71</td>
<td>1.90</td>
<td>1.52</td>
<td>0.80</td>
<td>0.90</td>
</tr>
</tbody>
</table>

a) Surface area of curdlan tablet.

where \( W_0 \) is the curdlan weight in the dry tablet and \( X_i \) is the theophylline amount remaining in the tablet at time \( t \).

**Disintegration Test** This method followed the disintegration test for the uncoated tablets in JP XII using a disintegration tester (Model NT-2F, Japan). The medium used was distilled water, kept at 37 ± 2°C.

**In Vitro Release Studies** To evaluate the utility of Tab S and Tab L as sustained-release preparations, the tablets were administered to five healthy volunteers (4 females, 1 male) from whom informed consent were obtained. One of them was a light smoker and the others were non-smokers. None of the subjects consumed any caffeine- or alcohol-containing food or drink for 2 d prior to and up until the end of the study. Before each dosage, the subjects fasted overnight. The smoker was permitted to smoke. A cross-over schedule was designed and a minimum interval of one week was allowed between each dosing. Blank saliva or blood samples were collected a few minutes before the administration of the drug. A single dose of 100 mg theophylline powder, or one or two tablets which contained 200 mg theophylline per tablet was administered with 200 ml of water. Saliva samples were collected at appropriate time intervals up to 48 h. Theophylline concentrations in saliva were determined by the slightly modified method of Chung and Shim. Each fresh saliva sample (about 1.5 ml) was centrifuged at 10000 rpm for 2 min (Eppendorf Centrifuge 5415). To 1.0 ml of a saliva supernatant, 50 µl of \( β \)-hydroxyethyl theophylline (80 µg/ml) was added as an internal standard. One-tenth (0.1 ml) of 0.1 M HCl solution and 7 ml of isopropyl alcohol/chloroform (5/95, v/v) were then added. The test tubes were shaken for 10 min and centrifuged at 3000 rpm for 5 min. Five ml of the organic layer was transferred to a test tube and evaporated under nitrogen gas in a water bath (60°C). The residue was dissolved in 200 µl of the HPLC mobile phase and filtered. Ten µl of the filtrate was injected into the column (ODS 120T, 4.6 mm × 150 mm, Tosoh, Japan). A high performance liquid chromatograph (LC-6A, Shimadzu, Japan) equipped with a spectrophotometric detector (SPD-6AV, Shimadzu, Japan, at 270 nm) was used. The mobile phase was acetonitrile/0.01 M acetic buffer (pH 4.0; 9/91, v/v) and the flow rate was 1.0 ml/min. Blood samples were taken at 2.5 and 6 h following oral administration. Theophylline concentrations in plasma were determined according to the method described above.

**Data Analysis** Saliva theophylline concentrations were converted to plasma theophylline concentrations by using a conversion factor determined for each subject. Good correlation between the theophylline concentrations in plasma and those in saliva was reported. The simultaneous sampling of saliva and blood was performed two times in each subject. The area under the calculated plasma theophylline concentration–time curve from time zero to \( t \) (\( AUC_{0→t} \)) was calculated by means of the trapezoidal method. The area from time \( t \) to infinity was estimated by \( C_i/k \), where \( C_i \) is the theophylline concentration at time \( t \) and \( k \) is the apparent elimination rate constant of the drug obtained from the slope of the log-linear portion of the curve by the least regression analysis. In summary, the area from time zero to infinity was calculated by the following equation:

\[
AUC_{0→∞} = AUC_{0→t} + C_i/k
\]

**RESULTS AND DISCUSSION**

**In Vitro Release of Theophylline from Curdlan Tablets Having Different Surface Areas** As shown in Fig. 1, theophylline release from Tab L was faster than that from Tab S. The actual drug amount in the tablet was determined to be the same as the theoretical value (200 mg). Although the drug release from Tab L was completed within 24 h, the release rate from Tab S was 93.0 ± 1.3% (\( N = 3 \)) of the content at 24 h and 100% (\( N = 3 \)) at 32 h. MDTs of Tab L and Tab S were 4.8 and 11.2 h, respectively. The release profiles in pH 1.2 medium were the same as in pH 7.4 medium (data not shown). We reported that the drug release rate of 300 mg curdlan tablets containing 100 mg theophylline in pH 1.2 was the same as that in pH 7.4 medium. In this study, very similar results were obtained for the tablets with a higher content (200 mg). Since curdlan is not water soluble, curdlan tablets used in this study maintained the cylindrical shape throughout dissolution experiment. In addition, neither of the tablets disintegrated over 3 h in the disintegration test of JP XII. However, the tablets swelled (Table 1), and the generalized equation, \( M_t/M_0 = K \cdot t^a \) or \( \log(M_t/M_0) = \)
log $K + n \cdot \log t$ was used to obtain information about the drug release mechanism from the curdlan tablet. $M_t/M_\infty$ is the fractional release from the drug at time $t$, $K$ is a constant and $n$ is the diffusional exponent characteristic of the release mechanism. A value of $n=0.45$ (cylinder shape) indicates Fickian diffusion, $n=0.89$ indicates zero-order release, and values in between are considered as non-Fickian (anomalous) transport. As shown in Fig. 2, log theophylline release vs. log $t$ correlated well in both tablets, and the $n$ values obtained from the slopes of Fig. 2 were 0.58 for Tab S and 0.59 for Tab L. Therefore, it can be said that the release mechanism is defined as non-Fickian, although Higuchi plots showed good linearity ($r \geq 0.993$, data not shown).

Upon immersion of the curdlan tablet in an aqueous medium, gradual gelation was observed from the outer surface of the tablet. Drug particles are essentially immobile in a dry polymer matrix. They then dissolve and their solution begins to diffuse out as the polymer swells by absorbing water. Thus, drug release depends on two simultaneous processes, water absorption of the tablet and drug diffusion through the gel layer.

Water uptake of the curdlan tablets was studied to evaluate the effect of water penetration in the tablet on drug release. As shown in Fig. 3, water uptake nearly reached a steady state at 24 h for Tab L and at 28 h for Tab S. The water absorption rate of Tab L was larger than that of Tab S, probably due to the difference in the tablet surface areas. If the drug release is controlled only by the water uptake, the percentage of drug release should be proportional to the water uptake; a plot should be linear with a unit slope and intercept at zero. Although the theophylline release (%) vs. water uptake ratio of each tablet was linear up to about 30% release of the total drug content, as shown in Fig. 4, the slope is much less than 1 and does not intercept at zero. Therefore, the water uptake was not a rate-limiting step for the drug release from these curdlan tablets.

**Bioavailability of Curdlan Tablets in Healthy Volunteers**

The saliva to plasma concentration ratio of theophylline in each subject is summarized in Table 2. Calculated drug concentration in plasma vs. time profiles of curdlan tablets and theophylline powder are shown in Fig. 5. Tablets with a larger surface area (Tab L) yielded a much higher plasma concentration than smaller tablets (Tab S) up to 32 h. The $AUC_{0-\infty}$ of Tab L was larger than that of Tab S (Table 2). The $AUC_{0-\infty}$ of Tab L showed smaller deviations among 5 subjects, while that of Tab S varied widely (Table 2). Moreover, the fact that the mean $AUC_{0-\infty}$ value of
Table 2. Pharmacokinetic Parameters Following Oral Administration of Curdlan Tablets or Theophylline Powder in 5 Healthy Volunteers

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>B.W. (kg)</th>
<th>S/P ratio</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (μg·mL&lt;sup&gt;-1&lt;/sup&gt;·h)</th>
<th>MRT (h)</th>
<th>VRT (h&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (μg·mL&lt;sup&gt;-1&lt;/sup&gt;·h)</th>
<th>MRT (h)</th>
<th>VRT (h&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (μg·mL&lt;sup&gt;-1&lt;/sup&gt;·h)</th>
<th>MRT (h)</th>
<th>VRT (h&lt;sup&gt;2&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>22</td>
<td>49</td>
<td>0.55</td>
<td>76.5</td>
<td>32.4</td>
<td>131.5</td>
<td>76.5</td>
<td>32.4</td>
<td>131.5</td>
<td>76.5</td>
<td>32.4</td>
<td>131.5</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>22</td>
<td>43</td>
<td>0.52</td>
<td>84.0</td>
<td>28.5</td>
<td>184.3</td>
<td>84.0</td>
<td>28.5</td>
<td>184.3</td>
<td>84.0</td>
<td>28.5</td>
<td>184.3</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>22</td>
<td>46</td>
<td>0.55</td>
<td>53.5</td>
<td>22.7</td>
<td>139.2</td>
<td>53.5</td>
<td>22.7</td>
<td>139.2</td>
<td>53.5</td>
<td>22.7</td>
<td>139.2</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>22</td>
<td>57</td>
<td>0.43</td>
<td>46.6</td>
<td>21.9</td>
<td>94.8</td>
<td>46.6</td>
<td>21.9</td>
<td>94.8</td>
<td>46.6</td>
<td>21.9</td>
<td>94.8</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>24</td>
<td>55</td>
<td>0.61</td>
<td>11.6</td>
<td>5.2</td>
<td>30.3</td>
<td>11.6</td>
<td>5.2</td>
<td>30.3</td>
<td>11.6</td>
<td>5.2</td>
<td>30.3</td>
</tr>
<tr>
<td>Mean (N)</td>
<td></td>
<td></td>
<td></td>
<td>0.53 (5)</td>
<td>53.2 (4)</td>
<td>11.1 (4)</td>
<td>123.9 (4)</td>
<td>62.5 (4)</td>
<td>26.2 (4)</td>
<td>573.5 (4)</td>
<td>21.9 (5)</td>
<td>5.2 (5)</td>
<td>37.8 (5)</td>
</tr>
<tr>
<td>S.D.</td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
<td>10.3</td>
<td>1.5</td>
<td>30.3</td>
<td>11.0</td>
<td>1.5</td>
<td>26.7</td>
<td>11.0</td>
<td>1.5</td>
<td>26.7</td>
</tr>
</tbody>
</table>

Note: a) Saliva to plasma ratio of theophylline. b) Two tablets were administered (400 mg as theophylline) in subjects 3 and 5.

Fig. 4. Relationship between Water Uptake Ratio and Theophylline Release Percent for Curdlan Tablets Having Two Different Surface Areas in Isotonic Phosphate Buffer (pH 7.4) at 37 ± 0.5°C

Tab L containing 200 mg theophylline was almost 2-fold that of powder (100 mg theophylline) indicates that almost complete absorption of the drug occurred from Tab L. However, it was suspected that there was incomplete absorption from Tab S due to the fact that the AUC ratio of Tab S to 100 mg powder was less than 2. The first moment of the calculated plasma concentrations vs. time curve, MRT, was 26.2 h for Tab S and 17.1 h for Tab L. As shown in Table 3, the calculated peak plasma concentration (C<sub>max</sub>) of Tab L (4.68 ± 0.84 μg/ml, N = 5) was higher than that of Tab S (2.00 ± 0.42 μg/ml, N = 4), while the time to reach C<sub>max</sub> (T<sub>max</sub>) of these tablets did not differ significantly (7.09 ± 1.16 h, N = 5 for Tab L; 6.28 ± 3.38 h, N = 4 for Tab S). On the other hand, the mean values of MRT, C<sub>max</sub>, and T<sub>max</sub> of Theo-Dur<sup>®</sup> of a commercially available sustained-release tablet containing theophylline in healthy volunteers were reported as 15—19 h, about 4 μg/ml (as 200 mg theophylline) and 7—8 h, respectively. Therefore, the bioavailability of Theo-Dur<sup>®</sup> seemed to be similar to that of Tab L. As shown in Table 2, the AUC of Tab L in subjects 3 and 5 increased by 2 times when the dose was increased from 200 to 400 mg, without a significant change in MRT. Theophylline can be absorbed even from the lower region of the colon, and the intestinal absorption rate of the drug was very fast judging from the plasma concentration vs. time curve of theophylline powder (Fig. 5). The mean absorption time of theophylline powder (MAT<sub>powder</sub>) was obtained by the following equation:

\[ MAT_{powder} = MRT_{powder} - 1/k_{powder} \]

where MRT<sub>powder</sub> is the mean residence time for theophylline powder, and k<sub>powder</sub> is obtained from the slope of the log-linear portion of the calculated plasma concentration–time curve for the powder. MAT<sub>powder</sub> was
small (0.11 ± 0.06 h, N = 4) in this study, as was generally believed. Consequently, the gastro-intestinal transit time (GIT) and the drug release rate from the tablet appeared to affect the bioavailability of curdlan/theophylline tablets. Sugito et al. reported the GITs of non-disintegrating dosage-forms (tablets, pellets, micro-particles) in healthy subjects using double isotope scintigraphy. The GITs of tablets with different sizes and different relative densities (d) (8 × 4 mm, d = 1.33 and 10 × 6 mm, d = 0.86) were almost identical under the non-fasting condition.12 Accordingly, it was expected that the GITs for curdlan tablets used in this study would be nearly the same. In order to evaluate the in vivo drug release from the curdlan tablets, the mean dissolution time in vivo (MDT\text{Tab, in vivo}) was calculated by the following equation:

\[
\text{MDT}_{\text{Tab, in vivo}} = \frac{\text{MRT}_{\text{Tab}}}{\text{MAT}_{\text{solution}} + \text{MRT}_{\text{in v}}}
\]

where MAT\text{solution} is mean absorption time of theophylline from the solution, which must be less than MAT\text{powder} (0.11 h), so that the MAT\text{solution} could be negligible. MRT\text{in v} is the mean residence time for intravenous administration, which was obtained from 1/k'. The parameter k' is the average of the slopes of the log-linear portion of the calculated plasma concentration–time curves for the powder and Tab L. In the case of subject 5, k' was obtained only from the data of Tab L. The MDT\text{Tab, in vitro} of Tab L and Tab S were 5.0 ± 2.1 (N = 5) and 13.9 ± 5.4 h (N = 4), respectively. These values were close to the MDT\text{Tab, in vitro} (4.8 and 11.2 h) in both tablets (Table 3). Although the k values of Tab L (0.0799 ± 0.0065 h\text{−1}, N = 5) and theophylline powder (0.0925 ± 0.0139 h\text{−1}, N = 4) did not differ significantly, the k value of Tab S (0.0438 ± 0.0069 h\text{−1}, N = 4) was much smaller than the other two. The lower availability from Tab S suggested that the drug release had not been completed during gastrointestinal transit. In conclusion, it was suggested that the curdlan tablet is applicable as a controlled release form for theophylline. The size of Tab L would be a better size for controlled release than that of Tab S.

REFERENCES