PREPARATION AND BIOPHARMACEUTICAL EVALUATION OF MICROCAPSULES OF AMPICILLIN

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(Received September 4, 1984)

Microencapsulation of ampicillin, an orally administered antibiotic, with different viscosity grades of ethyl cellulose was studied. The preparation of microcapsules was done as follows; the mixture of ethyl cellulose-CH₂Cl₂-ampicillin was dispersed in purified water containing 0.5% (w/w) sodium lauryl benzenesulphonate, and then CH₂Cl₂ was dried out by elevating the temperature. The dissolution curves for the release of ampicillin from microcapsules prepared using the four different viscosity grades of ethyl cellulose were quite different. The release of ampicillin increased with decreasing ethyl cellulose viscosity. The evaluation of prepared microcapsules was made using gastric-emptying-controlled rabbits. The plasma concentration of ampicillin obtained by the administration of microcapsules showed a significant sustained-release pattern. The area under the plasma concentration curve (AUC) of ampicillin obtained after a single oral administration of microcapsules prepared using 10 cps ethyl cellulose was 1.8 times greater than that obtained after double oral administration of powder. This fact will be caused by the delaying of gastric-emptying, intestinal-transit and dissolution of ampicillin there. It was confirmed that a large number of microcapsules still remained in the stomach and each microcapsules still contained ampicillin at 24 h after dosing from the experiment using gastric-emptying-controlled rabbits.

Keywords — microencapsulation; ampicillin; ethyl cellulose microcapsule; sustained-release formulation; dissolution, gastric-emptying; intestinal-transit; plasma concentration

INTRODUCTION

Ampicillin, an orally administered antibiotic, has a broad antibacterial spectrum, and is often used as the first choice in the treatments of various infectious diseases. In order to improve absorption, many derivatives have been developed, but most of the ampicillin derivatives cannot be substituted for ampicillin from various reasons, such as, its unpleasant taste (pivampicillin) or its high cost (amoxicillin). In the previous paper, it was reported that microcapsules of amoxicillin were prepared using gelatin and ethyl cellulose, and that the ethyl cellulose microcapsules containing 25% amoxicillin showed a significant sustained-release pattern of amoxicillin.

It has been reported that viscosity grades of ethyl cellulose influenced the release pattern of drugs from ethyl cellulose microcapsules prepared by the coacervation technique. However, the biopharmaceutical evaluation of microcapsules with different viscosity grades of ethyl cellulose has not yet been studied in detail.

The aim of this study was to establish a reproducible microencapsulation method for ampicillin using different viscosity grades of ethyl cellulose and to evaluate the microcapsules containing ampicillin biopharmaceutically.

MATERIALS AND METHODS

Materials — Ampicillin trihydrate (Sigma

* This paper forms Part X of a series entitled “Evaluation of Microcapsules.”

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Co., JP X grade) passing through a 100 mesh sieve (149 μm) was used. Four grades of ethyl cellulose (Tokyo Kasei Co.) having viscosity of 9–10 cps (mean molecular weight, $M_w$, 24000), 18–22 cps ($M_w$, 30000), 45–55 cps ($M_w$, 42000), 90–110 cps ($M_w$, 58000) for 5% (w/w) solution in toluene–ethanol mixture (80:20) were used. Other reagents were all of special reagent grade.

**Preparation of Microcapsules** — Ethyl cellulose microcapsules were prepared by the method described previously. The flow chart for microencapsulation is shown in Chart 1.

For the four viscosity grades of ethyl cellulose, we regulated both the components used for microcapsules preparation as well as the stirring rates of propeller following the dispersion of ethyl cellulose–CH₂Cl₂–ampicillin mixture in purified water containing 0.5% (w/w) sodium lauryl benzenesulphonate. The components used for the preparation of the microcapsules containing 20% (w/w) ampicillin and their relative contents are summarized in Table I. Figure 1 shows the typical size distributions of microcapsules prepared.

**Dissolution Studies** — The procedure and apparatus were essentially the same as described in JP X. Five hundred milliliters of dissolution medium (No. 2 solution, pH 6.8, used in the JP X disintegration test) was introduced in a beaker and stirred at 50 rpm. An accurately weighed amount of microcapsules (particle size, 350–500 μm) corresponding to 10 mg ampicillin was gently spread over the surface of the dissolution medium, which was maintained at a temperature of 37 °C. At appropriate intervals, 3 ml sample solution was filtered through a dried filter paper to remove any solid drug particles. Two ml of filtered sample solution was taken for analysis. The sample volume taken was replaced by an equivalent volume (3 ml) of fresh dissolution medium and the volume of the dissolution medium in the beaker was kept constant (500 ml). One-half ml of 0.4 M citric-buffer (pH 2) containing 7% (w/w) formaldehyde was added to 2 ml of the sample and heated in the boiling bath for 2 h. The sample solution was cooled and then 1 ml of 2 N NaOH was added. The mixture was measured by a fluorometric method (excitation wavelength, 346 nm; emission wavelength, 422 nm) using a Shimadzu spectrophotometer RF-500. The experimental results were plotted against time as a percentage of ampicillin extracted into the dissolution medium from the microcapsules.

**Gastric-Emptying-Controlled Rabbits** — The procedure for the gastric-emptying-controlled rabbits was the same as the previous studies.

**Single Intravenous Administration** — Ampicillin trihydrate (100 mg) was dissolved in 10 ml of glycine buffer (pH 11). The above solution was administrated as an intravenous infusion for a few minutes through the left ear vein. Blood samples (1 ml) were immediately centrifuged to obtained plasma (8000 rpm, 4 min) and 2 ml of 10% (w/w) trichloroacetic acid solution was added to plasma (0.5 ml) in the centrifuge tube. After shaking the centrifuge tube violently lengthwise, the mixture was centrifuged at 3000 rpm for 10 min. The clear supernatant (2 ml) was collected and used for analysis mentioned above.

**Oral Administration** — Single oral administration of microcapsules corresponding to 60 mg/kg of ampicillin trihydrate was made with 30 ml water and 22 g/kg of SD-3 which had been pre-
pared by adding 70 parts of water to 30 parts of the special solid diet (SD-1, Nihon Clea Co., prepared by removing alfalfa from commercial solid food for rabbits). Double oral administration of ampicillin trihydrate powder (30 mg/kg × 2) was made with 30 ml water and 22 g/kg of SD-3 at 0 and 12 h. Blood samples were withdrawn and analyzed in much the same way as shown in the preceding section, single intravenous administration.

<table>
<thead>
<tr>
<th>TABLE I. Components and Stirring Rates of Propeller for Preparation of Ethyl Cellulose Microcapsules Containing Ampicillin, and Contents of Ampicillin in Products</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microcapsules</strong></td>
</tr>
<tr>
<td>Ethyl cellulose (g)</td>
</tr>
<tr>
<td>Drug (g)</td>
</tr>
<tr>
<td>Stirring rates (rpm)</td>
</tr>
<tr>
<td>Contents (%)</td>
</tr>
</tbody>
</table>

**FIG. 1. Frequency Distribution for Various Microcapsules Containing Ampicillin**

The weight of particles lying within a certain size range is plotted against each size range. These four distribution, (1), (2), (3), and (4) are typical ones of 10 cps-ethyl cellulose(EC), 20 cps-EC, 50 cps-EC, 100 cps-EC, respectively.
Evaluation of Microcapsules of Ampicillin

Experiments for Withdrawing of Microcapsules from Stomach — A pentobarbital solution containing a corresponding amount of 85 mg/kg was injected to the gastric-emptying-controlled rabbits which had been administrated the ethyl cellulose microcapsules containing 20% (w/w) ampicillin 24 h before. The abdominal region was incised along the midline, the stomach was exposed and the contents in stomach were washed out using an isotonic NaCl solution. The gastric-emptying-rate of microcapsules was expressed as the ratio of the total number of microcapsules found in the stomach to the total administered number of microcapsules which were counted previously. The portion of microcapsules in the stomach was filtered, dried, and separated in order to check the residual contents of ampicillin in the microcapsules.

Electron Scanning Microscopy — The microcapsules were coated with pure gold using an Eiko Engineering Coater IB-3 type under a high vacuum, 0.1 Torr, high voltage, 800—1500 V, and 8 mA. Samples obtained by above procedure were examined with a scanning electron microscope, Hitachi-Akashi MSM-4 type.

Calculation of Rate Constants — The elimination rate constant, $K_0$ (h$^{-1}$), were calculated from the plasma-level data using a microcomputer. The Loo–Riegelman method was applied for calculation of the absorption rate constant, $K_a$ (h$^{-1}$).

RESULTS AND DISCUSSION

Preparation and Electron Scanning Micrographs of Ethyl Cellulose Microcapsules

Microcapsules containing 20% (w/w) ampicillin were prepared using four viscosity grades of ethyl cellulose. As shown in Table I, the loss of drug content on the preparation of microcapsules was increased with decreasing viscosity of ethyl cellulose from 100 to 10 cps. The stirring rates of propeller for the dispersion of ethyl cellulose–CH$_2$Cl$_2$–ampicillin mixture in purified water containing 0.5% (w/w) lauryl benzenesulfonate was controlled in order to have almost the same size distribution in the final products of microcapsules for four viscosity grades of ethyl cellulose. Figure 2 shows the appearance and surface of microcapsules containing 20% (w/w) ampicillin prepared using the four viscosity grades of ethyl cellulose.

Microcapsules prepared using 100, 50, and 20 cps ethyl celluloses were found to be almost spherical in shape. However, the microcapsules using 10 cps ethyl cellulose consisted of many distorted particles. In general, the coarseness of the surface of microcapsules increased with the viscosity of ethyl cellulose down to 10 cps. Especially, there was a considerable difference in the surface state between microcapsules using 10 cps ethyl cellulose and those using 100 cps ethyl cellulose. The surface of microcapsules using 100 cps ethyl cellulose was comparatively smooth and had small size pores. While, that using 10 cps ethyl cellulose was considerably coarse and had comparatively large size pores.

Releasing Rates of Ampicillin from Ethyl Cellulose Microcapsules

Figure 3 shows the dissolution patterns for the release of ampicillin from microcapsules prepared using four viscosity grades of ethyl cellulose. The release rates of ampicillin from various microcapsules were quite different and decreased with increasing viscosity of ethyl cellulose used. This phenomenon was the same as that described in the other reports concerning ethyl cellulose microcapsules prepared using the coacervation method. Figure 4 shows the first order process for the releasing of ampicillin from ethyl cellulose microcapsules prepared using four viscosity grades. After plotting on semilogarithmic graph, it was found that the experimental plots was almost a straight line.

Now, it is supposed that the difference of the releasing rates of ampicillin may be caused by difference of the characteristics of ethyl cellulose wall, that is, pore size and number of pores on the wall and coarseness of surface of microcapsules.

Ethyl cellulose microcapsules prepared in this experiment are thought as the matrix-multinuclear type and differ from the mononuclear type. Further experiments with regards to releasing mechanism of drugs from ethyl cellulose
FIG. 2. Scanning Electron Micrographs of Microcapsules Containing Ampicillin
(A) 100 cps-EC, (B) 50 cps-EC, (C) 20 cps-EC, (D) 10 cps-EC.
Evaluation of Microcapsules of Ampicillin

6.8 microcapsules will be necessary in future.

**Intravenous and Oral Administration of Ampicillin**

The plasma concentration of ampicillin after bolus intravenous administration (10 mg/kg) using gastric-emptying-controlled rabbits could be represented by Eq. 1 in this experiment.

\[
C_p(\mu g/ml) = 11.80 \exp(-1.866t) + 40.75 \exp(-7.061t)
\]  

**TABLE II. Biopharmaceutical Evaluation**

*a* for Sustained-releasing of Ethyl Cellulose Microcapsules Containing Ampicillin

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>[K_a] (h⁻¹)</th>
<th>[AUC_{0-24}] (h·μg·ml⁻¹)</th>
<th>[AUC_{7-24} ]</th>
<th>[C_{max}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder</td>
<td>0.255</td>
<td>23.8</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>10cps-EC</td>
<td>0.124</td>
<td>44.0</td>
<td>1.84</td>
<td>1.81</td>
</tr>
<tr>
<td>50cps-EC</td>
<td>0.108</td>
<td>31.9</td>
<td>1.34</td>
<td>0.85</td>
</tr>
<tr>
<td>100cps-EC</td>
<td>0.091</td>
<td>22.5</td>
<td>0.95</td>
<td>0.60</td>
</tr>
</tbody>
</table>

*a* Refer to Fig. 5. \[AUC_{0-24}\] means the area under the curve obtained by administration of microcapsule containing ampicillin during 0—24 h. \[AUC_{7-24}\] means the area under the curve obtained by administration of powder of ampicillin during 0—24 h. \[C_{max}\] means the maximum plasma concentration obtained by administration of microcapsule containing ampicillin. \[C_{max}\] means the maximum plasma concentration obtained by administration of powder of ampicillin. \(f\) For example, 10cps-EC means the microcapsule prepared with 10cps-ethyl cellulose.
where $C_p$ is the plasma concentration of ampicillin at time $t$.

The elimination of ampicillin from the body was very rapid, and no ampicillin could be detected 3 h after intravenous injection. Figure 5 shows the plasma concentration of ampicillin following single oral administration of microcapsules prepared using different viscosity grades of ethyl cellulose (10, 50 and 100 cps) and double oral administrations of ampicillin powder. Some biopharmaceutical values for the above oral administrations were summarized in Table II.

The absorption rate constant, $K_a$ (h$^{-1}$), calculated by plasma concentration data of ampicillin following double administration of ampicillin powder was much smaller than the elimination rate constant, $K_{10}$ (h$^{-1}$). Therefore, a flip-flop phenomenon in pharmacokinetic field was observed. This result coincided with the Tanigawara’s paper, in which the absorption process was proved to be a rate-determining step in the case of gastro-intestinal absorption of ampicillin.

On the other hand, the plasma concentration curves of ampicillin following single oral administration of microcapsules prepared using different viscosity grades of ethyl cellulose (10, 50 and 100 cps) showed a typical and an excellent sustained-release pattern. The pharmacokinetic values ($K_a$, $AUC$ and $C_{max}$) are mutually related to the releasing rate constant in three different viscosity grades of ethyl cellulose (10, 50 and 100 cps).

The area under the plasma concentration curve ($AUC$) obtained after the single oral administration of microcapsules prepared using 10 cps ethyl cellulose was nearly twice as large as that obtained after double administration of ampicillin powder.

Such a great increase in $AUC$ may be explained by the delay of gastric-emptying and intestinal-transit rates and the simultaneous eleva-

**TABLE III. Particle Number of Microcapsules$^a$ Staying in Rabbit Stomach at 24 h after Oral Administration (60 mg/kg)**

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Particle number Administration$^b$</th>
<th>Staying at 24 h$^b$</th>
<th>Staying ratio (%)</th>
<th>Remaining % of ampicillin in microcapsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>28420</td>
<td>7308</td>
<td>25.7</td>
<td>7.2</td>
</tr>
<tr>
<td>B</td>
<td>25380</td>
<td>6263</td>
<td>24.7</td>
<td>8.8</td>
</tr>
</tbody>
</table>

*a) Administered microcapsules were prepared with 100 cps-ethyl cellulose.

b) The values represent the numbers of particle.
tion of absorption efficacy. In general, ampicillin powder administered disperses and a portion of it dissolves in stomach and moves towards the small intestine within a relatively short time. In the case of microcapsules, if a delay of gastric-emptying rate of microcapsules take place and the microcapsules pass through absorption sites slowly and smoothly, there will be the possibility of an increase in $AUC$. If a delay of gastric-emptying and intestinal-transit rates occurs in the ethyl cellulose microcapsules containing ampicillin, the microcapsules will remain in the absorption sites of stomach and intestine, and continue to release ampicillin from the microcapsules there very slowly.

At 24 h after oral administration of microcapsules, the stomach of gastric-emptying-controlled rabbits was exposed and many particles were found remaining in the stomach as shown in Fig. 6. It was found that about 25% of the microcapsules administered orally remained in the stomach and the remained microcapsules still contained about 8% (w/w) ampicillin. The results are shown in Table III. Besides, the microcapsules remained throughout all the intestine. The results described above suggest the possibility of delay of gastric-emptying and intestinal-transit rates of microcapsules.

Recently, there have been more attempts to study the influence of specific gravity and particle size in pharmaceutical preparations on the transit times through gastro-intestinal tract. The new type preparation called “HBS (hydrodynamically balanced system),” which can float in the stomach for relatively long time by utilizing an excipient of a low density was developed in Roche Co.

When the microcapsules as a sustained-release preparation is designed, the releasing rates of drug from microcapsules considering transit times through gastro-intestinal tract will become an important problem in this field.

Acknowledgements The authors are grateful to Miss Yuuko Ishii for her excellent technical assistance.

REFERENCES