Sustained Release of Phenytoin Following the Oral Administration of Phenytoin Sodium/Ethylcellulose Microcapsules in Human Subjects and Rabbits

Ikuko Karakasa,1) Naomi Yagi,* Megumi Shibata, Harumi Kenmotsu, Hitoshi Sekikawa, and Masahiko Takada

Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University, Ishikari-Tobetsu, Hokkaido 061–02, Japan.
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Phenytoin sodium was microencapsulated with ethylcellulose (EC) by a coacervation-phase separation method from ethyl acetate solution to develop a prolonged release dosage form of phenytoin. Release of phenytoin from the microcapsules (phenytoin sodium/EC) was evaluated by the JP dissolution test in JP disintegration media No. 1 and No. 2. The release rates of phenytoin from phenytoin sodium powders were extremely rapid in both media, however, the release rates from the microcapsules were much more retarded. Following the oral administration of microcapsules to rabbits, prolonged plasma concentrations of phenytoin were obtained, while microcapsules orally administered to human subjects showed prolonged urinary excretion of phenytoin metabolites.

Keywords microcapsule; phenytoin; phenytoin sodium; ethylcellulose; sustained release

Phenytoin, an antiepileptic drug, has been the drug of choice for the treatment of most types of epileptic seizures except for petit mal. Therapeutic drug monitoring (TDM) of phenytoin has been done, because of the difficulty in phenytoin therapy to maintain effective therapeutic plasma levels (10–20 μg/ml).1)–3) In addition to the narrow therapeutic plasma levels, the drug has exhibited great variations in bioavailability following its oral administration to patients, because of its poor water-solubility.4) The dissolution characteristics of phenytoin were improved by preparing solid dispersion systems such as coprecipitates with polyvinylpyrrolidone5) or mechanochemical pulverization with crystalline cellulose.6) Phenytoin bioavailability was enhanced when such solid dispersion systems were administered to human subjects.5)–6) However, the plasma levels following the administration of these systems decreased rapidly. It might, therefore, be necessary for a patient to take the drug several times a day to maintain an effective therapeutic plasma level without side effects. Phenytoin sodium is used for injections or capsules. It is soluble in water, but if administered orally, parts of the phenytoin molecule may be recrystallized in the acidic stomach fluid.7) Moreover, phenytoin sodium swells with moisture in the air.8)

Many microencapsulation techniques have been developed for use in various fields including chemical, food, cosmetic, and pharmaceutical industries.9)–10) Various coating materials which are poorly water-soluble polymers have been used11)–14) for the sustained release of therapeutic agents. We studied the microencapsulation of phenytoin as a sodium salt containing ethylcellulose (EC) to enhance the drug's sustained release. Release patterns of phenytoin from microcapsules (phenytoin sodium/EC) were studied in vitro, and in vivo absorption studies were made in human subjects and rabbits.

MATERIALS AND METHODS

Materials Phenytoin (Aleviatin®, lot No. ML066MA) of JP grade was obtained from Dainippon Pharmaceutical Co., Osaka, Japan. Phenytoin sodium was prepared as follows: phenytoin and sodium hydroxide in equi-molar weights were dissolved in a minimum amount of redistilled water. Then the solution was filtered through filter paper and dried in vacuo at about 70 °C using a rotary evaporator. The obtained powder was further dried in vacuo at about 60 °C for 24 h. The resultant material was phenytoin sodium monohydrate. Anal. Caled for C15H13N2O4Na: C, 61.59; H, 4.45; N, 9.58. Found: C, 61.68; H, 4.33; N, 9.72. In the measurement with a differential scanning calorimeter (Thermal Analyzer DT-40, Shimadzu, Co., Ltd., Kyoto, Japan), an endothermic peak accompanied by the melting of phenytoin crystal was found at 295 °C. A broad peak was observed around 88 °C due to the dehydration, but no peak due to the melting of free phenytoin was found at 295 °C. The mean particle size of phenytoin sodium, as measured by optical microscopy (Olympus BH microscope) was 50.12 ± 18.02 μm (mean ± S.D., Green diameter). EC standard (lot No. 052-02765) with ethoxy content of about 49% was obtained from Wako Pure Chemicals Ind., Osaka, Japan. 5-(p-Hydroxyphenyl)-5-phenylhydantoin (HPH) and 5-(p-methylphenyl)-5-phenylhydantoin were obtained from Aldrich Chemicals Co., Milwaukee, Wis., U.S.A. Phenyl-trimethylammonium hydroxide (20–25% (w/v) methanolic solution, Tokyo Kasei Industrial Co., Tokyo, Japan) was diluted to 10–15% (v/v) by methanol. All other chemicals were of reagent grade.

Preparation of Microcapsules Microcapsules were prepared as follows. Phenytoin sodium corresponding to 80% (w/w) was suspended in 10% (w/v) EC solution in ethyl acetate. The suspension was stirred for several hours with a magnetic stirrer (constant torque high magnetic stirrer, Mitamura Riken Kogyo, Inc., Tokyo, Japan). n-Pentane was added dropwise to the suspension at a rate of 0.5–0.8 ml/min until the phase separation was completed and microcapsules were obtained. The microcapsules were collected on filter paper, dried in vacuo for 24 h at room temperature, and stored in a desiccator in vacuo.

Observation of Microcapsules by Scanning Electron
Microscope The microcapsules and phenytoin sodium were observed by scanning electron microscope (SEM, Model X-650, Hitachi, Co., Japan) to examine their shape and surface characteristics.

Release Studies The dissolution profiles of phenytoin from the microcapsules in JP XII disintegration media No. 1 (pH 1.2) and No. 2 (pH 6.8) were obtained by a beaker method at 37 ± 0.1°C in a constant-temperature water bath (Lo-Themp Bath Model BL-22, Yamato Scientific Co., Ltd., Tokyo, Japan). The beaker had a 1 liter capacity and was 105 mm in diameter. One liter of the test fluid in the beaker was placed in the water bath. A stainless, three-blade propeller (40 mm in diameter and each blade about 2 cm² in area) was immersed into the beaker to a depth of 30 mm from the bottom, and was rotated at 50 rpm by a constant torque stirrer (TR-5S, Toyama Sango Co., Ltd., Osaka, Japan). The amount of the test samples was 500 mg as phenytoin sodium equivalent. After sample powder was suspended in the fluid, 2 ml of the fluid was withdrawn with a syringe at predetermined time intervals. Two ml of fresh fluid was added to the beaker to maintain the original volume. The sample solution was filtered quickly through a membrane filter (pore size 0.2 µm, HA Type, Nihon Millipore Kogyo Co., Ltd., Yonezawa, Japan) and analyzed for phenytoin concentration by GC method.⁶

Plasma Levels of Phenytoin in Rabbits Male albino rabbits, weighing 2.8—3.7 kg, were used in this study following stomach-emptying-time controlling treatment.¹⁵ Phenytoin sodium (32.6 mg/kg, 30.0 mg/kg as phenytoin equivalent) or microcapsules (phenytoin sodium content of 54.6%) using JP No. 2 capsules were orally administered to rabbits with about 50 g of special soft diet (Clea Japan Inc., Tokyo, Japan), and water was allowed ad libitum. Blood samples were taken from the ear vein at 0, 1, 2, 3, 4, 6, 8, 12 and 24 h following administration of the sample using a heparinized syringe. Blood samples were centrifuged (3500 rpm, 20 min) and the obtained plasma samples were frozen and stored in a freezer until assayed. Rabbits were prevented from coprophagy by wearing a muzzle during the night. Phenytoin concentration in plasma samples was assayed by GC method.⁶

Urinary Excretion of the Metabolites of Phenytoin in Human Subjects Phenytoin sodium (271.8 mg) or microcapsules (271.8 mg as phenytoin sodium) was orally administered to three healthy male volunteers (from 23 to 29 years, weighing 50—62 kg). The amounts corresponded to 250 mg of phenytoin equivalent. The subjects fasted overnight and ingested the sample powder with about 200 ml of water. No food or beverage was given to them for 4 h thereafter, except for 60 ml of water every hour to maintain a normal urine volume. Urine was collected hourly for the first 9 h and thereafter at convenient times for up to 12 h after administration. The total amounts of HPPH and its glucuronic acid conjugate, the main metabolite in human urine,¹⁶ were assayed after acid hydrolysis by GC method.⁶

RESULTS AND DISCUSSION

Contents and Characteristics of Microcapsules Phenytoin sodium contents of the microcapsules were entrapped in amounts from 32.6 to 54.6% (w/w). Microcapsules containing 54.6% phenytoin sodium were used in this study. Phenytoin sodium in the microcapsules was also stable in tight glass containers for up to 3 months. Thermal analysis by differential scanning calorimeter did not show the endothermic peak of melting at 295°C (the melting point of phenytoin).

Figure 1 shows the shape of phenytoin sodium (a) and

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Figure 2. Release Profiles of Phenytoin from Microcapsules and Phenytoin Sodium in JP XII Disintegration Media No. 1 (a) and No. 2 (b)

○ microcapsules; ● phenytoin sodium.
phenytoin sodium/EC microcapsules (b) observed by SEM. The surface of the drug powder was covered by EC matrix in the microcapsules.

Release Profiles of Phenytoin from Microcapsules
Figure 2 shows the release profiles of phenytoin from phenytoin sodium crystal powder and the microcapsules in JP XII disintegration media No. 1 (a) and No. 2 (b).

Dissolutions of the drug from phenytoin sodium in media No. 1 and No. 2 were apparently completed at 2 and 1 h, respectively. In contrast, sustained release was observed from the microcapsules in both media. The dissolution or release of phenytoin from the samples was faster in medium No. 2 than in medium No. 1. A part of the molecule of phenytoin (pKₐ = 8.3) is considered to be ionized in medium No. 2 (pH 6.8) and this resulted in the faster dissolution of phenytoin from phenytoin sodium.

The release patterns of phenytoin from the microcapsules were in similar order to those found in the dissolution patterns of phenytoin sodium. This indicated that the release of phenytoin from the microcapsules depends upon the dissolution of phenytoin sodium from the matrix of the microcapsules. The medium might penetrate through the EC wall in the microcapsules, and phenytoin sodium in the core might then dissolve into the medium. The release of phenytoin from the microcapsules was considered to be dependent on pH of the medium. However, the percent of phenytoin released from the microcapsules was restrained in media No. 1 and No. 2 by 73 and 90%, respectively, for up to 8 h.

Sustained Plasma Concentration of Phenytoin Following the Administration of Microcapsules and Phenytoin Sodium in Rabbits
Maeda et al. reported good correlations between the plasma level–time curves of some drugs in the stomach-emptying-controlled rabbits and in human subjects. We utilized these rabbits as the model animal for the absorption studies of phenytoin.

Figure 3 shows the time course of plasma concentration of phenytoin following the administration of microcapsules and phenytoin sodium to rabbits. Table I summarizes the area under the plasma concentration–time curve (AUC), the mean residence time (MRT), the variance of residence time (VRT), the maximum plasma concentration (Cmax) and the time (Tmax) required to achieve the Cmax. The Tmax following the administration of microcapsules (4.16 h) was significantly prolonged compared to administration of phenytoin sodium (1.34 h). The MRT was significantly increased with microcapsules compared to phenytoin sodium. Significant difference was not observed in AUC values. However, these results showed larger AUC following administration of the microcapsules. The following speculation might be possible. Following

<table>
<thead>
<tr>
<th></th>
<th>AUC (µg·h/ml)</th>
<th>MRT (h)</th>
<th>VRT (h²)</th>
<th>Tmax (h)</th>
<th>Cmax (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal powders</td>
<td>92.78 ± 21.13</td>
<td>7.91 ± 0.91</td>
<td>57.50 ± 14.28</td>
<td>1.34 ± 0.21</td>
<td>10.13 ± 1.32</td>
</tr>
<tr>
<td>Microcapsules</td>
<td>110.32 ± 1.10</td>
<td>16.21 ± 1.35</td>
<td>215.60 ± 45.86</td>
<td>4.23 ± 0.23</td>
<td>5.74 ± 0.32</td>
</tr>
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Each data represents the mean ± S.E. of three rabbits. a) Significantly different from crystal powders of phenytoin sodium at p < 0.05.

Fig. 3. Plasma Concentration of Phenytoin Following the Oral Administration of Microcapsules and Phenytoin Sodium to Rabbits
Symbols are the same as Fig. 2.

Table 1. Pharmacokinetic Parameters Following the Oral Administration of Crystal Powders and Microcapsules of Phenytoin Sodium to Rabbits

Fig. 4. Urinary Excretion Rates (a) and Cumulative Amounts (b) of 5-(p-Hydroxyphenyl)-5-phenylhydantoin Following the Oral Administration of Microcapsules and Phenytoin Sodium to Human Subjects
Symbols are the same as Fig. 2.
the oral administration of phenytoin sodium, the salt might easily be transformed into free phenytoin in acidic stomach fluid. As free phenytoin is practically insoluble in water, its absorption might be incomplete in the gastrointestinal tract. On the other hand, while passing through the stomach, the volume of water penetrating into the microcapsules might be minimal. Most of the phenytoin sodium in the microcapsules then might not be converted into free phenytoin. The membrane of the microcapsules might protect phenytoin sodium from the acidic media.

**Urinary Excretion of the Metabolites of Phenytoin Following the Oral Administration of Microcapsules and Phenytoin Sodium to Human Subjects**

Figure 4 shows the time-courses of urinary excretion rates (a) and cumulative urinary amounts (b) of total HPPH following the oral administration of microcapsules and phenytoin sodium. A dose of 250 mg of phenytoin corresponded to 265.9 mg HPPH. In humans, major metabolites of phenytoin were HPPH and its glucuronic acid conjugate. Sulfate or unmetabolized phenytoin were not detected in the urine. Smith and Kinkel reported that following the oral administration of a single dose of 250 mg of phenytoin in tablet form, the peak plasma level of phenytoin was observed over the following 4–12 h. The maximum rate of urinary excretion of the metabolites was observed over 24 to 48 h. Dill et al. reported that in a normal adult subject receiving a single 250 mg intravenous dose of phenytoin, the maximum plasma level of HPPH occurred about 8 h after dosing. They found that a direct relationship existed between the plasma levels of HPPH and the rate of urinary excretion of total HPPH. Glazko et al. also reported that the maximum excretion rate of total HPPH was found to occur about 6 to 8 h after an intravenous dose. They mentioned that the delay of maximum excretion of HPPH was presumably due to an initial delay in the enzymatic processes of hydroxylation or conjugation prior to excretion of the metabolite. Yamamoto et al. reported the relationship between the plasma concentration of phenytoin and urinary excretion rate of total HPPH. They also found a delay in maximum urinary excretion of total HPPH after the peak phenytoin plasma levels. Although the urinary excretion rate of the metabolite was not proportional to levels of phenytoin in plasma, the larger urinary excretion rate of total HPPH reflected higher phenytoin plasma levels when phenytoin powder and a ground mixture of phenytoin with microcrystalline cellulose were administered to two human subjects. Sekikawa et al. also showed that the urinary excretion rates and cumulative amount of total HPPH following the oral administration of phenytoin–polyvinylpyrrolidone coprecipitate were greater than those of phenytoin alone.

Sustained urinary excretion of total HPPH following the administration of the microcapsules was observed in comparison with that of phenytoin sodium. The $T_{\text{max}}$ of the urinary excretion rates of total HPPH following the administration of phenytoin sodium and the microcapsules were found at 7 and 36 h post-administration, respectively. The excretion rates of total HPPH following the administration of the microcapsules were significantly lower up until 18 h, but were significantly higher after 60 h than those of phenytoin sodium. The metabolites continued to be excreted even at 120 h following oral administration of the microcapsules. Inter-subject variation of urinary excretion rates of total HPPH was quite limited when the microcapsules were administered orally.

The recoveries of total HPPH following the administration of phenytoin sodium and the microcapsules for up to 120 h were 79.0 and 80.0%, respectively (Fig. 4b). From the result of the urinary excretion rates (Fig. 4a), as total HPPH continued to be excreted 120 h after the administration of microcapsules, the recoveries were slightly larger than those following the administration of phenytoin sodium, although significant difference in recoveries was not observed between them. The mean times of 50% recovery of the metabolite excreted in urine following the administration of phenytoin sodium and microcapsules were 36 and 56 h, respectively. Saturation of phenytoin metabolism was found among the doses for the phenytoin therapy. When microcapsules of phenytoin sodium are appropriate for use in therapy, consideration of the saturation of metabolism might maintain the proper plasma levels of phenytoin.

Sekikawa et al. reported that the recoveries of total HPPH for 120 h following the oral administration of phenytoin and phenytoin–polyvinylpyrrolidone coprecipitates were 63.7 and 85.5%, respectively. The recoveries were smaller following phenytoin administration than those following phenytoin sodium administration, and inter-subject variation was much larger. When phenytoin sodium was administered orally, the absorption might be increased because of its rapid dissolution. Sodium salts of acidic drugs show higher dissolutions than free drugs, however, they are very unstable in the stomach to precipitate the free drugs which are poorly water-soluble. The salts of drugs which are water-soluble are surely dissolved. When the soluble salts of a drug are administered orally, there is rapid increase in plasma level of the drug. Rapid decrease in drug plasma level may mean that several administrations of the drug are required daily. The microencapsulation techniques of such salts may be applicable to obtain the sustained plasma level of a drug.

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**REFERENCES AND NOTES**

1) Present address: Department of Biochemistry, School of Medicine, Kitasato University, 1-chome 15-1, Kitasato, Sagamihara, Kanagawa 228, Japan.


