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Dissolution Properties and Bioavailability of Phenytoin from Ground Mixtures with Chitin or Chitosan[1)]

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With a view to application of chitin and chitosan to pharmaceutical preparations, the dissolution and bioavailability of ground mixtures of phenytoin with chitin or chitosan were investigated. Ground mixtures of phenytoin with chitin, chitosan and crystalline cellulose in 1:2 weight ratio were prepared by co-grinding in a ball mill. The X-ray diffraction patterns suggested that the size of crystals of phenytoin was decreased in the ground mixtures. The dissolution rate of phenytoin from the ground mixtures was significantly greater than that from physical mixtures or intact phenytoin powder. The ground mixture with chitosan showed fastest dissolution, followed by that with chitin and then that with crystalline cellulose. The dissolution of phenytoin from tablets of the ground mixture with chitin or chitosan was greater than that from tablets of the physical mixture, while that from tablets of the ground mixture with crystalline cellulose was significantly smaller than that from tablets of the physical mixture. It was confirmed that the ground mixture of phenytoin–chitosan gave enhanced bioavailability of phenytoin in beagle dogs.

Keywords——chitin; chitosan; ground mixture; phenytoin; dissolution rate; bioavailability; gastrointestinal absorption; blood level; anticonvulsant

Recently, chitin and chitosan have been reported to be useful for pharmaceutical preparations.[3,4)] The bone of cuttlefish (containing chitin)[3a)] was described to be used as an antacid and hemostatic.[5)] These effects of the bone of cuttlefish are considered to be related to the facilitating effect of chitin on wound healing,[6)] and to the acid-lowering effect of chitosan in the stomach.[7)]

When a poorly soluble drug is administered orally, the bioavailability, *i.e.*, the amount and the rate of the absorption, depends mainly on the dissolution rate in the gastrointestinal fluids. If the bioavailability of such a drug is enhanced, the dose required, and consequently the side effects, may be reduced. Therefore, great efforts have been made to increase the dissolution rate of such drugs.[4e,f,8)] For example, the dissolution and bioavailability of phenytoin (DPH), an anticonvulsant, were enhanced by using the ground mixture with crystalline cellose (MCC),[9)] by coprecipitation with polyvinylpyrrolidone,[10)] by solubilization with methylcellulose,[11)] by using the ground mixture with gelatin,[12)] and by a solvent deposition method,[13)] because it is difficult in DPH therapy to maintain an effective therapeutic blood level (10—20 μm/ml).[14)]

In this study, with a view to further application of chitin and chitosan to pharmaceutical preparations following studies on enhancement of the dissolution properties of griseofulvin[4e)] and prednisolone[4f)] by co-grinding with chitin or chitosan, the dissolution properties and bioavailability of ground mixtures of DPH with chitin or chitosan were investigated, for Nakai stated that these phenomena should be confirmed with many drugs.[15)]

Experimental

Materials——Chitin and chitosan, whose degree of deacetylation was calculated to be 92.7% from the amino group content, for fine chemical use were purchased from Kyowa Oil and Fat Co., Ltd. and were used after passage through a 200-mesh sieve. MCC of JP X grade, marketed as "Avicel PH 101," was used after passage through a 200-mesh sieve. DPH of JP X grade was supplied by Fujinaga Pharmaceutical Company,

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Ltd., Tokyo (Pulv. Hydantol,® lot XJ221).

Preparation of Ground Mixtures——Eighteen-gram samples of ground mixtures of DPH with chitin, chitosan and MCC in 1: 2 weight ratio were prepared by grinding in a ceramic ball mill for 24 h.

Preparation of Physical Mixtures——Physical mixtures of DPH with chitin, chitosan and MCC in 1: 2 weight ratio were prepared by simple blending in a ceramic mortar.

Powder X-Ray Diffraction Study——Powder X-ray diffractometry was carried out using a Rigaku Denki Geigerflex Model D-2 diffractometer with Ni-filtered Cu-$K\alpha$ radiation.

Dissolution Rate Study——Dissolution rates of DPH from the different powdered preparations into 300 ml of JP X disintegration medium No. 1 (pH 1.2) were measured at 37°C in a constant-temperature water bath. Each preparation containing 60 mg of DPH was transferred directly into the dissolution medium, which was stirred with a four-bladed stainless steel paddle at 300 rpm. Three ml of sample solution was withdrawn at appropriate intervals through a membrane filter (pore diameter, 0.45 μm) and immediately replaced with an equal volume of the test medium. As regards ground mixtures, each sample was immediately diluted with the test medium. Each sample was analyzed for DPH by the high performance liquid chromatography (HPLC) method.[16] Experiments were done in triplicate and the mean values were obtained.

Dissolution Rate from directly Compressed Tablets——Flat-faced tablets of 300 mg weight, 13 mm diameter and about 1.7 mm thickness were made by compressing a given amount of powder of DPH–excipient (1: 2) mixture directly under 200 kg/cm^2 for 30 s in a Shimadzu hydraulic press for KBr tablets for infrared spectroscopy. The dissolution rate of DPH from the different tablets was tested in a JP X dissolution test apparatus, Method I (rotating basket method) in 1 liter of JP X disintegration medium No. 1 (pH 1.2) at an agitation speed of 100 rpm at 37°C. A tablet containing 100 mg of DPH was used. Five ml of sample solution was withdrawn at appropriate intervals through a membrane filter (pore diameter, 0.45 μm) and immediately replaced with an equal volume of the test medium. The sample was analyzed for DPH by the HPLC method.[16] Experiments were done in triplicate and the mean values were obtained.

Measurement of Disintegration Time of Tablets——A Toyama Sangyo T-2HS type disintegration tester was employed according to the method in JP X using JP X disintegration medium No. 1 (pH 1.2) as the test fluid. Experiments were done in triplicate and the mean values were obtained.

***In Vivo* Absorption Study**——DPH powder ground alone in a ball mill for 24 h and DPH–chitosan (1: 2) ground mixture powder, each equivalent to 20 mg of DPH per kg, were administered orally to three male beagle dogs (body weight 10—12 kg, fasted for 12 h) by the two-way cross-over method with a two-week interval. Each powdered preparation was wrapped in two pieces of oblate. Three ml of heparinized blood sample was collected from the cephalic vein at 0, 1, 2, 3, 4, 6 and 8 h after the administration, and centrifuged to obtain the plasma, which was stored in a refrigerator at −10°C. Each sample was analyzed for unchanged DPH by the HPLC method.[16]

Results and Discussion

Powder X-Ray Diffraction Study

Powder X-ray diffraction patterns of DPH are shown in Fig. 1. The diffraction intensity of DPH in a ground mixture (GM), clearly observed at 11° diffraction angle, was smaller than that of the physical mixture (PM), suggesting that the size of crystals of phenytoin was decreased in the ground mixtures.

Dissolution Rate Study

Dissolution of DPH from the DPH–excipient (1: 2) mixtures is shown in Fig. 2 in comparison with that from DPH powder. The dissolution of DPH from mixtures was significantly greater than that from DPH alone, and the ground mixture with chitosan gave the fastest dissolution, followed by that with chitin and then that with MCC.

The solubility of DPH at 37°C is 30.1 μg/ml at pH 5.4.[17] Since the pK_a' of DPH is 8.06,[17] the solubility at pH 5.4 might not be significantly different from that at pH 1.2. Therefore, 60 mg of the drug in 300 ml of the dissolution medium corresponded to about 6.6 times its solubility. Thus, the initial stage in the dissolution profiles of ground mixtures shown in Fig. 2 is considered to correspond to a state of supersaturation. The concentration of DPH following dissolution from DPH–chitosan GM was almost twice the solubility of DPH. After reaching the peak, the concentration decreased gradually, indicating the occurrence of recrystallization. The supersaturation continued for a long period with the ground mixtures of DPH–chitin and DPH–chitosan, while the ground mixture

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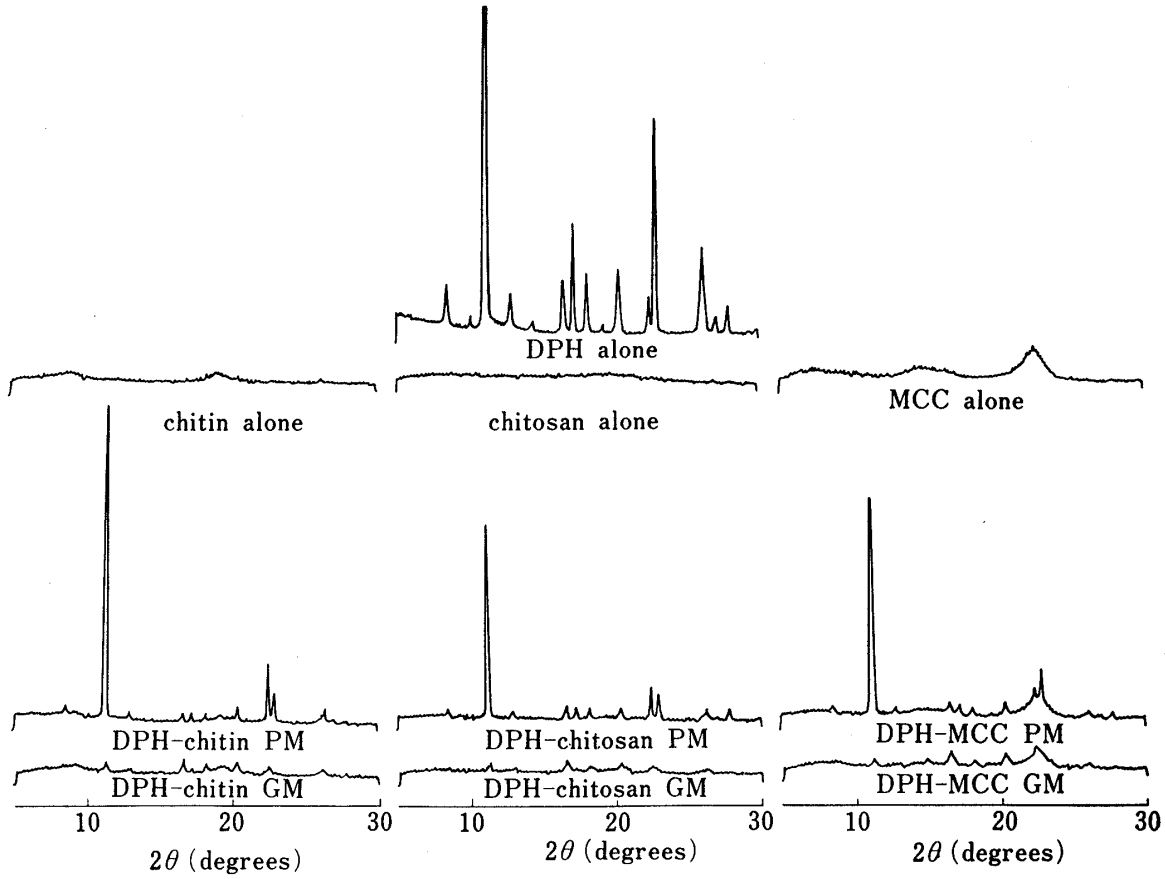

Fig. 1. Powder X-Ray Diffraction Patterns

(GM, ground mixture; PM, physical mixture).

of DPH–MCC reached the saturated concentration at 1 h, suggesting that chitin and chitosan were superior to MCC as regards enhancement of dissolution.

The difference of dissolution of DPH from ground mixtures and physical mixtures was attributed to the relative decrease in the size of crystals of DPH in the ground mixtures. The difference of dissolution of DPH from physical mixtures and DPH alone was considered to be simply attributable to the difference in wettability of DPH. This view was supported by the observation that DPH alone floats on the surface of the dissolution medium longer than the physical mixtures. However, this difference due to the difference in wettability of DPH might not contribute to the improvement of blood level of DPH, as was the case with DPH–gelatin physical mixture and DPH alone.[12]

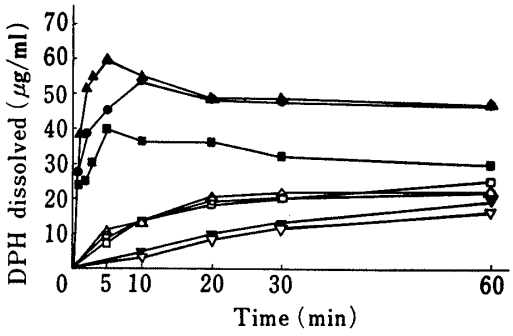

Fig. 2. Dissolution of DPH from Mixtures with Chitin, Chitosan and MCC in 300 ml of JP X Disintegration Medium No. 1 (pH 1.2) at 37°C

DPH–chitin GM (●), DPH–chitosan GM (▲), DPH–MCC GM (■), DPH–chitin PM (○) DPH–chitosan PM (△), DPH–MCC PM (□), DPH ground alone (▼) intact DPH (▽).

Dissolution Rate from Directly Compressed Tablets

The dissolution of DPH from the tablet of DPH–excipient (1 : 2) mixture is shown in

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Fig. 3. The dissolution rate of DPH from tablets of the ground mixture with chitin or chitosan was greater than that from tablets of the physical mixture. On the other hand, the tablets of the ground mixture with MCC retained the original form in the basket for more than 2 h, and the dissolution rate of DPH was significantly smaller than that from the tablets of the physical mixture.

This deterioration in the disintegration properties of the tablet made from DPH–MCC GM powder was attributable to the disappearance of capillaries of MCC in the grinding process, because the capillary of MCC was reported to play an important role in the disintegration process.[18] This was clearly observed for MCC in the disintegration test, as shown in Table I. DPH–chitosan tablets made from both PM and GM swelled, forming a gel on the mesh of the tester, and did not disintegrate within 30 min.

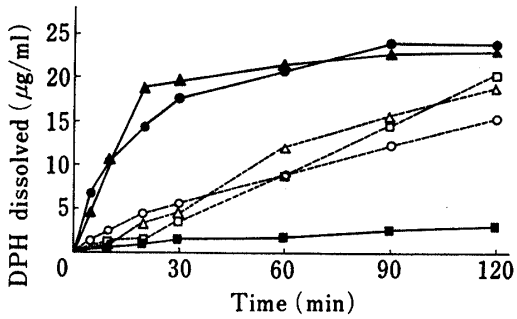

Fig. 3. Dissolution of DPH from Mixture Tablets in 1 liter of JP X Disintegration Medium No. 1 (pH 1.2) at 37°C

DPH–chitin GM (●), DPH–chitosan GM (▲), DPH–MCC GM (■), DPH–chitin PM (○), DPH–chitosan PM (△), DPH–MCC PM (□).

TABLE I. Disintegration Times of DPH Tablets containing Chitin, Chitosan and MCC

	DPH-Chitin	DPH-Chitosan	DPH-MCC
PM[a)]	1.6 min	>30 min	6.0 min
GM[b)]	10.7 min	>30 min	>30 min

a) Physical mixture.
b) Ground mixture.

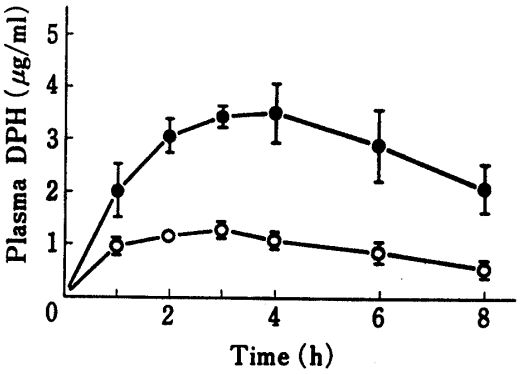

Fig. 4. Plasma DPH Levels after Oral Administation of DPH and Ground Mixture of DPH–Chitosan (1 : 2) in Beagle Dogs

DPH-chitosan GM (●), DPH ground alone (○), Each symbol represents the mean ± S.E. of three determinations.

In Vivo Absorption Study

DPH–chitosan GM, which gave the fastest dissolution, and DPH ground alone in a ball mill for 24 h were administred orally to three male beagle dogs. The plasma levels of DPH are shown in Fig. 4. Significantly high plasma levels of DPH were obtained for DPH–chitosan GM at 2, 3, 4, 6, and 8 h after the oral administration ($p<0.05$). The area under the curve (AUC) values in Fig. 4 with standard error were 21.88±2.37 and 7.40±0.93 (μg/ml)·h for DPH–chitosan (1 : 2) GM and DPH alone, respectively. A significantly higher (about 3 times greater) AUC (0—8 h) was obtained for DPH–chitosan GM than for DPH alone ($p<0.01$). The plasma levels of DPH in Fig. 4 were well correlated with the *in vitro* dissolution patterns. This result indicated that co-grinding with chitosan improves the bioavailability of DPH, suggesting that a similar enhancement of bioavailability can be expected for other poorly soluble drugs by co-grinding with chitin or chitosan.

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