Chem. Pharm. Bull. 28(2) 431—436 (1980)

Use of Rabbits for Absorption Studies on Polymorphs of Chloramphenicol Palmitate

Tadao Maeda,[1)] Hiroshi Takenaka, Yoshiya Yamahira, and Takeshi Noguchi

Formulation Research Department, Pharmaceuticals Division, Sumitomo Chemical Co., Ltd.[1)]

(Received June 8, 1979)

The α and β forms of chloramphenicol palmitate (CPP) crystals were successfully obtained in similar particle sizes by utilizing wet micronization.

Comparisons of the absorbability of CPP suspensions of the above crystals using conventionally fasted rabbits were unsuccessful because of the low plasma level after dosing the β form (less than 1 μg/ml).

Plasma levels were then compared by dosing CPP suspensions of the α and β forms to stomach-emptying-controlled (SE-controlled) rabbits in a crossover manner. Comparative absorption studies were also performed in the SE-controlled rabbits with a capsule form of CP and suspensions of CPP in the α and β forms. It was demonstrated that absorption occurred in the order CP>CPP (α form)>CPP (β form). The correlation between *in vitro* extent of hydrolysis and *in vivo* absorbability in terms of AUC is discussed.

These results suggest that the SE-controlled rabbit is a very useful animal model for preliminary bioavailability studies on oral dosage forms for human clinical use.

Keywords——chloramphenicol palmitate; polymorph; oral dosage form; animal model; bioavailability; stomach-emptying-controlled rabbit

Polymorphic forms of drugs have been investigated from various points of view:[2,3)] however, from the biopharmaceutical point of view, the effect of polymorphism on the absorption characteristics of drugs is of great interest. In the case of chloramphenicol palmitate (CPP), amorphous[4)] and three different crystal forms[5)] have been reported. Physicopharmaceutical studies on these polymorphs demonstrated significant differences in the heat of fusion and heat of solution between the α form (form B) and β form (form A).[6)] It is generally known that the polymorphic state of CPP greatly affects the gastrointestinal (GI) absorption of this drug.[7)]

Aguiar *et al.* also studied the *in vitro* enzymatic hydrolysis of CPP and stated that the enzymatic hydrolysis test could be used to predict blood levels obtainable from CPP suspensions if the particles were not highly aggregated.[7)] In the case of oral drug administration, however, many factors such as particle size, polymorphism, aggregation of particles and physiological variables may affect the absorbability of the drug. Therefore, it is almost impossible to predict GI absorbability of a drug preparation solely from the results of an *in vitro* test such as a dissolution or hydrolysis test. Thus an appropriate animal model with absorption characteristics similar to those of man is required for predicting the overall absorbability of a drug preparation.

In our previous paper[8)] it was shown that the stomach emptying-controlled (SE-controlled) rabbit is a useful animal model for GI drug absorption studies. Further studies have been

1) Location: *Kurakakiuchi 1-3-45, Ibaraki City, Osaka 567, Japan*; *a*) To whom inquiries should be directed.

2) J. Haleblian and W. McCrone, *J. Pharm. Sci.*, **58**, 911 (1969).
3) J. Haleblian, *J. Pharm. Sci.*, **64**, 1269 (1975).
4) T. Kimura and S. Hashimoto, Japanese Patent 60-5798 (1960).
5) A. Aguiar and J. Zelmer, *J. Pharm. Sci.*, **58**, 983 (1969).
6) H. Negoro, S. Ueda, T. Suyama, and T. Hoshi, *Takamine Kenkyusho Nempo*, **12**, 141 (1960).
7) A. Aguiar, J. Krc, Jr., A. Kinkel, and J. Samyn, *J. Pharm. Sci.*, **56**, 847 (1967).
8) T. Maeda, H. Takenaka, Y. Yamahira, and T. Noguchi, *J. Pharm. Sci.*, **66**, 69 (1977).

conducted to establish the usefulness of the rabbit in various bioavailability studies.[9] In our preceding paper,[10] the procedure for stomach-emptying control of rabbits was improved and the normality of the physiological state of SE-controlled rabbits was demonstrated. The purpose of this paper was to investigate SE-controlled rabbits further by using polymorphs of CPP, whose absorption characteristics have previously been demonstrated in man.[7]

Experimental

Materials and Equipment——Chloramphenicol palmitate (JP IX) was recrystallized from methanol-water and toluene-hexane following the method previously reported from this laboratory,[11] After purification, no impurity could be detected by thin-layer chromatography. Chloramphenicol (CP) was of JP IX grade. All other chemicals were of reagent grade. A Hitachi EPI-G type infrared spectrometer and a Shimadzu UV-200 spectrophotometer were used.

Preparation of CPP Polymorphs——The α and β forms of CPP were prepared using the method of Borka *et al.*[12] The polymorphs were characterized in terms of melting points and IR spectra. The polymorphs obtained were micronized in the wet state by agitation with glass beads to obtain uniform particles in the range of 1—5 μ diameter (microscopic measurement).

Preparation of the CPP Suspension——The suspension was prepared by dispersing 5.79 g of CPP in 100 ml of aqueous solution containing 1% (w/v) sodium carboxymethyl cellulose and 0.5% (w/v) polysorbate 80 by means of an ultrasonicator. The particle diameter of the suspended crystals was in the range of 1—5 μ.

Enzymatic Hydrolysis Test——The method reported by Yamamoto *et al.*[13] was applied with slight modifications. The snzyme solution was prepared by mixing 5 g of lipase (steapsin) powder with 100 ml of simulated intestinal fluid (the second fluid of the JP IX Disintegration Test) containing 0.005% (w/v) polysorbate 80. The pH of the solution was 7.5. The fluid was shaken mechanically for 15 min and then centrifuged. The supernatant was used as the source of the enzyme. CPP suspension equivalent to 20 mg of CPP was added to enzyme solution kept at 37° and the concentration of CP was determined periodically.

Animal Experiment——The stomach-emptying time of rabbits employed was controlled before the absorption study following the new procedure (cangue method) presented in our previous paper.[10] Two groups, each consisting of 3 male rabbits, were orally administered CPP suspensions of α or β form equivalent to 100 mg of CP in a strict crossover manner. The administration was performed through a stomach tube, and the tube was washed with 2 ml of water. A 2-week washout period was allowed between the administration of two preparations. For comparison, 5 additional rabbits were administered 100 mg of CP each, in a hard gelatin capsule.

In the study of conventionally fasted rabbits, 10 rabbits were used after fasting for 20 hr with free access to water. Blood specimens were taken by cardiac puncture using a heparinized syringe at predetermined times up to 8 hr. Plasma levels of CP were assayed as total nitro compounds following the method reported by Yamazaki *et al.*[14]

Results and Discussion

Aguiar *et al.*[7] studied drug absorption from suspensions containing CPP (α form) of two different particle sizes. Since little difference was observed, they concluded that the effect of particle size was less significant than that of polymorphic form. However, this conclusion was based on experiments using the α form of CPP and no comparative study was done with the α and β forms of CPP prepared to the same particle size. Recrystallized β form of CPP is usually larger in size than the α form. In our present study, wet micronization was introduced after preparation of the polymorphic forms. By this procedure the particle size difference was eliminated and the difference in bioavailability between the α and β forms could be investigated in isolation.

It is known that polymorphic transition proceeds from the α form to the β form irreversibly in the solid state.[15] In order to examine the effect of sonication during preparation of

9) T. Maeda, H. Takenaka, Y. Yamahira, and T. Noguchi, *J. Pharm. Sci.*, **68**, 1286 (1979).
10) T. Maeda, H. Takenaka, Y. Yamahira, and T. Noguchi, *Chem. Pharm. Bull.*, **27**, 3066 (1979).
11) M. Miyamoto, T. Kiyotaki, N. Kisoh, T. Mitsunaga, and T. Maeda, *Chem. Pharm. Bull.*, **21**, 1857 (1973).
12) L. Borka and K. Backe-Hansen, *Acta Pharm. Suecica*, **5**, 271 (1968).
13) K. Yamamoto, S. Matsuda, M. Nakano, T. Arita and Y. Nakai, *Yakugaku Zasshi*, **97**, 367 (1977).
14) M. Yamazaki, S. Shigeo, and A. Kamada, *Yakuzaigaku*, **34**, 97 (1974).
15) C. Tamura and H. Kuwano, *Yakugaku Zasshi*, **81**, 755 (1961).

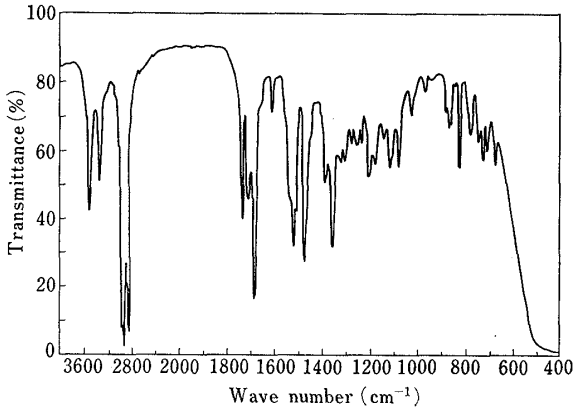

Fig. 1. Infrared Spectrum of α Form Crystals in Suspension Immediately after Preparation

The suspension was prepared by ultrasonication for 5 min, then filtered through a glass filter. The IR spectrum of the residue was examined by the KBr disc method.

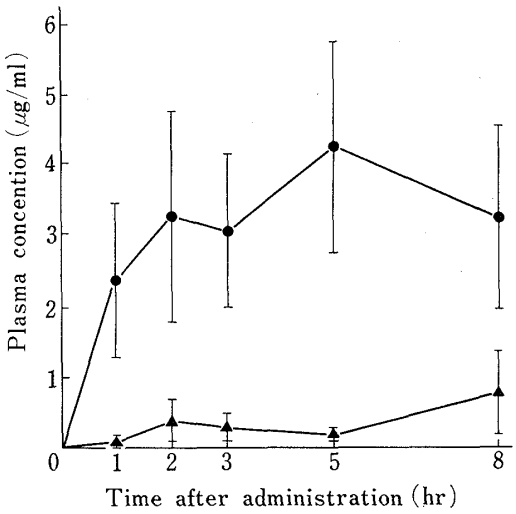

Fig. 2. Comparison of Plasma Levels in Conventionally Fasted Rabbits following Oral Administration of CPP Suspensions containing the α or β Form Polymorph Equivalent to 100 mg of CP

●, α form; ▲, β form.
Each point represents the mean ± S.E. of 5 rabbits.

the suspension on the polymorphic transition, the IR spectrum of α form CPP was measured just after sonication (Fig. 1). Examination of this spectrum showed that 99.8% of the crystals remained in the α form, so that polymorphic transition during preparation of the suspension could be neglected.

To compare the absorbability of CPP polymorphs, suspensions of the above preparations were administered orally to conventionally fasted rabbits; the results are shown in Fig. 2. The average plasma level of the β form was consistently less than 1 μg/ml plasma up to 8 hr after the administration. On the other hand, the peak plasma level of the α form was 4.3 μg/ml, and the average plasma level as well as AUC of the α form were significantly greater than those of the β form ($p<0.05$). However, the peak time and plasma pattern of the α form were quite different from those in humans,[7] suggesting slower absorption in conventionally fasted rabbits.

Next, the absorbability of CPP polymorphs was compared by the strict crossover method with SE-controlled rabbits using the cangue method[10] (Fig. 3 and Table I). As shown in table I, the results differed considerably from those in Fig. 2. The higher plasma level and faster peak time of SE-controlled rabbits compared to conventionally fasted rabbits are probably attributable to the faster gastric emptying of SE-controlled rabbits.[8,9]

In the case of SE-controlled rabbits, the average plasma level of the α form was consistently higher than that of the β form up to 8 hr. The average plasma levels at 1, 2, and 3 hr as well as the AUC of the α form were significantly greater than those of the β form ($p<0.05$). While the peak times of both the α and β forms were 1 hr, the plasma peak level of the former was almost twice that of the latter. Data for individual rabbits shown in Fig. 3 suggest that the α form has a higher peak level and AUC than the β form. These results clearly demonstrate that the suspension of the α form was superior to that of the β form as regards drug absorption.

Considering that the mean peak times are the same, and the subsequent plasma levels are in parallel for the two crystal forms, it appears that their stomach-emptying rates and/or intestinal transit times are similar. It is therefore suggested that the difference in absorption characteristics of the α and β forms probably arises form differences in the amounts absorbed from the small intestine.

Further studies were performed to compare the plasma levels of CPP and CP after oral administration to 5 additional rabbits. As shown in Fig. 4, the plasma level of CP was higher than that of CPP. The AUC ratio of CP: CPP (α form): CPP (β form) was 1.00: 0.50: 0.25. The ratio of plasma peak levels for CP: CPP (α form): CPP (β form) was 1.00: 0.40: 0.20, which is consistent with the AUC ratio. These data indicate good absorbability of CP compared with CPP.

Although the mechanism of intestinal absorption of CPP has not yet been clarified completely, it has been considered that CPP is absorbed as CP after hydrolysis of the ester bond

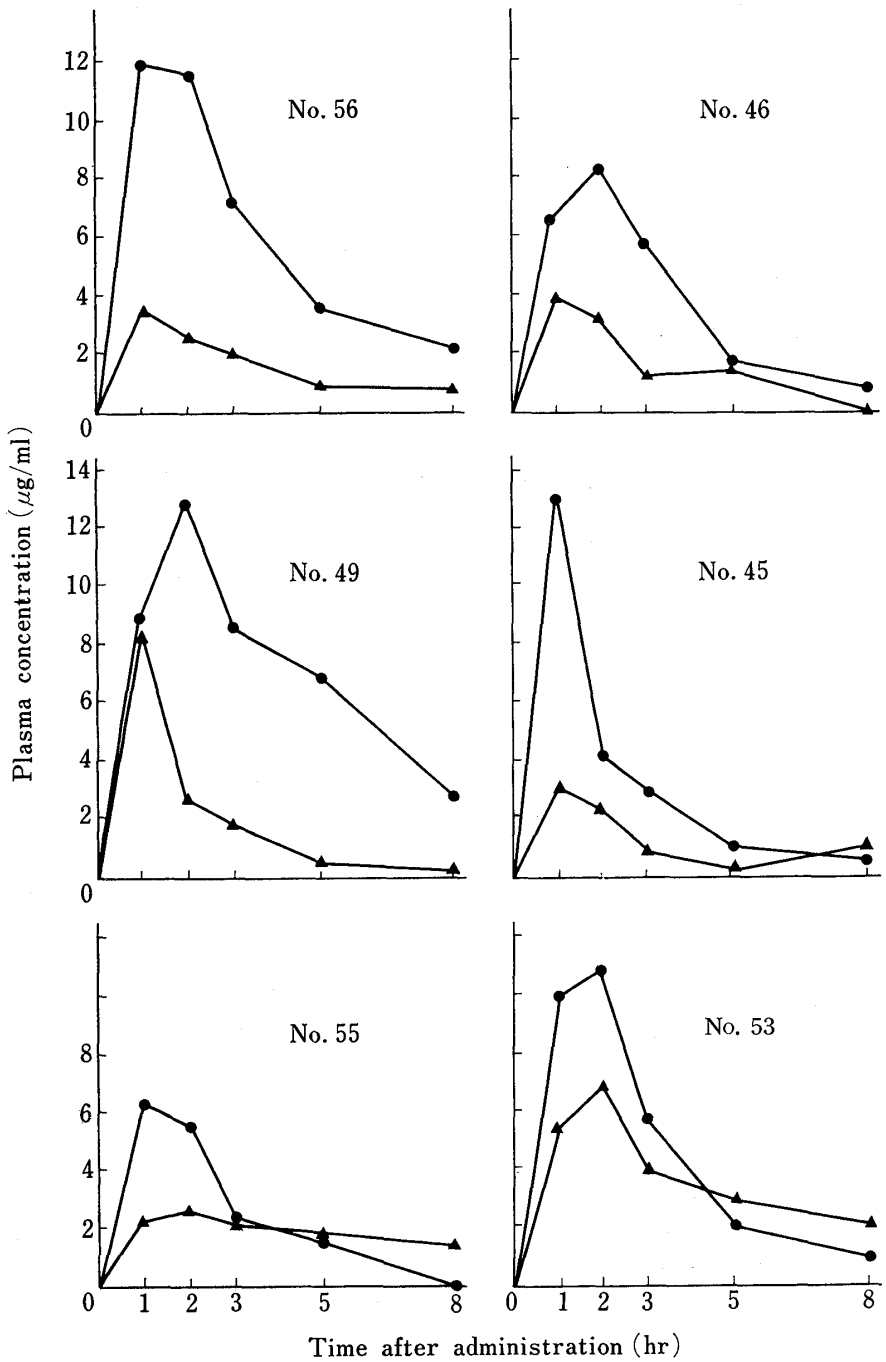

Fig. 3. Comparison of Plasma Levels in Individual SE-Controlled Rabbits following Oral Administration of CPP Suspensions Containing the α or β form Polymorph Equivalent to 100 mg of CP

●, α form; ▲, β form.

Table I. Plasma Levels and AUC of CP following Oral Administration of Suspensions of the α or β Form Polymorph to SE-Controlled Rabbits

Polymorph	Plasma level (μg/ml) at indicated sampling time (hr)					AUC (hr·μg/ml)
	1	2	3	5	8	
α	9.45±0.99[a)]	8.88±1.28[a)]	5.38±0.90[a)]	2.79±0.82	1.20±0.39	35.16±5.18[a)]
β	4.41±0.83	3.45±0.66	2.01±0.41	1.30±0.41	1.03±0.29	15.64±2.64

a) Significantly different from the β form ($p < 0.05$).
Each value is the mean±S.E. of 6 rabbits.

in the GI tract. Borka *et al.*[16] showed in their IR spectroscopic studies that the intramolecular H bonding of the OH group was stronger in the β form than in the α form, and they assumed that the degree of freedom of this group was a rate-determining factor in the solvation and/or hydrolysis process of CPP. Andersgaad *et al.*[17] investigated the correlation between absorption and hydrolysis rate and stated that hydrolysis was the rate-determining step.

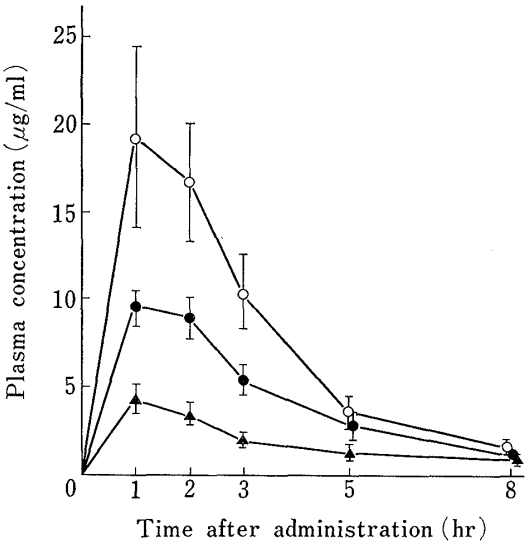

Fig. 4. Mean Plasma Levels of CP in SE-Controlled Rabbits receiving Equivalent Doses of CP or CPP Polymorphs

○, CP capsule; ●, CPP suspension (α form); ▲, CPP suspension (β form).
Each dose was equivalent to 100 mg of CP. Each point represents the mean±S.E. of at least 5 rabbits.

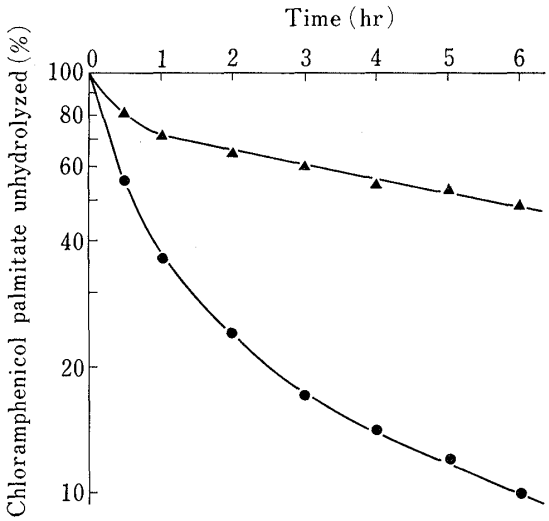

Fig. 5. Enzymatic Hydrolysis of CPP Suspensions at 37°

●, α form; ▲, β form.
Each point represents the mean of duplicate experiments.

In order to estimate the fraction of CP, which was considered to be available in the GI tract, apparent hydrolysis rates of CPP polymorph suspensions were determined *in vitro* (Fig. 5). The ratio of *in vitro* extent of hydrolysis of CPP suspensions during 6 hr was 9: 5 (α form: β form), which was in good accord with the *in vivo* absorbability in terms of AUC, shown in Table I. These results support the view of Menachenoff[18] that crystals of CPP hydrolyzable within 45 min were absorbed to give blood levels comparable to those obtained with CP *per se* at an equivalent dosage.

16) L. Borka and K. Bache-Hansen, *Acta Pharm. Suecica*, **5**, 525 (1968).
17) H. Andersgaad, P. Finfolt, R. Gjermundsen, and T. Hφyland, *Acta Pharm. Suecica*, **11**, 239 (1974).
18) E. Menachenoff, *Harokeach Haivri*, **10**, 300 (1964) [*Chem. Abstr.*, **63**, 6180 (1965)].

Consequently, it is clear that the SE-controlled rabbit (using the cangue method) is a useful animal model for studies of the absorbability of polymorphs of CPP as well as of the comparative absorption characteristics of CPP and CP. This again confirms that the SE-controlled rabbit is a suitable animal for preliminary bioavailability studies on oral dosage forms for human clinical use.

Acknowledgement The authors are grateful to Messrs. Takeshi Yoshida and Masahiko Yoshii for technical assistance.