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Effect of Polymorphism on the Dissolution Behavior and Gastrointestinal Absorption of Chlortetracycline Hydrochloride[1]

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The existence of the two crystalline forms of chlortetracycline hydrochloride (CTC-HCl) was confirmed by infrared spectroscopy and X-ray diffraction. From the dissolution studies with crystalline powder and compressed disk, an appreciable difference in the dissolution behavior in water was detected between the two forms. In order to determine the effect of polymorphism on the gastrointestinal absorption of CTC-HCl, blood plasma levels obtained in rabbits after intraduodenal administration and cumulative amounts excreted in human subjects after oral administration of the two forms were compared. The results indicated that polymorphic state of CTC-HCl significantly influences bioavailability of the CTC-HCl.

It is well known that the drug absorption is affected by polymorphism, and its effect on drug availability has been discussed recently.[3,4]

Karmarkar and Thirumalachar[5] reported that chlortetracycline hydrochloride (CTC-HCl) has shown polymorphic forms such as needles and prismatic plates. The characterization by physical analysis, however, has not been made and the physical-chemical properties of polymorphic forms have not been studied in detail. Furthermore, the effect of polymorphic forms on absorption following their administration has never been reported.

In the present study, the two crystal forms of CTC-HCl were prepared and the characterization of the two forms was established by infrared (IR) spectroscopy and X-ray diffraction. Furthermore, it is the purpose of this study to evaluate their physical-chemical properties and the effect of polymorphism on the bioavailability of CTC-HCl.

Experimental

Material——Anhydrous CTC-HCl was used, supplied by Lederle (JAPAN), LTD. All other materials were of J.P. VIII or reagent grade.

Preparation of the Two Crystal Forms——The α form was prepared by recrystallization of CTC-HCl from distilled water following the method described by Stephens, *et al.*[6] The β form was obtained by dissolving 1 g of CTC-HCl in 50 ml of hot anhydrous methanol, filtering the undissolved drug while hot, evaporating the filtrate to approximately a quarter volume at 40°, and allowing to stand overnight at 5°. The resulting crystals were filtrated and dried *in vacuo* at room temperature. For dissolution and absorption studies, the crystal size of the two forms was prepared to 170 mesh (88μ)—270 mesh (53 μ) by sieving.

Measurement of IR Spectra and of X-Ray Diffraction Patterns——IR spectra were recorded as mulls in liquid paraffin (Nujol) using a Nihon Bunko infrared spectrophotometer. X-ray powder diffraction patterns were obtained using a Rigaku Denki D-9C X-ray Diffractometer with nickel-filtered Cu-Kα radiation.

1) A part of this work was presented at the 91st Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, April 1971.
2) Location: *a) Kita-12, Nishi-6, Sapporo*: *b) 1-2-3, Kasumi, Hiroshima*; *c) 2-2-1, Oshika, Shizuoka.*
3) A.J. Aguiar, J. Kric, Jr., A.W. Krinkel, and J.C. Samyn, *J. Pharm. Sci.*, **56**, 847 (1968).
4) J.W. Poole, G. Owen, J. Silvwrio, J.N. Freyhof, and S.B. Rosenman, *Current Theraps. Res.*, **10**, 292 (1968).
5) S.S. Karmarkar and M.J. Thirumalachar, *Hindustan Antibiot. Bull.*, **6**, 9 (1963).
6) C.R. Stephens, L.H. Conover, R. Pasternack, F.A. Hochtein, W.Y. Moreland, P.P. Regna, F.J. Brunings, and R.B. Woodward, *J. Am. Chem. Soc.*, **76**, 3568 (1954).

Procedure for Dissolution Studies——a) Dissolution Behavior of Crystalline Powder: One milliliter portion of distilled water previously kept at the experimental temperature (15, 37, 55, and 70°) was added rapidly into individual 10 ml Erlenmeyer flasks, which were immersed in a constant temperature bath and contained an excess amount of samples, and they were immediately started to be mechanically shaken horizontally at a rate of 60±2 stokes/min. Sample solutions withdrawn at definite intervals were filtered through a 0.45 μ Millipore filter and the concentration was determined following the method described by Levine, *et al.*,[7] using a Hitachi Type EPU-2A spectrophotometer.

b) Dissolution Behavior of Compressed Disk: The Disk from crystalline powder was prepared by compressing 200 mg of sample under a pressure of 120 kg/cm² in 1.3 cm diameter die. When the sample was compressed to prepare the disk, the phase revertion was not observed by IR spectra. The disk was sticked to the one end of 1.2 cm diameter glass tube with adhesives.[8] The tube was introduced into the beaker containing 50 ml of distilled water maintained at 37°, being stirred by magnetic stirring bar at a high speed. Sample solutions were pipetted out periodically by a cotton-filter attached pipette. The concentration was determined following the Kohn's method[9] using a Hitachi Type 203 spectrofluorimeter. Fluorescein sodium at concentration of 0.04 μg/ml was used as a standard.

Procedure for Absorption Studies——a) Plasma Levels in Rabbits following Intraduodenal Administration: Male rabbits, weighing 2.8 to 3.2 kg, were fasted for 24 hr before the experiment. The animals were anesthetized by the intravenous injection of pentobarbital sodium (25 mg/kg body wt.). The upper part of the small intestine was exposed by a midline abdominal incision and 200 mg of sample dispersed in 10 ml of 0.9% NaCl was injected by means of a syringe near an opening of biliary duct in the duodenum lumen, followed by another 5 ml of 0.9% NaCl used to wash the container. The time between the addition of samples to 0.9% NaCl and the injection was 2 min. Blood samples were collected at regular intervals as shown in Fig. 6 during the experiment through polyethylene catheter inserted to the femoral artery and the plasma was separated immediately by centrifugation (2000 rpm).

b) Urinary Excretion in Human Subjects following Oral Administration: After an overnight fast, human subjects were given orally the β form in oblates with 50 ml of water at doses as shown in Table I. The α form was given after 1 week to the same subject under the same conditions as mentioned above. Urine collections were made at regular intervals as shown in Fig. 7 and the cumulative amount of CTC excreted in urine at each collection period was estimated.

Both plasma and urine samples were determined following Kohn's method.[9]

Result and Discussion

Characterization of the α and β Forms

Several methods were used to determine the crystal forms.

Microscopic examination showed the α and β forms to be platelets and needles, respectively, as shown by Karmarkar and Thirumalachar.[5]

The proof of the chemical nature of the β form was established by converting the β form to α form. The crystal form obtained by recrystallization of the β form from water was the α one of CTC-HCl, indicating that no chemical modification of CTC-HCl had occurred. In addition, the nuclear magnetic resonance (NMR) spectra[10] of the two forms in heavy water were essentially identical: this suggests that the two forms show the same nature in the water environment.

The elemental analysis, moisture determinations by the Karl Fisher method and weight loss studies (100°, 6 hr, *in vacuo*) of the two forms showed that 1 mole of the β form contained about 3/4 mole of water whereas water was absent in the α form. Drying the β form led to a loss of water, but totally anhydrous β form could not be obtained.

Buckley, *et al.*[11] and Balutsov[12] have reported that oxytetracycline hydrochloride exists in hygroscopic and nonhygroscopic forms and crystalline oxytetracycline hydrochloride, when crystallized from methanol, tends to absorb moisture to an appreciable extent. Thus, it may

7) J. Levine, E.A. Garlock, Jr., and H. Fishbach, *J. Am. Pharm. Assoc. Sci. Ed.*, **38**, 473 (1949).
8) Cemedine Super, Cemedine Co., Japan.
9) K.W. Kohn, *Anal. Chem.*, **23**, 862 (1961).
10) NMR spectra were run on a Hitachi H-60 NMR spectrometer in heavy water with Me_4Si as an external standard.
11) J.S. Buckley, Jr., Groton, and S.R. Stephens, Jr., Waterford, Conn., and R.I. Wagner, Jr., U.S. Patent 2867661 Jan. 6 (1959).
12) V. Balutsov, *Farmatsiya* (Sofia), **20**, 34 (1970) [through *C.A.*, **73**, 38500h (1970)].

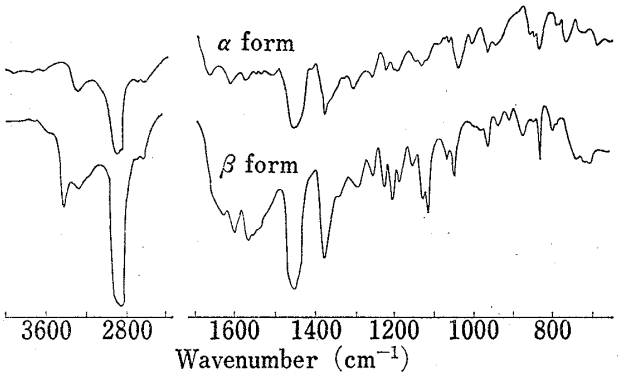

Fig. 1. Infrared Absorption Spectra of the α and β Forms of Chlortetracycline Hydrochloride in Nujol

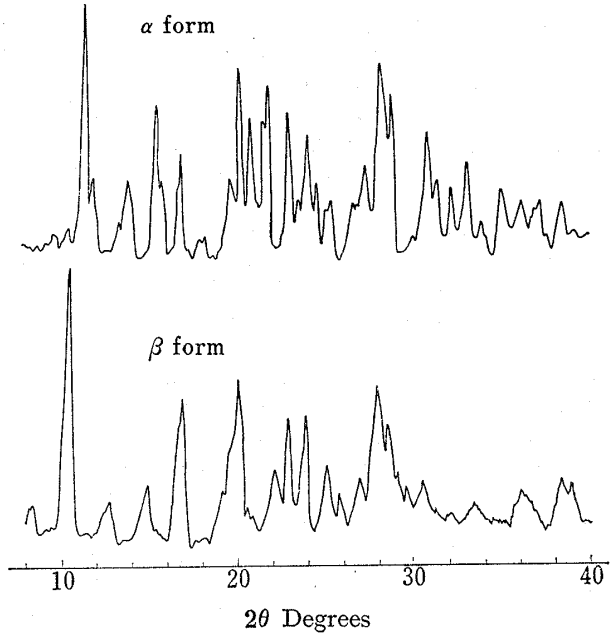

Fig. 2. X-Ray Diffraction Patterns of the α and β Forms of Chlortetracycline Hydrochloride

be suggested that the anhydrous β form is hygroscopic and when the anhydrous β form is exposed to a normal laboratory atmosphere, this form will rapidly take up moisture and revert to the crystal containing about 3/4 mole of adsorbed water.

The IR spectra and X-ray powder diffraction patterns are shown in Fig. 1 and 2. From these results, in which the α and β forms exhibited the features different from each other, the two forms were confirmed to be polymorphous.

Dissolution Behaviors of the α and β Forms in Water

The dissolution curves of the two forms of CTC-HCl from crystalline powder at various temperature are shown in Fig. 3 and 4, where the concentration of the drug in solution are plotted as a function of time. As indicated in these plots, each curve is drawn through points obtained during two experimental runs and the results were satisfactorily reproducible.

The distinct difference in dissolution behaviors was observed at 15 and 37°, the β form dissolving much faster than the α form and yielding a concentration supersaturated with respects to the α form in an initial stage of dissolution. However, a decrease in the amount

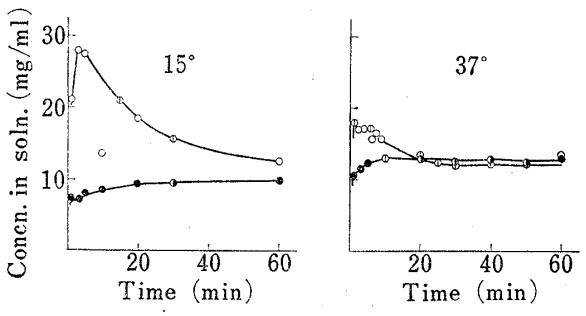

Fig. 3. Dissolution Curves of the α an β Forms of Chlortetracycline Hydrochloride from Crystalline Powder in Water at 15 and 37°

—●—◑—: α form —○—◑—: β form
(The two types of circles for each form represent successive experimental runs.)
sample amount: 40 mg per 1 ml of water

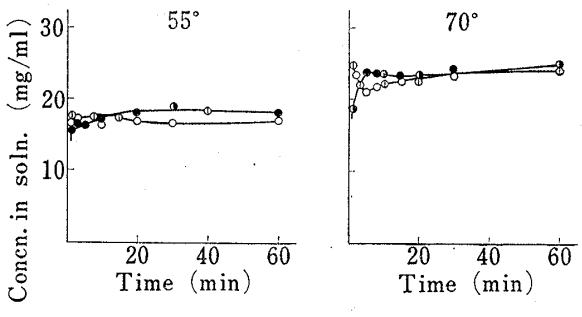

Fig. 4. Dissolution Curves of the α and β Forms of Chlortetracycline Hydrochloride from Crystalline Powder in Water at 55 and 70°

—●—◑—: α form —○—◑—: β form
(The two types of circles for each form represent successive experimental runs.)
sample amount: 50 mg per 1 ml of water

of the drug dissolved within a relatively short period was observed at 37°. The limiting value of this decrease was found to be solubility of the α form. The revertion of the β form to α form was confirmed by IR spectra and microscopic examination of the crystal isolated from the medium after equilibrium, whereas at 15° the complete reversion was not yet seen within 60 minutes.

On the other hand, the dissolution curves of the two forms were virtually identical at 55°, which may be considered to be near the transition temperature of this system. However, a supersaturation state for the α form was not found at 70° and no attempt was made to inquire further into this point.

In addition, the dissolution curves of the two forms obtained with compressed disks are illustrated in Fig. 5. As expected, the dissolution of the β form from compressed disks was shown to be faster than that of the α form. The apparent dissolution rates of the α and β forms from the slope of straight lines in an initial stage of dissolution were 3.5 and 28.0 mg/50 ml/min, respectively.

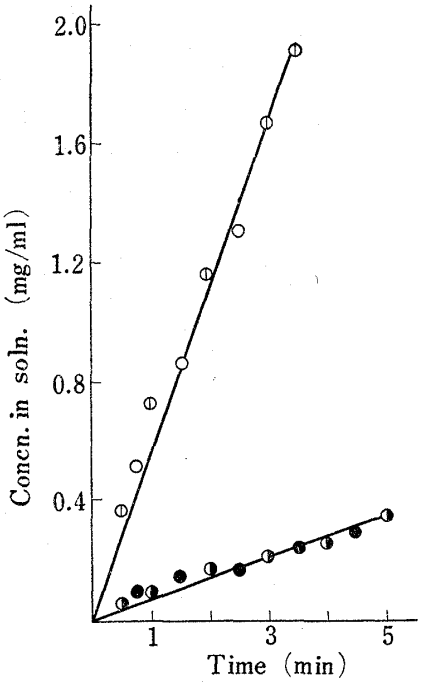

Fig. 5. Dissolution Curves of the α and β Forms of Chlortetracycline Hydrochloride from Compressed Disk in Water at 37°

—●—◑—: α form —○—◐—: β form
(The two types of circles for each form represent successive experimental runs.)

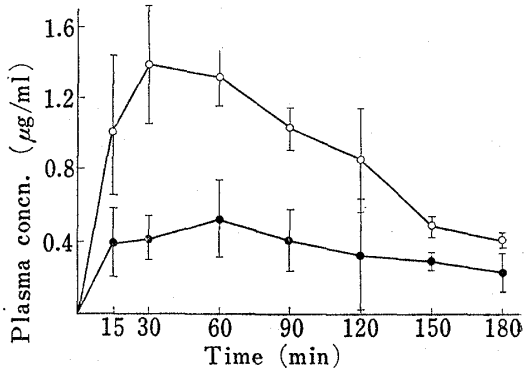

Fig. 6. Plasma Levels after Intradoudenal Administration of the α and β Forms of Chlortetracycline Hydrochloride to Rabbits

—●—: α form —○—: β form
dose: 200 mg
points represent mean levels attained; vertical bars indicate standard deviation. (4 experiments)

Effect of Polymorphism on the CTC Levels in the Body Fluids

On the basis of dissolution studies, an appreciable difference in the absorption of CTC-HCl would be expected. In order to ascertain the effect of polymorphism on the CTC levels in body fluids, the gastrointestinal absorption of the two forms was studied in rabbits and human subjects.

Fig. 6 shows the blood plasma levels of the two forms after the intraduodenal administration to rabbits. The results observed in this study are consistent with those of the dissolution studies. The administration of the β form gave a higher and earlier appearance of the peak plasma level than that of the α form. In fact, the peak plasma level attained was about 2.6 times that of the α form and reached within 30 min. The results indicate that the β

Table I. Recovery in Urine of Chlortetracycline Hydrochloride

Exptl. No.	Body wt. (kg)	Time (hr)	Dose (mg)	Recovery (%)	
				α form	β form
1	70	0—9	600	4.8	8.0
2	57	0—9	450	14.4	18.9

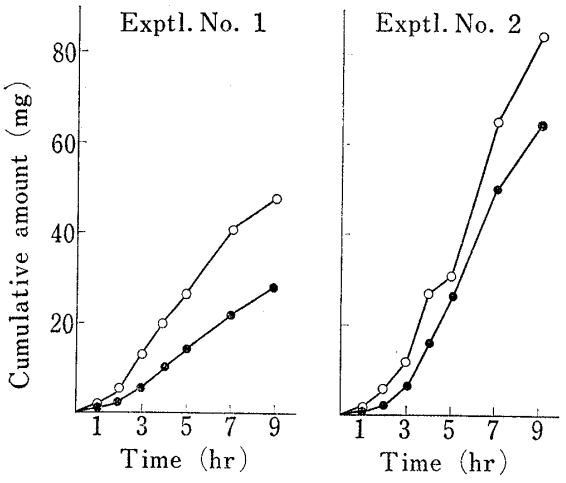

Fig. 7. Cumulative Excretion Curves after Oral Administration of the α and β Forms of Chlortetracycline Hydrochloride to Human Subjects

—●—: α form —○—: β form

form of CTC-HCl is more efficiently absorbed from the gastrointestinal tract of rabbits than the α form.

The similar results were noted after the oral administration of the two forms of CTC-HCl to human subjects. The results of this study are shown in Table I and Fig. 7. In Fig. 7, the cumulative amounts of CTC excreted in the urine are plotted as a function of time. These two experimental data indicate that the β form may exhibit greater bioavailability than the α form.

These results suggest that the more soluble β form is absorbed much faster than the α form from the gastrointestinal tract and that the solubility difference between the two forms has an effect on the bioavailability of the CTC-HCl.

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