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Bioavailability and inhibitory effect for stress ulcer of cimetidine polymorphs in rats

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Cimetidine, *N''*-cyano-*N*-methyl-*N'*-[2-[{(5-methyl-1H-imidazol-4-yl) methyl}thio]ethyl] guanidine, is a specific histamine H_2-receptor antagonist which inhibits the secretion of the histamine-stimulated gastric acid and is utilized in the treatment of peptic ulcer. It has four kinds of crystalline forms (polymorphs), three anhydrous (form A, B and C) and a monohydrate (form D) (Prodic-Kojic et al., 1979; Shibata et al., 1983; Hegedus and Gorog, 1985). In our previous paper (Shibata et al., 1983), we reported the structures of two cimetidine polymorphs (form C and D) by X-ray diffraction and the physicochemical properties of the four cimetidine polymorphs. In this study, the bioavailability on oral administration of cimetidine polymorphs (form A, B and C) and the inhibitory effect for stress ulcer of four cimetidine polymorphs were investigated. Furthermore, the relations between these results and in vitro dissolution behavior (Shibata et al., 1983) were discussed.

Four crystalline forms of cimetidine were prepared by slow evaporation, as described in our previous paper (Shibata et al., 1983). Form A was obtained by crystallization from its acetonitrile solution. Form B and D were obtained by crystallization from its aqueous solution at 20°C and form C was obtained by crystallization from its aqueous solution at 6°C. There were platelet (form A), needle (form B), pyramidal (form C) and cubic (form D) crystals. These crystalline forms were confirmed to coincide with those reported previously (Prodic-Kojic et al., 1979) by comparison of their respective IR spectra.

Wistar strain male rats, weighing 220–250 g for the bioavailability experiment and weighing 190–200 g for studying the inhibitory effect of stress ulcer, were fasted for 15 h before experiments but with access to water.

Cimetidine crystalline forms (44–74 μm in diameter) were administered orally to the rat stomach with 0.8 ml water at a dose of 100 mg/kg through a catheter. After pentobarbital sodium (50 mg/kg) anesthesia, blood samples were collected from the inguinal vein at the appropriate time. On the other hand, the cimetidine solution (in saline) was administered via a femoral vein at a dose of 100 mg/kg. The plasma concentration of cimetidine was measured by high-performance liquid chromatography (HPLC) using UV detector

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(220 nm), according to the method described by Randolph et al. (1977). The drug concentration area under the curve (AUC) was calculated by means of the trapezoidal rule.

The effect of the cimetidine polymorphs on rat gastric ulcers, produced by restraint and water immersion stress, was examined as described by Okabe et al. (1977). Powdered cimetidine (44–74 μm in diameter) was administered to rat stomach at a dose of 12.5, 25.0 and 50.0 mg/kg by the same manner of the bioavailability experiments. The rats were placed in the stress cages and immersed to the level of the xiphoid in a water bath at 21°C for 5 h. After taking the rats out of the stress cages, Evans' blue was injected via the tail vein to enhance the contrast of the gastric lesions. After 10 min, the stomach was removed, slightly inflated with 1% (w/v) formalin solution, and immersed in 10% (w/v) formalin solution for 10 min to fix the inner and outer layers of gastric walls. The area (mm^2) of each stress ulcer was measured under the dissection microscope. The inhibitory rate of cimetidine for stress ulcer was calculated as follows:

$$\text{Inhibition Rate}\ (\%) = (M_c - M_t)/M_c \times 100$$

where M_c is the mean of the total ulcer area in the control group (not administered cimetidine) and M_t in the test group. The Student's t-test was used to determine the statistical significance of data obtained.

Fig. 1 shows the time course of the plasma concentration of cimetidine following oral administration of cimetidine polymorphs (form A, B and C) in rats. The peak plasma levels of cimetidine were reached within 30 min after administration with each crystalline form; the absorption of these three forms was very rapid. The plasma concentration with form C was markedly higher than those of form A and B. The plasma concentration curves with form A and B were similar. The area under the curves (AUC_0^5) of forms A, B and C were 17.62, 19.10 and 27.24 $\mu g \cdot h/ml$, respectively. Absolute bioavailability of form A, B and C were 27.4, 30.9 and 42.4%, respectively, indicating the bioavailability of form C being 1.5 and 1.4 times larger than those of form A and B, respectively.

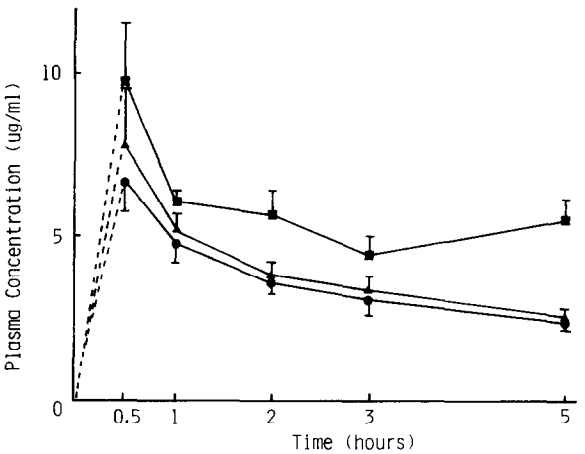

Fig. 1. The plasma concentration of cimetidine after oral administration of cimetidine polymorphs (●, form A; ▲, form B; and ■, form C) at a dose of 100 mg/kg in rats. Each value represents the mean ± S.E. of at least 5 rats.

Table 1 shows the effect of four crystalline forms on rat gastric ulcers produced by restraint and water-immersion stress. The inhibitory effect of form C, especially at the lower dose (12.5 mg/kg), was significantly high compared with

TABLE 1

Effect of 4 crystalline forms for stress ulceration in rats

Dose, (mg/kg)	Mean area of ulcer (mm^2)	Inhibition rate (%)
Control	22.3 ± 4.3	
Form A		
12.5	6.9 ± 2.1	69.1 ± 25.3
25.0	5.9 ± 2.4	73.7 ± 26.2
50.0	3.4 ± 2.3	84.7 ± 27.4
Form B		
12.5	8.7 ± 4.8	60.8 ± 31.1
25.0	3.2 ± 1.1	85.2 ± 25.8
50.0	3.6 ± 2.2	84.0 ± 27.1
Form C		
12.5	2.8 ± 1.7	87.4 ± 26.7
25.0	3.2 ± 1.1	85.8 ± 25.9
50.0	2.2 ± 0.9	90.4 ± 26.3 *
Form D		
12.5	8.0 ± 2.6	64.1 ± 25.7

All values represent the mean ± S.E. ($n = 6$).
* $P < 0.05$ relative to the same dose of forms A or B.

those of form A, B and D. However, there were no significant differences among the inhibitory rates of form A, B and C at doses of 25.0 and 50.0 mg/kg.

In our previous paper (Shibata et al., 1983), the apparent in vitro dissolution rate in deionized water for form C was 1.29, 17.0 and 1.90 times greater than those measured for forms A, D and B, respectively. The dissolution rates for forms A, B and C in neutral test solution (pH 6.8) were similar to those in deionized water. There was a similar relationship among crystalline forms with respect to bioavailability. Furthermore, it was comparable with the results of bioavailability and the dissolution rate that form C, the most effective form, had on inhibitory test for stress ulceration in rats.

In conclusion, form C, among the four kinds of cimetidine crystalline forms, had the highest bioavailability and inhibitory effect on stress ulceration.

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