Faculty of Pharmaceutical Sciences,
Nagoya City University,
Tanabe-dori, Mizuho-ku,
Nagoya 467, Japan

Norio Hashimoto
Toyohiko Aoyama
Takayuki Shioiri*

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Quantitative Analysis of Clebopride and Its Metabolites in Rat Blood by Acid Decomposition

6-Chloro-*m*-anisidine was obtained as one of the products by the acidic decomposition of elebopride and its main metabolites (total CP). This degraded product after derivatized with heptafluorobutyric anhydride was measured by use of mass fragmentography. A good calibration curve was obtained in the range 2—80 ng/ml of clebopride in rat blood. The concentration of total CP in rat blood after intravenous (0.1 and 0.5 mg/kg) and oral (0.5 mg/kg) administration was determined by this method.

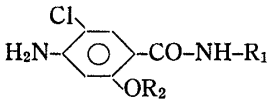
Keywords——clebopride; mass fragmentography; GC/MS; acid decomposition; benzamide drug; pharmacokinetics

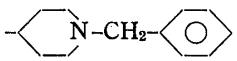
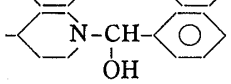
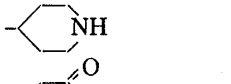
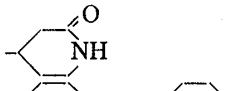
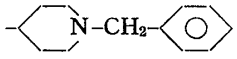
Clebopride (CP; N-(1′-benzyl-4′-piperidyl)-2-methoxy-4-amino-5-chlorobenzamide) is a new benzamide drug with potent antidopaminergic activity.[1] Therapeutic dose of CP is low (0.5 mg/kg). This drug is extensively metabolized as shown in Table I.[2]

Segura *et al.* recently reported an analytical procedure for CP in biological fluids[3]; however this procedure meet only to need for determination of the plasma level following doses more than 10 mg/kg in the experimental animals.

The present report describes mass fragmentographic determination of the unchanged CP and its main metabolites (total CP) in whole blood. This method is based on the fact that not only the unchanged CP but also its main metabolites having 6-chloro-*m*-anisidine (6-CA) ring have to be decomposed with hydrochloric acid to form 6-CA as one of the degraded

Table I. Structures of Clebopride, Its Main Metabolites and Internal Standard



	R_1	R_2
Clebopride		CH_3
Metabolite 1		CH_3
Metabolite 2		CH_3
Metabolite 3		CH_3
Internal standard		CH_2CH_3

products. This degraded product, after acylation, is sensitively detected by use of mass fragmentography.

The procedure is as follows; to 5 ml of heparinized whole blood sample 1 ml (200 ng) of aqueous solution of internal standard (IS; see Table I) was added and pH of the mixture was adjusted to 9.5 with 5 ml of borate buffer. The solution was extracted twice with 200 ml of chloroform and the extract was evaporated *in vacuo* to dryness. The residue was redissolved in 1.5 ml of the organic solvent, and the solution was transferred into a reaction-tube and evaporated by blowing air at 60° to dryness; the process was repeated four times with chloroform and once with methanol. The residue in the sealed reaction-tube was decomposed for 16 hr at 105° with 1 ml of 20% hydrochloric acid. The acid solution was made alkaline by addition of about 1 ml of 5.5 N sodium hydroxide. The alkalified solution was extracted twice with 6 ml of ethyl acetate and the organic layer was evaporated *in vacuo* to dryness. The residue was derivatized with 0.2 ml of heptafluorobutyric anhydride for 60 min at 60°. After 4 ml of water was added to the reaction mixture in order to decompose the residual reagent, the derivatized compounds were extracted with 6 ml of petroleum ether. The organic layer was washed with 4 ml of water and evaporated *in vacuo* to dryness. The residue was dissolved in 100 μl of *n*-hexane just before measurement. An adequate volume from 1 μl to 3 μl was injected into gas chromatograph-mass spectrometer [a Du Pont Dimaspec 321 GC/MS equipped with a Data System 320].

The gas chromatographic separation was carried out on a 0.5 m × 1.2 mm i.d. glass column packed with 2% OV-101 Chromosorb WH.P (100/120 mesh). The temperatures of the injector, column oven, jet separator and ion source were 160°, 135°, 200° and 180°, respectively. Helium was used as a carrier gas at a flow rate 25 ml/min. The mass fragmentography was carried out with use of the molecular ions ($M^{+\cdot}$) corresponding to the degraded total CP (*m/z* 353) and IS (*m/z* 367) derivatives.

A standard calibration curve was prepared by adding known amount of CP and IS to 5 ml of rat whole blood to give final CP concentration ranging from 2 to 80 ng/ml. The standard curve was linear throughout the above range with correlation coefficient greater than 0.998.

A selected ion recording of the standard sample extracted from whole blood was illustrated in Fig. 1.

This method was applied to study on pharmacokinetics of CP in the rat. The blood level of total CP in the rat after intravenous administration (0.5 and 0.1 mg/kg) was shown in Fig. 2(a). The concentration-time curves were expressed by the following bi-exponential equation; $C=A \exp(-\alpha t)+B \exp(-\beta t)$. These parameters for this equation obtained in 0.5 and 0.1 mg/kg doses, respectively, were as follows; A 207.3 and 41.8 ng/ml, B 88.6 and 26.6 ng/ml, α 0.178 and 0.559 min^{-1}, β 0.008 and 0.012 min^{-1}, and $T_{\beta 1/2}$ 86.6 and 57.7 min.

The blood level of total CP in the rat after oral administration (0.5 mg/kg) was shown in Fig. 2(b). Two peaks were observed at 15 min (mean 25 ng/ml) and 60 min (mean 43 ng/ml).

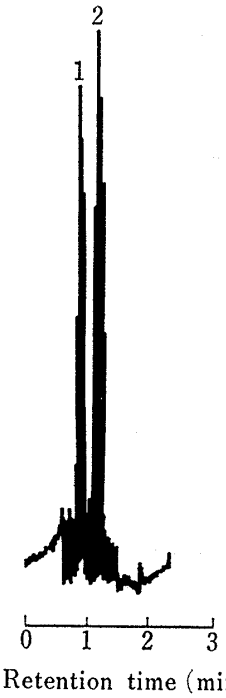


Fig. 1. Selected Ion Chromatogram with Two Peaks at *m/z* 353 and *m/z* 367

Sample; standard sample extracted from rat whole blood containing CP (20 ng/ml) and IS (40 ng/ml).
Peak 1; derived from CP (*m/z* 353), peak 2; derived from IS (*m/z* 367).

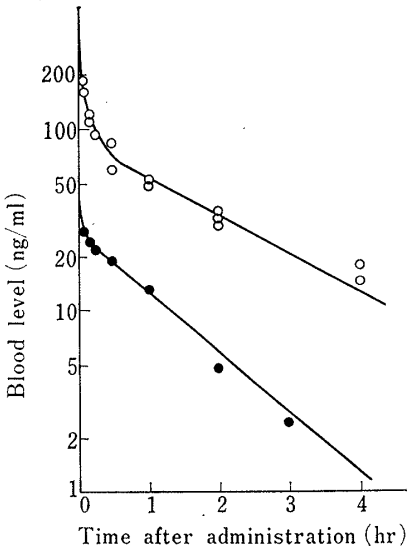


Fig. 2(a). Semilogarithmic Plot of Total CP Concentration in Whole Blood from Individual Rat Sacrificed at Different Time after Intravenous Administration

Each point (○; 0.5 mg/kg, ●; 0.1 mg/kg) represents the measured value of each animal. Concentration-time curves were calculated by bi-exponential equation.

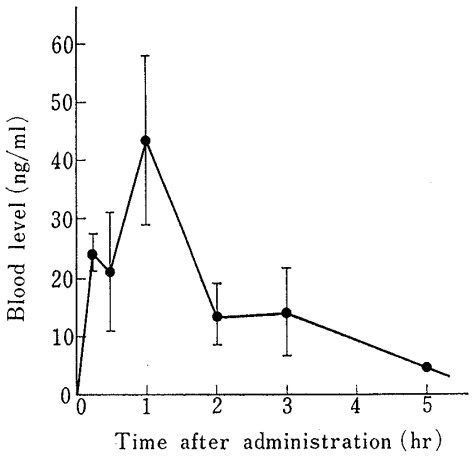


Fig. 2(b). Concentration of Total CP in Whole Blood from Individual Rat Sacrificed at Different Time after Oral Administration

Each point represents mean value ± S.D.

This pattern was the same as those with large dose (100 mg/kg).[4] The ratio of the area under blood concentration-time curve (between 0 and 300 min) after oral administration to that after intravenous administration (0.5 mg/kg) was about 0.5.

The sensitivity of this method was sufficient to determine total CP in whole blood 3 hr after intravenous administration of 0.1 mg/kg or 5 hr after oral administration of 0.5 mg/kg.

This method has to be further improved in order to determine the pg level of blood concentration of human being receiving therapeutic dose in clinical study.

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References and Notes

1) J. Prieto, J. Moragues, R.G. Spickett, A. Vega, M. Colombo, W. Salazar, and D.J. Roberts, *J. Pharm. Pharmacol.*, **29**, 147 (1977); P. Jenner, P.N.C. Elliot, A. Clow, C. Reavill, and C.D. Marsden, *J. Pharm. Pharmacol.*, **30**, 46 (1978).
2) G. Huizing, A.H. Beckett, and J. Segura, *Pharm. Weekblad Sci. Ed.*, **1**, 64 (1979); J. Segura, O.M. Bakke, G. Huizing, and A.H. Beckett, *Drug Metabol. Disp.*, **8**, 87 (1980); G. Huizing A.H. Beckett, J. Segura, and O.M. Bakke, *Xenobiotica*, **10**, 211 (1980); J. Segura and L. Borja, *Xenobiotica*, to be published.
3) G. Huizing, A.H. Beckett, and J. Segura, *J. Chromatogr.*, **172**, 227 (1979).
4) J. Segura, I. Garcia, L. Borja, E. Tarrus, and O.M. Bakke, *J. Pharm. Pharmacol.*, **33**, 214 (1981).

Laboratory of Biopharmaceutics,
Central Research Laboratories,
Meiji Seika Kaisha, Ltd.,
Morooka-cho, Kohoku-ku,
Yokohama, 222, Japan

Yoji Hayasaka*
Shinjiro Murata
Koshiro Umemura

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