Simultaneous Determination of Tiaramide and its Metabolites by High Performance Liquid Chromatography

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Received: August 30, 1984; accepted: December 27, 1984.

Abstract: A simultaneous quantitative HPLC assay was developed for tiaramide hydrochloride (4-[(5-chloro-2-oxo-3(2*H*)-benzothiazolyl)acetyl]-1-piperazine ethanol hydrochloride) and its main metabolites, namely, 1-[(5-chloro-2-oxo-3(2*H*)-benzothiazolyl)-acetyl]-piperazine (DETR) and 4-[(5-chloro-2-oxo-3(2*H*)-benzothiazolyl)acetyl]-piperazine acetic acid (TRAA). The assay was employed to determine plasma levels of tiaramide and its main metabolite, TRAA, after intravenous administration of tiaramide hydrochloride to rabbits.

The widely used basic anti-inflammatory drug, tiaramide hydrochloride, (4-[(5-chloro-2-oxo-3(2*H*)-benzothiazolyl)-acetyl]-1-piperazine ethanol (hydrochloride), strongly suppresses acute and subacute inflammatory edema (1).

With respect to the absorption, distribution, excretion and metabolism of tiaramide hydrochloride, Noda et al. (2) reported the results of experiments with the ^{14}C-labeled compound in rats and monkeys and with unlabeled tiaramide hydrochloride in man. The absorption of this drug is rapid after oral administration to rats, monkeys and men, and the drug reaches maximum blood concentrations within approximately one hour. In another report, Noguchi et al. (3) stated that tiaramide blood levels in rats followed a two-compartmental model. After oral administration the unchanged drug rapidly distributes to liver, kidneys and lungs to reach tissue levels four to six times higher than those in the blood. Similarly, Noguchi et al. (4) reported that there is a great difference among animal species in the composition of the metabolites in serum and urine (Fig. 1).

In the above reports, ^{14}C-labeled compound or gas chromatography was used for the quantitative determination of tiaramide hydrochloride. However, there has been no report on the quantitative determination of the drug using high-performance liquid chromatography, which is frequently applied to the quantitative determination of drug serum levels. Therefore, an HPLC method for the quantitative determination of tiaramide hydrochloride and its metabolites was established.

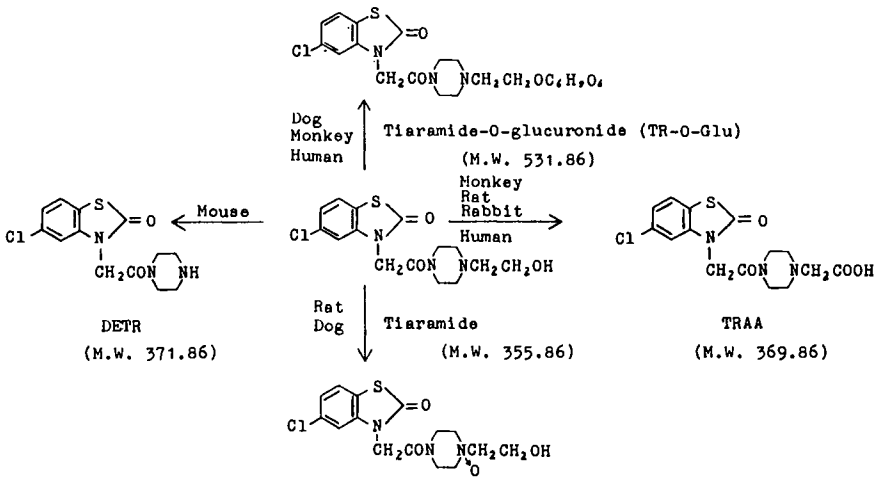

Fig. 1 Metabolism of tiaramide.

The procedure was applied to determine concentrations of tiaramide and its main metabolite in plasma after intravenous administration of tiaramide hydrochloride to rabbits.

Materials and Methods

Materials

The following reagents were used: tiaramide hydrochloride, 1-(5-chloro-2-oxo-3(2*H*)-benzothiazolyl)acetyl piperazine, 4-[(5-chloro-2-oxo-3(2*H*)-benzothiazolyl)acetyl]-1-piperazine acetic acid (Fujisawa Pharmaceutical Co., LTD., Osaka, Japan), monobasic sodium phosphate 2-hydrate, dibasic sodium phosphate 12-hydrate, monobasic potassium phosphate, propyl *p*-hydroxybenzoate, ammonia solution (sp. gr. 0.88) (Nakarai Chemicals Ltd., Kyoto, Japan).

Chromatographic conditions

Apparatus: High-performance liquid chromatograph LC-3A (Shimadzu)
Detector: SPD-2A (Shimadzu)
Column: Zorbax, ODS column (15 x 4.64 mm I.D., Dupont, U.S.A.)
Mobile phase: Methanol-phosphate buffer solution (0.015 M, NaH_2PO_4 and Na_2HPO_4, pH 8.00) = 55:45 (v/v)
Flow rate: 0.8 ml/min
Column pressure: 100 kg/cm^2
Column temperature: room temperature
Wavelength determined: 225 nm (ultraviolet)
Range: 0.04 Aufs
Chart speed: 2.5 mm/min
Internal standard solution: Twenty-five mg of propyl *p*-hydroxybenzoate were accurately measured, moved to a 50 ml-volumetric flask and diluted with the mobile phase. Then, 0.5 ml of this solution was transferred into another 50 ml-volumetric flask and diluted with the mobile phase to prepare the internal standard solution.

Thin-layer chromatographic conditions

Thin layer plate: pre-coated Kieselgel 60 (250 μm thick, 5 x 20 cm, Merck Co.)

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Development solvent: ethanol; ethyl acetate; ammonia solution (sp. gr., 0.88) = 60:40:6 (20 ± 1°C)
Detector: two-wavelength TLC scanner CS-900 (Shimadzu)
Sample λs = 295 nm
Reference λR = 440 nm
Width and height of the slit: 1.25 mm each
Sensitivity: absorbance x 2
Scan speed: 10 mm/min (Zig-zag)
Chart speed: 10 mm/min

Test Animals

Albino male rabbits (weighing 1.8–2.2 kg), fasted for 24 hours before the experiment, were fixed in a supine position. After cannulation with a silicone T-catheter (Okasan Kagaku, Japan) in the carotid artery under ether anesthesia, serial blood samples were collected with plastic disposable syringes. The blood samples were centrifuged at 3,000 r.p.m. for five minutes, and the plasma obtained was used for analysis.

The catheters, syringes and centrifugation tubes were moistened before use with 0.9 % physiological saline solution containing sodium heparin (90 U/ml) in order to avoid hemopexis.

Each group in each experiment consisted of five rabbits.

Intravenous Administration.

Tiaramide hydrochloride (63.7 μmole/kg) was dissolved in 2 ml physiological saline solution and rapidly injected into the rabbit auricular vein. Blood samples were collected at zero, 5, 10, 15, 25, 40, 60, 90, 120, 180, 240 and 300 minutes after administration.

Analytical Procedure

The plasma (0.5 ml) was placed in a small 15 ml stoppered test tube, mixed with 1.5 ml 0.5 M KH_2PO_4-NaOH buffer solution (pH 6.00), 1.5 g anhydrous sodium sulfate and 6 ml chloroform, and shaken for 15 minutes (250 strokes/min), followed by centrifugation (at 3,500 r.p.m. for five minutes). After removing the upper layer with an aspirator, the chloroform bed was filtered, and 3 ml of the filtrate was evaporated and dried in a water bath (at 65° C at atmospheric pressure). The mobile phase (0.4 ml) containing propyl *p*-hydroxybenzoate at a concentration of 5 μg/ml was added to the residue, mixed well and subjected to ultrafiltration (DUALEX TM®, 0.2-μm filter unit, Japan Millipore Corporation). For HPLC, 20 μl of the filtrate were injected.

Results and Discussion

For the separation of tiaramide and its metabolites by reversed-phase liquid chromatography on an octadecyl silane (ODS)-bonded column, similar conditions were employed as those described earlier for the quantitative assay of perphenazine, whose structure is similar to that of tiaramide hydrochloride (5) (i.e., an eluent mixture composed of methanol: phosphate buffer solution (0.05 M, pH 6.90) (53:47)). The optimum eluent system was found to be methanol: phosphate buffer solution (0.015 M, pH 8.00) (55:45) in order to separate tiaramide and its main metabolites, [(5-chloro-2-oxo-3(2*H*)-benzothiazolyl)acetyl]-piperazine (*N*-desethanol tiaramide; DETR) and 4-[(5-chloro-2-oxo-3(2*H*)-benzothiazolyl]-1-piperazine acetic acid (tiaramide acetic acid; TRAA) (Fig. 1). Figure 2 shows HPLC records of these three compounds and of propyl *p*-hydroxybenzoate, which was used as the internal standard. The method is capable of analyzing one specimen in 20 minutes. In previous reports (2, 3, 5) the basic drugs, tiaramide and DETR, and the acidic metabolite, TRAA, were separately extracted from plasma into organic solvents; the basic drugs and the acid compound were extracted under alkaline and acid conditions, respectively. We have determined the extraction yields of tiaramide, DETR and TRAA, at various pH into chloroform (Fig. 3 A) and found that these compounds can be simultaneously extracted at pH 6.0.

The HPLC eluents were monitored by UV absorption at 225 nm. Tiaramide showed a detection limit of 20 ng/ml plasma, and for DETR and TRAA the detection limit was approximately 200 ng/ml.

The calibration curves obtained by this method are shown in Figure 3 B). The coefficient of variance, [CV(%)] was less than 5 % at any one concentration.

Identification of Metabolites in Rabbit Plasma after Administration of Tiaramide Hydrochloride

At 15 minutes after intravenous injection of tiaramide hydrochloride

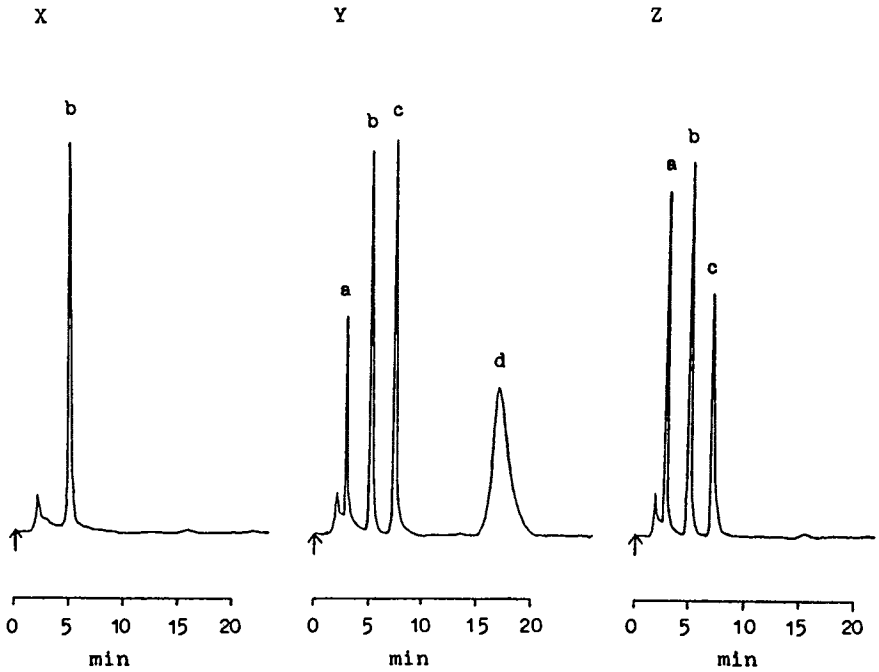


Fig. 2 High-performance liquid chromatograms of rabbit plasma extracts.
X, control plasma containing internal standard (propyl *p*-hydroxybenzoate) (b)
Y, plasma containing internal standard (b), TRAA (a), tiaramide (c) and DETR (d)
Z, plasma sample at 15 min after intravenous administration of 63.7 μmole/kg of tiaramide hydrocholide.

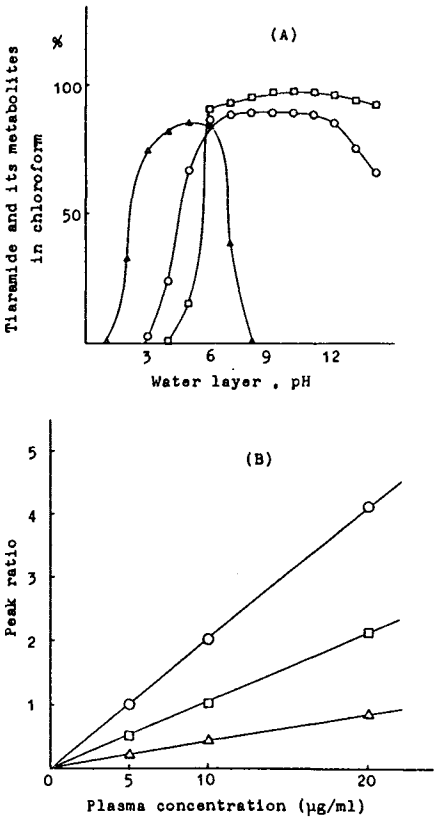


Fig. 3a Effect of pH on the extraction of tiaramide, TRAA and DETR from aqueous solution.
pH 2–5; 0.1M Na_2HPO_4-citric acid buffer
pH 6–8; 0.1M Na_2HPO_4-NaH_2PO_4 buffer
pH 9–11; 0.1M NH_4Cl-NH_4OH buffer
pH 12–13; 0.1M borax-NaOH buffer
b Calibration curve for tiaramide, TRAA and DETR extracted from plasma. Peak ratios were measured by the peak-height method (tiaramide and TRAA) or half-band width method (DETR). Linear regression lines were: tiaramide, y = 0.206x-0.046; TRAA, y = 0.0439x-0.0035; DETR, y = 0.110x-0.068.
Each point represents the mean of 5 determinations.
-○- tiaramide
-△- TRAA
-□- DETR

(63.7 μmole/kg), 1 ml of plasma was extracted and analyzed by HPLC. Tiaramide showed a peak at a 7.2 minutes retention time. In addition, a peak observed at 3.2 minutes, coincident with the elution time of authentic TRAA. There was no peak detectable at the position of DETR.

For further identification, tiaramide and its metabolites were first isolated by thin-layer chromatography (TLC). The spots of tiaramide, DETR and TRAA were detected at Rf values of 0.56, 0.34 and 0.13 respectively. At 15 minutes after intravenous injection of tiaramide hydrochloride, 5 ml of plasma was extraced and analyzed by TLC. There were spots at the Rf values of tiaramide and TRAA, but no spot was present at the Rf of DETR, as was also the case with HPLC analysis.

Rabbit plasma was also extracted at 30 minutes after oral administration of tiaramide hydrochloride (255 μmole/kg) and analyzed by HPLC and TLC. Tiaramide and TRAA were again readily detectable by both methods, and additionally, minor components that were absent in blank samples were observed at 5.6 minutes HPLC retention time and at 0.05 and 0.35 Rf values with TLC. However, these minor components were not further investigated.

Intravenous Injection of Tiaramide Hydrochloride

In order to obtain the pharmacokinetic parameters of tiaramide hydrochloride in rabbit blood, the drug was intravenously administered. After injection of the drug (63.7 μmole/kg), unchanged drug levels followed a two-compartmental model as shown on the semilogarithmic graph in Figure 4.

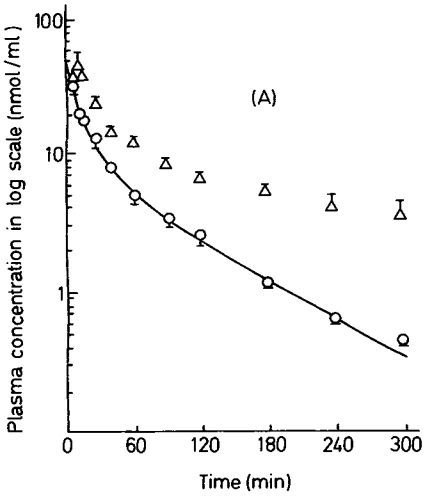
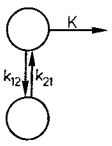


Fig. 4 Concentration of tiaramide and TRAA in plasma after intravenous administration (63.7 μmole of tiaramide HCl/kg). The solid line shows the calculated curve for tiaramide obtained from Eqn. 1 in Scheme 1. Pharmacokinetic parameters used for the calculated curve were as follows: α, 5.40 h^{-1}; β, 0.67 h^{-1}; k_{12} (from central to tissue compartment), 2.22 h^{-1}; k_{21} (from tissue to central compartment), 1.62 h^{-1}; Vc (distribution volume of central compartment) 2987 ml; k_a (absorption rate constant), 1.80 h^{-1}.
Each point represents the mean with the standard error.
o tiaramide
△ TRAA

The metabolites, TRAA, reached higher concentrations than the parent drug five minutes after intravenous injection and peaked 10 minutes after injection. Subsequently, TRAA levels decreased in a biexponential fashion. Since TRAA could not be injected intravenously because of the small available amount of pure TRAA, pharmacokinetic parameters of TRAA could not be directly determined. Therefore, in the present study, levels of the parent drug alone were analyzed pharmacokinetically according to the model depicted in Scheme 1. Estimates of the pharmacokinetic parameters are provided in the legend, Fig. 4.

(Model. 1)



(Eqn. 1)

$$A = \frac{A_0}{(\alpha-\beta)}\left[(k_{21}-\beta)e^{-\beta t} - (k_{21}-\alpha)e^{-\alpha t}\right]$$

A : central compartment
(injection site of tiaramide)
B : tissue compartment

Scheme 1

Acknowledgement

Part of the present work was supported by a Research Grant of the Development Committee of New Pharmaceutical Goods, Shiga Prefectural Pharmaceutical Commerce and Industry Association.

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