In vivo metabolism of 2-[1'-phenyl-3'-(3-chlorophenyl)-2'-propenylyden]hydrazino-3-methyl-4(3H)-quinazolinone in rats

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Received for publication: August 29, 2005

Keywords: 2-[1-phenyl-3-(chlorophenyl)-2-propenylyden]hydrazino-3-methyl-4(3H)-quinazolinone, HPLC, in vivo metabolism.

SUMMARY

The aim of this study was to investigate by HPLC the in vivo metabolism of 2-[1'-phenyl-3'-(3-chlorophenyl)-2'-propenylyden]hydrazino-3-methyl-4(3H)-quinazolinone as a substrate, and as a model compound in rats. The substrate was dissolved in DMSO/water (1:4) and administered intraperitoneally at a dose of 100 mg/kg in a volume of approximately 0.1 mL. Blood samples were taken before and 30 min, 1.5, 3, 6, 9, 11, 30 and 48 h after i.p. drug administration. The chromatographic separation of the substrate and its metabolites was performed using a stainless steel Novopak C18 column (150 x 4.6 mm i.d., 5-μm particle size). The optimal composition of the mobile phase was reached by introducing different mixtures of pure acetonitrile and water in a linear gradient system. Following the biotransformation of this compound, 2-hydrazino-3-methyl-4(3H)-quinazolinone (M1) and 3-(3-chlorophenyl)-1-phenylprop-2-en-1-one (M2) derivatives were identified together with substrate by comparing them to reference standards using HPLC-UV/DAD. In addition, the composition of these metabolites and substrate was confirmed by LC-MS in plasma.

INTRODUCTION

Hydrazone derivatives have been reported to be potent antibacterial [1–6], anticonvulsant [7–9], analgesic and anti-inflammatory [10, 11], antitumor [12–14] and antitubercular [15–18] agents. Hydrazones hydrolyze to their corresponding aldehydes or ketones and amines under acidic and basic conditions. Hydrolysis occurs near neutral pH, but at a slower rate. For example, pyridoxal isonicotinoyl hydrazone which has been identified as an effective iron chelator both in vitro and in vivo, hydrolyzed to pyridoxal and isonicotinoyl acid hydrazide in the serum with a half-life of 1.7 h [19]. However, among the numerous studies, only a few have investigated the metabolism of hydrazones: it has nevertheless been reported that hydrazone derivatives, i.e. hydrazide compounds, produce the corresponding hydrolytic products [20, 21]. We
therefore decided to investigate the in vivo metabolism of 2-[1'-phenyl-3'-(3-chlorophenyl)-2'-propenylden] hydrazino-3-methyl-4(3H)-quinazolinone containing a large quinazolinone moiety that had previously been synthesized and determined to be an effective analgesic and anti-inflammatory agent [22].

The aim of this study was to determine in the rat model whether hydrazone, containing a bulky group on the hydrazone moiety, led to the formation of hydrolytic products or not. The detection and characterization of in vivo metabolic products of 2-[1'-phenyl-3'-(3-chlorophenyl)-2'-propenylden]hydrazino-3-methyl-4(3H)-quinazolinone as a model substrate was performed by HPLC-UV/diode array detector (DAD) and light chromatography–mass spectrometry (LC–MS).

MATERIALS AND METHODS

2-[1'-Phenyl-3'-(3-chlorophenyl)-2'-propenylden] hydrazino-3-methyl-4(3H)-quinazolinone (substrate), 2-hydrazino-3-methyl-4(3H)-quinazolinone (M1) and 3-(3-chlorophenyl)-1-phenylprop-2-en-1-one (M2) were synthesized as reported previously [22]. All the reagents used for chromatographic separation were of analytical grade and obtained from Fluka and Merck.

Animals and collection of blood samples

The albino rats were purchased from the Department of Pharmacology’s Laboratory Animal Production Center, School of Medicine, Osman Gazi University, Eskisehir, Turkey. The animals were housed under a 12-hour light–dark cycle at a room temperature of between 20–22°C, with food and water available ad libitum. The experiments were conducted in accordance with the ethical guidelines for experimental animals, and were approved by the Ethical Committee for Animal Experimentation of Osman Gazi University, Eskisehir, Turkey. All compounds were dissolved in DMSO/water (1:4) and injected i.p. at a dose of 100 mg/kg in a volume of approximately 0.1 mL. The rats were anesthetized with ether, and 0.3-mL blood samples were taken by cardiac puncture according to a predetermined time schedule (i.e. 0, 30 min, then 1.5, 3, 6, 9, 11, 30, and 48 h after drug administration), and three different blood samples were taken at each time point. Plasma was separated and stored at −20°C until use.

Preparation of plasma samples

Three hundred μL of blood was withdrawn from the rats and centrifuged to separate out the plasma and blood cells. Plasma was prepared according to previously described methods [23, 24]. One mL of cold acetonitrile–methanol (50:50 v/v) was added to 100 mL plasma to precipitate plasma proteins, and centrifuged at 6000 rpm for 10 min. The upper phase was removed and evaporated under nitrogen. After evaporation, the residue was dissolved in 50 μL mobile phase, and a 20-μL aliquot was injected into the HPLC system.

Analysis of plasma samples by HPLC–UV/DAD and LC–MS

The chromatographic system used for sample analysis was a Hewlett-Packard 1100 model including a column at 22°C (G1316 A), a quaternary pump (G 1311 A), a manual injector (G 1328 B) and a DAD detector (G 13115 B) set at 220, 310 and 350 nm. To avoid any interference and to maximize the sensitivity of identification, acquisition wavelengths of 310 nm were used for the chromatograms shown in the figures. Data acquisition was performed using a chromatography software package (Agilent Chemstation, version 9.01 1206).

Chromatographic separation of the substrate and its metabolites was performed using a stainless steel Novopak C18 column (150 x 4.6 mm i.d., 5-μm particle size). The optimal composition of the mobile phase was achieved by different mixtures of pure acetonitrile and water. Acetonitrile was filtered through a 0.45-μm GH-membrane filter. The mobile phase consisted of elution at 1 mL/min with a 5-min linear gradient consisting of 30% acetonitrile/70% water, followed 1 minute later by 80% acetonitrile/20% water, then by 12 min 80% acetonitrile/20% water after which the initial conditions were restored (14 min), and the column was reequilibrated for 19 min before the next injection.

Metabolite peaks with constant retention times were observed, and the confirmation of these was elucidated by mass spectrometry. The MS analyses were performed on a Waters Micromass ZQ using the Electron Spray Ionization (ESI) method.

RESULTS AND DISCUSSION

The HPLC chromatogram of plasma samples showed sharp, symmetrical peaks at 4.034 (M1), 8.000 (M2) and 10.370 min (substrate) when compared with the blank plasma chromatogram (Fig. 1). Good separation of all metabolites was achieved by HPLC–UV/DAD using a reverse-phase C18 column and different mixtures of pure acetonitrile and water in a linear gradient system as mobile phase. Plasma samples were prepared by precipitating plasma proteins with acetonitrile/methanol (50:50 v/v).
The advantage of this study was that the precipitation of proteins was performed without an extraction step such as liquid–liquid extraction or solid phase extraction. Protein precipitation with acetonitrile/methanol (50:50 v/v) allowed the simultaneous determination of these compounds in the plasma.

The structure of the fractions obtained via HPLC was determined via the analysis of ESI mass spectra as well as by chromatographic, UV and mass spectrometric comparison with the synthesized reference substances.

The substrate was identified in the plasma at 1–11-h intervals, with a retention time of 10.604 min. The UV spectra of these peaks were also comparable with those of the synthetic substrate (Fig. 2). ESI–mass spectrometry of the collected fraction of the substrate showed a molecular ion peak at m/z 414 (Fig. 3).

Peaks corresponding to the retention times of M1 at 4.2 and 4.247 min were detected at 6 and 9 h in the plasma samples, respectively (Fig. 2). The mass spectrum of M1 displayed a molecular ion peak at m/z 190.20 (Fig. 4). The identification of M1 was further confirmed by HPLC–UV/DAD comparison of retention time and UV spectrum with the synthesized reference substance.

The analysis of plasma extracts by HPLC–UV/DAD showed another peak at 8.158 min over 9 h (Fig. 2). The UV spectrum of the peak was comparable to that for standard M2. The mass spectrum for M2 displayed a molecular ion peak at m/z 242.65 (Fig. 3). On the basis of the identical molecular ion peak, UV spectrum and retention time, M2 was identified as a chalcone derivative, i.e. a hydrolytic product of the substrate.

The aromatic hydroxylation of the substrate and the N-acetylation metabolite of the hydrazino derivative could be observed, but it was not possible to fully determine them under these chromatographic conditions (Fig. 4).

Results obtained from the in vivo study of hydrazone showed that in rats this compound was hydrolyzed into its corresponding hydrazine and chalcone derivatives (Fig. 1): Chromatograms of (A) blank plasma; (B) plasma spiked with 20 μg/mL standards 4.034 (M1), 8.000 (M2) and 10.370, 10.716 (substrate). Below: UV spectra of M1, M2 and substrate, respectively. Mobile phase consisted of acetonitrile:water in a linear gradient system at a flow rate of 1 mL/min.
Fig. 2: HPLC profiles of rat plasma extracts obtained (A) 1.5, (B) 3, (C) 6 and (D) 9 h respectively after the administration of substrate. Chromatographic conditions were the same as described in Figure 1.

4). Therefore hydrazone degradation occurs via the hydrolysis of imine bonds, as has been previously assumed [20, 21]. The weakly electron-withdrawing chloro-substituent on the phenyl ring also increased the hydrazone hydrolysis rate, irrespective of its position on the ring [19]. Thus the 3-chlorophenyl moiety on the hydrazone-bearing quinazolinone group promotes the hydrolysis of the imine bond as mentioned substrate. These data demonstrate the importance of the hydrolysis of hydrazone analogs and also that of designing clinically useful analogs, the efficacy of which is not limited by hydrolysis.

REFERENCES

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Fig. 3: UV and ESI mass spectra of metabolites obtained from the collected fractions with retention times at 4.200 min (metabolic M1), 8.158 min (metabolic M2) and 10.622 min (metabolic substrate). Chromatographic conditions were the same as described in Figure 1.


Fig. 4: Proposed metabolic pathways of the substrate (straight arrows: observed metabolites; dashed arrows: proposed pathways).