Note

**Benzbromarone Pharmacokinetics and Pharmacodynamics in Different Cytochrome P450 2C9 Genotypes**

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Summary: Benzbromarone is a uricosuric drug and has been shown to be metabolized predominantly by cytochrome P450(CYP)2C9 in vitro findings. This study aims to investigate the influence of the CYP2C9 genotype on plasma levels of benzbromarone and 6-hydroxybenzbromarone, as well as uric acid lowering effects. A single oral dose pharmacokinetic and pharmacodynamic trial of benzbromarone (100 mg) was performed in 20 healthy volunteers, which included 15 with CYP2C9*1/*1, 4 with CYP2C9*1/*3, and 1 with CYP2C9*3/*3. The oral clearance of benzbromarone in the CYP2C9*1/*1 genotype and CYP2C9*1/*3 genotype was 58.8 ± 25.2 L/hr/kg (mean ± SD) and 51.3 ± 7.9 L/hr/kg, respectively, whereas 8.58 L/hr/kg in the CYP2C9*3/*3 genotype. The metabolic ratio (6-hydroxybenzbromarone/benzbromarone) in urine was 38.6 ± 10.7 in the CYP2C9*1/*1 genotype, 35.4 ± 12.4 in the CYP2C9*1/*3 genotype and 12.9 in the CYP2C9*3/*3 genotype. Although benzbromarone significantly increased the urinary excretion and reduced the plasma concentration of uric acid, there were no significant differences in its effects for different CYP2C9 genotypes. These results suggest a critical role for CYP2C9 in the metabolism of benzbromarone in humans and a possible risk of toxicity in the CYP2C9*3 homozygote by lowering clearance of the drug. Further studies are required to assess the clinical impact of CYP2C9 on the metabolism of benzbromarone.

Keywords: benzbromarone; cytochrome P450 2C9; polymorphism; Pharmacokinetics; uricosuric effect

Introduction

Hyperuricemia is been associated with cardiovascular diseases in a variety of studies,1–5) and reported to be present in 25% to 50% of individuals with untreated primary hypertension, about five times the frequency found in normotensive persons.6) However, whether the level of uric acid in serum is an independent cardiovascular risk factor remains an item of debate.6,7) Benzbromarone is a potent uricosuric agent, and has been used for the treatment of hyperuricemia and gout for approximately 30 years in many countries, particularly those in Europe and Japan.8–10) However, hepatic dysfunction related to the use of benzbromarone has warned,11) to contribute to its withdrawal from the markets of many European countries in 2003. Currently, the drug remains available in some countries in Europe, as well as Brazil, Japan, and several other Asian countries.10)
It is known that the uricosuric effect of benzbromarone is based on its inhibition of renal reabsorption of uric acid by proximal tubular cells by inhibiting a urate transporter (URAT1, encoded by SLC22A12). It was initially thought that debrominate compounds, such as bromobenzarone and benzaron, are the predominant metabolites of benzbromarone. However, two main hydroxylated metabolites, 1'-hydroxybenzbromarone and 6-hydroxybenzbromarone, and not bromobenzarone and benzaron, have been detected in plasma and urine samples of volunteers after a single oral administration. It has been reported that the half-life of benzbromarone is short, while 6-hydroxybenzbromarone has a much longer half-life. Recently, in vitro results from a study using human cytochrome P450 (CYP)-expressing microsome samples revealed that benzbromarone is metabolized by CYP2C9 and the main metabolite formed was 6-hydroxybenzbromarone. However, it has not been shown that CYP2C9 is involved in the metabolic pathway of benzbromarone in humans in vivo. The existence of genetic polymorphisms of CYP2C9 has been recognized to influence the activity of the enzyme and clinical consequence, and human studies have demonstrated that the CYP2C9*3 (1075A>C, Ile359Leu) variant is associated with poor metabolism related to classic CYP2C9 substrates, such as tolbutamide, glibenclamide, and warfarin. The elimination of benzbromarone has been shown to have large inter-individual differences. However, the clinical impact of different CYP2C9 genotypes on the pharmacokinetics and uricosuric effect of the drug (i.e., pharmacodynamics) has not been clarified. Thus, the aims of the present study were to assess the clinical impact of CYP2C9 genotypes on the pharmacokinetics and pharmacodynamics of benzbromarone, and to evaluate whether CYP2C9 plays a critical role in its metabolism.

Materials and Methods

Subjects: CYP2C9 genotype status was screened in 144 healthy Japanese volunteers. After the screening, 20 healthy volunteers (age range, 20–30 years old; body weight range, 49.3–71.2 kg), which included 15 with CYP2C9*1/*1, 4 with CYP2C9*1/*3, and 1 with CYP2C9*3/*3, were enrolled in this study as subjects. The CYP2C9*2 allele was not detected in any of the genotyped volunteers. All subjects were in good health, as indicated by medical history, and routine physical examination and clinical laboratory test results. No medications including herbal drugs and beverages containing grapefruit products were permitted throughout the study period. The study protocol was approved by the Ethics Committee of Hamamatsu University School of Medicine and that of University of Shizuoka. All subjects gave written informed consent before enrollment.

Study design: A standardized meal was served from two days before the administration of benzbromarone until the final day of the study. Using an open-label study protocol, a single oral dose (100 mg) of benzbromarone (Urinorm; Torii Pharmaceutical Co. Ltd, Tokyo, Japan) was administered to the 20 subjects after overnight fasting. Blood samples were taken just before and at 1, 2, 4, 6, 8, 11, and 24 hr after administration, and the plasma levels of uric acid were analyzed before and 24 hr after benzbromarone administration. In addition, uric acid levels in 24-hr urine samples were determined before and after benzbromarone administration.

Genotyping: Deoxyribonucleic acid (DNA) was extracted from peripheral whole blood samples and CYP2C9 alleles were detected using a polymerase chain reaction (PCR)-restriction fragment length polymorphism, as previously described.

Determination of concentrations in plasma and urine: The concentrations of benzbromarone, 6-hydroxybenzbromarone, benzaron, and bromobenzaron were analyzed by high-performance liquid chromatography-tandem mass spectrometry. Briefly, urine was pretreated with β-glucuronidase. As an internal standard, indomethacin (20 ng) was added to plasma and urine samples, then extracted using diethyl ether/dichloromethane (7:3, v/v). High-performance liquid chromatography was performed using an analytical column (Symmetry C18, 2.1 x 150 mm, 5.0 µm, Waters, Milford, Mass), with the mobile phase (acetonitril/0.1% formic acid, 60/40, v/v) delivered at a flow rate of 0.2 ml/min. Mass spectrometry was performed with atmospheric pressure chemical ionization in negative ion detection mode. The ion transition monitored was mass-to-charge ratio, which was from 422.9 to 250.8 m/z for benzbromarone, 438.9 to 250.7 m/z for 6-hydroxybenzbromarone, 464.9 to 93.0 m/z for benzaron, 244.9 to 81.0 m/z for bromobenzaron, and 356.0 to 312.0 m/z for indomethacin. The limits of detection were 10 ng/ml for benzaron and 6-hydroxybenzbromarone, and 1.0 ng/ml for benzaron and bromobenzaron. The inter-assay coefficients of variation were <15.6% for all compounds.

Data analysis: The pharmacokinetic parameters of benzbromarone were calculated by non-compartmental analysis using WinNonlin (version 4.1, Pharsight, Mountain View, Calif). The metabolic ratio (ratio of amount for 6-hydroxybenzbromarone to that for benzbromarone) was calculated using the post-administration 24-hr urine samples. Changes in the urinary excretion and plasma concentration of uric acid were used to determine the pharmacodynamic effect of benzbromarone.

Statistical analysis: All data are presented as means and SD. GraphPad Prism (version 3.03, GraphPad Software, San Diego, Calif) was used for all statistical analyses. A Mann-Whitney test and paired t-test were used to...
assess differences between the CYP2C9*1/*1 and CYP2C9*1/*3 genotype groups, and between before and after administration, respectively. P-values <0.05 were considered significant.

Results

Pharmacokinetics of benzbromarone and 6-hydroxybenzbromarone: The plasma concentration of benzbromarone reached maximum at 2 hr after administration in subjects with CYP2C9*1/*1 and CYP2C9*1/*3, whereas the maximum plasma concentration was attained at 6 hr after administration in subject with CYP2C9*3/*3 (Fig. 1a). Plasma concentrations at 24 hr after administration in the subjects with CYP2C9*1/*1, CYP2C9*1/*3, and CYP2C9*3/*3 were 141 ± 154, 229 ± 176, and 3380 ng/ml, respectively. The plasma concentration of 6-hydroxybenzbromarone reached maximum at 4 hr after administration in subjects with CYP2C9*1/*1 and CYP2C9*1/*3, while it continued to increase until 24 hr in the subject with CYP2C9*3/*3 (Fig. 1b).

There were no significant differences in pharmacokinetic parameters of benzbromarone between the CYP2C9*1/*1 and CYP2C9*1/*3 genotypes (Table 1). However, in the subject with CYP2C9*3/*3, the area under the plasma concentration-time curve (AUC0–24) and elimination half-life (t1/2) of benzbromarone were 3.2- and 3.7-fold, respectively, which were greater than in those with CYP2C9*1/*1. Also, the oral clearance (CL/F) was only 15% in the subject with CYP2C9*3/*3 as compared to that in those with CYP2C9*1/*1. There was significant difference in t1/2 of 6-hydroxybenzbromarone between the CYP2C9*1/*1 and CYP2C9*1/*3 genotypes (Table 1). The metabolic ratio (6-hydroxybenzbromarone/benzbromarone) in urine was 38.6 ± 10.7 in the CYP2C9*1/*1 genotype, 35.4 ± 12.4 in the CYP2C9*1/*3 genotype and 12.9 (Fig. 2). Neither bromobenzamine nor benzamine were detected in plasma and urine samples from any of the subjects.

Pharmacodynamics: In comparison to the control condition before the administration of benzbromarone, the urinary excretions of uric acid increased by 125% after administration in all subjects (Fig. 3a). There were no differences in uricosuric effects of benzbromarone among subjects with different genotypes (127% increase in CYP2C9*1/*1, 130% in CYP2C9*1/*3, 75% in

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<th>Table 1. Pharmacokinetic parameters of benzbromarone and 6-hydroxybenzbromarone in subjects with CYP2C9*1/<em>1, CYP2C9</em>1/<em>3, and CYP2C9</em>3/*3 genotypes after a single oral administration of benzbromarone (100 mg)</th>
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Each value represents the mean ± S.D.

* Significant difference between CYP2C9*1/*1 and CYP2C9*1/*3, *P <0.05. Cmax: peak plasma concentration, t1/2: elimination half-life, CL/F: oral clearance and AUC0–24: area under concentration vs. time curve

\( t_{1/2} \) could not be calculated, because concentrations of 6-hydroxybenzbromarone in a subject with CYP2C9*3/*3 increased up to 24 hr after the administration.

**Fig. 1.** Plasma concentration-time curves of (a) benzbromarone and (b) 6-hydroxybenzbromarone in subjects possessing the CYP2C9*1/*1, CYP2C9*1/*3, and CYP2C9*3/*3 genotypes after a single oral dose (100 mg) of benzbromarone. Values are shown as means ± SD
CYP2C9*3/*3 (Fig. 3a). In addition, the plasma concentrations of uric acid were significantly reduced by 59% by the administration of benzbromarone (Fig. 3b), though there were no significant differences in the plasma uric acid-lowering effects among the subjects (60% reduction in CYP2C9*1/*1, 57% in CYP2C9*1/*3, 61% in CYP2C9*3/*3) (Fig. 3b).

**Fig. 2.** Metabolic ratio in urine in subjects possessing the CYP2C9*1/*1, CYP2C9*1/*3, and CYP2C9*3/*3 genotypes after a single oral dose (100 mg) of benzbromarone. Values are shown as means ± SD.

**Discussion**

Our results are the first to show that the oral clearance of benzbromarone is markedly reduced in individuals with the CYP2C9*3/*3 genotype. It was previously reported that there are large interindividual differences in regard to the elimination of benzbromarone. However, the underlying mechanisms to explain the effects of phenotype on plasma benzbromarone concentration have not been well elucidated. The present findings suggest that CYP2C9 plays a crucial role in the metabolism of benzbromarone and it is likely that individuals with a high plasma concentration possess CYP2C9*3/*3. Interestingly, there were no significant differences in the pharmacokinetic profile of benzbromarone between subjects with CYP2C9*1/*1 and CYP2C9*1/*3. A similar observation was reported for the unbound oral clearance of S-warfarin in Caucasian patients, as those with CYP2C9*1/*3 showed no discernible difference from those with CYP2C9*1/*1. Thus, the homozygous status of CYP2C9*3/*3 may be critical for the pharmacokinetics of benzbromarone.

In the present study, benzbromarone administration significantly increased the urinary excretion of uric acid, leading to reduction in uric acid in plasma, indicating a potent uricosuric effect of benzbromarone even in healthy individuals. In contrast to the pharmacokinetic differences among CYP2C9 genotypes, the subjects with

**Fig. 3.** Effects of oral benzbromarone (100 mg) on (a) urinary excretion and (b) plasma concentrations of uric acid in subjects possessing the CYP2C9*1/*1, CYP2C9*1/*3, and CYP2C9*3/*3 genotypes. Results are shown as means ± SD.
CYP2C9*1/*1 and CYP2C9*3/*3 did not significantly differ in the levels of plasma uric acid and urinary uric acid excretion. It is likely that the plasma concentrations of benzbrornarone in the CYP2C9*1/*1 genotype group were high enough to exert maximal uricosuric effect of the drug, while the plasma concentration of benzbrornarone in the CYP2C9*3/*3 genotype was not adequate to show additive pharmacodynamic effect. In addition, since 6-hydroxybenzbrornarone has a similar pharmacodynamic effect as benzbrornarone, both the reduced plasma concentration of 6-hydroxybenzbrornarone-1 to 6 h after the administration and increased concentration of benzbrornarone in the subject with the CYP2C9*3/*3 genotype could have caused the similar pharmacodynamic effect seen in subjects possessing CYP2C9*1/*1.

Hepatic dysfunction related to the use of benzbrornarone has warned recently. In agreement with previous reports, no de-halogenated compounds, including bromobenzarone and benzaron, were detected in the plasma and urine samples from any of the present subjects following a single oral administration of benzbrornarone. Although these compounds were previously proposed as candidate metabolites causing hepatitis, neither bromobenzarone nor benzaron seems to be a major metabolite of benzbrornarone. On the other hand, benzaron itself, a benzofuran derivative similar to amidarone, has been shown to cause mitochondrial toxicity and induce hepatic injury. Since the plasma concentration of benzbrornarone in the subjects with the CYP2C9*3/*3 genotype remained high even up to 24 hours after administration, it is reasonable to assume that a very high plasma concentration may occur in patients possessing CYP2C9*3/*3 and chronically treated with benzbrornarone. We consider that the pharmacokinetic profile of the subject with CYP2C9*3/*3 may be related to the causal factor for the toxic effect of the drug.

One of the limitations of this study was that only a single subject with CYP2C9*3/*3 was enrolled and we cannot exclude the possibility that the observed results were due to a subject-specific phenomenon. However, because the incidence of the CYP2C9*3 allele is very low (0.02) in Japanese as compared to Caucasian (0.06) populations, it is very difficult to find another subject with CYP2C9*3/*3 (1/2500). It is also true that hepatitis related to the use of benzbrornarone has been reported more often in European countries, where the ratio of CYP2C9*3/*3 carriers is greater than in Japan.

In conclusion, the present results suggest a critical role of CYP2C9 in the metabolism of benzbrornarone in humans. Additional prospective and epidemiological studies are required to assess the clinical impact of CYP2C9 in subjects treated with benzbrornarone.

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References


