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Determination of the stereochemical composition of the major metabolites of verapamil in dog urine with enantioselective liquid chromatographic techniques

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Abstract

The stereochemical composition of verapamil and seven of its basic-extractable metabolites, isolated from the urine of dogs administered oral racemic verapamil, was determined by HPLC, using an Ultron OVM (ovomucoid) column. One dog was given oral (*R*)-verapamil alone in order to discriminate the (*R*)- and (*S*)-enantiomers of the metabolites. Structure identification of the isolated verapamil metabolites was accomplished using a combination of HPLC–MS and FAB-MS–MS techniques. Six of the urinary verapamil metabolites, including verapamil, were predominantly of the (*R*)-configuration, whereas one of the metabolites was predominantly in the (*S*)-form. The remaining isolated metabolite was comprised of approximately equal amounts of the two forms.

1. Introduction

The calcium channel blocking drug verapamil (Fig. 1), an effective agent in the treatment of angina pectoris and supraventricular arrhythmias [1], is clinically used as a racemic mixture of equal amounts of two enantiomers, (*R*)-and (*S*)-verapamil. These enantiomers differ considerably in their pharmacological potency with (*S*)-verapamil being 10–20 times more potent than (*R*)-verapamil in slowing cardiac A-V conduction velocity in man [2], dog [3], and in the rabbit [4]. The pharmacokinetics of the verapamil enantiomers also differ substantially from each other in man [5,6] and in the dog [7]. These differences are most notable after oral dosing. In both species, the oral availability of the (*R*)-verapamil is 5–15 times greater than that of the more potent (*S*)-enantiomer, indicating that the presystemic metabolism of verapamil in man and in dog is highly stereoselective for (*S*)-verapamil. McIlhenny [8] previously studied the in-vivo metabolism of racemic verapamil in the dog; however, the stereochemical aspects of verapamil metabolism were not investigated. At least two verapamil metabolites, norverapamil

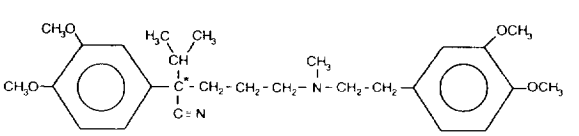

Fig. 1. Chemical structure of racemic verapamil. The * denotes the chiral carbon.

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and the primary amine of verapamil [9,10], when used as their respective racemic mixtures, have been shown to possess some negative dromotropic and/or vasodilator activity. However, their pharmacodynamic activities following the administration of racemic verapamil in vivo may differ vastly from that estimated when they are given directly as a racemic mixture due to stereoselective metabolism of verapamil.

The major aim of this study was to determine the stereochemical composition of the major basic-extractable metabolites of verapamil excreted in the urine. Previous studies of the in-vivo or in-vitro metabolism of verapamil enantiomers have relied on either sophisticated and expensive stable isotope mass spectrometric techniques or on the administration of the enantiomers separately [11–14]. These approaches have certain disadvantages, not the least of which is that only laboratories with expensive mass spectrometric equipment and/or sophisticated synthetic expertise are able to undertake such investigations. In this study, we used conventional achiral chromatography techniques to separate and purify verapamil and seven of its basic-extractable metabolites, and a combination of LC–thermospray-MS and FAB-MS–MS techniques to obtain the structures of the isolated metabolites. Separation of the enantiomers of the isolated metabolites was accomplished using a chiral (ovomucoid) column. Although the disposition of the enantiomers of a variety of racemic drugs and agents has been studied using a variety of chiral columns, few investigations have extended the utility of chiral columns to the enantiomeric separation of chiral metabolites.

2. Experimental

2.1. Chemicals

Racemic verapamil-HCl (Isoptin) and norverapamil-HCl were kind gifts from Knoll Pharmaceutical Co. (Whippany, NJ, USA). *R*-Verapamil-HCl (LU 33925) was a kind gift of Dr. Brode at Knoll AG (Luwigshafen, Germany). HPLC-grade potassium hydroxide, phosphoric acid, potassium phosphate, ammonium acetate, acetic acid, and ethanol were purchased from Fisher Scientific Co. (Pittsburgh, PA, USA). Glusulase (90 000 U of β-glucuronidase and 1000 U sulfatase/ml) was purchased from Du Pont Chemical Co. (Boston, MA, USA). Glass-distilled acetonitrile, pentane, and methylene chloride were purchased from Burdick and Jackson Laboratories (Muskegon, MI, USA). Glass-distilled water was used for all aqueous reagents.

2.2. Animals

Six female mongrel dogs (14–20 kg) were used in this study. Prior to racemic verapamil oral dosing, drug free urine was collected from each animal. Each dog was orally dosed with four doses of racemic verapamil (80 mg every 8 h) for a total dose of 320 mg. Urine was collected in flasks containing sodium bisulfite for up to 48 h after the last oral dose and was stored at −30°C. On a separate occasion, separated by three weeks, one of the animals was orally administered two doses, separated by 8 h, of 80 mg of (*R*)-verapamil. Urine was collected for 48 h after the last oral dose, and was stored at −30°C.

2.3. Extraction

Only a basic urine extract was used in these studies. The pH of 2-ml urine aliquots was adjusted to 11 with 5 *M* NaOH. The urine samples were extracted twice with 10 ml of pentane–methylene chloride (7:3, v/v). The organic phases were pooled and evaporated under nitrogen, and reconstituted in 200 μl of methanol. Control urine samples (verapamil-free) were treated in the same fashion. Aliquots (100 μl) were injected onto the achiral HPLC system.

To a separate set of 2-ml urine samples was added 0.5 ml of 0.1 *M* sodium acetate buffer (pH 4.5) and 100 μl of Glusulase (β-glucuronidase + aryl sulfatase). The samples were placed in a water bath at 37°C for 18 h. After incubation, the samples were extracted twice at pH 11 with 10 ml of pentane–methylene chloride (7:3, v/v), evaporated under nitrogen, reconstituted in methanol and subjected to achiral HPLC analy-

sis. Aliquots (2 ml) of verapamil-free dog urine were treated in the same manner.

2.4. Achiral HPLC separation of metabolites in basic extract

The HPLC system consisted of two Model 510 high-pressure pumps, a Model 720 system controller, a Model 730 data module (Waters Associates, Milford, MA, USA), and a Model 7125 sample injector (Rheodyne, Cotati, CA, USA). The HPLC column was an achiral 10-μm C_{18} column (25 cm × 10 mm I.D.; Alltech Associates, Deerfield, IL, USA). A flow-rate of 3 ml/min was used. The effluent from the column was monitored by a Model FS-970 fluorescence detector (Kratos Analytical Instruments, Ramsey, NJ, USA) at an excitation wavelength of 280 nm. A 340-nm cut-off filter was used to monitor the emission. For optimal separation of verapamil metabolites, two mobile phases (A) acetonitrile and (B) 0.05 *M* ammonium acetate (pH 4.5 adjusted with acetic acid) were used, employing a linear gradient elution in which the percent of solvent A increased from an initial 0% to 70% at a rate of 1%/min. Verapamil-related peaks were determined by comparison to the HPLC chromatogram of the basic extracts from control urine. The verapamil-related peaks were collected with a Gilson Model 203 fraction collector (Gilson Medical Electronics, Middleton, WI, USA) and were freeze-dried. The isolated metabolites were further purified by re-chromatographing them individually using the HPLC system described above, but using isocratic mobile phases made up of 0.05 *M* ammonium acetate (pH 4.5 adjusted with acetic acid) and varying (15–60%) amounts of acetonitrile. The peaks were collected, and freeze-dried for structure identification and stereochemical composition.

2.5. Structure identification of metabolites

HPLC–MS

The molecular mass information of the isolated metabolites was obtained using direct loop injection LC–thermospray-MS. The LC–thermospray-MS system consisted of a Model 510 pump (Waters Associates), a Model 7125 sample injector (Rheodyne), and a Finnigan MAT 4500 quadrupole mass spectrometer (San Jose, CA, USA) equipped with a Finnigan thermospray ion source, operating in the positive-ion mode. The vaporizer and source temperatures were 110°C and 350°C, respectively. The mobile phase consisted of acetonitrile–water (35:65, v/v) in 0.05 *M* ammonium acetate with 0.2 *M* acetic acid (pH 4.0) and the flow-rate was 1.2 ml/min.

FAB-MS with B/E linked-field scan

The isolated verapamil metabolites, including verapamil, were analyzed by positive ion FAB-MS–MS, using a JEOL HX 110 HF double-focusing forward geometry (E,B) mass spectrometer (Peabody, MA, USA), equipped with a high-field magnet, collision chamber in the first field-free region and a DEC LSI 11/73 data system. The accelerating voltage was 10 kV. FAB ionization using xenon gas, and a gun operating at 6 kV with thioglycerol matrix were used.

For B/E linked-field scans, the magnetic field (B) and the electric field (E) are scanned at a constant ratio. The scan range for static FAB was typically 100–600 amu. For B/E linked-field scanning, the scan range was 0–10% above the precursor ion mass. The scan cycle was typically 11 s. For B/E linked-field scan calibration sodium iodide and glycerol were used. A range of masses of ± 1% around the precursor ions, formed by FAB in the ionization source, were selected and were collisionally dissociated. The fragment ions formed in the first field-free region were detected. Mass spectra were recorded by the data system.

2.6. Resolution of enantiomers of isolated metabolites

The separation of the enantiomers of the metabolites and of verapamil was accomplished by HPLC. The system consisted of a Model 510 pump, a Model 720 system controller, a Model 730 data module (Waters Associates), and a Model 7125 sample injector (Rheodyne). The column used was a 15 cm × 4.6 mm I.D. chiral

OVM (ovomucoid) protein column (Mac-Mod Analytical, Chadds Ford, PA, USA). The flow-rate was kept at 1 ml/min for all analyses, and the detection was by means of fluorescence, using an excitation wavelength of 203 nm and a 270 nm emission cut-off filter. Discrimination between the (*S*)- and (*R*)-forms of each verapamil metabolite was accomplished by comparing the racemic chromatograms to that for the respective metabolite isolated from the animal that received only (*R*)-verapamil. The mobile phase used in the resolution of the enantiomers of all the metabolites, except for norverapamil (metabolite 7) and N-demethyl-O-demethyl verapamil (metabolite 3), consisted of a mixture of ethanol and 0.013 *M* KH_2PO_4 buffer. The pH of the buffer was adjusted to 7.0 with phosphoric acid. The percent of ethanol was 10% for the enantiomeric resolution of metabolites 1 and 2, and 18% for metabolites 4, 5, 6, and verapamil (8). For the optimal resolution of the enantiomers of norverapamil (7) and N-demethyl-O-demethyl verapamil (metabolite 3), the mobile phase consisted of acetonitrile and 0.013 *M* KH_2PO_4 (pH 7.0) (12:88, v/v). The *S*/*R* enantiomeric ratio was calculated as the ratio of the area under the peak for the (*S*)-enantiomer to the area under the peak for the (*R*)-enantiomer.

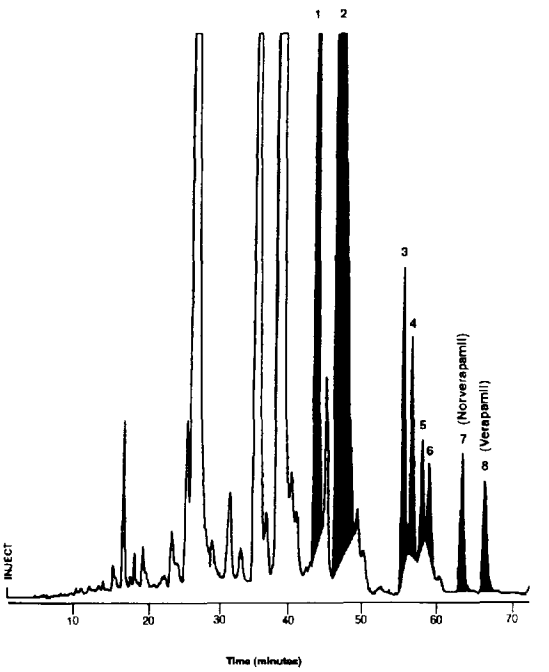

Fig. 2. HPLC-fluorescence tracing of a pH 11 pentane–methylene chloride (70:30, v/v) extract of dog urine after β-glucuronidase hydrolysis. Shaded peaks represent verapamil and its metabolites not present in control (verapamil-free) urine extract.

3. Results

3.1. HPLC isolation of basic extractable metabolites

Fig. 2 shows an HPLC-fluorescence chromatogram of a pH 11 urine extract of the 0–48 h urine sample from one of the dogs after enzymatic hydrolysis with glusulase. The shaded peaks, numbered 1–8 in Fig. 2, were absent in the control urine extract. Metabolites 7 and 8 had the same retention times as the norverapamil and verapamil synthetic standards, respectively. The major difference between the chromatograms of the hydrolyzed and nonhydrolyzed (not shown) samples was that metabolites 3–6 were absent (or substantially reduced) in the nonhydrolyzed samples, indicating that these metabolites were mostly excreted in the urine as glucuronic acid and/or sulfate conjugates. Although precise quantitation was not done in this study due to the lack of either radiolabeled verapamil or synthetic standards of most of the metabolites, based on fluorescence intensity, metabolites 1 and 2 appeared to be the most abundant of the verapamil metabolites contained in the basic extract. The fluorescent intensities of verapamil and norverapamil were approximately equal.

3.2. Structure identification of the verapamil metabolites

With the exception of verapamil and norverapamil, no synthetic standards of any of the known verapamil metabolites were available in order to confirm structure identification. Therefore, all structures of the isolated verapamil

metabolites derived in this investigation are putative structures. Structure identification relied on the combination of LC–thermospray-MS, FAB-MS–MS techniques, and on in-vivo verapamil metabolism data obtained by other investigators [8,15].

Identification of the structure of metabolites 1–8 was carried out using LC–thermospray-MS. $(M+H)^+$ ions were obtained, but due to the lack of fragmentation of the molecule, structural analysis was not possible by this method. The $(M+H)^+$ ions for each metabolite, which also happened to be the base ion, are listed in Table 1. Metabolites 1 and 2 had molecular masses of 276 and 290, respectively. Metabolite 3 and 4 had the same molecular mass of 426, and metabolites 5 and 6, and norverapamil (7) all had a molecular mass of 440. Verapamil gave a molecular mass of 454 on LC–thermospray-MS.

Structural identification of the isolated metabolites of verapamil was obtained using positive-ion FAB-MS with B/E linked-field scanning of the $(M+H)^+$ precursor ion. Fig. 3 shows the B/E linked-field scan of the $(M+H)^+$ ions for metabolites 1, 2, and 3; Fig. 4 shows the B/E linked-field scans for metabolites 4, 5 and 6. The fragmentation pattern of each metabolite is shown in each B/E linked-field scan spectrum. That fragmentation pattern suggested the structures of each metabolite. The identity of norverapamil (7) and verapamil (8) was confirmed by comparison of the B/E linked-field scans of these metabolites with synthetic standards.

Metabolite 1 ($M_r = 276$) was identified, based on its fragmentation pattern, as the primary amine of verapamil. Metabolite 2 ($M_r = 290$), which was the most abundant metabolite excreted in the urine, was identified as N-dealkylverapamil. Metabolites 3, 4, 5, and 6 all appeared to be O-demethylated metabolite products. Metabolites 3 and 4 both had a $M_r = 426$. The mass spectrometric fragmentation pattern suggested that metabolite 3 (Fig. 3) was (N-demethyl,O-demethyl)-verapamil, with the loss of the methyl group occurring from the dimethoxyphenyl-acetonitrile ring. Determination of whether the O-demethylation occurred at the *meta* or *para* position was not investigated. The structure of metabolite 4 (Fig. 4) appears to be O,O-demethylated verapamil, with O-monodemethylation occurring in each one of the two ring systems. Metabolites 5 and 6 (Fig. 4), both of which had $(M+H)^+$ ions at m/z 441 on LC–thermospray-MS analysis, appeared also to be isomers of O-monodemethyl verapamil. The spectrum for metabolite 5 suggests that the loss of the methyl group is from the dimethoxyphenyl-acetonitrile ring; for metabolite 6, the

Table 1
Molecular masses of verapamil and its metabolites as determined by LC–thermospray-mass spectrometry

Metabolite[a]	$(M+H)^+$ [b]	Molecular mass	Identity
1	277	276	N-Demethyl, N-dealkyl verapamil[c]
2	291	290	N-Dealkyl verapamil[c]
3	427	426	N-Demethyl, O-demethyl verapamil[c]
4	427	426	O,O-Demethyl verapamil[c]
5	441	440	O-Demethyl verapamil[c,d]
6	441	440	O-Demethyl verapamil[c,e]
7 (Norverapamil)	441	440	Norverapamil
8 (Verapamil)	455	454	Verapamil

[a] Metabolite number corresponds to achiral HPLC separation (Fig. 2).
[b] Data obtained from LC–thermospray-MS.
[c] Identity of metabolites as suggested by FAB-MS–MS analysis (Figs. 3 and 4).
[d] O-Demethylation occurred in the dimethoxyphenyl-acetonitrile portion of the molecule.
[e] O-Demethylation occurred in the substituted phenethylamine ring.

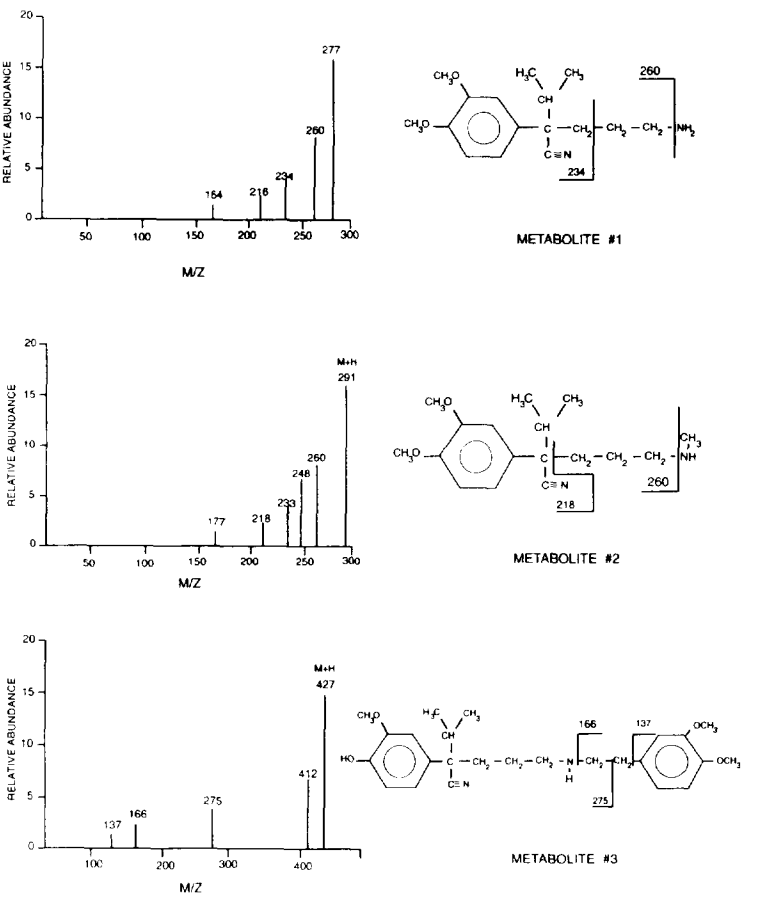

Fig. 3. B/E linked-field scan mass spectra of verapamil metabolites 1, 2, and 3.

loss of the methyl group from the substituted phenethylamine ring is suggested.

3.3. Chiral column separation of the enantiomers of the verapamil metabolites

Fig. 5 shows the chiral HPLC chromatograms of the resolution of the primary amine of verapamil (metabolite 1), N-dealkyl-verapamil (metabolite 2), and (N-demethyl,O-demethyl)- verapamil (metabolite 3). Fig. 6 shows the chiral HPLC chromatograms of the resolution of the O,O-demethyl verapamil (metabolite 4), and the two O-monodemethyl isomers of verapamil (metabolites 5 and 6). The chromatograms of the chiral separation of the enantiomers of norverapamil (7) and that of verapamil (8) are shown in Fig. 7. With the exception of (N-demethyl,O-demethyl)-verapamil (metabolite 3), the (*S*)-enantiomer of the all of the metabolites eluted prior to the (*R*)-enantiomer. The *S*/*R* enantiomeric ratio and the retention times for the *S*- and *R*-enantiomers of verapamil and its metabolites from the six animals are summarized in Table 2. The only metabolite that had a ratio substantially greater than 1 (3.36) was the primary amine of verapamil (metabolite 1). N-Dealkyl verapamil (metabolite 2), the most abundant metabolite based on fluorescence intensity, had a ratio of ca. 1. The remainder of

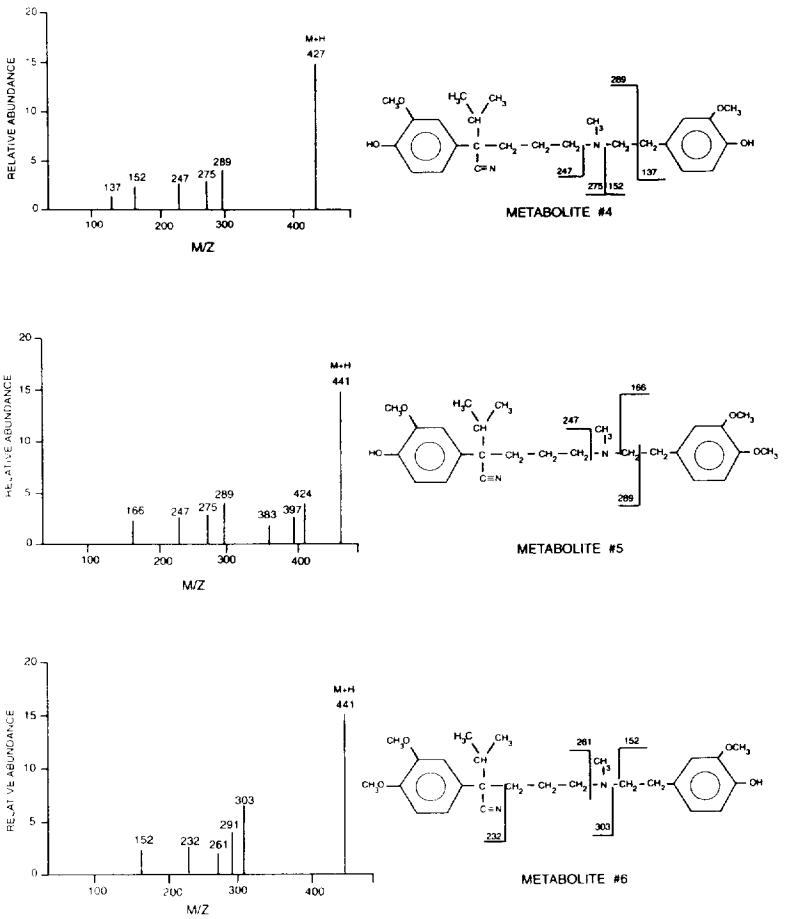

Fig. 4. B/E linked-field scan mass spectra of verapamil metabolites 4, 5, and 6.

the verapamil metabolites, including verapamil itself, had an *S*/*R* enantiomeric ratio substantially less than one.

4. Discussion

Over the past decade, there has been a growing interest in the stereochemical aspects of drug disposition and metabolism. Stereochemical considerations are extremely important in cases where the drug enantiomers differ in their pharmacological and/or pharmacokinetic behavior. Part of this growing interest over the years has been due to the recent development of new analytical methods and approaches, employing commonly available GC and HPLC equipment, to separate the enantiomers of a racemic drug. Prior to this time, much of the information on the disposition and metabolism of drug enantiomers has relied either on the separate administration of the drug enantiomers or on elegant stable-isotope mass spectroscopic techniques. Both of these techniques have certain disadvantages. A major disadvantage of the former approach is that it fails to directly evaluate possible enantiomer–enantiomer interactions that may occur when the drug is used as a racemic mixture. The latter technique, a highly sophisticated approach, requires a costly mass

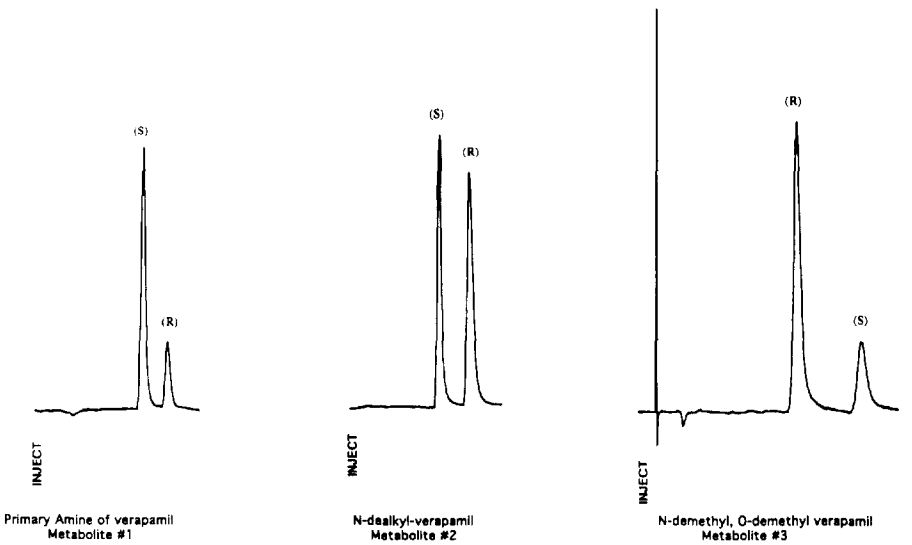

Fig. 5. HPLC-fluorescence tracings of the (*S*)-and (*R*)-enantiomers of verapamil metabolites 1, 2, and 3 using an Ultron OVM chiral column.

spectrometer and a high level of chemical expertise in order to synthesize the stable isotope-incorporated drug. The use of chiral-column chromatographic techniques overcomes these handicaps. However, chiral chromatographic techniques are not without certain disadvantages. One major drawback to the technique, as seen in the present study, is that it is labor-intensive, involving, at least, two sequential chromatographic procedures, one involved in the

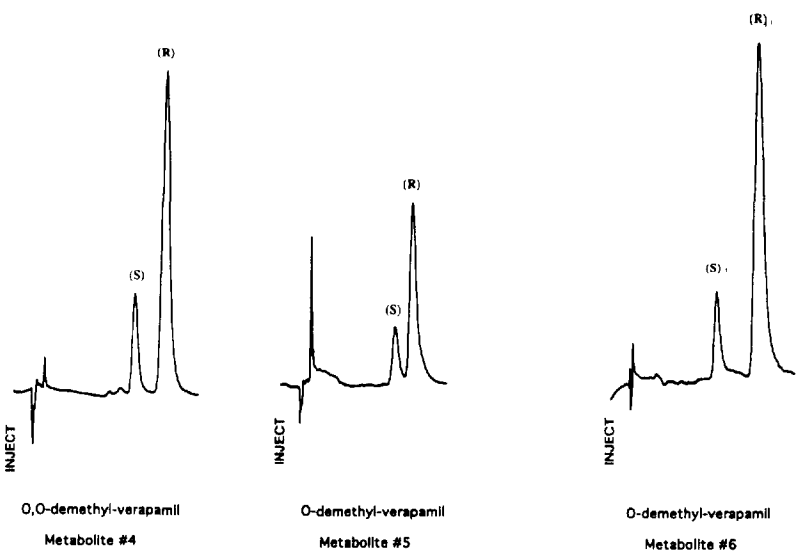

Fig. 6. HPLC-fluorescence tracings of the (*S*)-and (*R*)-enantiomers of verapamil metabolites 4, 5, and 6 using an Ultron OVM chiral column.

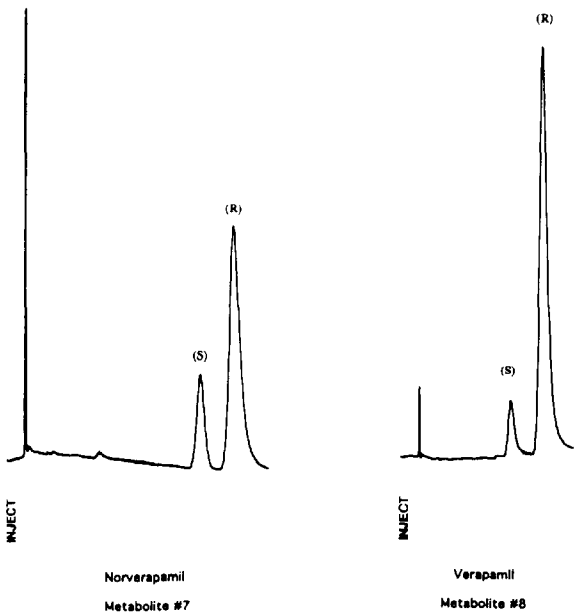

Fig. 7. HPLC-fluorescence tracings of the (*S*)-and (*R*)-enantiomers of norverapamil (metabolite 7) and verapamil (metabolite 8) using an Ultron OVM chiral column.

isolation and purification of the racemic agents and the other in resolving their enantiomers. This drawback can be partially overcome by using column-switching techniques, as has been previously demonstrated for verapamil and norverapamil [16,17]. However, as seen in the present study, when different mobile phases are required to resolve the enantiomers of numerous metabolites, the efficiency of column-switching techniques decreases and would be limited after single injections to resolving the enantiomers of substrates that employ the same mobile phase.

The most significant aspect of the present investigation was to extend the utility of chiral-column chromatographic techniques to resolve the enantiomers of the major metabolites of the calcium channel blocker verapamil. The vast majority of reports using chiral columns focused on the separation of the enantiomers of the parent racemic compound. There has been little documentation of their use to resolve the enantiomers of chiral metabolites.

Although definitive structural identification of most of the metabolites of verapamil isolated in the dog in this investigation could not be confirmed due to the lack of synthetic metabolite standards, the structures determined here, based on LC–thermospray-MS and especially on FAB-MS–MS analyses are remarkably similar to those found in vivo by McIlhenny [8] in the dog and by Eichelbaum et al. [15] in man. These metabolites consisted mostly of N-dealkylated, N-demethylated, and O-demethylated products. Based on in-vivo radioactivity studies these metabolites represent over 90% of the urinary metabolites of verapamil [8,15]. In agreement also with the other in-vivo studies, we found that

Table 2
Enantiomeric composition of verapamil and its basic extractable metabolites from urine of dogs given oral racemic verapamil

Metabolite	Identification	(*S*)-Retention time[a] (min)	(*R*)-Retention time[a] (min)	*S*/*R* Ratio[b]
1	N-Demethyl, N-dealkyl verapamil	12.3	15.5	3.36 ± 0.61
2	N- Dealkyl verapamil	12.8	17.3	1.02 ± 0.04
3	N-Demethyl, O-demethyl verapamil	25.7	18.2	0.58 ± 0.10
4	O,O-Demethyl verapamil	13.0	16.3	0.33 ± 0.08
5	O-Demethyl verapamil	12.7	14.7	0.21 ± 0.04
6	O-Demethyl verapamil	11.5	16.1	0.19 ± 0.04
7	Norverapamil	22.4	26.3	0.26 ± 0.05
8	Verapamil	12.6	16.2	0.12 ± 0.03

[a] On OVM (ovomucoid) column under the specified HPLC conditions described in Experimental section.
[b] Ratio calculated based on the areas under the (*S*)-and (*R*)-enantiomer chromatographic peaks.
Data expressed as mean ± S.D., $n = 6$.

most of the O-demethylated metabolites were mostly excreted in the urine as glucuronic acid and/or sulfate conjugates. However, extraction of the O-demethylated (phenolic) metabolites at pH 11, as employed in these studies, may have been incomplete.

One notable difference between the findings of the present investigation and McIlhenny's dog study [8] is that in his study norverapamil was not found, whereas definitive structure identification of its presence in the dog urine was found in this investigation. We have no explanation for this discrepancy. Previous verapamil pharmacokinetic studies in the dog [7] found norverapamil plasma concentrations equaling those of verapamil after oral verapamil administration. One other notable difference between the findings in the present study and those of both McIlhenny [8] and Eichelbaum et al. [15] was the apparent absence in our study of an O-monodemethylated metabolite of N-dealkyl-verapamil. This metabolite was found in the urine of both man and dog given verapamil [8,15]. It is possible that either this verapamil metabolite was not extracted at the pH used in this study or that it co-eluted with an endogenous substance in the urine and therefore could not be detected.

The oral availability of (*R*)-verapamil is substantially greater than that of the more pharmacologically active (*S*)-verapamil in man [6] and in dogs [7]. The mechanism for this effect is likely due to presystemic stereoselective hepatic metabolism of (*S*)-verapamil. If such is the case, then it stands to reason that at least one of the major metabolites of verapamil must exist predominantly in the (*S*)-configuration and be excreted as such. Of the seven metabolites of verapamil isolated from the urine, only the primary amine of verapamil (metabolite 1) had a greater amount of the (*S*)-enantiomer than of its optical antipode ($S/R = 3.36$). The major urinary excreted metabolite, N-dealkyl verapamil (metabolite 2), had an S/R enantiomeric ratio in the dog of ca. 1, whereas all the other metabolites, which include the O-demethylated isomers of verapamil (metabolites 5 and 6), O,O-demethyl verapamil (metabolite 4), the O-demethylated metabolite of norverapamil (metabolite 3), the active metabolite norverapamil, and verapamil itself were excreted predominately in the (*R*)-configuration. On the surface, these data suggest that the lower oral availability of (*S*)-verapamil compared to (*R*)-verapamil seen in the dog is due to the stereoselective metabolism of (*S*)-verapamil to the primary amine of verapamil. However, because the majority of the racemic verapamil dose (ca. 65%) in the dog undergoes biliary excretion [8], definitive conclusions concerning which metabolites or metabolic pathways are responsible for the stereoselective metabolism of verapamil in the dog based solely on urinary data cannot be made. Further studies directed to the stereochemical composition of the biliary-excreted metabolites of verapamil as well as to in-vitro verapamil metabolism in dogs will more clearly answer the question of which verapamil metabolite(s) or metabolic pathway(s) contribute to the stereoselective metabolism of (*S*)-verapamil. In addition, such studies will allow a more accurate comparison of the stereochemical composition of verapamil metabolites between dog and man.

It has been shown that norverapamil [9] and the primary amine of verapamil [10], when given as racemic mixtures, exhibit some vasodilator and electrophysiological activities similar to verapamil and they have been classified as active metabolites. Although these studies did not directly address the issue of active metabolites, the results of this study illustrate that potential errors can occur in assigning activities to metabolites if their stereochemical considerations are not taken into account. For example, assuming that most of the pharmacological activity of the verapamil metabolites reside with the (*S*)-configuration, then in the dog the contribution of norverapamil, which appears to be urinary-excreted mainly in the (*R*)-configuration ($S/R = 0.26$), would be lower than that predicted from its activity following intravenous administration of racemic norverapamil. The converse may be the case for the primary amine of verapamil, because it is predominantly excreted in the urine in the (*S*)-configuration.

In conclusion, we have demonstrated the utility of chiral column chromatographic techniques

to resolve directly the enantiomers of verapamil metabolites. These techniques can be utilized by a large population of laboratories interested in the disposition and metabolism of chiral compounds, because they employ commonly used LC equipment and commercially available chiral columns. Although the present investigation focused on verapamil metabolites, the utilization of these techniques is likely applicable to chiral metabolites of other racemic drugs.

Acknowledgement

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References

[1] D. McTavish and E.M. Sorkin, *Drugs*, 38 (1989) 19.
[2] H. Echizen, M. Manz and M. Eichelbaum, *J. Cardiovas. Pharmacol.*, 12 (1988) 543.
[3] K. Satoh, T. Yanagisawa and N. Taira, *J. Cardiovas. Pharmacol.*, 2 (1980) 309.
[4] J.C. Giacomini, W.L. Nelson, L. Theodore, F.M. Wong, D. Rood and K.M. Giacomini, *J. Cardiovas. Pharmacol.*, 7 (1985) 469.
[5] M. Eichelbaum, G. Mikus and B. Vogelgesang, *Brit. J. Clin. Pharmacol.*, 17 (1984) 453.
[6] B. Vogelgesang, H. Echizen, E. Schmidt and M. Eichelbaum, *Brit. J. Clin. Pharmacol.*, 18 (1984) 733.
[7] S.A. Bai, S.M. Lankford and L.M. Johnson, *Chirality*, 5 (1993) 436.
[8] H.M. McIlhenny, *J. Med. Chem.*, 14 (1971) 1178.
[9] G. Neugebauer,*Cardiovas. Res.*, 12 (1978) 247.
[10] K.E. Johnson, S.M. Balderston, J.A. Pieper, D.E. Mann and M.J. Reiter, *J. Cardiovas. Pharmacol.*, 17 (1991) 830.
[11] G. Mikus, M. Eichelbaum, C. Fischer, S. Gumulka, U. Klotz and H.K. Kroemer, *J. Pharmacol. Exp. Ther.*, 253 (1990) 1042.
[12] W.L. Nelson, L.D. Olsen, D.B. Beitner and R.J. Pallow, *Drug Metab. Dispos.*, 16 (1988) 184.
[13] W.L. Nelson and L.D. Olsen, *Drug Metab. Dispos.*, 16 (1988) 834.
[14] H.K. Kroemer, H. Echizen, H. Heidemann and M. Eichelbaum, *J. Pharmacol. Exp. Ther.*, 260 (1992) 1052.
[15] M. Eichelbaum, M. Ende, G. Remberg, M. Schomerus and H. Dengler, *Drug Metab. Dispos.*, 7 (1979) 145.
[16] Y. Oda, N. Asakawa, T. Kajima, Y. Yoshida and T. Sato, *Pharm. Res.*, 8 (1991) 997.
[17] Y.Q. Chu and I.W. Wainer, *J. Chromatogr.*, 497 (1989) 191.