

METABOLISM AND DISPOSITION OF THE ANTIHYPERTENSIVE AGENT MOXONIDINE IN HUMANS

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ABSTRACT:

The metabolism and pharmacokinetics of moxonidine, a potent central-acting antihypertensive agent, were studied in four healthy subjects after a single oral administration of approximately 1 mg (~60 μ Ci) of [¹⁴C₃]moxonidine. Moxonidine was rapidly absorbed, with peak plasma concentration achieved between 0.5 to 2 h post-dose. The maximal plasma concentration and the area under the curve of unchanged moxonidine are lower than those determined for radioactivity, indicating presence of circulating metabolite(s). The total recovery of radiocarbon over 120 h ranged from 99.6 to 105.2%, with 92.3 to 103.3% of the radioactivity excreted in the urine and only 1.9 to 7.3% of the dose excreted in the feces. Thus, renal elimination represented the principal route of excretion of radioactivity. Metabolites of moxonidine were identified in urine and plasma samples by high performance liquid chromatography

and liquid chromatography-tandem mass spectrometry. Oxidation of moxonidine on the methyl group or on the imidazoline ring resulted in the formation of hydroxymethyl moxonidine, hydroxy moxonidine, dihydroxy moxonidine, and dehydrogenated moxonidine. Metabolite profiling results indicated that parent moxonidine was the most abundant component in the urine. The dehydrogenated moxonidine was the major urinary metabolite as well as the major circulating metabolite. Moxonidine also underwent phase II metabolism, generating a cysteine conjugate. In summary, moxonidine is well absorbed after oral administration. The major clearance pathway for moxonidine in humans is via renal elimination. Furthermore, seven metabolites were identified with three metabolites unique to humans.

Moxonidine is a new antihypertensive agent that acts on central nervous system imidazoline receptors to decrease sympathetic nervous system tone (Ernsberger et al., 1992; Prichard and Graham, 1996). It has been marketed throughout Europe for the treatment of hypertension. The safety and efficacy of moxonidine in hypertension patients has been established (Ollivier et al., 1992; Sides et al., 1998). In comparison to clonidine and rilmenidine, moxonidine has fewer adverse effects such as dry mouth and sedation because it is considerably more selective for the I₁ receptor than the α_2 receptor that is associated with these indicated side effects (Yu and Frishman, 1996). In elderly patients, the clearance of moxonidine is reduced, and the area under the curve of unchanged drug is greater in comparison with younger patients suggesting the existence of an age-related decrease in metabolism (Theodor et al., 1996). Moxonidine has a relatively short half-life (about 2–3 h). However, it has long duration of effect and is given only once daily clinically. There is no clear correlation between the pharmacokinetics and pharmacodynamic effect of moxonidine.

To determine whether the long duration of moxonidine is attributed to the production of active metabolite(s), efforts were undertaken to identify and synthesize major metabolites of moxonidine and test their pharmacological activity. Moxonidine is very extensively metabolized in rats, and more than fifteen metabolites have been identified in different biological matrices (He et al., 2000). Despite the widespread clinical use of moxonidine, the metabolic fate of moxonidine in humans has not been fully investigated due to the limited analytical technologies available when the compound was originally developed. Two human metabolites of moxonidine were previously reported, but the data were preliminary and require further confirmation with current analytical technologies. The present study was conducted to characterize the metabolism and disposition of moxonidine in healthy subjects following a single oral administration of [¹⁴C₃]moxonidine (Fig. 1) and to establish a complete metabolic profile using HPLC¹

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¹ Abbreviations used are: HPLC, high performance liquid chromatography; LC/MS/MS, liquid chromatography-tandem mass spectrometry; MS, mass spectrometry; APCI, atmospheric pressure chemical ionization; RE, relative error, a measure of accuracy of a group of values; RSD, relative standard deviation, a measure of precision; AUC, area under the plasma concentration versus time curve; OCT, organic cation transporter; M1, dehydrogenated moxonidine; M2, putative *N*-oxide of dehydrogenated moxonidine; M3, hydroxy moxonidine; M4, dihydroxy moxonidine; M5, hydroxymethyl moxonidine; M6, cysteine conjugate minus chlorine; M7, unknown metabolite; GSH, glutathione; TLC, thin layer chromatography.

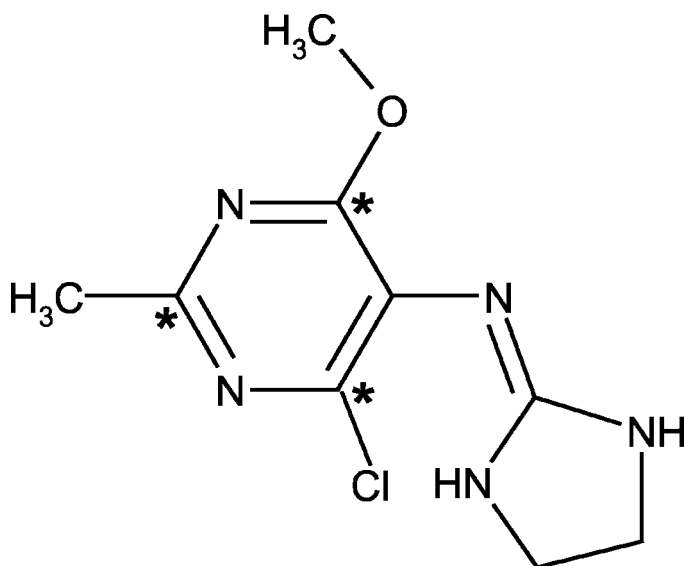


Fig. 1. Chemical structure of triple-labeled moxonidine (* indicates position of ^{14}C radiolabel).

and tandem mass spectrometry (LC/MS/MS). The pharmacokinetics and elimination profile of moxonidine were also determined in this study.

Materials and Methods

Chemicals and Materials. Unlabeled moxonidine with a purity of 99.9% (HPLC) was synthesized at Lilly Research Laboratories (Indianapolis, IN). [^{14}C]₃moxonidine was synthesized by the radiochemistry group in the Department of Drug Disposition, Lilly Research Laboratories. HPLC grade acetonitrile and methanol and grade ammonium acetate were purchased from J. T. Baker (Phillipsburg, NJ). American Chemical Society grade trichloroacetic acid was purchased from Fisher Scientific (Fair Lawn, NJ). Carbo-Sorb and Permafluor E+ liquid scintillant were purchased from Canberra Packard (Pangbourne, Berks). Milli-Q was purchased from Millipore Corp. (Bedford, MA). Varian C₁₈ solid phase extraction cartridge (Harbor City, CA) was used to extract metabolites from urine. LC-ABZ columns obtained from Supelco, Inc. (Bellefonte, PA) were used for chromatography.

Subjects, Dosing, and Sample Collections. Four healthy male subjects (55–65 years old) participated in the study. Each subject was given a single oral dose of 1 mg [^{14}C]₃moxonidine (~60 μCi). The dosing solution was administered orally along with 200 ml of water. Subjects were fasted from 10:00 PM the evening before, until 4.5 h after drug administration at 7:30 AM. Prescribed and over-the-counter medication were not permitted for 14 and 7 days, respectively, before dosing until after the subjects had been discharged from the unit. Blood samples (~7 ml) were obtained at predose and at 5, 10, 15, and 30 min, then 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, 48, and 72 h postdose. In addition, a larger volume of blood (~40 ml) was taken at 1, 3.5, and 7 h postdose for the purposes of metabolite identification. Urine samples were collected at 0- to 2-, 2- to 4-, 4- to 6-, 6- to 8-, 8- to 12-, 12- to 24-, 24- to 36-, 36- to 48-, 48- to 72-, 72- to 96- and 96- to 120-h postdose. Fecal samples were collected at 24-h intervals for the duration of the study period. Expired air samples were taken for approximately 5 min at predose and at 0.5, 1, 2, 3, and 4 h postdose. Plasma and urine samples were kept at approximately -20°C before they were analyzed for moxonidine concentrations. All other samples for metabolite identification were kept at approximately -70°C until analyzed.

Analysis of Moxonidine in Plasma. An LC/MS/MS assay was performed to determine concentrations of moxonidine in human plasma. Aliquots of human plasma (1 ml) were diluted with 1 ml of water while being chilled on ice. Each aliquot was fortified with 10 ng of internal standard (clonidine) and vortex mixed. Samples were applied under approximately 5 psi positive pressure to solid phase extraction columns (3M Empore extraction disk cartridges, 10 mm/6 ml) previously conditioned with methanol then water. The columns were washed with 15% methanol, and then the analytes were eluted

with a 3% trifluoroacetic acid in 95:5; methanol/water solution. The extracts were evaporated to dryness in a Turbo-vap at 45°C . The dried residues were reconstituted in 100 mM ammonium acetate solution (150 μl). One hundred and twenty-five microliters were injected onto the HPLC/MS/MS system. The mass spectrometry was performed on a PE Sciex API III+ (PerkinElmerSciex Instruments, Boston, MA) operated in the APCI ionization mode (heated nebulizer). The selected reaction monitoring (MS/MS) transitions were $242 \rightarrow 44$ (for moxonidine) and $230 \rightarrow 213$ (for internal standard clonidine). Moxonidine was quantitated over the concentration range of 0.05 to 8.00 ng/ml. Samples with concentrations greater than 8 ng/ml were diluted and reanalyzed. During the validation, the interassay precision (%RSD) was between 6.53 and 11.0%, and the interassay accuracy (%RE) was between -5.64 and 1.68%.

Analysis of Moxonidine in Urine. An LC/MS/MS assay was performed to determine concentrations of moxonidine in human urine. Aliquots of human urine (1 ml) were diluted with 1 ml of water. Each aliquot was fortified with 100 ng of internal standard (clonidine) and vortex mixed. Samples were applied to solid phase extraction columns (1 ml Bakerbond carboxylic acid; J. T. Baker) previously conditioned with methanol then water. The loaded samples were washed with 1.00 ml of water and then with 1 ml of a 1:1; methanol/water solution. Analytes were eluted with a 3% trifluoroacetic acid in 95:5; methanol/water solution. The extracts were evaporated to dryness in a Turbo-vap at 45°C . The dried residues were reconstituted in 100 mM ammonium acetate solution (150 μl). Sixty microliters were injected onto the HPLC/MS/MS system. The mass spectrometry was performed on a PE Sciex API III+ with ionization mode APCI (heated nebulizer). The selected reaction monitoring (MS/MS) transitions were $242 \rightarrow 44$ (for moxonidine) and $230 \rightarrow 213$ (for internal standard clonidine). Moxonidine was quantitated over the concentration range of 0.25 to 16.00 ng/ml. Samples with concentrations greater than 16 ng/ml were diluted and reanalyzed. During the validation, the interassay precision (%RSD) was between 3.23 to 7.99%, and the interassay accuracy (%RE) was between -6.88 and -2.19% .

Analysis of Radioactivity. Portions of urine, expired air trappings, and plasma were added directly to liquid scintillant and assayed by liquid scintillation counting. Feces were homogenized in an appropriate volume of deionized water and the homogenates reweighed. Portions of fecal homogenates and blood were added to Combusto-cones and combusted in oxygen using a Packard 306 series sample oxidizer (Canberra Packard, Pangbourne, Berks, UK). The carbon-14-combusted products were absorbed in Carbo-Sorb and mixed with Permafluor E+ liquid scintillant prior to liquid scintillation counting. Radioactive standards were combusted at the beginning of each day and at regular intervals throughout the day to check the carry over between samples and to determine the efficiency of combustion. All radioassays were performed in either duplicate or triplicate. Radioactivity was measured for 10 min using Beckman (Beckman Coulter Inc., High Wycombe, Bucks) or Packard Tri-Carb liquid scintillation counters (Canberra Packard) with the facilities for computing quench-corrected disintegrations per minute (dpm). The limit of detection for the analysis of each sample type was taken as twice the mean of the background disintegration rate obtained from the measurement of blank samples.

Pharmacokinetics. Pharmacokinetic analysis consisted of a noncompartmental assessment of unchanged moxonidine in plasma and radioactive plasma and blood concentration data, using WinNonlin professional network edition version 1.5 (Pharsight, Mountain View, CA).

C_{max} and t_{max} (time to reach maximum concentration) were determined directly from inspection of the individual concentration-time profiles. λ_z was calculated by linear regression of $\ln(C)$ versus time over the terminal phase of the log-linear concentration-time profiles. The start of the terminal phase for each subject was defined by visual inspection of the semilogarithmic plasma or blood concentration versus time profiles. AUC_{0-t} was calculated by the linear-logarithmic trapezoidal rule. $\text{AUC}_{0-\infty}$ was calculated according to eq. 1:

$$\text{AUC}_{0-\infty} = \text{AUC}_{0-t} + C_t'/\lambda_z$$

(where C_t' is the prediction for the last plasma or blood concentration above the lower limit of quantification).

The terminal half-life was calculated according to eq. 2:

$$t_{1/2} = 0.693/\lambda_z$$

The apparent systemic clearance (CL/F), renal clearance (CL_R), and non-renal clearance (CL_{NR}) were calculated using the following equations:

$$CL/F = \frac{\text{Dose}}{AUC_{0-\infty}}$$

$$CL_R = \frac{Ae_{ur}(0-12)}{AUC(0-12)} \quad CL_{NR} = CL/F - CL_R$$

Metabolite Identification. Urine and plasma sample preparation. Urine samples were prepared by either the solid phase extraction method or the concentration method before LC/MS/MS analysis. For solid phase extraction, approximately 5 ml of each urine sample was mixed with 1 ml of 20% trichloroacetic acid, and samples were centrifuged at approximately 3000 rpm at 4°C in a Beckman GPKR swinging bucket centrifuge for 5 min to sediment precipitated substances. The entire volume of supernatant from each sample was loaded onto a Varian C₁₈ solid phase extraction cartridge (3 ml, 200 mg solid/cartridge), which had been conditioned with 2 ml of methanol and equilibrated with 2 ml of 1% trichloroacetic acid in deionized water. Each cartridge was then rinsed with 2 ml of 1% trichloroacetic acid. Moxonidine-related substances were eluted with 2 ml of acetonitrile/25 mM ammonium acetate, pH 5.0 (50:50). The eluant was then evaporated to dryness under nitrogen gas in a 37°C water bath. The residue was reconstituted in 500 µl of 25 mM ammonium acetate (pH 5.0) prior to LC/MS/MS analysis. The recovery of this method was 75%. Urine samples were also prepared for analysis by concentrating the urine (concentration method). Samples were concentrated by adding an equal volume of organic solvent followed by evaporation. Approximately 5 ml of urine was mixed with 5 ml of acetonitrile and vortex mixed. The samples were then centrifuged at approximately 3500 rpm for 10 min at approximately 4°C. The entire supernatant was then transferred into four 16 × 100 silylated glass culture tubes, with equal volumes in each tube. The tubes were placed in a TurboVap solvent evaporator (Zymark Corporation, Hopkinton, MA) at approximately 37°C, and the liquid was evaporated to dryness under nitrogen gas. The contents of each tube were reconstituted with 100 µl of 25 mM ammonium acetate, pH 5.0. The reconstituted samples were then pooled into one tube for analysis. The recovery of radioactivity from patient urine samples by this method was 88 ± 12%. Plasma samples were pooled by time point from different subjects, then prepared by protein precipitation followed by concentration. The pooled plasma (~ 25 ml) was aliquoted in equal volumes to five glass culture tubes, and acetonitrile was mixed 1:1 (v/v) with each aliquot. After vortex mixing, aliquots of the pool were centrifuged in the culture tubes at 3500 rpm for approximately 15 min. The entire supernatant fraction was then removed from each tube, and the liquid was combined and evaporated to dryness. Samples were then reconstituted in 500 µl of 25 mM ammonium acetate, pH 5.0. The reconstituted pool was transferred to a 1.5 ml Eppendorf tube and centrifuged at approximately 14,000 rpm for approximately 10 min prior to LC/MS/MS analysis. The recovery of this method was 89%.

LC/MS/MS. Separation was performed using a C₁₈ Supelcosil LC-ABZ column (25 cm × 4.6 mm, 5µ) at a flow rate of 1 ml/min (Waters 600MS pump; Waters, Milford, MA). The flow was split to allow 250 µl to flow into the electrospray source and 750 µl to a Berthold LB 507A radiochemical detector with a solid flow cell (Berthold Technologies, Oak Ridge, TN), which provided simultaneous detection of radioactivity and mass spectrometry. A mobile phase gradient of (A) 25 mM ammonium acetate (pH 5) and (B) acetonitrile was programmed as follows: initiated with 100% solvent A and held for 2 min, changed to solvent A/solvent B at 96:4 over 2 to 22 min, held at 96:4 from 22 to 26 min, and changed to 100% solvent A from 26 to 27 min. The gradient was changed slightly to optimize the separation for each matrix. The characterization of the metabolites was performed on a Finnigan TSQ-700 Mass Spectrometer. The samples were introduced using atmospheric pressure ionization with electrospray. Samples were analyzed in the positive ion mode using a spray voltage of + 5000V, capillary heater temperature of 250°C, sheath gas of 80 psi (N₂) and an auxiliary gas flow (N₂) of 25 ml/min. For full scan analysis, the mass spectrometer was scanned from 150 to 600 atomic mass units in 1 s. MS/MS analysis was performed using a collision energy of -30 eV and collision gas pressure of 1.5 mTorr (argon).

HPLC profiling of metabolites. Determination of urinary metabolites was

performed using a Waters 600MS system, equipped with a reverse phase C₁₈ Supelcosil LC-ABZ column (25 cm × 4.6 mm, 5µ), a radiochemical detector (Ramona-92 with a 370-µl solid flow cell or Berthold LB 507A with a 100 µl cell; Bad Wildbad, Germany), and a fraction collector (LKB Bromma Superrac 2211 equipped with racks holding 20-ml scintillation vials; Pharmacia LKB Biotechnology, Uppsala, Sweden). A mobile phase gradient of (A) 50 mM ammonium acetate (pH 5.0) and (B) acetonitrile was programmed as follows: started with 100% solvent A, changed to solvent A/solvent B at 98:2 over 12 to 12.1 min, changed to solvent A/solvent B at 97:3 over 12.1 to 20 min, changed to solvent A/solvent B at 94:6 over 20 to 20.1 min, held at 94:6 from 20.1 to 40 min, and changed to 100% solvent A from 40 to 40.1 min. The metabolites and moxonidine were separated by the above gradient elution at a flow rate of 1 ml/min. The urinary radioactivity of moxonidine and its metabolites were estimated by collecting fractions (0.5 min/fraction) from HPLC and radioactive accounting of each fraction by liquid scintillation counting.

Results

Pharmacokinetics. The concentrations of moxonidine and radioactivity in plasma and whole blood from individual subjects are graphically depicted in Fig. 2. For subjects 1, 2, and 4, the plasma concentrations of moxonidine were below the limit of quantitation after 16 h, plasma or blood radioactivity levels were not detectable beyond 10 or 12 h postdose. Subject 3 had a different concentration versus time profile compared with the other subjects. The concentration of moxonidine and radioactivity in subject 3 remained detectable for up to 24 h. The concentrations of blood radioactivity were similar to plasma radioactivity for up to 5 h postdose in most subjects, suggesting the partitioning of moxonidine into the red blood cells. The partitioning of metabolites into the red blood cells was to a lesser extent at later time points, as the total radioactivity in the plasma at later time points is higher than in blood. The pharmacokinetic parameters for moxonidine and radioactivity in plasma and blood are summarized in Table 1. The C_{max} and AUCs of unchanged moxonidine are lower than those determined for radioactivity, indicating presence of circulating metabolites in plasma. The pharmacokinetic parameters of subject 3 were also different from the other subjects. The AUC values of moxonidine and radioactivity in subject 3 were at least two fold higher when compared with subjects 1, 2, and 4. The differences were more pronounced for the radioactivity than for the parent compound, indicating that the elimination of the moxonidine metabolite(s) was more heavily influenced in this subject. In addition, the elimination half-lives of moxonidine and radioactivity in subject 3 are longer than in other subjects.

Concentrations of moxonidine were also measured in urine samples. Renal clearance was determined over the interval 0 to 12 h postdose, because all subjects had measured moxonidine plasma concentrations for up to 12 h. The cumulative amount of moxonidine excreted in urine, expressed as a percentage of the administered dose (fe_{ur} (%)), was calculated. As shown in Table 2, the individual values ranged from 69 to 77% for subjects 1, 2 and 4. For subject 3, this percentage was only 46.5%. The renal clearance of subject 3 (4.62 l/h) was substantially lower than other subjects (24–30 l/h), although the nonrenal clearance seems to be comparable.

Radiocarbon Excretion. The recovery of total radioactivity ranged from 99.6 to 105.2% (Table 3). Since radioactivity was predominately excreted in the urine (92.3 to 103.3% of the dose), renal elimination represented the principal route of excretion of radioactivity. In subject 1, 2, and 4, the majority of the radioactivity was excreted in the urine within the first 24 h postdose and not detected beyond 72 h postdose. Radioactivity in feces was not detectable beyond 72 h postdose. Subject 3, however, had a more protracted excretion with levels of radioactivity detected in both urine and feces for up to 120 h postdose. The reason for the prolonged excretion with this subject was not clear.

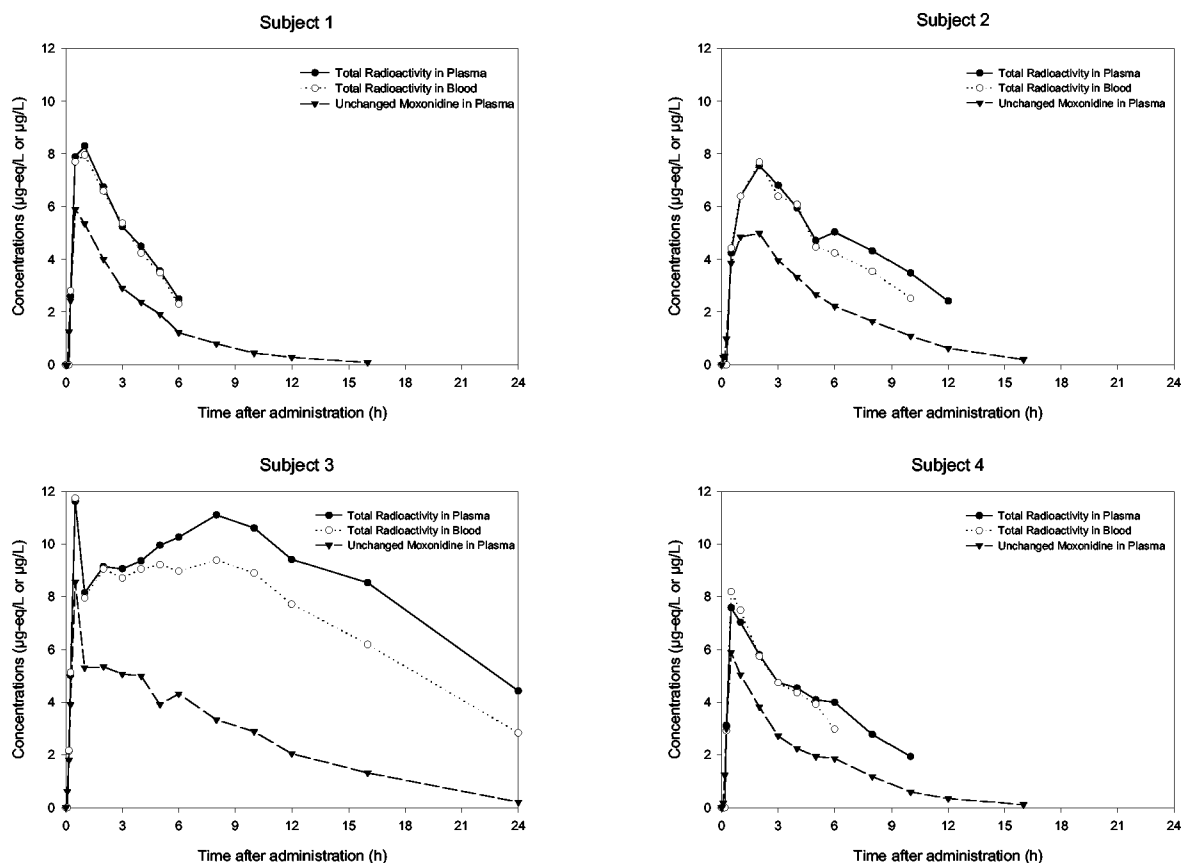


Fig. 2. Concentration versus time profiles of moxonidine in plasma and total radioactivity in plasma and blood after a single oral dose of [$^{14}\text{C}_3$]moxonidine in four healthy male subjects.

TABLE 1

Pharmacokinetic parameters of moxonidine or radioactivity following a single oral dose (1 mg) of [$^{14}\text{C}_3$]moxonidine to healthy male subjects

Subject No.	AUC_{0-t}	$\text{AUC}_{0-\infty}$	C_{\max}	t_{\max}	t
	ng or ng-eq · h/ml	ng or ng-eq · h/ml	ng or ng-eq/ml	h	
Unchanged Moxonidine Plasma					
1	23.0	23.4	5.90	0.5	2.58
2	31.0	31.7	4.99	2.0	2.57
3	59.6	60.8	8.57	0.5	3.72
4	24.6	25.0	5.89	0.5	2.44
Radioactivity in Plasma					
1	30.8	42.0	8.30	1.0	2.92
2	56.9	78.1	7.55	2.0	5.73
3	203.0	278	11.6	0.5	11.2
4	41.1	55.3	7.58	0.5	4.83
Radioactivity in Blood					
1	30.1	39.9	7.95	1.0	2.74
2	47.0	66.8	7.69	2.0	5.32
3	167	203	11.7	0.5	8.48
4	29.3	50.3	8.19	0.5	4.62

AUC_{0-t} , area under the concentration versus time curve from time zero to the last quantifiable time point; $\text{AUC}_{0-\infty}$, area under the concentration versus time curve from time zero to infinity; C_{\max} , maximum concentration; t_{\max} , time to reach maximum concentration; t , terminal half-life.

Radioactivity was not detected in the expired air collected from any subject at any time point.

Identification of Moxonidine Metabolites by LC/MS/MS. Urinary Metabolites. Urine samples were prepared by both solid phase extraction and concentration methods. Samples were analyzed by HPLC (with radiochemical detection), LC/MS and LC/MS/MS. Moxonidine was detected as the most abundant peak in all urine samples, based on radiochemical detection. In general, the same metabolites were detected in different subjects. There were differences in the relative amounts of metabolites between subjects or between urine

samples collected at different time points from the same subject. The characteristic product ions of moxonidine and the urinary metabolites are summarized in Table 4.

Metabolites M1 (m/z 240), M5 (m/z 258), and M6 (m/z 327) were positively identified as dehydrogenated moxonidine, hydroxymethyl moxonidine, and cysteine conjugate minus chlorine, respectively. These metabolites had been previously found in rat urine and bile (He et al., 2000). The identification of these metabolites in human urine samples was based on comparison of their product ion mass spectra to those of authentic standards. In urine samples, metabolite M1 was

TABLE 2

Renal and non-renal clearance and fraction of drug excreted unchanged in urine following a single oral dose (1 mg) of [$^{14}\text{C}_3$]moxonidine to healthy male subjects

Subject No.	CL _R	CL _{NR}	fe _{ur} 0-12 h
	l/h	l/h	% of dose
1	29.8	12.9	68.8
2	24.0	7.5	76.7
3	4.62	11.8	46.5
4	30.1	9.88	77.0

CL_R, renal clearance; CL_{NR}, non-renal clearance; fe_{ur}, fraction of drug excreted in urine.

detected in significantly higher concentrations compared with the other metabolites. Metabolite M6 was detected at low levels, but its concentration increased in the urine samples collected at later time points.

Metabolite M2 was identified as a putative *N*-oxide metabolite of dehydrogenated moxonidine. It has a protonated molecular ion of *m/z* 256, which was 16 atomic mass units higher than that of metabolite M1 (*m/z* 240). This metabolite was detected in both extracted and concentrated urine samples but often coeluted with moxonidine. This new *N*-oxide metabolite had not been detected previously in animal species. The analysis of an authentic standard would be required to confirm the identity of this metabolite.

Metabolites M3 (*m/z* 258) and M4 (*m/z* 274) were positively identified as hydroxy moxonidine and dihydroxy moxonidine, respectively. The hydroxy groups are located on the imidazolidine ring. Metabolite M3 was found in human urine samples but was absent in rat urine or bile samples in the previous study (He et al., 2000). Metabolite M4 is a human specific metabolite. It was not detected previously in any animal species *in vivo* or *in vitro*. The product ion mass spectra for M3 and M4 are shown in Fig. 3. The structural assignments for M3 and M4 were based on comparison of the characteristic product ions to those of authentic standards.

Metabolite M7 is also a new metabolite that had not been reported previously. This metabolite was consistently found in concentrated human urine samples but not detected in the extracted urine samples. M7 is a polar metabolite with a short retention time of approximately 5 min (Fig. 4). This early peak was analyzed by both positive and negative ion modes, and also by APCI, but no molecular weight determination could be ascertained for this metabolite. Other experiments were also conducted to characterize this metabolite. An HPLC fraction of this peak was collected and then incubated with β -glucuronidase at 37°C for approximately 16 h. This metabolite apparently was not hydrolyzed by the enzyme, suggesting that it was not a glucuronide conjugate. The identity of this metabolite remained to be determined.

Plasma metabolite. The plasma samples collected from four subjects were pooled by time point and the proteins were precipitated before analysis. The radiochemical trace only showed one peak, which was parent moxonidine. The positive ion mass chromatograms showed that dehydrogenated moxonidine (*m/z* 240) was detected at a low level in addition to parent moxonidine. No other metabolites were detected that could be confirmed using full scan MS/MS. It is possible that other metabolites may exist in plasma but their concentrations are too low to be identified by current analytical methods. The characteristic product ions of moxonidine and the dehydrogenated metabolite identified in human plasma are summarized in Table 4.

Metabolite Profiling. The levels of moxonidine and its metabolites were estimated in urine samples collected from four subjects for up to 24 h. Initial HPLC results indicated that most of the metabolites, besides moxonidine and dehydrogenated moxonidine, were not detected by the radiochemical detector when urine samples were in-

jected directly onto the HPLC. Therefore, urine samples were concentrated before analysis. HPLC with fraction collection (0.5 min/fraction) was performed for all urine samples available. All the fractions collected were evaluated for radioactivity by liquid scintillation counting. A representative graph indicating radioactivity in chromatographic fractions from 6- to 8-h urine sample collected from subject 1 is depicted in Fig. 4. The abundance of radioactivity peaks decreased over time, and the profile of the metabolites in urine samples also differed slightly over time. A complete urinary metabolite profile was obtained from subject 1 since this subject had samples available from all the time points. Table 5 shows the total recovery of moxonidine and its metabolites in urine samples collected from subject 1 over the 24 h period. The recovery was calculated based on urinary excretion data (Table 3). The results indicated that moxonidine was the most abundant radiolabeled component, representing 78% of the dose. The dehydrogenated moxonidine was determined as the major metabolite in all urine samples, accounting for 13% of the dose. All other metabolites were present at relatively low levels, accounting for approximately 8% of the dose.

Discussion

The aim of the current study was to characterize the metabolism and disposition of moxonidine in humans and identify metabolites in several biological matrices. Moxonidine is a very potent drug with therapeutic doses of 0.2 to 0.6 mg (Weimann and Rudolph, 1992). To identify low level metabolites, a triple-labeled [$^{14}\text{C}_3$]moxonidine with high specific radioactivity was prepared and was administered as an oral solution to healthy subjects during the study.

Moxonidine metabolites were identified using HPLC and LC/MS/MS. Interpretation of mass spectral data established the presence of seven metabolites (including an unknown metabolite) in human urine samples. The combined results from both extracted and concentrated urine samples provided a more complete profile of human metabolites. A proposed metabolic scheme for the metabolism of moxonidine in humans is shown in Fig. 5. Oxidation on either the methyl group (pyrimidine ring) or on the imidazolidine ring of moxonidine resulted in the formation of hydroxymethyl moxonidine (M5) or hydroxy moxonidine (M3). The hydroxy moxonidine may be further oxidized to dihydroxy metabolite (M4) or it lost water to form dehydrogenated moxonidine (M1), which can then be further oxidized to form a *N*-oxide (M2). All these phase I metabolites (except M2) have been positively identified and confirmed by authentic standards. The identification of metabolite M3 along with high level of metabolite M1 in human urine samples indicate that dehydrogenation (from moxonidine to M3 to M1) represents the major metabolic pathway in humans. Phase II metabolism of moxonidine in humans was also evident. The cysteine conjugate minus chlorine (M6) was identified in the urine samples from all subjects, and its structure was confirmed by an authentic standard. The identification of this metabolite suggests that in the presence of GSH, moxonidine formed a GSH conjugate minus chlorine, which was then hydrolyzed to generate the cysteine conjugate minus chlorine. Although cysteine conjugate minus chlorine was the only conjugate detected in humans, GSH conjugate minus chlorine, cysteinylglycine conjugate minus chlorine and cysteine conjugate minus chlorine were all identified in rats (He et al., 2000). Structurally, chlorine was absent from all these series conjugates. This indicated that moxonidine was attacked by the nucleophile GSH; possibly through direct displacement ($\text{S}_{\text{N}}2$) mechanism and the chlorine moiety was replaced by GSH, generating moxonidine GSH conjugate minus chlorine and then forming cysteinylglycine conjugate minus chlorine and cysteine conjugate minus chlorine through sequential hydrolysis. The absence of GSH conjugate minus chlorine,

TABLE 3

Summary of excretion of radioactivity following a single oral administration of [^{14}C]moxonidine to healthy male subjects

Matrix	Collection Period (h)	Percent of Administered Dose			
		Subject 1	Subject 2	Subject 3	Subject 4
Urine	0–2	49.89	N.S.	N.S.	29.79
	2–4	20.90	49.20	17.18	12.42
	4–6	10.44	12.47	N.S.	14.73
	6–8	7.750	9.306	N.S.	19.52
	8–12	7.807	15.76	8.239	12.63
	12–24	5.313	13.36	43.72	10.78
	24–36	0.024	1.945	17.20	0.169
	36–48	1.151	0.389	3.227	2.404
	48–72	N.D.	0.341	1.890	0.281
	72–96	N.D.	N.D.	0.542	N.D.
	96–120	N.D.	N.D.	0.336	N.D.
	Total Urine		103.3	102.8	92.32
Feces	0–24	0.395	0.046	N.S.	0.277
	24–48	1.040	0.928	1.166	1.753
	48–72	0.503	1.381	4.194	0.207
	72–96	N.D.	N.D.	1.692	N.D.
	96–120	N.D.	N.D.	0.245	N.D.
	Total Feces	1.938	2.354	7.298	2.237
	Total		105.2	105.1	99.62

N.D., not detected; N.S., no sample excreted.

TABLE 4

Moxonidine metabolites identified from human urine and plasma

Metabolite	[M + H] ⁺	Characteristic Product Ions	Identification	Urine	Plasma
		<i>m/z</i>			
N/A	242	44, 56, 149, 199, 206	Moxonidine	+	+
M1 ^a	240	56, 82, 135, 184, 189, 204	Dehydrogenated moxonidine	+	+
M2	256	56, 89, 128, 184, 199	Putative <i>N</i> -oxide of dehydrogenated moxonidine	+	N.D.
M3 ^a	258	60, 136, 174, 184, 199, 204, 240	Hydroxy moxonidine	+	N.D.
M4 ^a	274	57, 184, 199, 228	Dihydroxy moxonidine	+	N.D.
M5 ^a	258	44, 151, 192, 215, 228	Hydroxymethyl moxonidine	+	N.D.
M6 ^a	327	167, 206, 223, 240, 281	Cysteine conjugate minus Cl	+	N.D.
M7	N.D.	No spectrum available	Unknown metabolite	+	N.D.

N/A, not applicable; N.D., not detected.

^a Identification was confirmed by comparison with an authentic standard.

cysteinylglycine conjugate minus chlorine in humans (they may be present, but the concentration was too low to be detected) suggested that the enzyme involved in the hydrolysis of GSH conjugate minus chlorine was quite active, leading to the formation of final product, cysteine conjugate minus chlorine. There were no toxicological consequences associated with the formation of cysteine conjugate of moxonidine, since it was not involved in the formation of a reactive metabolite, rather, the chlorine underwent direct displacement mechanism during the GSH conjugation.

Two moxonidine metabolites, dehydrogenated moxonidine and guanidine metabolite, have been previously reported (Schaefer et al., 1998). In that study, which was conducted in early 1980s, moxonidine metabolites were not adequately identified due to limited analytical techniques available at that time (thin-layer chromatography with radiolabel detection). A large percentage of radioactivity in both urine and plasma samples remained uncharacterized. Also, the plasma and urine samples were extracted or prepared with very low recoveries. In the present study, moxonidine metabolites have been identified with modern analytical methods, which have much greater sensitivity. The results from the current study differed from the early study in two major aspects. First, all the metabolites identified in the present study except for dehydrogenated moxonidine were new human metabolites that had not been reported from the previous study. Second, the guanidine metabolite identified in the early study based on thin layer

chromatography results (R_f values) was not detected by LC/MS/MS in the present study, and it seems to have been misidentified. In fact, the retention time of the guanidine metabolite is very close to that of dihydroxy moxonidine. They both eluted between the hydroxymethyl moxonidine and hydroxy moxonidine in HPLC chromatogram. Therefore, the metabolite reported previously as the guanidine metabolite seems to actually be dihydroxy moxonidine.

The cytochromes P450 responsible for the metabolism of moxonidine in humans have not been determined although the ability of moxonidine to inhibit metabolism of marker catalytic activities for CYP3A, 2D6, 2C9, and 1A2 were examined. Moxonidine was found to be a competitive inhibitor of CYP2D6 but didn't seem to significantly inhibit the CYP3A, 2C9, and 1A2 (S. N. Binkley and S. A. Wrighton, unpublished data). In a recent study to determine the effect of quinidine on the renal clearance and pharmacokinetics of moxonidine, the renal clearance was not altered, but statistically significant increases in AUC and half-life of moxonidine (11% and 15% respectively) were observed after coadministration of quinidine (Wise et al., 2002). If moxonidine is also a substrate of CYP2D6, in addition to being an inhibitor, the inhibitory effect of quinidine on CYP2D6 could be responsible for the decrease in moxonidine clearance seen in the clinical study.

The metabolism and disposition of moxonidine in humans are different from that in rats (He et al., 2000). Most notably, the excretion patterns are different between humans and rats. In human sub-

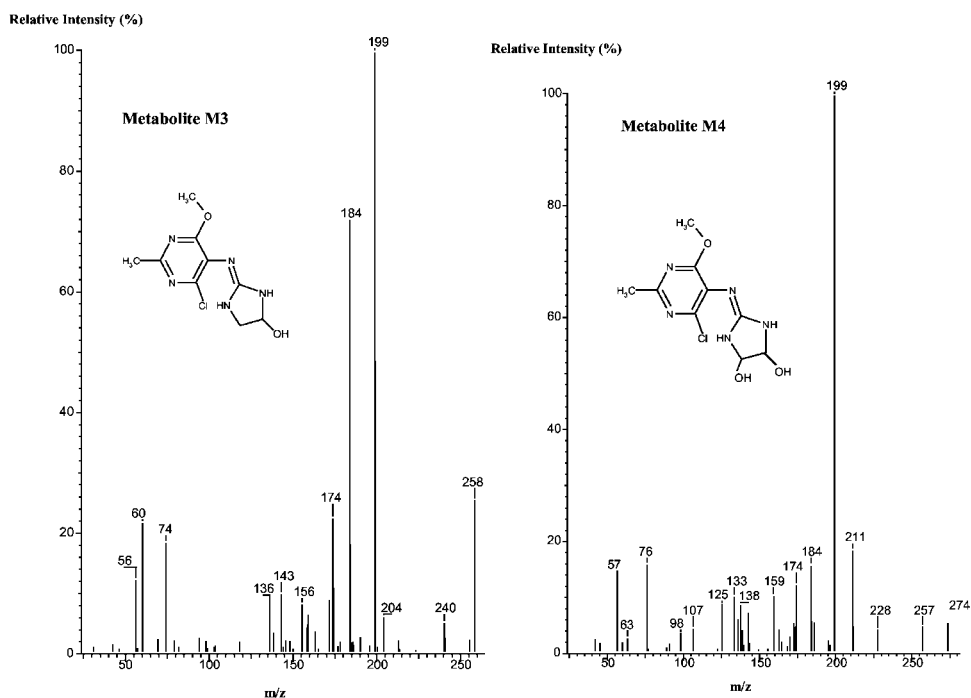


FIG. 3. Product ion mass spectra of hydroxy moxonidine (M3, m/z 258) (left) and dihydroxy moxonidine (M4, m/z 274) (right) from an extracted urine sample collected at 0 to 2 h from subject 1.

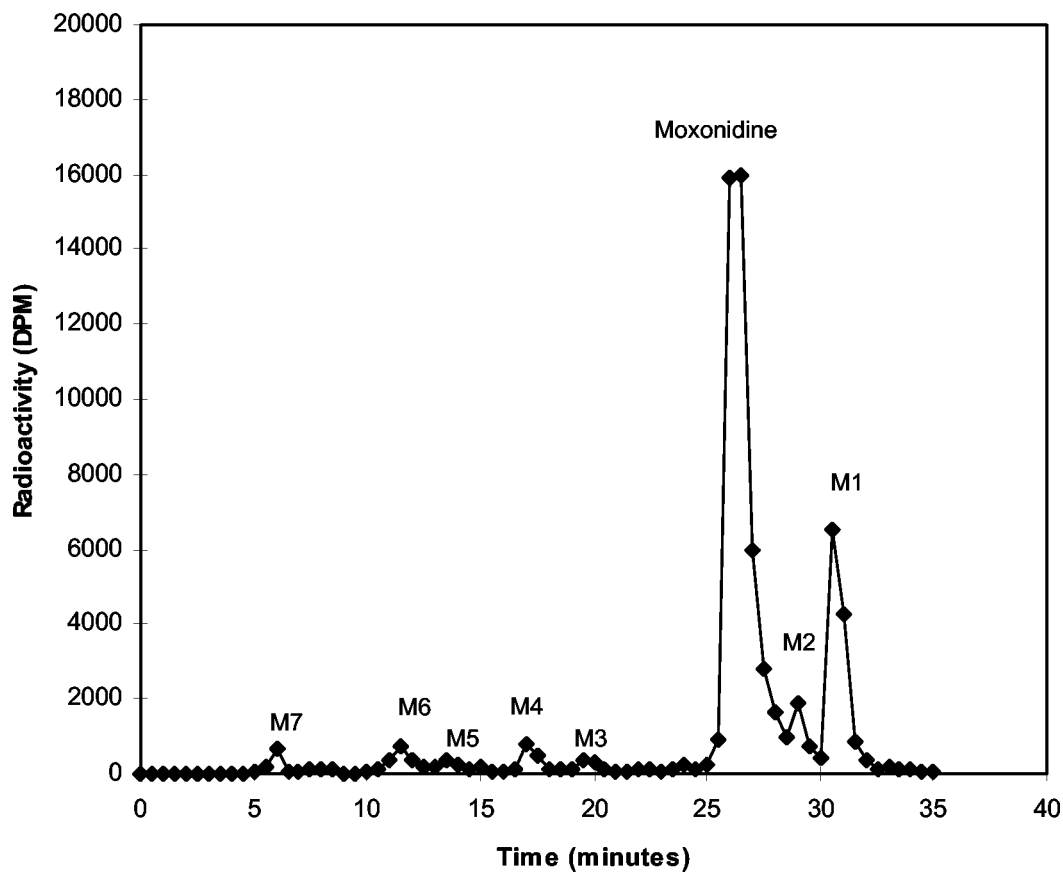


FIG. 4. Radioactivity in chromatographic fractions from an urine sample collected at 6 to 8 h from subject 1.

M1, dehydrogenated moxonidine; M2, putative *N*-oxide of dehydrogenated moxonidine; M3, hydroxy moxonidine; M4, dihydroxy moxonidine; M5, hydroxymethyl moxonidine; M6, cysteine conjugate minus chlorine; M7, unknown metabolite.

TABLE 5

Estimation of moxonidine and its metabolites in urine samples of subject 1

Peak Identification	% of Dose ^a
Moxonidine	77.7
Dehydrogenated moxonidine (M1)	13.0
¹ Putative N-oxide of dehydrogenated moxonidine (M2)	2.1
Hydroxy moxonidine (M3)	0.9
Dihydroxy moxonidine (M4)	1.9
Hydroxymethyl moxonidine (M5)	1.0
Cysteine conjugate minus chlorine (M6)	0.8
Unknown metabolite (M7)	0.7
Total	97.7

^a Sum of the percentage of dose from samples collected at 0- to 2-, 2- to 4-, 4- to 6-, 6- to 8-, 8- to 12- and 12- to 24-h post dose. The % of dose value at each interval was calculated based on the percentage of urinary radioactivity peak area, the sample preparation recovery, and the urinary excretion data from Table 3.

jects, most of the radioactivity was excreted in urine (92–103% of dose) with little eliminated in the feces. This contrasts to what was observed in rats where about 56% of dose was excreted in urine and 38% was in feces. The difference in fecal excretion between human and rats was due to differences in their elimination routes and not due to absorption. A separate excretion study in bile duct-cannulated rats resulted in 33% biliary excretion with little fecal excretion, indicating that moxonidine was well absorbed in rats (He et al., 2000). Second, moxonidine was determined as the major component in human urine samples, which was in contrast to what was observed in rats where parent moxonidine was detected as a minor component in the rat urine. Consistent with this, a total of seven metabolites was detected in humans whereas more metabolites (a total of fifteen) were identified in rats. Third and more important, several new metabolites were identified in humans. Metabolite M2, M4, and M7 were observed in

the present study, but they were not detected in rats or any other animal species, indicating the lack of these metabolic pathways in animals. Metabolite M3 was identified in the present study but not detected *in vivo* in rats. Interestingly, this metabolite was formed *in vitro* with rat liver microsomes, which indicated that *in vivo* M3 might be formed but rapidly converted to secondary metabolites in rats (He et al., 2000). Finally, the bioavailability determined in humans (88%; Schaefer et al., 1998) is higher than that in rats (5%; He et al., 2000), further demonstrating the important differences between rats and humans in the metabolism of moxonidine.

Most moxonidine metabolites (phase I) were synthesized and tested for pharmacological activity. The hydroxymethyl moxonidine (M5) possesses an antihypertensive and bradycardic activity in conscious unrestrained hypertensive rats but was less active and shorter acting than parent moxonidine (Wirth et al., 2002). Metabolites M1, M3, M4, and M6 were found to be inactive. Metabolite M2 and M7 have not been tested for activity. Since the current study demonstrates that hydroxymethyl moxonidine accounts for approximately 1% of the total dose, the vast majority of the pharmacological effect is attributed to the parent compound. Although three unique moxonidine metabolites were identified in humans (M2, M4, and M7), they are not significant enough to be of concern for the following reasons: 1) these metabolites are not major metabolites, 2) while if active, they were all detected at low levels (0.7–2.1% of the dose) and therefore would not contribute significantly in the overall activity, 3) their structures do not contain structural alerts for toxicity, and 4) there is substantial clinical data that has established the clinical safety of moxonidine.

The radiocarbon excretion profiles indicated that renal elimination is the primary route of elimination for moxonidine and its metabolites. The renal clearance ranged from 24.0 to 30.1 l/h (subject 1, 2, and 4),

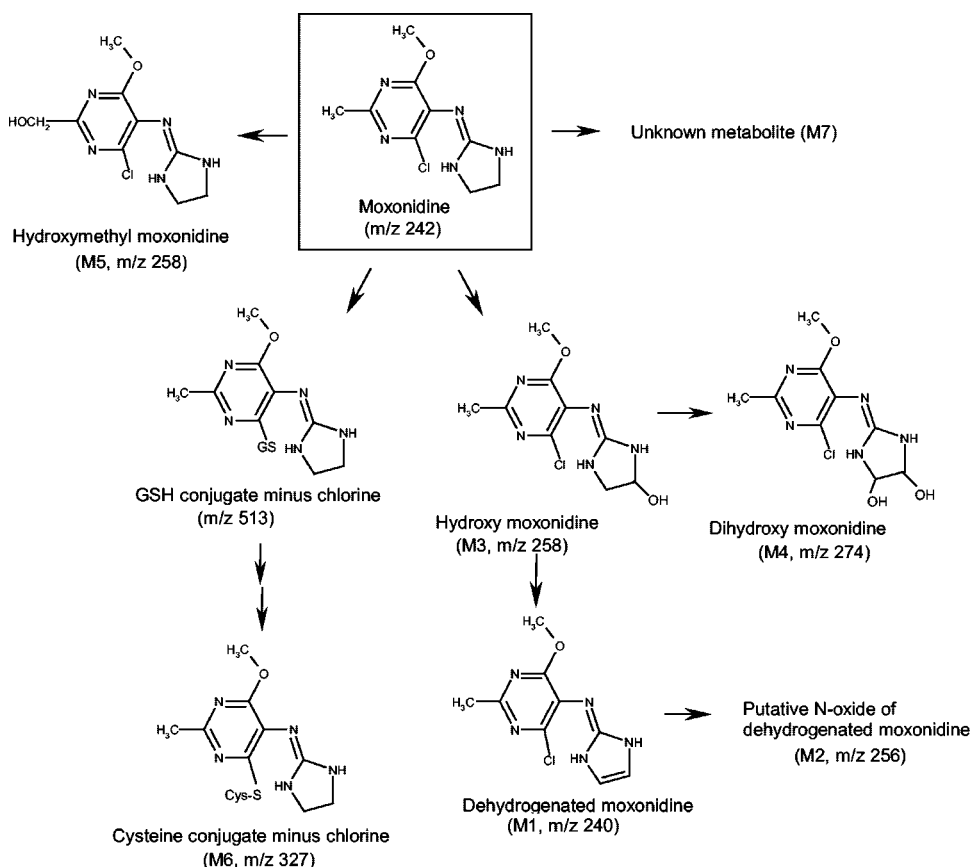


Fig. 5. Proposed metabolic scheme for metabolism of moxonidine in humans.

suggesting that active tubular secretion contributes in addition to glomerular filtration to the renal excretion of moxonidine, which was consistent with the previous observations (Kirsh et al., 1988). Subject 3 behaved differently as compared with the other three subjects. The plasma concentrations of moxonidine and total plasma radioactivity in subject 3 were much higher, and the half-lives were longer than others. The unusual excretion and pharmacokinetic profiles of subject 3 did not seem to be associated with an increase in the number, or severity of adverse events when compared with the other subjects. The creatinine clearance in this subject was comparable with the others, and there was no indication of impaired renal function. The metabolism of moxonidine in subject 3 did not seem to be different since the urinary metabolite profile in subject 3 was similar to that of the other subjects. In addition, the nonrenal clearance of moxonidine was comparable between subject 3 and the others. Therefore, the low renal clearance of moxonidine and metabolites seems to be responsible for the differences observed in the pharmacokinetic behavior between subject 3 and the other subjects. Since moxonidine undergoes active tubular secretion, one of the possible explanations is that the active secretion and active re-absorption were equal in subject 3. The other possible explanation is that subject 3 had an altered renal transporter expression or capacity that is critical for the active tubular secretion. The specific transport systems responsible for moxonidine active tubular secretion remain undetermined. It was reported that pharmacokinetics of moxonidine was not altered with coadministration of glibenclamide, which is a known P-glycoprotein inhibitor and substrate (Muller et al., 1993). A recent study also found that quinidine sulfate, a potent inhibitor of organic cation secretion as well as an inhibitor of P-glycoprotein, does not affect the renal clearance of moxonidine (Wise et al., 2002). These data suggest that active renal secretion of moxonidine does not involve P-glycoprotein or the renal organic cation transport system inhibited by quinidine. Other organic cation transporter systems may be involved in the clearance of moxonidine since there are different forms of human organic cation transporters (OCT1, OCT2, and OCT3). Also, two different types of transport systems for organic cations, a type 1 system mediating uptake of small hydrophilic organic cations and a type 2 system mediating uptake of hydrophobic organic cations such as quinine, have been reported (Inui et al., 2000). It is unknown whether different organic cation transporters are involved in the clearance of moxonidine metabolites.

In summary, in human moxonidine was well absorbed and rapidly eliminated after oral administration of [$^{14}\text{C}_3$]moxonidine solution.

Urinary excretion represents the primary route of elimination of moxonidine and its metabolites. Although several moxonidine metabolites have been identified, the parent compound was found to be the most abundant component in different biological matrices, indicating metabolism plays a modest role in the clearance of moxonidine in humans. The major metabolite found in urine and plasma was dehydrogenated moxonidine, and three human specific metabolites were also detected (M2, M4, and M7). The patterns of metabolism and excretion in human contrasts that previously reported in rats (He et al., 2000) where the elimination by urinary and fecal routes was much more balanced, and about twice as many metabolites were detected and bioavailability was significantly lower (88% for human versus 5% for rats).

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