*Br. J. clin. Pharmac.* (1986), **21**, 19S–25S

# The metabolism and kinetics of doxazosin in man, mouse, rat and dog

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**1** The metabolic fate of doxazosin was investigated in man, mouse, rat and dog using $^{14}C$-labelled compound. Bioavailability and pharmacokinetic studies were also conducted with non-labelled drug, using a specific h.p.l.c. method.

**2** Following both oral and intravenous administration, the major route of elimination of drug-related compounds was via the faeces for all species studied. Comparison of the oral and intravenous data show that doxazosin is completely absorbed in man, mouse and rat and is moderately well absorbed in dog.

**3** The drug is extensively metabolized, e.g. only about 5% of the dose was excreted unchanged in man. Metabolism in man mainly involves 6- and 7- *O*-demethylation and 6′ and 7′-hydroxylation. These and some minor products were common to the mouse, rat or dog and man.

**4** Plasma protein binding was high in all species studied, ranging from 95.3% in the rat to 98.3% in human patients.

**5** Oral bioavailability is 60% in dog and approximately 50% in the rat, which is similar to the value of 63% reported for man at therapeutic doses. Mean plasma clearance values were 13 ml $min^{-1}$ $kg^{-1}$ (dogs), 30 ml $min^{-1}$ $kg^{-1}$ (rats) and 1.2 ml $min^{-1}$ $kg^{-1}$ (human subjects). Mean plasma half-life values were 5 h in dogs and 1.2 h in rats: a value of 9 h was reported for human volunteers (*cf.* 2.5 h for prazosin). The long plasma half-life of doxazosin provides the basis for once-daily dosing.

**Keywords** bioavailability doxazosin half-life kinetics metabolites

## Introduction

Doxazosin, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzdioxan- 2-yl-carbonyl) piperazine, is a postsynaptic $\alpha_1$-adrenoceptor antagonist structurally related to prazosin (Alabaster & Davey, 1986; Singleton *et al.*, 1980; Timmermans *et al.*, 1980). Like prazosin, the compound is a potent antihypertensive agent which is effective when administered either orally or intravenously (Elliott *et al.*, 1982). Doxazosin is more slowly eliminated than prazosin in man and its relatively long half-life provides the basis for once-daily dosing, a therapeutic advantage (Vincent *et al.*, 1983).

Studies have been performed on the metabolism and kinetics of doxazosin in man and the species used in the evaluation of drug safety and efficacy. The metabolic fate of doxazosin has been investigated in man, mouse, rat and dog using drug labelled with $^{14}C$ in the 2-position of the quinazoline moiety. In addition, bioavailability and pharmacokinetic studies have been conducted using a specific h.p.l.c. method for drug assay in biological fluids.

## Methods

### *Preparation of [2-$^{14}C$]-quinazolinyl-doxazosin*

Radiolabelled doxazosin was custom synthesized from [2-$^{14}C$]-2-chloro-4-amino-6,7-dimethoxy-quinazoline (Amersham International) and converted to the methane sulphonate salt. Specific activities of batches ranged from 45 to 125 $\mu Ci$ $mg^{-1}$ and radiochemical purity was > 98% by t.l.c. in all cases.

*Preparation of [6′,7′-$^3$H]-doxazosin*

Tritiated doxazosin was prepared by tritiolysis of 6′,7′-dibromo doxazosin (Amersham International) and converted to the methane sulphonate salt. Specific activity was 97 mCi mg$^{-1}$ and radiochemical purity was 98% by t.l.c.

Unlabelled doxazosin was used to dilute the radiolabelled compound for dosing when appropriate.

*Synthetic standards*

Compounds II, III and V-VIII (Figure 1) which were considered to be potential metabolites of doxazosin were synthesized by unequivocal procedures in the Chemical Research Laboratories of Pfizer Central Research. Structural identity was established by elemental analysis, proton NMR, IR and electron impact mass spectrometry.

*Human studies*

Two healthy adult male volunteers, who had given their informed consent, participated in the study. Each subject received drug orally (2 mg gelatin capsule, 20 μCi) and intravenously (1 mg solution in lactate buffer 20 μCi) on a crossover basis. A capsule containing non-absorbable microspheres ($^{51}$Cr, 1.5 μCi) was also administered concomitantly with each dose of doxazosin. Urine and faeces were collected at intervals of up to 7 days and blood samples were taken up to 24 h after dosing. Subjects fasted on the night before and on the morning of each dose and did not ingest further food or fluids until at least 3 h after drug administration. They then subsisted on a bland diet (no spicy food or alcohol) for the duration of the study.

The protocol was agreed by a local Ethics Committee and by the Department of Health Advisory Panel on Radioisotope Studies.

*Animal studies*

Mice (Charles River CD1, *circa* 30 g), rats (Charles River, 150–300 g) and beagle dogs (Pfizer strain, 10–15 kg) were fasted overnight before receiving doxazosin. For intravenous administration the drug was dissolved in lactate buffer and for oral dosing was given as a suspension either in 1% aqueous carboxymethylcellulose or in 25% aqueous Cremaphor EL or as a powder mixed in gelatin capsules.

For metabolism studies, single doses of [2-$^{14}$C]-doxazosin were administered and dose levels expressed as mg base per kg body weight were, in mouse 5 mg kg$^{-1}$ i.v. and 10 mg kg$^{-1}$ p.o.; in rat 5 mg kg$^{-1}$ i.v. and 10 mg kg$^{-1}$ p.o.; and in dog 2 mg kg$^{-1}$ i.v. and 4 mg kg$^{-1}$ p.o. Amounts of radiolabelled drug used ranged from 5 μCi per animal in the mouse to 100 μCi per animal in the dog.

Unlabelled doxazosin was used in pharmacokinetic studies and dose levels used were 5 mg kg$^{-1}$

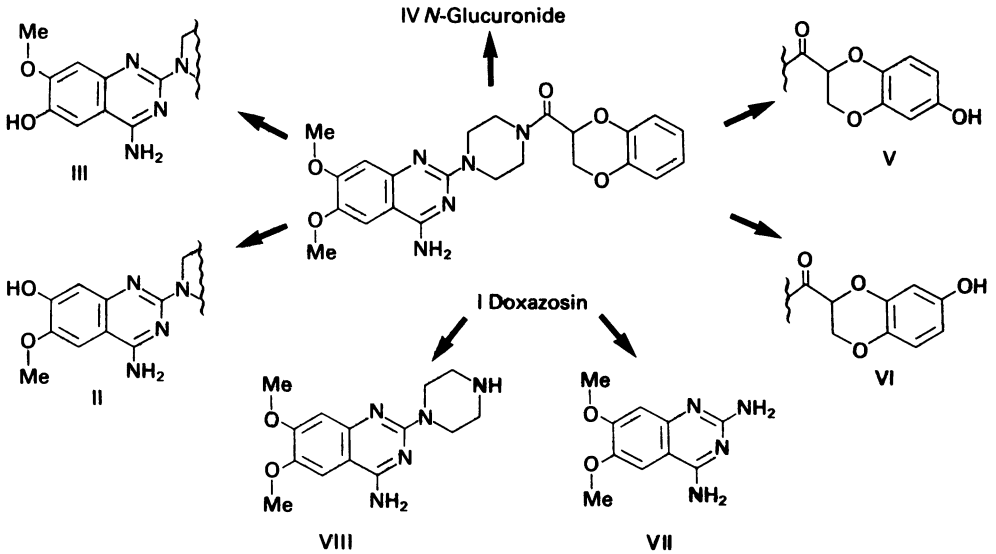


**Figure 1** The metabolites of doxazosin.

i.v. and p.o. in rat, and 0.05 mg $kg^{-1}$ i.v. and p.o. in dog.

Animals were housed in glass metabowls (Jencons) or stainless steel metabolism cages and urine and faeces were collected separately for up to 5 days. Animals had free access to food and water throughout the study.

Blood samples were taken at specified times into heparinized tubes and plasma prepared. Dogs were bled serially from the cephalic vein and groups of three rats were killed and exsanguinated.

### *Protein binding*

Plasma protein binding of [6′,7′-$^3$H]-doxazosin was determined for mouse, rat, dog and human patients, using drug concentrations of 5, 10 and 100 ng $ml^{-1}$. Plasma was dialysed against 0.1 M $Na_2HPO_4$ (pH 7.4) in a rotating dialyser (Dianorm, MSE) at 37°C. The extent of binding was calculated as

$$\% \text{ binding} = 100 \times \left(1 - \frac{\textit{conc. radioactivity in buffer}}{\textit{conc. radioactivity in plasma}}\right)$$

### *Analysis of samples*

The radioactive content of plasma, urine and other solutions (1–2 ml) was determined by liquid scintillation counting (Searle MK III counter) following the addition of Instagel (10 ml, Packard Ltd).

Faeces were homogenized and freeze-dried and the $^{14}C$ content of an aliquot (100 mg) was determined by combustion analysis (Packard 306 sample oxidizer). The $^{51}Cr$ content was determined by scintillation counting of aliquots (600 mg, Packard 5330 gamma spectrometer).

### *Assay for doxazosin in plasma*

Concentrations of doxazosin in plasma were determined by an h.p.l.c. method specific for drug and sensitive to concentrations greater than 1 ng $ml^{-1}$. Plasma (1 ml), to which had been added prazosin (10 ng) as internal standard, was made alkaline with 2 M NaOH (0.2 ml) and extracted with diethyl ether (5 ml). The dried organic phase was dissolved in mobile phase (500 μl) and an aliquot (20–50 μl) was injected onto the h.p.l.c. column. The column used, a Spherisorb 5μ ODS (12.5 x 0.5 cm), was attached to a 6000A pump (Waters Associates), equipped with a U6K injection system (Waters Associates) with detection by a FS970 spectrofluorometer (excitation 246 nm, emission 389 nm, Schoeffel Instruments). The mobile phase was a mixture of methanol and buffer (70:30 v/v). The buffer was an aqueous solution (pH 3.4) of tetramethyl ammonium chloride 0.02 M and sodium heptane sulphonate 0.01 M. Using a flow rate of 1 ml $min^{-1}$ typical retention times of 3.6 and 5.2 min were found for prazosin and doxazosin, respectively. Drug concentration was determined from the peak height ratio of drug and internal standard by reference to a calibration curve of peak height ratio *vs* known drug concentrations. Concentrations of doxazosin found in quality control samples were in good agreement with concentrations added, mean recovery 100 ± 8.7% ($n$ = 5).

### *Thin layer chromatography*

Appropriate samples of plasma and urine were applied to thin layer plates either undiluted or following solid phase extraction on a column of Amberlite XAD-2 resin. The column was eluted with water, heptane and methanol and the concentrated methanol residues were applied to the t.l.c. plates.

Suitable samples of faeces were extracted either directly with methanol or by dialysis from an aqueous slurry into methanol through a semi-permeable membrane (Visking, Scientific Instrument Centre Ltd). The concentrated methanol extracts were applied to the t.l.c. plates.

Both urine and faecal extracts were analysed by t.l.c. both before and after hydrolysis with β-glucuronidase (Ketodase, Warner-Lambert Ltd) and arylsulphatase (Sigma) by incubation at 37°C overnight.

Samples were applied to silica t.l.c. plates (Merck $60F_{254}$) and these were developed in three solvent systems: A, n-butanol/acetic acid/water (60:15:25, by vol); B, ethyl acetate/isopropylamine (95:5, v/v); and C, ethyl acetate/methanol/diethylamine (70:20:5, by vol). Radioactive components were detected by autoradiography using Osray M3 film (Agfa-Gevaert). The relative proportions of radiometabolites were determined either by removing discrete bands of silica and counting in methanol (1 ml) and Instagel (10 ml) or by quantitative scanning using a linear analyser data acquisition system (IM 3000, Lab-Logic Ltd).

### *High-performance liquid chromatography*

In some cases the urine and faecal extracts were further analysed for radiometabolites by high performance liquid chromatography. In this case a

Spherisorb 5μ ODS column was used (25 x 0.5 cm) in conjunction with the Waters equipment and a radiochemical detector (LB 503 HS, Berthold). The mobile phase was a mixture of methanol (45%) and a buffer (pH 6) consisting of 0.1 M tetramethyl ethylene diamine and phosphoric acid.

*Calculations*

Half-life of elimination, $t_{1/2}$, was determined by linear regression of the elimination portion of the log plasma concentration versus time curves.

The area under the plasma concentration versus time curve was calculated using the trapezoidal rule. The area was extrapolated to infinity by calculating the theoretical area under the last segment of the curve from

$$\text{Area} = \frac{C_o.t_{1/2}}{0.693}$$

Where $C_o$ is the last measurable plasma concentration.

Total clearance, CL, was defined

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

Systemic bioavailability (%) was defined as

$$\frac{AUC_{0-\infty}\ (\text{p.o.})}{AUC_{0-\infty}\ (\text{i.v.})} \times \frac{\text{dose (i.v.)}}{\text{dose (p.o.)}} \times 100$$

*Identification of radiometabolites*

The cochromatography of radiometabolites in urine and faecal extracts with doxazosin and synthetic standards II - VIII was determined on t.l.c. plates in solvent systems A, B and C.

In some instances, cochromatography of radiometabolites with the standards was also determined on h.p.l.c. using the radiochemical detector system (*vide supra*) in conjunction with an ultraviolet detector (λ = 248 nm, Pye Unicam).

Where possible, metabolites were isolated for spectroscopic investigation. Appropriate radioactive bands were eluted from t.l.c. plates with methanol. The concentrated residue was further purified on h.p.l.c. using a Spherisorb ODS column and a mobile phase consisting of a 50:50 mixture of methanol and 0.1 M $NH_4OCOCH_3$ (pH 5). Appropriate fractions were collected and freeze-dried. The residue was subjected to electron impact mass spectroscopy (LKB 9000S; 70 eV) by direct insertion probe and to proton NMR (Bruker WM 250 MHz) in $CD_3OD$. The metabolite spectra were compared to those of the synthetic standards.

The radiometabolite with an $R_F$ value of 0.4 on t.l.c. in solvent system A was sprayed with naphthoresorcinol to detect uronic acids (Dutton, 1966).

## Results

*Absorption and excretion*

The data in Table 1 show the recovery of radioactivity from animals and man following oral and intravenous administration of [2-$^{14}$C]-doxazosin. Table 1 also shows the proportion of the dose excreted as unchanged drug in the faeces. For all species and for both oral and i.v. doses the main route of elimination of radioactive compounds was via the faeces, urinary excretion being generally low. The extent of absorption was estimated by comparing the proportion of a radioactive dose which was excreted in the urine following oral administration with that following intravenous drug and by comparing the fraction of the dose excreted unchanged via the faeces following the two routes of administration. In human volunteers given a single radiolabelled dose of 1 or 2 mg, i.e. in the lower part of the therapeutic range, 9% of the dose was excreted in the urine after both oral and intravenous dosing. Furthermore, only about 5% of the dose was excreted as unchanged drug via the faeces following both routes of administration. Thus the data show that doxazosin is virtually completely absorbed by man following oral administration. By the same criteria, the data in the mouse and rat indicate that doxazosin is completely

**Table 1** The proportion of a radioactive dose (%) of doxazosin excreted in the urine and faeces of several species given oral drug and, in parentheses, the fraction of the dose (%) excreted unchanged in the faeces

| | | *Route* | |
|---|---|---|---|
| *Species* | *System* | *Oral* | *Intravenous* |
| Man | urine | 9 | 9 |
| | faeces[a] | 63(5) | 65(5) |
| Mouse | urine | 20 | 19 |
| | faeces | 74(1) | 73(4) |
| Rat | urine | 20 | 16 |
| | faeces | 79(8) | 66(14) |
| Dog | urine | 3 | 12 |
| | faeces | 78(44) | 73(4) |

[a]Figures in parentheses refer to % excreted unchanged.

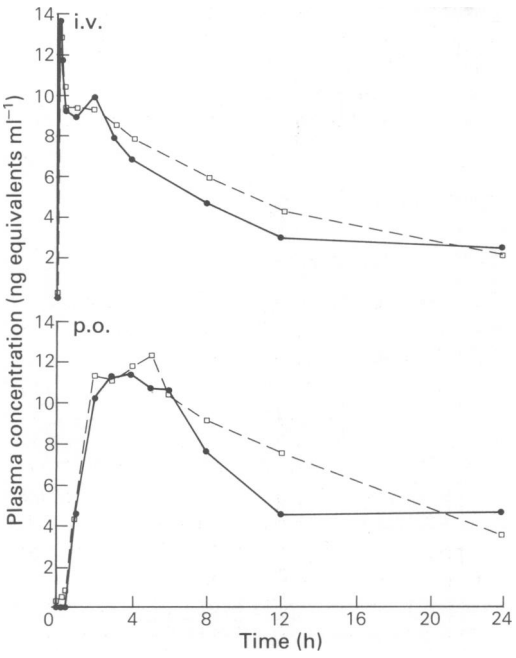


**Figure 2** Average plasma concentrations of doxazosin (●) and total radioactivity (□) in two human subjects given [2-$^{14}$C] drug.

absorbed in these species also. In the dog, given radiolabelled doxazosin 4 mg kg$^{-1}$ orally, urinary excretion of 3% of the dose was low compared to that following intravenous drug (12%). In addition following oral doxazosin, 44% of the dose was excreted unchanged in faeces compared to 4% following intravenous drug. Thus, the balance of doxazosin in the faeces, i.e. 40% of the dose, may represent unabsorbed drug and it is concluded that following oral administration at dose levels used in toxicity studies absorption may be 60%.

In the human study, plasma concentrations of radioactivity were compared with concentrations of doxazosin itself and these are depicted in Figure 2. The majority of plasma radioactivity comprised unchanged drug from both the oral and intravenous doses, which indicates that there were no major circulating metabolites, particularly during the first 4–6 h after dosing.

### *Bioavailability and pharmacokinetic studies*

Vincent *et al.* (1983) have reported the bioavailability and pharmacokinetic parameters for single doses of doxazosin in human volunteers. These data are shown in Table 2 along with the data for rat and dog in our studies. Bioavailability is high in man

**Table 2** The bioavailability and pharmacokinetic parameters of doxazosin in man, rat and dog

| | *Man* | *Rat* | *Dog* |
|---|---|---|---|
| Oral bioavailability (%) | 63 | 50 | 60 |
| Plasma half-life (%) | 9 | 1.2 | 4.7 |
| Plasma clearance (ml min$^{-1}$ kg$^{-1}$) | 1.2 | 30 | 13 |
| Volume of distribution (l kg$^{-1}$) | 0.97 | 3 | 5 |

(63%), dog (60%) and rat (50%). The relatively long half-life in man (9 h) is a consequence of a low plasma clearance (1.2 ml min$^{-1}$ kg$^{-1}$) and a moderate volume of distribution (0.97 l kg$^{-1}$). In the rat, high plasma clearance (30 ml min$^{-1}$ kg$^{-1}$) results in a short elimination half-life (1.2 h). In the dog, a plasma clearance of 13 ml min$^{-1}$ kg$^{-1}$ and a volume of distribution of 5 l kg$^{-1}$ resulted in a plasma elimination half-life value of 4.7 h.

### *Metabolism studies*

Absorbed drug was extensively metabolized in all species; little unchanged drug was excreted, less than 15% of the dose in all cases (Table 1). The large majority of metabolites were eliminated via the faeces and detailed studies were therefore performed mainly on faecal extracts. Metabolites were identified mainly by comparison with the synthetic standards on t.l.c. and h.p.l.c. (Figure 3) and in some cases by the matching of mass and NMR spectra of isolated substances with synthetic compounds. For example, for radiometabolite *V* (Figure 1) from dog given intravenous drug, peaks in the mass and NMR spectra matched those of the synthetic standard. Thus for the radiometabolite, peaks in the mass spectrum at m/$_z$ 467(M+), 316, 288, 259, 233, 221, 205 and 178 were also observed in the spectrum of the standard 6′-hydroxy doxazosin. Similarly, peaks at $\delta$ values of 3.95, 3.97, 3.7–4.5, 5.0–5.2, 6.34, 6.36, 6.78, 6.95 and 7.38 corresponded to peaks observed in the NMR of the synthetic material. Thus the metabolites shown in Figure 1 were shown to be present in differing relative proportions in the four species as shown in Figure 4. Metabolism in man mainly involves *O*-demethylation of the quinazoline substituents or hydroxylation of the benzdioxan moiety. The 6- and 7-*O*-desmethyl metabolites (III and II, Figure 1)

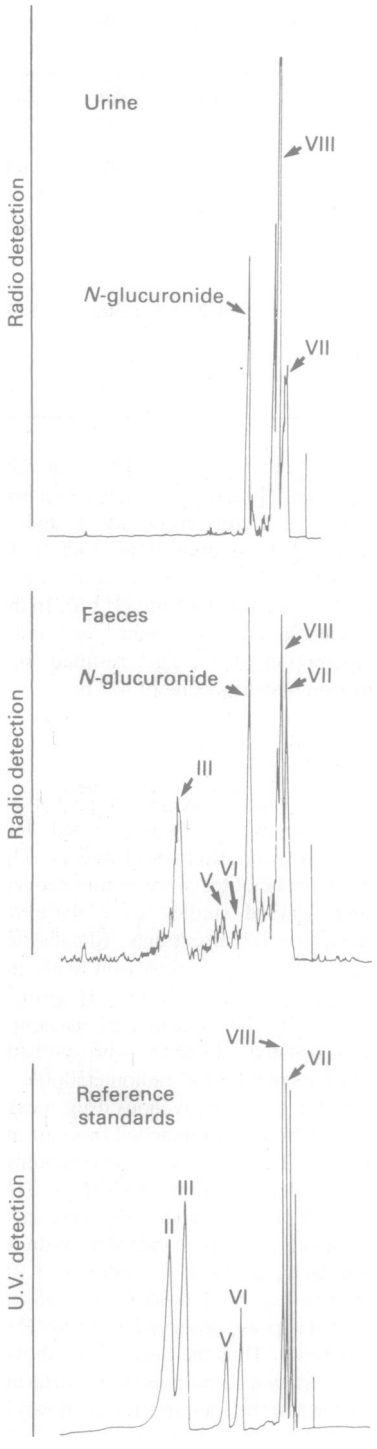


**Figure 3** Comparison by h.p.l.c. of reference standards with radiometabolites in urine and faeces of rats given [2-$^{14}$C]-doxazosin 10 mg kg$^{-1}$ orally.

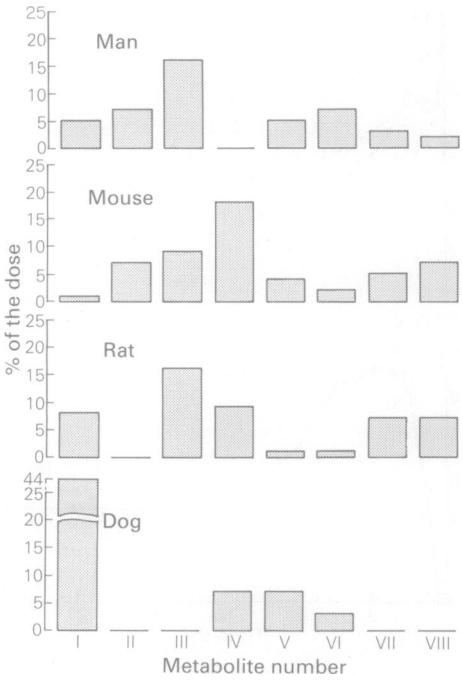


**Figure 4** The relative proportions of the metabolites of doxazosin in man, mouse, rat and dog.

comprise 16% and 7% of an oral dose and the 6′-hydroxy (V) and 7′-hydroxy (VI) products accounted for 5% and 7% of the dose, respectively. Minor products were the 2-piperazinyl (VIII) metabolite and the 2-amino compound (VII). The major metabolite in rat was the 6-*O*-desmethyl compound, as in man, and this accounted for 16% of the dose. All the metabolites were present in differing quantities in the rat and mouse and some were found in the dog. Interestingly, a metabolite which was not formed in man (metabolite IV) was present in the rat and dog and was major in the mouse, comprising 17% of the dose. This highly polar compound was resistant to β-glucuronidase but gave a purple colour with naphthoresorcinol, indicative of uronic acids (Dutton, 1966) and a mass spectrum identical to that of doxazosin. Thus, this compound was judged to be a glucuronide conjugate of doxazosin, presumably the *N*-glucuronide.

Plasma protein binding was high in all species studied, ranging from 95.3% in the rat to 98.3% in human patients. Thus the unbound fraction in man is approximately three-fold lower than in rat.

## Discussion

Doxazosin is completely absorbed in man when given at therapeutic doses and oral bioavailability is high (63%), as in rat (50%) and dog (60%). Absorbed doxazosin undergoes extensive biotransformation and metabolites are eliminated mainly in the faeces in man and the other animal species studied. The major route of metabolism in man and the rat was *o*-demethylation, as was the case with prazosin (Taylor *et al.*, 1977). Other metabolites in man were common to the mouse, rat or dog, which indicates that patients undergoing therapy will be exposed to the same chemical entities as the animal species used in toxicity tests.

Due mainly to low plasma clearance, doxazosin is eliminated from plasma with a moderately long half-life, approximately 9 h. This long half-life value, compared to 2.5 h for prazosin in man (Bateman *et al.*, 1979), provides the basis for once-daily dosing. The clearance value is low compared to hepatic blood flow in man (*circa* 25 ml $min^{-1}$ $kg^{-1}$, Greenway & Starck, 1971) and suggests that hepatic clearance would not be dependent on the changes in blood flow which may occur in cardiovascular disease, particularly since multiple pathways of metabolism are involved. Since bioavailability is high in man, then conversely first pass or pre-systemic metabolism is only moderate and would be expected to be less variable than for high extraction drugs. Overall these observations support the finding of low variation in half-life and in plasma concentration versus time profiles (Frick *et al.*, 1986). Since in subjects given radiolabelled drug the large majority of circulating radioactivity comprises doxazosin, the antihypertensive effect of the drug is judged to be due, in the main, to doxazosin itself.

We wish to acknowledge the skilled technical assistance of Mrs B. Gibson, Miss J. A. Norris and Mr J. L. Offerman and to thank Dr K. James, Dr Y. Noguchi and Mrs P. Searle for synthesis of reference standards.

## References

Alabaster, V.A. & Davey, M.J. (1986). The $\alpha$-adrenoceptor antagonist profile of doxazosin: preclinical pharmacology. *Br. J. clin. Pharmac.*, **21**, 9S–17S.

Bateman, D.H., Hobbs, D.C., Twomey, T.M., Stevens, E.A. & Rawlins, M.D. (1979). Prazosin, pharmacokinetics and concentration effects. *Eur. J. clin. Pharmac.*, **16**, 177–181.

Dutton, G.J. (1966). *Glucuronic Acid*, p. 116. London: Academic Press.

Elliott, H.L., Meredith, P.A., Sumner, D.J., McLean, K. & Reid, J.L. (1982). A pharmacodynamic and pharmacokinetic assessment of a new $\alpha_1$-adrenoceptor antagonist, doxazosin (UK-33,274) in normotensive subjects. *Br. J. clin. Pharmac.*, **13**, 699–703.

Frick, M.H., Halttunen, P., Himanen, P., Huttunen, M., Pörsti, P., Pitkäjärvi, T., Pöyhönen, L., Pyykönen, M.-L., Reinikainen, P., Salmela, P. & Saraste, M. (1986). A long-term double-blind comparison of doxazosin and atenolol in patients with mild to moderate essential hypertension. *Br. J. clin. Pharmac.*, **21**, 55S–62S.

Greenway, CV. & Starck, R.D. (1971). Hepatic vascular bed. *Physiol. Rev.*, **51**, 23–65.

Singleton, W., Saxton, C.A.P.D. & Hernandez, R. (1980). Alpha-blocking effect of prazosin, trimazosin and UK-33,274 in man. In *World Conference on Clinical Pharmacology and Therapeutics. Abstracts*, eds Turner, P. & Padgham, C. London and Basingstoke: Macmillan.

Taylor, J.A., Twomey, T.M. & Schach von Wittenau, M. (1977). The metabolic fate of prazosin. *Xenobiotica*, **7**, 357–364.

Timmermans, P.B.M.W.M., Kwa, H.Y., Karamat Ali F. & Van Zwieten, P.A. (1980). Prazosin and its analogues UK-18,596 and UK-33,274. A comparative study on cardiovascular effects and alpha-adrenoceptor blocking activities. *Arch. int. Pharmacodyn. Ther.*, **245**, 218–235.

Vincent, J., Elliott, H.L., Meredith, P.A. & Reid, J.L. (1983). Doxazosin, an $\alpha_1$-adrenoceptor antagonist: pharmacokinetics and concentration-effect relationships in man. *Br. J. clin. Pharmac.*, **15**, 719–725.