Stereoselective binding of doxazosin enantiomers to plasma proteins from rats, dogs and humans in vitro

Jia-an SUN1,2,*, De-zhi KONG1,8, Ya-qin ZHEN1, Qing LI1, Wei ZHANG1, Jiang-hua ZHANG1, Zhi-wei YIN1, Lei-ming REN1,*

1Institute of Chinese Integrative Medicine, Hebei Medical University, Shijiazhuang 050071, China; 2Bethune International Peace Hospital, Shijiazhuang 050081, China

Aim: (±)Doxazosin is a long-lasting inhibitor of α₁-adrenoceptors that is widely used to treat benign prostatic hyperplasia and lower urinary tract symptoms. In this study we investigated the stereoselective binding of doxazosin enantiomers to the plasma proteins of rats, dogs and humans in vitro.

Methods: Human, dog and rat plasma were prepared. Equilibrium dialysis was used to determine the plasma protein binding of each enantiomer in vitro. Chiral HPLC with fluorescence detection was used to measure the drug concentrations on each side of the dialysis membrane bag.

Results: Both the enantiomers were highly bound to the plasma proteins of rats, dogs and humans [(–)doxazosin: 89.4%–94.3%; (+)doxazosin: 90.9%–95.4%]. (+)Doxazosin exhibited significantly higher protein binding capacities than (–)doxazosin in all the three species, and the difference in the bound concentration (C_b) between the two enantiomers was enhanced as their concentrations were increased. Although the percentage of the plasma protein binding in the dog plasma was significantly lower than that in the human plasma at 400 and 800 ng/mL, the corrected percentage of plasma protein binding was dog>human>rat.

Conclusion: (–)Doxazosin and (+)doxazosin show stereoselective plasma protein binding with a significant species difference among rats, dogs and humans.

Keywords: doxazosin; enantiomer; stereoselectivity; plasma protein binding; equilibrium dialysis; species difference; pharmacokinetics; benign prostatic hyperplasia; lower urinary tract symptom; α₁-adrenoceptor

Original Article

Introduction

(±)Doxazosin is a long-lasting inhibitor of α₁-adrenoceptors that is widely used to treat benign prostatic hyperplasia and lower urinary tract symptoms to reduce the smooth muscle tone in the prostate and the bladder neck[1, 2]. Because (±)doxazosin is nonselective, the α₁-adrenoceptor blocking effects are not limited to the lower urinary tract and also affect other tissues, such as the vasculature; therefore, (±)doxazosin can cause vasodilator adverse effects (AEs), including dizziness and postural hypotension[3]. These vasodilator AEs can potentially lead to serious complications, such as falls and fractures[4]. Furthermore, a double-blind randomized multiple center clinical trial indicated that the use of (±)doxazosin was associated with a two-fold higher risk of congestive heart failure compared to chlorthalidone among high-risk hypertensive patients[5]. Doxazosin contains a chiral carbon, creating two enantiomers: (−)doxazosin and (+)doxazosin. Recently, we demonstrated that the chiral carbon atom in the molecular structure of doxazosin does not affect the therapeutic activity at α₁A-adrenoceptors in the rabbit prostate, but the chiral carbon significantly affects the inhibition of α₁D-adrenoceptors in the rat aorta[6]. In rat and rabbit heart tissues, (+)doxazosin significantly decreases the atrial rate and produces negative inotropic effects; however, (−)doxazosin produces positive inotropic effects in the atria via an α₁-adrenoceptor-independent mechanism[6].

Plasma protein binding plays a major role in drug therapy because the bound drug is difficult to pass through blood vessel walls and cell membranes, whereas the unbound drug can cross the capillary wall to reach the cellular target and metabolic tissues. A change in plasma protein binding significantly affects a drug’s pharmacokinetic and pharmacodynamic properties[7, 8], which is especially important for drugs with high protein binding affinities (such as doxazosin). Because the difference in the protein binding property between enantio-
moters often causes a difference in the pharmacokinetic character-
ers\textsuperscript{[9–12]}. Enantioselective protein binding studies are essential to understand the pharmacology, safety and clinical efficacy of these drugs. Hence, stereoselective detection and quantification of each enantiomer of (±)-doxazosin in biological media are essential.

Equilibrium dialysis has the advantage that non-specific adsorption can be compensated for if the concentration at equilibrium on each side is determined, which means that the adsorption will not affect the concentration ratio at equilibrium\textsuperscript{[13, 14]}. Consequently, equilibrium dialysis was used in the present study. Many studies have focused on the components of plasma proteins that are responsible for reversible drug binding. Although understanding the contribution of each isolated plasma protein to drug binding is important\textsuperscript{[15]}, the data obtained for the individual components of plasma are not applicable in a clinical situation. Therefore, whole plasma was used in this study.

The plasma protein binding for (±)-doxazosin in rats, dogs and humans has been reported to be greater than 90% (ranging from 95.3% in rats to 98.3% in humans)\textsuperscript{[16]}. However, no data are currently available showing the stereoselective binding of the doxazosin enantiomers to the plasma proteins of rats, dogs and humans. Therefore, we have applied chiral HPLC methods using a chiral stationary phase column in conjunction with equilibrium dialysis to simultaneously determine the protein binding properties of each doxazosin enantiomer in rat, dog and human plasma after the addition of (±)-doxazosin in vitro. The results demonstrate that the doxazosin enantiomers bind enantioselectively to the plasma proteins of rats, dogs and humans, which might partly explain the phenomenon that the plasma concentration of (–)-doxazosin is lower than that of (+)-doxazosin after administration of (±)-doxazosin.

**Materials and methods**

**Reagents**

(±)-Doxazosin mesylate, (+)-doxazosin mesylate and (–)-doxazosin mesylate with optical purities of more than 99.9% were synthesized and provided by the New Drug Research and Development Center of North China Pharmaceutical Group Corporation (Shijiazhuang, Hebei, China). Prazosin (>99.0% in purity, internal standard, IS) was purchased from Sigma Aldrich (St Louis, Missouri, USA). The dialysis membranes (molecular weight: cut-off 8000–12000, flat width: 10 mm) were purchased from Spectrum Laboratories, Inc (Los Angeles, California, USA). Acetonitrile and methanol (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA). All other chemicals and solvents were of analytical grade and were purchased from Tianjin Yongda Chemical Reagent Development Centre (Tianjin, China). Water was purified using a Nanopure (Thermo Fisher Scientific, Waltham, Massachusetts, USA) laboratory ultra-pure water system (0.2 µm filter).

Pooled human plasma (anti-coagulated with citrate) from three healthy individuals was obtained from the blood and transfusion center at Bethune International Peace Hospi-
tal (Shijiazhuang, Hebei, China). Pooled dog plasma was obtained from the blood of 12 male Beagle dogs, collected in citrate anti-coagulation tubes and centrifuged for 10 min at 1040×g at 4 °C (ScanSpeed 1580R, LaboGene, Lyngby, Denmark). Pooled rat plasma was obtained from the blood of 15 male Sprague-Dawley rats and prepared as described above. All of the plasma from humans, dogs and rats was stored frozen at -20 °C until use.

Phosphate-buffered saline (PBS) was prepared by dissolving 5.78 g NaCl, 4.00 g Na\textsubscript{2}HPO\textsubscript{4}, 2H\textsubscript{2}O, and 0.77 g NaH\textsubscript{2}PO\textsubscript{4}·H\textsubscript{2}O in 1 L of water and adjusting the pH to 7.4 with NaH\textsubscript{2}PO\textsubscript{4}. The PBS had anionic strength of 0.168, which is a value close to plasma.

**Instrumentations and conditions**

An Agilent 1260 liquid chromatography system (Agilent Technologies, Santa Clara, California, USA) equipped with a fluorescence detector, a quaternary solvent delivery system, an autosampler and a column compartment was used for all analyses. The chromatographic separations were performed on an Ultron ES-OVM column (150 mm×4.6 mm id, 5 µm, Shinwa, Kyoto, Japan) fitted with a refillable guard cartridge (Shinwa) packed with Ultron ES-OVM (10 mm×4.0 mm id, 5 µm). The mobile phase was phosphate buffer (20 mmol/L, pH 5.32)-acetonitrile (86:14, v/v). All separations were performed isocratically at a flow rate of 1.0 mL/min, and the column temperature was maintained at 30 °C. The detector was operated with an excitation wavelength of 255 nm and an emission wavelength of 385 nm. The retention times of prazosin, (–)-doxazosin and (+)-doxazosin mesylate were 6.2, 9.1, and 10.4 min, respectively.

**Assay of plasma protein concentration**

The protein concentration of the pooled plasma was determined using the BCA Protein Assay Kit (Multisciences Biotech Co Ltd, Hangzhou, Zhejiang, China). Bradford reagent (1.5 mL) was added to 50 µL of diluted plasma (diluted 100-fold) in a micro-tube. The reagent and the plasma were mixed in the plate for 30 min on an orbital shaker at 37 °C, and the ultraviolet (UV) absorption of each sample was measured with a TU-1901 UV-VIS Spectrophotometer (Purkinje General Instrument Co Ltd, Beijing, China) at a wavelength of 562 nm. A calibration curve prepared using known concentrations of BSA (bovine serum albumin) ranging from 0.2 to 10 mg/mL was used to quantify the protein concentration of each sample.

**Protein binding study**

**The process of equilibrium dialysis**

The dialysis membranes were prepared according to the guidelines provided by the supplier. Briefly, the membranes were soaked in boiling deionized water for 20 min, followed by a 20-min soak in 30% alcohol. After washing thoroughly with deionized water, a further 60-min soak in PBS buffer was needed.

A simple equilibrium dialysis device was used and is shown in Figure 1. First, 500 µL of the rat, dog, or human plasma...
was loaded in the 2-cm-long dialysis membrane bag that was ligated at both ends. The bag was then placed in a 5 mL microcentrifuge tube with 3 mL PBS buffer containing 200, 400, or 800 ng/mL of (±)doxazosin mesylate. The tube was capped tightly and incubated for 15 h at 37°C. After the dialysis was completed, the contents of the dialysis bag (free+bound) and the contents of the microcentrifuge tube (free) were collected and determined by HPLC. For each datum, the plasma protein binding was determined in five separate plasma samples.

**Characterization of the leakage**

A possible leakage of plasma into PBS buffer could have interfered with the measurements in the study. The degree of leakage was controlled by measuring the protein content of the PBS buffer using a turbidity comparison method. Briefly, the PBS buffer (0.5 mL) was sampled after equilibration in a transparent tube, and 0.2 mL of trichloroacetic acid (20%, in water) was added. The tubes were viewed horizontally against a dark background, and the data were rejected if a white precipitate appeared.

**Preparation of the samples in PBS buffer**

A 20-μL volume of internal standard solution (prazosin 1 μg/mL) was pipetted into 400 μL dialyzed fluid in a 1.5 mL tube. The mixture was extracted with 900 μL of hexane-ethyl acetate (1:1, v/v). After vortexing for 2 min, the samples were centrifuged for 4 min at 2500×g. Then, the organic layer was transferred to another tube and evaporated to dryness under a gentle stream of nitrogen. The residues were reconstituted with 200 μL of mobile phase. Finally, a 10 μL aliquot of the resulting solution was injected into the HPLC system to determine the total concentration (Ct) of each enantiomer of doxazosin.

**Statistical analysis**

The bound concentration (Cb) was calculated according to the equation of Cb=Ct−Cu, where Ct and Cu are the total concentration and the unbound concentration, respectively. The protein binding was calculated by the following equation:

$$P(\%) = \frac{C_b}{C_t} \times 100.$$

The statistical analysis was performed using Prism software 5.01 (San Diego, California, USA). A t-test was used to evaluate the difference in the bound drug concentration between (−)doxazosin and (+)doxazosin. A one-way ANOVA was used to evaluate the difference in the plasma protein binding of (−)doxazosin or (+)doxazosin among three species. All of the data were expressed as the mean±SD.

**Results and discussion**

**Method validation**

Figure 2 shows the representative chromatograms of the chiral doxazosin in blank plasma samples from rats, dogs and humans as well as in PBS buffer and spiked samples. The chiral HPLC methods for the separation of doxazosin enantiomers were highly selective. Prazosin, (−)doxazosin and (+)doxazosin were clearly resolved from the matrix components under the chromatographic conditions, and the two enantiomers of doxazosin were baseline resolved from one another (Figure 2).

**Optimization of the equilibrium dialysis conditions**

The time for doxazosin to reach equilibrium between plasma and isotonic PBS was investigated. Isotonic PBS spiked with (+)doxazosin (800 ng/mL) was dialyzed against pooled human plasma at 0, 4, 8, 15, and 20 h. (+)Doxazosin and (+)doxazosin

![Figure 1. The simple equilibrium dialysis device.](image-url)
crossed the dialysis membrane rapidly and reached a steady-state level in human plasma within 15 h at 37 °C (Figure 3). The equilibrium times for dog and rat plasma were the same as the equilibrium time for human plasma (data not shown). Therefore, the equilibrium dialysis duration was 15 h in the subsequent equilibrium dialysis experiments at incubation temperature of 37°C (body temperature).

**The difference in protein binding between the enantiomers**

In the concentration range between 200 and 800 ng/mL of (±)doxazosin, the percentages of plasma protein binding of the enantiomers in the three species were 89.4%–94.3% for (–)doxazosin and 90.9%–95.4% for (+)doxazosin. The results showed that either (–)doxazosin or (+)doxazosin was highly bound to plasma proteins, which is consistent with previous investigations that determined the binding of racemic doxazosin[16, 19]. Furthermore, the $C_b$ values of (–)doxazosin were significantly lower than those of (+)doxazosin ($P<0.01$) in the plasma from each of the three species at the used concentrations of (±)doxazosin (Table 1). As shown in Figure 4A to Figure 4C, the difference in the $C_b$ between the two enantiomers increased with higher concentrations of (±)doxazosin regardless of the species. Moreover, the stereoselectivity of

|          | Low      | Middle   | High     |
|----------|----------|----------|----------|
| (–)Doxazosin | 225.1±4.9 | 508.8±4.2 | 1004.0±19.6 |
| (±)Doxazosin | 256.0±5.2 | 577.8±5.3 | 1129.1±21.8 |
| (–)Doxazosin | 200.0±9.6 | 398.6±21.5 | 845.1±32.6 |
| (±)Doxazosin | 243.0±10.6 | 477.3±20.4 | 1006.8±40.6 |
| (–)Doxazosin | 218.7±8.4 | 481.0±4.5 | 996.4±51.3 |
| (±)Doxazosin | 242.8±7.4 | 531.9±3.4 | 1103.7±76.3 |

Table 1. Bound fraction of doxazosin enantiomers (ng/mL) in the plasma from rat, dog, and human when low (200 ng/mL), middle (400 ng/mL), and high (800 ng/mL) doses of racemic doxazosin were applied (mean±SD, n=5). $P<0.01$ vs (–)doxazosin.

---

**Figure 2.** Representative chromatograms of blank rat plasma (A), blank dog plasma (B), blank human plasma (C), PBS buffer (D), sample spiked with 12.5 ng/mL (±)doxazosin in PBS buffer (E), and the post-dialysis human sample after adding 200 ng/mL of (±)doxazosin (F) under the same chromatographic conditions. The enantiomers were resolved on an Ultron ES-OVM column after LLE (liquid-liquid extraction), with the mobile phase consisting of a mixture phosphate buffer (20 mmol/L, pH 5.32) and acetonitrile (86:14, v/v).

**Figure 3.** Effect of incubation time on unbound fractions of (–)doxazosin (▲) and (+)doxazosin (○) in equilibrium dialysis protein binding experiments in the PBS spiked with 800 ng/mL (±)doxazosin ($n=2$).
doxazosin in dog plasma was more significant than the stereoselectivity in rat plasma and human plasma (Table 1, Figure 4). The mean $C_b$ values (ng/mL) of (-)doxazosin vs (+)doxazosin for dog plasma in the PBS contained (+)doxazosin 200, 400, and 800 ng/mL were 200.0 vs 243.0, 398.6 vs 477.3 and 845.1 vs 1006.8, respectively. Overall, the enantioselective binding of doxazosin enantiomers to the plasma proteins from rats, dogs and humans was observed, and (+)doxazosin exhibited a higher protein-binding capacity than (-)doxazosin in all three species.

A difference in the plasma protein-binding capacity between the two enantiomers could lead to different pharmacokinetic behaviors. Drug clearance from the blood is directly proportional to the free fraction in the plasma, and the higher unbound (-)doxazosin concentration in human plasma observed in the present study might partly explain the phenomenon reported by Liu et al [20], that the human plasma concentration of (-)doxazosin is lower than that of (+)doxazosin after racemate administration [20]. In our laboratory, the different plasma concentrations of the two enantiomers in the dog and the rat were observed after administration of (-)doxazosin and (+)doxazosin (unpublished data). Therefore, the discrepancy for plasma protein-binding capacity between (-)doxazosin and (+)doxazosin could be a reason for the different pharmacokinetic parameters in rat, dog and human plasma [21, 22].

Difference in protein binding among three species
As shown in Table 2, no significant difference in the percentage of plasma protein binding among three species was found when 200 ng/mL of (+)doxazosin was added to PBS. However, the percentage of plasma protein binding in dog plasma was significantly lower than that in human plasma at 400 and 800 ng/mL of (+)doxazosin ($P<0.05$ and $P<0.01$, Table 2), which is consistent with the previous report that the plasma protein binding of (+)doxazosin in human plasma was higher than that in dog plasma [16].

Because the total protein concentrations of the pooled plasma from rats, dogs and humans were 61.81, 51.35, and 57.75 mg/mL, respectively, the percentage of plasma protein binding should be corrected with the value of the protein assay (Figure 5). The corrected percentage of plasma protein binding of the enantiomers at a given concentration was variable in different species: dog>human>rat ($P<0.01$).

Table 2. Percentage of plasma protein binding of (-)doxazosin and (+)doxazosin in rat, dog, and human plasma when low (200 ng/mL), middle (400 ng/mL), and high (800 ng/mL) doses of racemic doxazosin were applied (mean±SD, n=5). $^aP<0.05$, $^bP<0.01$ vs human plasma. $^cP<0.05$ vs dog plasma (one-way ANOVA test).

|       | (-)Doxazosin | (+)Doxazosin | (-)Doxazosin | (+)Doxazosin | (-)Doxazosin | (+)Doxazosin |
|-------|--------------|--------------|--------------|--------------|--------------|--------------|
| Low   | 89.35±1.30   | 90.97±1.19   | 92.31±0.19   | 93.73±0.20   | 92.34±0.43$^{aw}$ | 93.66±0.37$^b$ |
|       | 90.85±0.87   | 93.08±0.68   | 91.05±1.91$^a$ | 93.25±1.50$^b$ | 90.52±1.03$^c$ | 92.72±0.92$^c$ |
|       | 91.21±1.40   | 92.30±1.57   | 94.04±0.56   | 95.23±0.53   | 94.28±1.29   | 95.42±0.93   |

Conclusion
In this study, equilibrium dialysis was used to determine the plasma protein binding of the enantiomers of (+)doxazosin in rat, dog, and human plasma. Chiral HPLC with fluorescence detection was validated and used to measure the drug concentration on each side of equilibrium. The racemization test was
performed and showed no chiral inversion of the doxazosin enantiomers. The (−)-doxazosin and (+)-doxazosin enantiomers were baseline-resolved under chromatographic conditions. The results showed that either (−)-doxazosin or (+)-doxazosin was highly bound to plasma proteins. Moreover, the protein binding of the doxazosin enantiomers in human, dog, and rat plasma revealed a significant difference in the bound fractions, i.e., a higher protein-binding capacity of (+)-doxazosin than (−)-doxazosin, which could explain the difference in plasma concentration between (−)-doxazosin and (+)-doxazosin in human orally administered (±)-doxazosin[8]. Additionally, a significant difference in the plasma protein binding between species was found in this study, with a higher protein-binding capacity in humans than in dogs. Because the total protein concentrations of the plasma were significantly different among rats, dogs, and humans, the corrected percentage of plasma protein binding was calculated in the study, which indicated the order of dog>human>rat \(P<0.01\). Therefore, the findings of a stereoselective plasma protein binding between (−)-doxazosin and (+)-doxazosin and a significant species difference in the plasma protein binding of rats, dogs, and humans should be useful in understanding the pharmacokinetic characters of chiral doxazosin.

**Acknowledgements**

This work was supported by grants from the National Science & Technology Major Project “Key New Drug Creation and Manufacturing Program” of China (No 2011ZX 09102-011-04) and the National Program on Key Basic Research Project of China (973 Program) (No 2012CB518601).

**Author contribution**

Lei-ming REN and De-zhi KONG designed and supervised the research; Jia-an SUN Ya-qin ZHEN and Qing LI performed the research and analyzed the data; Wei ZHANG, Jiang-hua ZHANG, and Zhi-wei YIN assisted with part of the research; Jia-an SUN, De-zhi KONG, and Lei-ming REN wrote the manuscript.

**References**

1. Chapple CR. Selective alpha 1-adrenoceptor antagonists in benign prostatic hyperplasia: rationale and clinical experience. Eur Urol 1996; 29: 129–44.
2. Kirby RS, Andersen M, Gratze P, Dahlstrand C, Haye K. A combined analysis of double-blind trials of the efficacy and tolerability of doxazosin-gastrointestinal therapeutic system, doxazosin standard and placebo in patients with benign prostatic hyperplasia. BJU Int 2001; 87: 192–200.
3. Montorsi F, Moncada I. Safety and tolerability of treatment for BPH. Eur Urol Suppl 2006; 5: 1004–12.
4. Barendrecht MM, Koopmans RP, De La Rosette JJMCH, Michel MC. Treatment of lower urinary tract symptoms suggestive of benign prostatic hyperplasia: the cardiovascular system. BJU Int 2005; 95: 19–28.
5. ALLHAT Collaborative Research Group. Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone: The antihypertensive and lipid-lowering treatment to prevent heart attack trial (allhat). JAMA 2000; 283: 1967–75.
6. Zhao D, Duan LH, Wang FY, Wang M, Lu HG, Wu ZG, et al. Chiral recognition of doxazosin enantiomers in 3 targets for therapy as well as adverse drug reactions in animal experiments. Can J Physiol Pharmacol 2012; 90: 1623–33.
7. Williams K. Molecular asymmetry and its pharmacological consequences. Adv Pharmacol 1991; 22: 57–135.
8. Pacifici GM, Viani A. Methods of determining plasma and tissue binding of drugs. Pharmacokinetic consequences. Clin Pharmacokinet 1992; 23: 449–68.
9. Hong Y, Tang Y, Zeng S. Enantioselective plasma protein binding of propafenone: Mechanism, drug interaction, and species difference. Chirality 2009; 21: 692–8.
10. Sun DL, Huang SD, Wu PS, Li J, Ye YJ, Jiang HD. Stereoselective protein binding of tetrahydropalmatine enantiomers in human plasma, HSA, and AGP, but not in rat plasma. Chirality 2010; 22: 618–23.
11. Maddi S, Yamsani MR, Seeling A, Scriba GKE. Stereoselective plasma protein binding of amlodipine. Chirality 2010; 22: 262–6.
12. Tang YH, Wang JY, Hu HH, Yao TW, Zeng S. Analysis of species-dependent hydration and protein binding of esmolol enantiomers. J Pharm Anal 2012; 2: 220–5.
13. Howard ML, Hill JJ, Galluppi GR, McLean MA. Plasma protein binding in drug discovery and development. Comb Chem High Throughput Screen 2010; 13: 170–87.
14. Barre J, Chamouard JM, Houn G, Tillement JP. Equilibrium dialysis, ultrafiltration, and ultracentrifugation compared for determining the plasma-protein-binding characteristics of valproic acid. Clin Chim Acta 1985; 31: 60–4.
15. Mohamed N, Kuroda Y, Shibukawa A, Nakagawa T, El Gizaawy S, Askal H, et al. Enantioselective binding analysis of verapamil to plasma lipoproteins by capillary electrophoresis-frontal analysis. J Chromatogr A 2000; 875: 447–53.
16. Kaye B, Cussans N, Faulkner J, Stephe D, Reid J. The metabolism and kinetics of doxazosin in man, mouse, rat and dog. Br J Clin Pharmacol 2000; 875: 447–53.
Pharmacol 1986; 21 Suppl 1: 19S–25S.
17 Gewirtz DA, Holt SA. Protein binding as a component of drug interaction in cellular pharmacokinetic studies: Effects of probenecid on transport and accumulation of methotrexate in Ehrlich ascites tumor cells in vitro. Biochem Pharmacol 1985; 34: 747–54.
18 Yü TF, Dayton PG, Gutman AB. Mutual suppression of the uricosuric effects of sulfinpyrazone and salicylate: a study in interactions between drugs. J Clin Invest 1963; 42: 1330–9.
19 Young R, Brogden R. Doxazosin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in mild or moderate hypertension. Drugs 1988; 35: 525–41.

20 Liu K, Zhong D, Chen X. Enantioselective determination of doxazosin in human plasma by liquid chromatography-tandem mass spectrometry using ovomucoid chiral stationary phase. J Chromatogr B Analyt Technol Biomed Life Sci 2010; 878: 2415–20.
21 Mizushima H, Takanaka K, Abe K, Fukazawa I, Ishizuka H. Stereoselective pharmacokinetics of oxybutynin and N-desethyloxybutynin in vitro and in vivo. Xenobiotica 2007; 37: 59–73.
22 Brocks DR. Drug disposition in three dimensions: an update on stereoselectivity in pharmacokinetics. Biopharm Drug Dispos 2006; 27: 387–406.