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Comparing pharmacokinetics and metabolism of diltiazem in normotensive Sprague Dawley and Wistar Kyoto rats vs. spontaneously hypertensive rats in vivo

Pollen K.F. Yeung*, Angelita Alcos, Tanya Marcoux and Jinglan Tang

Pharmacokinetics and Metabolism Laboratory,
College of Pharmacy and Department of Medicine,
Faculties of Health Professions and Medicine, Dalhousie University Halifax, Nova Scotia, Canada

Abstract

Background: In order to identify a suitable rodent model for preclinical study of calcium antagonists, the pharmacokinetics and metabolism of one of the prototypes diltiazem (DTZ) in normotensive Sprague Dawley (SDR) was compared with Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) following 5 mg/kg twice daily for five doses given by subcutaneous injection.
Methods: Pharmacokinetic data were analyzed by standard procedures assuming a one-compartment model with first-order input using Rstrips®, and differences between the groups were considered significant when $p<0.05$.
Results: Plasma concentrations of DTZ were higher in the SHR than the normotensive SDR and WKY rats, although the differences did not reach statistical significance ($p>0.05$). Plasma concentrations of the active metabolites N-desmethyl DTZ (MA), deacetyl DTZ (M1) and deacetyl N-desmethyl DTZ (M2) were significantly higher in the SHR and WKY rats than the SDR, which was attributed to higher DTZ concentrations and also genetic factors.
Conclusions: Although the differences were mainly quantitative and very small, the study has shown for the first time that the metabolism profiles of DTZ in SHR and WKY rats were closer to humans than SDR, and they may be more preferable rat models to study pharmacokinetic and metabolism studies of DTZ or similar agents.

Keywords: Diltiazem (DTZ), metabolism; pharmacokinetics; spontaneously hypertensive rats (SHRs); Sprague Dawley rats (SDRs); Wistar Kyoto (WKY).

*Corresponding author: Pollen K.F. Yeung,
Pharmacokinetics and Metabolism Laboratory,
College of Pharmacy and Department of Medicine,
5968 College Street, Burbidge Building Dalhousie University,
Halifax, Nova Scotia, Canada B3H 4R2
Phone: +1 902 4943845, Fax: +1 902 4941396
E-mail: Pollen.Yeung@Dal.Ca
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Introduction

Diltiazem (DTZ) is one of the first prototype calcium channel-blockers (CCBs) still widely used in the treatment of angina and hypertension, and has the potential to treat atherosclerosis and prevent stroke (1–4). It is extensively metabolized in humans via deacetylation, *N*-demethylation, *O*-demethylation, and oxidative deamination yielding a host of metabolites, some of which have potent pharmacological activities. It has been shown that in dogs that the coronary vasodilating properties of deacetyl diltiazam (M1), *N*-monodesmethyl diltiazem (MA) and *N*-monodesmthyl deacetyl diltiazem (M2) were 50%, 20% and 17%, respectively, of DTZ (5). When comparing the effect on platelet aggregation and uptake of adenosine by erythrocytes in vitro, however, some of these metabolites (e.g., M1) were more potent than DTZ (6, 7). We have shown that both M1 and M2, when injected separately into rabbits, significantly lowered blood pressures (systolic blood pressure, SBP, and diastolic blood pressure, DBP). The hemodynamic effects of these metabolites were similar to DTZ, although their clearance and volume of distribution were greater in the rabbit model (8).

We have previously shown that pharmacokinetics and metabolism of DTZ differs quantitatively between animal species and humans, such that systemic clearance is higher in normotensive Sprague Dawley rats (SDRs), followed by rabbits, dogs and humans. In man and dogs, MA is the major metabolite following oral dose, whereas in SDRs, M2 is the most abundant one (9).

Increasingly animal models with a pathological phenotype are used for preclinical drug development (10), but pharmacokinetics and metabolism data in these models are very limited. Previous studies have shown that the hemodynamic responses to DTZ were significantly greater in spontaneously hypertensive rats (SHRs) and experimentally induced a higher renin rat model than in the SDR (11) (12), and that plasma concentrations of DTZ and its active metabolites were also higher in the SHR (13). Further, the metabolite profiles of SHR were also different from the SDRs as plasma concentrations of MA were higher than M2 and M1 in the SHR, whereas M2 was the most abundant metabolite in the SDRs (13).

In order to determine whether the difference in metabolite levels was attributed to higher plasma concentrations of DTZ or related to a genetic factor, the current study for the first time compares the pharmacokinetics and metabolism of DTZ between the SDR, SHR and Wistar Kyoto (WKY) rats, which is a normotensive strain similar to SDR but with a genetic background closer to SHR (14, 15) following repeated subcutaneous administration of DTZ in vivo.

Materials and methods

Chemicals

DTZ was received as gift from Biovail Corp. (Mississauga, ON, Canada), and its metabolites were generously donated by the Tanabe Seiyaku Co. (Japan). Solvents were high-performance liquid chromatography (HPLC) grade (BDH Chem., Halifax, N.S., Canada), and all other chemicals were reagent grade (Fisher Scientific, ON Canada).

Study protocol

The study protocol was approved by the Dalhousie University Committee on Laboratory Animals (UCLA). Male SDRs (n=8), SHRs (n=5) and WKYs (n=8 per group) were purchased from Charles River Laboratories (Wilmington, MA, USA) for the experiments. They were between 10 and 15 weeks old and weighed between 300 and 350 g for SHRs and WKYs, and between 350 and 450 g for the SDRs.

An indwelling catheter made of silastic® tubing (0.020″ ID×0.037″ OD, Dow Corning Corp., Midland, MI, USA) was implanted into the right carotid artery of each animal under general anesthesia for blood sample collection as described previously (16).

After recovery from the surgery (24 h), each animal received 5 mg/kg of DTZ by subcutaneous injection twice daily for five doses. Previous studies have shown that significant hemodynamic effect was obtained at this dosage, and that the pharmacokinetics and pharmacodynamics of DTZ could be adequately characterized following this regimen (11, 17).

Blood samples (0.3 mL each) were obtained at 0 (before the last dose), 0.25, 1, 2, 3, 4, 5, and 6 h post-dose. The plasma samples were immediately separated by centrifugation (4°C, 1720 × *g*, 5 min) and stored at –20°C until analysis. All the plasma samples were analyzed within 3 months after collection to avoid possible sample deterioration (18, 19). Plasma concentrations of DTZ and its major metabolites (MA, M1 and M2) were determined by a previously published HPLC method (20).

Data analysis

Pharmacokinetic parameters were calculated including area under the plasma concentration-time curve (AUC), half-life (T½), time to maximum plasma concentrations (t_{max}), systemic clearance (CL), maximum plasma concentration (C_{max}), mean residence time (MRT), and volume of distribution at steady-state (Vdss) if applicable by standard procedures (21, 22).

Due to the limited number of sampling points, the data were analyzed assuming a one-compartment model after a first-order or zero-order input using Rstrips® (V. 5, MicroMath, Saint Louis, MO, USA). The AUC from time 0 to the last sampling time (AUC_{0-last}) was calculated by the trapezoidal method and the AUC to the last sampling time ($AUMC_{0-last}$) and MRT by integration method. The bioavailability normalized clearance (CL/F) and volume of distribution at steady state (Vdss/F) of DTZ after the last dose were calculated using the equations D/AUC_{0-last} and $D\times AUMC_{0-last}/(AUC_{0-last})^2$, respectively.

The suitability of the model was assessed by graphical inspection, and was confirmed with the model selection criterion in Rstrips®, which gives the same rankings of fit as the Akaike information criterion (AIC). It is a normalized criterion and independent of scaling of data points (23).

Plasma concentration-time data were fitted individually for each rat as well as for the mean data. Differences in the pharmacokinetic parameters between different strains of rats (i.e., SDR, SHR and WKY) were evaluated using analysis of variance (ANOVA) followed by Tukey's multiple comparison test and considered significant when $p<0.05$ (Minitab® Inc. Release 16, State College, PA, USA).

Results

Plasma concentrations of DTZ was not detectable in the time 0 samples collected before the last dose for most of the SDRs, but it was detectable from the SHR and WKY rats. Plasma concentration of the metabolites was measurable in the time 0 samples from most of the rats studied (see Figure 1). Despite the limited number of sample time points (n=8), the plasma concentration-time curves of DTZ and most of its metabolites (except MA) were adequately described by a one-compartment open model following first-order input. The data for MA, however, were best characterized by one-compartment open model after zero-order input (see Figure 1).

Plasma concentrations of DTZ were considerably higher in the SHRs (see Table 1). However, due to the small number of animals involved and large inter-individual variation of the data (>50%), the differences did not reach statistical significance ($p>0.05$). There were also no significant differences in the CL/F and Vdss/F of DTZ between the different strains of rats (see Table 1).

Plasma concentrations of the active metabolites (MA, M1 and M2) were higher in the SHR and WKY rats, and the differences were significant for MA between SDRs and SHRs. The differences were also significant for M1 and M2 between SDR and WKY rats (see Table 1).

The apparent plasma half-life (T½) of MA was significantly longer for SHRs vs. SDRs ($p<0.05$, see Table 1), but there was no significant difference in the plasma T½ of DTZ or other metabolites. The pharmacokinetic parameters are summarized in Table 1.

The metabolite profiles were quantitatively different between the types of rats studied, such that M2 was the most abundant metabolite in the SDRs, whereas MA was found to be most abundant in the SHRs and WKYs (see Table 1). There were no significant differences ($p>0.05$) for the AUC ratios of the metabolite to DTZ between SDRs, WKY rats and SHRs (see Table 2).

Discussion

Plasma concentrations of DTZ were highest in the first sampling time for most of the rats studied, which suggests a rapid absorption following subcutaneous administration (see Figure 1). As reported previously, the pharmacokinetics of

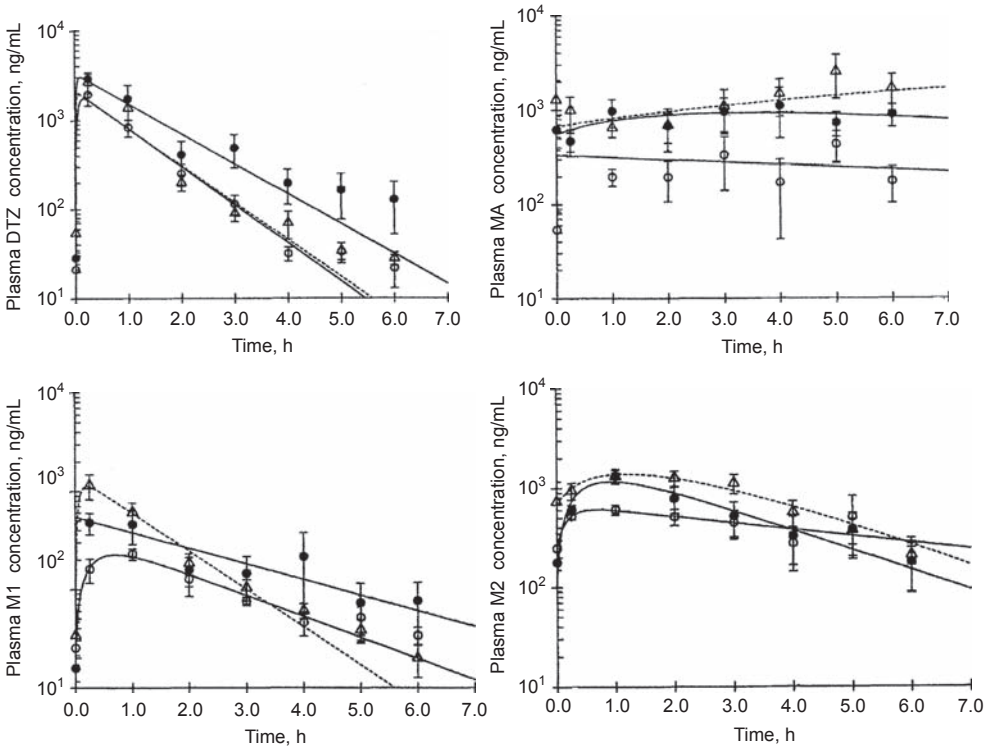

Figure 1 Mean plasma concentration-time profiles of DTZ and its active metabolites after 5 mg/kg subcutaneous DTZ twice daily for five doses in rats.
SHR ●, WKY rat Δ, SDR ○. Each plasma concentration-time point represents mean±SEM.

DTZ following subcutaneous injections estimated from eight sample time points collected over a period of 6 h could be adequately described by a two-compartment open model following intravenous bolus. The plasma concentration-time profiles of the metabolites could be characterized by a one-compartment open model following first-order input (13, 17, 24, 25).

We have found in the current study that similar results could be obtained by fitting the DTZ data with a one-compartment model following first-order input (see Table 1), although a two-compartment model may provide better fit for the terminal phase of the plasma concentration-time plot if there were more sample data collected beyond the 6 h. This would allow better characterization of both the ascending and descending portions of the plasma concentration-time profile of a two-compartment model (see Figure 1).

Despite the limitation, we have found fitting the DTZ data with either one compartment after first-order input or two compartments with intravenous bolus yielded comparable results (model selection criterion: 3.17 vs. 4.93). However, since the kinetics of the metabolites could only be described by a one-compartment model with first-order input, we chose to use the one-compartment model data of DTZ for comparison.

The t_{max} of DTZ calculated by this model were <30 min, which supports rapid absorption of DTZ after subcutaneous injection. Since the sampling times were sparse at the early phase of the plasma concentration-time curve, however, it was difficult to get an accurate estimate of t_{max}. As t_{max} is a hybrid parameter reflecting both absorption and elimination, the apparent difference observed for T½ between the different rat strains (albeit not statistically significant) could reflect a difference of either drug elimination and/or absorption because of the flip flop effect. The absolute bioavailability of DTZ following a subcutaneous injection had previously been shown to be >90% in rats (26), as such the pharmacokinetic parameters reported in this study, such as CL/F and Vdss/F, should be close approximates of CL and Vdss, respectively, following intravascular administration. It has been shown that the apparent plasma T½ of the metabolites MA, M1 and M2 were longer than the parent DTZ following a single dose, although they tended to become shorter after multiple exposure (17).

The results from the current studies indicate that there were no statistically significant differences in the apparent plasma T½ between the metabolites and DTZ after multiple doses in all three strains of rats, suggesting that elimination of the metabolites was rate-limited by their formation and not elimination after multiple doses of DTZ. Although the plasma T ½ of MA appears considerably longer than DTZ, particularly in the SHRs (147.5±139.9 vs. 4.11±6.37 h), the difference was not statistically significant ($p>0.05$), see Table 1. These data are consistent with the previous reports suggesting that the true T½ of MA may be longer than DTZ (13, 17), although it will need to be confirmed by injections of the metabolite directly.

The current study also showed that the T½ of MA was significantly longer in SHRs compared to SDRs (see Table 1). The difference observed should be interpreted with

Table 1 Pharmacokinetics of DTZ and its active metabolites in rats.

	DTZ	MA	M1	M2
C_{max}, µg/mL				
SDR	1.82±0.85[a]	0.42±0.25[b]	0.32±0.18[c]	0.70±0.24[c]
WKY	1.93±1.13	0.64±0.22	1.28±1.03	1.89±0.86
SHR	3.73±2.26	1.19±0.63	0.69±0.45	1.51±0.62
T_{max}, h				
SDR	0.25±0.19	1.16±1.04	0.54±0.42	1.09±0.95
WKY	0.37±0.17	1.14±1.49	0.30±0.21	1.09±0.68
SHR	0.25±0.13	2.71±0.97	0.27±0.16	0.76±0.49
AUC (last), µg^{-h}/mL				
SDR	1.64±0.75	1.34±1.09	0.59±0.20[c]	2.73±1.72[c]
WKY	3.03±1.96	5.03±4.91	1.53±0.88	5.05±1.49
SHR	3.90±2.13	4.78±2.53	1.23±0.86	3.47±1.66
T½, h				
SDR	0.58±0.094	15.6±28.5[b]	1.67±1.31	2.15±1.5
WKY	1.10±0.81	41.3±69.3	0.90±0.90	1.44±0.44
SHR	4.11±6.37	147.5±139.9	1.78±1.50	1.22±0.67
MRT, h				
SDR	0.95±0.17	2.40±1.21	1.91±0.66	2.37±0.69
WKY	1.28±0.51	2.74±0.84	1.20±0.61	2.20±0.38
SHR	1.10±0.80	2.95±0.28	1.67±0.71	1.84±0.55
CL/F (last), L/h/kg				
SDR	3.03±1.31			
WKY	1.90±0.89			
SHR	1.72±0.76			
Vdss/, L/kg				
SDR	2.85±1.21			
WKY	3.32±2.67			
SHR	1.83±1.59			

[a]Each value represents mean±standard deviation. [b]p<0.05 vs. SHR. [c]p<0.05 vs. WKY.

caution, however, because the plasma concentrations of MA were barely descending at the end of the 6 h experiment, which made it difficult to obtain a reliable estimate of the T½ (see Figure 1). Fitting individual data, however, yielded similar results as those from the mean data.

Plasma concentrations of DTZ measured over the 6 h period appeared to be higher ($AUC_{0\text{-last}}$ 3.90±2.13 vs. 1.64±0.75 µg^{-h}/mL) and the plasma T½ longer (4.11±6.37 vs. 0.58±0.094 h) in the SHRs than the SDRs, albeit the difference was not statistically significant (p>0.05). This was mainly attributed to the small number of animals included in the study and the large inter-individual variations in the data obtained, particularly in the SHRs (see Table 1). The large data variability is an inherent property of the pharmacokinetics and metabolism of DTZ, which is observed both in human and animal models (9, 17, 27–29). Previous studies have shown that there were no differences in the variability of plasma concentrations of DTZ or its metabolites between intra-arterial and subcutaneous administrations, nor between single dose vs. multiple doses (17, 24, 26). We employed multiple doses because the hemodynamic effects are more reproducible (11, 30) and would reflect a more relevant approach as most antihypertensive agents are intended for chronic use. For data with >50% variability, as seen in the SHRs, and a difference of 2 µg^{-h}/mL, as shown for the $AUC_{0\text{-last}}$, it would require 27 animals in each group to attain a margin of error below 20% (Minitab® Inc. Release 16, State College, PA, USA). Thus, the number of animals used (n=5 – 8 in each group) would not have sufficient power to detect the difference. As such, the results obtained from the current study should be considered pilot results and must be interpreted with caution.

Isanta and co-workers have previously shown that plasma concentrations of DTZ were higher in the SHR and suggested

Table 2 Ratios of area under the curve (AUC) of the metabolites to DTZ.

Type of rat	AUC MA/ AUC DTZ	AUC M1/ AUC DTZ	AUC M2/ AUC DTZ
SDR	0.86±0.57[a]	0.45±0.27	2.32±2.15
WKY	1.25±0.61	0.57±0.26	1.71±0.69
SHR	1.46±1.14	0.30±0.11	0.93±0.27

[a]Each value represents the mean±standard deviation.

that a reduction in clearance (CL) secondary to reduced blood flow was the main mechanism for the higher concentrations in the SHR (31), which was consistent with the results we report in this study.

It is interesting to note that plasma concentrations of DTZ in the WKY rats were closer to the SDRs, and yet the concentrations of the metabolites in the WKY rats were similar to the SHRs (see Figure 1). The higher plasma concentrations of metabolites could probably be attributed at least in part to the higher and more sustained DTZ concentrations, particularly in the SHRs, which may increase production of the metabolites, although the distribution and/or clearance of the metabolites ($CL_{(m)}$) may be other factors contributing to the difference. The fact that the metabolite concentrations were also higher in the WKY rats, which had similar DTZ concentrations to the SDRs, may suggest that both genetic and physiological or phenotypic factors could affect the pharmacokinetics and metabolism of DTZ and its metabolites, although the differences were not statistically significant ($p>0.05$), see Table 1.

Consistent with the results reported in previous studies (13, 17, 24, 25), the current study also indicates that M2 was the most abundant metabolite in the SDRs, followed by MA and M1 (see Table 1). While the plasma concentration-time profiles of the metabolites were similar (see Figure 1), MA was the most abundant metabolite in the SHRs and WKY rats, followed by M2 and M1 (see Table 1). Plasma concentrations of M1 and M2 were, however, higher in the WKY rats than SHRs and SDRs ($p<0.05$ vs. SDRs, see Table 1).

It has been shown in previous studies that there are quantitative differences in the pharmacokinetics and metabolism of DTZ between species (9). For example, in humans and dogs, MA is the most abundant metabolite found in plasma, as opposed to M1 and M2, which are the most abundant in rabbits and SDRs, respectively (9). To our knowledge, the current study is the first to demonstrate there is a difference in metabolism profiles among SDRs, WKY rats and SHRs, and that the metabolism of DTZ in the SHRs and WKY rats is closer to humans than in SDRs.

The ratio of AUC_{0-last} of the metabolites to parent DTZ, which is a measure of metabolic (or formation) clearance (CL_f) (21), also indicated that DTZ was metabolized more to MA in SHRs and WKY rats than in SDRs. Thus, SHRs and WKY rats may be more suitable models than SDRs for pharmacokinetic and metabolism studies of DTZ, albeit the difference is small.

DTZ is metabolized mainly by CYP3A4 and CYP2D6 (32–37) and an inducible deacetylase enzyme (38–40) leads to the formation of the basic metabolites MA, M1 and M2. DTZ is also metabolized by oxidative deamination to a series of acidic metabolites (41–44). It has been shown that the activities of CYP450 isozymes, such as CYP3As, are higher in the SHRs (45–47), which may be responsible – at least in part – for the higher plasma concentrations of the metabolites, particularly for MA (see Table 1). It is possible that the activity of some of these metabolic enzymes for DTZ may differ between the SDRs, WKY rats and SHRs. While pharmacokinetic and metabolism studies of DTZ and most other antihypertensive agents reported to-date have mostly used normotensive SDRs, future studies should attempt to determine whether the pharmacokinetics and pharmacodynamics of these agents are different in these different rat models, and whether any of these characteristics are more similar to humans. As SHR and other animal models with a pathologic phenotype and/or genotype are increasingly used in pre-clinical drug development, understanding the differences of drug disposition in disease and apparently normal models would help to select the most clinically relevant animal model for drug development.

Conclusion

In conclusion, despite the limitation of the small number of rats used for comparison, the current study has shown for the first time that plasma concentrations of DTZ and its main metabolites MA, M1, M2 appeared to be higher in the SHRs and WKY rats than in SDRs. The metabolism profiles in the SHRs and WKY rats also appear closer to humans than those of SDRs and as such they may be more favorable rodent models for preclinical studies. Additional studies using larger number of rats and longer sampling time are necessary, however, to confirm these preliminary findings. Further studies into the pharmacokinetics and hemodynamic effects of DTZ using these different rat strains are warranted to tease out the difference and determine the most suitable model for preclinical drug development.

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Conflict of interest statement

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References

1. Basile J. The role of existing and newer calcium channel blockers in the treatment of hypertension. J Clin Hypertens (Greenwich) 2004;6:621–29.
2. Grossman E, Messerli FH. Calcium antagonists. Prog Cardiovasc Dis 2004;47:34–57.
3. Ezeugo U, Glasser SP. Clinical benefits versus shortcomings of diltiazem once-daily in the chronotherapy of cardiovascular diseases. Expert Opin Pharmacother 2009;10:485–91.

4. Chen N, Zhou M, Yang M, Guo J, Zhu C, Yang J, et al. Calcium channel blockers versus other classes of drugs for hypertension. Cochrane Database Syst Rev 2010;CD003654.
5. Yabana H, Nagao T, Sato M. Cardiovascular effects of the metabolites of diltiazem in dogs. J Cardiovas Pharmacol 1985;7:152–7.
6. Kiyomoto, A, Sasaki, Y, Odawara, A, Morita, T. Inhibition of platelet aggregation by diltiazem. Circ Res 1983;52 (Suppl.I):115–9.
7. Yeung PK, Mosher SJ, MacRae DA, Klassen GA. Effect of diltiazem and its metabolites on the uptake of adenosine in blood: an in-vitro investigation. J Pharm Pharmacol 1991;43:685–9.
8. Yeung PK, Feng JD, Buckley SJ. Pharmacokinetics and hypotensive effect of diltiazem in rabbits: comparison of diltiazem with its major metabolites. J Pharm Pharmacol 1998;50:1247–53.
9. Yeung, PK, Mosher, SJ, Quilliam, MA, Montague, TJ. Species comparison of pharmacokinetics and metabolism of diltiazem in humans, dogs, rabbits, and rats. Drug Met Disp 1990;18:1055–9.
10. Muders F, Elsner D. Animal models of chronic heart failure. Pharmacol Res 2000;41:605–12.
11. Yeung P, Alcos A, Tang J, Casley W. Hemodynamic effects of diltiazem in spontaneously hypertensive rats vs. normotensive rats following multiple doses in vivo. Current Topics in Pharmacology 2008;12:39–44.
12. Bertera FM, Mayer MA, Opezzo JA, Taira CA, Hocht C. Increased sensitivity to diltiazem hypotensive effect in an experimental model of high-renin hypertension. J Pharm Pharmacol 2009;61:79–87.
13. Yeung P, Alcos A, Tang J, Casley W. Pharmacokinetics and metabolism of diltiazem following multiple doses: comparing normotensive rat vs hypertensive rat models in vivo. Drug Metab Let 2008;2:146–50.
14. Nabika T. [Congenic rats for hypertension studies]. Nippon Rinsho 2001;59:832–5.
15. Doroshchuk AD, Postnov AI, Afanas'eva GV, Budnikov EI, Postnov IuV. [Decreased ATP-synthesis ability of brain mitochondria in spontaneously hypertensive rats]. Kardiologiia 2004;44:64–5.
16. Tsui BC, Mosher SJ, Yeung PK. A reliable technique for chronic carotid arterial catherization in the rat. J Pharmacol Meth 1991;25:343–52.
17. Yeung P, Alcos A, Tang J, Casley W. Pharmacokinetics and metabolism of diltiazem in rats: comparing single vs. repeated subcutaneous injections in vivo. Biopharm Drug Disp 2007;28:403–7.
18. Caille G, Dube LM, Theoret Y, Varin F, Mousseau N, McGilveray IJ. Stability study of diltiazem and two of its metabolites using a high-performance liquid chromatographic method. Biopharm Drug Dispos 1989;10:107–14.
19. Yeung, PK, Mosher SJ, Klassen GA, McGilveray, IJ. Stability of diltiazem and its metabolites in plasma during storage. Therapeutic Drug Monitoring 1991;13:369–74.
20. Yeung, PK, Montague TJ, Tsui B, McGregor C. High performance liquid chromatographic assay of diltiazem and six of its metabolites in plasma: application to a pharmacokinetic study in healthy volunteers. J Pharm Sci 1989;78:592–7.
21. Rowland M, Tozer TN. Clinical Pharmacokinetics: Concepts and Applications. Baltimore: Lippincott Williams and Wilkins, 2011.
22. Shargel L, We-Pong S, Yu A. Applied Biopharmaceutics and Pharmacokinetics, 5th Edition, New York: McGraw-Hill (Medical Publishing Division), 2005.
23. RSTRIP. Rstrip Pharmacokinetic Analysis Handbook, Salt Lake City: MicroMath Inc., 1987.
24. Tsui BC, Feng JD, Buckley SJ, Yeung PK. Pharmacokinetics and metabolism of diltiazem in rats following a single intra-arterial or single oral dose. Eur J Drug Met Pharma 1994;19:369–73.
25. Tsui BC, Feng JD, Yeung PK. Pharmacokinetics and haemodynamic effect of diltiazem in rats: effect of route of administration. J Pharm Pharmacol 1998;50:183–8.
26. Tsui BC. Pharmacokinetics and Pharmacodynamics of Diltiazem and its Metabolites, Nova Scotia, Canada, Dalhousie University.
27. Caille G, Boucher S, Spenard J, Lakhani Z, Russell A, Thiffault J, et al. Diltiazem pharmacokinetics in elderly volunteers after single and multiple doses. Eur J Drug Met Pharma 1991;16:75–80.
28. Hoglund P, Nilsson LG. Pharmacokinetics of diltiazem and its metabolites after single and multiple dosing in healthy volunteers. Ther Drug Monit 1989;11:558–66.
29. Yeung PK, Buckley SJ, Hung OR, Pollak PT, Barclay KD, Feng JD, et al. Steady-state plasma concentrations of diltiazem and its metabolites in patients and healthy volunteers. Ther Drug Monit 1996;18:40–5.
30. Yeung P, Alcos A, Tang J, Tsui B. Pharmacokinetics and hemodynamic effects of diltiazem in rats following single and repeated subcutaneous injection in vivo. Clin Pharmacol Therap 2006;79:53(PII-66).
31. Isanta JR, Lasheras B, Fos D, Cenarruzabeitia E. Comparative diltiazem plasma clearance in normotensive and hypertensive rats. J Pharm Sci 1987;76:862–5.
32. Pichard L, Gillet G, Fabre I, Dalet-Beluche I, Bonfils C, Thenot J-P, et al. Identification of the rabbit and human cytochromes P-450IIIA as the major enzymes involved in the N-demethylation of diltiazem. Drug Met Disp 1990;18:711–9.
33. Molden E, Asberg A, Christensen H. CYP2D6 is involved in O-demethylation of diltiazem. An in vitro study with transfected human liver cells. Eur J Clin Pharmacol 2000;56:575–9.
34. Masica AL, Azie NE, Brater DC, Hall SD, Jones DR. Intravenous diltiazem and CYP3A-mediated metabolism. Br J Clin Pharmacol 2000;50:273–6.
35. Zhou S, Yung Chan S, Cher Goh B, Chan E, Duan W, Huang M, et al. Mechanism-based inhibition of cytochrome P450 3A4 by therapeutic drugs. Clin Pharmacokinet 2005;44:279–304.
36. Molden E, Johansen PW, Bøe GH, Bergan S, Christensen H, Rugstad HE, et al. Pharmacokinetics of diltiazem and its metabolites in relation to CYP2D6 genotype. Clin Pharmacol Ther 2002;72:333–42.
37. Sutton D, Butler AM, Nadin L, Murray M. Role of CYP3A4 in human hepatic diltiazem N-demethylation: inhibition of CYP3A4 activity by oxidized diltiazem metabolites. J Pharmacol Exp Ther 1997;282:294–300.
38. LeBoeuf E, Grech-Belanger O. Deacetylation of diltiazem by rat liver. Drug Metab Disposit 1987;15:122–6.
39. Lee YB, Koh IB. Effects of phenobarbital on the deacetylation of diltiazem in rats. Pharm Res 1996;13:S405.
40. Yeung PK, Buckley SJ, Cameron R, Feng JD, Jordan J. Effect of phenobarbital pre-treatment on the pharmacokinetics and metabolism of diltiazem in rats. Drug Metabol Drug Interact1996;13:29–39.
41. Chang J, Yerino P, Mooney J, Drees D, Hwang K. Comparative pharmacokinetics and metabolism of TA-3090 and its two acidic metabolites (MA2 and MA4) in the rat. Pharm Res 1992;9(Suppl.):S260.
42. Chang J, Yerino P, Mooney J, Drees D, Hwang K. Effect of diet and gavage on the absorption and metabolism of TA-3090 in the rat. Pharm Res 1992;9(Suppl.):S259.
43. Sugawara Y, Ohashi M, Nakamura S, Usuki S, Suzuki T, Ito Y, et al. Metabolism of diltiazem. I. Structures of new acidic and

basic metabolites in rats, dog and man. J Pharmacobio Dyn 1988;11:211–23.
44. Nakamura S, Ito Y, Fukushima T, Sugawara Y, Ohashi M. Metabolism of diltiazem.III. Oxidative deamination of diltiazem in rat liver microsomes. J Pharmacobio Dyn 1990;13:612–21.
45. Merrick BA, Davies MH, Cook DE, Holcslaw TL, Schnell RC. Alterations in hepatic microsomal drug metabolism and cytochrome P-450 proteins in spontaneously hypertensive rats. Pharmacology 1985;30:129–35.
46. Imaoka S, Funae Y. Hepatic and renal cytochrome P-450s in spontaneously hypertensive rats. Biochim Biophys Acta 1991;1074:209–13.
47. Ghosh SS, Basu AK, Ghosh S, Hagley R, Kramer L, Schuetz J, et al. Renal and hepatic family 3A cytochromes P450 (CYP3A) in spontaneously hypertensive rats. Biochem Pharmacol 1995;50:49–54.