EFFECT OF ADMINISTRATION ROUTE AND LENGTH OF EXPOSURE ON PHARMACOKINETICS AND METABOLISM OF DILTIAZEM IN DOGS

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SUMMARY

The objective of this study was to systematically determine the pharmacokinetics and metabolism of diltiazem (DTZ) after a single i.v. dose, and after single and multiple oral (p.o.) doses. Four mongrel dogs (3 M, 1 F), aged 1-3 years, body weight 19-25 kg, were each given a single 30 mg dose of DTZ as a solution by i.v injection, the same dose orally from an immediate release tablet (Cardizem®, Aventis Pharma, Canada, QC), and also t.i.d. for 10 doses. A 3-4 week washout period was allowed between each treatment. Blood samples (4 ml each) were obtained after each treatment from each animal via a cephalic vein at 0 (just before dosing), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, and 12.0 h post dose. Urine samples were collected for 24 h. The plasma samples were immediately separated by centrifugation and stored at -20°C until analysis. The results showed that the bio-availability after a single p.o. dose of DTZ was 26 ± 24%. Following a single i.v. dose, DTZ declined bi-exponentially with a terminal half-life ($t_{½}$) of 4.2 ± 1.7 h. *N*-Monodesmethyl DTZ (M_A), deacetyl DTZ

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(M_1), and deacetyl *N*-monodesmethyl DTZ (M_2) were the major metabolites. Contrary to the results observed in clinical studies, there were no increase of plasma concentrations of DTZ after repeated doses (accumulation factor $R = 0.94 \pm 0.51$). Plasma concentrations of M_1 decreased following repeated oral doses, accompanying by an increase of plasma concentrations of M_2, although these changes were not statistically significant ($p > 0.05$). This study cautions the use of mongrel dogs for direct extrapolation to humans, particularly for chronic pharmacokinetics studies of DTZ.

KEY WORDS

diltiazem, metabolites, pharmacokinetics, pharmacodynamics, dogs

INTRODUCTION

Diltiazem (DTZ) is a calcium antagonist widely used in the treatment of angina and hypertension /1-3/. It is extensively metabolized in humans via deacetylation, *N*-demethylation, *O*-demethylation, and oxidative deamination, yielding a host of metabolites (Fig. 1), some of which have potent pharmacological activities. It has been shown that in dogs, the coronary vasodilatating properties of deacetyl diltiazam (M_1), *N*-monodesmethyl diltiazem (M_A), and *N*-monodesmthyl deacetyl diltiazem (M_2) were 50%, 20% and 17%, respectively, of that of DTZ /4/. On the other hand, when comparing the effect on platelet aggregation and uptake of adenosine by erythrocytes *in vitro*, some of these metabolites (e.g. M_1) were more potent than DTZ /5,6/. More recently, it has been shown that both M_1 and M_2, when injected separately into rabbits, significantly lowered blood pressure (SBP and DBP). Their effects were comparable to DTZ, although clearance and volume of distribution of these metabolites were higher /7/.

The oral bioavailability of DTZ is about 40% in humans /8/, 30% in rabbits /9/, 15-30% in dogs /10,11/ and 15% in rats /12/. The systemic clearance following intravenous (i.v.) administration is about 14 ml/min/kg in humans /13/, 64 ml/min/kg in rabbits /9/, 45 ml/min/kg in dogs /10/, and 90-180 ml/min/kg in rats /12,14/. It has been shown in humans that plasma concentrations of DTZ increased

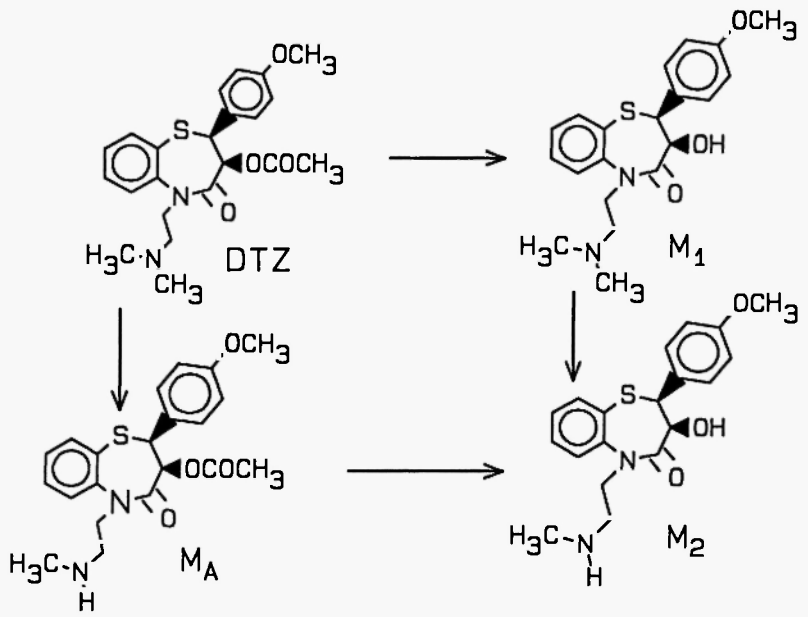

Fig. 1: Metabolism of diltiazem (DTZ). M_A = *N*-monodesmethyl DTZ; M_1 = deacetyl DTZ; M_2 = deacetyl *N*-monodesmethyl DTZ.

after multiple doses, accompanied by a greater increase of plasma concentrations of its major metabolites, such as M_A and M_1 /15,16/. The reason for the non-linear kinetics after chronic dosing is not fully understood, although it could be related to inhibition of CYP450 isozymes by diltiazem /17,18/. Metabolism profiles of DTZ are more similar between humans and dogs than in other animal species /19/. The current study used the dog as an animal model to evaluate the pharmacokinetics and metabolism of DTZ after single oral (p.o.), i.v., and repeated oral doses.

MATERIALS AND METHODS

Chemicals

DTZ and its metabolites were generously donated by the Tanabe Seiyaku Co. (Japan) via Aventis Pharma Canada (formerly Hoechst

Marion Roussel, Canada Research Inc., Laval, QC). Solvents were HPLC grade (BDH Chem., Halifax, N.S., Canada), and all other chemicals were reagent grade (Fisher Scientific, Ont., Canada).

Study protocol

The study protocol was approved by the Dalhousie University Committee on Laboratory Animals. Four mongrel dogs (3 M, 1 F), aged 1-3 years, body weight 19-25 kg (obtained via the Dalhousie Animal Care Centre) were used for the experiments. The study was a cross-over design with a 3-week washout period between each treatment. Each animal received a single 30 mg dose of DTZ intravenously as a solution, the same dose orally from an immediate release tablet (Cardizem®, Aventis Pharma, Canada), and also t.i.d. for 10 doses. Blood samples (4 ml each) were obtained after treatment from each animal at 0 (just before dosing), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, and 12.0 h post dose. Urine samples were collected for 24 h. The plasma samples were immediately separated by centrifugation (4°C, 1720 *g*, 5 min) and stored at -20°C until analysis /20/. All the samples (plasma and urine) were analyzed within 3 months after collection to avoid possible sample deterioration /21-23/.

Data analysis

Pharmacokinetic parameters were calculated using a computer-assisted non-linear curve-fitting program employing a two-compartment model following a bolus i.v. injection, and a one-compartment model following the p.o. dose (Rstrips®, MicroMath Scientific Software, Salt Lake City, UT, USA). Terminal half-lives ($t_{½}$) were calculated by non-linear regression. Areas under the plasma concentration-time curve from 0 to the last sampling time (AUC) were calculated by the trapezoidal method (Rstrip®, MicroMath, UT, USA). Absolute bioavailability (F) was calculated by the equation $F = AUC_{po}/AUC_{iv}$, where AUC_{po} and AUC_{iv} were the corresponding areas after i.v. and p.o. doses, respectively. Systemic clearance (Cl) was calculated from the equation $Cl = D/AUC$, where D was the i.v. dose /24/. The apparent volume of distribution at the $\exists$-phase ($V_{\exists}$) was equal to $Cl/\exists$ where $\exists$ was the slope of the terminal portion of the log-linear plasma concentrations-time curve after the i.v. dose /24/. Renal

clearance (Clr) was calculated from the equation A_e/AUC, where A_e was the amount excreted as the unchanged drug in the urine over 24 h, and AUC was the corresponding area /9,25/. Accumulation factor (R) was calculated by the ratio $(C_{ss})max/(C_1)max$, where $(C_{ss})max$ and $(C_1)max$ were the maximum plasma concentrations after the 10^{th} p.o. dose and the single p.o. dose study, respectively /24/. Due to the small number of animals used in this study and missing data, the differences between each dosage scheme were evaluated by ANOVA followed by the Tukey multiple comparison tests, and considered significant when $p < 0.05$ (Systat®, Systat, Inc., Evanston, IL, USA).

RESULTS

Following the single i.v. administration, plasma concentrations of DTZ declined bi-exponentially with an apparent terminal $t_{½}$ of 4.2 ± 1.7 h (Fig. 2, Table 1). The CI, Clr and V_3 of DTZ were 59 ml/min/kg, 0.13 ± 0.10 ml/min/kg, and 18 ± 10 l/kg, respectively. Plasma concentrations of the metabolites M_A and M_1 were measurable

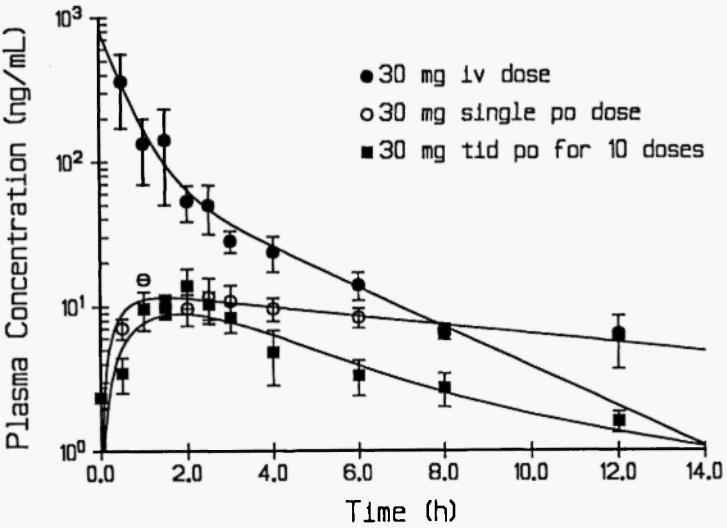

Fig. 2: Mean plasma concentration-time profile of diltiazem (DTZ) in dogs after DTZ. The solid lines represent concentrations predicted by the model using mean data.

TABLE 1

Pharmacokinetic parameters of DTZ in dogs after single and multiple p.o. doses, and single i.v. injection of 30 mg of DTZ

Parameters/ Route	Single dose i.v.	Single dose p.o.	Multiple dose p.o.	p
Apparent $t_{½}$ (h)	4.2 ± 1.7 [a]	8.8 ± 5.3	9.7 ± 4.6	NS
t_{max} (h)	ND	2.9 ± 1.8	1.9 ± 0.2	NS
AUC (ng-h/ml)	690 ± 590 [b]	91 ± 36	56 ± 19 [b]	<0.05 i.v. vs multiple p.o.
C_{max} (ng/ml)	ND	11 ± 5.8	9.5 ± 3.7	NS
Clr (ml/min/kg)	0.13 ± 0.10 [b]	0.21 ± 0.10	0.58 ± 0.36 [b]	<0.05 i.v. vs multiple p.o.

[a] Values represent means ± S.D.
[b] $p < 0.05$.
ND = not determined.
NS = not significant.

at most sampling times after the i.v. injection, with $t_{½}$ of 13 ± 12 and 17 ± 14 h, respectively (Fig. 3). The highest plasma concentrations (C_{max}) were M_1, followed by M_A, deacetyl *O*-monodesmethyl DTZ (M_4), and deacetyl *N,O*-didesmethyl DTZ (M_6), but M_2 was not detectable in plasma following the i.v. injection. The Clr of M_A was 0.46 ml/min/kg, but Clr could not be determined for the other metabolites because of insufficient data (Table 2).

Following the single p.o. dose, DTZ rose rapidly in plasma to reach a maximum concentrations by 1 h, and then declined with a $t_{½}$ of 8.8 ± 5.3 h (Table 1). The mean plasma concentration-time profiles of DTZ could be adequately characterized by an one-compartment model with first order input following oral dose, but a two-compartment model was needed to describe the disposition kinetics after i.v. administration (Fig. 3). The absolute oral bioavailability was 26 ± 24%. Compared to the i.v. dose, plasma concentrations of the metabolites, except M_1, appeared to be higher after the p.o. dose,

TABLE 2

Pharmacokinetic parameters of M_A in dogs after single and multiple p.o. doses, and single i.v. injection of 30 mg of DTZ

Parameters/ Route	**Single dose i.v.**	**Single dose p.o.**	**Multiple dose p.o.**	**p**
Apparent $t_{½}$ (h)	13 ± 12 [a]	10 ± 6.0	3.6 ± 2.4	NS
t_{max} (h)	2.4 ± 1.9	3.3 ± 2.6	2.7 ± 0.33	NS
AUC (ng-h/ml)	95 ± 35	110 ± 33	110 ± 65	NS
C_{max} (ng/ml)	11 ± 3.5	13 ± 3.6	14 ± 8.7	NS
Clr (ml/min/kg)	0.46 ± 0.30	0.35 ± 0.28	1.8 ± 1.4	NS

[a] Values represent means ± S.D.
NS = not significant.

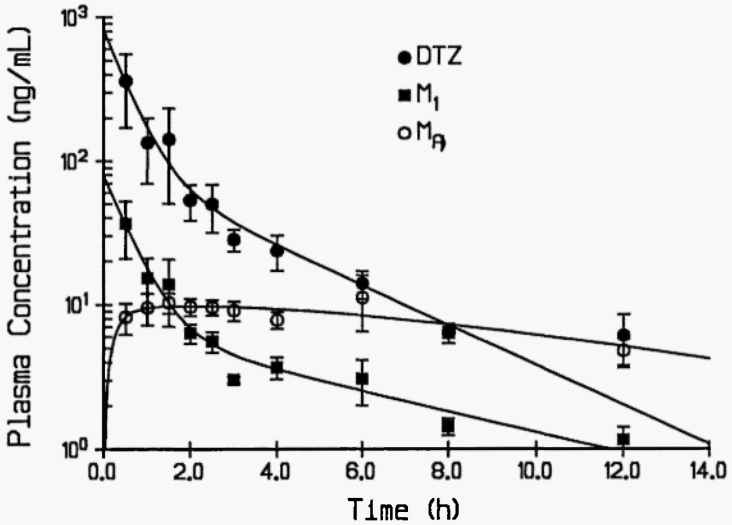

Fig. 3: Mean plasma concentration-time profile of diltiazem (DTZ) and its metabolites in dogs after a single 30 mg i.v. dose. The solid lines represent concentrations predicted by the model using mean data.

although the differences were not statistically significant ($p > 0.05$). The time to reach C_{max} (t_{max}) for M_1 was significantly longer after the single p.o. dose (4.7 ± 1.6 vs 0.10 ± 0.12 h, $p < 0.05$) (Table 3). The Clr of DTZ, M_A and M_2 were 0.21 ± 0.10, 0.35 ± 0.28 and 1.0 ± 1.1 ml/min/kg, respectively.

After repeated oral doses t.i.d. for 3 days, plasma concentrations of DTZ were lower than in the single p.o. dose study (AUC 56 ± 19 vs 91 ± 36 ng-h/ml), although the difference was not statistically significant. The mean accumulation factor (R) calculated from C_{max} was 0.94 ± 0.51. Renal clearance of DTZ was significantly higher after the multiple oral doses vs the i.v. dose ($p < 0.05$) (Table 1).

TABLE 3

Pharmacokinetic parameters of M_1 in dogs after single and multiple p.o. doses, and single i.v. injection of 30 mg of DTZ

Parameters/ Route	**Single dose i.v.**	**Single dose p.o.**	**Multiple dose p.o.**	**p**
Apparent $t_{½}$ (h)	17 ± 14 [a]	>30	>30	ND
t_{max} (h)	0.10 ± 0.12 [b]	4.7 ± 1.6 [b]	1.1 ± 1.3	< 0.05 i.v. vs single p.o.
AUC (ng-h/ml)	82 ± 56	53 ± 2.1	20 ± 4.3	NS
C_{max} (ng/ml)	130 ± 200	5.5 ± 1.2	2.6 ± 0.45	NS
Clr (ml/min/kg)	ND	ND	ND	ND

[a] Values represent means ± S.D.
[b] $p < 0.05$.
ND = not determined.
NS = not significant.

DISCUSSION

As reported previously for DTZ in humans and animal species, the pharmacokinetic data were highly variable /19,26/. Owing to the large variability of data and the small number of animals used in this study,

apparent differences in many observations did not reach statistical significance. The metabolism and disposition of DTZ in the dogs was similar to those reported earlier /10,27/. Following a single p.o. dose, M_A was the major metabolite, followed by M_1 and then M_2, which is similar to the metabolism profile observed in humans /19,26/. The $t_{½}$ of the metabolites was considerably longer than that of the parent DTZ (Fig. 3). These results are consistent with those reported previously in dogs /10,27/, and also in humans, rabbits and rats /12,14,19/. This suggests that the disposition of the metabolites could be rate limited by their elimination. However, when the metabolites, M_1 or M_2, were injected separately into rabbits, their $t_{½}$ was shorter than that of DTZ (4.5 vs 2.1 vs 2.8 h for DTZ, M_1 and M_2, respectively), but the volumes of distribution at steady-state (Vdss) were considerably larger (1.9 vs 5.9 vs 9.0 l/kg for DTZ, M_1 and M_2, respectively) /7/. Thus the apparently longer $t_{½}$ seen in the current study could be attributed to a larger volume of distribution of the DTZ metabolites.

Previous studies in rabbits and rats have shown that plasma concentrations of the metabolites were higher after an i.v. dose /9,12/. However, in the current study, with the exception of M_1, plasma concentrations of the metabolites were lower after the i.v. dose, although the differences were not statistically significant ($p > 0.05$). This could be attributed to a species difference in DTZ metabolism, particularly in extrahepatic sites, such as the blood and lung tissues, which are known to rapidly metabolize DTZ to M_1 in rabbits /28,29/. Extrahepatic metabolism of DTZ in other animal species has not been reported. Thus, an *in vitro* study to investigate systemically species differences in the metabolism of DTZ may be warranted to confirm this hypothesis.

In an earlier study /27/, the $t_{½}$ of DTZ following a single 90 mg oral dose of a sustained release DTZ (Cardizem® SR, Aventis Pharma, Canada, QC) in beagle dogs was about 5 h, which is shorter than the $t_{½}$ of 9 h determined in this study. The reasons for this discrepancy are not clear. It could be related to differences in dose, strain of animals used (beagles vs mongrels), formulation (sustained vs immediate release), or a combination of these factors. There is also evidence in this study to suggest that the kinetics of DTZ is non-linear following multiple oral doses. The mean C_{max} and AUC of DTZ following the single p.o. dose were higher than those after the multiple p.o. doses (Table 1). Although the differences were not statistically

significant, the data suggest that oral bioavailability of DTZ decreases after repeated doses. This is contrary to the increased oral bioavailability after multiple doses in mongrel dogs, as reported earlier by Maskasame and co-workers /10/. While the reasons for this discrepancy are not clear, it may be attributed at least in part to the size of the oral dose, which was 3 times higher than in the current study. It was also administered as a solution, as opposed to the tablets used in this study. An increase in oral bioavailability after chronic dosing has also been reported in clinical studies /15,16/. While the mechanism of altered bioavailability and the associated species differences after chronic dosing are not known, an inhibition/induction of metabolism could be an important attributing factor. Caille and co-workers /16/ have suggested that deacetylation of DTZ to M_1 may be increased in humans during chronic dosing. In the current study, however, the AUC of M_1 decreased from 53 ± 2.1 to 20 ± 4.3 ng-h/ml between single and multiple p.o. doses (Table 2). On the other hand, the AUC of M_2 rose from 36 ± 38 to 60 ± 28 ng-h/ml, although the differences were not statistically significant. Thus it appears that metabolism could be an important contributing factor to the non-linear behaviour when DTZ was continued from the single p.o. dose to the multiple doses in this animal species. Species comparison in single dose studies may not be adequate to identify a suitable animal model for pharmacokinetic studies.

In summary, the current study has confirmed the non-linearity and species differences of DTZ metabolism reported in earlier studies /10,19,27/, and cautions against the use of mongrel dogs for pharmacokinetic studies of DTZ, particularly for chronic experiments.

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REFERENCES

1. Medical Letter Consultants. Drugs for hypertension [Letter]. Med Lett 1999; 41: 23-28.

2. Medical Letter Consultants. Drugs for stable angina pectoris [Letter]. Med Lett 1994; 36: 111-114.
3. Weir M. Diltiazem: ten years of clinical experience in the treatment of hypertension. J Clin Pharmacol 1995; 35: 220-232.
4. Yabana H, Nagao T, Sato M. Cardiovascular effects of the metabolites of diltiazem in dogs. J Cardiovasc Pharmacol 1985; 7: 152-157.
5. Kiyomoto A, Sasaki Y, Odawara A, Morita T. Inhibition of platelet aggregation by diltiazem. Circ Res 1983; 52 (Suppl I): 115-119.
6. Yeung PKF 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: 685-689.
7. Yeung PKF, Feng JDZ, Buckley SJ. Pharmacokinetics and hypotensive effect of diltiazem in rabbits: comparing diltiazem to its major metabolites. J Pharm Pharmacol 1998; 50: 1247-1253.
8. Hermann PH, Rodger SD, Remones G, Thenot JP, London DR, Morselli PL. Pharmacokinetics of diltiazem after intravenous and oral administration. Eur J Clin Pharmacol 1983; 24: 349-352.
9. Yeung PKF, Mosher SJ, Pollak PT. Pharmacokinetics and metabolism of diltiazem in rabbits after a single intravenous or single oral administration. Eur J Drug Metab Pharm 1991; 16: 69-74.
10. Maskasame C, Lankford S, Bai S. A. The effects of chronic oral diltiazem and cimetidine dosing on the pharmacokinetics and negative dromotropic action of intravenous and oral diltiazem in the dog. Biopharm Drug Dispos 1992; 13: 521-537.
11. Murata K, Yamahara H, Noda K. Pharmacokinetics of diltiazem and its metabolites in dogs after oral administration of a multiparticulate sustained-release preparation. Pharmaceut Res 1993; 10: 1165-1168.
12. Tsui BCH, Feng JDZ, Buckley SJ, Yeung PKF. Pharmacokinetics and metabolism of diltiazem in rats following a single intra-arterial or single oral dose. Eur J Drug Metab Pharm 1994; 19: 369-373.
13. Hoglund P, Nilsson L. Physiological disposition of intravenously administered C(14)-labeled diltiazem in healthy volunteers. Ther Drug Monit 1988; 10: 401-409.
14. Lee YH, Lee MH, Shim CK. Pharmacokinetics of diltiazem and deacetyldiltiazem in rats. Int J Pharm 1991; 76: 71-76.
15. Hoglund P, Nilsson LG. Pharmacokinetics of diltiazem and its metabolites after repeated multiple-dose treatments in healthy volunteers. Ther Drug Monit 1989; 11: 543-550.
16. Caille G, Boucher S, Spenard J, Lakhani Z, Russell A, Thiffault J, Grace MG. Diltiazem pharmacokinetics in elderly volunteers after single and multiple doses. Eur J Drug Metab Pharm 1991; 16: 75-80.
17. Renton KW. Inhibition of hepatic microsomal drug metabolism by the calcium channel blockers diltiazem and verapamil. Biochem Pharmacol 1985; 34: 2549-2553.

18. Bottiger Y, Sawe J, Brattstrom C, et al. Pharmacokinetic interaction between single oral doses of diltiazem and sirolimus in healthy volunteers. Clin Pharmacol Ther 2001; 69: 32-40.
19. Yeung PKF, Mosher SJ, Quilliam MA, Montague TJ. Species comparison of pharmacokinetics and metabolism of diltiazem in humans, dogs, rabbits, and rats. Drug Metab Dispos 1990; 18: 1055-1059.
20. Yeung PKF, 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: 592-597.
21. 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: 107-114.
22. McLean AM, Cefali EA, Roden JS, Gonzalez MA, Bialer M. Stability of diltiazem in different biological fluids. Biopharm Drug Dispos 1991; 12: 327-334.
23. Yeung PKF, Mosher SJ, Klassen GA, McGilveray IJ. Stability of diltiazem and its metabolites in plasma during storage. Ther Drug Monit 1991; 13: 369-374.
24. Gibaldi M, Perrier D. Pharmacokinetics. 15. 82. New York: Marcel Dekker, Inc., 1982.
25. Fleishaker JC, Phillips JP, Smith TC, Smith RB. Multiple-dose pharmacokinetics and pharmacodynamics of adinazolam in elderly subjects. Pharmaceut Res 1989; 6: 379-386.
26. Yeung PKF, Prescott C, Haddad C, Montague TJ, McGregor C, Quilliam MA, Xei M, Li R, Farmer P, Klassen GA. Pharmacokinetics and metabolism of diltiazem in healthy males and females following a single oral dose. Eur J Drug Metab Pharm 1993; 18: 199-206.
27. Yeung PKF, Mosher SJ, Landriault H. Effect of multiple doses of ranitidine on the pharmacokinetics and metabolism of diltiazem in dogs. Biopharm Drug Dispos 1994; 15: 207-215.
28. Homsy W, Lefebvre M, Caille G, du Souich P. Metabolism of diltiazem in hepatic and extrahepatic tissues of rabbits: in vitro studies. Pharmaceut Res 1995; 12: 609-614.
29. Fraile LJ, Aramayona JJ, Bregante MA, Garcia MA, Abadia AR. Deacetylation of diltiazem by several rabbit tissues. Pharmaceut Res 1996; 13: 1875-1879.