BIOPHARMACEUTICS & DRUG DISPOSITION, VOL. 12, 327–334 (1991)

STABILITY OF DILTIAZEM IN DIFFERENT BIOLOGICAL FLUIDS

ANGUS M. McLEAN||, EUGENIO A. CEFALI||, JENNIFER S. RODEN||,
MARIO A. GONZALEZ*|| AND MEIR BIALER†‡

Schering-Plough Research||, Miami, 1500 SW 66th Avenue, Pembroke Pines, FL 33023, U.S.A.
The School of Pharmacy, Hebrew University of Jerusalem‡, Israel

ABSTRACT

The hydrolysis of diltiazem in biological fluids: whole blood, plasma, and gastric fluid was investigated under conditions considered close to the physiological situation. The most significant rate of hydrolytic degradation was found in whole blood (half-life of 27 h), followed by plasma (half-life of 88 h), while the least significant degradation rate was observed in gastric fluid (half-life 153 h). The kinetic profiles of diltiazem hydrolysis indicate that hydrolytic degradation in the biological fluids makes a minimal contribution to the clearance and disposition of the drug.

KEY WORDS Diltiazem Stability

INTRODUCTION

Diltiazem is a calcium channel blocking agent which is currently used in the treatment of angina pectoris, hypertension, and supraventricular tachyarrythmias.[1–4] Previous studies have established that diltiazem undergoes saturable first pass metabolism and that the total body clearance of diltiazem is high (11·6 ml min^{-1} kg^{-1} and higher).[4,5] Therefore, the total body clearance of diltiazem in these studies is most likely comprised of organ extraction as well as degradation in biological fluids.

Stability studies, conducted by Caille *et al.* at 25° C and colder, have established that diltiazem is converted to desacetyldiltiazem (DAD) rapidly in whole blood, less rapidly in plasma, and slowly in acidic media and phosphate buffer.[6] These stability data suggest that *in situ* degradation of diltiazem fluids could contribute to the high total body clearance of diltiazem.

Desacetylditiazem has been previously considered as the major metabolite of diltiazem.[2] Recently, Caille *et al.* reported that *N*-desmethyldiltiazem (MA) is the major metabolite of diltiazem.[6] These authors discussed the hydrolysis of diltiazem to DAD during sample handling and the risk of hydrolysis within the acidic environment of the stomach. Gastric hydrolysis of diltiazem is sup-

* Addressee for correspondence.
† Visiting Professor from the School of Pharmacy, Hebrew University of Jerusalem, Israel.

0142–2782/91/050327–08$05.00

Received 23 August 1990
Revised 11 December 1990

ported by low concentrations of DAD and MA observed following intravenous administration of diltiazem.[4,7] Similar degradation has been reported with other drugs containing acetyl moieties, such as aspirin and heroin, which undergo rapid deacetylation in biological fluids.[8,9] The total body clearance of these drugs is enhanced by the *in vivo* instability of the compound in the biological fluids. Furthermore, the gastric residence time of oral diltiazem formulations could allow for enhanced degradation of diltiazem by gastric fluid. Therefore, the hydrolysis of diltiazem in biological fluids, under conditions considered to be physiologic, was investigated.

The objectives of this study were:

1. to explore the contribution to total body clearance of diltiazem degradation in gastric fluid, plasma and whole blood;
2. to study diltiazem stability kinetics in biological fluids *in vitro* at 37° C which is close to normal physiologic conditions.

MATERIAL AND METHODS

Chemicals

The solvents used, acetonitrile and methyl *tert*-butyl ether, were HPLC grade from Burdick and Jackson (Muskeegan, MI). The sulfuric acid used was Ultrex grade from J.T. Baker (Philipsburg, NJ). Sodium phosphate, phosphoric acid, and triethylamine were ACS grade from Fisher Scientific (Pittsburgh, PA). Diltiazem hydrochloride was manufactured by Profarmaco (Milan); *N*-desmethyldiltiazem hydrochloride was provided by Interchem (Paramus, NJ); desacetyldiltiazem free base was synthesized by Radian (Austin, TX); loxapine succinate was a gift from Wyckoff Chemical Co. (Kalamazoo, MI).

Equipment

The HPLC equipment comprised a number of items manufactured by Waters Associates (Milford, MA): Datasystem 840, WISP autosampler, Model 510 pumps, UV detector Model number 481, and temperature control module. The LC-DB-8 guard column (2 cm) and the Supelcosil 5μ LC-8-DB (15 cm × 4·6 mm) chromatography column were purchased from Supelco (Bellefonte, PA). A Model TJ6 centrifuge from Beckman Instruments (Fullerton, CA) was used. A tube rotator was obtained from Glas-Col apparatus Co. (Terre Haute, IN). Incubation experiments were carried out using a Shaking Water Bath Model 129 from Fisher Scientific (Pittsburgh, PA). Fluid aliquots were made using the Brinkmann Dispensette from Baxter Healthcare Corporation (McGraw Park, IL). Culture tubes (16 × 25 mm) and 10 ml disposable centrifuge tubes were purchased from Fisher Scientific (Pittsburgh, PA).

Sample preparation and HPLC methodology

The sample preparation and HPLC analysis were carried out by a published procedure[6,10] which was validated for diltiazem, DAD, and MA. It was necessary to implement some minor adaptations of this method to suit our laboratory environment: the extraction of the drug from the plasma and the back-extraction were carried out using a rotating extractor, the chromatography column was a Supelcosil LC-8-DB; chromatography temperature was maintained at 40° C; flow rate was 1·3 ml min^{-1}; the mobile phase was pH 3,50 mM phosphate buffer and acetonitrile (66:34) containing 1·5 mM triethylamine. The HPLC method used in these experiments was capable of detecting small concentrations (2 ng ml^{-1}) of diltiazem, DAD, and MA. Only chromatography peaks coincident with the retention times of diltiazem and DAD were detected in the chromatograms for the incubated samples in all experiments. All reported assay values were the mean of duplicate determinations.

Clinical procedures

Gastric fluid was obtained from two healthy subjects during general anesthesia prior to elective surgery. Approximately 30–40 ml of gastric fluid was obtained from both subjects via nasogastric tube immediately after general anesthesia. Whole blood was obtained from a single volunteer and collected in 25 ml heparinized vacutainers.

Incubation of diltiazem with gastric fluid

The gastric fluid, adjusted to pH 1·3–1·5 with 0·1 M hydrochloric acid, and corresponding equal volumes of water were spiked with a standard solution of diltiazem hydrochloride, such that the final concentration of drug was 100 μg ml^{-1}. Aliquots (3 ml) were transferred to culture tubes and incubated. The incubation was terminated at 0·5, 1, and 2 h. Duplicate samples 0·2 ml) at each time point were diluted 10 fold prior to HPLC assay. The gastric fluid incubation experiment was repeated under the same conditions, but the incubation time was extended to 48 h, with sampling occurring at 0, 24, and 48 h.

Incubation of diltiazem with plasma, albumin, and phosphate buffer

Bovine serum albumin (BSA), 1 g, was weighed and transferred to a 25 ml volumetric flask. The volume of the albumin solution was then adjusted to 25 ml with pH 7·4, 0·05 M phosphate buffer. The albumin solution was then spiked with a standard solution of diltiazem hydrochloride, so that the final concentration of the hydrochloride sale was 400 ng ml^{-1}. Similarly, 0·05 M phosphate buffer and plasma was spiked to produce media at the same concentrations of diltiazem hydrochloride. Aliquots (2 ml) of each of the media were transferred

to culture tubes and incubated at 37° C. Duplicate samples were withdrawn at 0, 24, 73, and 96 h and analyzed by HPLC.

Incubation of diltiazem with whole blood

Whole blood was spike with a standard solution of diltiazem hydrochloride such that the final concentration was 600 ng ml^{-1}. Aliquots (5 ml) of whole blood were transferred to culture tubes and incubated at 37° C. Duplicate samples were removed from the shaking water bath at 0, 4, 8, and 12·8 h and centrifuged to yield 2 ml of plasma. These samples were also analyzed by HPLC using an adaptation of the published procedure.[6,10]

Quantitation of desacetyldiltiazem and diltiazem in gastric fluid

Calibration standards for the DAD assay were prepared at 0, 50, 100, 250, and 500 ng ml^{-1} of gastric fluid by spiking fluid with a standard solution of DAD free base. Additionally, each one of these standards was spiked with a standard solution of diltiazem hydrochloride, so that each one contained 10 μg ml^{-1} of diltiazem hydrochloride in gastric fluid. The quantitation of DAD and diltiazem hydrochloride was accomplished by simultaneous extraction and HPLC analysis of these standards with the incubated gastric fluid samples, using an adaptation of a published procedure.[6,10] Variance-stabilized least squares analysis[11] of the DAD calibration standards was used to determine the DAD concentrations in the incubated samples. The standard curve (y = 0·0028x + 0·2218) was linear in the range 50 to 500 ng ml^{-1} with a correlation coefficient of 0·9999. The diltiazem concentrations were calculated from the mean relative weight response[12] (1·31; CV = 1·5 per cent) obtained from the analysis of the five 10 μm ml^{-1} diltiazem standards.

Quantitation of diltiazem in phosphate buffer, albumin solution, and whole blood

Calibration standards for the assay of diltiazem were prepared at 0, 5, 25, 50, 100, 200, and 400 ng ml^{-1} by spiking the medium with a standard solution of diltiazem hydrochloride. The concentration of diltiazem hydrochloride in the incubated samples was determined by the simultaneous extraction and HPLC analysis of this set of standards together with the incubates using the same adapted assay procedure as above. When buffer was used as the incubation media, BSA was added after the incubation, but just prior to extraction, to maintain the concentration of protein the same as that of the incubated albumin samples. The data were analyzed by a recently described variance-stabilized regression analysis,[11] which described a linear relationship (y = 0·013915x − 0·01246) with a correlation coefficient of r = 0·9999.

In these laboratories, this assay has been shown to be linear across the range 5–800 ng ml^{-1} with $r > 0·999$. The intra-day precision of replicate analysis

($n = 7$) was 6 per cent (5 ng ml^{-1}), 1·7 per cent (25 ng ml^{-1}), 2·5 per cent (50 ng ml^{-1}) and 1·5 per cent (100 ng ml^{-1}).

Quantitation of diltiazem and desacetyldiltiazem in plasma

Calibration standards and data analysis for the assay of diltiazem were prepared as described above. Additionally, the calibrants were spiked with a standard solution of DAD free base, so that the calibrants contained not only the concentrations of diltiazem hydrochloride shown above, but also DAD free base at concentrations of 0, 10, 25, 50, 100, 200, and 300 ng ml^{-1}.

The HPLC analysis of the incubated samples, calibrants, and data analysis were as described above. The equation of the calibration line for diltiazem was $y = 0{\cdot}01521x - 0{\cdot}0052$ with a correlation coefficient of $r = 0{\cdot}9999$. The equation of the calibration line for DAD was $y = 0{\cdot}02558x - 0{\cdot}0068$ with a correlation coefficient of $r = 0{\cdot}9999$.

RESULTS

After 2 h of incubation in gastric fluid, 4·3 per cent of diltiazem was converted to DAD. In water, 8·3 per cent of diltiazem was converted to DAD; both the gastric fluid and water incubations were performed under the same conditions. Due to the low yield of DAD at 2 h, this experiment was repeated extending incubation in gastric fluid to 48 h. In the repeated experiment, 8·9 per cent of the diltiazem degraded in 24 h and the degradation increased to 19·7 per cent in 48 h. The degradation half-life of diltiazem from the extended gastric fluid experiment was calculated to be 152 h, assuming a first order rate of degradation.

Table 1. Concentration of diltiazem (ng^{-1} ml) in different biological media incubated at 37° C

Incubation time (h)	Blood*	Plasma	Buffer	Albumin
0	826 (100)†	410 (100)	417 (100)	425 (100)
4	580 (70·2)	–	–	–
8	466 (56·4)	–	–	–
12·8	353 (42·7)	–	–	–
24		230 (56·1)	359 (86·1)	379 (89·2)
73		159 (38·8)	240 (57·6)	312 (73·4)
96		143 (34·9)	208 (49·9)	261 (61·4)

* These are plasma concentrations separated from the blood at the different incubation times.
† Per cent diltiazem unchanged.

Table 1 describes the diltiazem stability data in whole blood, plasma, albumin

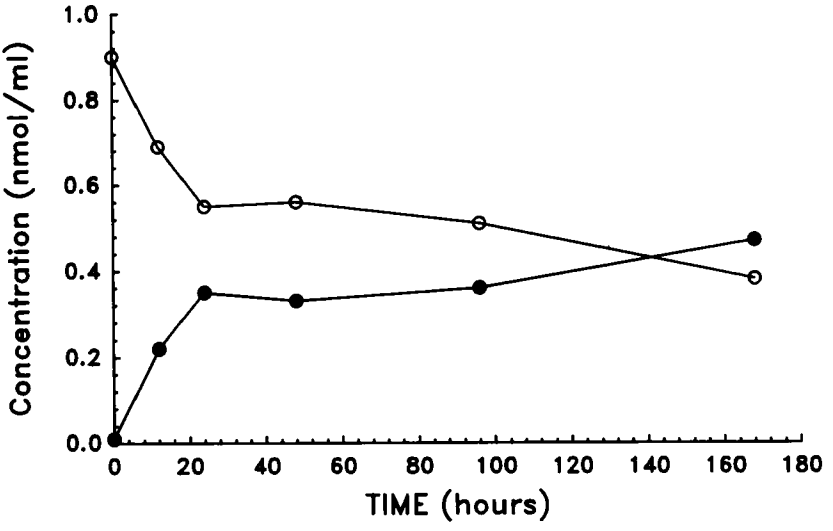

Figure 1. Mass balance plot of diltiazem hydrolysis to desacetyldiltiazem (DAD) in plasma at 37° C. O Diltiazem, ● DAD

solution, and phosphate buffer incubated at 37° C. The concentration of diltiazem fell to 42·7 per cent of the initial concentration by 12·8 h in whole blood. In plasma, 24 h were required to degrade 44 per cent of the diltiazem. Using an aqueous solution of albumin, 38·6 per cent of the diltiazem was degraded at 96 h. Degradation in phosphate buffer occurred slightly faster than in albumin. Assuming first order degradation, the diltiazem degradation half-life in these media were: 11 h in whole blood, 67 h in plasma, 93 h in phosphate buffer, and 143 h in the albumin solution. Figure 1 graphically depicts the mass balance attained in the degradation of diltiazem to DAD in plasma. This 1:1 stoichiometric conversion along with the absence of other chromatography peaks, demonstrates that only DAD was formed when these media were incubated at 37° C.

DISCUSSION

Smith *et al.* studied the single and multiple-dose pharmacokinetics of oral diltiazem in eight patients with paroxysmal atrial tachycardia.[13] They demonstrated a low single-dose extent of absorption (38% relative to an iv dose) which increased to 90 per cent on multiple dosing. Bianchetti *et al.* demonstrated that the bioavailability of diltiazem increases with dose while elimination half-line does not change.[14] These authors attributed this pharmacokinetic phenomena to saturable first-pass metabolism. Koelle *et al.* have estimated that the hepatic extraction ratio of diltiazem is 0·54 and that total body clearance of

diltiazem is 11·5 ml min^{-1} kg^{-1}.[5] Improper sample handling (i.e. exposure to UV radiation,[15] elevated temperature, and improper pH[16,17]) can artifactually decrease plasma diltiazem concentrations by ester hydrolysis, and inflate clearance calculations. The experiments described in this paper were designed to elucidate the contribution of ester hydrolysis, in different biological fluids under physiologic conditions, to the calculated total body clearance and first-pass metabolism of diltiazem.

Gastric residence of a diltiazem tablet or capsule was a concern, especially in the light of increased use of sustained-release diltiazem products. It was envisaged that prolonged exposure of the drug to gastric juice could result in significant degradation. According to S.S. Davis, the gastric transit of pellets or a single unit is less than 6 h under most circumstances.[18] The gastric fluid incubation experiments indicate that degradation in the stomach is of minimal consequence to the total body clearance of diltiazem. The longest expected transit time (approximately 10 h with a heavy breakfast) is much shorter than the estimated diltiazem degradation half-life in gastric fluid (153 h). These data suggest that less than 5 per cent of the diltiazem, would be degraded by gastric fluid in 10 h and less than 4 per cent in 6 h. Interestingly, the degradation of diltiazem seems greater in water than in gastric fluid under similar pH and temperature conditions. These observations demonstrate that DAD was formed primarily by a chemical conversion (due to the acidic pH[15–17]) and not by an enzymatic biotransformation. The data suggest that some constituent of gastric fluid protected diltiazem from hydrolysis to DAD.

Diltiazem was found to be least stable in whole blood under physiologic conditions (degradation half-life = 11 h). Red blood cell esterases might explain the larger rate of degradation in whole blood as compared to plasma and human albumin, since diltiazem degradation was much less in plasma and the albumin solution. Diltiazem half-life in man ranges from 3 to 7 h in most cases,[1–5,7,13] therefore, the contribution of diltiazem degradation in whole blood to total body clearance of diltiazem is small.

From the 25° C data published by Caille *et al.*,[6] we calculated degradation half-lives of 88 h in plasma and 27 h in whole blood assuming first order degradation kinetics. Caille's results at 25° C are very similar to the results found in this study at 37° C (67 and 11 h, respectively), especially considering the difference in temperature and between-laboratory assay variability.

Leboeuf and Grech-Belanger studied the deacetylation of diltiazem by rat liver.[19] These authors showed that the liver is the major metabolic site for the hydrolysis of diltiazem to DAD in rats. In rats, the diltiazem deacetylase activity is about 10 times higher in the liver than in whole blood. Assuming that the magnitude of the difference in deacetylase activity between blood and liver is true in humans, the difference between the biological half-life of diltiazem in humans and the degradation half-life in human whole blood supports the assumption that the metabolic deacetylation of diltiazem in humans occurs primarily in the liver and not in extrahepatic sites. The degradation experiments

conducted in whole blood, gastric fluid, plasma, and albumin also indicate that these media do not significantly contribute to the deacetylation of diltiazem in humans.

ACKNOWLEDGEMENTS

The authors thank Drs E. R. Gonzalez and C. Lathia for their assistance in obtaining the gastric fluid and conducting the gastric fluid incubations.

REFERENCES

1. K. E. Anderson, B. W. Johansson and S. O. Olson, *Acta Pharmacol. Toxicol.*, **57** (suppl.), 1 (1985).
2. M. Chaffman and R. N. Brogden, *Drugs*, **29**, 387 (1985).
3. M. Micaela, T. Buckley, S. M. Grant, K. L. Goa, D. McTabish and E. M. Sorkin, *Drugs*, **39**, 757 (1990).
4. P. Hermann, S. D. Rodger, G. Remones, J. P. Thenot, D. R. London and P. L. Morselli, *Eur. J. Clin. Pharmacol.*, **24**, 349 (1983).
5. E. U. Koelle, H. R. Ochs and K. O. Vollmer, *Arzneim. Forsch. Drug Res.*, **33**, 972 (1983).
6. G. Caille, L. M. Dube, Y. Theoret, F. Varin, N. Mousseau and I. J. McGilvery, *Biopharm. Drug Dispos.*, **10**, 107 (1989).
7. P. Hoglund and L. G. Nilsson, *Ther. Drug Monit.*, **10**, 401 (1988).
8. M. Rowland, S. Riegelman, P. A. Harris, and S. D. Sholkoff, *J. Pharm. Sci.*, **61**, 379 (1972).
9. E. R. Garrett and T. Gurkan, *J. Pharm. Sci.*, **69**, 1116 (1980).
10. L. M. Dube, N. Mousseau and I. J. McGilveray, *J. Chromatogr.*, **430**, 103 (1988).
11. A. M. McLean, D. A. Ruggirello, C. R. Banfield, M. A. Gonzalez and M. Bialer, *J. Pharm. Sci.*, **79**, 1005 (1990).
12. R. V. Smith and J. T. Stewart, *A Textbook of Biopharmaceutic Analysis*, Lea & Fabiger, Philadelphia, 1981, pp. 83–84.
13. M. S. Smith, C. P. Verghese, D. G. Shand and E. L. C. Pritchett, *Am. J. Cardiol.*, **51**, 1369 (1983).
14. G. Bianchetti, M. Regazzi and V. Ascalone, *Eur. J. Clin. Pharmacol.*, **36** Suppl, A242 (1989).
15. M. S. Suleiman, M. E. Abdulhameed, N. M. Najib and H. Y. Muti, *Int. J. Pharm.*, **50**, 71 (1989).
16. S. S. Davis, L. Illum, I. M. Triccas and K. N. Winchcomb, *Int. J. Pharm*, **30**, 29 (1986).
17. M. S. Suleiman, M. E. Abdulhameed, N. M. Najib and H. Y. Muti, *Drug Devel. Ind. Pharmacy*, **16**, 685 (1990.
18. S. S. Davis, *S.T.P. Pharma*, **2**, 1015 (1986).
19. E. LeBoeuf and O. Grech-Berlanger, *Drug Metab. Dispos.*, **15**, 122 (1987).