Deacetylation of Diltiazem by Several Rabbit Tissues

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Purpose. Diltiazem (DTZ) undergoes extensive metabolism yielding several metabolites, some of which retain a certain degree of pharmacological activity. N-demethylating activity has been detected mainly in the liver. Nevertheless, the organs involved in the formation of the deacetylated metabolite of DTZ (M1) have not been fully elucidated. In order to address this issue, we have carried out *in vitro* studies using the blood, lung, brain, small intestine, and liver as enzyme sources.
Methods. DTZ (1,000 ng/ml) was incubated in 10,000 × g supernatant homogenates of selected tissues or in whole blood for 240 minutes at 37°C. Multiple samples were withdrawn, and DTZ and its metabolite M1 were assayed by HPLC.
Results. The apparent degradation rate constant of DTZ was in the rank order blood > lung > brain > liver > small intestine. This trend can also be observed for the AUC and for the percentage of DTZ metabolized. In all the tissue homogenates examined there was a net production of the deacetylated metabolite. The M1 metabolite was also detected in the blood (500 ng/ml after 240 minutes of incubation).
Conclusions. The widespread distribution of the DTZ deacetylase activity described in this study suggests that extrahepatic metabolism of DTZ to M1 may play a relevant role in the overall pharmacokinetics of DTZ.

KEY WORDS: diltiazem; deacetyldiltiazem; metabolism; extrahepatic tissues.

INTRODUCTION

Diltiazem is a calcium channel blocker widely prescribed for the treatment of angina, hypertension, supraventricular cardiac arrhytmias and migraine (1). This drug has been shown to be extensively metabolized both in animals and humans, by at least five distinct pathways including deacetylation, N-demethylation, O-demethylation, ring hydroxylation and acid formation (2). These various reactions lead to the formation of at least 20 distinct metabolites. Some of them–deacetyldiltiazem (M1), N-demethyldiltiazem (MA), and N-demethyldeacetyldiltiazem (M2)–retain a potent pharmacological activity as antihypertensives and as coronary vasodilators (3). M1 and MA have been recognized as primary metabolites of DTZ, whereas M2 is assumed to be the product of a secondary reaction (Figure 1). Evidence has been presented that hepatic NADPH-dependent CYP IIIA is the major enzyme involved in N-demethylation of DTZ in rabbits and humans (4). Formation of M1 by a specific esterase has also been reported in rat, rabbit and humans (5). Nevertheless, the organs involved in M1 formation have not been fully elucidated, except in rats, in which a large formation of M1 in the liver has been demonstrated (6).

Although at present there is not doubt that the liver plays an important role in the overall metabolism of DTZ an extensive extrahepatic metabolism has been suggested and in some instances proved in selected animal species. Unfortunately, studies on extrahepatic DTZ metabolism have not been fully explanatory, and only marginal DTZ deacetylase activity has been found in organs such as the liver and the gut in rabbits (7).

In order to elucidate the presence of the enzyme responsible for DTZ deacetylation in liver and some extrahepatic tissues, *in vitro* studies were carried out using the blood, lung, brain, small intestine, and liver as enzyme sources. NADPH was not included trough the incubations to avoid formation of MA, and M1 to M2 degradation (Figure 1). In these experimental conditions, any formation of M1 throughout the incubations would be detected. Tissues were selected because of previously described occurrence of deacetylase activity (8,9). The rabbit was selected as the animal model, because high blood concentrations of M1 have been reported after either iv or po administration of DTZ (5).

MATERIALS AND METHODS

Chemicals

Diltiazem (DTZ) and imipramine were purchased from Sigma Chemical Co. (St. Louis, MO). Deacetyldiltiazem (M1) was received as a gift from Dr. R. Latini (Mario Negri Institute, Milan, Italy). All other reagents were of reagent or HPLC grade.

Animal Model

Nine New Zealand White rabbits (body weights 2.0–2.3 kg) were used in this study. They were provided by the Animal Experimentation Facility of the University of Zaragoza (Spain). All experimental procedures were performed accordingly to the Principles of Laboratory Animal Care. Rabbits were acclimatized to the laboratory for 8 days before the study. During this period, temperature (20°C), humidity (50%) and light cycle (12/12 hours) were maintained constants. Respiratory rate, rectal temperature, hematocrit, heart rate, blood gas parameters and ECG were evaluated just before experimentation. Food and water were supplied *ad libitum*.

Homogenate Preparation

Immediately before tissue collection, blood samples (15 ml) were drawn through the central ear artery for blood-plasma partition, and *in vitro* blood studies. Heparin (10 μl of a 150 UI/ml solution) had been previously added to the tubes to prevent blood clotting. Animals (n = 5) were anesthetized by intraperitoneal administration of sodium penthobarbital (50 mg/kg). Thereafter, the abdomen was opened and the portal vein was cannulated. Ice-cold isotonic saline was perfused through the cannula at a flow rate of 70 ml/minute. In order to minimize the blood content in organs, animals were bled via the abdominal

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ABBREVIATIONS: DTZ, Diltiazem; M1, Deacetyldiltiazem; MA, N-demethyldiltiazem; M2, N-demethyldeacetyldiltiazem.

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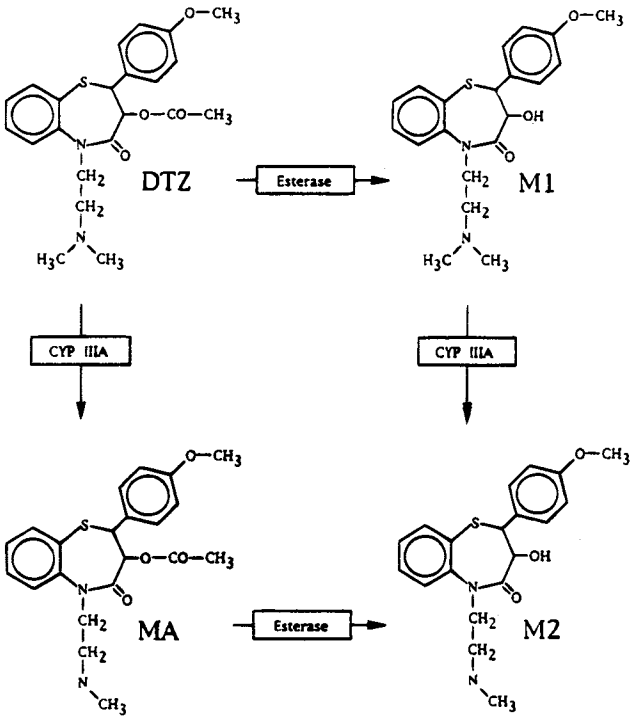

Fig. 1. Structural formulae of DTZ and of its active metabolites.

cava vein. When the colour of the liver became light tan (3–5 minutes), the liver, small intestine (0 to 30 cm beyond the pylorus), lungs and brain were extracted. The whole organs were excised, carefully dried, and then weighed. Feces remaining in the small intestine were removed by ice-cold saline perfusion. Exactly weighed tissue samples (in the range of 5 g) were minced and homogenized with 4 vol of ice-cold 1.15% (w/v) KCl-0.01 M phosphate buffer (pH 7.4) in a Potter-Elvehjem to yield 20% (w/v) homogenates. Each homogenate was centrifuged first at 1,000 × g for 10 minutes at 4°C and then at 10,000 × g for 20 minutes. The supernatant was then collected and stored frozen (−40°C) until use (not more than 24h).

In Vitro Formation of M1 in Blood and Homogenates

The kinetic studies were conducted at 37°C in a shaker bath (75 oscillations per minute). After preincubation of the tissue homogenates for 5 minutes, DTZ solution (100 μg/ml in saline) was spiked into the homogenates to yield a final concentration of 1,000 ng/ml. A similar study was performed using blood as the enzyme source. Aliquots of 1 ml were withdrawn from the incubation medium at 10, 30, 60, 120, 180 and 240 minutes. In order to stop the reaction, samples were transferred into tubes containing 20 ml of cold (−20°C) isobutanol/hexane extraction medium (see assay of DTZ and M1).

In addition, blood samples (15 ml) from four additional rabbits were drawn through the central ear artery and placed into heparinized tubes. Hematocrit was then measured. An aliquot (5 ml) was immediately separated and maintained at 5°C. Plasma was obtained from the remaining sample by centrifuging at 900 × g for 10 minutes and maintained at 5°C. Red blood cells were washed using an equal volume of isotonic saline. After centrifuging at 900 × g for 10 minutes the upper layer was removed and discarded. This procedure was repeated again. The resultant red blood cells pellet was diluted to the initial volume with normal saline. DTZ solution (100 μg/ml) was spiked to each specimen (whole blood, plasma, and resuspended red blood cells) to yield a concentration of 1,000 ng/ml and incubated for 30 minutes in the conditions described above. Aliquots of 1 ml were withdrawn at the end of the incubation period and assayed for M1 concentration.

Stability Studies

Stability of the enzyme responsible for the deacetylation of DTZ in the tissue homogenates and in whole blood was checked by adding DTZ to the homogenates or blood after either 60, 120, 180, and 240 minutes of preincubation (37°C) and then taking aliquots (1 ml) at 0 and 30 minutes. The initial DTZ concentration was 1,000 ng/ml. Samples were assayed for DTZ and M1 concentrations and results further compared with those obtained in the main experiment after a 5 minutes preincubation period.

In addition, a study on the stability of M1 in whole blood and homogenates was carried out. Incubation of M1 (37°C, 240 minutes) was performed at an initial concentration of 250 ng/ml. Aliquots of 1 ml were sampled from the incubation medium at 10, 60, 120, and 240 minutes and assayed for M1 concentrations.

Finally, in order to assess the chemical stability of DTZ and M1, two separate studies were carried out. The drugs were incubated (37°C, 240 minutes) separately in 1.15% (w/v) KCl-0.01 M phosphate buffer (pH 7.4). DTZ and M1 were added to the buffer to yield an initial concentration of 1,000 and 200 ng/ml, respectively. Serial samples were drawn at 10, 60, 120, and 240 minutes and assayed by HPLC.

Red Blood Cell-to-Plasma Ratio (RBC/P) of DTZ and M1

A 100 μl aliquot of a DTZ solution in saline was spiked to 3.5 ml of fresh heparinized blood to yield a concentration of 1,000 ng/ml and gently mixed by hand. The tubes were placed in a shaker bath at 37°C for 60 minutes (time required to reach equilibrium). After incubation, an aliquot of whole blood (1 ml) was separated and the remainder centrifuged at 6,000 × g for 5 minutes for plasma separation. RBC/P was calculated using the following equation (10): $RBC/P = (C^b - Cp(1 - Hc))/(Cp \times Hc)$ where C^b and Cp are the concentrations of DTZ or M1 in blood and plasma, respectively, and Hc is the hematocrit.

HPLC Assay of DTZ and M1

DTZ and its metabolite M1 were assayed in the blood and tissue homogenates, after isobutanol/hexane (2:98) extraction, with a previously published HPLC method (11). The HPLC system consisted of an isocratic pump (Model 145, Water Associates), a C_{18} reversed-phase column (Novapack, 5-μm silica, 150 × 3.9 mm id) and a UV absorbance detector working at 237 nm (Model 484, Water Associates). Quantitation was performed using a calibration curve of spiked blank blood or tissue homogenates over the range of concentrations assayed. The method was shown to be linear at concentrations from 10 to 5,000 and 10 to 1,000 ng/ml of DTZ, and M1, respectively. The detection limit was 10 and 5 ng/ml for DTZ and M1,

Table I. Percentage Metabolized Following the Incubation for 240 Minutes of M1 (250 μg/ml) in 10,000 × g Supernatant of Tissue Homogenates 20% (w/v) of Liver, Lung, Intestine, Brain, and in Whole Blood[a]

Tissue	M1
Liver	35.2 ± 7.8
Lung	5.7 ± 2.8
Small intestine	3.6 ± 1.9
Brain	6.3 ± 2.8
Blood	1.1 ± 0.2

[a] Values are expressed as mean ± standard deviation of five experiments.

respectively. Recovery was determined to be better than 90% for each drug. For DTZ, inter- and intra-assay precisions, determined by injection of control standards, were 7 and 3% for DTZ, and 9 and 4% for M1, respectively.

Data Analysis

The apparent degradation rate constant of DTZ in whole blood and homogenates (λz) was obtained from the slope of the log-linear portion of the curve by nonlinear regression analysis. The percentage of metabolized substrate was calculated using the following equation: % metabolized = $(C_0 - C_{240})/C_0$, where C_0 and C_{240} are the DTZ concentrations at 0 and 240 minutes. The area under the DTZ or M1 concentration-time curve ($AUC_{0\rightarrow240}$ DTZ and $AUC_{0\rightarrow240}$ M1, respectively) in the homogenates or in the blood was calculated by the trapezoidal rule.

Statistical Analysis

Values are presented as mean ± standard deviation. Differences between organs were assessed using analysis of variance. All other comparisons were performed using Student's unpaired t-test. In all instances, a p value of <0.05 was chosen as the level of statistical significance. Values obtained for whole blood were not statistically compared with those in 20% (w/v) homogenates because of the unequal composition of the incubation medium.

RESULTS

Controls for physiological variables measured before rabbit anesthesia showed not differences, compared with routine controls measured in our laboratory.

The production of M1 in the various tissue homogenates or whole blood was almost equivalent after either a preincubation period of 5 minutes or after 60, 120, 180 or 240 minutes of preincubation, indicating no appreciable degradation of the enzyme responsible for the deacetylation of DTZ over this period.

A certain amount of M1 was formed when DTZ (1,000 ng/ml) was incubated at 37°C for 240 minutes in 1.15% (w/v) KCl-0.01 M phosphate buffer. However, the DTZ degraded by chemical breakdown was less than 3% of the initial amount. In addition, any appreciable degradation of M1 was not observed when M1 was incubated without tissue for 240 minutes.

Liver homogenates metabolized around 35% of the initial amount of M1 (250 ng/ml) after 240 minutes of incubation (Table 1). In the remaining tissues only a small degradation of M1 was detected (1 – 6% of the initial amount in blood and brain, respectively).

The time course of DTZ in 10,000 × g tissue homogenates and in blood as a function of time is depicted in Figure 2. The *in vitro* metabolism of DTZ occurred monoexponentially in all the tissues examined, indicating apparent first-order degradation of DTZ at concentrations below 1,000 ng/ml. The DTZ concentration at time zero, calculated by regression from the time course of DTZ concentration curves, closely resembles that expected after DTZ was spiked (1,000 ng/ml) in all the homogenates but not in the blood (745 ± 22 ng/ml). Apparent degradation rate constants of DTZ (λz) in the homogenates are shown in Table 2. Degradation in the lung was faster than in the other organs examined ($p < 0.05$). The degradation rate constant calculated after incubation of brain homogenates was also statistically different from those calculated in small intestine and liver. A similar trend can also be observed for the $AUC_{0\rightarrow240}$ DTZ (Table 2). The percentage of DTZ metabolized throughout incubation of lung homogenates (36%) was statistically different from those observed in small intestine and liver homogenates.

Table II. Diltiazem (1,000 ng/ml) Metabolism in 10,000 × g Supernatant Fractions of 20% (w/v) Tissue Homogenates and in Blood[f]

Tissue	λz × 10^{-4} (min^{-1})	$AUC_{0\rightarrow240}$DTZ (μg min/ml)	% metabolized	$AUC_{0\rightarrow240}$M1 (μg min/ml)
Liver	5.28 ± 0.32[b,d]	226 ± 4[b,d]	13.6 ± 5.6[b]	15 ± 5[b,d]
Lung	18.41 ± 0.73[a,c,d]	182 ± 12[a,c]	35.9 ± 9.1[a,c]	50 ± 15[a,c]
Small intestine	4.26 ± 0.42[b,d]	228 ± 2[b,d]	9.3 ± 4.2[b]	22 ± 6[b,d]
Brain	10.97 ± 0.23[a,b,c]	210 ± 6[a,c]	23.7 ± 11.7	36 ± 12[a]
Blood	41.15 ± 2.26[e]	151 ± 3	61.0 ± 5.7	78 ± 13

[a] $p < 0.05$ compared to values in liver.
[b] $p < 0.05$ compared to values in lung.
[c] $p < 0.05$ compared to values in small intestine.
[d] $p < 0.05$ compared to values in brain.
[e] Values obtained in blood were not comparared with those in homogenates because different composition of the incubation medium.
[f] Values are expressed as mean ± SD of five experiments.

Table 2 shows the values calculated after whole blood incubations. As can be observed in Figure 2, there was a fast degradation of DTZ throughout the incubation period in this tissue. Nevertheless, results obtained for whole blood were not statistically compared with those in homogenates because of the unequal composition of the incubation medium.

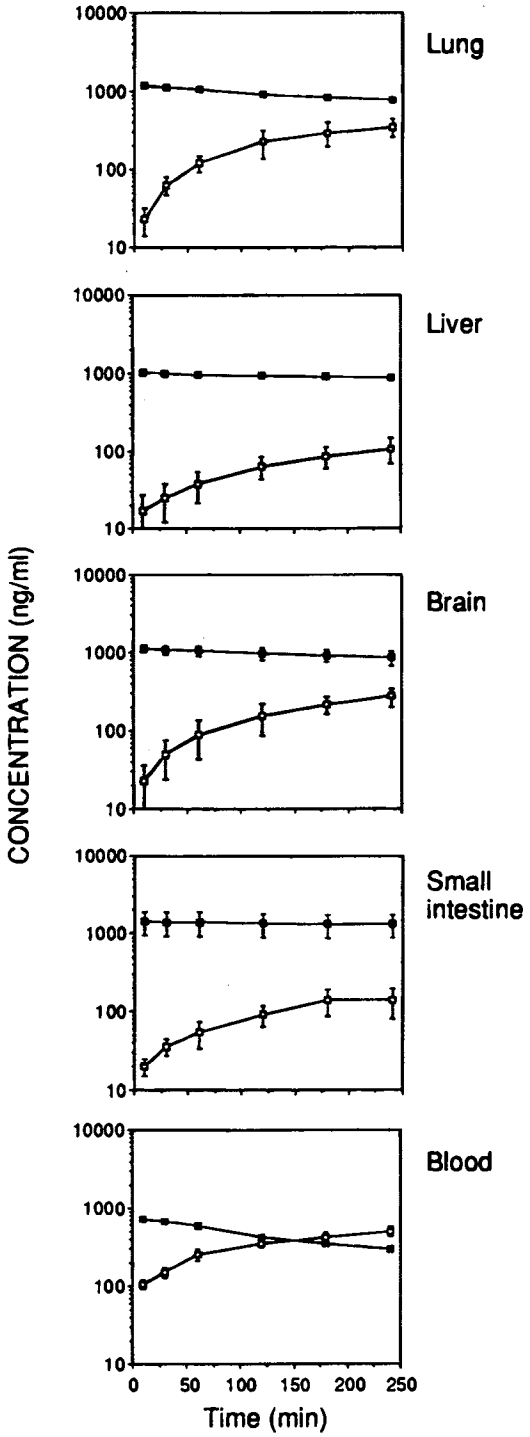

Fig. 2. *In vitro* degradation of DTZ (closed squares) and resulting formation of M1 (open squares) in 10,000 × g Supernatant Fractions of 20% (w/v) Tissue Homogenates and in Blood. Each point represent the mean ± SD of five experiments.

In all the tissue homogenates examined there was a net production of the M1 metabolite (Figure 2). M1 accumulates in the incubation medium according to an apparent first-order kinetic. Values obtained for the $AUC_{0\rightarrow240}$ M1 were in the rank order lung > brain > intestine > liver (Table 2). Except in liver homogenates, the faster the degradation rate for DTZ the higher $AUC_{0\rightarrow240}$ M1. M1 metabolite was also detected in blood with an approximate concentration of 500 ng/ml after 240 minutes of incubation.

The washed red blood cells were able to form 155 ± 21 ng/ml of M1 when incubated for 30 minutes with an initial DTZ concentration of 1,000 ng/ml. This value shows no differences with that found after incubation of whole blood (143 ± 32 ng/ml), but was statistically higher ($p < 0.01$) than that observed at the end of plasma incubations (56 ± 4 ng/ml).

The red blood cell-to-plasma ratio for DTZ (RBC/P) was determined as 1.48 ± 0.43, indicating that a substantial amount of this drug is able to penetrate into the blood cells. At the end of the incubation period (60 minutes) a certain amount of M1 was detected, and its RBC/P ratio could be calculated (1.01 ± 0.18).

DISCUSSION

DTZ metabolism is a complicated event in which the contribution of a certain number of phase I and phase II enzymes yield to the formation of almost 20 distinct metabolites (1). Nevertheless, attention has been paid to reactions forming the primary active metabolites MA and M1. MA formation is catalyzed by the NADPH-dependent CYP IIIA in rabbit and humans (4). In addition, it has been demonstrated that DTZ deacetylation is catalyzed by an specific esterase without the presence of any co-enzyme being required (6). Furthermore, in presence of NADPH, M1 could be the substrate of a secondary reaction to yield N-demethyldeacetyldiltiazem (Figure 1). Most *in vitro* studies on metabolic DTZ degradation, have been performed in presence of NADPH and, as a consequence, formation of M1 could have been pass unnoticed. We have designed an experiment in which NADPH has been excluded throughout the incubations, in an attempt to detect any formation of M1.

Because of the scarce deacetylase activity found in previous studies (12), unusual prolonged incubation periods (240 minutes) were needed. Thus, we have designed an experiment in which the stability of the enzyme responsible for the deacetylation of DTZ was checked. In this experiment, the activity displayed by the tissue homogenates and whole blood was not changed after either 5, 60, 120, 180 or 240 minutes of preincubation, indicating a negligible degradation of such enzyme over this period. Other source of variability is the consequence of the reported spontaneous degradation of DTZ in buffers and water at different pH values and temperatures (13). McLean et al, reported a degradation half-life for DTZ in phosphate buffer of 93 hours (13). Our results showed a degradation lesser than 3% of the initial amount over the period studied. Thus, assuming that a negligible DTZ degradation was occurring along the incubation period, corrections over the analytical results were not necessary.

In all the tissues examined in our study there was a net production of M1 (Table 2). In the lung, small intestine, and brain homogenates and in the whole blood, the sum of equivalents of DTZ plus M1 remained unchanged throughout

the incubations, indicating that most of the degraded DTZ throughout incubations was recovered as M1 and, as a consequence, that a unique reaction was occurring. However, after incubation of liver homogenates only 80% of the DTZ degraded was recovered as M1. This observation can have two interpretations. DTZ could be suffering a second reaction to yield an undetected metabolite. For example, the hepatic homogenates could contain a certain amount of NADPH which could permit the degradation of DTZ to MA. Alternatively, M1 could be the substrate of a secondary reaction and thus disappear from the incubation medium. Further results on M1 degradation in homogenates (Table 1) showed that liver homogenates metabolized around 35% of the spiked amount of M1 after 240 minutes of incubation, probably explaining the unrecovered amount in this tissue. Unfortunately, the present analysis does not allow a clear discrimination between these two possibilities and, therefore, the $AUC_{0\rightarrow240}$ M1 reported herein for liver homogenates may not accurately reflect the real DTZ deacetylase activity in this tissue.

The results reported here showed the lung and brain as the most active tissues in eliminating DTZ (Table 2) under conditions favoring the formation of M1. Using *in vitro* approaches, the true metabolic activity of an organ is very difficult to assess. Moreover, as mentioned above, NADPH was excluded from incubations, and DTZ metabolism derived to its deacetylation. It has been reported that, at usual doses, the lung is capable of accumulating significant amounts of DTZ (14) possibly contributing to increase the metabolic capability of this organ. For the first time we describe DTZ deacetylase activity in brain homogenates of rabbits. It is not usual to consider the brain as an organ involved in extrahepatic metabolism of drugs. The weight of this organ is very low (0.25% of the body weight) if compared with liver or blood. Moreover, its special histologic constitution does not permit an easy access for most drugs. It has been reported that after oral administration, DTZ can penetrate CNS reaching high concentrations in the brain (14). This could have a marked effect on the formation and ultimately on any accumulation of M1. The liver and small intestine homogenates showed low activity as compared to that of the lung (28 and 23%, respectively). Surprisingly, it has been reported a greater formation of M1 in liver and small intestine as compared to other organs like the lung or renal cortex (7). This apparent controversy can be explained by assuming that M1 could be the substrate of a secondary reaction. In fact, these results were obtained under incubation conditions (presence of a NADPH-generating system) that permit the formation of MA and, thus, the real capability of these tissues in forming M1 could be underestimated.

The blood turned out to be an active tissue in eliminating DTZ through the deacetylating pathway. Results showed DTZ deacetylase activity predominantly distributed in red blood cells. In addition, we have found that DTZ tends to accumulate in erythrocytes-a factor recognized as conditioning the distribution pattern of a drug-reaching high concentration levels inside the cells. These findings suggest that blood degradation of DTZ to its deacetylated metabolite could play an important role in the overall pharmacokinetics of DTZ in rabbits. Unlike tissue homogenates, the whole blood represent an unaltered tissue in which the reported DTZ degradation may be close to the real. After 240 minutes of incubation, we have not observed an appreciable degradation of M1. On the other hand, most of the DTZ disappeared during incubations was recovered as M1. These two findings point out that blood degradation of DTZ to MA does not exist or occurs slowly. The isozymes involved in the demethylation of DTZ are essentially membrane based enzymes. However, mature erythrocytes are transformed cells in which the smooth endoplasmic reticule and, in consequence, most of the enzymatic activity mediated by cytochromes is marginal.

In conclusion, the esterase responsible for the hydrolysis of DTZ to M1 was present in all tissues examined. The lung, blood and brain showing the maximal activity. The experimental conditions used during incubation do not allow an easy extrapolation to the in vivo situation. Under our experimental conditions, formation of MA and M2 was almost completely abolished. Thus, the *in vivo* route-dependent patterns of DTZ metabolism would not be present. Moreover, the *in vivo* disposition of M1 is the result of simultaneous production and degradation processes. In other words, in our experimental conditions the degradation of M1 (i.e. its demethylation) is not allowed and, as a result, it is conceivable that less M1 would have been produced in presence of other parallel elimination pathways. However, this does not limit the validity of the results in describing the presence of the esterase responsible for the deacetylation of DTZ in the tissues examined.

In vitro and *in vivo* studies have shown that extrahepatic metabolism is not a rare event, but happens with many drugs (15). The presence of drug-metabolizing enzymes in extrahepatic tissues indicates that different organs should be capable of detoxicating various xenobiotics and thus contribute to the overall elimination of a drug. In general, microsomal enzymes are found in greater concentrations in the liver, whereas cytosolic enzymes (including esterases) have almost even and widespread distribution throughout the organs (15). Our results largely agreed with these assertions. The widespread distribution of the DTZ deacetylase activity described in this study suggests that extrahepatic metabolization of DTZ to M1 may play an important role in the overall pharmacokinetics of DTZ.

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REFERENCES

1. M. T. Buckley, S. M. Grant, K. L. Goa, D. McTavish, and E. M. Sorkin. *Drugs* **39**:757–806 (1990).
2. Y. Sugawara, M. Oshashi, S. Nakamura, S. Usuki, T. Suzuki, Y. Ito, T. Kume, S. Harigaya, A. Nakao, M. Gaino, and H. Inoue. *J. Pharmacobiodyn.* **11**:211–223 (1988).
3. N. Yabana, T. Nagao, and N. Sato. *J. Cardiovasc. Pharmacol.* **7**:152–157 (1985).
4. L. Pichard, G. Gillet, I. Fabre, I. Dalet-Beluche, C. Bonfils, J. P. Thenot, and P. Maurel. *Drug Metab. Dispos.* **18**:711–719 (1990).
5. P. K. F. Yeung, S. J. Mosher, M. A. Quilliam, and T. J. Montague. *Drug Metab. Dipos.* **18**:2055–1059 (1990).
6. E. Leboeuf and O. Grech-Belanger. *Drug Metab. Dispos.* **15**:122–126 (1987).

7. W. Homsy, M. Lefebvre, G. Caille, and P. du Souich. *Pharm. Res.* **12**:609–614 (1995).
8. N. W. McCracken, P. G. Blain, and F. M. Williams. *Biochem. Pharmacol.* **45**:31–36, (1993).
9. F. J. Leinweber. *Drug Metab. Rev.* **18**:379–439 (1987).
10. P. Manitpisitsul and W. L. Chiou. *Biopharm. Drug Dispos.* **14**:555–556 (1993).
11. R. Bernasconi, S. Caliari, R. Latini, D. Leopaldi, S. Porzio, and A. Salimbeni. *Eur. J. Drug Metab. Pharmacokinet.* **17**:269–274 (1992).
12. Y. H. Lee, M. H. Lee, and C. K. Shim. *Pharm. Res.* **9**:1599–1606 (1992).
13. A. M. McLean, E. A. Cefali, J. S. Roden, M. A. Gonzalez, and M. Bialer. *Biopharm. Drug Dispos.* **12**:327–334 (1991).
14. S. Nakamura, T. Suzuki, Y. Sugawara, S. Usuki, Y. Ito, T. Kume, M. Yoshikawa, H. Endo, M. Ohashi, and S Harigaya. *Arzneim.-Forsch./Drug Res.* **37**:1244–1252 (1987).
15. D. R. Krishna. *Clin. Pharmacokinet.* **26**:144–160 (1994).