Metabolism of Diltiazem. III. Oxidative Deamination of Diltiazem in Rat Liver Microsomes*

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The main metabolites of diltiazem in rats are acidic metabolites having a carboxyl group which may be formed by oxidative deamination of the dimethylaminoethyl group of diltiazem. In order to identify the enzymes responsible for the deamination and formation of acidic and neutral metabolites [14C]diltiazem was incubated with microsomal and mitochondrial preparations from the liver of SD male rats. Both acidic and neutral metabolites were formed only in the presence of an nicotinamide adenine dinucleotide phosphate generating system. Their formation was remarkable, especially in the microsomes, and inhibited by SKF 525-A, but not by pargyline and iproniazid. The production of neutral metabolites surpassed that of acidic ones. Structural analysis by gas chromatography-mass spectrometry showed that the neutral metabolites are aldehydes which have not been detected in vivo. The results suggest that the dimethylaminoethyl group of diltiazem is oxidized to an aldehyde group by microsomal cytochrome P-450 in the liver. Subsequently, the aldehyde group would be dehydrogenated to the carboxyl group.

Keywords — diltiazem; drug metabolism; deamination; cytochrome P-450; monoamine oxidase; rat; liver

Introduction

(+-)(2S,3S)-2,3-Dihydro-3-acetoxyl-2-(4-methoxyphenyl)-5-(2-(dimethylamino)ethyl)-1,5-benzothiazepin-4(SH)-one hydrochloride (diltiazem·HCl) was introduced in 1971, and has been used as a Ca2+-channel blocker for therapy of angina pectoris and hypertension. Recently, new acidic metabolites (A1—A4), which have a CH2COOH group formed by deamination of the dimethylaminoethyl group of diltiazem, were found in rats, dogs and humans. These acidic metabolites accounted for 90% of the plasma metabolites in male rats.

Two pathways could be hypothesized for the deamination of diltiazem. These are oxidation processes by monoamine oxidase (MAO) in the mitochondrial membranes and by cytochrome P-450 in the microsomes, as has been reported for amphetamine, imipramine, and chlorpromazine.

The acidic metabolites (A1—A4) of diltiazem showed little effect on the cardiovascular system, and no other pharmacological activity has been found. Their toxicities are extremely low. However, the formation of acidic metabolites may affect the bioavailability of diltiazem because plasma and bile are abundant in these metabolites. Inhibition or induction by coadministered drugs of the deaminating enzymes could affect the metabolism of diltiazem. To predict the interaction of diltiazem with other drugs, it would be useful to determine whether deamination of diltiazem occurs by MAO or cytochrome P-450.

Materials and Methods

Chemicals — [14C]Diltiazem·HCl was synthesized from ([14C]ethyl)-dimethylaminoethyl chloride·HCl (2.102 GBq (56.8 mCi)/mmol, radiochemical purity: more than 93.5%, New England Nuclear, Boston, MA, U.S.A.). The specific radioactivity of [14C]diltiazem·HCl was

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** To whom correspondence should be addressed.
*** H. Yabana and T. Nagao, unpublished results.
**** T. Magaribuchi, unpublished results.
1.467 MBq (39.65 μCi)/mg and the radiochemical purity was more than 98%. Nicotinamide adenine dinucleotide phosphate (NADP) was purchased from Kohnin Co., Ltd. (Kumamoto, Japan). Glucose-6-phosphate (G6P), glucose-6-phosphate dehydrogenase (G6PD, type XV), pargyline, iproniazid and kynuramine were purchased from Sigma Chemical Co. (St. Louis, Mo. U.S.A.). SKF 525-A was synthesized in Organic Chemistry Research Laboratory of Tanabe Seiyaku Co., Ltd.

**Preparation of Sub-cellular Fractions** — The experiments were repeated 3 times and the enzyme preparations in each experiment were obtained from 2 rats. Sprague-Dawley male rats (8 weeks, Charles River Japan, Kanagawa) were fasted overnight before use. All procedures were carried out at 0—4 °C. The livers were obtained after perfusion with saline, cut with scissors, and homogenized in two volumes of a buffer solution containing 0.25 M sucrose, 25 mM KCl, 5 mM MgCl₂ and 50 mM Tris-HCl (pH 7.5) using a Potter Elvehjem type glass-Teflon homogenizer. The homogenate was passed through cotton gauze and used as homogenates, which were in turn centrifuged at 600 g for 10 min using an RS-18GL centrifuge (Tomy Seiko, Japan). The supernatant was centrifuged at 9750 g for 15 min. The precipitates were washed twice with the buffer solution described above (mitochondrial fraction). The mitochondrial fraction was suspended in 10 ml of 50 mM K-phosphate buffer (pH 7.5) before use. The postmitochondrial supernatant was centrifuged twice at 18000 g for 20 min (Tomy Seiko, RS-18GL). The resulting supernatant (S₃ fraction) was centrifuged at 106000 g for 60 min (Hitachi Koki, SCP85H). The precipitates were suspended in 50 mM K-phosphate buffer containing 0.15 M KCl and the suspension was centrifuged again at 106000 g for 60 min. The resulting precipitates (microsomal fraction) were suspended in 5 ml of 50 mM K-phosphate buffer (pH 7.5) before use.

Protein concentrations were determined by a modified Biuret method using Iatroace TPR-1 (Iatron, Japan). Absorption was measured at 546 nm.

**Experiments on in Vitro Metabolism and Its Inhibition** — Deamination of [¹⁴C]diltiazem-HCl was investigated using rat sub-cellular fractions with or without an NADPH generating system. The NADPH generating system was composed of 4 mM NADP, 40 mM G6P, 40 mM MgCl₂, and 2 units/ml of G6PD. Fractions of the homogenate, mitochondria, S₃ and microsomes were examined. After preincubation of these fractions in 50 mM K-phosphate buffer (pH 7.5) at 37 °C for 5 min (total 9 ml), 1 ml of an aqueous solution of [¹⁴C]diltiazem-HCl (10⁻⁴ M) was added. After incubation for 15 min the reaction was stopped by addition of 1 ml of 1 N HCl. Neutral and acidic metabolites were extracted with tert-butyl methyl ether (tBME). Acidic metabolites were extracted with an aqueous 0.01 M Na₂HPO₄ solution from tBME extracts. The aqueous Na₂HPO₄ solution was acidified with 1 N HCl, and acidic metabolites were extracted with tBME. The total quantity of neutral and acidic metabolites was determined from the sum of the radioactivities of each of the tBME fractions. The limit of determination for radioactivity was 80 cpm. Inhibitory actions of SKF 525-A (10⁻⁴ M), pargyline (10⁻⁴ M) and iproniazid (10⁻⁴ M) against production of neutral and acidic metabolites in the mitochondria and microsomes were examined in the presence of the NADPH generating system. The reaction and extraction of the products were carried out according to the same procedure as described above except for the addition of the inhibitors. The inhibitors were added 30 min before addition of the NADPH generating system and [¹⁴C]diltiazem-HCl.

**Measurement of MAO Activity** — MAO activity in the mitochondrial fraction was determined by a modification of the method of Weissbach using kynuramine. ⁸) The solution of 0.2 ml of mitochondrial fraction and 0.3 ml of 0.5 M K-phosphate buffer (pH 7.4) was made up to 2.7 ml with water. Reaction was started by addition of 0.3 ml of 1 mM kynuramine (final 10⁻⁴ M). The reaction was stopped by addition of 0.2 ml of 30% trichloroacetic acid at 0, 3, 5 and 7 min. The reaction mixture was centrifuged at 2500 rpm for 10 min. The supernatant was transferred to a test tube, made alkaline with KHCO₃ and the concentration of kynuramine was measured by its absorption at 360 nm. MAO
activity was calculated from the rate of decrease of kynuramine per minute (nmol/min/mg protein).

**High-Performance Liquid Chromatography (HPLC)** — A Hitachi 655-15 high-performance liquid chromatograph with a TSKgel ODS-80TM column (4.6 mm i.d. × 15 cm, 5 μm, Tosoh Co., Ltd.) was used. The metabolites were separated by the combination of isocratic and linear gradient elution in which the ratio of solvent acetonitrile and 0.1 M phosphate buffer (pH 2.2) were varied as follows, 30 : 70, 30 : 70, 50 : 50 and 50 : 50 (v/v) at 0, 1, 16 and 20 min after sample injection, respectively. The flow rate was 1 ml/min. The eluates were monitored by ultraviolet (UV) absorption at 238 nm.

**Gas Chromatography-Mass Spectrometry (GC-MS)** — The GC-MS analysis was performed in a positive ion electron ionization mode using a Hitachi M-80A gas chromatograph-mass spectrometer interfaced with a Hitachi M-003 data processing system. The dried neutral fractions were dissolved in acetonitrile, and subjected to GC-MS analysis. Some samples were treated with N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) or methoxylamine-HCl (a reagent for converting keto groups to methoxime) before measurement. The conditions were as follows: column, G-100 glass capillary column (methyl silicone, 1.2 mm i.d. × 40 m, film thickness 0.5 μm, Chemicals Inspection and Testing Institute, Tokyo, Japan); column oven temperature, 240—270 °C; carrier gas, helium, 20 ml/min; ionization energy, 20 eV; total emission current, 100 μA; ion accelerating voltage, 3 kV; ion source temperature, 180 °C.

**Measurement of Radioactivity** — Each sample was dissolved in a toluene-Triton X-100 based scintillation cocktail (2,5-diphenyloxazole 12 g, dimethyl-2,2'-p-phenylene-bis(5-phenylloxazole) 0.3 g, Triton X-100 1 l, toluene 2 l) and radioactivity was measured with a liquid scintillation spectrometer (Tri-Carb 460 CD, Packard Instrument Co., Inc., Chicago, Il, U.S.A.). Counting efficiency was determined by an external standard method.

**Results**

**Metabolism of Diltiazem in Rat Liver Preparations**

Productions of the acidic and neutral metabolites were examined after incubation of [¹⁴C]diltiazem-HCl with or without the NADPH generating system in the homogenates, the mitochondrial fraction, the 18000 g supernatant (S3 fraction) and the microsomal fraction of the liver of SD male rats. Table I shows the production rates of the acidic and neutral metabolites (pmol/min/mg protein) which were calculated from the radioactivities produced for the period. Production rates of both the acidic and neu-

**TABLE I. The Formation Rates of Acidic and Neutral Products from [¹⁴C]Diltiazem in the Sub-cellular Fractions of Rat Liver**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Acidic products</th>
<th>Neutral products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− NADPHᵇ</td>
<td>+ NADPHᵇ</td>
</tr>
<tr>
<td>Homogenates (13.1 mg/ml)</td>
<td>N.D.ᵃ</td>
<td>0.42ᵃ</td>
</tr>
<tr>
<td>Mitochondrial fraction</td>
<td>N.D.</td>
<td>2.60 ± 3.28</td>
</tr>
<tr>
<td>(1.5 mg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3 fraction (7.8 mg/ml)</td>
<td>N.D.</td>
<td>3.96 ± 0.52</td>
</tr>
<tr>
<td>Microsomal fraction (0.7 mg/ml)</td>
<td>N.D.</td>
<td>17.84 ± 5.83</td>
</tr>
</tbody>
</table>

*Each value represents mean ± S.D. (n = 3). The values in the parentheses are final protein concentration. a) 18000 g supernatant (see Methods). b) NADPH means NADPH generating system. c) Mean of 0.09 and 0.75 (n = 2). d) N.D. = not detected.*
tral metabolites were high in the presence of the NADPH generating system especially in the microsomes. Percentages of the production of the acidic and neutral metabolites from $[^{14}C]$diltiazem after incubation for 15 min were $1.81 \pm 0.18$ and $6.82 \pm 0.79\%$ ($n = 3$), respectively; most of the radioactivity was recovered as unchanged drug. By contrast, the amount of acidic metabolites was below the limit of detection in all the fractions examined without the NADPH generating system. In the neutral fractions from the reaction mediums, a small amount of radioactivity by contamination with unchanged drug was detected. But the amount was too small, so we regard the neutral product in the medium without the NADPH generating system as not detected (N.D.), and the values of the neutral products in the medium with the NADPH generating system were not corrected.

The MAO activity of the mitochondrial fraction in this study was $3.25$ nmol/min/mg protein. Since the value was as high as the one reported by Coomes et al. $^9$ ($3.40$ nmol/min/mg protein), low activity of the mitochondrial fraction on the metabolism of diltiazem suggests that diltiazem is not a good substrate for mitochondrial MAO under the conditions used for these experiments.

Inhibitory Effects of SKF 525-A, Pargyline and Iproniazid

In order to clarify which enzyme (cytochrome P-450 or MAO) is responsible for the metabolism of diltiazem, we examined the inhibitory effects of SKF 525-A (cytochrome P-450 inhibitor), pargyline (MAO inhibitor) and iproniazid (MAO inhibitor) on the production of both acidic and neutral metabolites. Results are shown in Table II.

In the microsomes SKF 525-A ($10^{-4}$ M) inhibited significantly the production of acidic and neutral metabolites by 85.9 and 52.1%, respectively. SKF 525-A also exhibited inhibition in the mitochondrial fraction, although percentages of inhibition were extremely low in comparison with those obtained in the microsomes. Slight inhibitory effects of pargyline and iproniazid on the production of acidic metabolites in the mitochondria may indicate the possible participation of MAO. However, it is considered that acidic metabolites in the mitochondrial fraction were produced by the microsomes contained in the mitochondrial preparation, since their

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Mitochondrial fraction (0.61 mg/ml)$^a$</th>
<th>Microsomal fraction (0.83 mg/ml)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$2.3 \pm 0.3$</td>
<td>$24.1 \pm 1.1$</td>
</tr>
<tr>
<td>SKF 525-A ($10^{-4}$ M)$^b$</td>
<td>$1.0 \pm 0.1^g$</td>
<td>$3.4 \pm 0.4^g$</td>
</tr>
<tr>
<td>Pargyline ($10^{-4}$ M)$^b$</td>
<td>$(56.5%)$</td>
<td>$(85.9%)$</td>
</tr>
<tr>
<td>Iproniazid ($10^{-4}$ M)$^b$</td>
<td>$2.0 \pm 0.1$</td>
<td>$22.3 \pm 2.1$</td>
</tr>
<tr>
<td>Neutral products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$19.0 \pm 1.2$</td>
<td>$82.6 \pm 3.3$</td>
</tr>
<tr>
<td>SKF 525-A ($10^{-4}$ M)$^b$</td>
<td>$16.1 \pm 1.0^g$</td>
<td>$39.6 \pm 0.9^g$</td>
</tr>
<tr>
<td>Pargyline ($10^{-4}$ M)$^b$</td>
<td>$(15.3%)$</td>
<td>$(52.1%)$</td>
</tr>
<tr>
<td>Iproniazid ($10^{-4}$ M)$^b$</td>
<td>$17.9 \pm 0.2$</td>
<td>$91.7 \pm 2.8^g$</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D. ($n = 3$) of the formation rates (pmol/min/mg protein). The values in the parentheses are % inhibition. $^a$ Final protein concentration. $^b$ Final inhibitor concentration. $^c$ Different from the control at $p < 0.01$ by Dunnett’s method.
production occurred only in the presence of NADPH generating system and was significantly inhibited by SKF 525-A. These results indicate that the enzyme responsible for the deamination of the side chain of diltiazem is microsomal cytochrome P-450.

**Acidic Metabolites**

Four acidic metabolites (A1—A4, see Fig. 4) in the acidic fraction were separated by HPLC (Fig. 1). These acidic metabolites were identified to the corresponding authentic samples by comparing their GC-MS and HPLC. The radiochromatograms were traced after measuring the radioactivity of aliquots of every 0.5 min fractions. Recovery of radioactivity through the HPLC procedure was quantitative. In the microsomes, A1 was most abundant, followed by A3 (O-demethylated acidic metabolite). The amounts of deacetylated acidic metabolites (A2 and A4) were small. A similar metabolite pattern was obtained in the mitochondria.

**Structures of Neutral Metabolites**

The structural analysis of neutral metabolites was performed using GC-MS. At first, mass chromatography was carried out by use of base peak ions (ion-b in Fig. 3) of the acidic metabolites (A1—A4). Aldehydes N1—N4 were postulated to correspond to the acids A1—A4. In the mass chromatogram of the neutral fraction, the main component was N1, followed by N3 (Fig. 2). The neutral fraction was contaminated by diltiazem probably due to latter’s high content in the reaction medium as an unreacted substrate.

Furthermore, mass spectra were compared among the main neutral metabolite (N1), diltiazem and an acidic metabolite A1 (Fig. 3). In the mass spectrum of diltiazem, major fragment ion peaks were (a) the base peak m/z 58
Fig. 3. Mass Spectra of Diltiazem (A), Trimethylsilyl (TMS) Derivative of A1 (B), a Neutral Metabolite N1 (C), TMS Derivative of N1 (D) and Oxime of N1 (E)

(CH₂ = N⁺(CH₃)₂) derived from the side chain, (b) m/z 150 (CH₃-O-CH = CH-OH⁺), (c) the tropilium ion m/z 121 derived from the CH of the position 2 of the benzothiazepin skeleton and the methoxyphenyl group at the position 2, and (d) m/z 136 (C₇H₆NS⁺) derived from the benzothiazepin skeleton. In the high mass area, the molecular ion peak (m/z 414) was noticed. In the mass spectrum of N1, no peak corresponding to the base peak ion (m/z 58) of diltiazem appeared. Therefore, the side chain of N1 is different from that of diltiazem. The other major
fragment ion peaks of diltiazem (b, c and d described above) appeared also in the spectrum of N1 as well as A1. This indicates that N1 has the benzothiazepin skeleton with a methoxyphenyl group at C-2 and an acetoxy group at C-3. In the mass spectrum of N1, the molecular ion peak appeared at m/z 385, and the ions which were produced by removal of acetic acid (60 amu) from the molecular ion appeared at m/z 325; the latter ion indicates the presence of an acetoxy group. The difference of the mass number of the molecular ions between N1 and diltiazem (m/z 414) was m/z 29 which accounts for the difference between CH₂N(CH₃)₂ and CHO groups. Therefore, N1 was estimated to be an aldehyde which was produced by deamination of the side chain of diltiazem. In the mass spectrum of N3, characteristic peaks which were 14 amu (OCH₃ - OH) lower than the molecular ions (M⁺ of N1, M⁺ - 60 ions, b and c ions, were seen, indicating that N3 is a O-demethylated compound of N1. An additional investigation on the structure of the neutral metabolites was carried out after treatment with BSTFA alone or treatment with methoxylamine·HCl and successively with BSTFA. After these treatments the peaks of the ions b, c and d remained (Fig. 3D and E). BSTFA reacted with the enol form of the aldehyde to give CH=CH-OTMS, affording the molecular ion of m/z 457. On the other hand, the molecular ion became m/z 414 after treatment with methoxylamine·HCl, a reagent for oximation of carbonyl compounds. The oximation of the CHO group of N1 (M⁺ is 385) might give rise to the CH= N-OCH₃ group; accordingly the molecular ion became m/z 414. These derivatizations introduce a double bond. Therefore, cis and trans isomers appeared in the mass-chromatogram (data not shown). Stereoisomers also appeared after oximation of N3.

These results indicate that the neutral metabolite N1 is the aldehyde produced by oxidative deamination of the CH₂CH₂N(CH₃)₂ group of diltiazem and N3 is the O-demethylated compound of N1.

Discussion

The main metabolites of diltiazem were acidic metabolites having a CH₂COOH group, which might be derived from a CH₂CH₂N(CH₃)₂ group by oxidative deamination.³,⁴ In addition, diltiazem undergoes N-

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Fig. 4. Possible Metabolic Pathways from Diltiazem to Acidic Metabolites (A1—A4) via Intermediate Neutral Metabolites (N1—N4) Which Are Assumed to be Formed by Liver Microsomal Cytochrome P-450

*: labeled positions with [¹⁴C], **: N2 and N4 were not detected in this study, Ac: an acetyl group. A and B indicate oxidation by cytochrome P-450.
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demethylation, O-demethylation and deacetylation. Deamination and N-dealkylation is basically identical. Namely, the attack of oxygen at the methyl group causes N-demethylation, whereas the oxidation of the methylene group initiates deamination. The definition depends on whether interest is focused on the formation of carbonyl compounds or on the new amines. Figure 4 shows possible metabolic pathways from diltiazem to acidic metabolites. The initial attack of oxygen must occur either at the carbon of the methylene or methyl group or at the nitrogen in the -CH₂N(CH₃)₂ of diltiazem. Tertiary amines might undergo N-demethylation prior to deamination in some cases. However, direct deamination of tertiary amines has been demonstrated in haloperidol, benspiride and isojurupidine; but these compounds have no N-alkyl group responsible for the production of secondary or primary amine intermediates by dealkylation. Furthermore, it has been suggested that imipramine produces carboxylic acid metabolites by α oxidation of the CH₂N(CH₃)₂ without preceding N-demethylation. Therefore, it is probable that the direct attack by oxygen at the methylene carbon of the CH₂N(CH₃)₂ causes deamination of diltiazem, although the possibilities of deamination of secondary (CH₂NCH₃) and primary amines (CH₂NH₂) could not be ruled out.

There are two possible enzymes for the deamination. One is an oxidation by MAO and another is one by cytochrome P-450. Diltiazem and its basic metabolites may be converted to the aldehydes by either MAO or cytochrome P-450, and then the aldehydes would be converted further to acidic metabolites by aldehyde dehydrogenase. Acidic metabolites were detected in the mitochondrial and microsomal fractions. In these fractions nicotinamide adenine dinucleotide (NAD⁺), which is the coenzyme of aldehyde dehydrogenase, may not be contained. Therefore, it was expected that the aldehydes, which had not been found in vivo, could be detected in this experiment using mitochondrial or microsomal fractions.

In this in vitro study, the amounts of neutral metabolites which were estimated to be aldehydes by GC-MS were higher than that of acidic ones (Table I). The main neutral metabolite was N1. In addition, a small amount of N3 was found. But none of the deacetylated compounds (N2 and N4) could be detected (Fig. 2). On the other hand, a small amount of deacetylated acidic metabolites, A2 and A4, were detected in the acidic fraction. A2 and A4 are assumed to be derived from A1 and A3 by deacetylation, but not from N2 and N4 because of their absence. No neutral metabolite (N1—N4) has been found in the urine and plasma of rats, dogs and men which are given diltiazem. In vivo the intermediate aldehydes are presumably converted to acidic metabolites immediately by aldehyde dehydrogenase.

NADPH is required for the activity of cytochrome P-450, but not for MAO activity. In this experiment, the acidic and neutral metabolites of diltiazem are markedly formed only in the presence of an NADPH generating system. The requirement of NADPH suggests the participation of cytochrome P-450. Furthermore, the production of the metabolites is inhibited by SKF 525-A, an inhibitor of cytochrome P-450, but not by MAO inhibitors (iproniazid and pargyline) (Table II). Therefore it is assumed that diltiazem was metabolized by cytochrome P-450 to acidic metabolites. The acidic or neutral products of diltiazem were not detected in the homogenate or S3 fraction without addition of an NADPH generating system. The generating system in these fractions is thought to be not enough to produce detectable amount of acidic or neutral metabolites.

Two forms of MAO, types A and B have been postulated. Pargyline is a specific inhibitor for type B MAO and inhibits the activity of type B MAO at low concentrations (lower than 10⁻⁷ M), although it inhibits the type A MAO activity also at the concentrations higher than 10⁻⁵ M. In this study pargyline did not show inhibitory effects on the deamination of diltiazem at 10⁻⁴ M, which is high enough to inhibit both types of MAO activities. Iproniazid, a non specific inhibitor for both types of MAO, did not exhibit inhibition at 10⁻⁴ M, which is the concentration required for the maximum inhibition of MAO activity.

In the mitochondrial fraction neutral and
acidic metabolites were detected, although the amount was small compared with that in the microsomal fraction. The MAO activity in the mitochondrial fraction determined using kynura- mine as a substrate was comparable to the reported value. Since the production of acidic metabolites was slightly inhibited by pargyline, a possibility of the participation of MAO in the deamination cannot be ruled out completely. However, the reaction required NADPH, and was inhibited by SKF 525-A very much more than by pargyline. These results suggest that the deamination detected in the mitochondrial fraction may be due to cytochrome P-450, and that a large quantity of the neutral and acidic metabolites in the mitochondrial fraction may be produced by contaminating microsomal P-450. The relatively low value of inhibition on the production of neutral metabolites in the mitochondrial fraction may be caused by dilution of radioactivity of the metabolites with that of substrate as a contaminant. It has been reported that the mitochondrial fraction contains a fair amount of microsomes when sub-cellular fractionation is done only by centrifugation.

This study shows that the main acidic metabolites of diltiazem in vitro is A1, followed by A3 (Fig. 1), and that the amounts of deacetylated metabolites (A2 and A4) are very small. On the other hand, the main in vivo metabolites in plasma were A2 and A4 in rats, and A2 in dogs.

Diltiazem is almost completely absorbed from the gastrointestinal tract, is metabolized in the liver to a high extent, and large amounts of the metabolites are excreted into the bile (the biliary excretion rate in rats was about 65%). The results in this study together with those so far reported indicate that most of diltiazem is metabolized in the liver by deamination, N-demethylation and O-demethylation. It has not determined whether deacetylation proceeds after or before these oxidative reactions.

No pharmacological activity has been found in the acidic metabolites (A1—A4) of diltiazem and their acute toxicities are extremely low. However, these metabolites account for most of the plasma metabolites in rats, and A2 is a main metabolite in dogs and men. Therefore, the factors affecting the formation of acidic metabolites would change the bioavailability of diltiazem. In this study, it is suggested that the deamination of diltiazem proceeds by the action of microsomal cytochrome P-450. Other metabolic reactions such as N-demethylation, O-demethylation and hydroxylation of the aromatic rings also should occur by the action of cytochrome P-450 in the liver endoplasmic reticulum.

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