PHARMACOKINETICS AND METABOLISM OF THE NOVEL ANTICONVULSANT AGENT N-(2,6-DIMETHYLPHENYL)-5-METHYL-3-ISOOXAZOLECARBOXAMIDE (D2624) IN RATS AND HUMANS

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ABSTRACT:

N-(2,6-dimethylphenyl)-5-methyl-3-isooxazolecarboxamide (D2624) belongs to a new series of experimental anticonvulsants related to lidocaine. This study was undertaken to understand the pharmacokinetics and metabolism of D2624 in rats and humans, with emphasis on the possible formation of 2,6-dimethylaniline (2,6-DMA). After oral administration of stable isotope-labeled parent drug to rats and GC/MS analysis of plasma samples, two metabolites were identified: D3017, which is the primary alcohol, and 2,6-DMA, formed by amide bond hydrolysis of either D2624 or D3017. In urine, three metabolites of D2624 were identified: namely D3017, 2,6-DMA, and D3270 (which is the carboxylic acid derivative of D3017). Based on plasma AUC analysis, D3017 and 2,6-DMA accounted for >90% of the dose of D2624. After oral administration, D2624 was found to be well absorbed (93%), but underwent extensive first-pass metabolism in the rat, thus resulting in 5.3% bioavailability. Rat and human liver microsomal preparations were capable of metabolizing D2624 to D3017 and 2,6-DMA. The formation of D3017 was NADPH-dependent, whereas 2,6-DMA formation was NADPH-independent and probably was catalyzed by amida(s) enzymes. In a single-dose (25–225 mg) human volunteer study, the parent drug (D2624) was not detected in plasma at any dose, whereas 2,6-DMA was detected only at the two highest doses (150 and 225 mg). D3017 was detected after all doses of parent drug, with approximate dose proportionality in AUC and a half-life of 1.3–2.2 hr. The metabolic behavior observed in humans suggests there is a marked species difference in the oxidative and hydrolytic pathways of D2624.

D2624 belongs to a new series of experimental N-aryl isooxazolecarboxamide anticonvulsants. These compounds are structurally related to the local anesthetic lidocaine, which is known to possess anticonvulsant activity (1). The anticonvulsant Drug Development Program at the National Institute of Neurological Disorders and Stroke (Bethesda, MD) found D2624 to have phenytoin-like activity in animal seizure models (2, 3) and to be devoid of the classical anticonvulsant and neurotoxic properties was suggested by the dependence of anticonvulsant potency in rodents on route of administration, with oral administration having a greater TD50/ED50 ratio than parenteral administration (3). As a lidocaine analog, D2624 possesses an amide bond that is susceptible to metabolic hydrolysis to yield 2,6-DMA, a known metabolite of lidocaine (5). Although D2624 was not found to be mutagenic in classical Ames and micronucleus tests, it is known that many simple aromatic amines, such as 2,6-DMA, produce a variety of species-dependent toxicities, such as nasal carcinomas and adenomas in rats (6, 7).

Based on these considerations, the objectives of this study were to: i) identify and quantify the metabolites of D2624, either in rats receiving the drug by different routes or in rat liver microsomal preparations; and ii) determine whether 2,6-DMA is formed in human liver microsomal preparations and whether it can be detected in the plasma of healthy subjects after single-dose administration.

Materials and Methods

D2624 and D3017 were synthesized using the method reported by Lepage et al. (3) and provided by Laboratoires Biocodex (Compiègne, France). [13C]D2624 (labeled in the xyridine methyl groups; isotopic purity: 99 atom% excess) was synthesized as outlined herein and provided by Laboratoires Biocodex. A sample of D3270 was prepared by synthesis, as outlined. 2,6-DMA was obtained from Aldrich Chemical Co. ( Milwaukee, WI). All other chemicals were of analytical grade and were obtained from Sigma Chemical Company.
Co. (St. Louis, MO). Solvents were of HPLC grade and were purchased from either Baxter Healthcare Corp. (McGaw Park, IL) or J. T. Baker (Phillipsburg, NJ).

Synthetic Procedures. Synthesis of D2624. This carboxylic acid derivative was prepared from the corresponding primary alcohol (D3017) in a two-step procedure, involving oxidation first to the aldehyde (8) and then to the acid (9). After these reactions, the methyl ester was analyzed by GC/MS. The mass spectrum of this material, which was fully consistent with the proposed structure, exhibited the following prominent ions: m/e 274 (M^+ 75%), 256 ([M-HOCH_2]^+ 15%), 246 ([M-CO_2]^+ 38%), 215 ([M-CO_3H]^+ 100%), 187 ([M-CO_3H]^+ 20%), 120 (ArNH_2 61%), and 105 (C_9H_7_7 ^+ 22%). With respect to purity of the synthesis of D2624, only a single peak was observed on GC analysis of the methyl ester.

Synthesis of ^13C_2/D2624. ^13C_2/D2624 labeled in the xyline methyl group was prepared in a three-step procedure. Labeled 2,6-dimethylacetanilide, synthesized from acetonilide and [1^3C_2]CH_3 according to the procedure of Tremont and Rahman (10), was hydrolyzed with aqueous potassium hydroxide, and the resulting labeled 2,6-DMA was condensed with 5-methyl-3-isoxazolacarboxylic acid to yield 54% [1^3C_2]D2624 labeled in the xylidineide, and the resulting labeled 2,6-DMA was condensed with 5-methyl-3-isoxazolacarboxylic acid to yield 54% [1^3C_2]D2624 labeled in the xyline methyl groups (99 atom% excess).

Instrumentation. H NMR Spectroscopy. This was performed on a Varian VXR 300 instrument (Varian Associates, Palo Alto, CA), operated at 300 MHz. Samples were analyzed in CDCl_3 solution, and chemical shifts are expressed relative to tetramethylsilane, which was added as the internal standard.

MS and GC/MS. MS was performed on a VG 70-70H double-focusing instrument (VG Instruments, Manchester, UK), operated in the electron impact mode at an ionizing energy of 70 eV and a resolving power of 10,000. Samples were introduced by the direct insertion technique, and data were acquired at a scan rate of 10 sec/decade. GC/MS analyses were conducted on a Hewlett-Packard 5970A MSD instrument, interfaced via a solvent divert valve to a Hewlett-Packard model 5890 GC equipped with a capillary splitless injector and a Hewlett-Packard 7673A autosampler. A fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness) coated with bonded stationary phase DB-5 was used for GC separations, and helium (head pressure 20 psi) served as carrier gas. Samples were injected in the splitless mode (injector temperature: 250°C) at a column temperature of 170°C and an interface temperature of 280°C. After a period of 5 min at 170°C, the oven temperature was increased linearly at 14°C/min to 280°C, and maintained at this temperature for 15 min. Metabolites were detected on the basis of the characteristic isotopic “doublets” that result from the presence of unlabeled and ^13C_2 mol-ecules in a 1:1 ratio, and were identified by comparing their GC and MS properties with those of the corresponding authentic reference compounds prepared by synthesis.

Biological Experiments. Metabolite Identification In Vivo. Adult male Sprague-Dawley rats (230–280 g) from Charles River Laboratories (Wilmington, MA) were housed in individual metabolic cages. Animals were dosed orally either with unlabeled D2624 (10 mg/kg; administered as a 5 mg/ml solution in saline/propanol glycol/ethanol, 5:4:1, v/v) or with an equimolecular mixture of unlabeled and [1^3C_2]D2624 (24 mg/kg total dose, administered in a solution of polyethylene glycol 400/saline, 2:1, v/v). Corresponding control animals received vehicle only. For the identification of metabolites in blood, rats were anesthetized with pentobarbital and provided with a jugular catheter 2–4 days before use and were allowed to recover before drug treatment. Blood samples (0.4 ml) were obtained via the indwelling catheter (using EDTA as anticoagulant) at the following time points: 0, 15, 30, 45, 60, 90, and 120 min postdose. Aliquots of whole blood (100 μl) were extracted with dichloromethane (5 ml), and extracts were evaporated to dryness under a stream of dry nitrogen. The residues then were reconstituted in the mobile phase (acetonitrile/water, 44:55, v/v) and analyzed by HPLC (Zorbax C8 column, 250 mm, 3 μm particle size, 4.6×250 mm) at a flow rate of 1.1 ml/min with UV detection at λ = 220 nm. In some cases, the previously described organic extracts were reconstituted with methanol (15–20 ml) and then distilled water (18–20 ml). Cartridges were washed with water (6–10 ml), and the aqueous eluates were collected. Lipophilic metabolites were then eluted with methanol (10–15 ml), and the methanol eluates were concentrated to approximately one-half of their original volume under reduced pressure. The latter organic phases were acidified with HCl (12 M) and extracted into dichloromethane (2 × 3 ml). The combined extracts were evaporated to dryness under a stream of dry nitrogen, taken up again in dichloromethane (2 ml), dried over magnesium sulfate, and concentrated to ~200 μl for analysis by GC/MS. A portion of each extract was methylated by treatment with a freshly prepared solution of ethereal diazomethane to facilitate the analysis of acidic metabolites of D2624. For the identification of 2,6-DMa as a urinary metabolite of D2624, the corresponding pentfluorobenzoyl derivative was prepared using a modification of the method reported by Coutts et al. (11) reconstituted in dichloromethane for analysis by GC/MS.

Metabolite Identification In Vitro. Hepatic microsomal preparations were obtained from the livers of male Sprague-Dawley rats (250 g body weight) using the procedure previously described by Thummel et al. (12). Incubations (total volume: 1 ml) contained microsomal protein (1 mg/ml), NADPH (1 mM), and D2624 (0.1 mM) in 100 mM phosphate buffer (pH 7.4), and were conducted at 37°C for 15 min. The incubation media then were extracted with dichloromethane (5 ml) and analyzed by HPLC (as previously described) and GC/MS. To analyze the major metabolite of D2624 (which was formed both in vivo and in vitro) by NMR spectroscopy, milligram quantities of the material were isolated by extracting a number of liver microsomal incubations and subjecting the pooled extracts to HPLC, as previously outlined.

In Vitro Metabolism of D2624 by Rat and Human Liver Microsomes. Three human livers (HL115, HL120, and HL125) were obtained from organ donors through the Solid Organ Transplant Program, University of Washington Medical Center, Northwest Organ Procurement Agency (Seattle, WA). Microsomes were prepared after a procedure similar to that used for rat liver microsomes, except that final resuspension was in 100 mM potassium phosphate buffer at a concentration of 6.6 mg protein/ml. Incubations were conducted at 37°C with a final incubation volume of 1 ml. Metabolite formation was investigated by incubating 1 mg rat or human microsomal protein with D2624 at 10 and 100 μM (rat) or 100 μM (human) for 10 min in the presence or absence of NADPH. Amidase-mediated metabolism of D2624 at 10 μM (rat) or 100 μM (human) was assessed by preincubating 1 mg rat or 0.3 mg human liver microsomal protein with bis(4-nitrophenyl)phosphate (an amidase inhibitor) and 1 mM NADPH for 10 min at 0–4°C. All incubations were performed in triplicate and were stopped either after 10 min by the addition of dichloromethane (4 ml) and 50 μl of internal standard (cyheptamide, 5 μg/ml) for the analysis of D2624 and D3017, or by the addition of acetonitrile (4 ml) and 50 μl of internal standard (cyheptamide, 5 μg/ml) for the analysis of 2,6-DMa. The organic layer was removed and evaporated to dryness under nitrogen. The residues then were reconstituted in a 100 μl mobile phase consisting of either acetonitrile/water (45:55, v/v), for the analysis of D2624 and D3017, or acetonitrile/0.01M potassium phosphate buffer (pH 5; 50:50, v/v), for the analysis of 2,6-DMa.

Pharmacokinetics of D2624 and Its Metabolites. Male Sprague-Dawley rats (230–300 g) were anesthetized with pentobarbital and provided with a femoral and a jugular catheter 2–4 days before drug treatment. Drug/vehicle was administered on a 2 ml/kg basis via the femoral vein. The following compounds were given at the doses indicated: D2624 at 4 mg/kg (17.4 μmol/kg; N = 6) or 12 mg/kg (52.2 μmol/kg; N = 9); D3017 at 12 mg/kg (48.8 μmol/kg; N = 6); and 2,6-DMa at 2 mg/kg (16.5 μmol/kg; N = 6). In some experiments, compounds were administered via oral gavage [D2624 at 52.2 μmol/kg (N = 6) or D3017 at 48.8 μmol/kg (N = 6)] as a solution in polyethylene glycol 400/saline (2:1, v/v). Serial samples (N = 10) of blood (300 μl) were taken at the following time points: 0, 3, 10, 20, 40, 60, 80, 120, 180, and 240 min postdose via the indwelling jugular vein catheter into heparinized tubes and stored at −70°C before HPLC analysis of D2624, D3017, and 2,6-DMa.

HPLC Analysis of D2624 and D3017. Blood samples (100 μl) were extracted with dichloromethane (5 ml) containing 50 μl cyheptamide solution (5 μg/ml; internal standard). After centrifugation at 600g for 15 min, the organic
phase was removed and evaporated to dryness under nitrogen. Residues were
reconstituted in 100 μl mobile phase (acetonitrile/water, 45:55, v/v) and
analyzed by reversed-phase HPLC (Waters Zorbax C4 column, 4.6 x 250 mm,
5 μm particle size) at a flow rate of 1.1 ml/min, with UV detection at λ = 210
nm.

**HPLC Analysis of 2,6-DMA.** Blood samples (100 μl) were extracted with
acetonitrile (4 ml) containing 250 μg cyheptamide (internal standard). After
centrifugation at 600g for 15 min at room temperature, the organic layer was
removed and concentrated under nitrogen to 100–200 μl at room temperature.
Care was taken during the latter step to ensure that samples did not evaporate
to dryness because of the volatile nature of 2,6-DMA. Extracts were analyzed
by HPLC using a Zorbax C4 column (4.6 x 250 mm, 5 μm particle size), a
mobile phase of acetonitrile and 0.01 M potassium phosphate buffer (pH 5;
50:50, v/v) at a flow rate of 1 ml/min, and UV detection at 210 nm.

**Human Studies.** The study protocol was approved by the Ethics Committee
in the region of Poitou-Charentes in accordance with the French law of
December 28, 1988 and the Declaration of Helsinki, Finland, of June 1964,
amended at the Third World Medical Assembly (1983), Venice. Six healthy
males aged 28 ± 2 year and weighing 69 ± 13 kg volunteered for the study after
giving their written informed consent. Before entering the study, the
subjects were found to be healthy by clinical examination, and none of the
subjects was on any chronic medication. Volunteers fasted overnight before the
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after oral dosing and were attributed to an absorption-rate limitation in elimination kinetics of both parent drug and metabolite (fig. 2B; table 1).

After intravenous administration of D3017 at 48.8 μmol/kg, the plasma profile of D3017 was best described by a one-compartment model, despite some curvature after three half-lives. The similar elimination half-lives of D3017 obtained after intravenous administration of D3017 (24.4 ± 4.1 min) and D2624 (30.7 ± 3.4 min) suggest that D3017 exhibited elimination rate-limited kinetics after D2624 administration. The steady-state volume of distribution of D3017 and its systemic clearance were significantly (p < 0.001) less than those of D2624. The plasma profile of 2,6-DMA following intravenous administration of 2,6-DMA (16.5 μmol/kg) was biexponential in four animals, whereas two animals showed a monoexponential decay. Values for elimination half-life of 2,6-DMA after intravenous administration (table 1) were significantly (p < 0.001) shorter than the corresponding values found after administration of either D2624 or D3017, thus suggesting formation-rate limited kinetics.

Signs of neurological side effects were not observed in rats after intravenous administration of D2624 at 17.4 μmol/kg or after oral administration of 52.2 μmol/kg. However, intravenous administration of D2624 at 52.2 μmol/kg caused mild sedation for a short period, and D3017 caused a mild transient muscular incoordination for 5–10 min after intravenous administration of 48.8 μmol/kg.

Oral Bioavailability of D2624 and D3017 in Rats. The AUC method was used to determine the F_Tot of D2624 and D3017 after oral
administration. The $F_{tot}$ of D2624 (5.3%) was much lower than that of D3017 (67.1%). Poor bioavailability of D2624 was attributed to either incomplete absorption from the gastrointestinal tract and/or extensive first-pass metabolism of the drug. The AUC of the metabolite (D3017) after oral and intravenous administration of the parent compound (D2624) was used to estimate the fraction of D2624 absorbed from the gut into the portal circulation using the equation $F_{abs} = (AUC_{m})_{poi}/(AUC_{m})_{iv}$. This AUC method indicated that absorption of D2624 was almost complete with $\approx 93\%$ of the dose appearing in the systemic circulation as the metabolite. Thus, the poor bioavailability of D2624 was most likely because of extensive first-pass metabolism. To quantify this first-pass effect, the fraction of the absorbed dose that crosses the liver unchanged ($F_{liv}$) was estimated by dividing the oral bioavailability by the fraction of dose absorbed from the gut based on metabolite AUC ($F_{liv} = F_{tot}/F_{abs}$). Using this equation, only 5.8% of the total dose absorbed was able to cross the liver unchanged.

**Fractional Metabolism of D2624 and D3017.** The formation of 2,6-DMA, after dosing with D2624, takes place via either arm of a “triangular metabolic” pathway, because both D2624 and its hydroxylated metabolite, D3017, possess an amide bond that, upon cleavage, can release 2,6-DMA (fig. 1). The fractional metabolism in each part of this triangular metabolic problem was determined using the plasma AUC method for the parent drug and its metabolites. The total fraction of D2624 converted to 2,6-DMA by i) direct hydrolysis to 2,6-DMA and ii) indirect metabolism via D3017 was calculated using the AUC ratio of 2,6-DMA after intravenous administration of D2624 to that obtained by dosing with 2,6-DMA and found to be 0.55 ($F_{m2}$). To determine the fraction of D2624 metabolized to 2,6-DMA via D3017 ($F_{m4}$), the fraction of D2624 converted to D3017 ($F_{m1}$) was estimated to be 0.42. The fraction of D3017 that undergoes metabolism to 2,6-DMA was calculated from the AUC ratio of 2,6-DMA after intravenous administration of both D3017 and 2,6-DMA, and estimated to be 0.16 ($F_{m3}$). The product of the fractions $F_{m1}$ and $F_{m3}$ indicated that only 6.6% of the dose of D2624 was metabolized to 2,6-DMA via D3017 ($F_{m4}$). Therefore, the fraction of D2624 metabolized directly to 2,6-DMA ($F_{m5}$) could be estimated by subtracting $F_{m4}$ from $F_{m2}$ and found to be 0.48. Thus, it seems that D3017 and 2,6-DMA are the main metabolites of D2624, because they account collectively for 90% of an intravenous dose.

![Fig. 2. Semilogarithmic plasma concentration time curves for D2624 and its metabolites in the rat.](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>Drug (Route)</th>
<th>N</th>
<th>Dose (mg/kg)</th>
<th>$CL_{D2624}$ (ml/min/kg)</th>
<th>$V_{SS_{D2624}}$ (liter/kg)</th>
<th>$T_{1/2_{D2624}}$ (min)</th>
<th>$CL_{D3017}$ (ml/min/kg)</th>
<th>$V_{SS_{D3017}}$ (liter/kg)</th>
<th>$T_{1/2_{D3017}}$ (min)</th>
<th>$CL_{2,6-DMA}$ (ml/min/kg)</th>
<th>$V_{SS_{2,6-DMA}}$ (liter/kg)</th>
<th>$T_{1/2_{2,6-DMA}}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2624 (iv)</td>
<td>6</td>
<td>4</td>
<td>2.43 (1.5)</td>
<td>17.4 (5.2)</td>
<td>38 (18)</td>
<td>82.3 (10.2)</td>
<td>2.11 (0.48)</td>
<td>17.4 (5.2)</td>
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<td>D2624 (po)</td>
<td>6</td>
<td>12</td>
<td>1.11 (0.31)</td>
<td>7.5 (1.2)</td>
<td>30.7 (3.4)</td>
<td>2.11 (0.48)</td>
<td>17.4 (5.2)</td>
<td>38 (18)</td>
<td>82.3 (10.2)</td>
<td>2.11 (0.48)</td>
<td>17.4 (5.2)</td>
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<tr>
<td>D3017 (iv)</td>
<td>6</td>
<td>12</td>
<td>20.8 (3.4)</td>
<td>1.47 (0.31)</td>
<td>33.8 (3.7)</td>
<td>20.8 (3.4)</td>
<td>1.47 (0.31)</td>
<td>33.8 (3.7)</td>
<td>20.8 (3.4)</td>
<td>1.47 (0.31)</td>
<td>33.8 (3.7)</td>
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<tr>
<td>D3017 (po)</td>
<td>6</td>
<td>12</td>
<td>24.4 (4.1)</td>
<td>1.11 (0.31)</td>
<td>23.6 (7.9)</td>
<td>24.4 (4.1)</td>
<td>1.11 (0.31)</td>
<td>23.6 (7.9)</td>
<td>24.4 (4.1)</td>
<td>1.11 (0.31)</td>
<td>23.6 (7.9)</td>
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<tr>
<td>2,6-DMA (iv)</td>
<td>6</td>
<td>2</td>
<td>54.1 (17.1)</td>
<td>16.5 (5)</td>
<td>11.7 (5.1)</td>
<td>54.1 (17.1)</td>
<td>16.5 (5)</td>
<td>11.7 (5.1)</td>
<td>54.1 (17.1)</td>
<td>16.5 (5)</td>
<td>11.7 (5.1)</td>
</tr>
</tbody>
</table>

Data are expressed as means ± (SD). CL, clearance; $V_{SS}$, volume of distribution at steady-state; $t_{1/2}$, half-life; ND, not determined.

* Dose in μmol/kg.

$\frac{CL}{F}$, where $F$ = oral bioavailability.
In Vitro Metabolism in Rat Liver Micr...bles D3017 by NADPH-dependent oxidative biotransformation to the alcohol metabolite D3017. However, the formation of 2,6-DMA was NADPH-independent, but could be inhibited by preincubating microsomes with BNPP, an amidase inhibitor.

In Vitro Metabolism in Rat Liver Micr...motes metabolized D2624 by NADPH-dependent oxidative biotransformation to the alcohol metabolite D3017. However, the formation of 2,6-DMA was NADPH-independent, but could be inhibited by preincubating microsomes with BNPP.

In Vitro Metabolism in Human Liver Micr...ble D3017 within 15 to 30
Discussion

By means of stable isotope labeling and GC/MS techniques, three metabolites of D2624 were identified in the rat. The most abundant metabolite, detected in both plasma and urine after oral administration, was D3017, which is the primary alcohol resulting from hydroxylation of the isoxazole methyl group. A second metabolite, which was detected only in methylated extracts of urine, was D3270, which is the carboxylic acid derivative formed from the corresponding primary alcohol (D3017). A third metabolite, which was present in plasma and urine, was identified as 2,6-DMA, which was formed by the hydrolysis of the amide bond of either D2624 or D3017 (fig. 1).

After intravenous administration of D2624, both metabolites had similar elimination half-lives that were significantly longer than that of the parent drug (fig. 2A). Intravenous metabolite studies were performed to determine whether metabolite disposition after parent drug administration was formation or elimination rate-limited. These studies indicated that D3017 exhibited elimination rate-limited kinetics after parent drug administration. However, the half-life of 2,6-DMA was significantly longer after intravenous administration of either D2624 or D3017 than after intravenous administration of 2,6-DMA, suggesting that 2,6-DMA exhibited formation rate-limited kinetics. 2,6-DMA exhibited a relatively high clearance (58.2 ml/min/kg), suggesting that further metabolism of 2,6-DMA may occur such that the calculated fractions of D2624 and D3017 metabolized to 2,6-DMA may represent underestimates.

After oral administration of D2624, the terminal half-life of D2624 increased 5-fold, which suggests that absorption became the rate-limiting step. This hypothesis was supported by the fact that D3017 half-life was increased 3-fold (fig. 2B). D2624 exhibited a much lower bioavailability than D3017. Using D3017 as a marker for absorption of the parent drug, it was found that D2624 was in fact almost completely absorbed from the gastrointestinal tract. Thus, it seems that D2624 acts as a prodrug for D3017. Because D3017 has potent anticonvulsant properties (3), it must contribute therefore to the activity observed after oral administration of D2624 (3). This may explain why greater efficacy against generalized tonic-clonic seizures was evident when D2624 was given orally, compared with intravenous administration (3).

Because D2624 and its hydroxylated metabolite D3017 possess an amide bond that, upon cleavage, can release 2,6-DMA, the metabolic source of 2,6-DMA was investigated. A stepwise approach, based on plasma AUC, was used to determine the fractional metabolism of D2624 and D3017 (fig. 1B). It is interesting to note that methyl hydroxylation of the D2624 structure at a site distant from the amide bond was associated with a marked reduction in susceptibility to hydrolysis. These quantitative metabolic studies also revealed that the two primary metabolites, D3017 and 2,6-DMA, accounted for 90% of a dose of D2624.

Rat and human liver microsomal preparations indicated that the two metabolites of D2624 are formed by different enzyme systems. The hydroxylation of the isoxazole methyl group to form D3017 was NADPH-dependent and therefore probably cytochrome P450-mediated, whereas the formation of 2,6-DMA was NADPH-independent, inhibited by BNPP, and probably catalyzed by amidaise enzymes. In human and rat liver microsomes, the formation of D3017 was the major route of metabolism of D2624, whereas 2,6-DMA formation was minor.

The in vitro human microsomal data suggested that D2624 would undergo extensive hepatic metabolism to D3017. Although the enzyme(s) responsible for the formation of D3017 were not identified and the Michaelis-Menten parameters not determined, the in vitro formation velocities of D3017 and 2,6-DMA seem to be in agreement with the in vivo findings [i.e. D3017 was present in plasma in high concentrations, whereas 2,6-DMA was detected in plasma at low concentrations (nM) and only at the highest two doses]. In general, 2,6-DMA concentrations were <3% of those of D3017 and suggest species differences in the oxidative and hydrolytic pathways of D2624. The detection of 2,6-DMA in human plasma and the very short half-life of the active metabolite (D3017) suggest that D2624 does not possess optimal characteristics for an antiepileptic compound. However, these studies have provided an insight into the metabolic fate and pharmacokinetics of a representative N-aryl isoxazolcarboxamide that should prove useful in the assessment of other candidate drugs from this structural class.

References