Tocainide Conjugation in Humans: Novel Biotransformation Pathway for a Primary Amine

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Abstract □ The metabolism of tocainide, an experimental antiarrhythmic drug, was studied in humans. Urinary excretion of unchanged drug was 28–55% in 24 hr after oral dosing. Urine hydrolysis with hydrochloric acid or β-glucuronidase increased tocainide recovery to 55–79%. Saccharo-1,4-lactone inhibited the β-glucuronidase-mediated tocainide recovery increase. Adjustment of urine to pH 13 produced a compound identified as 3-(2,6-xylyl)-5-methylhydantoin. Evidence suggests that it was derived from the same metabolite that formed the additional tocainide after acid or β-glucuronidase treatment. Tocainide carbamoyl *O*-β-D-glucuronide is the structure proposed for the metabolite. The suggested pathway for its formation involves the addition of carbon dioxide to the amino nitrogen of tocainide followed by uridine diphosphate glucuronic acid conjugation.

Keyphrases □ Tocainide—human metabolism, conjugation □ Antiarrhythmic agents—tocainide, human metabolism, conjugation □ Metabolism—tocainide in humans, conjugation

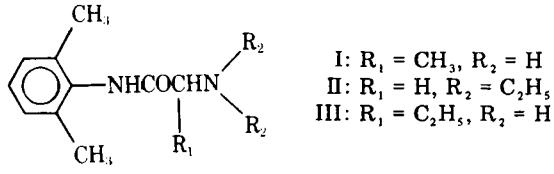
Tocainide, 2-amino-2′,6′-propionoxylidide (I), an analog of lidocaine (II), is an experimental antiarrhythmic drug undergoing clinical evaluation (1–6). Several studies of tocainide pharmacokinetics have been reported (2, 7, 8). In a human pharmacokinetic study, 90–100% bioavailability and 40% excretion of unchanged drug in the urine were reported (7). A novel metabolic pathway for the conjugation of this primary amine is described in this report.

EXPERIMENTAL

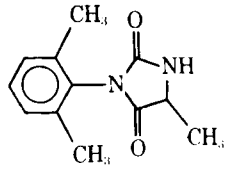
Chemicals—Tocainide (I), 3′,4′,5′-trideuterotocainide, and 2-aminobutyro-2′,6′-xylidide (III) were prepared as the hydrochloride salts by standard methods[1] (9).

Synthesis of 3-(2,6-xylyl)-5-methylhydantoin (IV) was carried out using a modification of a literature method (10). DL-Alanine ethyl ester hydrochloride was treated with phosgene in toluene to produce ethyl α-isocyanatopropionate, which then was treated with 2,6-xylidine. Cyclization was accomplished by treating the intermediate with 5 *N* NaOH. The crude product was purified by high-pressure liquid chromatography (HPLC). Compound IV was obtained as colorless crystals, mp 138.5–140.5°; NMR[2] ($CDCl_3$): δ 1.47 (d, J = 6.5 Hz, CH_3), 2.19, 2.17 (2s, 2 CH_3), 4.16 (q, J = 6.5 Hz, CH), and 7.16 (broad s, 3 aromatic, NH); electron-impact mass spectrometry[3]: 218 (M^+), 203 ($M - CH_3$), 157, 148, 147 (base peak), 132, 119, 113 (hydantoin ring), 105 (2,6-dimethylphenyl), 91, 77, 70, and 44; IR[4] (KBr): λ_{max} 3235 (NH), 1775 (C=O), 1703 (C=O), 1407, 1324, 1178, and 771 (3 adjacent H's) cm^{-1}.

Heptafluorobutyrylimidazole[5], mollusk β-glucuronidase (lyophilized; <0.7% arylsulfatase), and saccharo-1,4-lactone[6] were obtained from commercial sources. All other reagents were spectral or HPLC grade.



I: $R_1 = CH_3$, $R_2 = H$
II: $R_1 = H$, $R_2 = C_2H_5$
III: $R_1 = C_2H_5$, $R_2 = H$



IV

Mass Spectrum[3]—GLC–mass spectrometry conditions were: glass column, 1.8 m × 2 mm i.d., 3% JXR on 100–120-mesh Gas Chrom Q[7]; carrier gas, helium at 30 ml/min; injector temperature, 250°; column temperature, programmed from 150 to 250° at 8°/min; separator temperature, 250°, transfer line temperature, 225°; ion source temperature 125°; vacuum, 5×10^{-6} torr; ionization energy, 70 ev; emission current, 0.3 mamp; and electron multiplier, 2.5 kv.

Determination of Tocainide in Biological Fluids—Samples were extracted using an aliquot of urine or plasma, 1 ml of the internal standard (III, 5 μg/ml), 1 ml of 1 *N* NaOH, distilled water to bring the solution to a total volume of 5 ml, and 5 ml of methylene chloride. The solution was mixed for 10 min and centrifuged at 2000 rpm, and the aqueous layer was discarded. The organic layer was transferred to a clean tube. Heptafluorobutyrylimidazole, 20 μl, was added for derivatization, and the sample was evaporated to dryness in a 45° water bath. The dry residue was washed with 2 ml of methylene chloride and 1 ml of 0.1 *N* NaOH. The aqueous layer was discarded, and the methylene chloride was evaporated. Ethyl acetate, 20 μl, was added, and 1 μl was injected into a gas–liquid chromatograph equipped with a flame-ionization detector[8].

An electron-capture detector[9] also was used, in which case ethyl acetate was substituted for the methylene chloride in the final wash without evaporation. Concentration was determined by measuring the peak height ratio of drug to internal standard and relating this ratio to a calibration curve (0.5–20 μg).

The monoheptafluorobutyryl derivative was stable for 24 hr. The GLC conditions were: glass column, 1.8 m × 2 mm i.d., 3% OV-17 on 80–100-mesh Gas Chrom Q[7] or 3% PC-3210 Ultraphase on 80–100-mesh Chromosorb WHP[5]; and carrier gas, helium at 30 ml/min. The injector, column, and detector temperatures were 250, 205, and 275°, respectively, for the OV-17 column and 220, 165, and 210° for the PC-3210 column.

In addition, tocainide and IV were assayed by HPLC. The liquid chromatograph consisted of a high-pressure pump[10], a valve and a 25-μl loop injector[11], a 30 × 0.39-cm octadecylsilane reversed-phase column[12], and a variable-wavelength detector[13] set at 205 nm. The mobile phase consisted of 25% acetonitrile in 0.05 *M* $NaClO_4$ at pH 4.0 with a 2-ml/min flow rate.

A single extraction was used as described except for the heptafluorobutyryl derivatization prior to evaporation. The dry sample was dis-

[1] E. W. Byrnes, P. D. McMaster, H. S. Feldman, G. H. Kronberg, B. H. Takman, and P. A. Tenthorey, to be published.
[2] NMR spectra were recorded on a Perkin-Elmer model R-20 with tetramethylsilane as the internal standard.
[3] Electron-impact mass spectra were obtained on a quadrupole Finnegan 1015-D.
[4] IR spectra were obtained on a Perkin-Elmer 25 grating IR spectrometer.
[5] Pierce Chemical Co., Rockford, Ill.
[6] Calbiochem, La Jolla, Calif.
[7] Applied Science Laboratories, State College, Pa.
[8] Varian 2700, Palo Alto, Calif.
[9] Varian 3700 with a ^{63}Ni-detector, Palo Alto, Calif.
[10] Model M6000A, Waters Associates, Milford, Mass.
[11] Model CV-6-μHPa-N60, Valco Valve, Houston, Tex.
[12] μBondapak C_{18}, Waters Associates, Milford, Mass.
[13] Model SF 770, Schoeffel, Westwood, N.J.

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Table I—Tocainide Recovery before and after Hydrolysis

Subject	Daily Tocainide Hydrochloride Dose, mg	Percent Tocainide Dose Excreted Unchanged	Released by Acid Hydrolysis	Released by β-Glucuronidase
PS[a]	3200	55	19	15
SK[a]	2400	48	31	30
MB[a]	2400	47	21	15
DL[b]	300	37	23	—
NL[a]	1800	28	30	27
WC[a]	2400	42	16	28

[a] Chronic administration, 24-hr urine collection. [b] Single dose, 24-hr urine collection.

solved in 200 μl of the mobile phase, and 25 μl was injected. Urine also was diluted 10-fold with distilled water and injected directly. Tocainide, III, and synthetic IV eluted at 4.3, 5.3, and 7.4 min, respectively.

Isolation of IV from Urine—A urine aliquot was adjusted to pH 9 with sodium hydroxide and extracted five times with equal volumes of methylene chloride to remove the tocainide. The pH was adjusted to 13 by the addition of sodium hydroxide, leading to the formation of IV. Hydrochloric acid was used to return the pH to 9, and the sample was extracted with methylene chloride. Preparative HPLC[14] was used to purify the crude IV. The other chromatographic conditions included a 1.0-ml injection loop[11], a 1.2-m × 0.95-cm porous silica column[15] (37–75-μm particles), and a 1% ethanol in chloroform mobile phase. The column was purged thoroughly with methanol each morning before use.

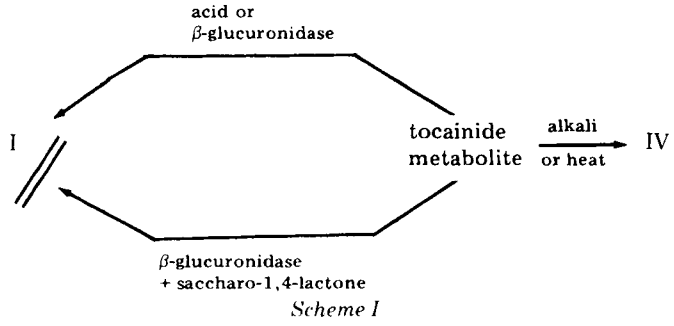
Drug Administration—Twenty-four-hour urine collections were obtained from five patients (two females and three males, ages 40–60) undergoing long-term treatment with tocainide hydrochloride. Individual oral doses ranged from 1.8 to 3.2 g/day in divided doses. A male volunteer received a single 300-mg oral dose of tocainide hydrochloride following an overnight fast. A second volunteer received a 300-mg dose as a 50:50 (w/w) mixture of tocainide and 3′,4′,5′-trideuterotocainide. Twenty-four-hour urine samples were collected from both volunteers.

Urine Hydrolysis—The acid hydrolysis involved mixing of 5.0 ml of urine with 5.0 ml of 4 *N* HCl, followed by incubation for 1 hr at 100°. An aliquot was taken for tocainide analysis by GLC and HPLC after cooling.

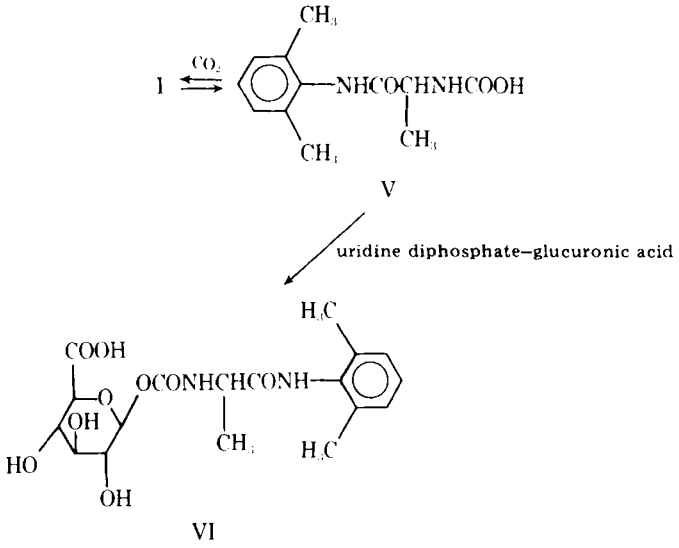
For the enzyme hydrolysis, a 1.0-ml urine aliquot adjusted to pH 4.0 with 0.5 *N* acetic acid was incubated with β-glucuronidase (pH 4, 25,000 Fishman units) in a water bath at 37°. After 12 hr, an additional 25,000 units of β-glucuronidase was added, and the incubation was continued for another 12 hr. Aliquots were assayed for tocainide as described. An identical set of samples was subjected to enzyme hydrolysis with 2 mg of saccharo-1,4-lactone, a specific inhibitor of β-glucuronidase, added with each addition of enzyme.

RESULTS AND DISCUSSION

Either acid hydrolysis or treatment with β-glucuronidase increased the tocainide amount recovered from the urine (Table I). Based on the five patients who received multiple tocainide doses, 44% of the oral dose was excreted as free tocainide. An acid or β-glucuronidase hydrolyzable metabolite accounted for an additional 23% of the dose. The excretion



Scheme I



Scheme II—Metabolic pathway for tocainide uridine diphosphate–glucuronic acid conjugation.

pattern for the volunteer (Subject DL) who received a single dose was similar.

The basis for elucidating the metabolite structure is described in Scheme I. There was essentially complete recovery of the free tocainide with the five methylene chloride extractions at pH 9.0. These methylene chloride extracts did not contain IV. The remaining aqueous phase was adjusted to pH 13 and then readjusted to pH 9 prior to a methylene chloride extraction. This methylene chloride extract contained a significant amount of IV but not tocainide. Typically, the urine tocainide concentrations ranged from 200 to 600 μg/ml, and the IV concentrations were approximately half of that. These concentrations indicated that IV was formed at pH 13, that it could be extracted at pH 9, and that it was not derived directly from tocainide.

Direct injection of urine onto the HPLC column confirmed the absence of IV in the untreated urine. However, direct injection of urine, from which free tocainide had been removed, onto the GLC column resulted in a peak for IV, demonstrating its thermal formation. After acid hydrolysis or β-glucuronidase treatment of the urine, it was not possible to generate IV at pH 13. Furthermore, after extraction of IV from the alkaline urine, acid hydrolysis of the remaining urine did not yield additional tocainide.

The synthetic IV and the IV isolated from urine had identical retention times upon GLC analysis. Further confirmation of identity was obtained by IR, NMR, mass spectral, mixed melting-point, and elemental analyses. The mass spectrum of IV for the subject who had ingested tocainide with 3′,4′,5′-trideuterotocainide exhibited doublets with each ring fragment, confirming that IV arose from ingested tocainide.

The formation of tocainide upon acid- or β-glucuronidase-catalyzed hydrolysis suggested that the metabolite was a glucuronide conjugate of tocainide or of a labile compound that easily formed tocainide. The presence of a glucuronide conjugate was confirmed when simultaneous treatment with saccharo-1,4-lactone prevented enzyme-catalyzed tocainide release. For example, the tocainide concentrations in untreated urine, β-glucuronidase-treated urine, and urine treated with β-glucuronidase and saccharo-1,4-lactone were 199, 395, and 209 μg/ml, respectively. This specific inhibitor of β-glucuronidase was used since commercial β-glucuronidase preparations often contain other hydrolytic enzymes. Also, preliminary electrophoretic studies[16] indicated that the metabolite was negatively charged at pH 4.6 and neutral at pH 2.2. The metabolite on the electrophoretogram was identified by treatment with alkali, resulting in IV, and by acid hydrolysis, resulting in tocainide.

The simplest interpretation of these data was to hypothesize an *N*-glucuronide of tocainide for the metabolite structure. However, the formation of IV from the metabolite under strongly basic conditions or upon thermal decomposition could not be reconciled with the *N*-glucuronide structure.

An extra carbonyl group clearly was present in IV that was not present in tocainide. Compound IV definitely was a product of the metabolite

[14] Model ALC 202/401, Waters Associates, Milford, Mass.
[15] Porasil B, Waters Associates, Milford, Mass.
[16] Electrophoretic studies were conducted as described previously (11) using a HV-5000A power supply and FP-30S flat plate (Savant Instruments, Hicksville, N.Y.).

since tocainide did not produce IV when treated with alkali or heat. The explanation is that a carbonyl group is present in the metabolite between the amino nitrogen and the glucuronide moiety.

A proposed reaction for metabolite formation *in vivo* is presented in Scheme II. Based on the proposed metabolite structure, the data can be explained easily. Acid or enzyme hydrolysis initially would yield the carbamic acid (V), which would then easily generate tocainide. A carbamic acid is in a constant and rapid equilibrium with an amine. A change in the pH or the carbon dioxide–bicarbonate concentration can easily shift the equilibrium. Conjugation of V with glucuronic acid produces the proposed metabolite (VI), tocainide carbamoyl *O*-β-D-glucuronide. This structure also provides a reasonable explanation for the facile conversion to IV by attack of the amide nitrogen on the carbonyl carbon with displacement of the glucuronide moiety as a leaving group. The uncharged species at pH 2.2 found in the electrophoretic studies also can be explained since the inclusion of the carbonyl group would result in a loss of basicity of the nitrogen atom.

The reaction of tocainide with carbon dioxide has ample precedent in the transport mechanism for carbon dioxide by hemoglobin; hemoglobin carbamic acid accounts for ~12% of the carbon dioxide transported by the blood of resting humans (12). Glycine and glycylglycine with pKa values of ~8.0 often have been used as models for the reaction of hemoglobin with carbon dioxide (12); tocainide has a pKa of 7.8. Among other factors, the degree to which any amine combines with carbon dioxide will depend on its pKa, the pH, and the carbon dioxide concentration (12).

In summary, a pathway for the conjugation of an amine has been proposed. It was fortunate that tocainide carbamoyl *O*-β-D-glucuronide yielded a characteristic degradation product (IV). Whether this pathway applies to amines other than tocainide can be resolved only by further research.

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Secondary Products of Itanoxone

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Abstract □ Itanoxone synthesis by Friedel–Crafts reaction between itaconic anhydride and 2-chlorobiphenyl was studied. Five isomers corresponding to possible impurities were prepared and studied to perfect a reliable and practical method to detect these impurities in itanoxone.

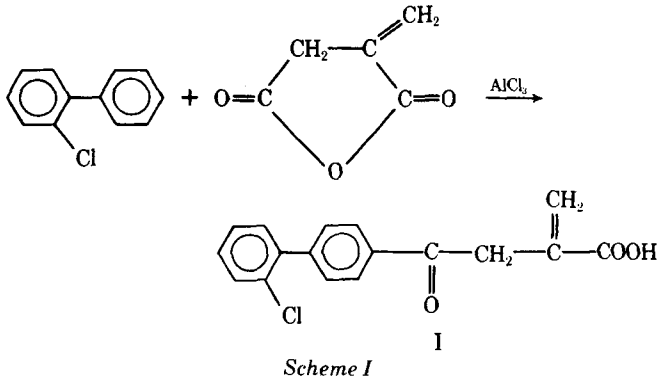
Keyphrases □ Itanoxone—synthesis, synthesis and detection of impurities □ Hypolipidemic agents—itanoxone, synthesis, synthesis and detection of impurities □ Hypouricemic agents—itanoxone, synthesis, synthesis and detection of impurities

The Friedel–Crafts reaction between itaconic anhydride and an aromatic derivative was reported (1–3). This reaction was adapted to many aromatic substrates and led to the synthesis of a chemical series with interesting pharmacological properties (4, 5). Pharmacological and toxicological studies yielded a new compound, 4-[4′-(2-chlorophenyl)phenyl]-4-oxo-2-methylenebutanoic acid (F 1379) (I), whose International General Designation is itanoxone. Compound I is a powerful hypolipidemic and hypouricemic agent (6–8).

The impurities that might be found in I were defined, isolated, or synthesized. With both chemical and physicochemical data of secondary products and samples, a detection method for these impurities in the end-product was investigated.

BACKGROUND

The Friedel–Crafts reaction between itaconic anhydride and 2-chlorobiphenyl gave I as the main product as reported previously (4, 5) (Scheme I).



I

Scheme I

0022-3549/80/0100-0049$01.00/0