TERTIARY N-GLUCURONIDES OF CLOZAPINE AND ITS METABOLITE DESMETHYLCLOZAPINE IN PATIENT URINE

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ABSTRACT:

In experiments with expressed human UDP-glucuronosyltransferase 1A4 (UGT1A4), the antipsychotic clozapine proved to be conjugated to two different glucuronides, one of which was identified as the quaternary ammonium glucuronide derivatized at the N-methylpiperazine group; this compound had previously been isolated from patient urine. An additional glucuronide produced in larger quantity was assumed to be conjugated at the secondary nitrogen of the central ring to form 5-N-glucuronide, but this was not proven. The analogous olanzapine 10-N-glucuronide was found to make a major contribution to urinary metabolites in human volunteers. In the present investigation, tertiary 5-N-glucuronides were isolated from incubations of clozapine and desmethylclozapine with human liver microsomes fortified with UDP-glucuronic acid, and their structures were confirmed by mass and \(^1\)H NMR spectrometry. The same conjugates could also be purified from patient urine. Their approximate quantities in urine from four patients ranged between 0.1 and 0.5% of the dose, as did those of the quaternary amonium glucuronide of clozapine. Analogous to olanzapine 10-N-glucuronide, the tertiary clozapine 5-N-glucuronide was resistant toward enzymatic hydrolysis but was labile under acidic conditions.

The atypical antipsychotic clozapine is known to undergo a large number of biotransformation reactions in humans. Most of these are oxidative and comprise N-demethylation, N-oxidation (Gauch and Michaels, 1971), aromatic hydroxylation, and exchange of aromatically bound chlorine against hydroxy or methylthio groups (Stock et al., 1977; Dain et al., 1997; Schaber et al., 2001). In addition, analyses of patient urine and experiments in vitro have revealed the possibility of direct conjugation of clozapine at nitrogen atoms. Luo et al. (1994) succeeded in isolating from patient urine clozapine N\(^{+}\)-glucuronide, the quaternary ammonium glucuronide resulting from conjugation at the N-methylpiperazine group. Its mass spectrum was identical with that of a synthetic reference compound. The same glucuronide was the minor product when clozapine was incubated with expressed human UGT1A4 in the presence of UDP-glucuronic acid (Green and Tephly, 1996). For the major conjugate, structural proof was not possible, but glucuronic acid attachment at the secondary nitrogen (N-5) of the central ring was suggested because an analogous conjugate was not formed from loxapine in which the secondary nitrogen is lacking. The assumption became more probable when the biotransformation of olanzapine, an atypical antipsychotic closely related to clozapine, was investigated in human volunteers (Kassahun et al., 1997). In urine and feces, a tertiary N-glucuronide, 10-N-glucuronide, was found to be a major metabolite, whereas a quaternary ammonium glucuronide represented a smaller fraction of the dose. The authors reported that the quaternary, but not the tertiary N-glucuronide, could be hydrolyzed enzymatically, whereas the latter was acid-labile.

The present experiments were performed to answer the question whether glucuronidation at the secondary nitrogen atom plays a part in the metabolic fate of clozapine in vivo. For this purpose, unambiguous identification of the tertiary 5-N-glucuronides of clozapine and desmethylclozapine was achieved by their isolation from incubates with human liver microsomes and patient urine and measurement of their mass and NMR spectra. A procedure was developed for the quantitation of the tertiary and quaternary N-glucuronides in patient urine, and the susceptibility of clozapine 5-N-glucuronide toward enzymatic and acid hydrolysis was investigated.

Experimental Procedures

Substances. Clozapine and N-desmethylclozapine were kindly provided by Novartis (Basel, Switzerland). The disodium salt of UDP-glucuronic acid and \(\beta\)-glucuronidase from \(E.\ coli\) K 12 were purchased from Roche Diagnostics (Mannheim, Germany). \(\beta\)-glucuronidase from \(Helix\ pomatia\) was obtained from Sigma (Deisenhofen, Germany). Clozapine N\(^{+}\)-glucuronide was prepared by a modification (Schaber et al., 2001) of the procedure of Luo et al. (1992).

Preparation of N-Glucuronides by Microsomal Incubation. Human liver microsomes prepared by conventional methodology (Breyer-Pfaff and Nill, 1995) were incubated for 1 h at 37°C at 1 mg/ml with (final concentrations) 25 mM saccharose, 50 mM Tris-HCl, pH 8.0, 10 mM MgCl\(_2\), 2 mM UDP-glucuronic acid, and 0.5 mM clozapine or desmethylclozapine in a total volume of 3 ml. After cooling, protein was precipitated with 0.3 ml of 150 mM barium hydroxide, followed by 0.3 ml of 150 mM zinc sulfate. From the supernatant, unreacted drug was largely removed by extraction with 1 and 2 ml of tert-butyldimethyl ether in the case of clozapine and by three extractions with 3 ml in the case of desmethylclozapine. Following extraction with 1 ml of hexane, the aqueous solution was passed through a cartridge with 100 mg of C\(_{18}\)-silica gel (Bond Elut; Analytichem International, Harbor City, CA). After
washing with 1.5 ml of water, adsorbed compounds were eluted with 1.5 ml of methanol and 1.5 ml of methanol/25% ammonia (9:1, v/v). The combined organic phases were evaporated, and the residue was subjected to thin layer chromatography in 1-butanol/acetone/25% ammonia/water (2:5:2:5:1:0.5, v/v) on sheets precoated with silica gel with a fluorescent indicator (Alugram Sil G/UV254, Macherey-Nagel, Düren, Germany). When clozapine had been incubated, a weak UV-absorbing band was present at Rf 0.31 (identical with the Rf of clozapine N'-glucuronide) and a stronger band at Rf 0.52 assumed to contain the tertiary N-glucuronide. In extracts from incuba-
tions of desmethyl-clozapine, the substrate was present at Rf 0.77, and an additional band probably containing N-glucuronide was present at Rf 0.46. The glucuronide bands were removed and extracted three times with 2 ml of methanol. The extract residues were homogeneous in HPLCC; for NMR and mass spectrom-
etry, the extracts were dissolved in water, adsorbed on C18-silica gel cartridges, eluted with methanol, and evaporated.

For an estimate of the quantity formed, the UV spectrum of an aliquot of the purified compound was measured in methanol and compared with that of clozapine at a defined concentration. The quantities per milliliter of incubate purified compound was measured in methanol and compared with that of clozapine 5'-N-glucuronide was found at Rf 0.46. The glucuronide

**Isolation of Tertiary N-Glucuronides from Urine.** Urine was obtained from a male patient of 31 years who received 400 mg/day clozapine as a monotherapy and had serum concentrations of 331 ng/ml clozapine and 283 ng/ml desmethylclozapine (Rf 0.22) from microsomal incubations. The combined aqueous phase containing polar compounds were incubated at pH 7 for 2 or 8 h, respectively, and served as controls, with the small clozapine or desmethy-
ilclosupronine quantities measured in them being subtracted from those in hy-
drolysed samples. Recovery experiments were carried out by adding about 1.5 nmol of one of the N-glucuronides to 10 ml of urine from drug-free volunteers. The samples were processed in the same way as those from patients in parallel or along with reference samples of N-glucuronides in which the contents of the conjugates were measured without initial solid-phase extraction. Reference solutions of tertiary N-glucuronides were initially extracted with tert-butylmethylether at alkaline pH to remove unconjugated compounds originating from spontaneous hydrolysis upon storage. Recoveries were 93 to 102% (mean 97%) for clozapine 5-N-glucuronide, 88 to 107% (mean 98%) for its desmethyl analog, and 93 to 98% (mean 96%) for clozapine N'-glucuronide (n = 3–4). Due to the imprecision of standardization by UV spectrometry and to the lability of the tertiary N-glucuronides, data measured in urine have to be regarded as semiquantitative with an error up to 20%.

**Enzymatic and Acid Hydrolysis.** The pH stability of clozapine 5-N-glucuronide was investigated by incubating 5 μM solutions in buffers composed of 20 mM each of acetic acid, MES, potassium dihydrogen phosphate, Tris, and glycine and adjusted to pH 4.5 to 7 with HCl or NaOH. Aliquots were drawn after 2 h and directly injected for HPLC analysis in system 2.

For enzymatic degradation, aqueous solutions of 1.5 to 2 nmol clozapine 5-N-glucuronide or clozapine N'-glucuronide were incubated with about 2000 Fishman units/ml of β-glucuronidase from *H. pomatia*, pH 6 or 7, in a total volume of 0.24 to 0.7 ml, aliquots being drawn after 1 to 4 h. Analogous experiments were performed with 2 U/0.5 ml of β-glucuronidase from *E. coli*, pH 7, within up to 8 h. The aliquots of 0.1 to 0.2 ml were alkalized with 0.05 ml of 2 N ammonia and twice extracted with 0.7 ml of tert-butylmethylether. The residue of the organic phases was dissolved in 0.1 ml of eluent for HPLC system 2.

**HPLC Analyses.** System 1 comprised a 250-× 4.6-mm column with C18-silica gel (Prodigy 5 μm ODS; Phenomenex, Hönshausen, Germany), the eluate being monitored at 290 nm and data being registered by the MT2 integration program (Kontron, München, Germany). The eluant was 50 mM ammonium acetate/acetonitrile (80:20, v/v) 1 ml/min for clozapine 5-N-glucuronide (kT, 9.3 min) and desmethyloclozapine 5-N-glucuronide (kT, 3.2; RF, 9.3 min). The quantitation of clozapine (kT, 11.3; RF, 23.3 min) and desmethyloclozapine (kT, 7.9; RF, 17 min) was based on peak areas and per-
formed with 10 mM perchloric acid adjusted to pH 2.5 with NaOH/acetonitrile (72/28, v/v) 1 ml/min as the eluent.

In system 2, a 200-× 4.6-mm column filled with C18-silica gel 5 μm (Nucleosil 5 C18; Macherey-Nagel) was run with 0.02 M ammonium acetate, 0.9 M acetic acid (pH 3.0)/methanol (50:50, v/v) 1 ml/min. The absorption of the eluate was measured at 290 nm, and peak heights served for quantitation of clozapine 5-N-glucuronide (kT, 2.3; RF, 7.5 min) and clozapine (kT, 4.2; RF, 11.8 min). Desmethyloclozapine and its N-glucuronide had kT values of 3.1 and 1.9 (RF, 9.3 and 6.5 min), respectively.

**Mass Spectrometry.** A TQ 700 triple quadrupole mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) and Finnigan acquisition software were used for spectra in the electrospray ionization (ESI) and collision-induced dissociation (CID) modes. The samples were dissolved in methanol/water (9:1) to concentrations of 10 to 20 mg/ml and infused into the ion source via a syringe pump at a flow rate of 1.5 μl/min. The electrospray needle voltage was 4500 V. The temperature of the heated transfer capillary was set to 120°C. Sheath gas was nitrogen. Spectra were acquired over the mass range 100 to 800 amu in 2 s during an acquisition time of 1 min. In the CID mass spectrometry mode, argon was used as collision gas. The collision cell pressure was 1.9 to 2.1 mtorr, and the collision offset voltage was -29 eV. The scan range was 20 to

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1 Abbreviations used are: HPLC, high-pressure liquid chromatography; MES, 4-morpholineethanesulfonic acid; ESI, electrospray ionization; CID, collision-in-
duced dissociation; amu, atomic mass unit; MS, mass spectrosopy.
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TABLE 1

<table>
<thead>
<tr>
<th>Clozapine δ</th>
<th>Clozapine N°-Glucuronide</th>
<th>Clozapine 5-N-Glucuronide</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppm</td>
<td>ppm</td>
<td>Hz</td>
</tr>
<tr>
<td>H-1</td>
<td>7.27 d</td>
<td>7.34 d dd</td>
</tr>
<tr>
<td>H-2</td>
<td>7.03 t</td>
<td>7.07 dt</td>
</tr>
<tr>
<td>H-3</td>
<td>7.36 t</td>
<td>7.38 dt</td>
</tr>
<tr>
<td>H-4</td>
<td>7.00 d</td>
<td>7.01 dd</td>
</tr>
<tr>
<td>H-6</td>
<td>6.81 d</td>
<td>6.82 d d</td>
</tr>
<tr>
<td>H-7</td>
<td>6.85 dd</td>
<td>6.89 dd</td>
</tr>
<tr>
<td>H-9</td>
<td>6.97 d</td>
<td>6.99 d</td>
</tr>
<tr>
<td>H-12/15 eq</td>
<td>3.43 s broad</td>
<td>3.62–3.78 m</td>
</tr>
<tr>
<td>H-13/14 eq</td>
<td>2.54 s broad</td>
<td></td>
</tr>
<tr>
<td>H-13/14 ax</td>
<td>4.14 t and 3.96 m si*</td>
<td>9.5</td>
</tr>
<tr>
<td>H-15/15 eq</td>
<td>3.78–3.90 si and 3.95 si</td>
<td></td>
</tr>
<tr>
<td>H-16</td>
<td>2.33 si</td>
<td>3.3 si</td>
</tr>
<tr>
<td>H-1'</td>
<td>4.96 d</td>
<td>8.9</td>
</tr>
<tr>
<td>H-2'</td>
<td>3.83 t</td>
<td>8.6</td>
</tr>
<tr>
<td>H-3'</td>
<td>3.48 t si</td>
<td></td>
</tr>
<tr>
<td>H-4'</td>
<td>3.55 t</td>
<td>8.5 + 8.9</td>
</tr>
<tr>
<td>H-5'</td>
<td>3.83 d</td>
<td>9.7</td>
</tr>
</tbody>
</table>

si, superimposed; d, doublet; dd, double doublet; s, singlet; t, triplet; dt, double triplet; m, multiplet.

Results

When human liver microsomes were incubated with 0.5 mM clozapine for 1 h, about 1% was converted to the quaternary ammonium glucuronide and about 4% to a metabolite presumed to be the 5-N-glucuronide. Desmethylclozapine was conjugated by about 1%. The UV spectra of all N-glucuronides in methanol resembled those of clozapine in water or methanol. Therefore, an alteration of the chromophore by substitution of the tertiary N-10 in the diazepine ring was not probable.

Distinct differences were apparent between the fragmentation patterns in CID mass spectrometry of clozapine 5-N-glucuronide and clozapine N°-glucuronide (Fig. 1). In the latter, [M + H]⁺ with m/z 503 for the 35Cl isotope showed the four parallel fragmentations described previously (Schaber et al., 2001), which involve a loss of 100 amu (N-methylpiperazine) that must be preceded by an intramolecular rearrangement. A degradation of the piperazine ring by a loss of 57 amu took place from the base ion m/z 327 (clozapine) only but not from the protonated molecular ion. In contrast, such a degradation did occur with clozapine 5-N-glucuronide, resulting in m/z 446 in parallel to eliminations of two H₂O resulting in m/z 485 and 467, of 92, 134 (fragmentation processes of the glucuronosyl residue), and 176 amu (glucuronic acid – H₂O). Identical ESI-MS and MS/MS spectra were obtained with the tertiary glucuronides isolated from microsomal incubates and from patient urine (Fig. 1).

Desmethylclozapine 5-N-glucuronide from microsomes showed [M + H]⁺ 489, which in MS/MS lost two H₂O, 92, 134, and 176 amu, as did the clozapine analog. The ESI-MS and MS/MS spectra of the compounds produced by microsomes and excreted by a patient were identical (Fig. 2).

In 1H NMR (Table 1), the axial and equatorial protons in the piperazine ring of clozapine exhibited broad signals around 2.54 ppm (H-13 and H-14) and 3.43 ppm (H-12 and H-15) (Schaber, 1998). This is indicative of rapid equilibration of different chair forms of the piperazine ring. In contrast, one of these conformations is fixed in clozapine N°-glucuronide, which can be concluded from the signals of equatorial protons (H-13 and H-14) at 4.14 ppm (triplet, J = 9.5 Hz; broad, 1 H) and 3.96 ppm (multiplet, broad, superimposed, 2 H, including one H-13/14 eq). The aromatic protons of clozapine N°-glucuronide were minimally influenced by the glucuronosyl residue, and no basic N-CH₃ group was detectable. The HH rotating frame nuclear Overhauser enhancement spectroscopy spectrum showed coupling of the anomic proton H-1’ of the glucuronosyl group with other glucuronide protons and with those of the piperazine ring, but none with aromatic protons.

In clozapine 5-N-glucuronide, the piperazine ring protons H-13 and H-14 formed two broad singlets at 2.52 and 2.62 ppm, whereas H-12 and H-15 were probably present at 3.47 and 3.57 ppm, but with superposition. Of the glucuronosyl protons, only H-1’ could be assigned to a doublet at 4.61 ppm (J = 8 Hz) and a broad singlet at 4.52 ppm (integrated intensities together corresponding to 1 H). The other signals were extremely broad between 3.35 and 3.85 ppm. The signals of H-4 and H-6 were shifted down-field by 0.8 and 1.0 ppm, respectively, relative to those in clozapine. Together with the unchanged signals of a basic N-CH₃ group at 2.35 ppm and those of the CH₂ groups in the piperazine ring, this indicates that the zwitterion is probably protonated at N-10 with the possibility of mesomeric structures carrying a positive charge at N-5 (Fig. 3). The resonances of all aromatic protons were broadened, pointing to relatively slow inversion of the seven-membered ring. Consequently, signals generated by the glucuronosyl protons stem from at least three conformers and those of the piperazine ring protons from two times two conformers. The structures confirmed for the 5-N-glucuronides of clozapine and desmethylclozapine are shown in Fig. 3.

When clozapine N°-glucuronide was incubated with β-glucuronidase from E. coli at pH 7, the liberation of clozapine was completed within 2 h, and the quantity corresponded to that expected on the basis of UV photometry. The same applied upon incubation with the enzyme from H. pomatia for 1 or 2 h at pH 6 or 7. In contrast, no clozapine was liberated from clozapine 5-N-glucuronide by E. coli.
β-glucuronidase at pH 7 within 2 and 4 h, and deconjugation with β-glucuronidase from *H. pomatia* amounted to 1% within 2 h. The acid hydrolysis rate of clozapine 5-N-glucuronide exhibited a steep increase between pH values of 6 and 4.5 (Fig. 4). Although substrate loss at pH 7 amounted to less than 10% within 28 h, it was 33% at pH 6 in the same time and 63 and 92% within 8 h at pH 5 and 4.5, respectively. Correspondingly, estimated half-lives varied from 190 h at pH 7 to 2 h at pH 4.5. In the latter case, a similar half-life (1.6 h) resulted for the appearance of clozapine in the incubate. The hydrolysis of desmethylclozapine 5-N-glucuronide was measured at pH 4.5 only; the conjugate disappeared with a half-life of 2.4 h, and the aglycone concentration increased with a half-life of 2.1 h.

In urine samples from four patients under clozapine monotherapy, the three N-glucuronides were assayed by solid-phase extraction, separation from unpolar compounds, and enzymatic or acid hydrolysis, respectively, followed by HPLC. In each one of the samples, all three conjugates were present, although their sum only accounted for about 0.36 to 1.3% of the clozapine dose (Table 2). The lowest percentages of 5-N-glucuronides occurred in the two samples with pH 6.0, whereas higher values were measured in samples with pH values of 6.9 and 7.35.

**Discussion**

In the present investigation, the structure of the major clozapine conjugate produced in human liver microsomes in the presence of UDP-glucuronate was shown to be that of the 5-N-glucuronide. It can be supposed to be identical with "clozapine metabolite I" formed on incubation with expressed UDP-glucuronosyltransferase 1A4 (Green and Tephly, 1996). This enzyme concomitantly produced clozapine N-glucuronide at about a 5-fold lower rate in agreement with the present findings in liver microsomes. Green and Tephly had also observed glucuronidation of desmethylclozapine, and this could now be demonstrated to occur at N-5 of the seven-membered ring. None of the tertiary N-glucuronides had previously been detected in human urine. In the investigation by Schaber et al. (2001), this was due to the occurrence of acidic conditions in the work-up procedure, which lead to rapid hydrolysis of the 5-N-glucuronides. For an identification by a single HPLC separation (Dain et al., 1997), excreted quantities are

![Fig. 1. ESI-MS/MS spectra of clozapine N-glucuronide (A) and of clozapine 5-N-glucuronide from microsomal incubates (B) and from patient urine (C).](image1)

![Fig. 2. ESI-MS/MS spectra of desmethylclozapine 5-N-glucuronide from microsomal incubates (A) and from patient urine (B).](image2)

![Fig. 3. Structural formulas of clozapine N-glucuronide and of the 5-N-glucuronides of clozapine and desmethylclozapine.](image3)
N-glucuronides of clozapine and desmethyloclozapine

Probably too small. In contrast, the most abundant metabolite of olanzapine in urine and feces of volunteers given a single dose was the 10-N-glucuronide, which corresponds to clozapine 5-N-glucuronide. Its formation was estimated to account for 21 to 25% of the dose, with smaller quantities appearing as the quaternary N-glucuronide (Kassahun et al., 1997). This difference between the two structurally related drugs may be due to the predominance of oxidative attack in clozapine at the chlorine-substituted aromatic ring (Stock et al., 1977; Dain et al., 1997; Schaber et al., 2001) that is missing in olanzapine. Alternatively, the structure of olanzapine is favorable for glucuronidation at the secondary nitrogen of the central seven-membered ring. The two drugs are examples for regioselective N-glucuronidation reactions with tertiary and quaternary conjugates being produced in parallel in humans. The first observations on regioselectivity in N-glucuronide formation were made when substituted triazoles were incubated with human liver microsomes, and isomeric tertiary N-glucuronides were detected (Huskey et al., 1994).

The tertiary N-glucuronides of clozapine and olanzapine not only differ by their quantities excreted but apparently also by their acid stability. Incubation of plasma with an equal volume of 1 or 2 N HCl for 1 h at 50°C was required for 60 to 75% hydrolysis of olanzapine 10-N-glucuronide, whereas the corresponding clozapine glucuronide was decomposed to the same degree within 3 h at pH 4.5 and 37°C. This lability toward acidic conditions may be one of the reasons for the low percentage of the clozapine dose represented by urinary 5-N-glucuronide, namely 0.1 to 0.5% of the dose, the highest values being found in urine samples with higher pH values. The N-glucuronide, which was formed in vitro at markedly lower rates, represented 0.15 to 0.32% of the dose in urine, and an amount of 3% of the dose was reported for feces (Dain et al., 1997). In view of the higher formation rate of the tertiary N-glucuronide in vitro, one would have expected higher excreted quantities, but these were not found. Discrepancies between relative quantities formed in vitro and determined in urine were also observed with regard to desmethyloclozapine 5-N-glucuronide. Its urinary excretion was at least as high as that of clozapine 5-N-glucuronide, whereas its biosynthesis was much slower and its acid lability very similar.

Few examples of tertiary N-glucuronide formation from other secondary aromatic amines have been published (Chiu and Huskey, 1998). One of these is the formation of a pimobendan glucuronide, which in contrast to that of clozapine was not detected in vitro, but was among the major metabolites in human urine (Pahernik et al., 1995). Although data on the stability of the tertiary N-glucuronides (except those derived from hydroxylamines) are lacking, conjugates of primary aromatic amines are known to be very acid-labile (Green and Tephly, 1998). Glucuronidation principally is a reversible reaction in vivo, and this applies to quaternary N-glucuronides, too (Breyer-Pfaff et al., 1990). Because of their chemical stability (Mey et al., 1999; Kowalczyk et al., 2000), enzymatic hydrolysis must be the main factor. In contrast, olanzapine 10-N-glucuronide proved to be resistant toward β-glucuronidase from either E. coli or H. pomatia (Kassahun et al., 1997), and the same was observed with clozapine 5-N-glucuronide. Unless a β-glucuronidase with different substrate specificity is operative in humans, losses are probably due to the acid lability of this conjugate. These may result from uptake into intracellular compartments with low pH or following glomerular filtration when urine is acidified. The majority of clozapine liberated in kidney tubules will be reabsorbed (Schaber et al., 1998) such that formation of the tertiary N-glucuronide may initiate a futile cycle. On the other hand, since desmethyloclozapine is in balance not reabsorbed, filtration of its 5-N-glucuronide and decomposition in tubular fluid will augment the apparent tubular secretion of desmethyloclozapine (Schaber et al., 1998).

In conclusion, structural proof was obtained for tertiary N-glucuronides of clozapine and desmethyloclozapine, and it was demonstrated that, besides being synthesized in human liver microsomes, they were excreted by patients receiving clozapine. Estimation in urine was achieved by measuring aglycones liberated on acid hydrolysis of polar fractions. Acid lability steeply increased between pH values 7 and 4.5 and probably was a reason for the small percentage (0.2–1%) of the clozapine doses found in the form of tertiary N-glucuronides in patient urine. Quantities of N-glucuronides in urine do not always reflect their formation rates in liver microsomes.

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FIG. 4. Time-dependent decrease of clozapine 5-N-glucuronide concentrations during incubation at 37°C and different pH values.

TABLE 2
Approximate quantities of the 5-N-glucuronides of clozapine and desmethyloclozapine and of clozapine N-glucuronide in urine of four patients under clozapine monotherapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Urine pH</th>
<th>Clozapine Dose (mg/day)</th>
<th>5-N-Glucuronide of Clozapine</th>
<th>Desmethyloclozapine N-glucuronide</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.J.</td>
<td>m</td>
<td>34</td>
<td>6.0</td>
<td>175</td>
<td>0.11</td>
</tr>
<tr>
<td>O.M.</td>
<td>m</td>
<td>34</td>
<td>6.0</td>
<td>300</td>
<td>0.1</td>
</tr>
<tr>
<td>S.B.</td>
<td>m</td>
<td>31</td>
<td>6.9</td>
<td>400</td>
<td>0.48</td>
</tr>
<tr>
<td>E.M.</td>
<td>f</td>
<td>27</td>
<td>7.35</td>
<td>550</td>
<td>0.15</td>
</tr>
</tbody>
</table>

m, male; f, female.
spectra and to Dr. A. Ding and Dr. K. Wagner for the opportunity to use the analytical instruments.

References


