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Urinary Excretion of Amitriptyline *N*-Oxide in Humans

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Abstract □ The excretion rate of amitriptyline *N*-oxide was measured in healthy human subjects after the administration of a single oral dose of amitriptyline. The total amount of this metabolite excreted accounted for about 1% of the dose. It appears in the urine soon after administration of the parent compound but has practically disappeared by the 9th hr after treatment. The role of this metabolite in amitriptyline degradation in humans is discussed.

Keyphrases □ Amitriptyline—excretion of *N*-oxide urinary metabolite in humans □ Metabolism—urinary excretion of amitriptyline *N*-oxide in humans □ Biotransformation—urinary excretion of amitriptyline *N*-oxide in humans

Excretion of the major metabolites of amitriptyline and related drugs has been extensively studied in humans (1, 2). There exists, however, an interesting minor metabolic route, that of *N*-oxidation. The mechanism of formation, metabolism, and excretion of these *N*-oxides was investigated (3–6) both *in vitro* and *in vivo* in rats and guinea pigs. However, in humans, studies of the excretion of these compounds have, thus far, been limited to imipramine *N*-oxide (7, 8).

Because of previous work in this laboratory on amitriptyline metabolism, interest also existed in its metabolism to the *N*-oxide (9). According to Bickel and coworkers (3–6), some reasons why this minor pathway is of general interest are:

1. The natural occurrence of *N*-oxides in plant and animal tissues has posed interesting problems as to the biochemistry and function of these compounds in biological systems.

2. *N*-Oxides are metabolites of many tertiary amine drugs and have been postulated as intermediates in *N*-dealkylation.

3. Certain *N*-oxides possibly formed in mammalian tissues act as antimetabolites or carcinogens and are postulated as possible inducers of spontaneous cancer (10).

This paper reports studies on the excretion rate of amitriptyline *N*-oxide in humans. On the basis of these data and the results of previous work (9) on amitriptyline metabolism in humans, the probable role of this metabolite in the degradative pathway of the drug is discussed.

EXPERIMENTAL[1]

Synthesis of Amitriptyline *N*-Oxide—The synthesis of amitriptyline *N*-oxide was performed according to the method of van Steen (11), with the following modifications. Five grams of amitriptyline base, 10,11-dihydro-*N*,*N*-dimethyl-5*H*-dibenzo[*a*,*d*]cycloheptene-$\Delta^{5\gamma}$-propylamine, was dissolved in 20 ml of anhydrous methanol. Then 5 ml of 30% H_2O_2 was added to the solution, dropwise with stirring, and the solution was kept at 0° for a few days. After the further addition of 3 or 4 ml of 30% H_2O_2 and subsequent storage at 0° for 5 days, the excess hydrogen peroxide was cautiously decomposed with an aqueous suspension of 10% Pt black. This mixture, kept in ice, was freed from Pt black by filtration, and the filtrate was concentrated under reduced pressure at 30°. The solid residue was twice recrystallized from a mixture of methyl chloride–anhydrous ether (1:1) and colorless crystals of pure amitriptyline *N*-oxide, mp 114–115°, were obtained in an 88% yield. Amitriptyline *N*-oxide in the original method of van Steen melts at 99–106°. The IR spectrum of amitriptyline *N*-oxide was identical to that of amitriptyline except for the absence of the signal of the NH-band between 2400 and 2550 cm^{-1}. The UV spectrum of the compound, dissolved in methanol, showed the absorption maximum at 238 nm characteristic of amitriptyline.

Characterization of Amitriptyline *N*-Oxide—*TLC*—Glass plates (10 × 20 cm) were spread with a mixture of silica gel G[2]–fluorescent indicator[3]–distilled water (1:0.04:2); the thickness was 0.3 mm. Standards of amitriptyline *N*-oxide, amitriptyline, and the other amitriptyline metabolites known to occur in humans (1) (10-hydroxyamitriptyline, nortriptyline, desmethylnortriptyline, and 10-hydroxynortriptyline) were chromatographed by the ascending technique in the following solvent systems: A, isopropanol–ethyl acetate–concentrated ammonium hydroxide (45:5:1); B, chloroform–methanol (4:1); and C, benzene–dioxane–water–concentrated ammonium hydroxide (62.5:35:1.8:0.7).

The detection of amitriptyline and its metabolites was performed with a UV light (254 nm); the R_f values are listed in Table I. In all solvent systems tested, amitriptyline *N*-oxide possessed

[1] All chemicals and reagents (analytical grade) were obtained from Merck, Darmstadt, Germany. Amitriptyline, nortriptyline, desmethylnortriptyline, 10-hydroxyamitriptyline, and 10-hydroxynortriptyline were supplied by Merck, Sharp & Dohme, Pavia, Italy.
[2] Merck, Darmstadt, Germany.
[3] Woelm, Eschwege, Germany.

Table I—R_f Values of Amitriptyline and Its Metabolites in Solvent Systems A, B, and C[a]

	A	B	C
Amitriptyline	0.80	0.75	0.77
10-Hydroxyamitriptyline	0.75	0.48	0.52
Nortriptyline	0.49	0.32	0.25
Desmethylnortriptyline	0.52	0.30	0.24
10-Hydroxynortriptyline	0.29	0.16	0.14
Amitriptyline *N*-oxide	0.08	0.27	0.02

[a] A, isopropanol–ethyl acetate–concentrated ammonium hydroxide (45:5:1); B, chloroform–methanol (4:1); and C, benzene–dioxane–water–concentrated ammonium hydroxide (62.5:35:1.8:0.7).

different R_f values from those of amitriptyline and the other amitriptyline metabolites.

Reduction with Titanium Trichloride to Amitriptyline—Amitriptyline *N*-oxide (1 mg/ml water) was mixed with 1 ml of 2 *N* HCl and 0.6 ml of 13% (w/v) $TiCl_3$ and incubated for 30 min in the dark at room temperature. At the end of the reaction, the mixture was adjusted to pH 10 with 5 *N* NaOH and extracted with five volumes of 1,2-dichloroethane. The extract was concentrated to dryness *in vacuo*, taken up in 500 μl of methanol, and submitted to chromatographic analysis in the various solvent systems. Only the spot of amitriptyline was present on the chromatogram, indicating that the *N*-oxide had been quantitatively reduced to amitriptyline.

Solvent Extraction—For the extraction of amitriptyline *N*-oxide from the aqueous phase, the following organic solvents were tested: 1,2-dichloroethane, chloroform, ether, and *n*-heptane. The compound was extracted from 0.1 *M* buffered solutions; the buffers used were: acetate, pH 5; phosphate, pH 7; and glycine, pH 9 and 11.

Previous to extraction, the organic phases were presaturated by shaking with the same volume of the corresponding aqueous phase for 10 min. Amitriptyline *N*-oxide was dissolved in 10 ml of aqueous buffer solution (10 μg/ml) and extracted by shaking with 10 ml of the organic solvent for 30 min at 20°. Drug concentrations were determined by UV spectrophotometry at 238 nm (the absorption maximum of amitriptyline *N*-oxide) in the aqueous phase before extraction and in both phases after extraction. The concentrations were evaluated with a linear standard curve. Five experiments were run for each buffer–solvent combination.

Figure 1 shows that the passage of amitriptyline *N*-oxide from the aqueous solutions into the organic solvents varied over a wide range (6–97%) depending on the solvent. The extractability of amitriptyline *N*-oxide into the organic solvents did not seem to be influenced by the pH of the aqueous phase.

Of the four solvents tested, chloroform extracted the most amitriptyline *N*-oxide, but it also extracted other compounds (*i.e.*, urinary pigments) which interfered in the chromatographic separation of the metabolite. Thus, the urines were extracted with 1,2-dichloroethane, which extracts a high proportion (82%) of this metabolite and less of the impurities.

Isolation of Amitriptyline *N*-Oxide from Urines—Immediately after collection, 50 ml of each urine sample was acidified with acetic acid and buffered with 0.1 *M* acetate buffer to pH 5. Then the samples were twice extracted with five volumes of 1,2-dichloroethane. The organic layers were collected, dried over anhydrous sodium sulfate, filtered, and concentrated to dryness *in vacuo* at 30°. The residue was taken up in 1 ml of methanol. The extract was subjected to two-dimensional TLC in Solvent Systems A and B.

For two-dimensional separation, Solvent System A was run first and the plate was air dried before developing in the second dimension with the other solvent system. The spot corresponding to amitriptyline *N*-oxide, by the criterion of R_f values, was eluted with water and its identity was confirmed by reduction by titanium trichloride and UV and IR spectra.

Quantitative Determination and Percent Recoveries from Urines—The quantitative determination of amitriptyline *N*-oxide isolated from urines was performed as follows. After the chromatographic run, the silica gel spot of amitriptyline *N*-oxide was scraped off and extracted by shaking for 15 min with 2.5 ml of 0.1 *M* acetate buffer (pH 5)–methanol (3:1). After centrifugation for 5 min (4000 rpm), the absorbance at 238 nm of the extract was determined *versus* a silica gel blank. The corresponding concentration was determined from a calibration curve of known amounts of the synthesized standard that had been applied to silica gel plates. This curve was linear in the range from 0.5 to 10 μg/ml; 5 μg of amitriptyline *N*-oxide had an absorbance of about 0.08. This quantitation method proved highly reproducible. When amitriptyline *N*-oxide was added to normal urine in a concentration of 1 μg/ml and incubated for 6, 12, or 24 hr at 37°, recovery was 94 ± 1.6% (mean ± *SE* of five experiments).

Table II—Urinary Excretion of Amitriptyline *N*-Oxide by Human Subjects

Subject	Sex	Weight, kg	Age, years	12 hr[a]	24 hr[a]
1	M	74	23	0.92	1.04
2	M	70	30	0.99	1.08
3	M	79	24	0.88	1.00
4	M	71	22	0.94	1.05
Mean ± *SE*		73.5	24.7	0.93 ± 0.02	1.04 ± 0.02
5	F	60	22	0.98	1.14
6	F	56	26	1.06	1.24
7	F	54	24	1.05	1.18
8	F	65	27	0.96	1.05
Mean ± *SE*		58.7	24.7	1.01 ± 0.02	1.15 ± 0.04[b]

[a] Data in the last two columns represent the total amount of the metabolite excreted (expressed as percent of the dose of amitriptyline, 50 mg, oral) by the 12th or 24th hr after administration. [b] $p < 0.05$ when compared with the male value; Student's *t* test.

Drug Administration and Urine Collection—Eight volunteers, four females and four males (Table II), with normal gastroenteric, circulatory, hepatic, and renal functions (as determined by routine clinical tests) participated in the study. The subjects had not taken any drug for at least 4 weeks before the study. Amitriptyline hydrochloride[4] was administered orally as a single 50-mg

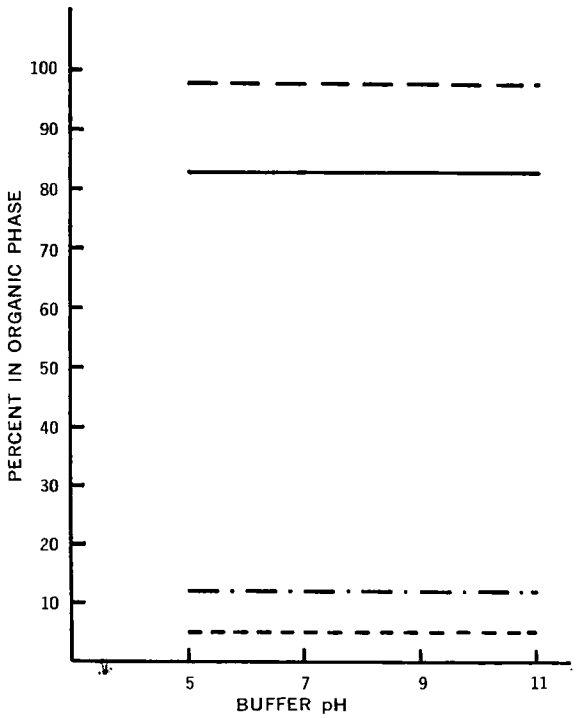

Figure 1—*Extractability of amitriptyline* N*-oxide by various solvents. Amitriptyline* N*-oxide (10 μg/ml) in various aqueous buffers (see* Experimental*) was extracted with an equal volume of 1,2-dichloroethane* (——), *chloroform* (— — —), *ether* (—·—·—), *or* n*-heptane* (— — — —).

[4] Tryptizol, Merck, Pavia, Italy.

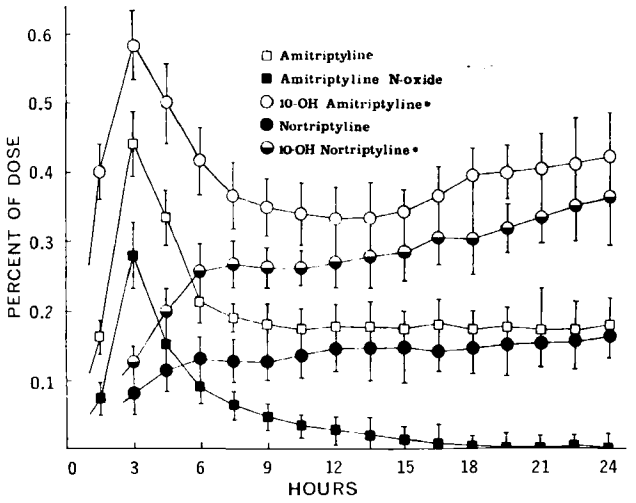

Figure 2—*Urinary excretion of amitriptyline and its metabolites. The data cover the first 24 hr after administration of amitriptyline (50 mg po). Each bar represents the percent of the dose (mean ± SE from four male subjects) present as each metabolite at the given time points. Key: *, conjugated + unconjugated fraction.*

tablet. The drug was always given in the morning to the fasted subjects.

The urine from each volunteer was collected at time intervals of 1.5 hr on the day of drug administration and then at intervals of 24 hr up to 180 hr. Each urine sample was divided into two portions, one for the determination of amitriptyline *N*-oxide and the other for the determination of unmodified amitriptyline and the related principal metabolites known to occur in humans (1) (10-hydroxyamitriptyline, nortriptyline, desmethylnortriptyline, and 10-hydroxynortriptyline). These determinations were done by methods reported previously (12).

RESULTS AND DISCUSSION

The urinary levels of amitriptyline *N*-oxide in male and female volunteers 12 and 24 hr after administration are reported in Table II. The total amount of amitriptyline *N*-oxide excreted by the subjects represents 1.04 ± 0.02% (in males) and 1.15 ± 0.04% (in females) of the administered dose of amitriptyline. Of this amount, 90% is excreted during the first 12 hr. The difference between males and females, although statistically significant, is small enough to be attributable to differences in distribution of the drug within subjects of different body weights. Therefore, there is no clear indication of a sex difference in synthesis of this metabolite. These findings are in agreement with results of studies with drugs chemically related to amitriptyline. Christiansen *et al.* (8) and Goldenberg *et al.* (13) observed that, in humans, about 1% of administered imipramine and promazine was excreted as the respective *N*-oxide. The extremely low excretion of amitriptyline *N*-oxide, when compared to that of the other amitriptyline metabolites (Table III), demonstrates that the *N*-oxidation of amitriptyline is a minor metabolic pathway of the drug in humans.

Figure 2 shows the excretion rate patterns, for four male subjects, of amitriptyline, amitriptyline *N*-oxide, and other amitryptyline metabolites during the first 24 hr after drug administration. The hydroxylated and *N*-demethylated metabolites of amitryptyline represent the products of the major metabolic pathways of the drug in humans (1).

The most polar metabolite is 10-hydroxyamitriptyline, which appears early in urine and is almost entirely glucuronide conjugated. Similarly, amitriptyline *N*-oxide appears early and this behavior correlates with its polar character due to the N—O bond (10). Unlike the other compounds, however, this metabolite soon disappears completely from urine, although considerable levels of the parent drug are still present in the body and all of the other amitriptyline metabolites continue to be excreted.

Table III—Excretion[a] of Amitriptyline and Its Metabolites

Amitriptyline	5.0 ± 0.4
10-Hydroxyamitriptyline	27.6 ± 1.6
Nortriptyline + desmethylnortriptyline	6.2 ± 0.5
10-Hydroxynortriptyline	41.4 ± 4.1
Amitriptyline *N*-oxide	1.04 ± 0.02

[a] Data are expressed as percentages (mean ± *SE* for four male subjects) of the administered dose (50 mg amitriptyline po) excreted over 180 hr after administration.

The disappearance of amitriptyline *N*-oxide from urine may be interpreted in various ways:

1. Formation of this metabolite ceases when the concentration of the parent drug in the body falls below a critical level. If so, the *N*-oxidation could represent an "emergency" route of amitriptyline metabolism in humans.
2. Amitriptyline *N*-oxide is continuously formed in the presence of the parent drug in an amount proportional to its concentration in the body, but most of it undergoes further metabolic transformations so that only a small fraction of the amount formed appears in the urine.

In either case, one might expect to see an "escape" by this metabolite into the urine early after administration; this could correspond to the first passage of the parent compound through the liver when both the concentration in this organ and the rate of metabolism would be maximal. Once the concentration of the parent compound declines due to metabolism and distribution throughout the body, the rate of formation of amitriptyline *N*-oxide would also decline and less of it might escape further metabolism.

Amitriptyline *N*-oxide is known to undergo further metabolism in the rat (5). It has been reported that this compound, which is formed by microsomal enzymes, undergoes reduction to amitriptyline or *N*-dealkylation to nortriptyline, either within the cell or in the circulating blood. If these processes take place also in humans, amitriptyline *N*-oxide should be considered as an intermediate product rather than as a terminal metabolite in amitriptyline metabolism.

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