Metabolites of Octoclotheptin Eliminated in Human and Rat Urine

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1. Four metabolites and unchanged octoclotheptin were extracted with dichloroethane from the urine of humans given octoclotheptin. These substances were isolated and purified by column and thin-layer chromatography. 2. By chromatographic, spectrophotometric and polarographic analysis, unchanged octoclotheptin and three of the metabolites were identified (noroctoclotheptin, noroctoclotheptin *S*-oxide and octoclotheptin *S*-oxide). 3. The presence of glucuronides in human urine was proved. 4. The same metabolites and unchanged octoclotheptin were also found in rat urine by chromatography.

During systematic studies of 10,11-dihydrodibenzo[*b,f*]thiepin, Protiva, Jílek and their associates synthesized perathiepin {10-(4-methylpiperazino) - 10,11 - dihydrodibenzo[*b,f*]thiepin} (Jílek, Seidlová, Svátek & Protiva, 1965; Protiva & Jílek, 1966) and its 8-chloro derivative octoclotheptin {8-chloro-10-(4-methylpiperazino)-10,11-dihydrodibenzo[*b,f*]thiepin} (Protiva *et al.* 1965) (Scheme 1). Octoclotheptin is a very potent neuroleptic agent whose activity surpasses those of perathiepin and chlorpromazine (Metyšová, 1966; Metyšová & Metyš, 1967).

The metabolism of perathiepin has been previously studied (Queisnerová, Svátek, Macek & Metyšová, 1968), and in human and rat urine unchanged perathiepin, two identified metabolites (norperathiepin and perathiepin *S*-oxide) and two unidentified metabolites were found. It was therefore of interest to know whether the 8-chloro derivative of perathiepin, namely, octoclotheptin, would undergo similar metabolic changes in man and in laboratory animals. Human and rat urine was collected after oral administration of octoclotheptin, and unchanged octoclotheptin and its metabolites were isolated and purified by column chromatography and t.l.c. and compared with corresponding synthetic standards by using chromatographic, spectrophotometric and polarographic methods.

METHODS

Synthetic standards, analytical grade, were prepared at the Chemical Synthesis Department of our Institute (Jílek, Metyšová, Pomykáček & Protiva, 1968). Some characteristics are given in Table 1.

The urine of ten inpatients (male and female) receiving daily oral doses of 40–80 mg. of octoclotheptin was collected for 24 hr. The pooled urine (12 l.) was immediately filtered, adjusted to pH 10 with NaOH and extracted twice with an

equal volume of dichloroethane. The combined extracts were concentrated under reduced pressure to a syrup, which was then dried in a desiccator.

Unconjugated metabolites. The crude extract was partitioned by chromatography on a Sephadex LH-20 column (15 mm. × 200 mm.). Portions (100 mg.) were dissolved in 1.5 ml. of chloroform–ethanol (9:1, v/v) and put on the column. The same solvent also served as eluent. Fractions (5 ml.) were collected and evaporated to dryness, and the residues were chromatographed. In this way concentrates of five compounds (I–V) were obtained. Each concentrate was then purified by t.l.c. on silica gel G (E. Merck A.-G., Darmstadt, Germany) on glass plates (100 mm. × 200 mm., or 200 mm. × 200 mm.) with chloroform–ethanol (4:1, v/v). The spots were detected with a low-pressure mercury discharge tube (Chromatolite type) and with Dragendorff reagent. The zones identified by comparison with the synthetic standards were eluted with chloroform, the eluates were evaporated to dryness under reduced pressure and the dry residues were analysed.

The crude extracts and purified substances were compared with the corresponding synthetic standards, first by descending paper chromatography on Whatman no. 4 paper impregnated with ethanolic 40% formamide (E. Merck A.-G.) containing ammonium formate (5%). The following solvents were employed: *S*₁, benzene–chloroform (1:1, v/v); *S*₂, benzene–cyclohexane (1:1, v/v); *S*₃, chloroform. The detection was performed as described for t.l.c.

The i.r. spectrophotometry of the substances isolated by chloroform extraction from the thin layers and of the synthetic standards was carried out in KBr pellets with a Unicam SP.200G spectrophotometer.

The same compounds were also polarographed in 0.1 M-HCl containing ethanol (20%) on an LP55A polarograph.

Glucuronides. The hydrolysis with β -glucuronidase was carried out in human urine after removal of unconjugated metabolites and in control urine. The urine was filtered through a sintered-glass bacterial filter and acidified with acetic acid to pH 4.5. Ox-liver β -glucuronidase (Betaglucuronidase; SPOFA, Prague, Czechoslovakia) was added (1000 Fishman units/ml. of urine) and the

samples were incubated at 37° for 24 hr. The urine was then adjusted to pH 9 and extracted with dichloroethane. The extracts were evaporated to dryness and the dry residues chromatographed.

In the animal experiments two groups of Wistar albino rats weighing 140–180 g. were used; one served as a control group, and each animal in the other group received a single oral dose of octoclotheptin (50 mg./kg. body wt., expressed in terms of the active base). The urine was then collected in metabolic cages for 24 hr. and processed as described for the human urine.

In pharmacological tests, octoclotheptin, octoclotheptin *S*-oxide, noroctoclotheptin and noroctoclotheptin *S*-oxide were studied. Acute intravenous and oral toxicity of these compounds was tested in mice. Deaths were recorded over a period of 48 hr. or 7 days respectively. The median lethal doses (LD_{50}) were determined graphically by the method of Litchfield & Wilcoxon (1949). The ability of mice to remain on a rotating rod was determined (Metyšová, Metyš & Votava, 1963). The median effective doses (ED_{50}) at the time of the maximal effect were assessed (Litchfield & Wilcoxon, 1949). Cataleptic action was studied in rats by the method described by Boissier & Simon (1963). The median effective doses (ED_{50}) were determined by the method of Litchfield & Wilcoxon (1949) at regular intervals over a period of 5 hr. Strain H female albino mice weighing 17–21 g. and female albino Wistar rats weighing 110–140 g. were used. Each dose was tested in groups of ten animals each. All the substances were administered in doses calculated as the base of the active substance. Octoclotheptin and octoclotheptin *S*-oxide were administered intravenously (dissolved in 5% glucose solution or 0.9% NaCl solution), and noroctoclotheptin and noroctoclotheptin *S*-oxide, which are less soluble, were administered orally (in suspension in 25% gum acacia).

RESULTS

Paper chromatography of the urine extracts with solvents S_1 , S_2 and S_3 revealed five substances

(I–V). Column chromatography on Sephadex LH-20 yielded concentrates of these substances, which were then completely purified by t.l.c. on silica gel G.

Since relatively low doses of octoclotheptin were used and few (ten) patients were examined, only small amounts of each substance were obtained, so that recrystallization was not possible.

The purified substances (I)–(V) were compared with synthetic standards by using paper chromatography, i.r. spectrophotometry, polarography and certain colour reactions.

From the R_F values of the substances in solvents S_1 , S_2 and S_3 and comparison with synthetic standards, substance (I) was shown to be identical with unchanged octoclotheptin {8-chloro-10-(4-methylpiperazino) - 10,11 - dihydrodibenzo[*b,f*]thiepin}, substance (II) with octoclotheptin *S*-oxide {8-chloro-10-(4-methylpiperazino)-10,11-dihydrodibenzo[*b,f*]thiepin *S*-oxide}, substance (III) with noroctoclotheptin (8-chloro-10-piperazino-10,11-dihydrodibenzo[*b,f*]thiepin) and substance (IV) with noroctoclotheptin *S*-oxide (8-chloro-10-piperazino-10,11-dihydrodibenzo[*b,f*]thiepin *S*-oxide) (Table 1 and Scheme 1). Substance (V), which exhibited the strongest polar character, was not identified. All these substances were Dragendorff-positive and, with the exception of the sulphoxides, quenched u.v. light of about 250 nm. The strongly polar substance (V) also reacted with diazotized sulph-anilic acid, indicating the presence of phenolic hydroxyl groups.

The identity of the substances (I)–(IV) was further established by i.r. spectrophotometry and comparison with synthetic standards. The i.r. spectra of substance (IV) and synthetic norocto-

Table 1. *Selected characteristics of octoclotheptin, its metabolites and corresponding synthetic standards*

Chromatography was carried out on Whatman no. 4 paper impregnated with ethanolic 40% formamide containing ammonium formate (5%); solvents S_1 , S_2 and S_3 are defined in the text.

Substance	Melting point (Kofler)	Colour reaction		Solvent ...	100 × R_F		
		Dragendorff	Sodium nitroprusside		S_1	S_2	S_3
Octoclotheptin	99–101° (base)	Orange	Negative		90	88	99
Octoclotheptin <i>S</i> -oxide	186–188° (maleate)	Orange	Negative		83	42	95
Noroctoclotheptin	135° (base)	Orange	Blue		75	09	83
Noroctoclotheptin <i>S</i> -oxide	175–176° (maleate)	Orange	Blue		12	00	48
Substance (I)	Not done	Orange	Negative		90	88	97
Substance (II)	Not done	Orange	Negative		85	44	95
Substance (III)	Not done	Orange	Blue		75	09	84
Substance (IV)	Not done	Orange	Blue		12	00	48
Substance (V)	Not done	Orange	Negative		06	00	35

Scheme 1. OctoclothePIN and three of its metabolites.

clothePIN *S*-oxide are coincident throughout the range 650–1800 cm.⁻¹. The i.r. spectrum of substance (V) exhibits a broad absorption band typical of OH groups, but no synthetic standard was available for comparison.

The identities of substances (II) and (IV) with synthetic standards was also shown by polarography in 0.1 M-hydrochloric acid containing ethanol (20%). Substance (II) and synthetic octoclothePIN *S*-oxide, and substance (IV) and synthetic noroctoclothePIN *S*-oxide, exhibited coinciding reduction waves ($E_1 = -0.605$ v against a saturated calomel electrode). Under the conditions described the sulphoxides of octoclothePIN yielded the best-developed waves. The other substances studied exhibited no characteristic reduction waves.

Both nor derivatives of octoclothePIN (substances III and IV) give a blue colour, characteristic of secondary amino groups, with sodium nitroprusside and acetaldehyde in 20% sodium carbonate. The presence of chlorine was proved in unchanged octoclothePIN and its metabolites by the Beilstein flame reaction (copper wire with a sample of the compound is brought into a flame; volatile copper chlorides impart a green colour to the flame).

In urine the substances were present in different amounts. In the crude urine extract they occurred in the diminishing order: noroctoclothePIN *S*-oxide > octoclothePIN *S*-oxide > unchanged octoclothePIN > noroctoclothePIN $\approx$ substance (V).

To ascertain whether the metabolites were also present as glucuronides, the urine, after removal of

the unconjugated metabolites with dichloroethane, was treated with β -glucuronidase. Extracts from treated and untreated urine samples were then compared by chromatography in solvent *S*₃. In the extracts from the treated urine two strongly polar substances were found, one with *R*_F corresponding to that of substance (V), but they were not isolated.

In experiments on rats the metabolites present in the urine were compared with those in human urine. Extracts of rat and human urine, prepared similarly, were compared by paper chromatography in solvents *S*₁, *S*₂ and *S*₃. Identical *R*_F values were obtained, showing the presence of the same metabolites and of unchanged octoclothePIN in both human and rat urine. Their proportions, however, were different. In rat urine, as in human urine, noroctoclothePIN *S*-oxide was the major metabolite; then followed unchanged octoclothePIN and its sulphoxide in roughly equal amounts, and then substance (V). NoroctoclothePIN, in contrast with human urine, was present in trace amounts only.

In pharmacological tests octoclothePIN exhibited a high sedative activity both in mice and in rats (Metyšová, 1966; Metyšová & Metyš, 1967; Metyš & Metyšová, 1969). Table 2 gives the results of these pharmacological tests, which include the disturbing effect of the substances on the motor co-ordination in the rotating-rod test in mice, the cataleptic effect on rats and the acute toxicity in mice.

Table 2 shows the high sedative activity of octoclothePIN observed in mice after administration by either route and its high cataleptic effect on rats

Table 2. *Selected pharmacological data for octoclotheptin and its metabolites*

Test methods are given in the text. Median effective doses after intravenous and oral administration were assessed at the time of the maximal effect of each substance.

Substance	Route ...	Acute toxicity in mice (LD ₅₀ , mg./kg.)		Rotating-rod test results in mice (ED ₅₀ , mg./kg.)		Catalepsy in rats (ED ₅₀ , mg./kg.)
		Intravenous	Oral	Intravenous	Oral	Oral
Octoclotheptin		46.3	78	0.06	2.2	4.3
Octoclotheptin <i>S</i> -oxide		10.0		0.36		
Noroclotheptin			120		5.6	30.0
Noroclotheptin <i>S</i> -oxide			115		Weakly active	Inactive

after oral administration. The sedative effect of octoclotheptin *S*-oxide is about one-sixth of that of octoclotheptin when the substances are administered intravenously; noroclotheptin administered orally has about half the effect; noroclotheptin *S*-oxide has practically no sedative activity. The cataleptic effect of noroclotheptin on rats is substantially less than that of octoclotheptin but noroclotheptin *S*-oxide has no such effect. In mice the oral toxicity of noroclotheptin and noroclotheptin *S*-oxide is lower than that of octoclotheptin, but octoclotheptin *S*-oxide injected intravenously exhibits a substantially higher toxicity.

DISCUSSION

In man and the rat octoclotheptin is excreted in the urine partly as unchanged octoclotheptin and as four unconjugated metabolites, and as glucuronides. Chromatographic, spectrophotometric and polarographic methods and comparison with synthetic standards show the substances excreted to be unchanged octoclotheptin, noroclotheptin and two sulphoxides (octoclotheptin *S*-oxide and noroclotheptin *S*-oxide). Similar results were obtained in our previous study of the metabolism of perathiepin (Queisnerová *et al.* 1968), except that we had no certain proof of the existence of norperathiepin *S*-oxide. During the metabolism of both these neuroleptics demethylation on the piperazine ring, or oxidation of the sulphur atom of the thiepin nucleus, or both, occur; in this last case noroclotheptin *S*-oxide is formed. Demethylation and oxidation of a sulphur atom, occurring during the metabolism of drugs, have been described frequently, e.g. with the well-known neuroleptic chlorpromazine (Fishman & Goldenberg, 1963; Posner, Culpan & Levine, 1963).

In the fourth metabolite found (substance V) we assume, on the basis of polarographic and i.r.

spectrophotometric analyses, that only hydroxylation on the benzene nuclei occurred.

Pharmacological tests show that the metabolites of octoclotheptin exhibit lower sedative and cataleptic activities than the parent drug. Noroclotheptin and noroclotheptin *S*-oxide also exhibit lower oral toxicities, but octoclotheptin *S*-oxide shows a substantially higher intravenous toxicity. Similar elevation of the intravenous toxicity in mice was also found with perathiepin *S*-oxide compared with perathiepin (Queisnerová *et al.* 1968).

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