Biochemical Pharmacology, Vol. 19, pp. 1443–1448. Pergamon Press. 1970. Printed in Great Britain

BIOCHEMISTRY OF DRUGS—XV

THE FATE OF OCTOCLOTHEPIN [10-(4-METHYLPIPERAZINO)-8-CHLORO-10,11-DIHYDRODIBENZO (b,f) THIEPIN] IN THE ANIMAL ORGANISM

Z. FRANC, O. HOREŠOVSKÝ, B. PELC, V. FRANCOVÁ and P. KRAUS

Research Institute for Pharmacy and Biochemistry, Prague, Czechoslovakia

(*Received* 3 *September* 1969; *accepted* 18 *October* 1969)

Abstract—Octoclothepin is absorbed well from the intestinal tract of mice; while in rats, absorption is slow. If given intravenously, highest concentrations of the drug were found in the kidneys and in the lungs and the lowest in the spleen, skin and muscle. Elimination is slow and depends on the route of application and on the animal species. The urine of rats treated with ^{35}S-octoclothepin contained ^{35}S-octoclothepin, ^{35}S-octoclothepin sulphoxide, ^{35}S-sulphate, ^{35}S-sulphate esters and unidentified metabolites. Twenty per cent of the intravenously administered ^{14}C-(methyl)-octoclothepin was eliminated as $^{14}CO_2$.

OCTOCLOTHEPIN® was prepared by Jílek and coworkers.[1, 2] It was pharmacologically examined by Metyšová and Metyš.[3] Clinical trials revealed a remarkable antipsychotic effect.[4–7] The absorption, excretion, distribution and biotransformation of labelled octoclothepin in rats and mice is reported in this paper.

EXPERIMENTAL

Preparation of ^{35}S-octoclothepin

^{35}S-octoclothepin was prepared from ^{35}S-*p*-chlorothiophenol and *o*-iodophenylacetic acid in the same way as described in the original method.[1, 2] ^{35}S-*p*-chlorothiophenol was prepared from ^{35}S and *p*-chloro-bromobenzene with use of the Grignard's reaction. ^{35}S-octoclothepin used in our experiments had a specific activity 0·2 μc/mg.

Preparation of ^{14}C-(methyl) octoclothepin

This compound was prepared by the methylation of 10-(4-piperazino)-8-chloro-10,11-dihydrodibento(b,f)thiepin: 9 mg of this substance were dissolved in 0·25 ml of tetrahydrofuran and mixed with a solution of 3·75 mg (500 μc) of ^{14}C-methyl iodide. The mixture was heated in a sealed tube for 4 hr at 80° and then allowed to cool. Next day, 1 ml of 25% ammonium hydroxide was added and the solution was repeatedly extracted with chloroform. The organic layers were collected, washed three times with water and dried with anhydrous sodium sulphate. The solvent was then evaporated. To 12 mg of the dry residue, 203 mg of octoclothepin were added as a carrier, together with 70 mg of maleic acid dissolved in 90% ethanol. The solution was heated for 1 hr under reflux and then allowed to crystallize. 179 mg of ^{14}C-(methyl)-octoclothepin maleate were obtained. Specific activity of the final product was 1 μc/mg.

The animals

Adult male Wistar rats and white mice (strain "H") were used. The animals were maintained on a Larsen diet with a free access to water. Labelled octoclothepin was given either orally by a gastric tube or intravenously into the tail vein.

The determination of radioactivity in the tissues

Approximately 1 g of the tissue was weighed and dissolved in 25% ethanolic KOH. The final volume was made up to 4·0 ml. To 0·1 ml of this solution or to 0·1 ml of the urine, 10 ml of the scintillation fluid were added and the counts were taken. Quench correction as determined by the external standard method applied to individual samples.

RESULTS

The distribution of ^{35}S-octoclothepin in the organism and its elimination

There were no essential differences between the findings in both animal species when the drug was given intravenously. The digestive tract, which comprises only 8 per cent of the body weight, contained about 40–50 per cent of the total radioactivity.

Except for the short initial period, the excretion of ^{35}S-octoclothepin in the urine is very slow. There was no significant excretion by the faeces in the first 3 hr after the intake of the drug (Fig. 1).

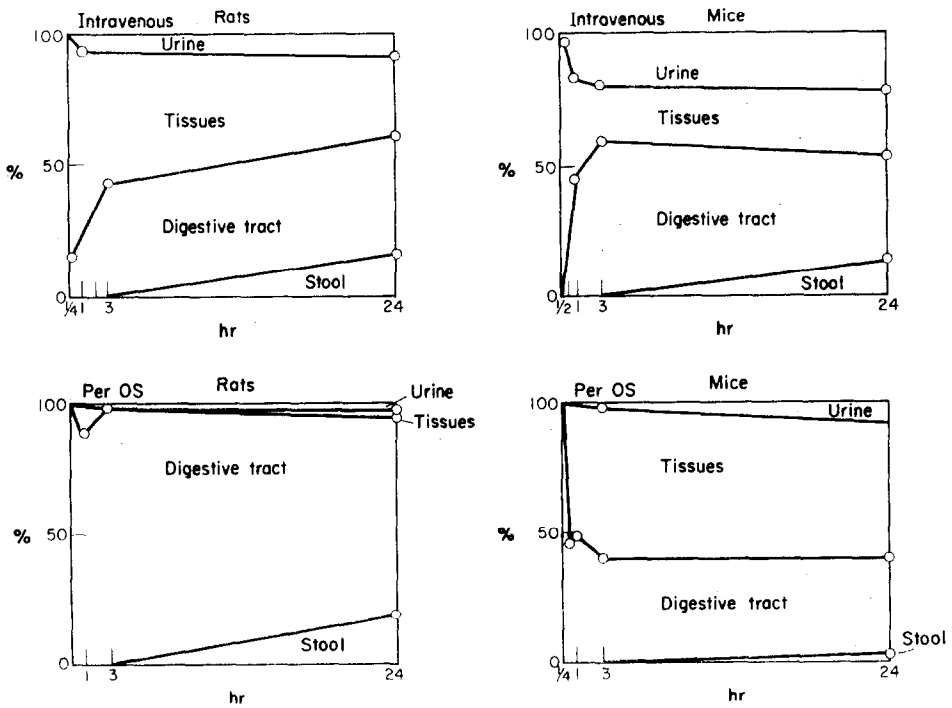

FIG. 1. The distribution of ^{35}S in rats and mice after oral and intravenous application of ^{35}S-octoclothepin. The animal received 25 mg/kg of labeled octoclothepin orally or intravenously. They were killed at various intervals after the intake of the drug and the radioactivity in the organism as well as in the excrements was determined. The term "tissues" denotes the entire body except the digestive tract. "Digestive tract" comprises the tissue and the contents inside the tract.

After the peroral application, ^{35}S-octoclothepin is rapidly absorbed from the murine intestinal tract. More than 50 per cent of the dose were found in the tissues. In rats, ^{35}S-octoclothepin remains almost entirely in the digestive tract and is gradually eliminated by the stool. The amount of the drug which passes into the tissues does not exceed 10 per cent of the dose. These differences between the absorption rate in both studied animal species may be one of the reasons why octoclothepin is significantly less toxic in rats after the peroral applications.

In another set of experiments, the elimination of ^{35}S-octoclothepin was followed up for 72 hr. As may be seen in Table 1, the excretion of this drug depends on the route of application and on the animal species used for the study.

The amount of octoclothepin and its metabolites in tissues

The total amount of the drug (free plus metabolites) in various tissues was deter-

TABLE 1. ELIMINATION OF OCTOCLOTHEPIN

Animal species	Route of application	Number of animals	Elimination of ^{35}S (in % of the dose)		
			urine	stool	total
Mice	Intravenous	5	34 ± 1·3*	29 ± 5·1	65 ± 5·2
Mice	Peroral	6	24 ± 2·8	38 ± 3·1	63 ± 4·6
Rats	Intravenous	5	21 ± 4·6	45 ± 2·5	66 ± 4·4
Rats	Peroral	6	19 ± 0·8	71 ± 2·4	90 ± 2·4

The animals received 25 mg/kg of ^{35}S-octoclothepin, and were placed in metabolic cages. Urine and faeces were collected separately and stored under a toluene layer in a refrigerator until used for the determination of radioactivity.

* Mean ± S.E.M.

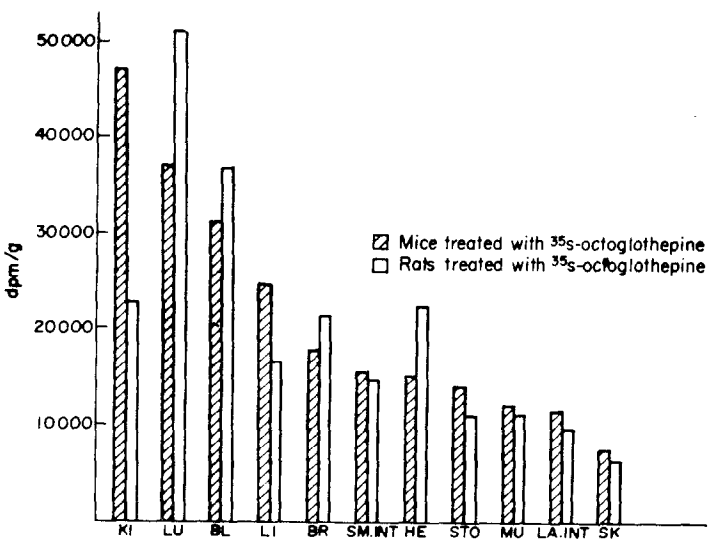

FIG. 2. The distribution of radioactivity in the organs of mice and rats after a single dose of ^{35}S-labeled octoclothepin. The animals received 25 mg/kg of labeled octoclothepin intravenously and were killed 6 min later. Tissue samples were treated as described in the Experimental section. Presented data are mean values from five determinations. Ki—kidneys, Lu—lungs, Bl—blood, Li—liver, Br—brain, Sm. int—small intestine, He—heart, Sto—stomach, Mu—muscle, La. int.—large intestine, Sk—skin.

mined at the 6th min after an intravenous application. The results are presented in Fig. 2. In some tissue samples, the amount of true octoclothepin apart of its metabolites was determined in the following way: The samples were dried under an infrared lamp and pieces weighing 5 g were extracted in the Soxhlet's apparatus with the mixture chloroform–ethanol–25% ammonium hydroxide 70:20:5. The extraction lasted 6 hr. The solvent was evaporated, the dry residue was dissolved in 0·5 ml of ethanol and analyzed by the thin-layer chromatography on aluminium hydroxide using a mixture of petrolether–diethyl ether–benzene–ethanol (20:10:10:1) as a solvent. The spots were detected in the ultraviolet light (260 nm). The compounds were identified by comparing their R_f values with these of synthetic standards. The areas corresponding to different metabolites were scraped off and their radioactivity was determined by the liquid scintillation method. It was seen that octoclothepin is metabolized to a considerable extent (see Table 2).

TABLE 2. OCTOCLOTHEPIN AND ITS METABOLITES IN THE ORGANS OF RATS

Organ	Number of determinations	Octoclothepin (%)	Octoclothepin sulphoxide (%)	Unidentified (%)
Brain	4	52	17	31
Kidney	3	40	24	36
Liver	4	47	22	31
Lung	3	22	15	63

The rats received 25 mg/kg of ^{35}S-octoclothepin intravenously and were killed 6 min later. Tissue samples were treated as described in the experimental section. Presented data are mean values.

The metabolites of octoclothepin in the urine of rats

The urine was collected for 72 hr after the oral intake of 25 mg/kg of ^{35}S-octoclothepin. Ten ml of the filtered urine were acidified with diluted acetic acid to pH 3 and extracted three times with 10 ml of diethyl ether. The organic phase was evaporated under reduced pressure and the residue was dissolved in 0·2 ml of ethanol (Fraction I). The aqueous phase was alkylated with 1·0 ml of 25% ammonium hydroxide and extracted three times with chloroform. The extracts were mixed together, the solvent was evaporated and the residue was dissolved in 0·2 ml of ethanol (Fraction II). The aqueous phase was neutralized and mixed with an excess of the benzidine reagent.* The solution was allowed to stand 2 hr at 4°. The precipitate of benzidine sulphate was collected on a Buchner funnel, washed three times with ethanol and dried on the air (Fraction III). To the filtrate, concentrated hydrochloric acid was added in a quantity sufficient to obtain a final concentration of 6 N. Sulphate esters were hydrolyzed by refluxing for 2 hr and precipitated by the benzidine reagent (Fraction IV). Fraction II was further analyzed using two-dimensional thin-layer chromatography on Silufol.† The following solvent systems were used: (1) benzene–ethyl acetate–diethylamine (7:2:1); (2) chloroform–acetone–diethylamine (87:3:10). The compounds were detected by autoradiography, by iodine vapors or by ultraviolet light at 260 nm. Usually four distinct spots were detected by the former method (Fractions II_1–II_4).

* 5 g of benzidine hydrochloride were dissolved in 40 ml of 1 N HCl and mixed with 200 ml of ethanol.

† Silica gel layer on the aluminium foil. It is a produce of Kavalier, Czechoslovakia.

The radioactivity of all fractions was determined by the liquid scintillation method. From the results of three experiments, the following average amounts of ^{35}S were found in different fractions (the data are related to the total radioactivity of the urine): Fraction I,* 2%; Fraction II_1 (octoclothepin) 5%; Fraction II_2 (octoclothepin sulphoxide) 9%; Fraction II_3* 4%; Fraction II_4* 12%; Fraction III (sulphates) 3%; Fraction IV (sulphate esters) 19%.

The expiration of $^{14}CO_2$ by the rats treated with ^{14}C-(methyl) octoclothepin

The rats received 12·5 mg/kg of ^{14}C-(methyl)-octoclothepin intravenously and were placed in metabolic cages aerated with CO_2-free air. The effluent air passed through a saturated solution of barium hydroxide. At various intervals, ^{14}C barium carbonate was collected on a Buchner funnel, washed three times with water and three times with ethanol. An aliquot was taken for the determination of radioactivity. As may be seen

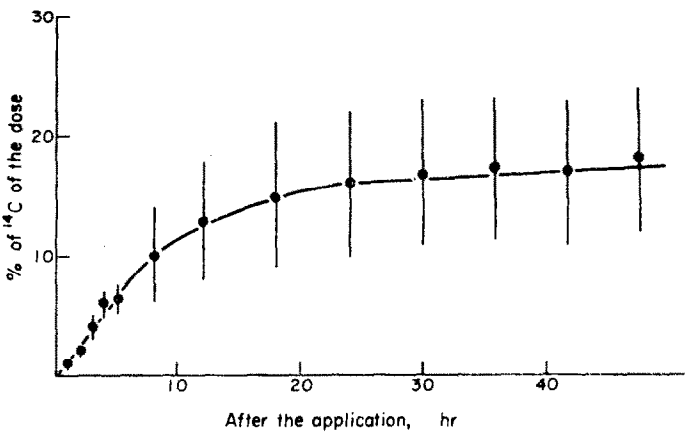

FIG. 3. The expiration of $^{14}CO_2$ by rats treated with ^{14}C (methyl)-octoclothepin. The animals received 25 mg/kg of ^{14}C (methyl) octoclothepin and were placed in air-tight met bolic cages. The expired $^{14}CO_2$ was trapped into barium hydroxide. The results are presented as means ± S.E.M. from six determinations.

in Fig. 3, the rats eliminated about 20 per cent of the given radioactivity as $^{14}CO_2$. Although the attempts to detect the demethylated octoclothepin in the urine or in the tissues were unsuccessful, the presence of $^{14}CO_2$ in the expired air of rats treated with ^{14}C-(methyl)-octoclothepin may be considered as evidence that octoclothepin is demethylated in the animal organism.

* Unidentified.

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