Distribution and Elimination of o-^{125}I-Benzoate in Rats

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Abstract. ^{125}I-labelled o-iodobenzoate (OIB) was prepared by means of an isotopic exchange reaction; its distribution and excretion were determined and its pharmacokinetic parameters in rats were calculated. The calculated value of the half-life of OIB elimination was 38.7 ± 0.7 min, the distribution volume was 278.2 ± 53.5 ml.kg^{-1}. The rate of elimination activity in urine was in agreement with the above values. On the basis of the developed technique of separation of OIB metabolites by thin-layer chromatography, their relative proportion in rat urine was determined; within 24 h 50% of the eliminated activity was in the original form (as OIB) and the metabolites of o-iodohippurate and o-iodobenzoylglucuronide formed approximately 25% of the activity eliminated in urine each.

Key words: Pharmacokinetics — Elimination — Metabolism — Iodobenzoate

Introduction

The radioisotope ^{131}I-labelled o-iodobenzoate (OIB) was proposed by Eysselt and Dohnálek (1967, 1968, 1973) in the sixties to examine the detoxication function of the relative proportion of the individual OIB metabolites in urine. According to Eysselt and Dohnálek (1968), the principal biotransformation actions take place in the liver: conjugation of OIB with glycine (preceeded by binding to coenzyme A assisted by the enzyme glycine-N-acylase) to o-iodohippurate (OIH), or conjugation with uridinediphosphoglucuronic acid (assisted by the enzyme uridinodiphospho-glucuronyl-transferase) to o-iodobenzoylglucuronide (OIBG). A certain proportion does not undergo any metabolism in the body and is excreted in an unchanged form as OIB. In liver disease, such as cirrhose or chronic hepatitis, as well as in malignant tumours, hyperthyreosis and psoriasis, the proportion of OIBG is increased (Dohnálek et al. 1966a; Kolářová et al. 1968). Changes in the relative proportion of OIB metabolites in urine under physiological conditions were also

studied in other papers (Tubis et al. 1964; Dohnálek et al. 1966b; Ando et al. 1973).

The hitherto published papers concerning the above problems have dealt primarily with the examination of the relative proportion on the individual OIB metabolites in urine within a more detailed investigation of their distribution and pharmacokinetics. The present paper deals with the problems of ^{125}I-labelled OIB, its distribution in rats, and with the evaluation of basic pharmacokinetic parameters in a comprehensive manner. Also, a simple technique was developed to separate the basic OIB metabolites on a thin layer.

Material and Methods

Preparation of ^{125}I-labelled OIB

The preparation was performed by means of an isotope exchange reaction between non-radioactive OIB and radioactive sodium iodide (Na^{125}I). One hundred mg of chromatographically pure OIB were dissolved under warm conditions in 0.35 ml of 1 mol/l solution of NaOH. The solution was diluted with 3 ml of purified water and its pH was adjusted to 4.6. After three min tempering on a boiling water bath, 0.5 ml of Na^{125}I (total activity 450 MBq) were added and the reaction mixture was heated for 30 min on a boiling water bath. After cooling, the reaction mixture was poured through a column filled with a mixture of silver chloride and washed sea sand (1:3) to remove unreacted Na ^{125}I. After filtration, 0.15 ml of 10^{-2} mol/l solution of the sodium salt of ethylenediamine tetra-acetic acid were added to the solution and the pH was adjusted with 0.1 mol/l NaOH to 7.0. The yield of the exchange reaction was 98.7%, and after re-purification on the column the product contained 0.4% of the activity of ^{125}I in the form of iodide. Radiochemical purity was checked by means of chromatography using Whatman 4 paper in descending arrangement and the solvent system benzene-concentrated acetic acid-water (2:2:1).

Animals

Wistar male rats weighing 170—220 g were used for biological experiments.

Distribution studies

Rats were fastening for 18—24 h before the experiment, they had free access to water. OIB was administered intravenously into the exposed femoral vein under light ether anesthesia induced using open circuit inhalation (the stage of visually uninfluenced projection of abdominal breathing). The animals were fixed lying on the back, and 0.5 mg/kg OIB was administered within three minutes. The supply of the anaesthetic agent was stopped and the animals were distributed one by one in cages. Just before the end of the selected time interval for sampling, the carotic artery was exposed under newly induced light ether anesthesia and a blood sample was taken. The animals were then killed by exsanguination. The liver, kidney, brain, spleen, gonads, stomach, small intestine, colon were taken together with a sample of the femoral muscle for ^{125}I activity determination. The activity was measured in standard 20 ml scintillation flasks. The sample volume was adjusted with water to 10 ml. The activity of ^{125}I was measured using the beta-gamma spectrometer NE 8312 (Nuclear Enterprises Ltd., Edinburgh), and compared with the activity of standard samples of ^{125}I-OIB.

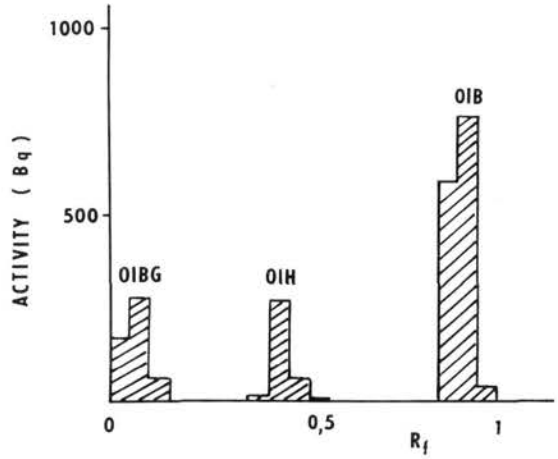

Fig. 1. Separation of OIB metabolites by thin-layer chromatography on Silufol using the solvent system benzene-concentrated acetic acid-water (65:25:1). (R_f for OIB is 0.90—0.05; for OIH, 0.45—0.50; for OIBG, 0.05—0.1).

Elimination of OIB and its metabolites in the stools and urine

OIB was administered to animals in the same manner as described above (distribution studies). Following OIB administration the animals were placed one by one in glass metabolic cages construction of which allows reliable separation of urine from solid excretions. The animals were kept in cages and had free access to standard diet and water. Two hours after OIB administration, the animals were forced to empty their urinary bladders into the metabolic cages. The inside of the cages was then washed with 50 ml of water to obtain all the eliminated activity. The volume of the liquid in the collecting flask was measured, and total eliminated activity and the relative proportion of the individual metabolites were determined. Rats were again placed into the same cages, and urine was repeatedly taken 24, 48 and 72 hours after OIB administration using the same withdrawal procedure. Also, the proportion of ^{125}I activity eliminated in solid excretions was determined in the same intervals.

Separation of OIB metabolites from rat urine

To separate OIB metabolites, a method was developed consisting of ascending chromatography on a thin layer Silufol (Kavalier, Votice) in an unsaturated chamber using the system benzene-concentrated acetic acid-water (65:25:1) as developer. An example of separation of OIB metabolites from rat urine and values of the retention factors R_f are shown in Fig. 1.

Pharmacokinetic analysis

The values of ^{125}I activity in the blood in the time interval up to 120 min were used to calculate the basic pharmacokinetic parameters. With regard to the approximately mono-exponential decrease in the blood levels during this time interval, a one-compartment open model (Gibaldi and Perrier 1975) was used, and the respective parameters were calculated using linear regression after semilogarithmic transformation of concentrations.

Table 1. Tissue distribution of ^{125}I activity (expressed in percents of the administared activity per organ) in rats given (^{125}I)OIB.

Tissue	Time — interval						
	1 min	5 min	15 min	30 min	60 min	120 min	24 h
Blood (total)	29.6	28.2	20.9	18.9	8.3	4.0	0.11
	±3.6	±1.9	±3.2	±0.7	±1.7	±1.0	±0.06
Liver	8.4	6.1	5.3	5.1	2.7	1.1	0.02
	±0.9	±1.0	±0.5	±0.7	±0.9	±0.2	±0.01
Kidney	5.1	5.1	8.1	9.6	4.3	1.7	0.02
	±1.7	±1.6	±2.5	±3.0	±2.2	±0.2	±0.01
Brain	0.13	0.17	0.19	0.18	0.12	0.06	0.0005
	±0.01	±0.02	±0.01	±0.03	±0.04	±0.01	±0.0005
Spleen	0.30	0.26	0.26	0.25	0.09	0.05	0.002
	±0.06	±0.03	±0.02	±0.03	±0.03	±0.01	±0.001
Gonads	0.25	0.24	0.38	0.59	0.42	0.22	0.008
	±0.04	±0.05	±0.06	±0.06	±0.10	±0.04	±0.002
Stomach	0.68	0.63	0.57	0.86	0.53	0.55	0.15
	−0.18	−0.14	−0.12	−0.33	−0.10	−0.13	−0.08
Small intestine	4.0	3.6	3.3	3.5	3.9	2.2	0.09
	±0.2	±0.4	±0.3	±0.3	±1.0	±0.8	±0.04
Colon	1.4	1.5	1.6	1.8	1.5	1.3	0.12
	±0.3	±0.1	±0.2	±0.2	±0.3	±0.1	±0.04
Muscle (total)	34.4	29.4	29.5	30.7	13.6	5.8	0.28
	±10.2	±3.5	±2.6	±2.9	±9.4	±1.8	±0.07

(^{125}I)OIB was given intravenously in a dose of 0.5 mg/kg. The values are means of eight determinations ±S.D. The calculation of the activity distribution in total blood and total muscle was based on the assumption that the blood and muscles constitute 8.15%, and 40% of the total body mass, respectively.

Results and Discussion

The values of ^{125}I activity in the blood (expressed in per cents of the administered dose per organ) are shown in Table 1. From the point of view of the distribution, the liver, kidney, the gastrointestinal tract (GIT) and muscle seem to be the most important organs. In other organs (brain, spleen and gonads) less than 1% of the administered activity was found. The decrease in hepatic activity nearly paralleled that in the blood, while in the kidney activity cumulation was observed in the initial time intervals (up to 30 min) with a rapid decrease thereafter. In GIT the activity level did not practically change over 120 min.

Values of activity smaller than 1% of that found in the first experimental time interval (1 min after administration) were found 24 hrs after the administration in all organs examined but in GIT. With regard to the radiochemical purity of OIB (98.7%), the activity found 24 hours after the administration may however have been due to a considerable extent to radiochemical impurities, rather than to OIB or its metabolites.

Table 2. Pharmacokinetic parameters of OIB in rats

	M ± SD*
Elimination half-life $T_{1/2}$ (min)	38.7 ± 0.7
Apparent distribution volume V_D (ml.kg^{-1})	278.2 ± 53.5
Total blood clearence Cl_T (ml.min^{-1}.kg^{-1})	4.99 ± 1.05

* Optimalized values ± standard deviations;

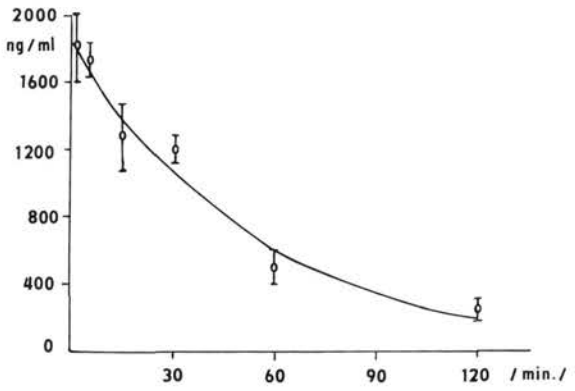

Fig. 2. Decrease in blood OIB level — a comparison of experimental values with the theoretical curve.

The values of ^{125}I activity in the blood converted into values of OIB concentration served to calculate the basic pharmacokinetic parameters (Table 2). The theoretical course of OIB concentration in the blood calculated on the basis of the above parameters is shown in Fig. 2, together with experimental values.

The calculated value of the half-life of elimination of the ^{125}I activity from the blood, $T_{1/2} = 38.7 \pm 0.7$ min, is in agreement with the results obtained in examining ^{125}I elimination in urine and stools (Fig. 3): 2 h after OIB administration a substantial portion of the administered activity has been excreted via urine. It is obvious from the results shown in Table 1 and Fig. 3 that the kidney is the principal organ of elimination for OIB and its metabolites, and that the proportion of radioactivity excreted in the stools is very low (less than 3% in 72 h). The half-life of urinary excretion of activity is thus practically identical with the biological one.

Within the time interval 2—24 h the relative proportion of OIB metabolites in rat urine (Fig. 4) is partially shifted in favour of OIH (the proportion of OIB is decreased) as compared with the interval 0—2 h; over the whole interval 0—24 h roughly 50% of the activity is eliminated in the original form (as OIB), and each of the metabolites (OIH and OIBG) makes up approximately a quarter of the total activity excreted in urine.

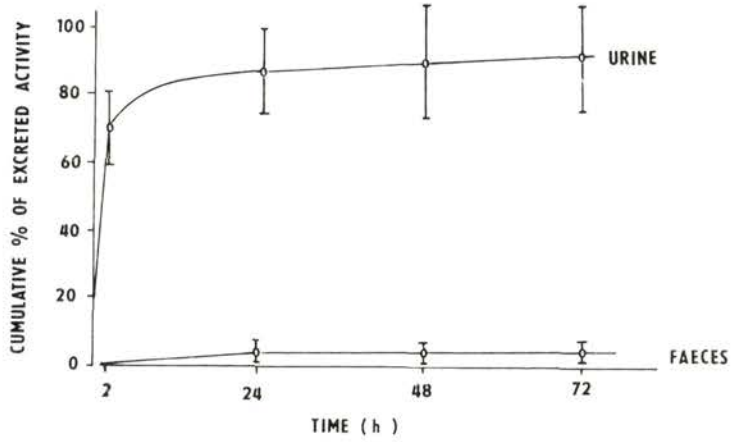

Fig. 3. Excretion of total radioactivity in the urine and faeces of male rats after i.v. administration of OIB. Each point represents mean ±S.D. od seven animals.

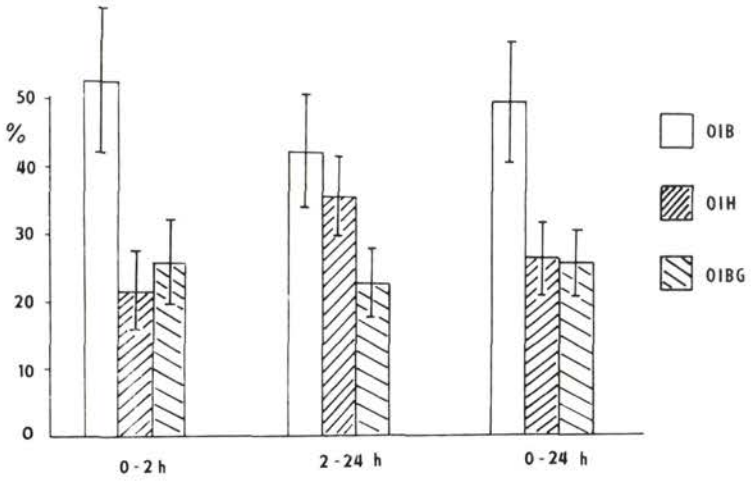

Fig. 4. The relative proportion of OIB metabolites in rat urine (mean ±S.D.).

After oral administration of OIB to rats Eysselt (1965) found 37% OIB, 30% OIH and 33% OIBG in urine, i.e., a higher relative proportion of both metabolites as compared with our results obtained after intravenous administration. Similar differences in the relative proportion of the metabolites of OIB after its oral and intravenous administration were also observed in rabbits (after i.v. administration, 60.3% OIB, 8.1% OIH, and 31.6% OIBG; after p.o administration, 48% OIB, 4% OIH, and 48% OIBG) (Eysselt 1965).

The relatively rapid urinary excretion of OIB and thus a short biological half-life of the drug suggests that the effective half-life of OIB will be influenced to a very limited extent by the substantially longer physical half-life of the radio-iodine ^{131}I or ^{125}I. The use of ^{125}I-labelled OIB as compared with the ^{131}I-labelled preparation will therefore produce a considerable decrease in the radiation dose (resulting from more favourable nuclear characteristics of ^{125}I) (Chervu et al. 1974) and a prolongation of the exspiration period of the preparation.

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References

Ando A., Hisada K., Ando I. (1973): Metabolism of iodum-131 labelled iodobenzoic acid in rats. Radioisotopes **22,** 632—637

Chervu L. R., Freeman L. M., Blaufox M. D. (1974): Radiopharmaceuticals for renal studies. Semin. Nucl. Med. **4,** 3—22

Dohnálek J., Eysselt M., Martínek K., Polášková A. (1966a): Detoxication of the OIB-^{131}I acid in some hepatic and renal disease. Vnitřní Lék. **12,** 1056—1060 (in Czech)

Dohnálek J., Eysselt M., Polášková A. (1966b): Über den Metabolismus der o-Jodbenzosäure-J-131 bei Menschen. Scripta Med. Brun. **39,** 189—203

Eysselt M. (1965): Metabolism of the o-J-benzoic acid (in Czech). Scripta Med. Fac. Med. Univ. Brun. Purkynianae **38,** 239—252

Eysselt M., Dohnálek J. (1967): Einfluß der Leberschädigung auf dem o-Jodbenzoesäure-^{131}J Stoffwechsel bei Ratten. Scripta Med. Brun. **40,** 71—77

Eysselt M., Dohnálek J. (1968): Metabolic pathways during the OIB acid test. Vnitřní Lék. **14,** 631—636 (in Czech)

Eisselt M., Dohnálek J. (1973): Our hepatic detoxication capacity test using o-iodobenzoic-I^{131} acid glucuronidation determination. Čas. Lék. Čes. **112,** 581—586 (in Czech)

Gibaldi M., Perrier D. (1975): One-compartment model. In: Pharmacokinetics (Ed. J. Swarbrick), pp. 2—27, Marcel Dekker, Inc., New York

Kolářová O., Staníček J., Dohnálek J., Eysselt M. (1968): Excretion of radioiodine-labelled OIB acid metabolites in gynecologic cancer. Čas. Lék. Čes. **107,** 151—153 (in Czech)

Tubis M., Blahd W., Endow J. S., Rawalye S. S. (1964): The Metabolism of Ortho-I^{131}Iodobenzoic Acid. J. Nucl. Med. **5,** 532—541

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