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SYNTHESIS OF N-ISOPROPYL-p-^{123}I-AMPHETAMINE AND BIODISTRIBUTION IN RATS

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The described labelling and purification preparation of N-isopropyl-p-^{131}I-amphetamine /^{131}I-IMP/ represents a fast and efficient method to obtain a compound that fulfils all criteria of purity for its in-vivo application. After isotope exchange and quality control ^{131}I-IMP could be obtained with radiochemical yields in the range between 68% and 78% with a radiochemical purity of 98-99%. As demonstrated in animal experiments the cerebral affinity of IMP offers a possibility for the diagnosis of brain diseases in clinical studies when the product is labelled with ^{123}I.

INTRODUCTION

Acting as chemical mediators, amines play an important role in brain functions including cerebral disorders such as schizophrenia and maniac depressive psychoses. In their studies with various N-alkylphenylamines Winchell

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et al.[1] found that iodination in para position of the aromatic ring generally leads to a higher brain uptake of the compound than the corresponding ortho isomer in a time interval up to 60 min after administration. However, in the case of N-isopropyl-iodobenzylamine this effect was observed with the substrate iodinated in meta position. In these studies the authors demonstrated the ideal properties of N-isopropyl-p-I-amphatemine /IMP/ for the study of brain functions due to its high cerebral uptake and brain-to-blood ratio.

IMP is a lipophilic compound with a fast blood clearance and a slow washout of radioactivity accumulated in the retina of certain animals[2-4], an effect which could limitate its application in clinical studies. Holman et al.[5] verified these findings in monkeys but could not confirm them in humans.

The aim of this work was to study the pharmacokinetics of ^{131}I-IMP in rats within a time interval of 24 h after administration. With respect to a future labelling with ^{123}I for clinical diagnosis of cerebral diseases accumulation in brain, eyes and brain-to-blood rations in dependence on time were of special interest[6].

MATERIALS AND METHODS

N-isopropyl-p-I-amphetamine /IMP/ was obtained from EMKA-Chemie /D-7145 Markgröningen-Talhausen, FRG/. All other chemicals were of analytical quality.

Labelling

Synthesis of ^{131}I-IMP was performed as follows:

A solution of 20 µl Na_2SO_4 /4 mg ml^{-1} H_2O/ and the desired amount of ^{131}I-radioactivity in 0.01N aqueous

NaOH was evaporated under vacuum to complete dryness at 70 °C. 200 µg of IMP in 100 µl of glacial acetic acid were added and the solution was heated for 20 min at 170 °C in an oil bath. After removing the solvent /70 °C, vacuum/ 2 ml of water was added and the reaction flask was swirled in order to dissolve the residue.

Purification

After the labelling step ^{131}I-IMP was purified by means of reverse phase /RP-18/ extraction columns /BAKER, 100 mg resin/ which had to be washed with 1 ml methanol, 2 ml 0.01N NaOH, 3 ml water, 1 ml methanol, 4 ml diethylether, 1 ml methanol and 2 ml water before use. By means of a vacuum pump the ^{131}I-IMP containing solution was sucked through the column followed by the elution of free $^{131}I^-$ with 1 ml NaI solution /0.5 mg ml^{-1} 0.01N NaOH/ and 3 ml H_2O. ^{131}I-IMP was eluted with 100 µl ethanol and 3 ml diethylether into a flask containing 100 µl of glacial acetic acid. The solvents were removed by vacuum evaporation /25-70 °C/. ^{131}I-IMP was dissolved in isotonic saline solution and sterilized by Millipore filtration /0.22 µm/.

Radiochemical control

Radiochemical quality of the product was checked by 2 methods:

- Electrophoresis with a buffer solution of acetic acid/acetate /pH 4.5/ and 300 V during 40 min.
- Paper chromatography with Whatman paper Nr. 1 and 3MM /2x25 cm/ with following solvents:
  methanol/water = 75/25 /vol/,
  chloroform/methanol/acetic acid = 85/15/1 /vol/,
  ethanol/ethylacetate = 1/1 /vol/.

TABLE 1

Results of quality control by electrophoresis after preparation and column purification of ^{131}I-IMP

n=6	^{131}I-IMP	$^{131}I^-$	$^{131}IO_3^-$
Yield, %	98.8 ± 0.8	1.0 ± 0.7	0.12 ± 0.07
Rf-value	0.21	0.7	0.5

Animal experiments

Biodistribution of ^{131}I-IMP was studied with male Wistar rats /mean body weight 250 g/. Each animal was anaesthesized with a solution of urethane /100 mg/100 g body weight, i.p./ followed by i.v. injection of 2.96 MBq /80 mCi/ ^{131}I-IMP in isotonic saline solution. 5, 15, 30, 60, 240 min and 24 h after administration the animals were sacrificed by decapitation and the following organs removed and washed: heart, brain, eye, kidneys, lungs and liver. After determination of their radioactivity and weights the results of accumulation could be expressed as % dose/g organ.

RESULTS AND DISCUSSION

Radiochemical yields of ^{131}I-IMP were between 67 and 87% /range of 8 individual experiments/.

Results of quality control of the product after column purification are shown in Tables 1, 2 and 3. It can be seen that analysis of the product fraction checked by electrophoresis and paper chromatography with different papers and solvent mixtures show product purities of >98% and small amounts of free ^{131}I-iodide and ^{131}I-

TABLE 2

Results of quality control by paper chromatography after preparation and column purification of ^{131}I-IMP /n=6/

Solvent	Methanol 75 Water 25	Ethanol 1 Ethylacetate 1	Chloroform 85 Methanol 15 Acetic acid 1
Paper	What.3MM What.1	What.3MM What.1	What.3MM What.1
^{131}I-IMP	98.90 ± 0.2	98.00 ± 0.1	98.70 ± 0.3
$^{131}I^-$	0.92 ± 0.04	1.70 ± 0.3	0.70 ± 0.2
$^{131}IO_3^-$	0.23 ± 0.1	0.34 ± 0.25	0.60 ± 0.01

TABLE 3

Rf-values of ^{131}I-IMP, $^{131}I^-$ and $^{131}IO_3^-$ under various chromatographic conditions /n=6/

Solvent	Methanol 75 Water 25	Ethanol 1 Ethylacetate 1	Chloroform 85 Methanol 15 Acetic acid 1
Paper	What.3MM What.1	What.3MM What.1	What.3MM What.1
^{131}I-IMP	0.90 ± 0.03	0.96 ± 0.05	0.98 ± 0.02
$^{131}I^-$	0.76 ± 0.01	0.10 ± 0.03	0.06 ± 0.01
$^{131}IO_3^-$	0.40 ± 0.01	0.49 ± 0.01	0.53 ± 0.03

iodate. These results indicate that the purification procedure by reverse phase columns is well suited to obtain a product with high radiochemical purity within a time of only several minutes.

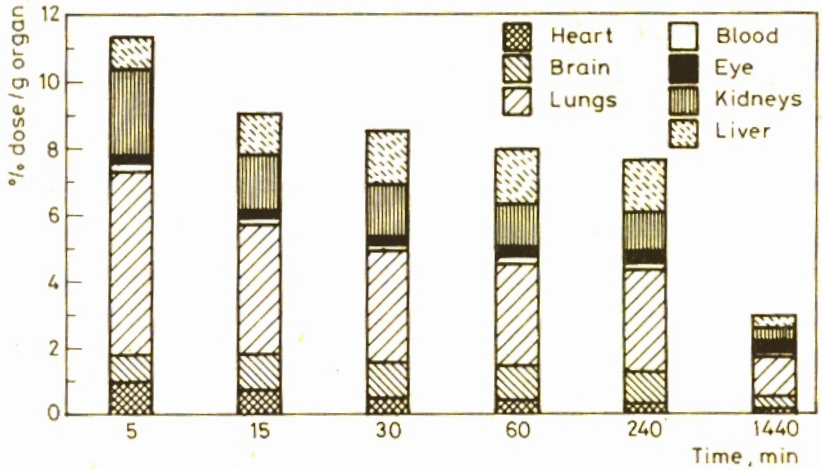

Fig. 1. Organ distribution of ^{131}I-radioactivity /% dose/g/ as function of time after i.v. injection of ^{131}I-IMP in rats /n=7/

Pharmacokinetics

Table 4 and Fig. 1 show the organ distribution of ^{131}I-radioactivity expressed as % dose/g organ in various organs between 5 min and 24 h after administration of ^{131}I-IMP. In brain an increase of accumulation is observed within 15 min remaining constant at a plateau of about 1 %/g up to 4 h p.i. and decreasing to 0.33 %/g 20 h later. High uptake of ^{131}I-IMP takes place in the lungs immediately after injection of the tracer followed by a decrease from 5.52 %/g at 5 min to a constant value of about 3 %/g between 15 and 240 min and a further decrease to 1.24 %/g one day later.

The efficient extraction of ^{131}I-IMP from blood is shown by the low dose of 0.19 %/g already 5 min after injection remaining constant for 4 h and decreasing to 0.07 %/g 24 h later.

TABLE 4

Organ distribution of ^{131}I-radioactivity /% dose/g/ and brain-to-blood ratio as function of time after i.v. injection of ^{131}I-IMP in rats /n=7/

Organ \ Time, min	5	15	30	60	240	1440
Heart	0.99 ±0.21	0.72 ±0.25	0.45 ±0.12	0.34 ±0.08	0.27 ±0.11	0.13 ±0.04
Brain	0.81 ±0.13	1.06 ±0.22	1.08 ±0.20	1.05 ±0.14	0.92 ±0.15	0.33 ±0.06
Eye	0.22 ±0.04	0.24 ±0.04	0.25 ±0.05	0.29 ±0.03	0.41 ±0.07	0.39 ±0.11
Kidneys	2.63 ±0.38	1.72 ±0.21	1.61 ±0.20	1.37 ±0.35	1.17 ±0.53	0.34 ±0.05
Lung	5.52 ±2.71	3.93 ±0.94	3.38 ±1.0	3.11 ±0.29	3.10 ±0.60	1.24 ±0.33
Liver	0.94 ±0.14	1.23 ±0.49	1.57 ±0.29	1.59 ±0.32	1.55 ±0.61	0.38 ±0.44
Blood	0.19 ±0.03	0.14 ±0.04	0.13 ±0.03	0.16 ±0.05	0.16 ±0.07	0.07 ±0.02
Brain/Blood	4.26	7.57	8.31	6.56	5.75	4.71

In contrast to all organs studied, an increase of ^{131}I-radioactivity takes place in the eye within 24 h starting from 0.22 %/g /5 min p.i./ to a nearly constant value of about 0.4 %/g between 4 h and 24 h after administration, an effect which has already been described by Sarget et al. in dogs and monkeys[2].

Continuous excretion of ^{131}I-radioactivity takes place via the kidneys as can be shown by the slow decrease starting from 2.63 %/g down to 0.34 %/g between 5 min and 24 h. On the other hand, the liver exhibits storage effects for ^{131}I-IMP and/or its metabolites as is demonstrated by an increase of accumulation up to 30 min and a long plateau phase between 30 and 240 min.

Table 4 also contains brain-to-blood ratios as function of time within 24 h p.i. Starting at 5 min with a value of 4.26 nearly a doubling /8.31/ is observed 25 min later slowly decreasing to 4.71 after 24 h.

CONCLUSION

The described labelling technique via isotope exchange in IMP and purification by extraction columns offers a possibility for a fast and efficient synthesis of ^{131}I-IMP with radiochemical yields between 67% and 87% and a radiochemical purity of 98%-99% as was checked by electrophoresis and paper chromatography under various conditions. Pharmacokinetics in rats within a time interval of 24 h after administration of the tracer demonstrate a fast extraction of ^{131}I-IMP from blood and a cerebral accumulation of about 1 %/g within 4 h. The long retention of ^{131}I-IMP in brain and the favourable brain-to-blood ratios between 4 and 8 within 24 h make IMP labelled with the short-lived radioisotope ^{123}I to a radiopharma-

ceutical well-suited for in-vivo diagnosis of cerebral diseases by single-photon-emission-computed-tomography /SPECT/.

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