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PHARMACOKINETICS OF 125-I-LABELLED META-IODO-BENZYL-GUANIDINE (MIBG) ON NMRI MICE AND WISTAR RATS

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ABSTRACT

The study of some pharmacokinetics providing the mechanism of uptake and metabolism parameters for the 125-mIBG is described. NMRI mice are used for plasma binding study; the animals are killed by decapitation after intravenously (IV) injection of 125-I-mIBG. Wistar rats are used in urinary excretion study. After IV injection, animals are placed in metabolic cages to collect urine. For biodistribution, the rats are killed at different time intervals. The considered organs are removed. The radioactivity of all parameters was performed by gamma counter. The results show that the blood clearance is very high after several hours post injection. The plasma binding is very low one hour post injection and very high after 72 hours. Furthermore, we observe a rapid excretion of radioactivity 24 hours post injection. However, we observe that 72 hours after injection, the radioactivity is important relatively. Concerning the bio distribution, the radioactivity per gram of different organs was normalized according to the adrenal glands. Also, we note, the adrenal glands may be the only target organs 48 hours post injection.
These results confirm that 125-I-mIBG has a high affinity for the adrenergic innervation›s organs (adrenal glands, salivary glands, heart and spleen).

KEY WORDS

- Adrenergic receptor ligands,
- Meta-iodo-benzyl-guanidine,
- Uptake,
- Storage,
- Neuroblastoma cells.

INTRODUCTION

The aralkylguanidine radio iodinated meta-iodo-benzyl-guanidine, a physiological analogue of the adrenergic-neuron-blocking agents (Fig.1), has been developed for diagnostic and therapeutic evaluation of the neuroendocrin tumours [1-6].

It was shown that the mechanism of mIBG uptake in catecholamine storage granules in sympathetic nerve endings and the adrenal medulla is almost identical to that of norepinephrine (NE) [7-12].

However; the biological half life of NE is only a few hours in the sympathetic nerve endings while that of radio iodinated mIBG in adrenergic tumours is several days 6.

Furthermore, it was found that in vivo, mIBG and NE uptake were inhibited by the same drugs [13].

The mechanism of uptake of mIBG was studied in vitro in comparison with norepinephrine NE [8]. Using cultured bovine adrenomedullary cells, it has been shown that mIBG and NE are transported by the same carrier involved in a sodium dependent system [9, 10].

Evaluation of excretion and metabolism of radio iodinated mIBG has been made at the University of Michigan [14]. Up to 55% of the injected radioactivity is recovered in the urine in the first 24 hours after infusion Up to 90% is excreted by four days. The use of radiopharmaceuticals in vivo must satisfy the biological and physiological parameters.

We report in this paper pharmacological studies providing better understanding of the mIBG uptake mechanism storage and excretion.

MATERIALS AND METHODS

1- Radiopharmaceutical preparation

1.1. Synthesis of unlabelled compound

The meta-iodo-benzyl-guanidine sulphate was synthesised according to a modified Wieland and al method [4,12], by condensing cyanamide Fluka with meta-iodo-benzyl-guanidine hydrochloride Aldrich followed by the transformation of the obtained salt in hydrogen carbonate then in sulphate.

The final product was purified by recrystallization from a mixture of ethanol and water 80/20, v/v and identified by analysis methods mass spectra, RMN, IR .

1.2. Synthesis of radio labelled compound

The cold molecule was labelled with iodine-125 Amersham by solid state transfer STT isotopic exchange reaction in presence of ammonium sulphate according to a modified Mangner et al. method [13,14]. The exchange occurs in acidic conditions created by in-situ decomposition of ammonium sulphate NH4. But not necessary in oxidant environment inert atmosphere, N_2 [12,15]. The radioactivity from Na125-I and 125I labelled compound was quantified with a radioisotope calibrator Robotron M27013 and gamma counter Berthold Mag 315. Both instruments were calibrated. Against an NBS standard solution of Na125I. Radio iodination may be achieved with 125I or 131I.

The radiochemical yield was ranged from 60% to 90%. The specific activity was 1,8 mCi / mgm of mIBG (0,6 mCi / mmol).

1.3. Purification of radio labelled compound.

The purification of radio labelled compound was achieved by dissolving the reaction mixture in sodium acetate buffer pH (4,2) and passing the solution through a silver chloride filter [12] to remove unreacted radioiodine.

$$AgCl + 125\text{-}I \rightarrow Ag\ 125\text{-}I + Cl^-$$

1.4. Purity determination

Radiochemical purity for 125-I-labelled compound was greater than 95% determined by radio-TLC on 2x12 cm silica gel coated tinfoil (Silufol-Kavalier) using two solvent systems and by radio-HPLC on a liquid chromatograph (Waters Millipore) equipped with ultraviolet (254 nm) detector using a bondapack C-18 (4.6×250mm). In this case the radioactivity was achieved by a gamma counter after collecting fractions of mIBG [4, 12].

1.5. Pharmacology study

The study concerns some pharmacological parameters of radio iodinated mIBG. The animals in each case were allowed free access to water and food throughout the experiment.

All injections were carried using aseptic techniques. The injectable solution of 125-I-mIBG was formulated in bacteriostatic 0,9% saline and has a radioactive concentration for 0,2 mCi per ml. The final product was sterilized by filtration over 0,22 um Millipore filter prior to injection. A volume of 200 µl and 500 µl of the preparation under studies corresponding to an activity of 0,04 mCi and 0,1 mCi of 125-I was injected intravenously via the lateral tail vein, to each animal mice and rats respectively.

Experimental

a) Blood clearance

Males NMRI mice** (N : 6) are used, volume of µl of 125-I-mIBG corresponding to an activity of 40µCi (1,48 MBq) of 125I, injected intravenously to each mouse. At different intervals (0,6, 3, 24 and 72 hours), the animals are killed by decapitation and their blood activity was measured by gamma counter.

b) Plasma binding

Males NMRI mice are used according to the same protocol of blood clearance. The animals are killed by decapitation and their blood was incubated in Marie bath (Bioblock-Polystat IV 86271) at 37°C for one hour. The binding percentage of the 125-I-mIBG to plasma proteins was studied by trichloroacetic acid (20%) precipitation. After centrifugation of the plasmatic proteins, their radioactivity was measured by a gamma counter.

c) Urinary excretion

Males Wistar rats** (N : 3) weighting 250-300 gm are used. A volume of 500 µl of the preparation, corresponding to an activity of 100µCi of 125 I, was injected intravenously.

The animals are placed in metabolic cages to collect urine in course of time (24, 48, 72, and 144 hours). Gamma radioactivity was determined and the quantity of urine excreted at different time intervals was expressed at percentage of the injected activity.

d) Tissue biodistribution studies

Biological tissues biodistribution studies were performed on male Wistar rats (N : 3), weighting 250-300 gm, injected intravenously with 100 µCi of 125I-mIBG in a volume of 500µl. The distribution of 125I-mIBG was determined at time intervals of 0,5, 2, 6, 24, 48, 72 and 144 hours after the administration of the labelled compound. The animals are killed under ether anaesthesia, then tissues and organs of interest are removed, placed into test tubes which are sealed and their weights determined. The representation samples of tissues were counted in a gamma counter with corrections made for background and counter efficiency.

Resultats and discussion

a) Blood clearance

The results of blood clearance study of 125I-mIBG are expressed in figure 1. The curve shows that the blood clearance is very high several hours post injection. It was found that the blood clearance of radio labelled mIBG in human was also rapid [16].

b) Plasma binding

The results of plasma binding are shown in table 1. These results show, upon the curve, that the plasma binding is very low one hour post injection. However it is very high 72 hours post injection.

c) Urinary excretion

The results of the renal elimination at different time intervals are shown in table 2. We note a rapid excretion of radioactivity 24 hours post injection upon the curve presented on figure 2. However, we observe that 72 hours post injection, the radioactivity is important relatively, Kline et al found that the mean urinary excretion was 64% (range 53-70%) over the first 24 hr [16].

d) Tissues Biodistribution studies

The distribution of 125I - mIBG on nine selected tissues of the rat, at 72 hr after injection, was summarized in a table and expressed on the histograms shown in figure 3.

The radioactivity (cpm) per gram of tissues and organs was normalized according to the adrenal glands (cpm/ gm tissue / cpm/gm Adrenal glands) [12, 17]. These results show that the largest adsorbed dose from 125-mIBG is delivred to the adrenal gland.

However in the myocarde, the radioactivity is more important two hours post injection compared to the amount of activity in other organs.

The adrenal glands will be the target tissue 48 hours post injection. Also, the amount of free iodine released in vivo by the labelled compound no stocked is important in unblocked thyroid. The normal distribution of radioiodinated mIBG has been investigated in detail [15, 18]. As shown in figure 4, the estimated radiation dosimetry calculated from animal distribution studies using mIBG tagged with 125I and 131I, was 0,82 rads/mCi for the adrenal medulla, 0,14 rads/mCi for the heart and 0,03 rads/mCi for the whole body [19].

These data were applied to the standard MIRD formula. Further, the time of maximum uptake for all organs and tissues (% Kg dose/g) was calculated by the University of Michigan group [19].

CONCLUSION

The mIBG appears to follow the biological uptake and release pathways of norepinephrine and is localised within intracellular chromaffin storage granules. However the preliminary pharmacokinetics results obtained in this study show a rapid blood clearance of 125-I-mIBG, a low plasma binding one hour post injection and the majority of 125-I-mIBG is excreted in urine unchanged in the first 24 hours following administration. mIBG is taken up by a range of normal tissue, particularly those with a rich sympathetic innervation.

FOOT NOTES

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TABLES

Table 1 : *Plasma binding of 125I-mIBG in % dose of injected activity for different time intervals.*

Table 2 : *Urinary Kinetic in % dose of injected activity in urine*

FIGURES CAPTION

Fig. 1. *Comparison of the molecular structure of bretylium, guanethidine, norepinephrine and metaiodobenzyleguanidine.*

Fig. 2. *PLC Analysis after exchange radioiodination.*

Fig. 3. *Blood Clearance (a), Plasma Binding (b) and Urinary Excrection (c) of 125-I-mIBG.*

Fig. 4. *Histograms of distribution of 125-I-mIBG in rat unblocked (72 hours). The values of concentrations in count/min/mgm are normalized according to adrenal glands.*

Table 1

Time (hours)	0.6	03	24	72
Blood (x104-)	6.05±	3.62±	1.32±	0.054±
Supernatant (x104-)	0.327±	0.329±	0.218±	0.381±
Precipitate (x104-)	0.109±	0.654±	0.054±	0.763±

Table 2

Collecting times of urine (hours)	0 – 24	0 – 48	0 – 72	0 – 144
% dose of activity	16.83	1.52	4.69	1.25

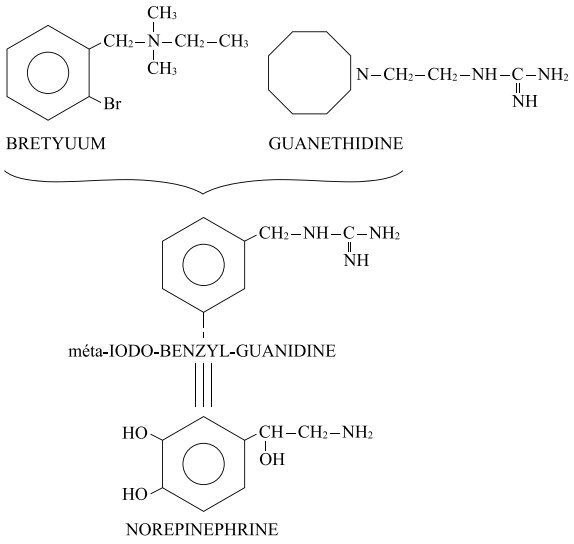

Fig. 1

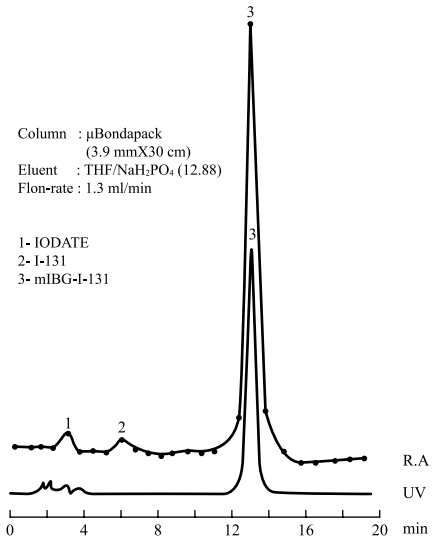

Fig. 2

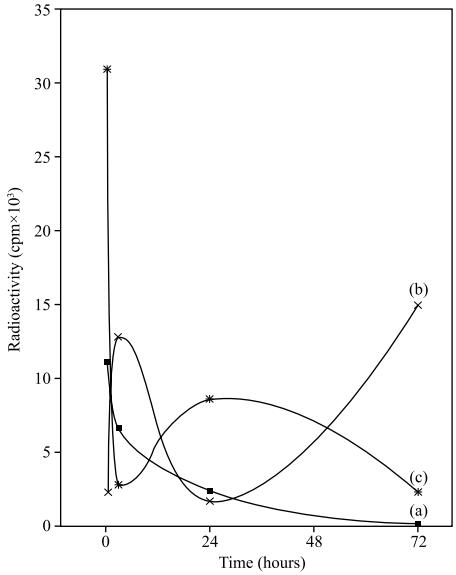

Fig. 3

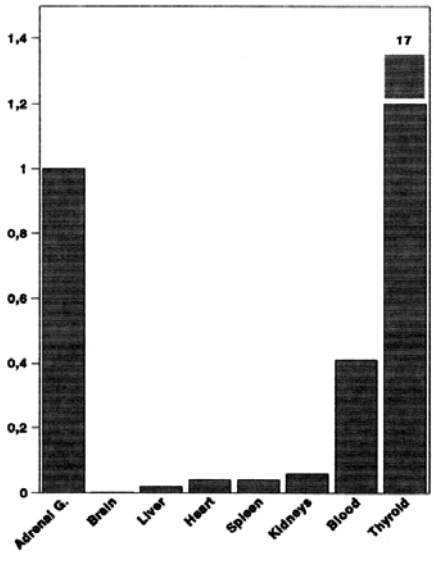

Fig. 4