TECHNETIUM-99m-DIMETHYLGLYOXIME (^{99m}Tc-DMG) AS RENAL IMAGING AGENT

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SUMMARY.- Dimethylglyoxime (DMG) labelled with ^{99m}Tc is here presented as a renal imaging agent. The behaviour of this complex was analysed at different pH by means of UV spectral data and using DMG-calcium chloride as a reference complex. Biokinetic data were evaluated in two biological models, Sprague-Dawley rats and *Didelphis albiventris* argentine opossum. Biodistribution in rats demonstrated fast and specific renal excretion. Time-activity values over both kidneys could be quantified for this complex. Renographic studies led to mean time-to maximum values on twelve assays of 2.0 ± 0.1 min and a mean relative function of 53.0 ± 2.3 and 47.0 ± 3.2 for right and left kidneys, respectively. ^{99m}Tc-DMG showed specificity for the renal excretion pathway and therefore seems to be a very useful radiopharmaceutical for renal function studies.

Keywords: Dimethylglyoxime, Technetium, Kidneys.

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INTRODUCTION

Measurement of renal flow using suitable radiolabelled compounds, renal gammagraphy and radiorenograms constitute the actual contribution of nuclear medicine and radiopharmacology to the evaluation of renal function (1-3). In fact, functional and morphologic studies may be carried out using radiopharmaceuticals. o-Iodohippuric acid (OIHA) labelled with I^{131} or I^{123} is currently used for tubular secretion studies.

In the past ten years the level of radiopharmaceutical chemistry and product development has risen rapidly. Research for a ^{99m}Tc compound as potential substitute of OIHA led to N,N'-bis(mercaptoacetyl)ethylenediamine (^{99m}Tc-DADS), which was however inferior to OIHA in specificity and excretion rate, specially in patients with impaired kidney function (4). Chemical changes in the ligand improved its clinical use but only for one of the stereoisomers (5).

A new ligand mercaptoacetyltriglycine (MAG3) as a hippuran replacement was also satisfactorily developed (6). This was the first ^{99m}Tc complex which showed biokinetic data close to OIHA. However, some problems occur with the kit formulation of this radiopharmaceutical. A recent biological investigation of ^{99m}Tc-MAG3 and the impurities produced during the complexation reaction allowed the quantification of biokinetic data (7). Moreover, C-methylsubstituted derivatives of ^{99m}Tc-MAG3 revealed interesting characteristics with regard to the determination of relative functional renal mass for one of the isomers (B) of the ^{99m}Tc-mercaptoacetylglycyl-L-alanylglycine (^{99m}Tc-MAGAG-LB)(8).

Also, ^{99m}Tc-diethylenetriaminepentacetic acid (^{99m}Tc-DTPA) (9) is still a suitable radiopharmaceutical for determination of glomerular filtration rate, possessing in great extent the "ideal" properties of a functional renal radiopharmaceutical.

As seen above, different organic ligands have been used in the development of radiopharmaceuticals for renal studies. In this paper, we describe a simple ligand dimethylglyoxime (DMG) labelled with ^{99m}Tc as a renal imaging agent. The crystal data for the ^{99}Tc-Sn-DMG complex (10), and the preparation

of a series of ^{99m}Tc-dioxime complexes, their structures, their physico-chemical parameters as well as their stability as a function of pH (11) have been previously reported. The biological behavior under labelling controlled conditions is here evaluated. The use of labelled oximes is not new in nuclear medicine, several derivatives were synthesized, among them a neutral boronic acid adduct of technetium dimethylglyoxime complex (BATO) (12), but they were used in diagnosis of cerebral function (12-14).

MATERIALS AND METHODS

Dimethylglyoxime (DMG) was purchased from ALDRICH, USA, and was purified from side-products (2,3-butanedione monoxime) by semipreparative HPLC (Fig. 1 A). Chemical purity of the ligand was analysed by HPLC with an isocratic system (LKB 2221 pump and integrator) using reverse phase columns (250 x 5mm, RP 18 Varian Micropak, 10 μm). The columns were eluted at a flow rate of 1 mL/min with methanol-water (80:20). The eluent was monitored with an UV detector (Pharmacia LKB VWM 2141) at 254 nm. $^{99m}TcO_4^-$ was eluted from a commercial generator (Medgenix Diagnostics).

Kit preparation: Solution A: DMG (1 mg) was added to a physiological saline solution (2 ml) and heated until complete dissolution.
Solution B: sodium tartrate (40 mg), and stannous chloride (0.2mg) were added to physiological saline solution (2 ml) and heated until complete dissolution.
Solution C: Solution A and 3 drops of solution B were heated and pH was adjusted to 9.5, 555 MBq (15 mCi) of $^{99m}TcO_4^-$ (6.5ml) were added to solution C

Labelling was performed at different selected pH (6.0, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10). pH was adjusted to the required value using 5% NaOH solutions.

Labelling efficiency was determined at all above mentioned pH by ascendent chromatography on paper strips (Whatman 1) using physiological saline and methylethylketone (MEK), respectively, as mobile phases. The chromatograms were scanned for radioactivity with an ionization chamber detector. The purity of the ^{99m}Tc-DMG complex was controlled by analytical HPLC under the same conditions as described above but using a scintillograph Alphanuclear E II

coupled to the chromatograph (Fig. 1 B).

Animal Experiments:

Biodistribution studies were performed at pH 8.5, with Sprague-Dawley rats (both sexes, 200-300 g body weight), in groups of 6 rats each, anaesthetized by i.p. injection of sodium thiopental (60 mg/kg). Each animal was placed in a supine position and the radiopharmaceutical (0.15 ml) was i.c. bolus injected. At different times post-administration (2, 5, 10, 15, 30, 60, 120 min) animals were sacrificed by cervical disarticulation. Liver, brain, kidneys, urine and blood were counted with an automatic gamma detector (Clinigamma Pharmacia) and compared to injected activity determined by a dose calibrator (Vexal).

Radiorenographic studies were performed with the argentine opossum (*Didelphis albiventris*, Marsupialia- Didelphidae) (3.5 - 5 kg body weight; 12 animals). Anaesthesia was induced by i. m. injection of ketamine chlorhydrate (40 mg/kg), followed by i. p. injection of sodium thiopental (60 mg/kg). Each animal was placed in a supine position under the gamma camera (OHIO 410) fitted with a pinhole divergent collimator in an exactly defined distance (3 cm). After i. c. bolus injection of 55.5 MBq (1.5 mCi; 0.6 mL) of the technetium -DMG complex, twenty minute images were recorded at 30 sec intervals, using an on-line data processing system (Alphanuclear programm and computer). A static acquisition was obtained at 60 min post-administration.

Regions of interest (ROI) were placed over heart, kidneys and urinary bladder. The counts/ROI were corrected for physical decay of the radionuclide, soft tissue absorption and geometric factors of the collimator/camera system. From these data time-activity curves were calculated for each ROI.

RESULTS AND DISCUSSION

Chemical purity of ligand: Purity determined by HPLC accounted for 99.9%. Commercial DMG was previously purified from butanedione monoxime by semipreparative HPLC (Fig. 1 A). This is an important step in order to obtain reliable biological results.

Radiochemical purity: All preparations were higher than 90 % radiochemical

yield and remained stable over 24 h. With the labelling method tested neither reduced hydrolyzed technetium nor free pertechnetate were detected (Fig. 1B).

Biodistribution: Biodistribution results are presented as histograms in Fig 2. to 5, values of the percent uptake at 2, 30, 60, 120 min post administration were comparatively plotted. Fig. 2 shows uptake in liver, Fig. 3 in urine, Fig. 4 in blood and Fig 5 in brain.

The data show a fast kinetic of renal excretion of ^{99m}Tc-DMG with activity in urine accounting for about 60 % of injected dose as soon as 120 min post injection. Hepatic excretion is at a low level and a very small percentage remains in blood, both indicating a high specificity for the renal excretion pathway. The complex showed no significant accumulation of activity in brain, similarly in lungs, during the period of investigation.

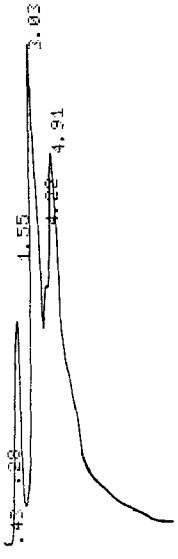

Fig. 1A. HPLC of commercial DMG

t_R 1.55 : Butanedione monoxime;

t_R 3.03, 4.22, 4.91 min: E/Z isomers of DMG

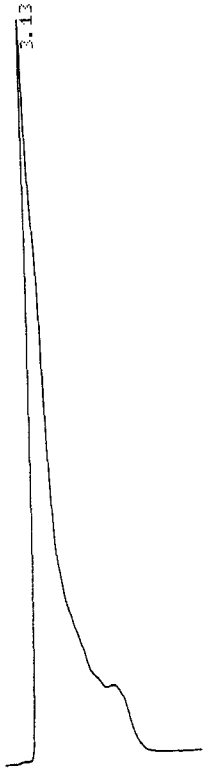

Fig. 1B. HPLC of ^{99m}Tc-DMG complex

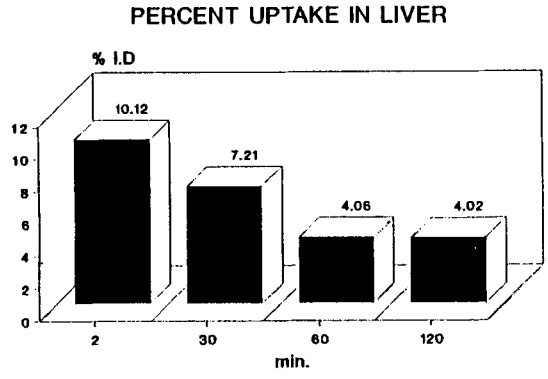

Fig. 2

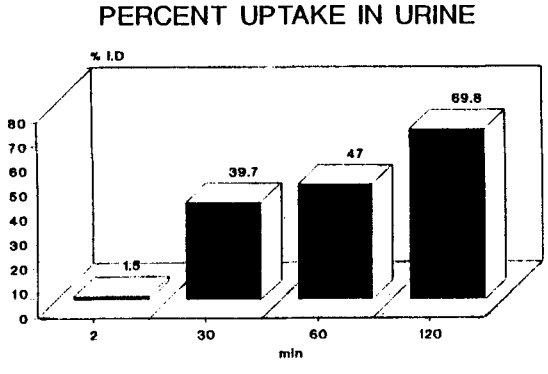

Fig. 3

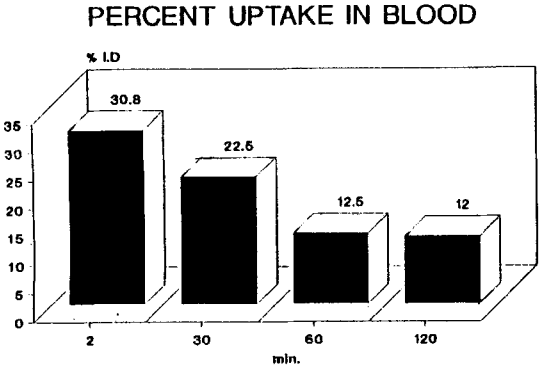

Fig. 4

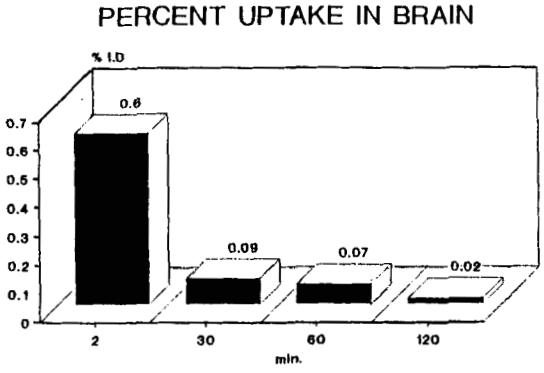

Fig. 5

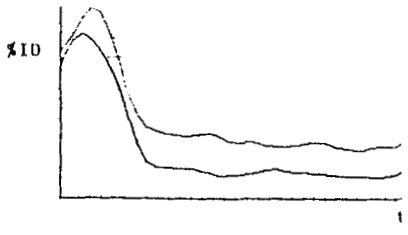

Fig. 6. Renogram of ^{99m}Tc-DMG

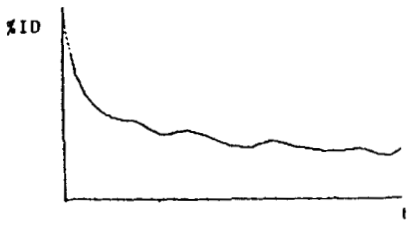

Fig. 7. Ventricular elimination of ^{99m}Tc-DMG

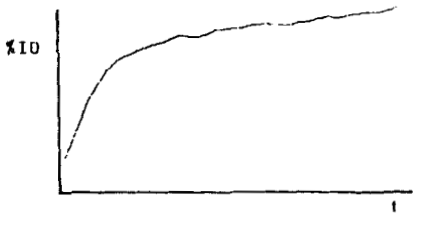

Fig. 8. Cumulative excretion of ^{99m}Tc-DMG into the urinary bladder of opossum

Radiorenographic studies: Fig 6 exhibits a renogram acquired with the ROI-technique as example of the renograms obtained. Mean values of 12 renograms provide a time to maximum 2.0 ± 0.1 min and a relative function of 53.0 ± 2.3 %, and 47.0 ± 3.2 % for right and left kidneys, respectively. Fig 7 shows the ventricular elimination curve or plasmatic clearance for a clearance value of 50 for 2.5 ± 0.8 min. Fig. 8 shows bladder filling accounting for renal clearance with an accumulation value of 50 for 30.0 ± 0.9 min.
In Fig. 9 the scintiphotos of ^{99m}Tc-DMG are shown. The complex is excreted nearly exclusively through the kidneys into the urinary bladder.

The opossum renograms revealed a maximal renal accumulation at 2.0 ± 0.1 min post injection for ^{99m}Tc-DMG versus about ^{99m}Tc-MAG3 at 1.6 ± 0.09 min post injection. Bladder was not visualized before 2.0 min post injection whereas hepatic activity was nearly absent.

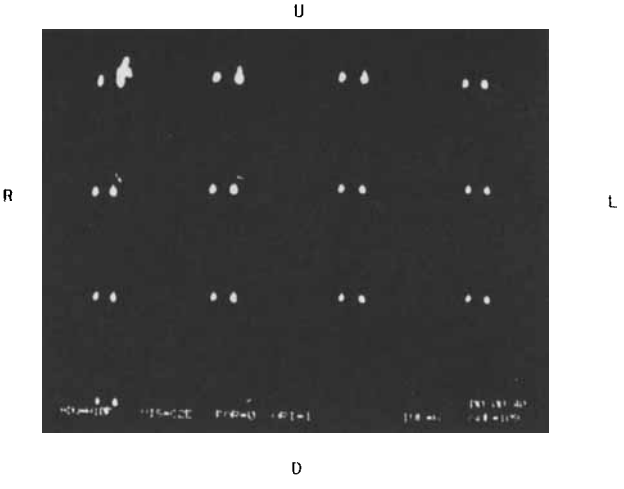

Fig. 9. Scintiphotos in opossum after intracardiac administration of ^{99m}Tc-DMG (55.5 MBq) from head to abdomen.

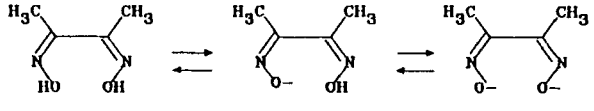

Fig. 10. Structures of DMG at different pH.

Images with excellent kidney to background ratio were obtained due to the pronounced accumulation in the kidneys and the high plasma clearance.

The renal elimination of ^{99m}Tc-DMG as determined from the renograms and the excretion pattern is comparable to that of ^{99m}Tc-MAG3 (or ^{99m}Tc-DTPA) under similar conditions. Whether this behaviour is the result of glomerular filtration or is caused by a low tubular extraction rate has not yet been investigated.

CONCLUSIONS

^{99m}Tc labelling of DMG is pH dependent (11). According to the pKa1 = 10.6, and pKa2 = 11.9 values for ionisation of DMG (15), three structures **A**, **B** and **C** are proposed (Fig.10). Thereafter at the labelling pH (9.5) tested herein in the biological models prevails **A** as ligand.

Concerning the structure of the ^{99m}Tc-DMG complex formed, this is in agreement with that proposed by Salako and Theobald (11), a Tc (V) complex with the core *trans*-TcO_2^+, anionic nature and further stabilized by H-bond bridges involving the oxime groups. Although Deutsch *et al.* (10) (tris oxime ^{99}Tc-Sn-DMG complex of Tc V) and Linder *et al.*(16) (neutral tris oxime complexes of Tc III) have proposed structures based on X-ray data, these complex-structures probably change in solution as suggested by Salako and Theobald (11). Moreover, complexes in water are expected to contain H-bond bridges involving the oxime groups. Further studies on the chemical nature of these and related complexes are being carried out in our laboratories.

According to the structure and size of the complex, supported by the biodistribution and scintigraphic data, ^{99m}Tc-DMG has a higher and faster renal accumulation without interference of other organs, allowing immediate imaging after injection. The radiation dose is also lower due to the faster urinary excretion.

Radiorenograms also exhibited the renal maximum followed by an excretion phase comparable to those of ^{99m}Tc-MAG3. Therefore ^{99m}Tc-DMG is a suitable renal function radiopharmaceutical. Moreover, the availability of DMG as ligand increases the possibilities of the use of this compound in other biological studies that are currently in progress.

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