^{99m}Tc-d,l HMPAO. KIT FORMULATION AND QUALITY CONTROL

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ABSTRACT

The d,l diasteroisomer of hexamethylpropyleneamine oxime (HMPAO) labelled with ^{99m}Tc is an efficient cerebral perfusion imaging agent that is widely used in Nuclear Medicine. The synthesis of d,l HMPAO was developed in Demokritos Laboratories. Lyophilized kit of HMPAO was formulated (500µg d,l HMPAO and 12.5µg $SnCl_2$ anhydrous) and labelled with ^{99m}Tc, with different volumes and different activities. The radiochemical composition of ^{99m}Tc- HMPAO was determined in three chromatographic systems: 1) ITLC-SG, 0.9% NaCl; 2) Whatman 1, MEK and 3) Whatman 1, 50% acetonitrile. Radiochemical purity was assayed 5, 15, 30 and 60 minutes after labelling and, the stability of lyophilized kit, kept at 4° C, was evaluated until 6 months. Biodistribution was performed in Swiss Albino mice . The results were expressed as % dose/g and % dose/organ. The yield of labelling were 93.99; 94.47; 92.00 and 89.58 % at 5, 15, 30 and 60 minutes, with an stability no more than 30 minutes after the addition of ^{99m}Tc. The kit was stable until 6 months (94.5 %). Biological studies in mice showed a brain uptake of 4.4, 4.53, 4.80 and 6.39 % dose/g at 5, 15, 30 and 60 minutes after i.v. dose, respectively.

INTRODUCTION

The main biological requeriments for new rCBF tracers labelled with ^{99m}Tc are the abilities to cross the intact blood brain barrier (BBB) and to distribute in the brain proportionally to blood flow. Once in the brain, it should retain a fixed regional distribution for a time to permit imaging acquisition. This time is 20-30 minutes for a rotating gamma camara system (SPECT).

A ^{99m}Tc complex in order to penetrate BBB has to be relatively small (< 50 dalton), lypophilic an with net charge of zero. Loberg et al. [1-2] and Oldendorf [3-4] proposed a kind of radiopharmaceuticals with these characterics. The approach to this problem is to prepare a neutral stable complex of ^{99m}Tc with suitable physical and biological properties (such stability, lipid solubility).

Troutner, Volker et al. [5-6] have studied the ^{99m}Tc-complex of propylene-amine-oxime (PnAO), a neutral and lypophilic agent. A large number of derivates of PnAO were synthetized and evaluated. The d,l HMPAO labelled with ^{99m}Tc is an efficient cerebral perfusion imaging agent that is widely used in Europe and has received FDA aproval for use in the USA [7]. This agent is particularly valuable because does not redistribue within the brain for several hours after its initial localization. The uptake within the brain is believed to result from the inherent instability. However, the "in vitro" instability is a major limitation to the potential clinical aplication of ^{99m}Tc-d,l HMPAO in Nuclear Medicine.

The aim of this study was the development of HMPAO lyophilized kit; the pure d,l HMPAO was obtained in Demokritos Laboratories [8]. Lyophilized kits were formulated and labelled with ^{99m}Tc with different volumes (1, 3 and 5 ml) and different activities (37, 111, 370 and 1110 MBq). This paper describes the radiochemical studies and biological assays in mice.

MATERIALS AND METHODS

The synthesis and methods used for the preparation of the d,l HMPAO was based on method previously reported by Neirinckx [9] with some modifications [8] in Demokritos Laboratories. Technetium-99m was obtained from IPEN-TEC / SP (Brasil) generator.

1- Formulation of the instant freezed dried kit: 25 mg of pure d,l HMPAO was mixed under magnetic stirring with 18 ml oxigen free water. The mixture was cooled in ice bath, nitrogen was bubbled and pH was adjusted to 1.0 with 5N HCl. After complete dissolution of HMPAO, the pH was adjusted to 5.5 with 2N NaOH. Then 0.3 ml of SnCl2 solution (45 mg SnCl2 anhydrous in 1.0 ml conc. HCl (boilled) and diluted with 20 ml water) was added with stirring. The pH was adjusted to 9.5 with 2N NaOH and the solution was diluted to 25 ml by 0.01M phosphate buffer. The stock solution was divided (0.5 ml) in vials and lyophilized ("INTERFRIGO") at 0°C during 24 hr. The vials were sealed under vacuum.

2- Radiochemical quality control: A combination of three chromatographic system was used for a characterization of the radiochemical composition of d,l HMPAO kit, based on Neirinckx et al. report [9] with some modification. Figure 1 shows the Rf values of the ^{99m}Tc components on ITLC-SG (Fluka) and Whatman N. 1 strips. The difference is that the strips used in 50% ACN and MEK are Whatman 1.

Eluated of ^{99m}Tc generator was taken, and 1, 3 and 5 mL (1 - 2 mCi/mL) were injected into the vial. The vial was shaken until complete dissolution and the radiochemical purity (rate of decomposition) was assayed 5, 30, 60 and 120 minutes after labelling for the stabillity determination. Self-life of the kit was studied up to 6 months after preparation.

A drop test sample was applied 1 cm from the base of the chromatography strips. The ascending chromatograms were developed in flask containing fresh solvents (2 - 3 mL). The strips were dried and the radioactivity distribution was analysed in gamma counter (ANSER-ABBOTT).

The quantitative assessment of the radiochemical composition was determined using the following relationship:

$\%TcO_2$ = % activity remaining at the origin in the system 3

$\%TcO_4$ = % activity in the front of system 1

% secondary complex = % activity in the origin of the system 2 minus % TcO_2 system 3

3- Biological distribution in mice: Biodistribution studies were performed in Swiss-Albino mice (25-35g). Each animal was injected with 20 - 40 µCi / 0.1ml test preparation through the tail vein. Radiochemical quality control was performed, and at all times (5 - 30 min.), the purity of ^{99m}Tc - complex was not less than 92.00 %.

At different periods after the i.v. injection (5, 15, 30 and 60 minutes) the animals were sacrified sample of blood were colleted and the other organs were removed intact and weighed. The radioactivity was determined in an automatic gamma counter (BERTHOLD). The % dose/g and % dose/organ, were evaluated by comparison of tissue radioactivity level to standard (0.1ml test sample in 10 ml water).

RESULTS

Pure d,l HMPAO was formulated as instant dried kit. Formulation was performed under inert atmosphere N_2 at low temperature, in order to minimize the formation of chemical impurities.

Addition of $^{99m}TcO_4$ to a freeze dried HMPAO kit produces a mixture of radiochemical species wich changes with time. In addition to the desired lipophilic complex, there are free pertechnetate, reduced-hidrolized technetium (TcO_2) and an unidentified secondary complex which less liphophilic than ^{99m}Tc-HMPAO [8]. ^{99m}Tc-HMPAO rapidly decomposes in aqueous medium, the presence of buffer is essential due the ^{99m}Tc eluates have quite different pH values and the buffer brings a constant pH. This fact allows formulation of more stable kit based on Volkert et al. observation [10 - 11].

Table 1 shows radiochemical purity of ^{99m}Tc-d,l HMPAO as a function of pertechnetate volume (1 to 5 ml), 93.68 - 92.87 % respectively. Labelling efficient was over 96% when pertechnetate activities were 111 - 370 Mbq (Table 2). Complex is stable for 30 minutes after labelling (92.00%), formation of secondary complex and $^{99m}TcO_2$ increased in function of time (Table 3) .Stability studies of the freezed dried kit after storage at 4° C for a period of 6 months, demostrated satisfactory labelling efficient (94.5%). The use of ^{99m}Tc-HMPAO with 85% of labelling has been reported and the validity of this product could be until 60 minutes after labelling. Otherwise, it is convenient to follow the pattern method, injecting in animals between 15 - 30 minutes (Table 3).

The biodistribution date in mice of 99mTc- d,l HMPAO are shown in Table 4 and 5, significant uptake and retention into brain tissue were observed: 4.4: 4.53: 4.80 and 6.39 % dose/g at 5, 15, 30 and 60 minutes after i.v. dose, respectively.

TABLE 1 - Effect of ^{99m}Tc (pertechnetate) volume on the radiochemical purity (%) of ^{99m}Tc-d,l HMPAO

^{99m}Tc-compounds / Volume(ml)	1	3	5
$^{99m}TcO_4^-$	0.20	0.67	0.48
$^{99m}TcO_2$	4.34	3.25	1.75
Secondary complex	1.78	2.66	4.90
^{99m}Tc-d,l HMPAO *	93.68	93.42	92.87

* (n = 4) 15 minutes after labelling

TABLE 2 - Effect of ^{99m}Tc (pertechnetate) activities on the radiochemical purity (%) of ^{99m}Tc-d,l HMPAO

^{99m}Tc-compounds / Activities (MBq)	37	111	370	1110
$^{99m}TcO_4^-$	0.21	0.19	0.32	2.77
$^{99m}TcO_2$	2.54	1.24	1.52	2.22
Secondary complex	1.67	2.33	2.14	1.40
^{99m}Tc-d,l HMPAO *	95.58	96.24	96.06	93.61

* (n = 4) 5 minutes after labelling

TABLE 3 - Radiochemical purity (%) of ^{99m}Tc-d,l HMPAO in function of time (min.) after labelling

^{99m}Tc-Compounds / Tempo (min.)	5	15	30	60
$^{99m}TcO_4$	0.19	0.06	0.41	0.41
$^{99m}TcO_2$	1.11	1.52	2.26	2.61
Secondary complex	4.71	3.95	.4.97	7.75
^{99m}Tc-d,l HMPAO *	93.99	94.47	92.00	89.58

* (n = 4) 37 - 74 MBq / 1 - 2 ml ^{99m}Tc

TABLE 4 - Biological distribution of ^{99m}Tc-d,l HMPAO in mice (n = 4), expressed in % dose /g

Organs / Time (min.)	5	15	30	60
Kidney	16.50	14.85	11.96	13.44
Liver	9.73	8.53	7.67	7.46
Heart	8.49	7.73	6.30	8.72
Lung	26.39	29.00	18.99	25.00
Spleen	4.89	2.99	3.36	3.31
Stomach	7.33	6.60	5.91	5.04
Intestine	4.83	7.14	7.95	12.07
Muscle	2.04	1.97	1.74	1.96
Brain	4.44	4.83	4.76	6.39
Blood	0.86	0.74	0.65	0.69

TABLE 5 - Biological distribution of ^{99m}Tc-d,l HMPAO in mice (n= 4), expressed in % dose/ organ

Organs / Time (min.)	5	15	30	60
Kidney	4.80	4.45	3.60	4.04
Liver	9.70	8.46	7.06	6.73
Heart	0.85	0.78	0.68	0.89
Lung	5.24	4.76	3.45	3.84
Spleen	0.26	0.19	0.23	0.17
Stomach	1.32	1.12	0.78	0.78
Intestine	7.80	9.36	11.66	16.49
Muscle	0.25	0.22	0.20	0.21
Brain	1.64	1.83	1.83	2.21
Blood	0.86	0.74	0.65	0.69

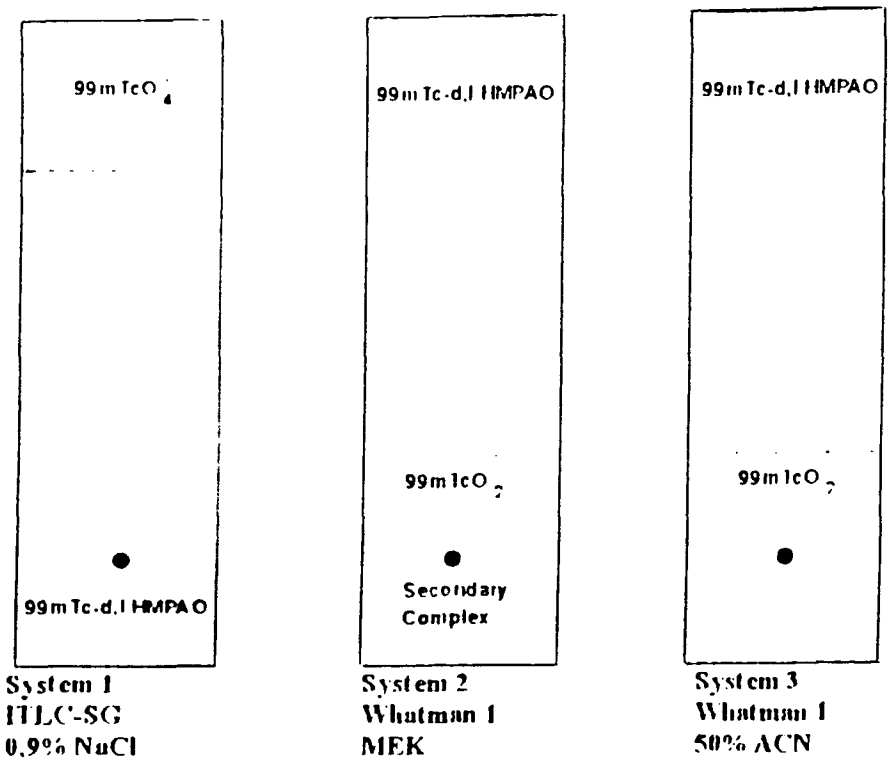

FIGURE 1 - Rf values of ^{99m}Tc-d,lHMPAO in different chromatographic systems

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