Intracellular Localization of ^{99m}Tc -*d,l*-HMPAO in Rat Blood

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Key Words

HMPAO · Blood · Intracellular · Localization · Rat

Abstract

Objective: The objective of this study was to investigate the intracellular localization of technetium ^{99m}Tc -hexamethylpropylene amine oxime (^{99m}Tc -*d,l*-HMPAO) in rat red blood cells (RBCs) and plasma. **Materials and Methods:** Sprague-Dawley rats ($n = 36$) were injected with 129.5 MBq ^{99m}Tc -*d,l*-HMPAO. Using a heparinized syringe, 2-ml blood samples were withdrawn from the rats 10, 20, 30, 40, 50 and 60 min after ^{99m}Tc -*d,l*-HMPAO injection. Blood was centrifuged for 10 min at 2,240 rpm to separate RBCs from plasma. A chloroform/methanol/distilled water mixture was added to the RBCs and plasma in separate tubes to extract ^{99m}Tc -*d,l*-HMPAO into aqueous (Aq) and lipid (Lp) phases. The ^{99m}Tc -*d,l*-HMPAO activities in Aq and Lp phases were measured separately using a dose calibrator. The data were analyzed using Student's *t* test and a one-way ANOVA. **Results:** More than 70% of the injected dose of ^{99m}Tc -*d,l*-HMPAO was retained in the RBCs for up to 60 min, compared to less than 20% in plasma. The uptakes in the Aq for plasma were 88.77 ± 1.04 , 94.23 ± 2.42 , 90.15 ± 1.88 , 98.59 ± 0.89 , 96.42 ± 1.50 and $97.59 \pm 1.92\%$ at 10, 20, 30, 40, 50 and 60 min after injection, respectively. The corresponding uptakes in the Lp phases were: 11.23 ± 0.04 , 5.77 ± 0.07 , 9.85 ± 0.06 , 1.41 ± 0.08 , 3.58 ± 0.04 and 2.41 ± 0.06 , respectively. Similar val-

ues ($\% \pm \text{SE}$) and trends were obtained in the Aq and Lp phases for RBCs. A highly significant difference between the Aq and Lp phases at each time point studied was observed ($p < 0.0001$). There was no statistically significant difference between the time points in the Aq phase, but there was a significant difference ($p < 0.001$) between time points in the Lp phase. **Conclusion:** This study demonstrated that most of the intracellular localization of ^{99m}Tc -*d,l*-HMPAO was in the Aq phase compared to Lp phase in both RBCs and plasma up to 60 min.

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Introduction

Technetium ^{99m}Tc -hexamethylpropylene amine oxime (^{99m}Tc -*d,l*-HMPAO) is a lipophilic radiotracer that is used in cerebral perfusion scanning to detect variations in regional brain blood flow [1]. ^{99m}Tc -*d,l*-HMPAO is thought to accumulate in the brain through its intracellular conversion from a lipophilic to a hydrophilic form within the brain parenchyma [2–4]. In addition, it has been used to label leukocytes for detection of inflammation [5]. Cell labeling can provide information of clinical importance, which depends on the uptake and/or the intracellular localization of ^{99m}Tc -*d,l*-HMPAO. One study investigated the intracellular localization of ^{99m}Tc -*d,l*-HMPAO in the rat brain [6], and a prior study investigated the uptake of

^{99m}Tc -*d,l*-HMPAO in rat red blood cells (RBCs) and plasma. Therefore, in the present study, we investigated the intracellular localization of ^{99m}Tc -*d,l*-HMPAO in rat RBCs and plasma.

Materials and Methods

Materials

An HMPAO pharmaceutical kit (exametazime) was purchased from GE Healthcare (Chalfont St. Giles, UK). ^{99m}Tc -pertechnetate ($^{99m}\text{TcO}_4^-$) was eluted from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator (GE Healthcare). All other chemicals used in this study were supplied by Sigma-Aldrich (Gillingham, UK).

Experimental Animals

Sprague-Dawley rats ($n = 36$) of 300 ± 50 g weight and bred at the Animal Facility of the Faculty of Medicine, Kuwait University, were used in this study. The animals had free access to water and food and were handled in accordance with ethical standards, approved by the Institutional Ethics Committee as recommended by the Helsinki Declaration.

Preparation of ^{99m}Tc -*d,l*-HMPAO

Fresh elutes of ^{99m}Tc -pertechnetate were used at each time point to prepare ^{99m}Tc -*d,l*-HMPAO, following the manufacturer's instructions and recommendations [1]. In brief, 2,960 MBq of ^{99m}Tc -pertechnetate in 5 ml saline was added to a freeze-dried exametazime kit to prepare ^{99m}Tc -*d,l*-HMPAO. ^{99m}Tc -pertechnetate was eluted less than 3 h earlier from a generator which had been eluted less than 24 h previously [1, 7].

Experimental Protocol

Each rat was anaesthetized by intraperitoneal (i.p.) injection with 20 mg/kg intraval sodium (thiopentone sodium; Rhone Merieux, Dublin, Ireland) 10 min before starting the experiment. An intravenous (i.v.) line was placed in the tail vein of each rat for 10- to 15-min injection time of ^{99m}Tc -*d,l*-HMPAO. Then, 129.5 MBq of ^{99m}Tc -*d,l*-HMPAO was injected within 30 min of ^{99m}Tc -*d,l*-HMPAO preparation, and followed by a saline push administered via the fixed i.v. line. Five-milliliter syringes flushed with 0.2 ml 5,000-IU/ml heparin sodium (Harlan Sera-Lab, Leicestershire, UK) were used to withdraw 2-ml blood samples from rats 10, 20, 30, 40, 50 and 60 min after ^{99m}Tc -*d,l*-HMPAO injection. The number of rats was 6 for each time point. Whole blood volume was determined and activity was measured using a dose calibrator (Atomlab 100; Biodex Medical Systems, Shirley, N.Y., USA). Then, the blood was centrifuged for 10 min at 2,240 rpm to separate plasma from RBCs, and the activity was determined separately.

Aqueous-Lipid Extraction Method

The aqueous-lipid extraction procedure was modified from Bligh and Dyer [8] and Al-Saeedi et al. [9]. In brief, the activity in either RBCs or plasma samples was extracted using a chloroform/methanol/distilled water solvent system. Methanol (1 ml) and chloroform (0.5 ml) were added to each RBC or plasma sample and left at 4°C for 5 min, after which chloroform (0.5 ml) was

added to the suspension followed by distilled water (0.5 ml) with thorough mixing. The suspension was centrifuged for 10 min at 2,240 rpm and 4°C. The upper (aqueous, transparent or Aq) and the lower (lipid, cloudy or Lp) phases were separated, and their activities were determined separately using the dose calibrator. Measured activities from the separated Aq or Lp phases were normalized to volumes, and then expressed as a percent of the total activity in Aq and Lp RBCs or plasma.

Data Presentation and Statistical Analysis

All data, unless otherwise stated, are expressed as means $\pm$ SE. Student's *t* test was used to determine statistical differences between the Aq and Lp phases' ^{99m}Tc -*d,l*-HMPAO uptake, while the Kruskal-Wallis non-parametric one-way ANOVA was used to evaluate differences between time points. Statistical analysis was performed using SPSS version 13.0 software (Chicago, Ill., USA).

Results

The results showed that more than 70% of the injected dose of ^{99m}Tc -*d,l*-HMPAO was retained in the RBCs for up to 60 min, while less than 20% was in plasma. The total whole blood activity expressed in kBq and that in separated RBCs and plasma are given in table 1. The amount activity of plasma and RBCs at 10- to 60-min time points corrected for decay are given in table 2. The percentages retained in the Aq phase for plasma were 88.77 ± 1.04 , 94.23 ± 2.42 , 90.15 ± 1.88 , 98.59 ± 0.89 , 96.42 ± 1.50 and $97.59 \pm 1.92\%$ at 10, 20, 30, 40, 50 and 60 min after radiotracer, respectively. The corresponding percentages for Lp phases were 11.23 ± 0.04 , 5.77 ± 0.07 , 9.85 ± 0.06 , 1.41 ± 0.08 , 3.58 ± 0.04 and 2.41 ± 0.06 . Similar values and trends were obtained in the Aq and Lp phases for RBCs. We observed a highly significant difference between the Aq and Lp phases at each time point studied ($p < 0.0001$). There was no statistically significant difference between the time points in the Aq phase ($p = 0.416$), but there was a significant difference ($p < 0.001$) between time points in the Lp phase. In addition, there was a trend of decreasing activity uptake in the total blood activity as well as in the RBCs and its plasma and the Aq uptake; this was not as pronounced for the Lp uptake throughout the 60 min.

Discussion

In this study, the intracellular localization of ^{99m}Tc -*d,l*-HMPAO in rat blood was determined using the Aq-Lp extraction method. This method was useful in determining the intracellular localization of this tracer in both Aq

Table 1. Activity in Aq and Lp phases for plasma and RBC at different time points corrected for decay

	Plasma activity, kBq		RBC activity, kBq	
	Aq	Lp	Aq	Lp
<i>Time (min)</i>				
10	380.72 ± 2.94 (88.77 ± 1.04)	48.18 ± 0.49 (11.23 ± 0.04)	1,599.04 ± 12.34 (88.77 ± 0.74)	202.34 ± 2.07 (11.23 ± 0.06)
20	375.72 ± 13.35 (94.23 ± 2.42)	23.00 ± 0.30 (5.77 ± 0.07)	1,578.04 ± 56.08 (94.23 ± 3.03)	96.62 ± 1.26 (5.77 ± 0.09)
30	297.10 ± 8.17 (90.15 ± 1.88)	32.46 ± 0.18 (9.85 ± 0.06)	1,247.80 ± 34.33 (90.15 ± 1.93)	136.34 ± 0.74 (9.85 ± 0.14)
40	268.64 ± 2.14 (98.59 ± 0.89)	3.85 ± 0.27 (1.41 ± 0.08)	1,128.29 ± 8.99 (98.59 ± 0.88)	16.17 ± 1.12 (1.41 ± 0.05)
50	191.91 ± 7.02 (96.42 ± 1.50)	7.13 ± 0.25 (3.58 ± 0.04)	806.04 ± 29.49 (96.42 ± 1.44)	29.95 ± 1.03 (3.58 ± 0.07)
60	173.34 ± 5.20 (97.59 ± 1.92)	4.27 ± 0.13 (2.41 ± 0.06)	728.02 ± 0.59 (97.59 ± 1.01)	17.95 ± 0.54 (2.41 ± 0.03)

Figures in parentheses are percentages.

Table 2. Activity in whole blood, RBCs and plasma at different time points corrected for decay

	Blood, kBq	RBCs, kBq	Plasma, kBq
<i>Time (min)</i>			
10	3,641.91 ± 27.01	2,861.20 ± 21.87 (78.56)	657.08 ± 9.43 (18.04)
20	3,394.01 ± 59.2	2,535.66 ± 33.00 (74.71)	658.82 ± 7.88 (19.41)
30	3,642.28 ± 12.21	2,719.36 ± 73.55 (74.66)	512.77 ± 5.67 (14.08)
40	3,264.88 ± 82.14	2,359.77 ± 41.20 (72.27)	432.88 ± 6.23 (13.26)
50	3,061.01 ± 78.44	2,283.72 ± 108.48 (74.60)	308.02 ± 7.69 (10.06)
60	3,021.05 ± 51.06	2,285.12 ± 6.15 (75.64)	258.60 ± 3.45 (8.56)

Figures in parentheses are percentages.

and Lp phases of the RBCs or the plasma of rat blood. This method also can result in optimum lipid and non-lipid extraction, as previously demonstrated [8].

Our results are partially in agreement with a previous study that reported that more than 15% of the injected dose of $^{99m}\text{Tc-}d,l\text{-HMPAO}$ was retained in the plasma or RBCs after 10 min [10]. However, we demonstrated that the retention of $^{99m}\text{Tc-}d,l\text{-HMPAO}$ in RBCs was more than the double that of plasma and more than 70% of the injected dose at 60 min; thus, confirming the study of Nakamura et al. [11] in which approximately 60% of activity was retained of patients with $^{99m}\text{Tc-}d,l\text{-HMPAO}$.

In this study, the retained $^{99m}\text{Tc-}d,l\text{-HMPAO}$ in both RBCs as well as plasma was demonstrated to be mostly as water-soluble metabolites, and thus mostly found in the Aq phase compared to the Lp phase. An experimental study, similar to our study, investigated the accumulation and retention mechanisms of $^{99m}\text{Tc-}d,l\text{-HMPAO}$ in cultures of dissociated rat cerebellum upon chloroform extraction and distribution between Aq and Lp phases. This study reported 69.1% of the retained $^{99m}\text{Tc-}d,l\text{-HMPAO}$

in the Aq phase, 6.8% in the Lp metabolites phase and 24.1% in the other metabolites phase of the rat cerebellum [12]. Although the study was different in the type of tissue used (we used whole blood samples), our results have similar values of %Aq and %Lp uptake and are in agreement that the $^{99m}\text{Tc-}d,l\text{-HMPAO}$ uptake was demonstrated to be mostly as water-soluble metabolites.

$^{99m}\text{Tc-}d,l\text{-HMPAO}$ is currently used for cerebral perfusion studies. The accepted mechanism by which $^{99m}\text{Tc-}d,l\text{-HMPAO}$ activity accumulates in the brain is through its intracellular conversion from a lipophilic form to more hydrophilic derivatives within the brain parenchyma [13]. In this study, the accumulation of $^{99m}\text{Tc-}d,l\text{-HMPAO}$ in both RBCs and plasma could also be due to conversion of lipophilic $^{99m}\text{Tc-}d,l\text{-HMPAO}$ to the hydrophilic form that binds to blood proteins [13]. There was no statistically significant difference between the time points studied for the %Aq phases, but there was a significant difference for the %Lp phases. This is because the Lp phase was in the lower part of the glass tube used for sampling, and thus it was difficult to obtain the correct amount of Lp,

especially during sampling, which may have led to loss of Lp volumes and the normalized activities also leading to the appearance of irregular %Lp uptake between the time points studied. In addition, some chloroform evaporated during the separation method, but this was not serious provided the decrease in volume of some samples was not too great [8]. This may also suggest lipid-soluble metabolite changes and products, but these changes are still not well explained. Further investigations are needed to explain these changes.

^{99m}Tc -*d,l*-HMPAO has been used in other clinical applications such as a pure indicator of infiltration in the bone marrow [14], a breast cancer cell lines imaging agent [15] and as labeling antigen-loaded dendritic cells for cancer therapy [16]. Our results in this study provide a suggestion for further work to investigate the potential pharmacodynamic changes of liposomes and tumor or infection imaging when coupled with targeting antibodies or lipids [17].

Conclusion

This study demonstrated that most of the intracellular distribution and localization of ^{99m}Tc -*d,l*-HMPAO are in the Aq phase rather than in the Lp phase in both RBCs or plasma at each of the time points studied. Our result may provide a foundation for future investigations of ^{99m}Tc -*d,l*-HMPAO as a biomarker to other types of cells, such as cancer cells or liposomes.

Acknowledgements

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References

- 1 Neirinckx RD, Canning LR, Piper IM, Nowotnik DP, Pickett RD, Holmes RA, Volkert WA, Forster AM, Weisner PS, Marriott JA, Chaplin SB: technetium ^{99m}Tc -*d,l*-HMPAO: a new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. *J Nucl Med* 1987;281:191–202.
- 2 Kalra R, Wade KE, Hands L, Styles P, Campbelljohn R, Greenall M, Adams GE, Harris AL, Radda GK: Phosphomonoester is associated with proliferation in human breast cancer: a ^{31}P MRS study. *Br J Cancer* 1993; 67:1145–1153.
- 3 Lveill J, Demonceau G, Walovitch RC: Intrastudy comparison between technetium- ^{99m}Tc -ECD and technetium- ^{99m}Tc -HMPAO in healthy human subjects. *J Nucl Med* 1992; 33:480–484.
- 4 Zerarka S, Pellerin L, Slosman D, Magistretti PJ: Astrocytes as a predominant cellular site of (^{99m}Tc)-HMPAO retention. *J Cereb Blood Flow Metab* 2001;21:456–468.
- 5 Uslu H, Varoglu E, Kadanali S, Yildirim M, Bayrakdar R, Kadanali A: ^{99m}Tc -HMPAO labelled leucocyte scintigraphy in the diagnosis of pelvic inflammatory disease. *Nucl Med Commun* 2006;27:179–183.
- 6 Costa DC, Lui D, Sinha AK, Jarritt PH, Ell PJ: Intracellular localization of ^{99m}Tc -*d,l*-HMPAO and 201Tl-DDC in rat brain. *Nucl Med Commun* 1989;10:459–466.
- 7 Ceretec package insert: GE Healthcare, Chalfont St. Giles, UK, 2008.
- 8 Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911–917.
- 9 Al-Saeedi F, Welch AE, Smith TA: [Methyl- ^3H]Choline incorporation into MCF7 tumour cells: correlation with proliferation. *Eur J Nucl Med Mol Imaging* 2005;32:660–667.
- 10 Hayashida K, Nishimura T, Imakita S, Uehara T: Validation to eliminate vascular activity on ^{99m}Tc -HMPAO brain SPECT for regional cerebral blood flow (rCBF) determination. *Nucl Med Commun* 1991;12:545–550.
- 11 Nakamura K, Tukatani Y, Kubo A, Hashimoto S, Terayama Y, Amano T, Goto F: The behavior of ^{99m}Tc -hexamethylpropyleneamineoxime (^{99m}Tc -HMPAO) in blood and brain. *Eur J Nucl Med* 1989;15:100–107.
- 12 Suess E, Huck S, Reither H, Hrtnagl H, Angelberger P: Uptake mechanism of technetium- ^{99m}Tc -*d,l*-HMPAO in cell cultures of the dissociated postnatal rat cerebellum. *J Nucl Med* 1992;33:108–114.
- 13 Neirinckx RD, Burke JF, Harrison RC, Forster AM, Andersen AR, Lassen NA: The retention mechanism of technetium- ^{99m}Tc -HM-PAO: intracellular reaction with glutathione. *J Cereb Blood Flow Metab* 1988; 8(suppl 1):S4–S12.
- 14 Mariani G, Molea N, La Civita L, Porciello G, Lazzeri E, Ferri C: Scintigraphic findings on ^{99m}Tc -MDP, ^{99m}Tc -sestamibi and ^{99m}Tc -HMPAO images in Gaucher's disease. *Eur J Nucl Med* 1996;23:466–470.
- 15 Ballinger JR, Duncan J, Hua HA, Ichise M: Accumulation of ^{99m}Tc -HMPAO and ^{99m}Tc -ECD in rodent and human breast tumor cell lines in vitro. *Ann Nucl Med* 1997;11:95–99.
- 16 Blocklet D, Tungouz M, Kiss R, Lambertmont M, Velu T, Duriau D, Goldman M, Goldman S: ^{111}In -oxine and ^{99m}Tc -HMPAO labelling of antigen-loaded dendritic cells: in vivo imaging and influence on motility and actin content. *Eur J Nucl Med Mol Imaging* 2003;30:440–447.
- 17 Suresh M, Cao Y: A simple and efficient method for radiolabeling of preformed liposomes. *J Pharm Pharm Sci* 1998;1:31–37.