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SYNTHESIS OF Tc-D,D-HMPAO AND Tc-L,L-HMPAO AND THEIR COMPARISON OF CHEMICAL AND BIOLOGICAL PROPERTIES

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Abstract: Tc-D,D- and Tc-L,L-HMPAO were synthesized. The stability of Tc-D,D- and Tc-L,L-HMPAO in vitro is similar to that of d,l-isomers by the spectrophotometric and three strips methods. Cerebral uptake (%D in brain) for the L,L-isomer is higer than the D,D- and d,l-isomer in rats. Delayed studies shows that Tc-L,L-HMPAO reveals less washout and reflects a higher cerebral deposition properties than the D,D- and d,l-isomer.

Over the last decade, several brain imaging radiopharmaceuticals have been developed for routine cerebral blood flow imaging by single photon emission computed tomography (SPECT)[1], which include N-isopropyl-*p*-^{123}I-iodoamphetamine (IMP)[2]; *N,N,N'*-trimethyl-*N'*-2-hydroxy-3-methyl-5-^{123}I-iodobenzyl-1,3-propane-diamine (HIPDM)[3], ^{201}Tl-dimethyldithiocarbamate (DDC)[4], ^{99m}Tc-d,l-hexamethyl propylene-amine oxime (HMPAO)[5], and ^{99m}Tc-l,l-ethyl cystinate dimer (ECD)[6].

Tc-99m-d,l-HMPAO, the mixture of Tc-99m-L,L-HMPAO and Tc-99m-D,D-HMPAO, is widely used as an efficacious cerebral perfusion imaging agent all over the world[7]. It is unstable in the body and readily converts from primary highly lipophilic to a hydrophilic species[8]. The rapid intracellular conversion that prohibits fast diffusion readily across cell membranes is thought to be the reason of its retention in the brain and various organs. It is trapped in the brain due to the rapid hydrolysis to more polar ionization species that is believed to result from the inherent instability of Tc-99m-d,l-HMPAO[9]. However, the inherent instability also leads to relatively rapid decomposition in vitro. It means that Tc-99m-d,l-HMPAO must be administrated within half an hour from its formation and this is a major limitation for its potential clinical use. Although Tc-99m-d,l-HMPAO was widely studied, there was much less attention paid to which isomer has higher biological evaluations. Therefore, we synthesized Tc-D,D-HMPAO and Tc-L,L-HMPAO at both the carrier and non-carrier-added level and achieved by reconstituting a lipophilized kit with pertechnetate. The chemical and biological properties of the Tc-99m-L,L-HMPAO, Tc-99m-D,D-HMPAO and Tc-99m-d,l-HMPAO were investigated and compared. In addition, cerebral images of rats were obtained to study the L,L-, D,D- and d,l-isomer tracer distribution within the brain and the cerebral uptake (%D in brain) for their isomer were measured.

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The spectrophotometric and three strips methods[10] allow the analysis of Tc-99m-D,D-HMPAO, Tc-99m-L,L-HMPAO and Tc-99m-d,l-HMPAO to determine the relative rates in vitro under all condition, which shows that the stability of Tc-99m-L,L-HMPAO and Tc-99m-D,D- HMPAO is similar to that of Tc-99m-d,l-HMPAO (Table 1). The properties of Tc-99m-D,D-HMPAO, Tc-99m-L,L-HMPAO and Tc-99m-d,l-HMPAO measured by the electrophoresis(on Sepharose III in a phosphate buffer pH 7.0) and partition coefficient(a n-octanol / phosphate buffer pH 7.4 system) are neutral and lipophilic, A summary of the radioanalytical data is presented in Table 2.

Table 1. The rate constant of decrease in the concentration of 5.0 x 10^{-4} M Tc-99m-D,D-HMPAO, Tc-99m-L,L-HMPAO and Tc-99m-d,l-HMPAO (pH 6.0) by u.v. visible spectrophotometric and three strips Methods

Method	Rate constant $k_d(h^{-1})$		
	Tc-99m-D,D-HMPAO	Tc-99m-L,L-HMPAO	Tc-99m-d,l-HMPAO
u.v. visible spectrophotometric method	0.0015±0.003	0.0014±0.002	0.0015±0.004
three strips method	0.0014±0.003	0.0015±0.003	0.0014±0.002

Table 2. Radioanalytical of data of 5.0 x 10^{-4} M Tc-99m-D,D-HMPAO, Tc-99m-L,L-HMPAO and Tc-99m-d,l-HMPAO (pH 6.0)

Compound	Partition coefficient (PC_o)	Electrophoretic results	
		bound	TcO_4^{-1}
Tc-99m-D,D-HMPAO	28.4	94.0	3
Tc-99m-L,L-HMPAO	27.9	96.3	2
Tc-99m-d,l-HMPAO	27.8	93.8	3

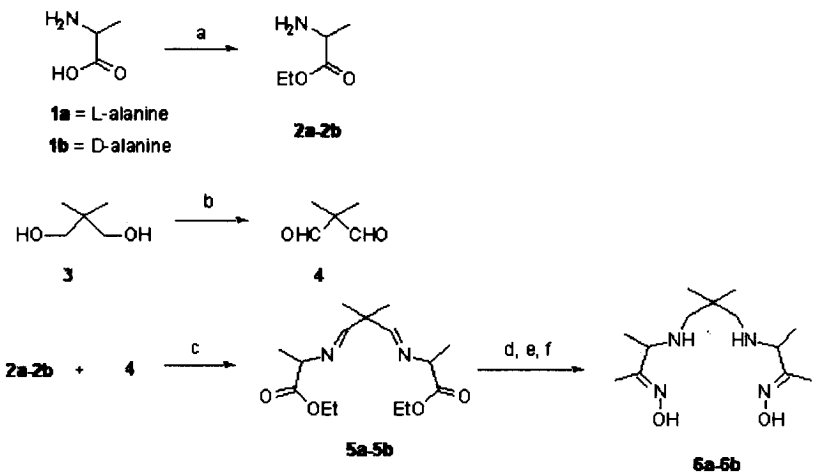
In this study, the dose of 0.3 mCi per Kg was injected in a rat. We found that cerebral uptake (%D in brain) obtained for Tc-99m-L,L-HMPAO (2.03±0.20, 5.02±0.25, 4.74±0.25, 4.81±0.25, 4.63±0.25 and 4.07±0.62, at 15s, 20, 30, 60, 180, and 270 min., respectively) is statistically different from that obtained for

Tc-99m-d,l-HMPAO (1.96±0.18, 3.74±0.28 and 2.96±0.31, 2.76±0.18, 2.43±0.27, and 1.69±0.24 at 15s, 20, 30, 60, 180, and 270 min., respectively), and the value for Tc-99m-D,D-HMPAO (1.95±0.13, 2.05±0.14, 1.89±0.19, 1.67±0.13, 1.42±0.19, 1.09± 0.15 at 15s, 20, 30, 60, 180, and 270 min., respectively) is lower than either the d,l-mixture or L,L-isomer. Cerebral radiography in rats demonstrates initial(15s pi) agreement in L,L-, D,D- and d,l-isomer tracer distribution within the brain. Delayed studied (60 min pi) showed that images of Tc-99m-D,D-HMPAO and Tc-99m-d,l-HMPAO reveal more washout and observable loss of structural detail than that of Tc-99m-L,L-HMPAO. These results suggest that Tc-99m-L,L-HMPAO ,Tc-99m-D,D-HMPAO and Tc-99m-d,l-HMPAO are the same stable, neutral and lipophilic complexes, which readily cross the blood brain barrier and retained in the brain. Furthermore, Tc-99m-L,L-HMPAO is intracellularly converted more rapidly to the hydrophilic Tc-99m-species in brain than D,D- and d,l- isomer, and the retention time of Tc-99m-L,L-HMPAO is longer than that of Tc-99m-D,D-HMPAO and Tc-99m-d,l-HMPAO. It seems that the Tc-99m-L,L-HMPAO decomposes faster than Tc-99m-D,D-HMPAO and Tc-99m-d,l-HMPAO in the brain and the cerebral deposition properties exhibited by Tc-99m-L,L-HMPAO should reflect a higher contribution

Experimental Procedure

Synthesis procedure of D,D-HMPAO and L,L-HMPAO were described in scheme 1: The esterification of L- or D- alanine **(1a-1b)** produce to be **2a-2b**. Diol **3** was oxidated by PCC to generate dialdehyde **4.** Then dialdehyde **4** reacted with 2a-2b to form diimine **5a-5b**. Diazadioxime **6a-6b** was prepared in situ by treating **5a-5b** with $NaBH_4$ followed by methyllithium and NH_2OH.

Tc-L,L-HMPAO and Tc-D,D-HMPAO were prepared by the method described in the literature[10] (Jurisson et al., 1986) from diazadioxime **6a-6b**.



Scheme 1.(a). EtOH /c-H_2SO_4, heat. (b). P.C.C. /CH_2Cl_2 (c). EtOH. (d). $NaBH_4$ /EtOH (e). CH_3Li /Et_2O (f). NH_2OH, HCl

Mature female rats (Wistar) were anesthetized with thiopental and injected with 25μl of Tc-99m-HMPAO complex solution (the dose of 0.3 mCi per Kg) via tail vein. At various time periods after the administration, animals were killed by blood withdrawal from the aorta, then selected organs were isolated, weighed and counted.

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