SYNTHESIS CHARACTERIZATION AND BIOLOGICAL EVALUATION OF A NOVEL MIXED LIGAND ^{99m}Tc COMPLEX AS POTENTIAL BRAIN IMAGING AGENT

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Abstract

One approach in the design of neutral oxotechnetium complexes is based on the simultaneous substitution of a tridentate dianionic ligand and a monodentate monoanionic coligand on a $[Tc(V)O]^{+3}$ precursor. Following this "mixed ligand" concept, a novel ^{99m}Tc complex with N,N-bis(2-mercaptoethyl)-N´N´-diethylethylenediamine as ligand and 1-octanethiol as coligand is prepared and evaluated as potential brain radiopharmaceutical. Preparation of the complex at tracer level was accomplished by using ^{99m}Tc-glucoheptonate as precursor. The substitution was optimized and a coligand/ligand ratio of 5 was selected. Under this conditions the labeling yield was over 80% and a major product (with radiochemical purity > 80%) was isolated by HPLC methods and used for biological evaluation. Chemical characterization at carrier level was developed using the corresponding rhenium complex as structural model. The Re complex was also prepared by substitution method and isolated as a crystalline product. The crystals were characterized by UV-vis and IR spectra and elemental analysis. Results were consistent with the expected ReOLC structure. X ray crystallographic study demonstrated that the complex adopts a distorted trigonal bipyramidal geometry. The basal plane is defined by the SS atoms of the ligand and the oxo group, while the N of the ligand and the S of the coligand occupy the two apical positions. All sulphur atoms underwent ionization leading to the formation of a neutral compound. ^{99}Tc complex was also prepared. Although it was not isolated due to the small amount of reagents employed, the HPLC profile was identical to the one observed for the rhenium complex suggesting the same chemical structure. Biodistribution in mice demonstrated early brain uptake, fast blood clearance, excretion through hepatobiliary system and a brain/blood ratio that increased significantly with time

1.- Introduction

The development of neutral lipid soluble ^{99m}Tc complexes capable of penetrating the intact blood brain barrier is still a major goal in radiopharmaceutical research, as brain perfusion imaging allows detection of various cerebral vascular abnormalities (stroke, transient ischemia) and

benefits management of neurological disorders (Alzheimer, epilepsy,Parkinson) .

Two ^{99m}Tc brain perfusion agents are extensively used in clinical Nuclear Medicine: ^{99m}Tc- d,l-hexamethylpropyleneamineoxime (^{99m}Tc- HMPAO) (1,2) and ^{99m}Tc-l,l-ethylenecysteine- dimer (^{99m}Tc-ECD) (3). ^{99m}Tc- HMPAO is based on a N_4O_2 ligand, which loses two amine protons and an oxime proton on coordination to the Tc(V) monooxocore, resulting in a neutral complex. The complex is taken up by brain and then decomposes to a more hydrophilic species that is unable to diffuse back out of the brain. Instability, responsible of prolonged brain retention, occurs also *"in vitro"* and is considered a major disadvantage for routine assessment of brain perfusion (3). The ligand of ^{99m}Tc-ECD belongs to the family of the diaminedithiols (DADTS) (4). The $[Tc(V)O]^{3+}$ core coordinates with 2 nitrogen and 2 sulphur donor atoms to form a complex with 3 5-membered rings which is very stable *"in vitro"*, easily crosses the blood brain barrier, and distributes in the brain according to regional blood flow. The retention mechanism of ^{99m}Tc-ECD has been identified as an specific enzymatic process occurring in the brain of the primates.

Both ^{99m}Tc-HMPAO and ^{99m}Tc-ECD are formed by the action of tetradentate ligands on the $[Tc(V)O]^{3+}$ core. Another approach in the design of neutral oxotechnetium complexes is based on the simultaneous substitution of a tridentate dianionic ligand and a monodentate monoanionic coligand on a $[Tc(V)O]^{3+}$ precursor (5,6). The advantage of the "mixed ligand concept " in the design of radiopharmaceuticals lies in the variety of possible tridentate/monodentate complexes that can be synthesized by modifying either the ligand or the coligand.

Various TcOLC complexes, where L is a tridentate aminothiol and C is a monodentate aromatic monothiol, have already been synthesized and

characterized (7,8) . In the present study a novel mixed ligand ^{99m}Tc complex with N,N-bis(2-mercaptoethyl)-N',N'-diethylethylenediamine as ligand and 1-octanethiol as coligand is prepared, characterized and evaluated as potential brain perfusion radiopharmaceutical.

2.- Materials and Methods

2.1.- Synthesis of the ligand

The ligand was synthesized by reacting N,N-diethylethylenediamine with ethylene sulfide in toluene at 110°C, following a previously described method (9) . Purification was achieved by high vacuum distillation. Commercially available 1-octanethiol was used as coligand.

2.2.- Preparation of ^{99m}Tc complex

Preparation of the mixed ligand complex at tracer level was accomplished by using ^{99m}Tc-glucoheptonate as precursor. A vial containing a lyophilized mixture of 200 mg calcium glucoheptonate and 0.2 mg $SnCl_2$ was reconstituted with 5 mL water and 0.5 mL of this solution was mixed with 0.5-1 mL $^{99m}TcO_4^-$ (Elumatic III generator , Cis Biointernational) with an activity of 5 - 50 mCi (185 - 1850 MBq). Substitution was performed at different coligand/ligand molar ratios (1, 5 and 10) by adding the precursor (with radiochemical purity > 95%) to a centrifuge tube containing the ligand (0.02 moles) and the coligand . The mixture was agitated in a vortex mixer and left to react at room temperature for 10 minutes. The lipophilic species were extracted with CH_2Cl_2 and the organic layer dried with $MgSO_4$, filtered and analyzed by paper chromatography (Whatman 1, ethyl acetate) and normal phase HPLC (Bondclone-Si column,

metanol:dicloromethane 50:50, 0.5 mL/min). Activity measurements were performed either in a Dose Calibrator (Carpintec CRC- 5R) or in a scintillation counter (3"x3" NaI (Tl) crystal detector associated to an ORTEC monochanel analyzer). HPLC analysis was developed using a LC-10 AS Shimadzu Liquid Chromatograph coupled to a scintillation counter.

2.3.- Preparation of rhenium analog

Chemical characterization was developed at carrier level using the corresponding rhenium complex as structural model. The oxorhenium precursor trans-oxotrichlorobistriphenylphosphine rhenium (V) $[ReOCl_3(PPh_3)_2]$ was reacted with a mixture of ligand (0.2 mmoles) and coligand (molar ratio 1:1:5) in methanol under reflux, until green-yellow color of the precursor turned to green. After being cooled at room temperature the reaction mixture was extracted with CH_2Cl_2 and the organic layer dried and filtered. The solvent was removed under reduced pressure to aprox. 5 mL and 5 mL of isopropanol were added. Slow evaporation of the solvents afforded the product as green crystals. HPLC analysis of the reaction mixture and the crystals (using the same conditions as at tracer level) was also performed. Detection was accomplished with a photodiode array detector (SPD-M10A, Shimadzu) that recorded UV-vis spectra on flux.

IR spectrum was obtained from KBr pellets in the range 4000 to 400 cm^{-1} on a Bomen MB-102 FT-IR spectrophotometer. Elemental analysis was performed on a Carlo Erba EA 1108 analyzer.

2.4.- Crystallographic studies

Diffraction measurements were made on a Crystal Logic Dual Goniometer diffractometer using graphite monochromated Mo radiation. Unit cell dimensions were determined and refined using the angular settings of automatically centered reflections in the range $11 < 2\theta < 23$. Intensity data were recorded using a θ - 2θ scan to 2θ (max) = 50 deg. with scan speed 1.5 deg/min and scan range 2.4 plus $\alpha_1\alpha_2$ separation. Three standard reflections monitored every 97 reflections showed less than 3% variation and no decay.

Table I.- Summary of Crystal,Intensity Collection and Refinement Data

	Complex ReOLC
Formula	$C_{18}H_{39}N_2OS_3$
FW	581.92
Temp, K	298
Wavelength	Mo Ka 0 .71073
Space group	$P2_1/n$
a (Å)	16.506 (9)
b (Å)	9.997 (5)
c (Å)	15.339 (8)
β, deg	103.03 (1)
V ($Å^3$)	2466
Z	4
D_{calcd} (Mg m^{-3})	1.567
Abs coeff, (μ), mm^{-1}	5.19
Scan mode/speed (deg/min)	θ - 2θ/1.5
Scan range (deg)	2.4 +$\alpha_1\alpha_2$ separation
θ range (deg)	50
Reflections collected	4796
Independent reflections	4335 [R(int) = 0.0199]
Range of h, k , l	0 -13, 0 - 13, 20 - 19
F (000)	1168
$[\Delta/\sigma]_{max}$	0.802
$[\Delta\rho]_{max}$ $[\Delta\rho]_{min}$ ($c/(Å^3)$)	1.24 and - 1.06
Refinement method	Full matrix least squares on F^2
Data/ parameters	4335/235
Goodness of fit on F^2	1.100
R indices [4335 refs $I > 2\sigma(I)$][(2)]	R1 = 0.0530 wR2 = 0.1618
R indices (all data)	R1 = 0.0773 wR2 = 1618

(1) R1 based on F's, wR2 based on F^2

Lorenz polarization correction and psi-scan absorption corrections were applied using Crystal Logic software. Symmetry equivalent data were averaged with R= 0.0199 to give 4335 independent reflections from a total of 4796 collected. The structure was solved by direct methods using SHELXS-86 (10) and refined by full-matrix least squares techniques on F^2 with SHELXL-93 (11) using 4335 reflections and refining 235 parameters. All hydrogen atoms were introduced at calculated positions as riding on bonded atoms. All non-hydrogen atoms were refined anisotropically. The final values for R_1, wR_2 and GOF for observed data are 0.0530, 0.1618 and 1.100 and for all data are 0.0773, 0.1618 and 1.097 respectively. The maximum and minimum residual peaks in the final difference map were 1.24 and - 1.06 e/ $Å^3$. The largest shift/esd in the final cycle was 0.802. A summary of crystal, intensity collection and refinement data is presented in Table I.

2.5.- Preparation of ^{99}Tc complex

A solution of stannous chloride (11.3 mg) in HCl (1N, 1 mL) was added to an aqueous solution of $K^{99}TcO_4$ (Amersham International, 4 mg) and sodium gluconate (20 mg) to obtain ^{99}Tc-gluconate. The pH of the solution was adjusted to 7.5 with 0.1 N NaOH before adding it with stirring to a mixture of ligand (0.02 mmoles) and coligand (0.1 mmoles) in methanol. After 20 minutes stirring the solution was extracted with CH_2Cl_2. The organic phase was separated, dried over $MgSO_4$, filtered and analyzed by HPLC.

2.6.- Biodistribution studies

Normal mice (CD1, 25-30 g, 4 animals per group) were injected via tail vein with aprox. 100 μCi (3.7 MBq) of the HPLC purified ^{99m}Tc complex. At

different intervals after injection (1 to 60 minutes) the animals were sacrificed by neck dislocation. Whole organs, total urine volume and samples of blood and muscle were collected, weighted and assayed for radioactivity. Corrections by different sample geometry were applied when necessary.

3.- Results and Discussion

Simultaneous action of the tridentate ligand N,N-bis(2-mercaptoethyl)-N',N'-diethylethylenediamine and the monodentate coligand 1-octanethiol on the ^{99m}Tc(V) glucoheptonate at different coligand/ligand molar ratios, produced the formation of ^{99m}Tc lipophilic species, which were extracted by CH_2Cl_2 with high yield (> 80% in all cases). The organic layer was analyzed by paper chromatography and HPLC in order to study the different ^{99m}Tc species formed upon substitution. Paper chromatography demonstrated the presence of at least 2 species (Rf=0 and Rf=1 respectively) whose percentage correlated with the coligand/ligand ratio. HPLC analysis showed a major peak with a retention time of about 7 minutes and a secondary one of about 12. Besides part of the activity was not eluted from the column. The retained activity was approximately equal to the percentage of activity with Rf = 0 in paper chromatography. A molar ratio coligand/ligand of 5 yielded 80% activity elution from the HPLC column as well as 80% purity of the complex with 7 minutes retention time and was consequently selected to continue the study.

Chemical characterization was developed at carrier level using the corresponding rhenium complex as structural model. Rhenium, as technetium's third row congener, exhibits many of the chemical properties of technetium. ^{99}Tc complexes and Re complexes with identical ligands have essentially identical coordination parameters and Re has environmentally preferable non

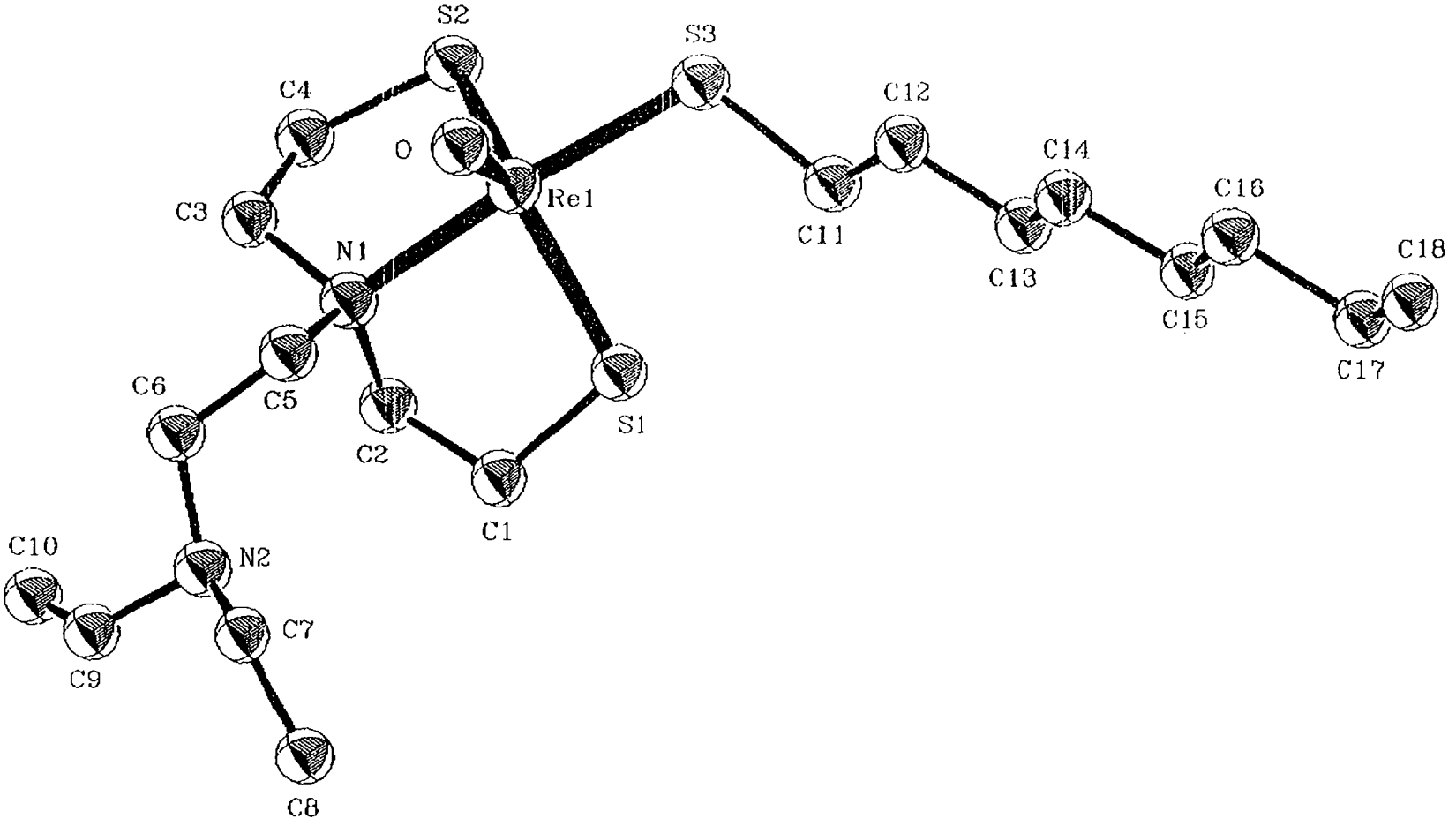

Figure 1.- ORTEP diagram of ReOLC with 50% thermal ellipsoid probability .

Table II.- Selected bond distances (Å) and angles (degrees) of ReOLC

	Complex ReOLC
Re - O1	1.682 (7)
Re - S1	2.277 (3)
Re - S2	2.271 (3)
Re - S3	2.277 (4)
Re - N1	2.188 (11)
O1 - Re - S1	119.97 (31)
O1 - Re - S2	117.55 (30)
O1 - Re - S3	105.70 (34)
O1 - Re - N1	95.38 (37)
N1 - Re - S1	82.48 (21)
N1 - Re - S2	83.94 (23)
N1 - Re - S3	158.77 (22)
S1 - Re - S2	121.74 (11)
S1 - Re - S3	88.96 (14)
S2 - Re - S3	84.14 (16)

radioactive isotopes. Substitution of ligand and coligand on the $[Re\ (V)O]^{3+}$ precursor in a molar ratio 1:5 yielded only one complex according to HPLC analysis. The retention time was identical to the major ^{99m}Tc complex. Furthermore, when ^{99m}Tc and Re complexes were coinjected, both radioactivity (for tracer) and UV-vis detectors exhibited identical chromatographic profiles, suggesting the same chemical structure was formed under both chelating conditions. The rhenium complex was obtained as a crystalline product. It is neutral, stable and lipophilic, as indicated by its quantitative extraction from the

aqueous to the organic layer during isolation. ReO stretching vibration frequency (942.2 cm^{-1}) is consistent with other reported for monooxo rhenium(V) complexes and demonstrated the presence of the $[Re\ (V)O]^{3+}$ core. UV-vis spectrum exhibited an intense band at aproximately 398 nm, probably due to S ⟶ Re charge transfer transition. Additional absorptions at shorter wavelenghts (235,264 nm) correspond to ligand and coligand. Elemental analysis results were consistent with the presence of one molecule ligand and

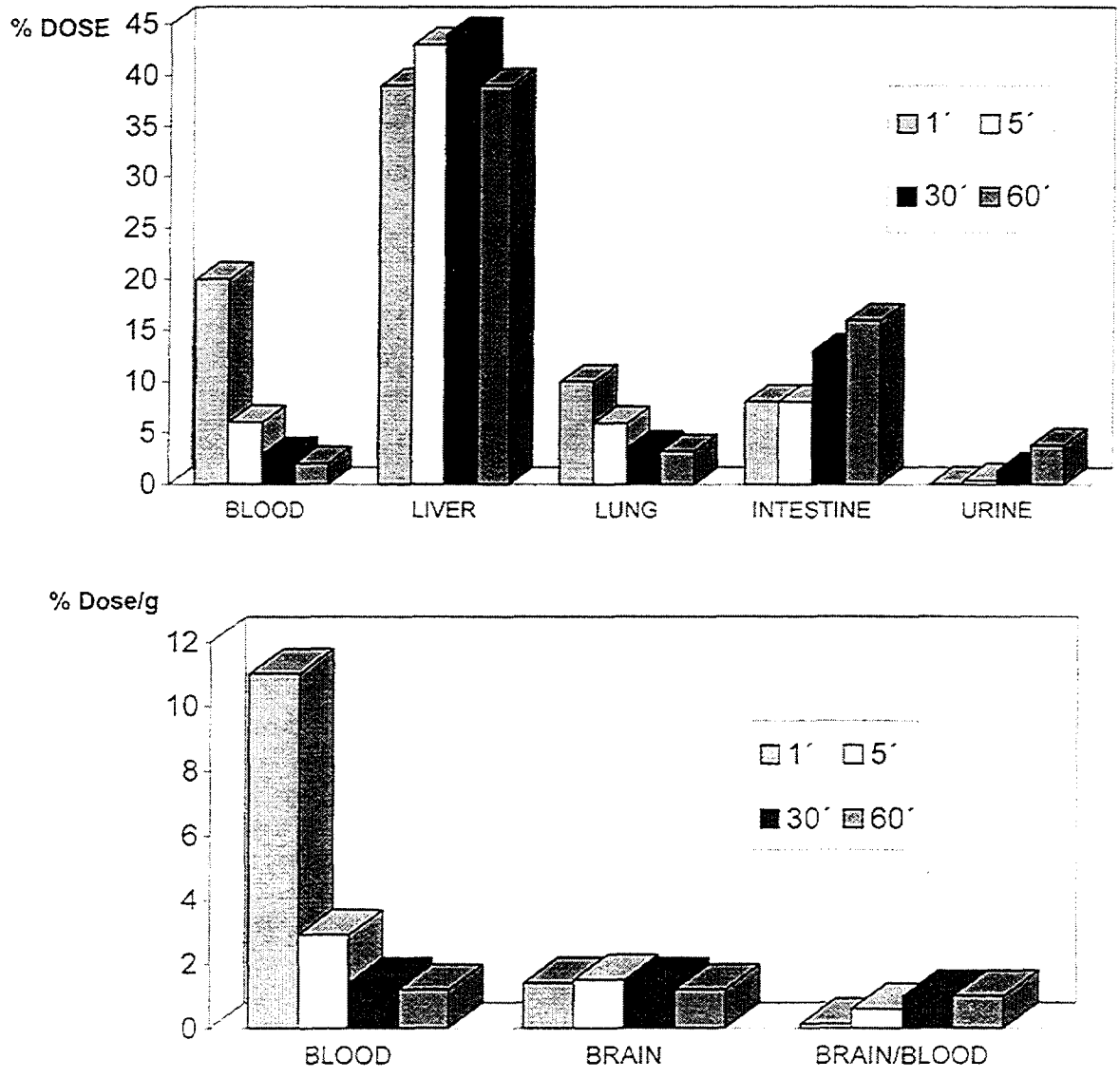

Figure 2.- Biodistribution results from ^{99m}TcOLC in mice

one of coligand per complex molecule. Figure 1 shows the ORTEP diagram for the complex. The coordination geometry is distorted trigonal bipyramidal, the calculated trigonality index, τ, being 0.62 . The basal plane is defined by the SS atoms of the ligand and the oxo group, while the N of the ligand and the S of the coligand occupy the two apical positions. Table II shows selected bond distances and angles. All sulphur atoms underwent ionization leading to the formation of a neutral compound.

^{99}Tc complex was also prepared by exchange method using ^{99}Tc-gluconate as precursor. Although the mixed ligand complex could not be isolated due to the small amount of reagents employed (0.02 mmoles), HPLC profile was identical to the one observed for the rhenium complex suggesting the same chemical structure.

Biodistribution studies of the major ^{99m}Tc complex (eluted from HPLC) were also performed. Figure 2 shows activity (%D/organ) for selected organs and tissues, as well as brain and blood uptake and retention (% Dose/g of organ) as a function of time. The complex demonstrated high initial blood, lung and liver uptake (20 ± 5%,10 ± 2% and 39 ± 3% respectively at 1 min. post injection), as expected from a lipophilic complex. Blood and lung clearance was rather fast while liver activity remained essentially constant during the period of study. Brain initial uptake was not notably high (0.51 ± 0.08% at 1 min. post - administration) but remained constant with time (until 60 minutes). Excretion occurs mainly through hepatobiliary system as demonstrated by high intestinal activity (16 ± 1 % at 60 min. post-adm.), urinary excretion being very low (3.8 ± 0.8 % at 60 min.). Stomach and thyroid values were within acceptable levels (0.12 ± 0.01 % and 2.0 ± 0.5 % respectively at 60 min.), indicating no "in vivo" decomposition. Prolonged brain retention together with fast blood clearance

determined a brain/ blood ratio that increased significantly with time (0.13 ± 0.01 % 1 min. post -administration and 1.0 ± 0.3 % at 60 min) .

4.- Conclusions

Neutral mixed-ligand oxotechnetium complexes with N,N-bis(2-mercaptoethyl)-N',N'-diethylethilenediamine as ligand and different aromatic thiols as coligands have already been successfully evaluated as potential brain radiopharmaceuticals. This study presents a novel compound in which the coligand is an aliphatic thiol.

Its coordination chemistry has been extensively studied through the rhenium model. The elucidated structure was similar to other mixed complexes with the same ligand but a molar ratio coligand/ligand of 5 was neccesary to obtain the complex with high yield, suggesting that aliphatic thiols have less binding ability than aromatic thiols. Other aliphatic thiols should be studied to support this hypothesis. ^{99m}Tc complex was also formed in a reproducible way. Corroboration of the structure was achieved by comparing high performance liquid chromatographic profiles of rhenium/^{99}Tc/^{99m}Tc complexes. The results suggest that in this case rhenium complex is an adequate model to study the coordination chemistry at carrier level.

Biological evaluation demonstrated prolonged brain retention as well as high brain/blood ratios that make this complex promising for further development of brain perfusion imaging agents.

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REFERENCES

[1] NEIRINCKX, R. .D., et al., Technetium 99m d, l HmPAO: A new radiopharmaceutical for Spect imaging of regional cerebral blood perfusion,. J. Nucl. Med. **28** (1987) 191-202.

[2] JURISSON, S. et al., Synthesis and characterization an X-ray structural determinations of technetiumV-oxotetradentate amine oxime complexes, Inorg. Chem. **25** (1986) 543-549.

[3] WALOVITC, R.C. et al., Characterization of Tc-99m-L,L ECD for Brain Perfusion Imaging, Part I: Pharmacology of Tecnetium-99m ECD in Nonhuman Primates, J. Nucl. Med. **30** (1989) 1892-1901.

[4] HUNG, J.C. et al., Kinetic analysis of technetium 99m-d,l HmPAO decomposition in aqueous media, J. Nucl. Med. **29**, (1988) 1568-1576.

[5] KUNG, H.F. et al., Synthesis and biodistribution of neutral lipid soluble Tc-99m complexes that cross the blood brain barrier, J. Nucl. Med **25** (1984) 326-332.

[6] MASTROSTAMATIS, S.G. et al., Tridentate ligands containing the SNS donor atom set as a novel backbone for the development of technetium brain imaging agents, J. Med Chem **37** (1994) 3312-3218.

[7] SPYRIOUNIS, D.M. et al., Synthesis and characterisation of oxotechnetium(V) complexes with aza substituted 2,6 Dimethyl-4-azaheptane-2,6 dithiols ligands and benzylmercaptan as coligand, Inorg. Chem. **34** (1995) 1077- 1082.

[8] PAPADOPOULOS, M. et al., Synthesis and characterisation oxorhenium(V) complexes of tripodal NS_3 containing a monodentate ligand as coligand In: Nicolini, Bandolli,G. Mazzi, U (Eds.), Technetium

and Rhenium in Chemistry and Nuclear Medicine,4. SG Editoriali Padova, (1995) 223.

[9] PIRMETTI, I.C., et al., Synthesis and Characterization of oxotechnetium(V) Mixed-ligand complexes containing a Tridentate-N-substituted Bis (2-mercaptoethyl)amine and a monodentate thiol, Inorg Chem. **35** 5 (1995) 1685-1691.

[10] CORBIN, J.L. et al., Preparation and properties of tripodal and linear etradentate N,S-donor ligands and their complexes containing the MoO_2^{2+} core, Inorg. Chim. Acta. **90** (1984) 41-51

[11] SHELDRICK ,G.M., SHELXS-86: Structure Solving program, University of Goettingen (1986), Germany.

[12] SHELDRICK, G.M. , SHELXL-93: Crystal Structure Refinement, University of Goettingen (1993), Germany.