Labelling of ECD with ^{99m}Tc: Preliminary Distribution Studies in Animals

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Although ^{99m}Tc-HMPAO represents a major advancement for routine assessment of brain perfusion, the *in vitro* instability of the complex complicates its ready use in certain clinical situations. Cheesman *et al.* developed a number of ^{99m}T-complexes of ester-derivatized DADT ligands. Of these complexes, ^{99m}Tc-Ethyl Cysteinate Dimer (ECD), a radiochemically stable brain perfusion agent, is a reportedly good marker for regional cerebral blood flow (rCBF). In this work we report on the labelling conditions of ECD

with ^{99m}Tc and on preliminary biodistribution studies in animals.

The N,N′-1,2-ethylenediylbis-L-cysteine, diethyl ester dihydrochloride (L,L-ECD) was prepared by a published procedure and its MP, infrared spectrometry and elemental analyses were determined. The labelling conditions studied for 1 mg L,L-ECD, 360 μg of $Na_2EDTA \cdot 2H_2O$ and 24 mg of mannitol were: $SnCl_2 \cdot 2H_2O$ mass (1-1000 μg); pH (4.0 and 7.5); ^{99m}Tc activity (37-3700 MBq); final volume (1-6 mL) and incubation time (5-45 minutes). Radiochemical purity was determined by ITLC-SG in 20% NaCl and RP-HPLC on a Nucleosil C_{18} column (250 × 46 mm; 5 μ) eluted with a gradient mixture of phosphate buffer (pH 2.5) and ethanol. A kit formulation was also developed and consisted of two serum-capped, 8.5 mL vials: vial I was freeze-dried under *vacuum* and contained 1.0 mg L,L-ECD, 125 μg $SnCl_2 \cdot 2H_2O$, 360 μg $Na_2EDTA \cdot 2H_2O$ and 24 mg mannitol; vial II contained 1 ml of pH 7.5 phosphate buffer (0.02 M) under an air headspace. The complex was formed by adding 1.2 mL of saline into vial I (pH 2-4); one milliliter of this solution was injected into vial II. The generator eluate was injected into vial II and reacted for at least 30 minutes (pH 7.0-7.5). Biodistribution was evaluated in mice after i.v. administration of the complex. The animals were sacrified at different time and the radioactivity contents of blood samples and of some organs were determined. Planar scintigraphic images were acquired in the dog.

The compound L,L-ECD was obtained with relative high yield: MP 184°C, ν_{MAX} (KBr, cm^{-1}) 2975-2172 ($NH_2 \cdot HCl$), 1737 (ethyl ester), 1552 (NH). Analytical calculated formula for $C_{12}H_{26}N_2O_4S_2Cl_2$: C 36.26; H 6.60; N 7.05; found: C 35.46; H 6.42; N 7.09.

Studying the ECD/$SnCl_2 \cdot 2H_2O$ mass ratio, radiochemical purity was higher than 98% with $SnCl_2 \cdot 2H_2O$ between 100 μg and 125 μg. Radiochemical purity was higher than 98% when the final volume was 1 to 4 mL. For 5 and 6 mL, this value decreases to 94.45 ± 0.32 and 89.61 ± 0.24, respectively. An incubation time longer than 15 minutes was necessary for a radiochemical purity higher than 98%. Labelling with 37 to 3700 MBq showed satisfactory radiochemical purity (98%), with stability until 4 hours post-labelling. HPLC analysis showed a single peak with Rt = 48 minutes both at pH 4.0 and at pH 7.5. The kit formulation was stable during a storage period longer than 4 months in ideal conditions (4 to 8°C). Stability of the lyophilized kit was also correlated with the pH of formulation, being greater in acid (pH 2.0-4.0) than in neutral conditions (pH 7.5).