Trapping of technetium-99m albumin macroaggregate and other four radiopharmaceuticals by blood clots in vitro

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Abstract

Radiopharmaceuticals are known to interact with the blood components (i.e. the red blood cells, serum proteins etc) but so far, there have been no data regarding their purely mechanical trapping in thrombi. The experiments presented in this communication provide evidence that the technetium-99m labeled albumin macroaggregate (^{99m}Tc-MAA), apparently due to its particle size, can be almost quantitatively retained in the *in vitro* model described. These results can be extrapolated *in vivo* and offer a plausible explanation for either the "hot spot" artifact, occasionally seen in lung perfusion imaging or for the partial trapping of ^{99m}Tc-MAA by a thrombus at the tip of a subclavian catheter, as has been recently reported. Control experiments were also run using ^{99m}Tc-methylene diphosphonate (^{99m}Tc-MDP), ^{99m}Tc(III)-dimercaptosuccinic acid (^{99m}Tc(III)-DMSA), ^{99m}Tc-methoxyisobutyl isonitrile (^{99m}Tc-MIBI) and sodium pertechnetate ($Na^{99m}TcO_4$), in order to study the extent of trapping of these radiopharmaceuticals under identical incubation conditions. ^{99m}Tc-MDP and ^{99m}Tc(III)-DMSA exhibited the lowest blood clot uptake (partially non-specific and partially mechanical trapping), while in the case of ^{99m}Tc-MIBI and $Na^{99m}TcO_4$, besides mechanical and non-specific clot-trapping, transport and retention inside the red blood cells was also observed.

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Introduction

In the lung perfusion scans performed with technetium-99m macroaggregated albumin (^{99m}Tc-MMA), hot spots often occur [1-3]. It is therefore recommended in clinical practice, not to withdraw the patient's blood in the syringe containing ^{99m}Tc-MMA, prior to injection. It has been reported recently that the administration of ^{99m}Tc-MAA, via a double lumen (Hickman) subclavian line, resulted in trapping almost half of the injected dose, in the right atrium, at the tip of the subclavian catheter in a large intra-atrial thrombus [4]. Due to the importance of identifying, during a routine scan, silent and potentially life-threatening thrombi, we have studied *in vitro,* whether various radiopharmaceuticals, namely ^{99m}Tc-MAA, ^{99m}Tc-methylene diphosphonate (^{99m}Tc-MDP), ^{99m}Tc(III)-dimercaptosuccinic acid (^{99m}Tc(III)-DMSA), ^{99m}Tc-methoxyisobutyl isonitrile (^{99m}Tc-MIBI) and sodium pertechnetate ($Na^{99m}TcO_4$), can be trapped into recently formed clots *in vitro*.

Materials and methods

Whole blood was collected from healthy individuals taking no medication and added into Lavender tubes containing K_3 ethylene diamine tetraacetic acid (K_3EDTA) (15%, w/w) as an anticoagulant. Portions of 1ml of the anticoagulated blood were added into polystyrene tubes containing 200 μL of each of the above five radiopharmaceuticals in a dose of 7.4-25.9 MBq as counted in a Capintec CRC-12 radioisotope calibrator (Ramsey, NJ, USA). Blood without anticoagulant in a volume of 1 mL was added in another set of tubes containing the same volume and activity of the radiopharmaceuticals as mentioned above. All tubes were left to incubate at room temperature for 30 min. In the tubes containing blood without anticoagulant and ^{99m}Tc-MAA, a plastic Pasteur pipette was also inserted, to facilitate detachment from the walls and washing of the thrombus, since centrifugation precipitates both, the labeled albumin macroaggregates and the red blood cells and it is not therefore recommended in this experiment as a method of separation. All other samples were washed three times with the repeated addition of 3 mL of normal saline and centrifuged at 2500 rpm for 10 min at room temperature. The concentration of the radiopharmaceuticals used in these experiments was

well above the usual dose administered to patients. The radiopharmaceuticals were purchased as follows: $Na^{99m}TcO_4$, from a $^{99}Mo/^{99m}Tc$ generator as Elumatic III™, Cisbio, Schering AG, Germany, ^{99m}Tc-MAA as MAASOL™, Amersham Health S.r.l., Italy, ^{99m}Tc-MDP from Amerscan™ Medronate II agent, GE Healthcare Ltd, United Kingdom, ^{99m}Tc(III)-DMSA as Renocis™, Cisbio, Schering AG, Germany and ^{99m}Tc-MIBI as Cardiolite™, Bristol-Myers Squibb S.r.l., Italy. The radiopharmaceuticals were prepared according to the instructions of the manufacturers and were subjected to quality control by thin layer chromatography. The experiments were run in triplicates for ^{99m}Tc-MDP, ^{99m}Tc(III)-DMSA, ^{99m}Tc-MIBI and $Na^{99m}TcO_4$ and in quadruplicates for ^{99m}Tc-MAA. The percent of added radioactivity, corrected for the ^{99m}Tc decay and background activity, bound both to the blood clot and to the non coagulated blood containing mainly red blood cells, was measured and expressed as mean ± Standard Deviation (SD).

Results

Results are shown in Table I. The data indicate that for the ^{99m}Tc-MDP and the ^{99m}Tc(III)-DMSA, the bound radioactivity in the non-coagulated blood containing mainly red blood cells, was minimal (< 2%), as expected. However, clotted blood showed higher levels of bound radioactivity, indicating a significant mechanical trapping of the radiopharmaceutical in the clot. This higher level of bound radioactivity was approximately 10% for ^{99m}Tc-MDP and ^{99m}Tc(III)-DMSA, after the non-specific binding (≈2%) was considered and subtracted. ^{99m}Tc-MIBI and $Na^{99m}TcO_4$ exhibited much higher clot binding due to both, specific and non-specific mechanisms. ^{99m}Tc-MAA was practically all trapped inside the thrombus (≈95 %), due to purely mechanical factors.

Discussion

^{99m}Tc-MDP and ^{99m}Tc(III)-DMSA do not cross red blood cells membrane (anionic charge-lack of transporter) and the amount of bound radioactivity in this case represents true non-specific binding, in close agreement with the results obtained for ^{99m}Tc-diethylene-triamino-penta acetic acid (^{99m}Tc-DTPA) and reported elsewhere [5]. However, under the aforementioned incubation conditions, $^{99m}TcO_4^-$ and ^{99m}Tc-MIBI appear to be transported and partially retained inside the red blood cells. $^{99m}TcO_4^-$ uses the band-3 protein to cross the red blood cell membrane [6], while the ^{99m}Tc-MIBI uses the red blood cell membrane Na+/K+-ATP-ase and due to its positive charge, it is retained by them, due to their negative intracellular potential [7].

The significantly higher percentage of bound radioactivity for the ^{99m}Tc-MIBI in the blood clot experiments is explained on the basis of the known hydrophobicity of this radiopharmaceutical and the presence of the hydrophobic fibrin in the clot, formed during the coagulation process. It is known [8, 9], that the fibrin precursor (fibrinogen) is hydrophilic and therefore it is expected to exhibit relatively minimal ^{99m}Tc-MIBI binding, while the fibrin formed during the coagulation process is hydrophobic. ^{99m}Tc-MIBI shows a strong binding affinity towards fibrin. Practically all ^{99m}Tc-MAA is mechanically trapped in the thrombus (≈ 95 %). The findings of this paper appear for the first time in the literature and shed light in known, but so far unproved imaging findings.

Table I. *Results of incubation experiments of whole blood (with or without anticoagulant) with the radiopharmaceutical indicated. Results are expressed as % bound of the added radioactivity (mean ± SD). The number of experiments is shown in parenthesis*

Radiopharmaceutical	RBC-K_3EDTA (% bound of added radioactivity)	Blood clot (% bound of added radioactivity)
^{99m}Tc-MDP	1.1 ± 0.2 (n=3)	12.5 ± 4.7 (n=3)
^{99m}Tc(III)-DMSA	1.4 ± 0.5 (n=3)	8.7 ± 5.1 (n=3)
$^{99m}TcO4^-$	6.6 ± 0.6 (n=3)	21.2 ± 2.9 (n=3)
^{99m}Tc-MIBI	8.2 ± 0.6 (n=3)	31.7 ± 2.9 (n=3)
^{99m}Tc-MAA	NA	95.9 ± 1.4 (n=4)

NA: Not applicable, SD: Standard deviation

In conclusion, our *in vitro* results indicate almost complete mechanical trapping of ^{99m}Tc-MAA into blood clots. This trapping was inferred to take place also *in vivo*, according to previous observations [1-4]. Mechanical and non-specific trapping was also observed, to a lesser extent, for all the other radiopharmaceuticals studied (^{99m}Tc-MDP, ^{99m}Tc(III)-DMSA, $^{99m}TcO_4^-$ and ^{99m}Tc-MIBI), although in the $^{99m}TcO_4^-$ and ^{99m}Tc-MIBI cases, specific retention mechanisms also seemed to operate.

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