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Research Article

Preparation and biological evaluation of ^{99m}Tc-stannous fluoride colloid-labelled-leucocytes in rats

^{99m}Tc-stannous fluoride-labelled-leucocytes in rats

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Summary

High splenic activity is always visible in ^{99m}Tc-stannous fluoride (SnF_2)-labelled-leucocytes scans. In an attempt to reduce this activity, this study investigated the effect of pre-injected SnF_2 colloid on the distribution of ^{99m}Tc-SnF_2 colloid, ^{99m}Tc-SnF_2-labelled-leucocytes, and opsonized ^{99m}Tc-SnF_2 colloid in rats. The radiopharmaceuticals ^{99m}Tc-SnF_2 colloid and ^{99m}Tc-SnF_2-leucocytes were each found to exhibit identical biodistributions in separate experiments. SnF_2 colloid pre-injection (26 μg) resulted in reduced splenic uptake of ^{99m}Tc-SnF_2 colloid (38%) and ^{99m}Tc-SnF_2-labelled-leucocytes (30%), but not for opsonized ^{99m}Tc-SnF_2 colloid. This indicates that the level of opsonization of radiocolloid is rate limiting rather than the phagocytic capacity of liver and spleen macrophages. There is a low level of ^{99m}Tc-SnF_2-labelled-leucocytes dominated by unopsonized radiocolloid in the *ex vivo* whole blood dose. Following administration of this dose, free radiocolloid is present *in vivo* that predominantly localizes in the liver and spleen. This uptake can be challenged with non-radioactive stannous fluoride

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colloid pre-injection, where splenic activity can be significantly reduced by up to 52%. Copyright © 2003 John Wiley & Sons, Ltd.

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1. Introduction

Leucocytes radiolabelled with either ^{111}In or ^{99m}Tc are still the radiopharmaceutical agents of choice for the diagnosis of focal bacterial infection and inflammation. Initial studies identified ^{111}In-oxine as a promising agent for leucocyte labelling,[1] and numerous studies thereafter have confirmed its useful clinical role as well as ^{111}In-tropolonate labelled leucocyte scans in the detection of infection and inflammation. In inflammatory bowel disease, ^{111}In-leukocyte scans accurately identified the disease site with 94% sensitivity.[2] A good correlation was observed between the intensity of uptake on the scan with severity of the inflammation, and the degree of faecal excretion of ^{111}In-leucocytes with degree of inflammation in Crohn's disease and ulcerative colitis patients.[3] Unfortunately, the isotope ^{111}In is not produced in Australia, making ^{111}In-radiopharmaceuticals expensive to obtain. Studies using ^{99m}Tc-HMPAO labelled neutrophils resulted in better image resolution and reduced radiation dosimetry, and these were shown to be as sensitive as ^{111}In-leukocyte scans in the diagnosis of acute sepsis and inflammatory bowel disease.[4] The ^{99m}Tc-label does dissociate from the neutrophil to a small extent, resulting in biliary and urinary excretion of secondary hydrophilic complexes, as well as non-specific bowel activity at approximately 3 h post injection.

^{99m}Tc-stannous fluoride (SnF_2) colloid labelled leucocyte scans have been performed in Australia for more than 15 years, successfully detecting sites of infection and inflammation. The cell labelling technique is inexpensive and simpler than the others, and like with ^{111}In-leucocytes, this radiopharmaceutical has the advantage of no normal bowel activity or excretion of tracer. As such, ^{99m}Tc-SnF_2 labelled white cells have proven to be particularly useful in the assessment of inflammatory bowel disease.[5,6] However there are still unanswered questions concerning which cells are labelled, and the reason for increased liver and spleen uptake seen with this technique. Radiolabelling is achieved by incubating ^{99m}Tc-SnF_2 colloid with a

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whole blood sample *ex vivo*, in a procedure where no additional cell separation steps are required to isolate the leucocytes. The radioactive blood is administered back into the same patient, and ^{99m}Tc-neutrophils follow their normal course in blood circulation to participate in the acute phase response at an inflammatory site. The mechanism is believed to depend upon phagocytic engulfment of appropriately sized ^{99m}Tc-SnF_2 colloid particles[7] by neutrophils, rendering the ^{99m}Tc isotope internally localised, but this radiocolloid has also been reported to be bound to the surface of erythrocytes, lymphocytes and eosinophils in labelled samples of whole blood.[8] These scans normally result in more liver and spleen uptake than the other leucocyte labelling methods, which may be due to unbound radiocolloid also present in the dose.[9] This activity does not usually interfere with the diagnosis of inflammatory lesions located in the distal small bowel or ascending and descending colon, but may limit identification of inflammatory foci in proximal small bowel, in the hepatic and splenic flexures, the transverse colon and focal abscesses in the liver and spleen.

If excess ^{99m}Tc-SnF_2 colloid is present in blood after intravenous administration, then it could be phagocytosed by liver and splenic macrophages to result in increased splenic and hepatic radioactivity. It is possible that this could be reduced by competitive binding with non-radioactive or 'cold' colloid administered first. Although it is not clear how free ^{99m}Tc-SnF_2 colloid is present *in vivo*, we were interested in the possibility of challenging its reticuloendothelial uptake. In particular, this study has investigated the effect of a pre-injection of cold stannous fluoride colloid on the biodistribution of (i) ^{99m}Tc-SnF_2 colloid and (ii) ^{99m}Tc-SnF_2 labelled leucocytes in a rat model.

2. Materials and methods

Sodium ^{99m}Tc-pertechnetate was obtained from the daily milking of a $^{99}Mo/^{99m}Tc$-generator (Gentech, Australian Radioisotopes, Sydney, Australia). Radioactive samples were counted in either a validated counting unit (Atomlab 100^+ Dose Calibrator, Biodex Medical Systems, New York, USA), a large volume counter (Biosentry; AEI-EKCO, Australia) linked to a multichannel analyser (Model 3100; Canberra Industries Inc; USA), or a gamma counter (Packard Auto-Gamma 5650; Hewlett Packard) over a ^{99m}Tc-window (70–210 KeV),

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depending on radioactive concentration. Inbred female rats (Sprague-Dawley, 160–180 g) were used for the rodent studies.

2.1. Radiolabelling

2.1.1. Radiocolloid and colloid preparations. ^{99m}Tc-stannous fluoride colloid was prepared using LWC Kit [A + B] (RAH Radiopharmacy; Adelaide; Australia). The Kit comprises of vial A [sodium fluoride (6.25 mg) in water for injection (5 ml)], and vial B [SnF_2 (0.64 mg) in water for injection (1 ml)]. Vial A (4 ml) was added to vial B, and after mixing for 20 s, the entire colourless liquid was filtered (0.2 μm) into a sterile vial. This filtered dispersion (0.5 ml) was added to a syringe (5 ml) containing ^{99m}Tc-pertechnetate (40–180 MBq) in saline (0.9%; 2.5 ml), and then mixed by rotation (~40 rpm) at room temperature for 50–60 min using a rotation apparatus (RSM6 suspension mixer; Ratek Instruments; Victoria; Australia). Radiochemical purity (RCP) of the radiopharmaceutical was determined by ascending thin layer chromatography on paper (ITLC-SG; Gelman Sciences; Ann Arbor; USA) with normal saline as the eluent. ^{99m}Tc-tin fluoride colloid remained at the origin ($R_f = 0.0$) and ^{99m}Tc-pertechnetate migrated with the solvent front ($R_f = 1.0$). Radioactive paper sections were counted in a gamma counter, and % RCP was calculated as 100% – % ^{99m}Tc-pertechnetate activity. Radiocolloid with RCP exceeding 98% was used for all experiments. The unlabelled or 'cold' colloid was prepared as above except that no radioactivity was used during rotation of the filtered dispersion. The level of cold colloid in the pre-injection dose is based on the mass (μg) of initial stannous fluoride present.

2.1.2. Leucocyte radiolabelling procedure. Using a procedure similar to labelling human blood,[10] ^{99m}Tc-stannous fluoride colloid (1.0 ml) prepared above was added to rat blood (5.0 ml) in a syringe (10 ml) containing heparin (30 units), and the syringe was rotated (~40 rpm) for 45–50 min at room temperature. The radiolabelled blood was then transferred into a sterile syringe and used within 10 min of preparation for the rodent studies.

2.2. Radiotracer localization in rats

Experiments performed with the rats complied with '*The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*

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NHMRC' and according to a protocol approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Sciences, Adelaide. Plasma for the opsonization experiments was obtained by centrifuging heparinized rat blood at 300 g for 10 min. All doses were administered intravenously to rats via the tail vein with a syringe (0.5 ml) containing a 29 gauge needle (0.33 mm × 12.7 mm). Rats were sacrificed by asphyxiation using Halothane. Results are reported as percentage injected dose (id) of organ or % id per gram of blood as specified. Percentage id was calculated as 100 × (organ activity divided by the total activity), and % id/g was calculated as % id divided by mass of the respective organ.

2.2.1. ^{99m}Tc-SnF_2 colloid. In a modification of the British Pharmacopoeial test for ^{99m}Tc-colloidal tin,[11] a group of rats ($n = 5$) were injected with ^{99m}Tc-SnF_2 colloid (0.2 ml; 0.5 MBq) and then they were sacrificed 20 min later. The lungs, liver and spleen were removed, rinsed in running water, blotted dry and counted in a large volume counter. Blood was also obtained from rats ($n = 3$) by intra-cardiac puncture, weighed, then counted to determine % id/g using a dose equivalent standard.

2.2.2. Cold SnF_2 colloid and ^{99m}Tc-SnF_2 colloid. The procedure was repeated as above except that cold colloid (0.2 ml; 26 μg; $n = 6$ rats) was injected first, followed by injection of ^{99m}Tc-stannous fluoride colloid (0.2 ml; 0.5 MBq) into the same rats 20 min later. The level of cold colloid in the pre-injection was varied (0.1 ml, 13 μg; $n = 3$ rats; and 0.4 ml, 52 μg; $n = 3$ rats) in separate experiments.

2.2.3. Cold SnF_2 colloid and opsonized ^{99m}Tc-SnF_2 colloid. In a sterile vial, fresh rat plasma (5 ml) was added to ^{99m}Tc-SnF_2 colloid (1.0 ml, 2.5 MBq) and mixed to form a homogeneous dispersion. This vial was allowed to stand at room temperature where the opsonization reaction ensued over 5–10 min.The opsonized radiocolloid (0.2 ml; 0.5 MBq) was injected intravenously as the second injection, 20 min after cold colloid (0.2 ml) injection into the same rats ($n = 3$) using the procedure above.

2.2.4. ^{99m}Tc-SnF_2 leucocytes in whole blood. A group of rats ($n = 4$) were injected intravenously with ^{99m}Tc-leucocytes in whole blood (0.2 ml, 1 MBq) and then after 20 min they were sacrificed. The lungs, liver and spleen were removed and counted.

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2.2.5. Cold SnF_2 colloid and ^{99m}Tc-SnF_2 leucocytes in whole blood. The procedure was repeated as above except that cold colloid (0.2 ml; 26 μg) was injected 20 min before ^{99m}Tc-SnF_2 leukocytes in whole blood (0.2 ml; 1 MBq) was injected into the same rats ($n = 4$).

2.2.6. ^{99m}Tc-red cells. Using a literature procedure[12] the *in vivo* labelling method was employed. A stannous pyrophosphate kit (RAH Radiopharmacy, Adelaide, Australia) [sodium pyrophosphate (15.0 mg) and stannous chloride (3.2 mg) in water for injection (1 ml)] was diluted with saline (9 ml). Doses of stannous solution (0.04 ml) were prepared in saline (0.1 ml), and then injected into rats ($n = 3$). After allowing 20 min for the red cells to become 'tinned', ^{99m}Tc-pertechnetate (0.2 ml; 0.8 MBq) was injected. Rats were sacrificed 20 min later, then the lungs, liver, spleen and a blood sample were removed and counted as above. Blood was obtained by intra-cardiac puncture, weighed, then counted to determine % id/g using a dose equivalent standard.

2.3. Statistical analyses

Results are reported as mean ± standard error, and groups were compared using paired sample *t*-tests or ANOVA. Statistical significance was defined as a *p* value less than 0.05.

3. Results

3.1. Effects of colloid pre-injections on the distribution of ^{99m}Tc-SnF_2 colloid

Rat physiological distributions of ^{99m}Tc-SnF_2 colloid as the sole injection or after cold colloid pre-injections, are summarized in Table I. Following injection of ^{99m}Tc-SnF_2 colloid alone there was 3.1% uptake in the lungs, 85.7% in the liver and 7.6% in the spleen. These results are consistent with the product specification for ^{99m}Tc-Colloidal Tin Injection, where $\geq$80% of the radioactivity is found in the liver and spleen, and $<$5% in the lungs.[11]

When cold stannous fluoride colloid containing levels of 13, 26 or 52 μg in the dose was given as a pre-injection to ^{99m}Tc-SnF_2 colloid, a slight increase in liver uptake (2–5%) and a decrease in spleen uptake (relative to no cold colloid pre-injection) by 39, 38 and 53%,

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Table I. ^{99m}Tc-stannous fluoride colloid distribution in rats at 20 min post injection with or without pre-injection of cold SnF_2 colloid

Organ	Percentage injected dose Mass of SnF_2 colloid in pre-injection				
	0 μg	13 μg	26 μg	52 μg	26 μg[a]
Lungs[b]	3.1 ± 0.6	1.4 ± 0.0	2.0 ± 0.1	1.6 ± 0.1	2.6 ± 0.1
Liver[b]	85.7 ± 0.7	87.9 ± 0.7	90.3 ± 0.3	88.2 ± 0.7	84.3 ± 0.7
Spleen[b]	7.6 ± 0.3	4.6 ± 0.3	4.7 ± 0.3	3.6 ± 0.2	7.8 ± 0.3
Blood[c]	0.021 ± 0.001	—	0.041 ± 0.002	—	0.020 ± 0.006
Sample size	$n=5$	$n=3$	$n=6$	$n=3$	$n=3$

[a] pre-opsonized ^{99m}Tc-SnF_2 colloid.
[b] $p<0.05$ for 0 vs 13 μg, 0 vs 26 μg, 0 vs 52 μg, 26 vs 26 μg[a]; $p>0.09$ for 0 vs 26 μg.
[c] All values ($n=3$) reported as % id/g.

respectively, was observed. The altered uptake by lungs, liver and spleen due to the pre-injection of cold colloid, was not observed when the second injection was opsonized ^{99m}Tc-SnF_2 colloid.

The results of ^{99m}Tc-SnF_2 colloid activity in the blood shown in Table I, indicated that no effect was observed when either cold SnF_2 colloid pre-injection or opsonized ^{99m}Tc-SnF_2 colloid was administered. The average mass of these rats was 180 g. Assuming a total blood volume of 11.5 ml for a rat mass of 180 g[13] and a blood density ~1.1 g/ml,[14] then at 20 min post radiocolloid injection there is 0.2–0.5% of the injected dose in the total blood volume of a rat. A very low whole blood activity indicates a high extraction of colloid from the blood by the liver, spleen and bone marrow during this period, and that radiocolloid is not associated with any circulating red cells. Furthermore, any residual blood in the excised organs can have little effect on the organ counts. As most of the injected dose is accounted for in the liver, lungs and spleen, the remaining carcass activity is attributed to bone marrow uptake (4.3 ± 0.6%, range 2–7%).

3.2. Distribution of ^{99m}Tc-SnF_2 labelled neutrophils in whole blood

The physiological distribution of ^{99m}Tc-neutrophils in whole blood, and the effect of a SnF_2 colloid pre-injection dose is shown in Table II. The organ uptake values are consistent with a previous study in Staphylococcus aureas infected rats where liver (83%) and spleen (12%) activity totalled >90% of the injected dose of ^{99m}Tc-SnF_2 labelled leucocytes at 1 h post injection.[10] Pre-injection of SnF_2 colloid resulted in a significant increase in liver activity and a 30% decrease in spleen activity. ^{99m}Tc-neutrophils in whole blood gave an identical

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Table II. The organ distribution in rats of ^{99m}Tc-SnF_2 labelled neutrophils in whole blood or ^{99m}Tc-red cells (via *in vivo* method) at 20 min post injection

Organ	Percentage injected dose		
	^{99m}Tc-SnF_2 labelled neutrophils		^{99m}Tc-red cells
	Mass of SnF_2 colloid in pre-injection		
	0 μg	26 μg	
Lungs	2.7 ± 0.0	2.8 ± 0.1	1.0 ± 0.0
Liver	85.4 ± 0.0^a	88.3 ± 0.3^a	6.7 ± 1.2
Spleen	8.7 ± 0.2^a	6.1 ± 0.3^a	0.3 ± 0.0
Stomach	—	—	0.5 ± 0.1
Blood[b]	—	—	8.9 ± 0.2
Sample size	$n=4$	$n=4$	$n=3$

[a] $p<0.05$ for 0 versus 26 μg.
[b] All values reported as % id/g.

distribution to ^{99m}Tc-SnF_2 colloid (with no cold colloid pre-injection) for the lungs ($p=0.260$), liver ($p=0.162$) and spleen ($p=0.202$). Over 96% of the injected dose was accounted for by the lungs, liver and spleen, and the remaining activity attributed to bone marrow uptake.

3.3. Distribution of ^{99m}Tc-red cells labelled in vivo

From the results shown in Table II, the *in vivo* labelling method resulted in very low stomach uptake, indicating no free ^{99m}Tc-pertechnetate present, or quantitative formation of ^{99m}Tc-red cells. After the 20 min localization period, most of the activity was associated with the circulating blood. In these experiments, the average mass of rats was 160 g. Assuming a total blood volume of ~10 ml for a rat mass of 160 g, then at 20 min post injection approximately 100% of ^{99m}Tc-red cells are in the blood pool. This is supported experimentally, where a single rat blood sample of 4.5 ml contained 39.6% of the injected dose. There was insignificant uptake by spleen, and low uptake in the lungs and liver. The liver activity is most probably due to residual intact ^{99m}Tc-erythrocytes[15] that remained in the vascular bed since the organ was not perfused with water after dissection, although any damaged ^{99m}Tc-erythrocytes would also deposit there.

4. Discussion

The use of ^{99m}Tc-SnF_2 colloid labelled leucocytes in whole blood to image inflammatory sites, has the advantage of avoiding cell

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separation steps in the labelling procedure, where only phagocytic cells are labelled. Although the nature of the radiocolloid interaction with blood cells is not accurately known, this radiopharmaceutical has found routine application imaging patients with inflammatory disorders.[16] Our results in rats indicate that $^{99m}Tc\text{-}SnF_2$-neutrophils in whole blood gave low lung uptake with higher uptake in liver and spleen, yielding an identical biodistribution to the corresponding organ values found for $^{99m}Tc\text{-}SnF_2$ colloid alone when no cold stannous fluoride colloid pre-injections were used. A similar organ distribution pattern is observed clinically in patients with inflammatory bowel disease after receiving a whole blood dose of ^{99m}Tc-neutrophils prepared by the same colloid method.[17] These results support the likely existence of unbound $^{99m}Tc\text{-}SnF_2$ colloid *in vivo* when leucocyte scans are performed using this method.[9] Rather than excess free colloid being present in the original dose, it has been shown that approximately 75% of the radioactivity is associated with the erythrocytes.[8] However surface adherence is reversible in the presence of leucocyte rich plasma,[18] where there is an abundance of opsonizing proteins, as well as leucocytes that can 'steal' radiocolloid particles.[8] Thus, the radioactive dose is mainly composed of $^{99m}Tc\text{-}SnF_2$ colloid-bound-erythrocytes, ^{99m}Tc-neutrophils, and to a lesser extent ^{99m}Tc-monocytes.

It was proposed earlier that excessive erythrocyte labelling might contribute to splenic uptake.[8] In this study, the difficulty in isolating purified $^{99m}Tc\text{-}SnF_2$ colloid-bound-erythrocytes was avoided, in favour of preparing ^{99m}Tc-red cells via a standard procedure.[12] This procedure results in tightly bound ^{99m}Tc to erythrocytes from an interaction with intracellular haemoglobin, different to $^{99m}Tc\text{-}SnF_2$ colloid-bound-erythrocytes where radiocolloid is bound onto the outer membrane surface. Our results showed the level of spleen uptake by ^{99m}Tc-red cells is extremely low (0.3%) and that after a 20 min localization period, most of the activity is still in the circulating blood pool.[19] This is in contrast to the ^{99m}Tc-colloid distribution studies which revealed no circulating blood activity. It is unlikely that the radioactive red cells will sequester in the spleen at a significant level, implying the radiocolloid dissociates from erythrocytes *in vivo*. Ultimately phagocytosis within the spleen would be facilitated with smaller opsonized particles[20] rather than $^{99m}Tc\text{-}SnF_2$ colloid-bound-erythrocytes. The physico-chemical properties of the antigen are important,[21,22] and if the antigen is an erythrocyte, it needs to be substantially altered

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such as derivation from a different species[23] or containing other modifications.[24–26] It was concluded that the spleen activity observed with the ^{99m}Tc-neutrophils distributions is therefore due to radiocolloid plus ^{99m}Tc-neutrophils.

An initial pre-injection dose of SnF_2 colloid (26 μg) resulted in decreased splenic uptake of ^{99m}Tc-SnF_2 colloid and ^{99m}Tc-SnF_2 neutrophils by 38 or 30%, respectively. This suggests a blockage of ^{99m}Tc-colloid uptake occurs because excess cold colloid saturates spleen macrophage function.[27] There was no observed change in liver uptake for either of these ^{99m}Tc-agents, attributed to the far higher concentration of phagocytic Kupffer cells in the liver compared to macrophages in the sinus basement of the spleen. The proportion of total reticuloendothelial cells in the body was reported as 80–90% in the liver, 5% in spleen and 5% in bone marrow.[28] Hence, a decrease of $\sim$3% id of radiocolloid after a cold colloid pre-injection is substantial for the spleen but low for the liver (88–90% id).

A dose response effect was observed, where an increasing level of pre-injected cold colloid resulted in a decreasing splenic uptake. The extent of the decrease for spleen activity was approximately equal to the increase in liver activity, suggesting that the phagocytic function of liver compensates for the depressed function of the spleen. The best level of ^{99m}Tc-SnF_2 colloid blockage in the spleen was 53% when an initial dose of 52 μg SnF_2 colloid was administered. ^{99m}Tc-sulfur colloid has a particle size range of 0.1–1.0 μm when prepared by the thiosulfate method.[29] and after intravenous administration, 97% of the dose is removed from the blood by liver and spleen within 10–15 min.[27] ^{99m}Tc-SnF_2 colloid has a larger particle size of 1.5–2.1 μm[30] and it yielded similar levels of extraction (93%) by the same organs after 20 min. In general, larger particles have higher retention in the liver and spleen [31] than in the bone marrow.

The introduction of particulate antigens into the blood system initiates a process of opsonization, where there is binding of antibodies or specific plasma proteins such as ligand binding protein (LBP), immunoglobulins (IgM, IgG),[32,33], C3bi[34] or streptoccocal C5a peptidase[35] to the antigen surface. Opsonic receptor molecules (i.e. CD14) anchored onto the macrophage cell surface, bind the opsonized-particles,[36] and this interaction subsequently activates signals via other receptor molecules[37] to finally result in phagocytic engulfment. Engulfment of unopsonized particles occurs by alveolar[38] but not splenic macrophages. When an initial cold colloid dose of 26 μg was

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administered to rats, opsonized $^{99m}Tc\text{-}SnF_2$ colloid resulted in significantly lower liver and higher spleen activity when compared to non-opsonized $^{99m}Tc\text{-}SnF_2$ colloid ($p < 0.05$). However for both of these organs, uptake of $^{99m}Tc\text{-}SnF_2$ was not significantly different when the opsonized colloid was compared to the non-opsonized form with no cold colloid pre-injection ($p > 0.09$). These results can be explained by the pre-injection of cold SnF_2 colloid substantially depleting the circulating opsonin concentration in rats, thereby decreasing the opsonization rate of subsequently administered unopsonized $^{99m}Tc\text{-}SnF_2$ colloid. When a second injection of opsonized $^{99m}Tc\text{-}SnF_2$ colloid was given, no decrease in spleen uptake was observed, where phagocytosis commenced immediately because opsonization was unnecessary. The rate limiting step appears to be the level of opsonization in blood rather than phagocytic capacity of the macrophages of liver and spleen.[39]

With the $^{99m}Tc\text{-}SnF_2$ neutrophils dose, the small whole blood sample (5 ml) used for radiolabelling *ex vivo* has a finite concentration of opsonins, sufficient to coat only a small portion of the total $^{99m}Tc\text{-}SnF_2$ particle population. Because of the low concentration of opsonized radiocolloid, a limited amount of phagocytosis ensues, resulting in a low percentage of ^{99m}Tc-neutrophils also present in the same dose. While unopsonized colloid is associated with erythrocytes,[8] our results suggest that in the rat, this excess colloid is somehow detached from red cells *in vivo* to become opsonized and yield an identical physiological distribution to $^{99m}Tc\text{-}SnF_2$ colloid. At the molecular level, the interaction between radiocolloid and the red cell surface is undefined, as is the mechanism of dissociation. Following injection of the radiocolloid-attached cells, detachment of the $^{99m}Tc\text{-}SnF_2$ colloid may be promoted by the excess opsonins present in the circulation, high shear at vessel walls during turbulent blood flow, or mechanical disruption as cells distort to permit their transit through the finer lung capillaries. We are currently investigating which of these phenomena explains the cellular detachment.

The high liver and spleen activity seen in $^{99m}Tc\text{-}SnF_2$ leucocyte scans limits the detection of inflammatory and infective foci within and close to these organs. These results in this study suggest that a pre-injection of cold colloid may reduce splenic activity and potentially improve detection rates of inflammatory and infective foci in the left upper quadrant of the abdomen.

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5. Conclusion

The biodistribution results in rats show that there is excess non-opsonized radiocolloid present in a dose of ^{99m}Tc-neutrophils prepared by the stannous fluoride colloid method. There is a saturation of reticuloendothelial cell capacity in the spleen of rats rather than liver, evident from experiments with pre-injected cold stannous fluoride colloid. However the more important process appears to be opsonization of the radiocolloid prior to phagocytic engulfment, which depends on the opsonin concentration in circulating blood. The effect of opsonization of radiocolloids is also being investigated by this laboratory. A pre-injection of unlabelled stannous fluoride colloid may reduce splenic uptake in ^{99m}Tc-SnF_2 colloid labelled white cell scans.

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