

COMMONWEALTH DEPARTMENT OF HEALTH



Australian Radiation Laboratory

**Ligand-Free, Protein-Bound Technetium-99m.
Evidence for Tumour Localisation.**

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**[Presented in part at the Australian and New Zealand Society of Nuclear
Medicine, 14th Annual Scientific Meeting, Perth, Western Australia,
May, 1983.]**

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ABSTRACT

A hypothesis that cations accumulate in tumours independent of ligand is tested. A preparation of technetium-99m known to be "ligand-free" (that is, the technetium is protein bound and no other ligand is injected) has been shown to accumulate in a T-cell lymphoma.

INTRODUCTION

Several radiopharmaceuticals based on technetium-99m, such as glucoheptonate (1), MDP (2), pyrophosphate (3), citrate (4) and others, have been found to concentrate in tumours. In addition, the use of citrates of other cations (for example ^{54}Mn , ^{111}In , ^{169}Yb and ^{65}Zn) has also been suggested (5) for detection of tumours, while gallium-67 citrate is currently the radiopharmaceutical of choice (6). The metal in each of these agents (including those based on technetium-99m) is weakly complexed and we suggest that in each case it is the ligand-free cation (bound to a suitable protein) that is transported to, and eventually accumulates in, the tumour in a manner analogous to gallium cations (7).

Recently we described (8) the biodistribution in mice and rabbits of technetium-99m labelled transition metal borides. Initially, the bulk of the activity was found, as expected, in the liver and lungs of the animal (depending on the particulate size of the preparation). Within an hour, however, the radioisotope was being excreted in the urine. No significant activity in the stomach or thyroid was detected eliminating the possibility of the technetium being converted in vivo to pertechnetate. Since no other ligand had been administered, it appeared that the technetium was transported in the blood as a cation-protein complex and, as such, this system would be an excellent test for our "protein-bound cation tumour localisation" hypothesis.

Injection of technetium-99m labelled nickel "boride" into mice with a T-cell lymphoma in the thigh showed preferential retention of the label in the tumour.

MATERIALS AND METHODS

Iron-dextran (50mg/ml) was obtained commercially (Imferon, Fisons Australia). An EDTA solution was prepared by dissolving sodium EDTA (20mg) in saline (1ml). Nickel "boride" was prepared and labelled with technetium-99m as previously reported (9).

Animal experiments were performed on female (CBA x B6) F1 mice weighing 25-30 gm in which a T-cell lymphoma (EL4) had been previously implanted in the left rear thigh. Eight days after implantation, the tumour weighed about 0.5gm. At this time the mice were injected intravenously with a labelled suspension of nickel "boride" ($200-1000\mu\text{Ci } ^{99\text{m}}\text{Tc}$) and sacrificed after 7.5, 52 and 72 hours. The activity in their weighed organs was determined using a

Packard Auto-Gamma scintillation spectrometer. Alternatively, activity distribution was monitored in live animals using a Searle Pho γ 3HP gamma camera.

The effect of iron dextran on tumour localisation of technetium-99m was determined by injecting the tumoured mice with iron dextran (0.1 ml) intraperitoneally 4 hours after intravenous injection of labelled nickel "boride" suspension. These mice were sacrificed 3.5 hours later and the biodistribution of the activity compared with control mice sacrificed 7.5 hours after intravenous injection of the labelled suspension.

The effect of EDTA on the biodistribution of the isotope was determined in healthy, white, male mice (25-30 gm). The mice were injected intravenously with labelled nickel "boride" suspension and after 6 and 7 hours respectively were injected intraperitoneally with EDTA solution (0.1 ml). All mice were sacrificed 7.5 hours after the original injection of the isotope. The biodistribution of the activity was compared with control mice sacrificed 7.5 hours after injection of the labelled suspension.

RESULTS

The biodistributions of the activity in selected organs at 7.5, 52 and 72 hours (expressed in organ % dose/gm relative to tumour % dose/gm) are shown in Table 1.

The results indicated that, while the activity gradually accumulated in the tumour and muscle over 72 hours, the blood activity dropped sharply. The tumour would therefore be expected to be more clearly delineated as time progressed and gamma camera scans implied this to be true. Activity in the stomach remained relatively low while kidney activity was high. The results indicated that after initial localisation of the activity in the liver and lungs, the technetium-99m was not dispersed as pertechnetate but rather was transported in the blood and excreted. The findings confirmed our earlier observations (8). Neither EDTA nor iron dextran had any significant effect on the biodistributions set out in Table 1.

TABLE 1. Biodistribution of labelled nickel "boride" in mice with EL4 tumour.

	Tumour to organ ratios ^{1,2} following IV administration (hours)		
	7.5	52	72
Tumour/blood	0.70 ± 0.30	1.38 ± 0.12	1.91 ± 0.35
Tumour/muscle	2.77 ± 1.53	2.78 ± 1.34	4.39 ± 3.52
Tumour/skin	1.22 ± 0.55	1.02 ± 0.39	1.08 ± 0.16
Tumour/heart	1.12 ± 0.43	1.64 ± 0.15	1.74 ± 0.39
Tumour/lungs	0.56 ± 0.28	0.20 ± 0.08	0.18 ± 0.10
Tumour/liver	0.61 ± 0.14	0.36 ± 0.09	0.25 ± 0.05
Tumour/stomach	0.33 ± 0.13	0.62 ± 0.26	1.14 ± 0.52
Tumour/GI tract	0.58 ± 0.16	1.32 ± 0.45	1.98 ± 0.50
Tumour/spleen	1.11 ± 0.35	0.76 ± 0.20	0.96 ± 0.90
Tumour/kidneys	0.14 ± 0.07	0.17 ± 0.04	0.19 ± 0.03

1 Expressed as tumour (% dose/gm) to organ (% dose/gm) ratios

2 Mean of 5 mice ± SD.

DISCUSSION

The results in Table 1 lend weight to the hypothesis that protein bound cations, independent of ligands, are transported to tumours and that in this respect, technetium-99m is no exception. The labelled nickel "boride" preparation is the first potential source of uncomplexed technetium-99m cations to be tested in vivo. All other technetium based radiopharmaceuticals either employ the stannous ion alone and result in a labelled colloid which is irreversibly localised in the liver (or lungs) or, alternatively, employ ligands which, it may be argued, play a role in the localisation of the isotope in the target organ.

The finding that EDTA (which is known to complex with technetium, resulting in its rapid urinary excretion) had no effect on the background activity, implies that no free technetium cations are actually circulating in the blood stream. The technetium is therefore strongly protein-bound.

Sephton and co-workers (7) reported that iron dextran administration enhanced gallium-67 uptake by tumours by competitive interaction with the protein carrier transferrin. The failure of iron dextran to influence tumour localisation of technetium suggests either that gallium and technetium localise in tumours by different mechanisms or that the effect of iron on the biodistribution of gallium citrate is incidental to the actual tumour uptake mechanism. (For example, an interaction between iron and citrate freeing cationic gallium and promoting its binding to the protein carrier).

Clinically, the preparation had no use for detection of tumours. The continuous "elution" of activity from the reticuloendothelial system led to a high blood pool background in the short term (24-48 hours). Only after long periods, impractical for scans employing technetium-99m, was a steady state reached where retained tumour activity was significantly above that of the blood background.

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