Impurities in a $^{99m}$Tc Lung Imaging Kit

J. R. McLean

Radiation Protection Bureau, Ottawa, Canada

Paul Wise

Ottawa General Hospital, Ottawa, Canada

We investigated the nature and significance of three radiochemical impurities that were found in a lung imaging kit using $^{99m}$Tc-tin-macroaggregated albumin. The most abundant radiochemical impurity was a soluble $^{99m}$Tc-labeled protein. Free pertechnetate ($^{99m}$TcO$_4$) and reduced and hydrolyzed technetium ($^{99m}$TcO$_2$) were present in relatively low radioconcentrations. The presence of a soluble $^{99m}$Tc-labeled protein in the preparation at the time of injection modified the biologic performance of the product. We suggest that, to be meaningful, a quality control program must employ assay procedures which are capable of identifying and quantitating all the significant radiochemical impurities that are present in a preparation.

Radiochemical impurities in a preparation of $^{99m}$Tc-tin macroaggregated albumin ($^{99m}$Tc-Sn-MAA) can arise from the presence of $^{99m}$Tc in various oxidation states or from reagents or chemical impurities that can compete with the intended carrier molecule for available $^{99m}$Tc. In addition, the quantity and oxidation state of tin in the preparation is critical for efficient labeling (1.2). The biologic performance of the preparation should be within the parameters that have been established by previous batches of the product if the most significant radiochemical impurities are maintained at low levels.

The current quality control practice for many $^{99m}$Tc-labeled radiopharmaceuticals is almost exclusively restricted to monitoring the preparation for $^{99m}$TcO$_2$ and the labeling efficiency for the process is defined in these terms. This is adequate if the main radiochemical impurity is $^{99m}$TcO$_2$. Several assay procedures may be required, however, if other less obvious radiochemical impurities are present in the preparation in significant quantities. In theory, it should be possible to predict the in-vivo performance of a preparation from a knowledge of the nature and concentration of its radiochemical constituents, since each has a characteristic biologic distribution. The use of radiochemical assays for this purpose, however, is limited by the absence of rapid and reliable techniques for identifying all relevant entities and for defining the biologic behavior and significance of each.

Various formulations of $^{99m}$Tc-Sn-MAA have been evaluated in the literature and each product appears to have similar radiochemical parameters, as defined by the level of $^{99m}$TcO$_2$, but diverse biologic behavior (3-7). Studies on the metabolism of $^{99m}$Tc microspheres (8), $^{99m}$Tc-Fe(OH)$_3$ (9), and $^{99m}$Tc-Sn(OH)$_2$ adsorbed onto freshly prepared MAA (10) suggest that the clearance of radioactivity from the lungs occurs independently from the metabolism of the macroparticle. The $^{99m}$Tc-Sn label dissociates as $^{99m}$TcO$_4$ and $^{99m}$TcO$_2$, which may be subsequently oxidized to $^{99m}$TcO$_2$ and possibly $^{99m}$Tc-HSA or some other protein fragment (8). The rate at which $^{99m}$Tc-Sn dissociates from the MAA particles depends, in part, on the denaturing conditions that were used to form the particles (11). A $^{99m}$Tc-Sn-MAA radiopharmaceutical tends to have a relatively short biologic half-life if the particles have been formed under "soft" conditions of low denaturing temperatures and short denaturing times (3,8,11). A short biologic half-time is a desirable product attribute because the radiation dose received by the critical organs is minimized. The biologic half-life of different $^{99m}$Tc-Sn-MAA preparations will vary because there are many methods for producing the Sn-MAA coprecipitate, each one employing slightly different conditions for particle formation. It is possible that denaturation of albumin may be incomplete for some procedures, especially when the preparation is found to have a short effective half-life in the target organ. The radiochemical data reported for some of the products evaluated in the literature (4-7) are very similar, showing less than 5% $^{99m}$TcO$_2$ at the time of injection. The level of $^{99m}$TcO$_2$ was never reported and no attempt was made to detect the presence of a soluble $^{99m}$Tc-protein or protein fragment. Since soluble and partially denatured albumin, or albumin fragments, could also be present in MAA kits, this study was undertaken to determine if a soluble $^{99m}$Tc-protein was present in sufficient quantities to affect the biologic performance of the preparation.
Materials and Methods

The ⁹⁹ᵐTc-Sn-MAA kit (Pulmolite, New England Nuclear, North Billerica, MA; lot No. 7061) was supplied in the form of vials each containing 1.5 mg of denatured human serum albumin, 10 mg of normal serum albumin (HSA), 10 mg of NaCl, and a maximum of 0.07 mg of stannous chloride, all in lyophilized form. The vials were stored at 4°C until use. The preparation of ⁹⁹ᵐTc-Sn-MAA for the radiochemical and biologic assays was performed by slowly adding 3 ml of Na⁹⁹ᵐTcO₄ (NEN generator) into the vial. Labeling efficiency of the preparation was determined by several techniques. Paper chromatography using Whatman No. 4 as the stationary phase and 70% methanol as the mobile phase (USP XIX) was used to detect and quantitate the presence of ⁹⁹ᵐTcO₄. Paper chromatography using Whatman No. 1—pretreated with a 1% solution of HSA and 0.15 M NaCl as the mobile phase was used to monitor for ⁹⁹ᵐTcO₄ and for soluble labeled protein fragments (12). Technetium-⁹⁹m-tin human serum albumin (C.E. Frosst, lot 2166W) was also chromatographed by this method as a reference protein. The centrifuge assay (USP XIX) was used to quantify all radioactivity that was dissociated from the macroparticles. A modified centrifuge assay was performed by treating a 0.1-ml aliquot of the preparation with 15% trichloroacetic acid (TCA). The mixture was centrifuged at 2,000 rpm for 20 min at 4°C in a Sorvall RC-2B centrifuge. This procedure left the radioactivity due to ⁹⁹ᵐTcO₄ and ⁹⁹ᵐTcO₂ largely in the supernatant, while acid-insoluble labeled proteins were precipitated with the macroparticles.

Tissue distribution studies were done using 20–25-g male Swiss Webster/HPB strain mice at various time intervals following the iv administration of about 20 μCi of ⁹⁹ᵐTc-Sn-MAA in a volume of 0.1 ml. Groups of three mice were sacrificed at 2-, 5-, 10-, 30-, and 60-min intervals. Lungs, liver, kidneys, and spleen were completely excised and blotted free of blood before being assayed for radioactivity. The radioactivity was then expressed as percent of administered dose per whole organ. Triplicate 20–μl samples of blood were collected from the heart in Sahli pipettes. Blood was estimated at 8% of body weight.

In patients, lung imaging was performed using a Searle Radiographics Pho/Gamma IV scintillation camera immediately following iv injection of 2 mCi of ⁹⁹ᵐTc-Sn-MAA, which was reconstituted with 2 ml of Na⁹⁹ᵐTcO₄ (NEN generator) just before use. Immediately after routine lung imaging, a rough estimate of the relative radioactivity in the kidneys was obtained by comparing the times required to accumulate 50,000 counts over the lungs and over the kidneys. The radioactivity outside the field of interest was shielded in each case. Both procedures were completed within 25 min of the administration of the ⁹⁹ᵐTc-Sn-MAA.

### Results and Discussion

The results of radiochemical assays (Table 1) indicate that the initial concentration of ⁹⁹ᵐTcO₂ in the preparation was about 2.5%. This level increased to about 7.2% at 6 h after preparation (Table 2). Results from HSA-treated paper-saline chromatography and from the centrifuge assay (USP XIX) indicate, respectively, that about 16.5% and 14.6% of the radioactivity in the preparation was dissociated from the macroparticles. On HSA-treated paper-saline chromatograms [Fig. 1(A)], two radiochemical impurities were detected at Rr 0.75 and 0.85, corresponding to ⁹⁹ᵐTcO₂ and ⁹⁹ᵐTc-protein (8,12). A HSA-treated paper-saline chromatogram [Fig. 1(B)] of ⁹⁹ᵐTc-Sn-HSA showed a similar pattern of peaks, indicating that the radiochemical impurity observed in Fig. 1(A) was the ⁹⁹ᵐTc-labeled albumin. The modified TCA centrifuge assay indicated that 3.6% of the radioactivity was acid soluble and this probably represented the combined ⁹⁹ᵐTcO₂ and ⁹⁹ᵐTcO₄ components. The most significant radiochemical impurity present in the preparation was a ⁹⁹ᵐTc-protein which represented 12–14% of total radioactivity. This was calculated from Table 1 by taking the difference between the USP XIX centrifuge assay value (16.5%) and the TCA modified centrifuge assay value (3.6%), or by the difference between the radioactivity in the interval Rr 0.65–0.95 on HSA paper-saline chromatograms (14.5%) and the ⁹⁹ᵐTcO₂ detected on paper-methanol chromatograms (2.5%).

The in-vitro assay data suggest that a relatively large

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<tr>
<th>TABLE 1. Radiochemical Assays of ⁹⁹ᵐTc-Sn-MAA (NEN)</th>
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<tbody>
<tr>
<td>Paper methanol chromatography (USP XIX)</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
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<tr>
<td>Radiochemical Impurity Detected</td>
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<tr>
<td>Percent of total radioactivity</td>
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<td>Replicates</td>
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*By paper methanol chromatography; mean of three values ± sd.

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<th>TABLE 2. Percent ⁹⁹ᵐTcO₂ at Various Times After Preparation*</th>
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<td>Time after preparation (h)</td>
</tr>
<tr>
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</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
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*By paper methanol chromatography; mean of three values ± sd.
proportion of the radioactivity present in the preparation would not be retained in the lungs at the time of injection but would remain circulating in the blood like a serum protein. The small quantities of $^{99m}$TcO$_4^-$ and $^{99m}$TcO$_2$ would be rapidly cleared from the blood by the kidneys and liver, respectively (13,14). Organs with large blood supplies would therefore be subjected to higher radiation exposure and the resolution of the target organ could be degraded by elevated levels of background radiation in contiguous tissues. In the case of liver and kidneys, the radiation dose would be further increased by the presence of breakdown products of $^{99m}$Tc-Sn-MAA occluded in the lungs. The extent of this dose would be determined by the length of time that the labeled protein remained in the blood, which would largely depend on its molecular weight (14-16), and on the rate at which $^{99m}$Tc-Sn dissociated from the macroparticles, which is known to depend, in part, on the denaturing conditions under which the albumin particles were formed (8-10).

The results of animal studies (Table 3) indicate a qualitative radionuclide distribution profile that is in agreement with what could be inferred from the radiochemical data. At 2 min, 88% of the injected dose was retained in the lungs. Initially a portion of this radioactivity was rapidly eliminated, but the rate of clearance appeared to decrease significantly at 10 min after injection. The presence of a short-lived component implies that at least a portion of the $^{99m}$Tc was weakly associated with the macroparticles. The steady rise of radioactivity in the blood, liver, and kidneys during the time interval studied indicates that the breakdown products probably included a $^{99m}$Tc-labeled protein in addition to the expected $^{99m}$TcO$_4^-$ and $^{99m}$TcO$_2$ metabolites (7).

The radiochemical and animal data therefore suggest that high levels of radioactivity in the blood from the $^{99m}$Tc-protein and a rapid dissociation of $^{99m}$Tc-Sn from the occluded particles would be reflected, in humans, by visualization of the kidneys, which have a high blood flow and which excrete $^{99m}$TcO$_4^-$ (14). This was confirmed

<table>
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<th>Organ</th>
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<th>5</th>
<th>30</th>
<th>60</th>
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<tbody>
<tr>
<td>Lung</td>
<td>88.2 ± 1.2</td>
<td>83.7 ± 2.8</td>
<td>44.5 ± 2.0</td>
<td>41.9 ± 1.6</td>
</tr>
<tr>
<td>Blood</td>
<td>8.0 ± 1.2</td>
<td>5.7 ± 0.2</td>
<td>11.9 ± 0.8</td>
<td>13.0 ± 1.0</td>
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<tr>
<td>Liver</td>
<td>2.4 ± 0.9</td>
<td>2.2 ± 1.1</td>
<td>5.4 ± 1.0</td>
<td>5.2 ± 1.1</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>4.2 ± 0.9</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>Spleen</td>
<td>—</td>
<td>—</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
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*Mean of three animals ± sd.

FIG. 1. (A) $^{99m}$Tc-Sn-MAA chromatographed in N$_2$ atmosphere on HSA-saturated Whatman No. 1 using aqueous N$_2$-purged 0.15 M NaCl as solvent. Peaks at R$_s$ of 0.74 and 0.85 correspond to $^{99m}$TcO$_4^-$ and $^{99m}$Tc-protein, respectively. (B) $^{99m}$Tc-Sn-HSA chromatographed under same conditions, with R$_s$ of 0.00, 0.75, and 0.85 corresponding to $^{99m}$TcO$_4^-$, $^{99m}$TcO$_2^-$, and $^{99m}$Tc-HSA, respectively.

FIG. 2. (A) $^{99m}$Tc-Sn-MAA lung scan at about 15 min after administration. (B) Visualization of kidneys immediately after completion of lung scanning. Activity in kidneys was 8.4 ± 3.7% at 25 min.
in 11 patients who were investigated immediately after the completion of routine lung imaging. The radioactivity in the kidneys relative to that in the lungs was found to be 8.4 ± 3.7% at about 25 min after injection (Fig. 2).

The most significant radiochemical impurity, the soluble $^{99m}$Tc-protein, is probably an artifact of the production process. The use of a single radiochemical assay, such as paper-methanol chromatography for $^{99m}$TcO$_4^-$, would not be adequate as a quality control procedure because it would not detect the most abundant impurity, and the radiochemical parameters so defined would show little correlation with the biologic performance of the preparation.

We will investigate kits of other manufactures for the presence of this $^{99m}$Tc-protein impurity, and the results will be reported in future articles.

References


(Editor's note: A reply to this paper from New England Nuclear appears on p. 35 of this issue.)
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