jnm / PRELIMINARY NOTE

TECHNETIUM-99m LABELING BY MEANS OF STANNOUS PYROPHOSPHATE: APPLICATION TO BLEOMYCIN AND RED BLOOD CELLS

A. Bardy, H. Fouyé, R. Gobin, J. Beydon, G. de Tovar, C. Pannecière, and M. Hégésippe

CIS, DR/SPRR. Cen-Saclay, Foch Hospital, Suresnes, and American Hospital, Neuilly, France

A new technique of technetium labeling using stannous pyrophosphate instead of stannous chloride as reducing agent for pertechnetate has been applied to red blood cells and bleomycin. Results are so encouraging that this technique could be extended to other compounds capable of forming stable complexes with reduced technetium. No saline washes of red cells are necessary before or after the addition of pertechnetate. No purification step is performed after labeling of bleomycin.

In order to use ^{99m}Tc as a tracer for the visualization of organs or regions, the radionuclide should be in a reduced state for labeling various compounds. Among the agents used to reduce pertechnetate into a lower valence (*1–3*), stannous chloride is the most widely used. All the corresponding techniques must include the following: (A) daily preparation of an acid solution of stannous chloride, (B) mixing of the compound to be labeled with the previously mentioned solution followed by an adjustment of pH, and (C) in order to prevent the presence of colloidal tin, the compound must always be a complexing agent of stannous ion or excess tin should be eliminated before adding pertechnetate as is generally the case for red cell labeling.

The present work is a tentative proposal to simplify these problems. If the acid solution of stannous chloride could be replaced by a weak stannous complex stable in the neutrality zone and forming a rather labile complex with "reduced technetium," it would be sufficient to add this complex to the compound to be labeled just before or after the addition of $^{99m}TcO_4^-$ to obtain a complete labeling. Neutralization and purification steps would then be unnecessary.

We arrived at this idea by studying the technetium–tin–pyrophosphate complex (*4*). Mesmer, et al (*5*) have shown that, according to the concentration of pyrophosphate, the complexes formed with Sn^{2+} were different in their constitution, stable in the pH range 5–7, and of relatively weak force ($K_1 = 6 \times 10^5$, $K_2 = 2 \times 10^8$). A simple qualitative method of determining the weakness of a technetium complex is to follow its behavior on a Sephadex G-25 column. Hégésippe, et al (*6*) have already shown that in the case of technetium–tin–pyrophosphate, the radioactivity is almost completely adsorbed on the column top (> 95%). The Sephadex gel in this particular case destroys the complex. Valk, et al (*7*) and Richards, et al (*8*) have complained recently that the polysaccharide gel not only fixes the free reduced technetium but also displaces it from weak radioactive complexes. The replacement of stannous chloride by a weak stannous complex of this type could be applied to the labeling of noncomplexing tin compounds. This assumption has not yet been experimentally verified.

Choosing an appropriate concentration ratio between stannous pyrophosphate and the agent to be labeled, we have tried to label some compounds, i.e., DTPA, bleomycin, and also some red blood cells.

MATERIALS AND METHODS

Stannous pyrophosphate solutions. A commercial kit of stannous pyrophosphate can be used, i.e., the CIS kit which contains 0.28 mg tin per milliliter.

Red cell labeling. For 2 ml of blood (or 1 ml of red cells) we used the following quantities of stannous pyrophosphate containing: (A) 280 μg of tin, corresponding to 1 ml of the kit; (B) 28 μg of tin, corresponding to 0.1 ml of the kit; and (C) 7 μg of tin, corresponding to 1 ml of a 1/40 dilution of the kit.

For smaller quantities of stannous pyrophosphate, a convenient kit has been prepared so as to obtain 0.28 μg of tin, corresponding to 0.1 ml of this new kit.

Bleomycin labeling. For the bleomycin labeling we

Received Aug. 19, 1974; original accepted Dec. 15, 1974.

For reprints contact: Michel Hégésippe, CIS, DR/SPRR, Cen-Saclay, B.P. 8-91190 Gif-sur-yvette, France.

TABLE 1. TECHNETIUM-99m FIXED ON THE RED BLOOD CELLS AS FUNCTION OF STANNOUS PYROPHOSPHATE QUANTITY AND OF NUMBER OF WASHINGS PERFORMED BEFORE ADDITION OF TcO_4^-

Washings (No.)	Percent technetium fixed on the red blood cells for different quantities of stannous pyrophosphate expressed by their tin content (μg)										
	280	28	7	2.8	1.4	0.56	0.28	0.14	0.028	0.014	0
None	5.6	32.4	44.5	73.3	77.8	94.3	98.3*	96.4	81.9	31.9	5.6
1	25.8	81.5	86.9	89.4	91.2						
3	96.8	98.1	98.3	98.3	98						

* Mean value of 18 assays.

used the following quantities: (A) 4.2 μg tin, (B) 7 μg tin, and (C) 11 μg tin, corresponding, respectively, to 0.3–0.5–0.8 ml of a 1/20 dilution of the CIS kit.

Labeling procedure. *Red blood cells.* Two milliliters of blood were withdrawn from the patient into a sterile tube with heparin as anticoagulant. Two labeling techniques and a system of determining labeling yield have been employed.

1. The first technique used the CIS kit as a source of stannous pyrophosphate. The quantities of stannous pyrophosphate mentioned before were added under sterile conditions to the tube containing the sample. The mixture was incubated 5 min at room temperature after short and slight mixing by hand. Centrifugation was then performed and, after separation from the supernate, the cells were washed one to three times with 5 ml of 0.9% NaCl. The red cells were resuspended in 2 ml of a sterile pertechnetate solution containing the desired quantity of radioactivity (Table 1).
2. The second technique employed the new kit of stannous pyrophosphate. After adding the necessary quantity of stannous pyrophosphate to the tube containing the sample, the suspension was gently mixed by hand. After 5 min incubation time, centrifugation was performed, the supernate discarded, and radioactive pertechnetate solution added without any subsequent centrifugation or washing steps (Table 1).
3. The labeling yield was determined by a separate measurement of the supernate and the cells. The quality of technetium labeling was ascertained by measuring the radioactivity of the supernate obtained by centrifugation after saline washes (5 ml) of the labeled blood cells.

Bleomycin. The contents of a 15-mg vial of lyophilized bleomycin (distributed by Laboratoire Roger Bellon, Paris) were dissolved with pertechnetate solution (15–30 mCi) obtained from a CIS sterile generator or a ready-to-use sterile pertechnetate solution. Then the diluted stannous pyrophosphate previously described was added to the mixture.

The analytical methods used were paper chromatography with 85% methanol solvent on Whatman No. 1 paper to determine the free TcO_4^- percentage and thin-layer chromatography on alumina or silica gel with 10% ammonium acetate/methanol 1/1 (v/v) to separate bleomycin A and B (only with silica gel plate) and also the sum of reduced technetium + pyrophosphate–technetium (Table 2).

RESULTS AND DISCUSSION

Red cells. Data reported in Table 1 show that, as the tin pyrophosphate quantity decreases, the yield of labeling increases, passing by a maximum between 0.1 μg and 0.5 μg of tin. Furthermore, washings may be avoided. For example, with 0.28 μg of tin, it is no longer necessary to remove either excess reducing agent or unreduced pertechnetate (less than 1.5%). This procedure is short and the results are reproducible.

The stability of such a diluted stannous pyrophosphate solution during storage is good if very careful precautions have been taken in its preparation and in the conditioning of the kits, insuring the presence of nitrogen atmosphere during preparation, the use of freshly prepared materials, and the storage of the complex in lyophilized form under nitrogen.

The pyrophosphate complex seems in this particular case to be more stable than the citrate mentioned by Richards, et al (*9*). Our results show at present a shelf-life of over 2 months. Nevertheless, the data mentioned in Table 1 show that a more concentrated pyrophosphate solution can be used if three washes of red cells are performed before the addition of pertechnetate. The technique is longer but is convenient for use in a small hospital.

Whatever the labeling procedure, the ^{99m}Tc is firmly bound within the cell. Less than 1.5% of radioactivity can be separated from the cells after two saline washes. Technetium-99m is supposed to be bound to hemoglobin as in the other labeling techniques (*10*).

TABLE 2. TECHNETIUM-99m LABELING OF BLEOMYCIN AS A FUNCTION OF BLEOMYCIN QUANTITY, PERTECHNETATE VOLUME, AND STANNOUS PYROPHOSPHATE QUANTITY

				Percent of ^{99m}Tc fixed on the different species‡				
Bleomycin (mg)	TcO_4^- solution (ml)	Stannous pyrophosphate* (ml)	Tin (μg)†	Reduced technetium	Bleomycin A	Bleomycin B	Bleomycin total	TcO_4^-
0	1.0	0.5	7.0	98.8				1.2
15	0.5	0.3	4.2	4.8	43.1	51.0	94.1	2.1
15	1.0	0.5	7.0	6.4	35.0	54.8	89.8	3.8
15	1.5	0.8	1.2	8.7	41.0	45.5	86.5	4.8

* Dilution 1/20 of CIS kit.

† Stannous pyrophosphate quantities are expressed by their tin content.

‡ The analytical methods used were: (A) paper chromatography with 85% methanol on Whatman No. 1 paper made to determine free TcO_4^- percentage (R_f, 0.6–0.7); (B) thin-layer chromatography with 10% ammonium acetate/methanol 1/1 (v/v); either on alumina to evaluate separately reduced technetium (R_f, 0.08), technetium-bleomycin (R_f, 0.6–0.7) and TcO_4^- (R_f, 0.8), or on silica gel to evaluate separately technetium-bleomycin A (R_f, 0.7–0.8) and technetium-bleomycin B (R_f, 0.4–0.5).

Bleomycin. Recent studies published on the problem of bleomycin labeling describe different procedures, nearly all requiring a purification step due to the low yield [less than 50% of bleomycin-technetium (*11–13*)]. The present method allows quantitative labeling. Data reported in Table 2 show that the radioactivity of the obtained solution is composed of more than 85% bleomycin-technetium and a small quantity of radioactivity at $R_f = 0$, not yet chemically defined. Clinical experiments have never shown any bone fixation (*14*).

We have not yet analyzed the nature of the technetium–bleomycin bond and possible chemical differences between our complex and those obtained with stannous chloride methods. However, we have experimentally found by using $SnCl_2$ that it is possible to obtain comparable labeling yield with much less reducing agent than the amount mentioned in the literature (reduced Tc 9% for 1.3 μg Sn^{2+}). The reduced technetium fraction ($R_f = 0$) increases with the quantity of tin. This low quantity requires daily preparation of stannous chloride. Stannous pyrophosphate is consequently more convenient.

This new technique has been applied to other compounds capable of forming with reduced technetium "stronger complexes than Tc-pyrophosphate" (*15*).

In conclusion, we think that the data reported here clearly demonstrate that in some cases stannous pyrophosphate could advantageously replace chloride in ^{99m}Tc labeling. Furthermore, it has the advantage of decreasing the content of tin in radiopharmaceuticals.

REFERENCES

1. HARPER PV, LATHROP KA, GOTTSCHALK MS, et al: Pharmacodynamics of some technetium 99m preparations. In *Radioactive Pharmaceuticals*, Andrews GA, Kniseley RM, Wagner HN, eds, Symposium Series 6, CONF-651111, Springfield, Va, National Bureau of Standards, 1966, p 335

2. ECKELMAN WC, MEINKEN G, RICHARDS P: The chemical state of ^{99m}Tc in biomedical products. II. The chelation of reduced technetium with DTPA. *J Nucl Med* 13: 577–581, 1972

3. STEIGMAN J, ECKELMAN WC, MEINKEN G, et al: The chemistry of technetium labeling of radiopharmaceuticals by electrolysis. *J Nucl Med* 15: 75–80, 1974

4. HÉGÉSIPPE M, BEYDON J, BARDY A, et al: Stannous pyrophosphate labeled with technetium 99m for skeletal imaging. *J Nucl Biol Med* 17: 3, 93–96, 1973

5. MESMER RE, IRANI RR: Metal complexing by phosphorus compounds. *J Inorg Nucl Chem* 28: 493–502, 1966

6. HÉGÉSIPPE M, BEYDON J, BARDY A, et al: Trousse "single step" de pyrophosphate d'étain marqué au technetium 99m pour scintigraphie osseuse—Preparation, etudes chimiques et biologiques. In *Radiopharmaceuticals and labelled compounds*, vol 1, Vienna, IAEA, 1973, pp 109–118

7. VALK PE, DILTS CA, MCRAE J: A possible artifact in gel chromatography of some ^{99m}Tc-chelates. *J Nucl Med* 14: 235–237, 1973

8. RICHARDS P, STEIGMAN J: Chemistry of technetium as it is applied to radiopharmaceuticals. In *Radiopharmaceuticals*, Rhodes BA, Subramanian G, Cooper JF, et al, eds, New York, The Society of Nuclear Medicine: to be published

9. RICHARDS P, SMITH TD: Recent advances in Technetium-99m Radiopharmaceuticals. Invited Presentation at the 11th International Annual Meeting of the German Society of Nuclear Medicine, BNL 18258, Athens, Greece, September 24–29, 1973

10. NOUEL JP, BRUNELLE P: Le marquage des hématies par le technetium 99m—Son intérêt en clinique. *Presse Med* 2: 73–74, 1970

11. THAKUR ML, MERRICK MV, GUNASEKERA SW: Some pharmacological aspects of a new radiopharmaceutical ^{111}In-Bleomycin. In *Radiopharmaceuticals and labelled compounds*, vol 2, Vienna, IAEA, 1973, pp 183, 193

12. LIN MS, GOODWIN DA, KRUSE SL: Bleomycin as a ^{99m}Tc carrier in tumor visualization. *J Nucl Med* 15: 338–342, 1974

13. KATO S, KURATA K, IKEDA I, et al: A simple device for preparation of various ^{99m}Tc compounds in sterilized form by an electrolytic method. *J Nucl Med* 14: 415, 1973

14. PANNECIÈRE C: personal communication, 1974

15. Procédé de marquage au Technetium 99m. French patent 74-27249, 1974