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ANALYSIS

PREPARATION AND RADIOCHEMICAL CONTROL OF ^{99M}TC LABELED BLOOD POOL AGENT FOR IN VIVO LABELLING OF THE RED BLOOD CELLS

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Abstract: Our aim was to prepare cheap blood pool imaging kits by simplified method to overcome the burden on purchase department of MINAR, Nishtar Hospital, Multan, Pakistan. Secondarily, prompt supply of kits should save the time of patient during transportation. A total of 24 subjects selected for this study were equally divided into two groups. Mixture of stannous chloride and sodium pyrophosphate solution at pH 7 was injected to these subjects. Various concentrations (ranging from 200 to 800 µg) of stannous chloride dihydrate were injected to group one, followed by intravenous administration of technetium-99m (^{99m}Tc) pertechnetate at 30 min interval in 12 subjects. Labeling percentage of each sample was calculated afterwards followed by imaging under γ camera. Each parameter was tested on three different patients and average of these three was calculated. In second set of experiments done on group two the same procedure was repeated in another 12 subjects, while keeping the concentration of Sn PYP constant at 400 µg. In this case, ^{99m}Tc was administered at different time intervals in different subjects ranging from 15 to 120 min (15, 30, 60 and 120 min) followed by calculation of labeling percentage and imaging under γ camera. In group one, average percentage values of binding of red blood cells with ^{99m}Tc were 23.24, 84.88, 83.78 and 60.33% for concentrations of 200, 400, 600 and 800 µg, respectively. In group two, average percentage binging values of 22.26, 84.36, 55.54 and 28.67% were calculated at time intervals of 15, 30, 60 and 120 min, respectively. It is concluded from the results that the best blood pool imaging under γ camera was observed for the concentration of 400 µg and the time interval of 30 min. The maximum percentage binding of red blood cells with ^{99m}Tc was calculated at concentration of 400 µg after 30 min interval that also correlated with imaging results.

Keywords: radiochemical control, ^{99m}Tc labeled blood pool agent, *in vivo* labeling, percentage binding of red blood cells with ^{99m}Tc

Technetium-99m (^{99m}Tc) labeled red blood cells (TlRBC) have become the radiopharmaceutial of choice for blood pool scintigraphy because of the convenience of *in vivo* labelling procedure of Paval et al. (1). As the clinical use expanded to include studies of intermittent GIT bleeding, variable amount of gastric, urinary and colonic activities were seen with red blood cells (RBC) labeled *in vivo* (2). The non-imaging procedures used in past have met with limited success, but in radionuclide imaging, such as ^{99m}Tc sulfur colloid and ^{99m}Tc labeled red blood cells have wider applications. Both radiopharmaceutials provide similar information and their sensitivity appears to be significantly higher than that of angiography for detection of lower GIT bleeding (3). A similar modification by Bauer et al. was shown to improve image quality (4). Bearn et al. labeled red blood cells with ^{99m}Tc for *in vivo* study but the results showed higher bone affinity in experimental subjects (5).

^{99m}Tc RBC scintigraphy can play a useful role in the preoperative localization of unexplained gas-

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trointestinal bleeding in hospital with nuclear medicine facilities. Blood pool scintigraphy is routinely done at all nuclear medicine centres of Pakistan Atomic Energy Commission using imported blood pool agent in the form of kits, which are quite expensive, i.e., approximately US $ 25/kit (containing only 5 vials).

This study was done in an attempt to replace high cost radiopharmaceuticals with the locally produced kits to save high foreign exchange. We developed *in vivo* labeling of RBC with ^{99m}Tc for blood pool scintigraphy with excellent clinical results. The calculated cost for the local preparation is not more than US $ 5/per kit having 5 vials (total cost includes cost of chemicals, staff salaries and related expenditure etc.). This technique was evaluated in term of variation in the concentration of stannous chloride and the time interval between stannous chloride and ^{99m}Tc injection, radiochemical binding and its safety.

MATERIALS AND METHODS

Materials

Technetium-99m and stannous chloride solution were obtained from Gamma Chemicals, Karachi, Pakistan. They prepared two solutions, i.e., solution A consisted of Na pyrophosphate solution (20 mg/10 mL saline) which acts as chelator, while solution B consisted of stannous chloride solution (20 mg/5 mL HCl). Then, the solution A (1 mL) was mixed with the solution B (0.25 mL) and the obtained total volume was 1.25 mL. Its pH was adjusted to 7.3 with NaOH. Total volume was brought up to 4 mL by saline and injected to patient. After it, 20 mCi (^{99m}Tc) was injected to the patient. Then, blood samples of patients were taken to check binding to RBCs after specific time intervals and total activity was recorded. Then, plasma and RBCs were separated. Now again, activity in plasma and RBCs was measured. RBCs activity was calculated as percentage binding of total injected activity. Asuming possible binding mechanism, chelator ruptures the RBC membrane and then ^{99m}Tc binds to protein in RBC hemoglobin. Binding can be calculated in terms of percentage of total activity injected (activity found in plasma + activity found in RBCs or hematocrit).

METHODS

This prospective study was carried out at Multan Institute of Nuclear Medicine and Radiotherapy (MINAR). Twenty four healthy volunteers (14 males and 10 females) participated in this study. An informed consent was obtained from all volunteers. The subjects were divided into two groups and each group was further divided into four subgroups each with three volunteers.

Appropriate volume of prepared stannous chloride solution (3 µg/kg body weight) at pH 7 was injected intravenously to male and female volunteers, six each. After 15 min, ^{99m}Tc was withdrawn from ^{99m}Tc sterile generator and its activity was checked by radioisotope calibrator. ^{99m}Tc was injected intravenously to all 12 volunteers as 0.1 mCi/kg body weight. After 15 min in each experiment, 5 mL of blood sample was obtained from each volunteer in a disposable syringe. Red blood cells and plasma were separated by centrifugation at 3000 rpm for 15 min in a centrifuge tube. Then, the packed red blood cells were washed with normal saline. Plasma and red blood cells were transferred into separate γ counting vials and counted in γ well counter. Then, the percentage labeling of each sample was calculated. Similarly, the labeling efficiency of red blood cell was determined after 30, 60 and 120 min of injection of ^{99m}Tc.

In group 1, the effect of concentration of stannous chloride was studied while keeping the time between stannous chloride and ^{99m}Tc injections constant. In this group, different concentrations of stannous chloride solution were given to each subgroup (200, 400, 600 and 800 µg to subgroup 1a, 1b, 1c and 1d, respectively) and average percentage binding of red blood cells with ^{99m}Tc of each subgroup was calculated after fixed time interval i.e., 30 min. In group 2, the effect of time between stannous chloride solution injection and ^{99m}Tc injection was studied while keeping the concentration of stannous chloride solution constant. In this group, average percentage binding of each subgroup was calculated at the following intervals: (15, 30, 60 and 120 min for subgroups 2a, 2b, 2c and 2d, respectively).

Clinical study

Blood pool images of all 24 patients were acquired using single headed Siemens Orbiter Gamma Camera interfaced with ICON computer system. The clinical procedure used for this study is outlined as follows: firstly, stannous pyrophosphate was intravenously injected, followed by intravenous injection of ^{99m}Tc-pertechnetate, then static imagings of brain, heart, great vessels, aortic bifurcation and lower extremities were obtained.

Table 1. Percentage binding of RBCs with ^{99m}Tc for various concentrations of Sn-PYP.

Subgroup	Concentration of Sn-PYP (µg)	Percentage binding of ^{99m}Tc with red blood cells (%)			Average percentage binding (%)
		1	2	3	
1(a)	200	22.64	23.68	23.40	23.24
1(b)	400	85.92	83.52	85.20	84.88
1(c)	600	84.80	83.62	82.92	83.78
1(d)	800	60.36	61.30	59.33	60.33

Table 2. Red blood cells binding with ^{99m}Tc for various time intervals between Sn-PYP and ^{99m}Tc injections.

Subgroup	Time interval between Sn-PYP and ^{99m}Tc injections (min)	Percentage binding of ^{99m}Tc with red blood cells (%)			Average percentage binding (%)
		1	2	3	
2(a)	15	21.52	22.91	22.35	22.26
2(b)	30	86.86	84.44	81.78	84.36
2(c)	60	54.37	54.38	57.87	55.54
2(d)	120	29.31	27.33	29.37	28.67

RESULTS AND DISCUSSION

The study was performed on a total of 24 subjects. Mean age was 36 years (standard deviation = 11.3 years) and range was 19–58 years. Percentage binding of ^{99m}Tc with red blood cells (%) was studied in both groups and the results of group 1 are given in Table 1. The result showed that maximum binding percentage (84.88%) is calculated at the concentration of 400 µg. Table 2 exhibits the results of group 2. The result showed that maximum binding percentage (84.36%) is calculated at a time interval of 30 min between the injection of stannous chloride solution and ^{99m}Tc. Best blood pool images were obtained at the concentration of 400 µg of Sn-PYP and at the lag time of 3 min between Sn-PYP injection and ^{99m}Tc-pertechnetate injection.

In the present study, 400 µg was found to be the minimum concentration of stannous chloride that resulted in satisfactory RBC labeling. This dose is less than the previously reported dose for the same procedure [6]. The resulting scan was also of good quality without any significant artifacts. It was also observed that RBC labeling with ^{99m}Tc depends upon the time interval between inactive stannous chloride injection and ^{99m}Tc. A time interval of 30 min was selected, based on the dual consideration that this time interval provided better red blood labeling than longer time interval, and also because 30 min is very convenient interval for the patient and staff in busy nuclear medicine department. A shorter interval of 15 min has not provided good quality image as stannous chloride ions take some time to penetrate into the red blood cells. If this time is significantly less than 30 min, the penetration of stannous chloride into red blood cells becomes insufficient and hence labeling efficiency also decreases. If the concentration of stannous chloride or time interval is increased or decreased beyond the above limits, the percentage of bound fraction of technetium decreases in red blood cells as is evident from the images and statistics provided. The results of this study suggest that ^{99m}Tc-labeled RBC by *in vivo* technique is a superior blood pool imaging agent compared to any other preparations.

CONCLUSION

The technique is described for in-house production of blood pool agent for nuclear medicine studies. This methodology produces low-cost kit

that can be used in any study requiring labeling of ^{99m}Tc with red blood cells.

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