The Effects of Selected Antineoplastic Agents on the Labeling of Erythrocytes with Technetium-99m Using the UltraTag® RBC Kit

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Objective: Adverse affects of various drugs on the labeling efficiency of RBCs with ^{99m}Tc-pertechnetate have been known for several years. This study presents data on the ability of the UltraTag® RBC kit to label RBCs with pertechnetate in the presence of various antineoplastic drugs.

Methods: Five different antineoplastic drugs, either alone or in combination, were incubated for 30 min at 37° C with 2-mL samples of whole blood obtained from normal volunteers. Each sample was labeled with pertechnetate and the radiochemical purity determined according to the UltraTag RBC product package insert. Doxorubicin was specifically tested in molar ratios with stannous ion of greater than 1:1 to determine if there was any significant chelation effect that would affect the ability of the kit to label RBCs. In addition, patients were given a bolus injection of doxorubicin and a blood sample was drawn at 30 min to test whether the metabolites had any effect on labeling.

Results: The ability of the UltraTag RBC kit to label RBCs with pertechnetate was not adversely affected by the antineoplastic drugs when they were present alone or in combination. Likewise, doxorubicin metabolites did not interfere with the labeling efficiency of ^{99m}Tc RBCs using the UltraTag RBC kit. Molar ratios of doxorubicin-to-tin that exceeded 1:1 also had no adverse effects on the labeling efficiency of the UltraTag RBC kit.

Conclusion: When performing nuclear medicine exams involving the labeling of RBCs with pertechnetate on patients who have received doxorubicin, as well as certain other antineoplastic agents, a high RBC labeling efficiency can be obtained if the UltraTag RBC kit is used.

Key Words: UltraTag® RBC kit; antineoplastics; labeling interference

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Since 1967 (*1*) nuclear medicine has been experimenting with various methods to label erythrocytes (RBCs) with ^{99m}Tc. The most widely used technique for labeling RBCs with ^{99m}Tc involves the use of stannous (Sn^{+2}) ion. In 1975, Bardy et al. (*2*) reported the use of a stannous pyrophosphate kit to label RBCs in vitro with ^{99m}Tc. The same article also reported the importance of using the correct amount of Sn^{+2} ion to obtain a reasonable percent of labeled ^{99m}Tc RBCs. Since that time there have been several articles (*3–9*) that have dealt directly with how much Sn^{+2} ion is required for efficient labeling.

As early as 1971 (*10*), problems relating to the labeling of RBCs were reported. The literature now contains many articles (*11–18*) which have dealt with multiple factors that affect the labeling of RBCs with ^{99m}Tc. Some of these factors include tinning time, ^{99}Tc interference, hematocrit levels, methods of injection (direct ''stick'' versus intravenous tubing), length of time that ^{99m}Tc is incubated with RBCs, volume of blood radiolabeled, quantity of stannous ion used to tin RBCs, and source of normal saline used to reconstitute the pyrophosphate reagent kit. Particularly with the advent of the in vivo method of labeling RBCs, problems concerning the interference of various pharmaceuticals began to appear in the literature (*7–9,12,18–29*). One drug frequently reported to have an adverse affect on RBC labeling with ^{99m}Tc is doxorubicin (*8,9,12,18,20,24,26*). Doxorubicin appears to adversely affect both in vivo and in vitro labeling techniques. It has been suggested that doxorubicin as well as other drugs (*26*) chelate the Sn^{+2} ion, thus limiting its availability for intracellular reduction of ^{99m}Tc.

The purpose of this study was fourfold. The first purpose was to determine if the presence of doxorubicin in whole blood, at the manufacturer's suggested maximum levels, had an adverse affect on the ability of the UltraTag® RBC kit (Mallinckrodt, Inc., St. Louis, MO) to label RBCs with ^{99m}Tc. Since doxorubicin is rarely, if ever, used alone as a chemotherapy agent, the second purpose was to determine if other antineoplastic agents either singularly or in combination with doxorubicin had any adverse effects on labeling. The third purpose was to determine if metabolites of doxorubicin had any adverse effects on the labeling of RBCs using the UltraTag RBC kit. A fourth purpose was to determine if the purported chelation effect of doxorubicin would have an impact on the efficacy of the UltraTag RBC

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kit when doxorubicin-to-tin is present in more than a 1:1 molar ratio.

MATERIALS AND METHODS

Phase 1

Blood samples were labeled using the UltraTag RBC kit and ^{99m}Tc in the form of sodium pertechnetate. The effect of 5 different antineoplastic drugs, alone and in combination, on the radiochemical purity of ^{99m}Tc RBCs was tested. The drugs included in this research protocol were doxorubicin, cyclophosphamide, cisplatin, 5-fluorouracil and vincristine sulfate. Each drug used, as well as controls, was tested in groups of 5 replicated samples.

The amount of each drug tested was based on the manufacturer's maximum recommended dose per meter squared body surface area as specified in the respective product package inserts. All calculations were based on a 70-kg human having a body surface of 1.73 m^2 and 5000 mL of whole blood. The maximum recommended doses as well as the mass of each drug used are listed in Table 1.

A 2-mL sample of blood was drawn from normal volunteers, incubated at 37° C for 30 min with a specified quantity of drug (Table 1), and then labeled with ^{99m}Tc-pertechnetate using the UltraTag RBC kit procedure, according to the product package insert instructions. Blood used for the controls and experimental portion of the study was obtained from the same donors.

Radiochemical purity for all samples was determined by removing 0.5 mL of ^{99m}Tc-labeled whole blood and adding it to 1 mL of normal saline in a centrifuge tube. The sample was then centrifuged at 1000 G for 4 min. The total activity in each tube was measured, then the supernate was pipetted off leaving the packed red blood cells. The labeling efficiency (% ^{99m}Tc bound to RBCs) was determined by dividing the amount of radioactivity associated with the packed RBCs by the total activity in the sample and multiplying by 100.

Phase 2

According to the manufacturer's package insert, doxorubicin is 70% metabolized within the first 30 min after injection. The metabolites of doxorubicin are not available commercially, thus in this phase, blood was drawn from 3 patients 30 min postbolus injection of doxorubicin. The blood was labeled and tested for radiochemical purity according to the procedure described in Phase 1.

TABLE 1
Amount of Each Drug Added Per Sample of Blood Tested*

Drug	Maximum recommended dose	Mass of drug per 2 mL whole blood
No drug		
Doxorubicin	75 mg/m^2	50 μg
5-Fluorouracil	1 g	400 μg
Vincristine	1.4 mg/m^2	1 μg
Cyclophosphamide	1.8 g/m^2	1.25 mg
Cisplatin	120 mg/m^2	83 μg
Combination of all 5 drugs per sample		As above for each

*Based on the manufacturer's maximum recommended dose diluted in 5000 mL blood.

TABLE 2
Doxorubicin-to-Tin Molar Ratios Tested in Phase 3 of Methodology

Doxorubicin mass	Drug-to-tin molar ratios
100 μg	0.83:1 (based on minimum Sn^{+2})
190 μg	0.83:1 (based on theoretical Sn^{+2})
350 μg	1.50:1 (based on theoretical Sn^{+2})

Phase 3

In addition to the values given in Table 1, doxorubicin was incubated with samples of blood at concentrations higher than those used clinically. This portion of the protocol was designed to determine if doxorubicin, in sufficient quantity, diminishes the amount of stannous ion available to reduce pertechnetate (possibly through a chelation effect). Specifically, 3 molar ratios (doxorubicin-to-tin) were evaluated as outlined in Table 2. The values given in Table 2 represent drug concentrations both below and above a 1:1 doxorubicin-to-tin molar ratio. The product package insert for the UltraTag RBC kit lists both a minimum and a theoretical quantity of stannous ion included in the reagent kit. Therefore the molar ratio referred to above takes into account both minimum and theoretical amounts of stannous ion. Samples were incubated, radiolabeled and tested for radiochemical purity as described in Phase 1.

RESULTS

The results of the Phase 1 radiochemical purity testing are presented in Table 3, the results of Phase 2 are presented in Table 4, and the results of Phase 3 are presented in Table 5. A one-way ANOVA test was conducted on the data for all phases of this study. The advantage of this type of analysis is related to the fact that pooling of the sample SDs results in a more precise estimate of the population SD. This consideration is very important when the sample size is small, as is the case with this study.

TABLE 3
Technetium-99m-RBC Radiochemical Purity Results for Control Blood and Blood Incubated with Antineoplastic Drugs

Drug	Mean ± SD	n
Control (no drug)	97.79 ± 0.16	5
Doxorubicin	99.47 ± 0.19	5
5-Fluorouracil	98.48 ± 0.16	5
Cyclophosphamide	99.07 ± 0.31	5
Vincristine	98.77 ± 0.46	5
Cisplatin	98.55 ± 0.36	5
All drugs	98.87 ± 0.21	5

TABLE 4
Technetium-99m-RBC Radiochemical Purity Results for Blood Drawn from Patients 30 Min Postbolus Injection of Doxorubicin

Drug	Mean ± SD	n
Doxorubicin metabolites	97.20 ± 1.37	3

An assumption of a 5% significance level was made for the statistical tests. For the combined data in Tables 3 and 4, the ANOVA test produced an F-ratio of 10.41 (critical ratio of $F = 2.33$; $P < 0.0000$). Hence, for the combined data of Phases 1 and 2, there is a statistical difference in the labeling efficiency of samples containing antineoplastic medications and the sample containing the doxorubicin metabolites compared to the control. However, from a clinical point of view, this difference may not be relevant.

For the data in Table 5, the ANOVA test resulted in an F-ratio of 0.4855 (critical ratio of $F = 6.94$). For Phase 3, there is no statistical difference between the mean radiochemical purity of the samples and the mean radiochemical purity of the control. Thus, it can be established that the varying doxorubicin-to-tin molar ratios tested in Phase 3 had no significant effect on radiochemical purity of the ^{99m}Tc RBCs.

DISCUSSION

The results of this study indicate that the in vitro method of labeling RBCs using the UltraTag RBC kit is resistant to interference from selected antineoplastic medications and doxorubicin metabolites as tested in an in vitro model of the vascular pool. These results are consistent with the findings of Gleue et al. (*7*) who examined the effects of cyclosporine, as well as the data collected by Mallinckrodt (*30*) during Phase III clinical trials, in which 7 other drugs were tested.

Several previous authors have investigated the effects of doxorubicin on in vivo, in vivo/in vitro and in vitro methods of labeling RBCs with ^{99m}Tc (*8,9,12,18,20,24,26*). There are 3 differences between our findings and the results of the these investigators:

1. The quantities of doxorubicin used in Phase 1 of our study mimicked those present in the vascular pool immediately after injection of the manufacturer's highest recommended dose. Moreover, Phase 2 tested RBC labeling at near-peak levels of doxorubicin metabolites. If there is no interference from doxorubicin or its metabolites at these levels, it is unlikely that lower levels of the drug would be interfering. Thus it appears that the UltraTag RBC kit could be used as a monitoring tool for cardiotoxicity of doxorubicin at any point during the course of therapy. On the contrary, all of the above-referenced studies have found that doxorubicin interfered with their respective method of RBC labeling.
2. Phase 3 of our study revealed that when doxorubicin-to-tin molar ratios exceeded 1:1, there was no adverse effect on RBC labeling. These results indicate that doxorubicin either does not bind to extracellular tin or, if it does: (a) it does not interfere with the RBC labeling process; or (b) the chelation process occurs at doxorubicin levels toxic to patients. This data contradicts the suggestion of Dewanjee (*26*) that doxorubicin interferes through a chelation effect.
3. This is the first study to investigate the effects of multiple antineoplastic drugs, both alone and in combination. Previous studies have solely examined the effect of doxorubicin.

TABLE 5
Technetium-99m-RBC Radiochemical Purity Results for Blood Incubated with Varying Doxorubicin-to-Tin Molar Ratios

Doxorubicin-to-tin molar ratios	Mean ± SD	n
0.83:1 (based on minimum Sn^{+2})	99.08 ± 0.43	2
0.83:1 (based on theoretical Sn^{+2})	99.22 ± 0.22	2
1.50:1 (based on theoretical Sn^{+2})	98.90 ± 0.38	3

Certain literature suggests that it is critical to have the proper amount of stannous ion for efficient labeling (*2–9*). The exact quantity of tin to be used is likely to be dependent on the efficiency of the method for removing the stannous ion that is not bound intracellularly. Many of the drugs reported to interfere with other RBC labeling techniques have not been found to interfere with the UltraTag RBC method (*7,30*). It is probable that the UltraTag RBC method provides consistently high labeling yields, even in the presence of various medications, due to: (a) the large quantity of stannous ion that allows for maximum cell ''tinning;'' and (b) the extremely high efficiency with which hypochlorite oxidizes extracellular tin.

CONCLUSION

When incubated with whole blood at concentrations equivalent to the maximum recommended dosages, the medications doxorubicin, cyclophosphamide,5-fluorouracil, cisplatin and vincristine sulfate, alone or in combination, do not interfere with the labeling efficiency of ^{99m}Tc RBCs using the UltraTag RBC kit. Likewise, doxorubicin metabolites cause no interference with the labeling of ^{99m}Tc RBCs using this commercially available kit.

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