Effect of Triphenyl Tetrazolium Chloride Staining on the Distribution of Radiolabeled Pharmaceuticals

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Myocardial tissue is routinely exposed to the vital stain triphenyl tetrazolium chloride (TTC) to delineate infarction in conjunction with myocardial isotope research. However, it is unknown whether TTC has a direct effect on tracer deposition. We evaluated this possibility in rabbit hearts injected with either teboroxime, sestamibi or ^{201}Tl. The hearts were excised and treated as follows: (1) TTC or normal saline was perfused through the heart and the residual activity monitored; (2) hearts were sliced into 0.5-cm thick sections, counted and incubated in either TTC or normal saline for 10 min then recounted; and (3) the circumflex artery was ligated postmortem and TTC perfused. Autoradiographic images were produced from 30-μm slices to depict any disparity in activity concentration from the selective perfusion of TTC. Both perfusion and incubation by TTC resulted in a significant activity loss of both ^{201}Tl and sestamibi, but not teboroxime, compared to normal saline. An independent octanol extraction experiment measured the change in the partition coefficient of labeled teboroxime and sestamibi induced by the addition of TTC. TTC was shown to liberate the radiolabel from sestamibi, but not from teboroxime. We conclude that histochemical staining techniques involving TTC can alter the distribution of radiolabeled pharmaceuticals. As a result, experiments using TTC with ^{201}Tl and sestamibi should be interpreted cautiously.

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Compounds labeled with thallium-201 (^{201}Tl) (*1–5*) and the newer technetium-99m (^{99m}Tc) (*6–10*) are useful agents for assessing myocardial blood flow. The determination of the extent of myocardial salvage is based on an understanding of the uptake characteristics of these agents. The question of uptake into areas of infarction, in relation to flow, is often based on microsphere counting interpreted in conjunction with vital stains (*11–14*) such as triphenyl tetrazolium chloride (TTC) (*15–18*). However, if the collective work in experimental models of coronary occlusion and reperfusion is to be useful in determining the relationship between microsphere flow, diffusible tracers and myocardial viability, we must be certain that the process of vital staining does not alter myocardial distribution of the tracer. The assessment of tracer distribution in the presence of tetrazolium salts is valid only insofar as the vital stain is inert to that tracer. We hypothesized that TTC stain could alter the ex vivo distribution of diffusible myocardial perfusion tracers. We applied standard methods of triphenly tetrazolium chloride staining, incubation and perfusion to rabbit hearts injected with either ^{201}Tl or one of two ^{99m}Tc radiolabeled lipophilic perfusion compounds: teboroxime (chloro-[tris-(cyclohexanedionedioxime)-methyl]-boronic acid) or sestamibi (2-methoxyisobutyl isonitrile). We then determined the effect of this vital stain on tracer deposition by using a radioisotope calibrator and by autoradiography. Finally, to further evaluate the effect of a TTC solution on the stability of the radiolabel, we measured the change in the partition coefficient by octanol extraction of labeled sestamibi and teboroxime induced by the addition of TTC.

MATERIALS AND METHODS

Radiopharmaceuticals

Teboroxime and sestamibi were obtained as lyophilized kits (Squibb Diagnostics, Princeton, NJ, and E.I. du Pont de Nemours and Co., Wilmington, DE, respectively). Up to 200 mCi of ^{99m}Tc pertechnetate in 1 ml of saline was added to each vial. The vials were heated in a water bath at 100°C for 10 min (sestamibi) or 15 min (teboroxime), then cooled to room temperature. Radiochemical purity was confirmed by paper chromatography (teboroxime) or thin-layer chromatography (sestamibi) to exceed 90% in all kits.

Triphenyl Tetrazolium Chloride

TTC was obtained in powder form (J.T. Baker Chemical Co., Phillipsburg, NJ). A 1% solution of TTC in either 0.1 *M* phosphate buffer or 0.1 *M* Tris buffer was mixed to a pH of 8.0 and warmed to approximately 37°C.

Perfusion

Rabbits (N = 18, weight >1.7 kg) were anesthetized with sodium pentobarbital (30–50 mg/kg IU) via an intravenous injection. Either ^{201}Tl (1 mCi), sestamibi (8–10 mCi) or teboroxime (8–15 mCi) was then administered intravenously and allowed to

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circulate for 2 min (teboroxime) or 5 min (^{201}Tl and sestamibi). Cardiac arrest was then induced by an intravenous injection of KCl. The hearts were quickly removed, rinsed with normal saline, weighed and counted in preparation for retrograde aortic perfusion.

The hearts were mounted on a perfusion apparatus and suspended in a warm saline bath (37°C). A three-way connecter allowed continuous monitoring of the aortic pressure as the perfusate was introduced. The perfusion rate was varied to maintain a constant aortic pressure (65 mmHg). The hearts were perfused with either normal saline or a 1% solution of TTC in 0.1 *M* phosphate buffer in aliquots of 40 ml via a syringe pump (Orion model 341 A, Orion Research, Inc., Cambridge, MA). Coronary sinus flow and regurgitant aortic valve drainage were monitored from right and left ventricular catheters, respectively. After each 40-ml perfusion, the preparation was removed from the perfusion apparatus and the activity measured in a radioisotope calibrator (Squibb CRC-16). To prevent premature necrosis from taking place, the time for this process was limited to 30 sec.

Incubation

Three saline-perfused hearts from each group were sliced into 0.5-cm thick sections, perpendicular to the long axis of the heart. Each section was weighed and counted. Control slices were incubated in normal saline at 37°C. The remaining slices were incubated in a 1% solution of TTC in phosphate buffer at 37°C. After a 10-min incubation period, the slices were rinsed with saline, then recounted for activity.

Autoradiography

To ascertain if selective postmortem TTC perfusion could alter tracer distribution in a normal heart, we performed the following experiment. The heart of one rabbit was excised and mounted on a perfusion apparatus as described above after administration of ^{201}Tl (1 mCi). A large branch of the circumflex artery and of the vein were ligated proximally. The heart was perfused with 60 ml of a 1% solution of TTC in phosphate buffer.

The heart was removed from the perfusion apparatus and rinsed with saline and the atria excised. The ventricles were filled with embedding medium (O.C.T., Tissue-Tek, Miles, Inc., Elkhart, IN), then rapidly frozen in liquid nitrogen. Ultrathin sections (30 μm) were collected on tape (Type 820, 3M, St. Paul, MN) in a cryomicrotome (PM 2250, LAB Instruments, Gaithersburg, MD) at −20°C. The mold was photographed before each cut to record the TTC image. The sections were air-dried at room temperature and mounted face-up on cardboard.

The sections were placed directly on a sheet of singled-coated x-ray film (MRM-1, Eastman Kodak, Rochester, NY) then placed in a black plastic bag (Cronex, DuPont, Wilmington, DE). The bag was vacuum sealed, providing close apposition (2π geometry) between the film and sample. After an appropriate exposure period (1 day for ^{99m}Tc and 5 days for ^{201}Tl), the film was developed in an automatic x-ray film processor (RP X-OMAT, Eastman Kodak, Rochester, NY). The autoradiographic images were compared to their corresponding TTC photographs. This procedure was repeated for both sestamibi and teboroxime.

Octanol Extraction

To obtain an independent measure of the effect of TTC on tracer dissociation, we measured the octanol partition coefficients for sestamibi and teboroxime in different TTC and buffer solutions. One millicurie of sestamibi was placed in each of five preweighed test tubes containing 2 ml of either normal saline, a 1% solution of TTC in Tris buffer, a 1% solution of TTC in phosphate buffer, Tris buffer or phosphate buffer. The mixtures were thoroughly sonicated and allowed to settle overnight. The octanol and aqueous phases were separated and the weight and activity of each phase determined.

Given the activity concentration in the octanol phase (c_A) and aqueous phase (c_B), the partition coefficient K (*19,20*) was obtained as follows:

$$K = \frac{c_A}{c_B}.$$

A total of five trials were performed and the average partition coefficient ($\bar{K}$) calculated. This procedure was repeated for teboroxime.

TABLE 1
Myocardial Activity Retention after TTC or Saline Perfusion

Radiopharmaceutical	TTC perfusion	Saline perfusion
^{201}Tl	11.7 ± 6.8*	43.5 ± 5.0
Sestamibi	18.1 ± 1.3†	72.3 ± 11.4
Teboroxime	71.9 ± 14.4	78.4 ± 18.3

*p = 0.015 versus normal saline.
†p = 0.001 versus normal saline.
The activity retention after perfusion by 200 ml of TTC or saline expressed as the average percentage of the original activity (N = 3).

Data Analysis

All results are expressed as the mean ± 1 s.d. Statistical comparisons were made using an analysis of variance and appropriate t-test. A p value less than 0.05 was considered significant.

RESULTS

Perfusion

The effect of perfusion by 200 ml of TTC on each radiopharmaceutical is summarized in Table 1. The average activity retention of ^{201}Tl and sestamibi cardiac activity after TTC perfusion was 11.7% ± 6.8% and 18.1% ± 1.3%, respectively, compared to 43.5% ± 5.0% and 72.3% ± 11.4%, respectively, after perfusion by normal saline. No significant difference was observed between TTC and normal saline perfusion in liberating teboroxime activity from normal cardiac tissue. It is interesting to note that saline perfusion reduced the myocardial retention of all three agents, but had its greatest effect on ^{201}Tl.

Figure 1 displays the percentage of the initial activity in the heart after each 40-ml perfusion, plotted as a function of perfusate volume. In most cases, the activity washout was mono-exponential. On average, for any volume of perfusate used, TTC affected the retention of both ^{201}Tl and sestamibi to a greater degree compared to saline. As the perfusate volume increases, the difference between normal saline and TTC perfusion quickly approaches significance.

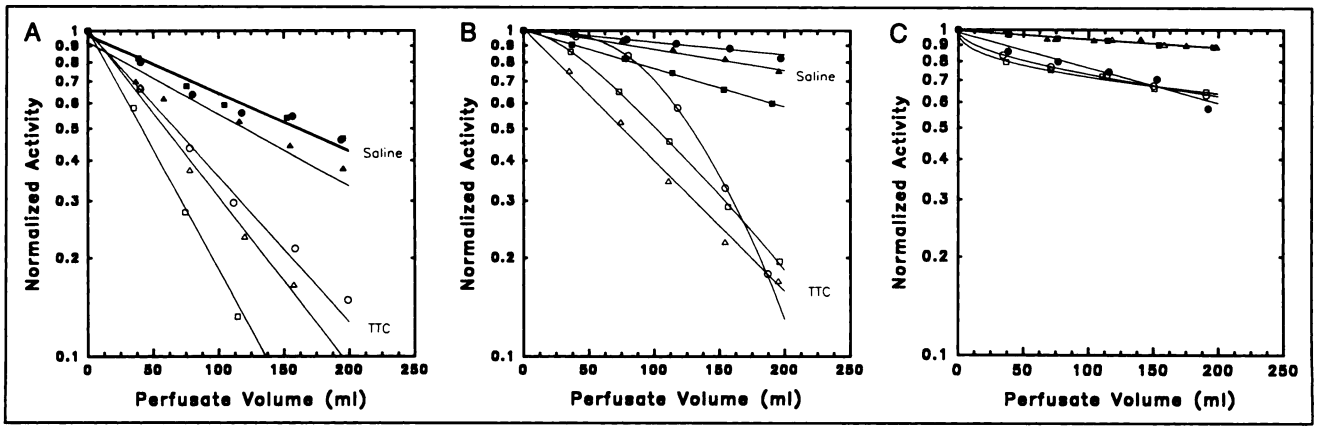

FIGURE 1. The activity retention of (A) ^{201}Tl, (B) sestamibi and (C) teboroxime after each 40-ml perfusion of TTC or normal saline, plotted as a function of normalized initial activity. Hollow symbols represent TTC perfusion; solid symbols represent normal saline perfusion.

Incubation

Table 2 summarizes the results of the incubation experiment. The average activity retention of ^{201}Tl and sestamibi was 62.9% ± 3.8% and 68.7% ± 4.3%, respectively, after TTC incubation, compared to 77.3% ± 4.2% and 81.8% ± 1.2%, respectively, after saline incubation. As in the perfusion experiment, no significant difference was observed between TTC and normal saline incubation in liberating teboroxime activity from normal cardiac tissue. Note that saline incubation reduced myocardial retention of both sestamibi and ^{201}Tl but had a lesser effect on teboroxime.

Autoradiography

Ligation of the circumflex artery was performed postmortem, followed by TTC perfusion. If the presence of TTC alters tracer deposition, then the region of the heart protected by the occlusion (TTC negative region) would be left unaffected by TTC perfusion. Therefore, lower activity concentrations are expected in TTC positive regions compared to unstained myocardium protected from TTC exposure by the occlusion. Any disparity in activity concentration would be depicted on an autoradiograph.

The autoradiographic image of teboroxime showed uniform tracer intensity across the TTC positive and negative zones (Fig. 2). Therefore, myocardial teboroxime distribution is inert to TTC perfusion. However, uniform tracer distribution was not retained for ^{201}Tl and sestamibi. Autoradiographic images showed a selective loss of tracer intensity in the TTC positive regions of both the ^{201}Tl and sestamibi labeled hearts (Figs. 3 and 4).

Octanol Extraction

The octanol extraction experiment measured the stability of a radiolabeled lipophilic compound in a suspension medium. If a component in the incubation solution removed the radiolabel from the compound, then a lower partition coefficient would be measured compared to incubation in a saline control.

Table 3 summarizes the results of the octanol experiment. Sestamibi incubated in the normal saline control yielded an average partition coefficient of 8.7 ± 0.61. A lower partition coefficient of 2.9 ± 0.4 was measured when sestamibi was incubated in a phosphate buffer, but no significant difference was measured after Tris buffer incubation. However, the addition of TTC to the incubation medium resulted in a further significant decrease in the partition coefficient. This suggests that TTC will dissociate the technetium label from this isonitrile independent of the choice of buffer.

For teboroxime, no significant dissociation of the ^{99m}Tc atom from the compound was observed between incubation in normal saline and the other four media.

DISCUSSION

In this study, histochemical staining techniques involving TTC were shown to alter the distribution of radiolabeled pharmaceuticals. Postmortem perfusion or incubation of tissue samples with TTC was shown to liberate both ^{201}Tl and sestamibi from normal myocardium, while myocardium containing teboroxime was unaffected. Consistent findings were observed by autoradiographic analysis of hearts regionally perfused with TTC in that autoradiographic images showed lower intensity values of ^{201}Tl and sestamibi in regions exposed to this vital stain. In contrast to ^{201}Tl and sestamibi, uniform intensity distribution of teboroxime was observed despite selective perfusion with TTC.

TABLE 2
Myocardial Activity Retention after TTC or Saline Incubation

Radiopharmaceutical	Weight (N = 3) (g)	Incubation solution	Activity retention (%)
^{201}Tl	1.194 ± 0.355	Normal saline	77.3 ± 4.2
	1.395 ± 0.452	TTC	62.9 ± 3.8*
Sestamibi	1.262 ± 0.347	Normal saline	81.8 ± 1.2
	1.193 ± 0.440	TTC	68.7 ± 4.3*
Teboroxime	1.169 ± 0.393	Normal saline	97.2 ± 0.9
	1.093 ± 0.192	TTC	92.8 ± 3.6

*$p \leq 0.012$ versus normal saline.

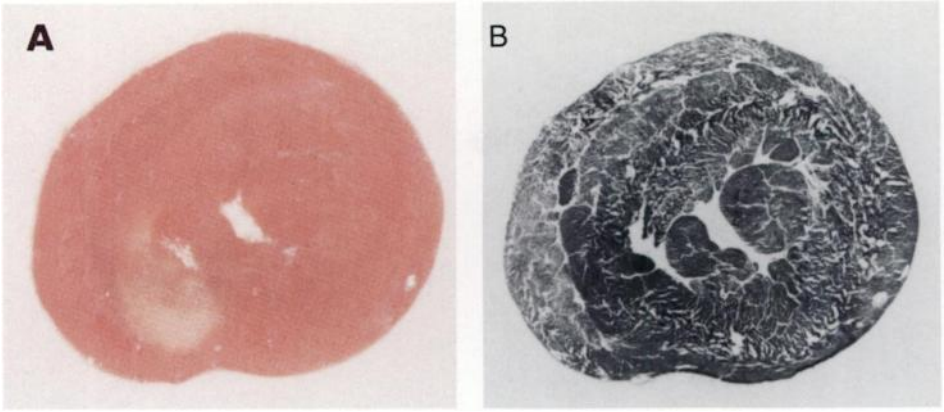

FIGURE 2. (A) Myocardial image after regional perfusion of TTC and (B) corresponding teboroxime autoradiograph. The isotope was injected prior to coronary occlusion and TTC perfusion. Technetium-99m activity is relatively unaffected by TTC staining.

TTC Effect on Myocardial Tracers

Postmortem perfusion and incubation of tissue samples with normal saline reduced the myocardial retention of all three agents, but had its greatest effect on ^{201}Tl. Given the difference in myocardial transport studies (*21,22*) comparing these three agents, it is not surprising that the most soluble one, ^{201}Tl, shows the fastest clearance during saline perfusion. Both sestamibi and teboroxime are large, lipophilic compounds and therefore are less influenced by normal saline infusion.

The octanol experiment offered insight on how TTC affects radiolabeled compounds. In an octanol-aqueous solution, lipophilic compounds such as sestamibi and teboroxime are attracted to the octanol phase and carry their radiolabel with them (*20,23–25*). If, however, a component in the solution removed the radiolabel from the compound, then activity would appear in the aqueous phase. As a result, the partition coefficient K would decrease. A significant decrease in the partition coefficient was observed after sestamibi was incubated in a 1% solution of TTC in either Tris or phosphate buffer. When sestamibi was incubated in the phosphate buffer alone, the partition coefficient was significantly higher than that observed with TTC but was still lower than the normal saline values. The octanol experiment also demonstrates that, at least for sestamibi, the effect is due to TTC and is independent of the choice of buffer solution. Therefore, a percentage of the sestamibi activity liberated from the myocardium may be attributed to free ^{99m}Tc rather then intact sestamibi. The octanol experiment involving teboroxime showed no significant difference in the partition coefficient between normal saline and the other four media. This finding is consistent with the observed inert behavior of TTC on tissue samples containing teboroxime.

Comparison to Previous Work

Based on the findings of this study, the assessment of the performance of sestamibi obtained in studies involving selective myocardial perfusion with TTC could be subject to alternative explanations. In their evaluation of sestamibi, both Sinusas et al. (*26*), using dogs, and Freeman et al. (*27*), using swine, incorporated a selective postmortem perfusion technique in their assessment of a coronary occlusion-reperfusion model in which TTC was perfused directly into the area at risk. Therefore, only the area at risk (occlusion zone) was exposed to TTC. Myocardial sestamibi activity was later correlated with microsphere blood flow. Regions of necrosis, which stained TTC negative, demonstrated low sestamibi activity in spite of reperfusion measured by microspheres. Therefore, both studies concluded that myocardial uptake of sestamibi has a strong dependence on cellular viability and not just reperfusion flow. Our findings suggest that these studies should be interpreted cautiously because TTC could have affected the postmortem distribution of sestamibi in these experiments. Consequently, if myocardial uptake of sestamibi

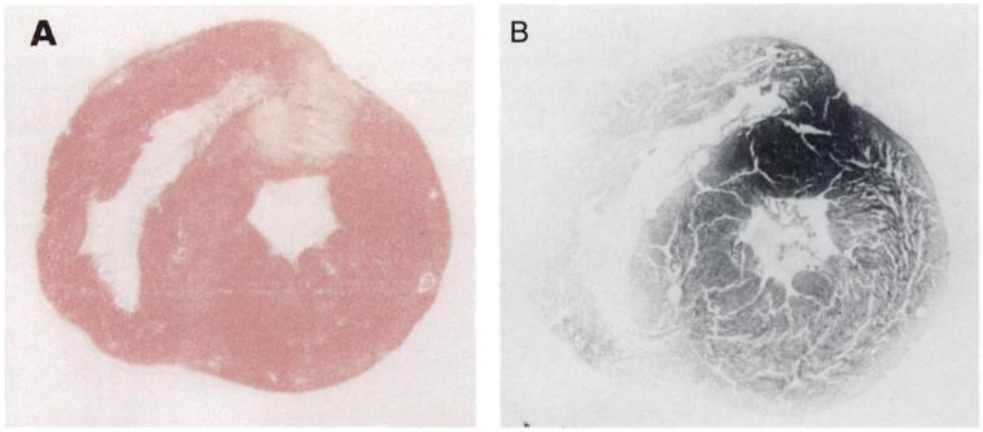

FIGURE 3. (A) Myocardial image after regional perfusion of TTC and (B) corresponding ^{201}Tl autoradiograph. The isotope was injected prior to coronary occlusion and TTC perfusion. Thallium-201 activity is markedly reduced in areas stained with TTC (red regions).

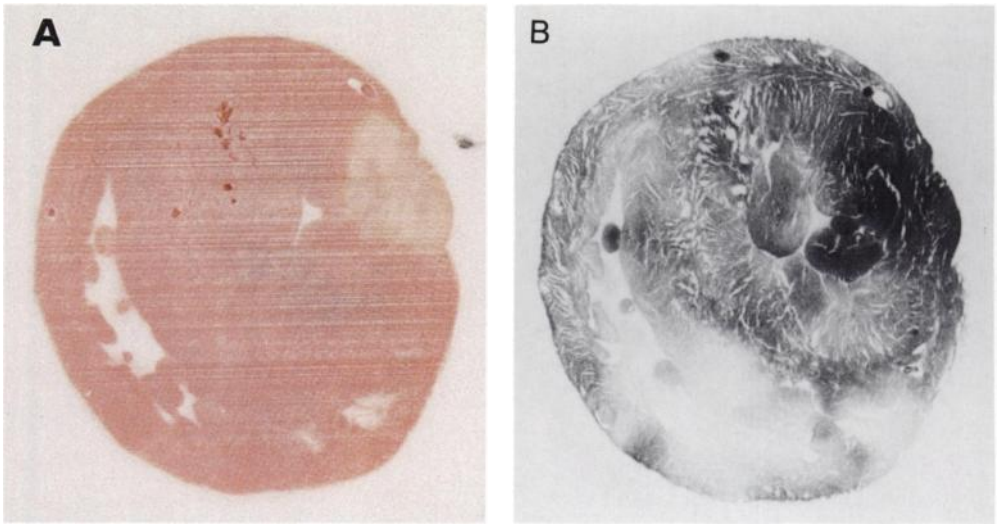

FIGURE 4. (A) Myocardial image after regional perfusion of TTC and (B) corresponding sestamibi autoradiograph. The isotope was injected prior to coronary occlusion and TTC perfusion. Technetium-99m activity is markedly reduced in areas stained with TTC (red regions).

had been performed in the absence of TTC staining, these authors may have found that its tissue distribution reflected flow (at the time of tracer administration) more clearly than viability.

Incubating tissue samples with TTC was shown to liberate more than 30% of both ^{201}Tl and sestamibi from normal myocardium. Using 20-μm tissue sections in their evaluation of ^{14}C-IMPPA, Humbert et al. (*28*), asserted that TTC would wash out all the ^{201}Tl activity from their sample, enabling an acquisition of a pure ^{14}C autoradiographic image. Therefore, the extent of activity washout of ^{201}Tl as well as sestamibi may be a function of sample thickness and duration of TTC exposure.

In an occlusion-reperfusion canine model, Melin et al. (*29*) studied the uptake characteristics of ^{201}Tl. Tissue samples 1–1.5 cm in thickness were incubated for 30 min in a TTC bath prior to the measurement of ^{201}Tl and microsphere activity. They concluded that ^{201}Tl uptake is an unreliable indicator of myocardial cell viability and that reperfused necrotic tissue may have high levels of ^{201}Tl uptake. In contrast, a similar study by Maddahi et al. (*3*) concluded that ^{201}Tl perfusion imaging allows immediate assessment of myocardial viability. In their study, 1-cm thick tissue samples were first imaged under a scintillation camera then incubated in TTC. Activity distributions from the scintigraphic images were later compared to TTC photographs. Melin et al. (*29*) attribute these differences to the imaging techniques employed by Maddahi et al. (*3*) as being insufficiently sensitive and quantitative to detect uptake by nonviable myocardium. Our findings offer an alternative hypothesis. Surface contamination with ^{201}Tl resulting from a 30-min TTC bath may account for the ^{201}Tl activity found in nonviable myocardium. Therefore, in this example, the assessment of ^{201}Tl distribution may have been an artifact caused by exposing tissue samples to TTC.

TABLE 3
Octanol Partition Coefficients

Incubation Media	Sestamibi	Teboroxime
Normal saline	8.7 ± 0.61	23.0 ± 4.4
TTC in phosphate buffer	0.48 ± 0.01*†	19.4 ± 1.7
TTC in tris buffer	2.3 ± 0.8*†	17.3 ± 2.5
Phosphate buffer	2.9 ± 0.41	19.0 ± 3.2
Tris buffer	8.9 ± 0.67	17.9 ± 2.8

*$p < 0.0004$ versus normal saline.
†$p < 0.0004$ versus buffer alone.

Study Limitations and Implications

Our study was limited to the effect of TTC staining on normal myocardial tissue samples containing ^{201}Tl, sestamibi or teboroxime. Soaking of tissue samples or postmortem heart perfusion with TTC with this agent (*30–36*) has been utilized in the evaluation of other cardiac radiopharmaceuticals. Still other protocols involve different stains and dyes, either in conjunction with or independent of TTC staining (*27,35,37*). No reference was made to the possible effects these agents might have on the radiopharmaceutical being investigated. In addition, several reports utilize vital stains in ischemic, infarcted or normal tissue. Our observations in normal tissue samples may be similar, enhanced or depressed when evaluated in damaged myocardial cells.

Our findings clearly demonstrate that vital tissue stains should not be assumed to be inert in all situations. We suggest that a pilot study similar to the one presented be performed and reported in studies using dyes and stains in myocardial tissue samples that will subsequently be analyzed for radiopharmaceutical distribution. If the stain is shown to alter the tracer's retention, then tracer distribution should be determined prior to staining. Alternatively, adjacent tissue slices should be stained, assessed and compared for tracer concentration. This would ensure that the interpretation of myocardial tracer distribution is not compromised by artifacts introduced by tissue staining.

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REFERENCES

1. Strauss HW, Harrison K, Langan JK, Lebowitz E, Pitt B. Thallium-201 for myocardial imaging: relation of thallium-201 to regional myocardial perfusion. *Circulation* 1975;51:641–645.
2. Markis JE, Malagold M, Parker A, et al. Myocardial salvage after intracoronary thrombolysis with streptokinase in acute myocardial infarction. *N Engl J Med* 1981;305:777–782.
3. Maddahi J, Ganz W, Hinomiya K, et al. Myocardial salvage by intracoronary thrombolysis in evolving acute myocardial infarction: evaluation using intracoronary injection of thallium-201. *Am Heart J* 1981;102:664–674.
4. Schuler G, Schwartz F, Hofmann M, et al. Thrombolysis in acute myocardial infarction using intracoronary streptokinase: assessment by thallium-201 scintigraphy. *Circulation* 1982;66:658–664.
5. Kayden DS, Sigal S, Soufer R, et al. Thallium-201 for assessment of myocardial viability: quantitative comparison of 24-hour redistribution imaging with imaging after reinjection at rest. *J Am Coll Cardiol* 1991;18:1480–1486.
6. Wackers FJT, Berman DS, Maddahi J, et al. Technetium-99m hexakis 2-methoxy-isobutyl isonitrile: human biodistribution, dosimetry, safety, and preliminary comparison to thallium-201 for myocardial perfusion imaging. *J Nucl Med* 1989;30:301–311.
7. Di Rocco RJ, Rumsey WL, Kuczynski BL, et al. Measurement of myocardial blood flow using a co-injection technique for technetium-99m-teboroxime, technectium-96-sestamibi and thallium-201. *J Nucl Med* 1992;33:1152–1159.
8. Bonow RO, Dilsizian V. Thallium-201 and technetium-99m-sestamibi for assessing viable myocardium. *J Nucl Med* 1992;33:815–818.
9. Piwnica-Worms D, Chiu ML, Kronauge JF. Divergent kinetics of ^{201}Tl and ^{99m}Tc-sestamibi in cultured chick ventricular myocytes during ATP depletion. *Circulation* 1992;85:1531–1541.
10. Meerdink DJ, Leppo JA. Experimental studies of the physiologic properties of technetium-99m agents: myocardial transport of perfusion imaging agents. *Am J Cardiol* 1990;66:9E–15E.
11. Nachlas MM, Shnitka TK. Macroscopic identification of early myocardial infarcts by alterations in dehydrogenase activity. *Am J Path* 1963;17:379–405.
12. Cox JL, McLaughlin VW, Flowers NC, Horan LG. The ischemic zone surrounding acute myocardial infarction. Its morphology as detected by dehydrogenase staining. *Am Heart J* 1968;76:650–659.
13. Badir B, Knight B. Fluorescence microscopy in the detection of early myocardial infarction. *Forensic Sci Inter* 1987;34:99–102.
14. Chorpra P, Sabherwal U. Histochemical and fluorescent techniques for detection of early myocardial ischemia following experimental coronary artery occlusion: a comparative and quantitative study. *Angiology* 1988;39:132–140.
15. Fishbein MC, Meerbaum S, Rit J, et al. Early phase acute myocardial infarct size quantification: validation of the triphenyl tetrazolium chloride tissue enzyme staining technique. *Am Heart J* 1981;101:593–600.
16. Freeman I, Grunwald AM, Robin B, Rao PS, Bodenheimer MM. Effect of early reperfusion on use of triphenyltetrazolium chloride to differentiate viable form non-viable myocardium in area of risk. *Circ Res* 1990;24:109–114.
17. Ebrahimi S, Fishbein MC, Kobayashi S, et al. Overestimation of myocardial infarct size by histologic measurement in a model of occlusion followed by reperfusion. *Arch Pathol Lab Med* 1990;114:1218–1222.
18. Lie JT, Pairolero PC, Holley KE, Titus JL. Macroscopic enzyme-mapping verification of large, homogeneous, experimental myocardial infarcts of predictable size and location in dogs. *J Thorac Cardiovasc Surg* 1975;69:599–605.
19. Vogel AI. Extraction with solvents. In: *Practical organic chemistry: including qualitative organic analysis. third ed.* London: Lowe and Brydone LTD.; 1972:44–45.
20. Cheng SW, Shanker R, Lindenbaum S. Thermodynamics and mathematical modeling of the partitioning of chlorpromazine between n-octanol and aqueous buffer. *Pharm Res* 1990;7:856–862.
21. Leppo JA, Meerdink DJ. Comparison of the myocardial uptake of a technetium-labeled isonitrile analogue and thallium. *Circ Res* 1989;65:632–639.
22. Stewart RE, Schwaiger M, Hutchins GD, et al. Myocardial clearance kinetics of technetium-99m-SQ30217: a marker of regional myocardial blood flow. *J Nucl Med* 1990;31:1183–1190.
23. Betageri GV, Rogers JA. Correlation of partitioning of nitroimidazoles in the n-octanol/saline and liposome systems with pharmacokinetic parameters and quantitative structure-activity relationships (QSAR). *Pharm Res* 1989;6:399–403.
24. Andersen AR, Friberg H, Lassen NA, Kristensen K, Neirinckx RD. Assessment of the arterial input curve for [^{99m}Tc]-d,l-HM-PAO by rapid octanol extraction. *J Cereb Blood Flow Metabol* 1988;8:S23–S30.
25. Atkinson HC, Begg EJ. Relationship between human milk lipid-ultrafiltrate and octanol-water partition coefficients. *J Pharm Sci* 1988;77:796–798.
26. Sinusas AJ, Trautman KA, Bergin JD, et al. Quantification of area at risk during coronary occlusion and degree of myocardial salvage after reperfusion with technetium-99m methoxyisobutyl nitrile. *Circulation* 1990;82:1414–1437.
27. Freeman I, Grunwald AM, Hoory S, Bodenheimer MM. Effect of coronary occlusion and myocardial viability on myocardial activity of technetium-99m-sestamibi. *J Nucl Med* 1991;32:292–298.
28. Humbert T, Luu-Duc C, Comet M, Demenge P. Evaluation of cellular viability by quantitative autoradiographic study of myocardial uptake of a fatty acid analogue in isoproterenol-induced focal rat heart necrosis. *Eur J Nucl Med* 1991;18:870–878.
29. Melin JA, Becker LC, Bulkley BH. Differences in thallium-201 uptake in reperfused and nonreperfused myocardial infarction. *Circ Res* 1983;53:414–419.
30. Khaw BA, Fallon JT, Beller GA, Haber E. Specificity of localization of myosin-specific antibody fragments in experimental myocardial infarction. *Circulation* 1979;60:1527–1531.
31. Jansen DE, Corbett JR, Buja LM, et al. Quantification of myocardial injury produced by temporary artery occlusion and reflow with technetium-99m-pyrophosphate. *Circulation* 1987;75:611–617.
32. Zalutsky MR, Garg PK, Johnson SH, Utsunomiya H, Coleman RE. Fluorine-18-antimyosin monoclonal antibody fragments: preliminary investigations in a canine myocardial infarct model. *J Nucl Med* 1992;33:575–580.
33. Miller DD, Gill JB, Livni E, et al. Fatty acid analogue accumulation: a marker of myocyte viability in ischemic-reperfused myocardium. *Circ Res* 1988;63:681–692.
34. Endo T, Kiuchi K, Sato N, Hayakawa H, Okumura H. Does the extent of the zone at risk after coronary artery occlusion influence the percentage of the zone that evolves to infarction? *Cardiology* 1990;77:112–120.
35. Morguet AJ, Munz DL, Klein HH, et al. Myocardial distribution of indium-111-antimyosin Fab and technetium-99m-sestamibi in experimental nontransmural infraction. *J Nucl Med* 1992;33:223–228.
36. Khaw BA, Gold HK, Leinbach RC, et al. Early imaging of experimental myocardial infarction by intracoronary administration of ^{131}I-labeled anticardial myosin (Fab)'2 fragments. *Circulation* 1978;58:1137–1142.
37. Verani MS, Jeroudi MO, Mahmarian JJ, et al. Quantification of myocardial infarction during coronary occlusion and myocardial salvage after reperfusion using cardiac imaging with technetium-99m hexakis 2-methoxyisobutyl isonitrile. *J Am Coll Cardiol* 1988;12:1573–1581.