Interaction of technetium 99m–labeled teboroxime with red blood cells reduces the compound's extraction and increases apparent cardiac washout

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Background. **^{99m}Tc–labeled teboroxime shows high myocardial extraction in both in vivo animal and in vitro cell culture and isolated heart studies. Whereas in vivo studies show rapid myocardial clearance of teboroxime, in vitro cell culture and isolated heart studies show slower washout comparable to that of ^{201}Tl. Binding of teboroxime to blood components may contribute to these conflicting results.**

Methods and Results. **We measured teboroxime extraction in the isolated blood-perfused rabbit heart after injection in saline solution, brief incubation in red blood cell perfusate, or 4-hour incubation with human red blood cells. Teboroxime in saline solution showed high extraction (E_{max} = 0.89 ± 0.02; E_{net} = 0.69 ± 0.02), whereas brief incubation in perfusate (E_{max} = 0.60 ± 0.06; E_{net} = 0.48 ± 0.05) or prolonged incubation with human red blood cells (E_{max} = 0.43 ± 0.09; E_{net} = 0.38 ± 0.07) resulted in reduced extraction. Teboroxime clearance was similar for all groups and was slower than ^{201}Tl clearance. Analysis of total residual cardiac teboroxime (comparable to external imaging) showed that teboroxime clearance was biexponential. Reduced extraction of teboroxime in red blood cells resulted in an increased size of the rapidly clearing (unextracted) fraction, giving the appearance of rapid myocardial washout.**

Conclusions. **Teboroxime has a high myocardial extraction. Binding to blood components reduces teboroxime extraction and increases the rate of cardiac teboroxime clearance. (J NUCL CARDIOL 1994;1:270-9.)**

Key Words: teboroxime · thallium · technetium 99m · rabbit · isolated heart

^{99m}Tc–labeled teboroxime (^{99m}Tc-teboroxime) is a neutral, lipophilic compound developed for myocardial perfusion imaging. In vitro cell culture, in vivo animal, and most isolated heart studies have shown that teboroxime has a high myocardial extraction that permits accurate assessment of coronary blood flow even during pharmacologic hyperemia.[1-6] In vivo animal studies have shown that this high initial extraction of teboroxime is followed by a rapid myocardial clearance that may be related to the lipophilicity of the compound.[4-8] Therefore cardiac imaging ideally should be completed within several minutes of injection. In contrast, cell culture data show a myocardial cellular washout of teboroxime that is slower than that of ^{201}Tl.[1,2] In addition, isolated heart studies show conflicting results, with teboroxime washout being slightly faster[3] or slower[9] than ^{201}Tl washout.

Because some ^{99m}Tc-labeled perfusion agents have been shown to bind to blood components,[10,11] we hypothesized that the differing measurements of extraction in the isolated heart might be related to an interaction of ^{99m}Tc-teboroxime with perfusate. Pre-

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liminary observations suggested that interaction with red blood cells could significantly reduce the myocardial extraction of ^{99m}Tc-teboroxime.[12] We also hypothesized that teboroxime binding to human red blood cells could reduce the compound's myocardial extraction and affect its cardiac clearance. Accordingly, we studied the effect of red blood cell incubation on the extraction and retention of teboroxime in the isolated rabbit heart model.

METHODS

In Vitro Binding of ^{99m}Tc-Teboroxime to Red Blood Cells

^{99m}Tc-teboroxime used in all experiments was prepared by adding ^{99m}Tc-labeled pertechnetate in saline solution to kits supplied by Bristol Myers-Squibb (Princeton, N.J.). Radiochemical purity was determined by paper chromatography according to standard methods and was always greater than 90%.[13] ^{99m}Tc-teboroxime was incubated for variable times with samples of washed human red blood cells suspended in normal saline solution. After incubation, the red blood cells were centrifuged and washed in saline solution three times to measure the bound fraction of ^{99m}Tc-teboroxime. ^{99m}Tc activity bound to red blood cells and in the saline supernatant, corrected for background and decay, was measured in a gamma well counter.

Surgery and Perfusion

Isolated, isovolumically contracting hearts from New Zealand white rabbits were perfused according to established methods.[14] Hearts were mounted on a perfusion apparatus and perfused retrogradely through the aorta with Krebs-Henseleit buffer enriched with washed bovine red blood cells (RBC/KH perfusate). A catheter and temperature probe were placed in the right ventricle through the right atrium, and a silicone rubber catheter was placed in the right ventricle through the pulmonary artery to determine coronary flow and collect coronary sinus drainage for indicator dilution experiments. A plastic tube in the left ventricular apex was used to collect thebesian vein flow and aortic valve leakage. Left ventricular pressure and its first derivative were monitored constantly with a saline-filled latex balloon inserted into the left ventricle through the left atrium. The heart was placed in a water-jacketed chamber filled with saline solution, maintained at 37° ± 1° C, and paced to at least 180 beats/min.

The RBC/KH perfusate was oxygenated with 4% CO_2/96% air as it passed through a membrane oxygenator. Oxygen was supplemented as needed, and appropriate adjustments were made to maintain blood pH, partial pressure of oxygen, and partial pressure of carbon dioxide in the physiologic range. Lactate, glucose, and insulin (2 mU/ml) were provided as substrate for myocardial metabolism.

Experimental Protocol

The injected isotopes consisted of 6 μCi ^{111}In–labeled diethylenetriamine pentaacetic acid/albumin,[15] 20 μCi ^{201}Tl-labeled chloride, and 35 μCi ^{99m}Tc-teboroxime. To assess the effect of red blood cell binding on cardiac teboroxime extraction, three sets of indicator-dilution experiments were performed. A fourth experiment was designed to assess the effect of perfusion technique on myocardial extraction. The four experimental protocols are illustrated in Figure 1.

Experiment A. ^{99m}Tc-teboroxime was incubated for 4 hours with bovine red blood cells (the cells used in the perfusate for the rabbit heart model) in saline solution versus control incubation of teboroxime in saline solution. In these experiments ^{99m}Tc-teboroxime in saline solution or bovine red blood cells was mixed with ^{111}In-labeled albumin (^{111}In-albumin) and ^{201}Tl in standard RBC/KH perfusate and injected 2 to 3 minutes later into the aortic inflow of the isolated heart. Paired control versus red blood cell injections were performed in six hearts at constant coronary flow.

Experiment B. ^{99m}Tc-teboroxime was incubated for 4 hours with human red blood cells in saline solution versus saline control. In these experiments ^{99m}Tc-teboroxime in saline solution or human red blood cells was mixed with ^{111}In-albumin and ^{201}Tl in saline solution injected immediately into the aortic inflow of the isolated heart. A total of eight pairs of saline control versus red blood cell injections were performed in four hearts at constant coronary flow. An important difference between experiments A and B is that the control injections for experiment A used isotopes mixed in RBC/KH perfusate just before injection, whereas the control injections for experiment B used isotopes mixed in saline solution.

Experiment C. Because control teboroxime suspended in RBC/KH perfusate for 2 to 3 minutes showed a lower extraction than teboroxime in saline solution, we performed another series of experiments to assess the effect of brief exposure to RBC/KH perfusate on teboroxime extraction. ^{111}In-albumin, ^{201}Tl, and ^{99m}Tc-teboroxime were mixed in standard RBC/KH perfusate and injected 2 to 3 minutes later versus injection in saline solution. In this set

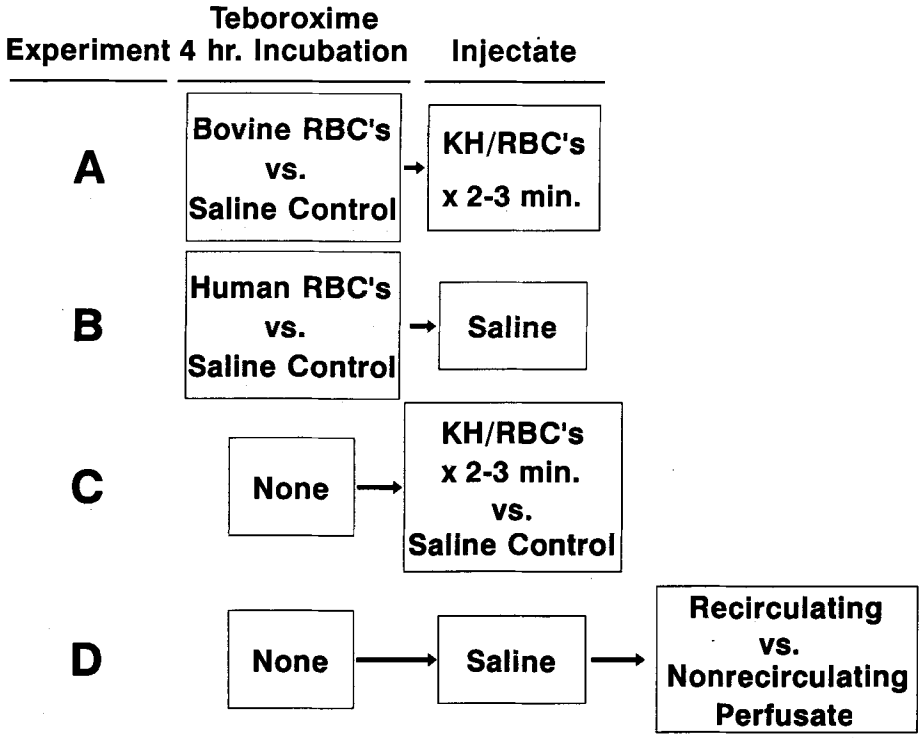

Figure 1. Summary of isolated heart experiments. Experiments A through C assess effect of red blood cells *(RBCs)* on teboroxime extraction. Experiment D assesses effect of perfusion technique on myocardial tracer extraction.

of experiments extraction of ^{99m}Tc-teboroxime in RBC/KH perfusate was measured in five hearts and control extraction in saline solution was measured in five separate hearts that were matched for the level of coronary flow.

The above experiments were performed with isolated rabbit hearts perfused with continuously recirculating RBC/KH perfusate (except during coronary venous collections after tracer injection). Because other studies that used nonrecirculating RBC/KH perfusate have suggested that perfusion technique may affect tracer extraction,[14,16] experiment D was performed to determine whether nonrecirculating versus recirculating perfusate could affect tracer extraction.

Experiment D. ^{99m}Tc-teboroxime and ^{201}Tl extractions were measured in four hearts receiving nonrecirculating perfusate, and the results were compared with those of four separate hearts receiving recirculating perfusate and matched for the level of coronary flow. All injections for this experiment used isotopes mixed in saline solution with no incubation.

Isotope Injection and Collection

The isotopes were mixed thoroughly and a 0.3 ml bolus was quickly loaded into an injection loop that ran parallel to and joined with the aortic inflow with three-way valves. The isotopes were injected by turning the three-way valves so that the bolus was distributed as homogeneously as possible to both coronary arteries. Collection of the coronary venous effluent into preweighed plastic tubes (1 to 3 seconds each; 3 to 4 minutes total) was timed so that each tube contained approximately 0.2 ml). After each tube was weighed, activities in the samples and a 0.1 ml aliquot of injectate were determined in a gamma well counter. Appropriate corrections for energy crossover, background, and decay were made for each isotope, and activities were expressed as counts per minute per milliliter.

Myocardial extraction and retention of ^{99m}Tc-teboroxime and ^{201}Tl were compared by the multiple indicator-dilution technique.[17-21] With this method, diffusible compounds (^{201}Tl and ^{99m}Tc-teboroxime) were coinjected into the aorta together with a reference tracer (^{111}In-albumin) that remains in the intravascular space. Samples of venous flow were then collected from the coronary sinus, and the measured isotope activities were used to plot indicator-dilution venous outflow curves for each isotope. For each injection, normalized outflow dilution curves [h(t)] were calculated for each of the coinjected tracers: ^{111}In-albumin (reference) [$h_R(t)$] and ^{201}Tl or ^{99m}Tc-teboroxime (diffusible) [$h_D(t)$] by use of the following equation: $h(t) = F \cdot C(t)/q_0$, where F is coronary flow

(in milliliters per minute), t is time (in seconds) after injection, C(t) is isotope activity (in counts per minute per milliliter), and q_0 is injected dose (in counts per minute); the units of h(t) are seconds^{-1}.[21,22] Therefore h(t) is the fraction of the injected tracer activity that is collected from the coronary sinus at time t.

Instantaneous extractions [E(t)] of the diffusible tracers were then calculated as $E(t) = 1 - h(t)_D/h(t)_R$, where $h(t)_D$ is the venous outflow dilution curve of the diffusible tracer ^{201}Tl or ^{99m}Tc-teboroxime, and $h(t)_R$ is the transport function of albumin, the intravascular reference. E_{max} was taken as the highest value of E(t) up to the peak of the albumin h(t) curve and is the best estimate of fractional tissue extraction for each diffusible tracer.

Net tissue extraction [$E_{net}(t)$] of a diffusible compound reflects the integral balance of both extraction and clearance up to time t. E_{net} was calculated as follows:

$$E_{net}(t) = \frac{\int_0^t [h(\lambda)_R - h(\lambda)_D]d(\lambda)}{\int_0^t h(\lambda)_R d(\lambda)}$$

where λ is a dummy variable for integration. $E_{net}(t)$ was determined at time t (in seconds), when 99.99% of the reference albumin had emerged in the collected coronary sinus flow.

Residual cardiac activity, representing both intravascular and extracted myocardial activity, can be calculated at time t as $R(t) = 1 - H(t)$, where $H(t) = \int_0^t h(\lambda)$. Time-activity curves generated by external imaging devices are equivalent to R(t) because an external camera cannot separate intravascular and myocardial activity.[20] To compare the present teboroxime data with those of in vivo studies, R(t) cardiac washout curves were calculated for teboroxime injected in saline solution or RBC/KH perfusate and teboroxime incubated with human red blood cells for hearts perfused with recirculating perfusate. R(t) curves were also calculated for teboroxime injected in saline solution for hearts perfused with nonrecirculating perfusate. Cardiac washout data were then fitted to biexponential curves, and rates of clearance for the rapid and slow teboroxime fractions were calculated.

All data are expressed as means ± SD. Continuous data were compared with *t* tests. Nonlinear regression was used to fit data to biexponential curves with BMDP 3R software (BMDP Statistical Software, Inc., Los Angeles, Calif.).

RESULTS

Binding of Teboroxime to Human Red Blood Cells. The fraction of ^{99m}Tc activity that remained in the red blood cell pellet despite triple saline washing is shown in Figure 2. Teboroxime showed rapid binding to human red blood cells that ranged from 61% to 71% of total activity. Even at 10 minutes most teboroxime was bound to the human red blood cells. These data represent the minimum fraction of teboroxime bound to the red blood cells because loosely bound teboroxime may have been removed by triple washing.

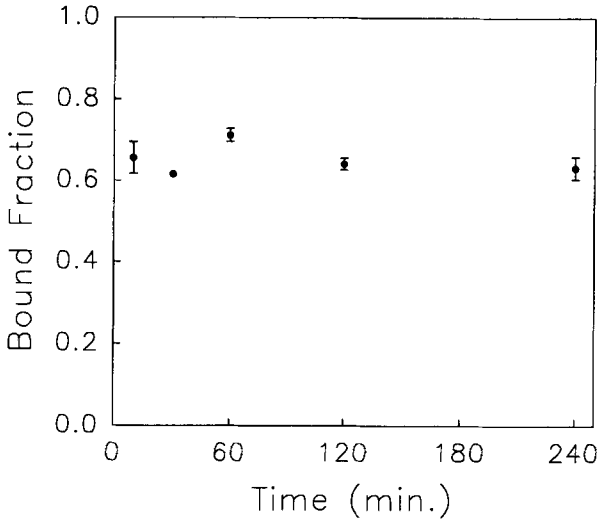

Figure 2. Fraction of teboroxime bound to human red blood cells after varying incubation times.

Extraction of Teboroxime After Incubation With Red Blood Cells. Table 1 shows the results for all hearts comparing extraction of teboroxime in saline solution, in RBC/KH perfusate, or after red blood cell incubation. To evaluate the effect of red blood cell binding on cardiac extraction of teboroxime, we performed six pairs of injections with teboroxime in RBC/KH perfusate (control) and after 4 hours of bovine red blood cell incubation in six hearts (Table 1). Similarly, we performed eight pairs of injections with teboroxime in saline solution (control) and after 4-hour human red blood cell incubation in four hearts (Table 1). The data show that incubation with either bovine or human red blood cells significantly reduced the extraction of teboroxime in the isolated rabbit heart. In addition, extraction of teboroxime injected in RBC/KH perfusate (control, experiment A, Table 1) appeared to be lower than teboroxime in saline solution (control, experiment B, Table 1).

Extraction of Teboroxime After Brief Incubation in RBC/KH Perfusate. To assess the effect of brief mixing of teboroxime with red blood cells, we injected teboroxime and ^{201}Tl in saline solution into five hearts with a coronary flow of 2.4 ± 0.5 ml/min · gm and injected ^{201}Tl and teboroxime after 2 to 3 minutes in RBC/KH perfusate into five hearts with a coronary flow of 2.5 ± 0.4 ml/min · gm. The data in Table 1 show that even brief mixing in

Table 1. Extraction of teboroxime and ^{201}Tl

		Teboroxime		^{201}Tl		
	n	E_{max}	E_{net}	E_{max}	E_{net}	Flow (ml/min · gm)
Group A						
Teboroxime in RBC perfusate	6	0.59 ± 0.09	0.49 ± 0.08	0.71 ± 0.08	0.48 ± 0.06	2.6 ± 0.5
Teboroxime with bovine RBCs		0.16 ± 0.06*	0.16 ± 0.04*	0.68 ± 0.02	0.45 ± 0.08	2.6 ± 0.5
Group B						
Teboroxime in saline solution	8	0.81 ± 0.03	0.67 ± 0.04	0.67 ± 0.05	0.51 ± 0.06	1.9 ± 0.5
Teboroxime with human RBCs		0.43 ± 0.09*	0.38 ± 0.07*	0.70 ± 0.05	0.54 ± 0.04	1.9 ± 0.4
Group C						
Teboroxime in saline solution	5	0.83 ± 0.02	0.69 ± 0.03	0.69 ± 0.06	0.53 ± 0.08	2.4 ± 0.5
Teboroxime in RBC perfusate		0.60 ± 0.06*	0.48 ± 0.05*	0.67 ± 0.06	0.48 ± 0.04	2.5 ± 0.4
Group D						
Teboroxime in saline solution	4	0.82 ± 0.01	0.69 ± 0.02	0.69 ± 0.04	0.54 ± 0.02	1.7 ± 0.4
Teboroxime in saline solution (non-RC perfusate)		0.89 ± 0.02*	0.71 ± 0.04	0.80 ± 0.05*	0.60 ± 0.02*	1.7 ± 0.4

n, Number of pairs of injections; *RBC*, red blood cell; *non-RC perfusate*, nonrecirculating perfusate.
*$p < 0.05$ versus paired control.

KH/RBC perfusate significantly reduced the myocardial extraction of teboroxime compared with injection in saline solution.

Effect of Recirculating Versus Nonrecirculating Perfusate. Table 1 shows that teboroxime extraction was higher in hearts perfused with nonrecirculating perfusate versus perfusion with recirculating perfusate. All of the hearts for these injections received teboroxime in saline solution to minimize any interaction with blood components. In addition, extraction of ^{201}Tl was also greater in hearts receiving nonrecirculating perfusate.

Figures 3 and 4 illustrate instantaneous and net extractions for teboroxime and ^{201}Tl. Figures 3 and 4 also illustrate that the highest extraction of teboroxime occurred when the tracer was injected in saline solution (Figures 3, *A* and *B*, and 4, *A* and *B*). Even brief exposure to RBC/KH perfusate resulted in reduced E_{max} and E_{net} (Figures 3, *C*, and 4, *C*), whereas the lowest extraction was seen after prolonged incubation of teboroxime with red blood cells (Figures 3, *D*, and 4, *D*).

Teboroxime R(t) Clearance Curves. R(t) was calculated for hearts injected with teboroxime in saline solution (nonrecirculating and recirculating perfusate), in perfusate, or after red blood cell incubation. Clearance data from three hearts (matched for coronary flow) in each of the four experimental groups were fitted with biexponential curves. Figure 5 illustrates cardiac washout data and fitted curves from one heart in each group. Exposure of teboroxime to RBC/KH perfusate or red blood cells (Figure 5, *C* and *D*) resulted in more rapid cardiac clearance compared with injections of the compound in saline solution (Figure 5, *A* and *B*). Table 2 shows that cardiac teboroxime clearance had a rapidly clearing fraction with a half-life ($t_{1/2}$) of 25 to 38 seconds, whereas the slower fraction had a clearance $t_{1/2}$ of 19 to 26 minutes. Interaction with red blood cells resulted in an increased size of the rapidly clearing component and a reduction in the slowly clearing fraction. Incubation of teboroxime with red blood cells or injection in RBC/KH perfusate resulted in lower extraction, whereas extracted teboroxime (Figures 3 and 4; Table

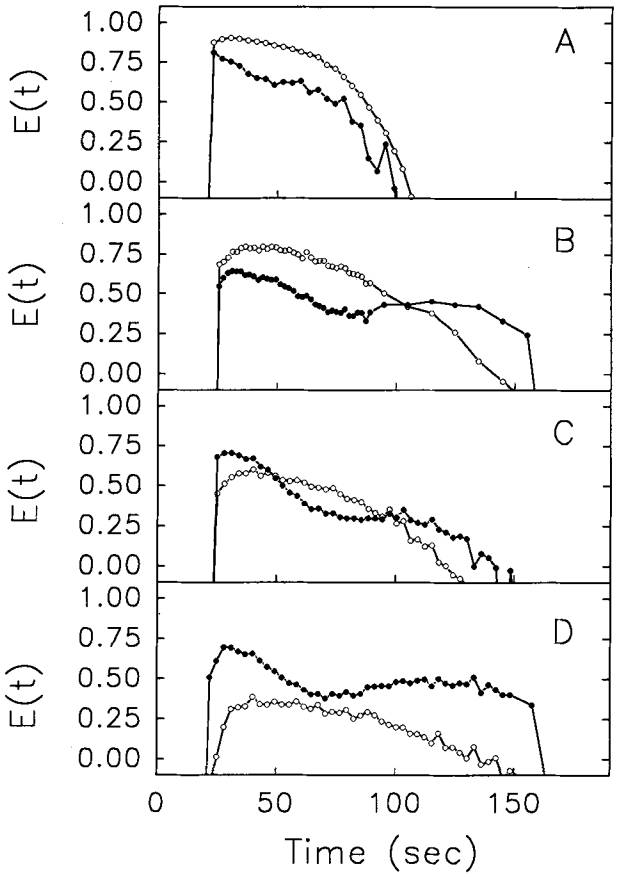

Figure 3. E(t) of teboroxime *(open circles)* and ^{201}Tl *(closed circles)*. E_{max} for each tracer is highest early value (time < 50 seconds). **A,** tracers injected in saline solution in heart with nonrecirculating perfusate; **B,** tracers injected in saline solution, hearts *B* through *D* treated with recirculating perfusate; **C,** tracers injected in perfusate; **D,** teboroxime incubated for 4 hours with human red blood cells. There is reduced teboroxime extraction for injection *C* in perfusate and *D* with red blood cells compared with injections *A* and *B* in saline solution.

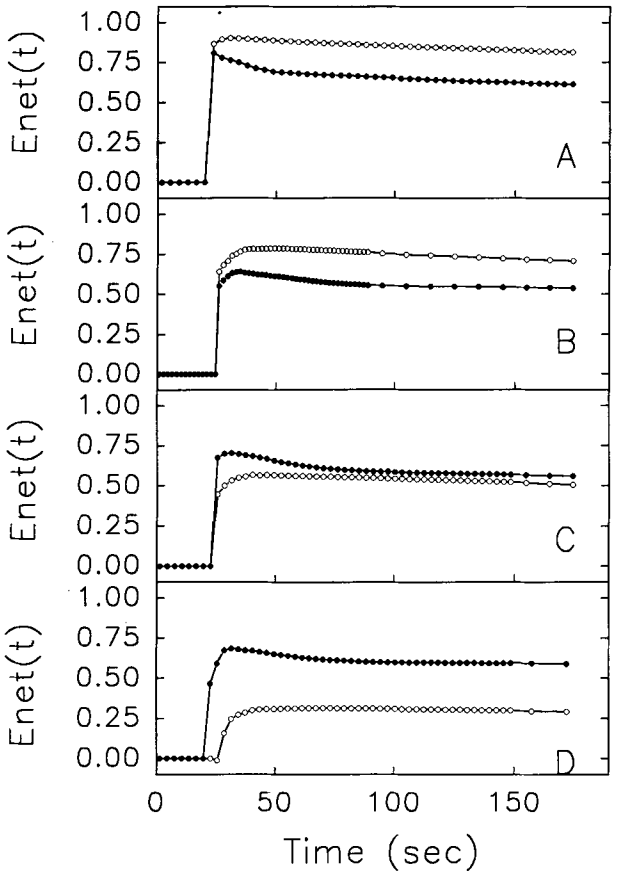

Figure 4. $E_{net}(t)$ for teboroxime *(open circles)* and ^{201}Tl *(closed circles)*. **A,** tracers injected in saline solution in heart with nonrecirculating perfusate; **B,** tracers injected in saline solution, hearts *B* through *D* treated with nonrecirculating perfusate; **C,** tracers injected in perfusate; **D,** teboroxime incubated for 4 hours with human red blood cells. Rate of myocardial clearance for extracted teboroxime and ^{201}Tl are similar in each heart.

1) showed a similar rate of myocardial clearance for all groups, and the rate of clearance of extracted teboroxime was similar to that of ^{201}Tl. Therefore the rapidly clearing teboroxime fraction appears to represent unextracted tracer rather than rapid myocardial washout.

DISCUSSION

If the myocardial deposition of a radiolabeled compound is to be used for the quantitative assessment of myocardial blood flow, the extraction of the compound must be very high to prevent underestimation of high flows during coronary hyperemia. Both in vitro and in vivo studies of teboroxime show that this lipophilic compound has a high myocardial extraction.

Teboroxime Extraction. Maublant et al.[2] have shown that cultured rat myocardial cells accumulate teboroxime such that the intracellular/extracellular ratio of teboroxime is 585. With cultured chick myocytes, Kronauge et al.[1] have shown a similarly high intracellular accumulation for teboroxime. In both studies the accumulation of teboroxime was 3.7 to 4 times greater than that of ^{201}Tl or sestamibi.

Our isolated rabbit heart study shows that teboroxime has a myocardial extraction that is greater than that of ^{201}Tl. When injected in saline solution, the E_{max} and E_{net} of teboroxime (0.82 ± 0.01 and 0.69 ± 0.02, respectively) were significantly higher than the E_{max} and E_{net} of ^{201}Tl (0.69 ± 0.04 and

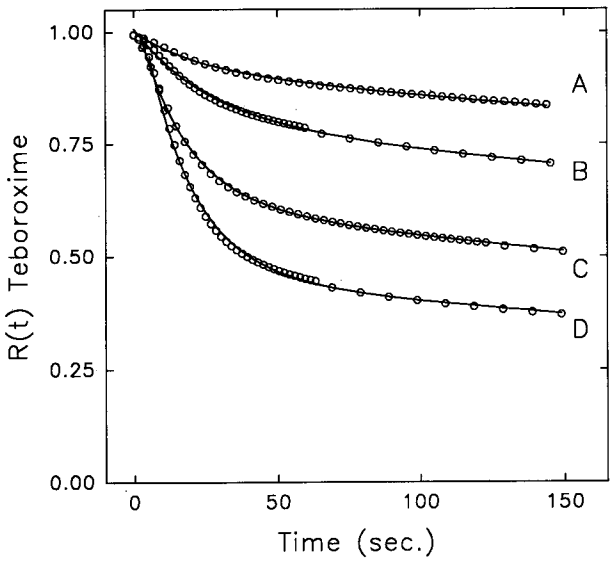

Figure 5. R(t) for injections of teboroxime in saline solution (*A* and *B*), perfusate *(C),* or human red blood cells *(D).* Hearts were treated with nonrecirculating *(A)* or recirculating perfusate (*B* through *D*). Teboroxime in saline solution has slowest clearance. Teboroxime in perfusate or human red blood cells shows much faster clearance because of reduced extraction. *Open circles* are teboroxime data and *solid lines* are fitted biexponential curves.

0.54 ± 0.02). Because an isolated rabbit heart model that uses nonrecirculating RBC/KH perfusate may result in a higher extraction of radiolabeled perfusion agents than a model that uses recirculating perfusate,[14,16] we also measured teboroxime and ^{201}Tl extraction in hearts with nonrecirculating perfusate. Isolated hearts perfused with nonrecirculating perfusate showed even higher extractions for both teboroxime and ^{201}Tl; however, teboroxime extraction (E_{max} 0.89 ± 0.02; E_{net} 0.71 ± 0.04) remained higher than myocardial extraction of ^{201}Tl (E_{max} 0.80 ± 0.05; E_{net} 0.60 ± 0.02).

In an in vivo canine model, Stewart et al.[6] measured myocardial teboroxime activity after intracoronary injection with external sodium iodide detection; teboroxime extraction was determined from time-activity curves according to the intercept (B)/peak activity (A) method. This in vivo study also showed teboroxime to have a high myocardial extraction, or retention fraction, of 0.90 ± 0.04, which is comparable to the E_{max} of 0.89 in our study. Previous estimates of myocardial ^{201}Tl extraction, which have ranged from 82% to 88%,[23,24] are also comparable to the ^{201}Tl E_{max} of 80% in this study.

In a previous study of teboroxime extraction in the isolated rabbit heart, Leppo and Meerdink[3] also found teboroxime to have a higher extraction than ^{201}Tl. The somewhat lower value of 0.72 ± 0.09 for teboroxime E_{max}, compared with that of this study, is probably related to injection of teboroxime in RBC/KH perfusate. Using a similar isolated heart model, Marshall et al.[9] found teboroxime to have an even lower E_{max} of 0.62 ± 0.12, which was lower than the E_{max} of 0.67 ± 0.11 for ^{201}Tl. These divergent results might also be explained by teboroxime-blood interaction. The E_{max} of 0.62 for teboroxime reported by Marshall et al. is similar to the 0.60 value for E_{max} that we observed when teboroxime was injected in RBC/KH perfusate. In addition, our E_{max} for teboroxime in RBC/KH perfusate was also lower than the value for ^{201}Tl (Table 1).

We have shown that binding to red blood cells reduces extraction of teboroxime. With a buffer-perfused isolated rat heart model, Rumsey et al.[25] previously reported a progressive fall in teboroxime extraction when the compound was exposed to rat red blood cells and whole blood. Extractions of ^{201}Tl and sestamibi were not affected by blood binding. In addition to red blood cell binding, a second mechanism for reduced teboroxime extraction was chlorohydroxyl exchange, which was accelerated by exposure to blood. The hydroxy metabolite showed a much lower myocardial extraction than native teboroxime. Studies by Kronauge et al.[1] and Leppo and Meerdink[3] have previously shown that substitution of a hydroxyl for a methyl group on teboroxime also results in reduced uptake by cultured cardiac myocytes, reduced extraction in the isolated rabbit heart, and poor imaging of the human heart.

Therefore teboroxime has a high myocardial extraction, as shown by both in vitro and in vivo experimental models. Studies showing a lower teboroxime extraction may be explained by blood interaction because rat, bovine, and human blood reduce myocardial extraction of the compound. Chemical transformation of teboroxime by chlorohydroxyl exchange is an additional cause of reduced extraction.

Teboroxime Clearance. In vitro studies of teboroxime show not only a high myocardial extraction but also a myocardial clearance that is as slow or slower than that of ^{201}Tl. Studies by Maublant et al.[2] and McCall et al.[26] with isolated rat myocytes showed clearance $t_{1/2}$ of 5 to 6 minutes for ^{201}Tl. In contrast, teboroxime clearance $t_{1/2}$ was 13 minutes, whereas sestamibi showed a much slower clearance with a $t_{1/2}$ of 28 minutes. With chick myocytes, Kronauge et al.[1] observed a biexponential cellular clearance for teboroxime with a rapid, early-component $t_{1/2}$ of 4 minutes. Although ^{201}Tl clearance was not reported

Table 2. Effect of red blood cells and perfusate on myocardial clearance of teboroxime

Injectate	a	$T_{1/2}$a (sec)	b	$T_{1/2}$b (min)	Flow (ml/min · gm)
Saline solution (NRCP)	0.08 ± 0.07	38 ± 16	0.89 ± 0.03	26 ± 10	1.8 ± 0.4
Saline solution	0.18 ± 0.02	31 ± 10	0.82 ± 0.02	22 ± 5	1.8 ± 0.5
Perfusate	0.43 ± 0.12	23 ± 5	0.61 ± 0.10	18 ± 2	1.8 ± 0.4
Human red blood cells	0.61 ± 0.09	23 ± 4	0.47 ± 0.07	17 ± 4	1.8 ± 0.4

Injectate refers to the solution in which teboroxime is mixed before aortic injection; *NRCP*, nonrecirculating perfusate, all other hearts received recirculating perfusate; *a*, fraction of teboroxime with rapid clearance; *b*, fraction of teboroxime with slower clearance.
Each value represents the mean of three injections; hearts in each experimental group were matched for level of coronary flow.

for this model, sestamibi clearance showed a $t_{1/2}$ of 8 to 10 minutes. By 20 minutes, cellular clearances for sestamibi and teboroxime were equivalent. Therefore cell culture data suggest that intrinsic cellular clearance of teboroxime is comparable to that of ^{201}Tl.

Leppo and Meerdink[3] assessed washout of teboroxime and ^{201}Tl in the isolated rabbit heart by comparing E_{max} with final retention, measured by E_{net}. The relative fall in extraction ($E_{max} - E_{net}/E_{max}$) gave an estimate of tracer washout. In this study teboroxime washout was 23.6% of initial E_{max} compared with 19.2% for ^{201}Tl. However, even though teboroxime washout was slightly faster than that of ^{201}Tl, such a small difference between teboroxime and ^{201}Tl in this model does not explain the rapid teboroxime washout that is seen in vivo.[4,6,7]

When the data of this study were used to determine washout ($E_{max} - E_{net}/E_{max}$), teboroxime washout was comparable or slightly slower than ^{201}Tl washout. When injected in saline solution or perfusate, teboroxime washout ranged from 16% to 20% compared with 23% to 33% for ^{201}Tl. Neither this study nor that of Leppo and Meerdink[3] suggests that teboroxime has an extremely rapid myocardial washout from the isolated rabbit heart.

Marshall et al.[9] have also studied teboroxime extraction in the isolated rabbit heart with the multiple indicator-dilution technique, with a 40- to 60-minute collection protocol designed to measure tracer washout. Their data also showed that teboroxime had a myocardial clearance that was slower than that of ^{201}Tl. In addition, preliminary data from other researchers who used a buffer-perfused rat heart model, with external NaI detection of cardiac activity, have also shown the rate of myocardial teboroxime clearance to be similar to that of ^{201}Tl.[27,28] Therefore in vitro cell culture and isolated heart studies consistently show that intrinsic teboroxime clearance is not significantly faster than that of ^{201}Tl. In contrast, in vivo studies show a much more rapid myocardial washout for teboroxime.

After a high initial extraction of intracoronary teboroxime (in an in vivo canine model), Stewart et al.[6] observed a biexponential myocardial clearance of tracer with 63% clearing with a $t_{1/2}$ of 2.3 minutes and 19% with a $t_{1/2}$ of 20 minutes. After intravenous injection, myocardial teboroxime clearance was monoexponential with a $t_{1/2}$ of 20 minutes and a faster clearance $t_{1/2}$ of 13 minutes during dipyridamole infusion. In a subsequent canine study, Stewart et al.[7] found a control teboroxime clearance $t_{1/2}$ of 12 minutes and a faster $t_{1/2}$ of 9 minutes during adenosine or dipyridamole infusion. Gray and Gewirtz[4] showed a similar teboroxime clearance $t_{1/2}$ of 10 minutes during adenosine infusion in a swine model. In contrast, in vivo studies show a much slower rate for myocardial ^{201}Tl clearance. Intrinsic myocardial ^{201}Tl clearance after intracoronary injection shows a $t_{1/2}$ of 54 to 100 minutes, whereas intravenous ^{201}Tl injection results in a much slower cardiac clearance with a $t_{1/2}$ of 7 hours.[24,29] Therefore in vitro studies suggest similar clearance rates for teboroxime and ^{201}Tl, whereas in vivo teboroxime clearance is much faster than that of ^{201}Tl.

In view of the differing estimates of teboroxime clearance from in vitro and in vivo studies, what are the factors that may account for these contrasting results? One mechanism for the rapid in vivo clearance of teboroxime is tracer binding to blood components. Our data in Table 1 show that injection of teboroxime with red blood cells reduces its myocardial extraction. Although blood interaction does not change the clearance rate of extracted teboroxime, an external imaging system measures both extracted and intravascular tracer. A larger unextracted fraction of teboroxime results in the appearance of more rapid

washout. As shown in both Figure 5 (graphically) and Table 2 (clearance $t_{1/2}$), myocardial teboroxime clearance is biexponential. The rapidly clearing fraction has a $t_{1/2}$ of 23 to 38 seconds, whereas the slower fraction shows a clearance $t_{1/2}$ of 17 to 26 minutes. Injection of teboroxime in blood results in an increased size of the rapidly clearing (unextracted) fraction that appears as rapid washout with external imaging.

An additional mechanism for rapid teboroxime washout caused by blood interaction is unopposed clearance after intravenous injection. The principal factor determining the rate of myocardial ^{201}Tl clearance after intravenous injection is the rate of decline in ^{201}Tl blood levels.[24,29,30] Okada et al.[29] have previously shown that the rate of myocardial ^{201}Tl clearance is virtually identical to the rate of blood clearance after intravenous injection. After intracoronary injection, ^{201}Tl shows a much faster myocardial clearance when recirculating blood levels are very low. Because teboroxime binds to blood cells or undergoes chemical (Cl-OH) transformation, it is unavailable for continued extraction during recirculation (analogous to ^{201}Tl after intracoronary injection). Therefore despite comparable blood levels of tracer activity after intravenous injection,[6,7,24,29] teboroxime clearance would be faster than ^{201}Tl clearance.

Apart from the effect of blood on teboroxime extraction and washout, this study shows that the method of isolated heart perfusion can affect the extraction of perfusion agents. Although the use of recirculating RBC/KH perfusate has the advantage of limiting the volume of bovine blood that must be harvested, the higher values for teboroxime and ^{201}Tl extraction in hearts with nonrecirculating RBC/KH perfusate are closer to those measured in vivo. Therefore the nonrecirculating model may be preferable for the study of myocardial perfusion agents. Finally, when a potential myocardial perfusion agent is studied in the isolated heart model, measurement of tracer extraction after incubation in blood may improve the prediction of the compound's in vivo kinetics.

SUMMARY

Teboroxime is a neutral compound with a high myocardial extraction. Both in vivo animal data and in vitro cell culture and isolated heart data confirm the high extraction of this perfusion agent. Teboroxime clearance is not faster than ^{201}Tl clearance with in vitro cell culture or isolated heart models, whereas in vivo animal studies show rapid myocardial clearance of teboroxime. These divergent results may be explained by the effect of whole-blood components on teboroxime extraction. The fall in teboroxime extraction after exposure to blood results in a larger unextracted fraction that can be imaged externally as a faster net clearance of myocardial teboroxime activity.

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References

1. Kronauge JF, Chiu ML, Cone JS, et al. Comparison of neutral and cationic myocardial perfusion agents: characteristics of accumulation in cultured cells. Nucl Med Biol 1992;19:141-8.
2. Maublant JC, Moins N, Gachon P. Uptake and release of two new Tc-99m labeled myocardial blood flow imaging agents in cultured cardiac cells. Eur J Nucl Med 1989;15:180-2.
3. Leppo JA, Meerdink DJ. Comparative myocardial extraction of two technetium-labeled BATO derivatives (SQ30217, SQ32014) and thallium. J Nucl Med 1990;31:67-74.
4. Gray WA, Gewirtz H. Comparison of ^{99m}Tc-teboroxime with thallium for myocardial imaging in the presence of a coronary artery stenosis. Circulation 1991; 84:1796-807.
5. Beanlands R, Muzik O, Nguyen N, Petry N, Schwaiger M. The relationship between myocardial retention of technetium-99m teboroxime and myocardial blood flow. J Am Coll Cardiol 1992;20:712-9.
6. Stewart RE, Schwaiger M, Hutchins GD, et al. Myocardial clearance kinetics of technetium-99m–Q30217: a marker of regional myocardial blood flow. J Nucl Med 1990;31:1183-90.
7. Stewart RE, Heyl B, O'Rourke RA, Blumhardt R, Miller DD. Demonstration of differential post-stenotic myocardial technetium-99m–teboroxime clearance kinetics after experimental ischemia and hyperemic stress. J Nucl Med 1991;32:2000-8.
8. Johnson G III, Glover DK, Hebert CB, Okada RD. Early myocardial clearance kinetics of technetium-99m–teboroxime differentiate normal and flow-restricted canine myocardium at rest. J Nucl Med 1993;34:630-6.
9. Marshall RC, Leidholdt EM Jr, Zhang D-Y, Barnett CA. The effect of flow on technetium-99m–teboroxime

(SQ30217) and thallium-201 extraction and retention in rabbit heart. J Nucl Med 1991;32:1979-88.
10. Deutsch E, Ketring AR, Libson K, Vanderheyden J-L, Hirth WW. The Noah's ark experiment: species dependent biodistributions of cationic ^{99m}Tc complexes. Nucl Med Biol 1989;16:191-232.
11. Gerundini P, Savi A, Gilardi MC, et al. Evaluation in dogs and humans of three potential technetium-99m myocardial perfusion agents. J Nucl Med 1986;27:409-16.
12. Dahlberg ST, Gilmore MP, Siwko R, Leppo JA. Incubation with red blood cells reduces the extraction of technetium-99m teboroxime in the isolated rabbit heart [Abstract]. J Nucl Med 1991;32:910.
13. Narra RK, Nunn AD, Kuczynski BL, Feld T, Wedeking P, Eckelman WC. A neutral technetium-99m complex for myocardial imaging. J Nucl Med 1989;30:1830-7.
14. Leppo JA, Meerdink DJ. Comparison of the myocardial uptake of a technetium-labeled isonitrile analogue and thallium. Circ Res 1989;65:632-9.
15. Hnatowich DJ, Layne WD, Childs RL. The preparation and labeling of DTPA-coupled albumin. Int J Appl Radiat Isot 1982;33:327-32.
16. Marshall RC, Leidholdt EM Jr, Zhang D-Y, Barnett CA. Technetium-99m hexakis 2-methoxy-2-isobutyl isonitrile and thallium-201 extraction, washout and retention at varying coronary flow rates in rabbit heart. Circulation 1990;82:998-1007.
17. Bassingthwaighte JB, Holloway GA. Estimation of blood flow with radioactive tracers. Semin Nucl Med 1976;6:141-61.
18. Bassingthwaighte JB, Raymond GM, Chan JI. Principles of tracer kinetics. In: Zaret BL, Beller GA, eds. Nuclear cardiology: state of the art and future directions. St Louis: Mosby–Year Book, 1993:3-23.
19. Kuikka JT, Bassingthwaighte JB, Henrich MM, Feinendegen Le. Mathematical modeling in nuclear medicine. Eur J Nucl Med 1991;18:351-62.
20. Bassingthwaighte JB, Goresky CA. Modeling in the analysis of solute and water exchange in the microvasculature. In: Renkin EM, Michel CC, eds. Handbook of physiology: the cardiovascular system, vol 4: the microcirculation. Bethesda, Maryland: American Physiological Society, 1984:549-626.
21. Bassingthwaighte JB. Physiology and theory of tracer washout techniques for the estimation of myocardial blood flow: flow estimation from tracer washout. Prog Cardiovasc Dis 1977;20:165-89.
22. Bassingthwaighte JB, Chinard FP, Crone C, et al. Terminology for mass transport and exchange. Am J Physiol 1986;250:H539-45.
23. Weich HF, Strauss HW, Pitt B. The extraction of thallium-201 by the myocardium. Circulation 1977;56:188-91.
24. Grunwald AM, Watson DD, Holzgrefe HH Jr, Irving JF, Beller GA. Myocardial thallium-201 kinetics in normal and ischemic myocardium. Circulation 1981;64:610-8.
25. Rumsey WL, Rosenspire KC, Nunn AD. Myocardial extraction of teboroxime: effects of teboroxime interaction with blood. J Nucl Med 1992;33:94-101.
26. McCall D, Zimmer LJ, Katz AM. Kinetics of thallium exchange in cultured rat myocardial cells. Circ Res 1985;56:370-6.
27. Stone JA, Dawood F, Wen W-H, McLaughlin PR, Liu PP. Is the myocardial uptake of teboroxime viability dependent? [Abstract]. Circulation 1992;86:I-707.
28. Beanlands RS, Palser A, Hartman N, Aung M, Ruddy TD. Are the kinetics of Tc-99m-teboroxime altered in the postischemic myocardium? (Abstract). Circulation 1993;88:249.
29. Okada RD, Jacobs ML, Daggett WM, et al. Thallium-201 kinetics in nonischemic canine myocardium. Circulation 1982;65:70-7.
30. Gewirtz H. Differential myocardial washout of technetium-99m–teboroxime: mechanism and significance. J Nucl Med 1991;32:2009-11.