Myocardial Extraction of Teboroxime: Effects of Teboroxime Interaction with Blood

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The isolated perfused rat heart preparation was used to determine whether the interaction of blood with either ^{99m}Tc-teboroxime, ^{99m}Tc-sestamibi or ^{201}Tl affects the extraction of these myocardial perfusion agents. Hearts were retrogradely perfused at 72 cm H_2O with Krebs-Henseleit buffer equilibrated with O_2:CO_2 (95:5). The hearts were paced at 5 Hz. Single-pass extraction of ^{99m}Tc-teboroxime (96% ± 1%) was greater than that of ^{99m}Tc-sestamibi (15% ± 1%) or ^{201}Tl (30% ± 5%). Extraction of the hydroxide form of ^{99m}Tc-teboroxime was only 43% ± 4%. When arterial blood obtained from rats administered ^{99m}Tc-teboroxime was injected into the perfused heart, extraction of ^{99m}Tc-teboroxime decreased progressively as its time in circulation was lengthened. Similar experiments using either ^{99m}Tc-sestamibi or ^{201}Tl showed that extraction of these agents was neither affected by the presence of blood nor residence in circulation. For ^{99m}Tc-teboroxime, extraction was 99.5% ± 0.5%, 57% ± 13%, 20% ± 2% at 1, 5, and 60 min postinjection, respectively. In separate experiments, HPLC analysis of blood at 5, 15 and 60 min postinjection indicated that only 34% ± 4%, 13% ± 2%, and 2% ± 1%, respectively, of the total ^{99m}Tc-teboroxime was free and was associated with extraction values of 44% ± 7%, 28% ± 5%, and 19% ± 3%, respectively. The percentage of this free radioactivity that converted from the chloro to the hydroxide form was 9% ± 2%, 6% ± 2%, and 2% ± 1%, respectively. The results indicate that teboroxime is highly extracted on the first pass, however, extraction from subsequent passes may be affected by the binding characteristics of teboroxime to blood cells and plasma proteins.

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Clinical noninvasive diagnosis of coronary artery disease is performed by imaging the heart using either SPECT or planar imaging with ^{201}Tl as the probe. The better availability, more suitable photon energy, and the shorter half-life of ^{99m}Tc (6 hr) has resulted in the development of ^{99m}Tc radiopharmaceuticals. It has been reported that at least one of these new technetium agents, i.e., ^{99m}Tc-teboroxime, undergoes a chemical transformation in vitro, exchanging a chloro atom for a hydroxide (*1*). The extraction of this metabolite has not been determined. Moreover, the lipophilic nature of teboroxime and another ^{99m}Tc radiopharmaceutical, ^{99m}Tc-sestamibi (Cardiotec, DuPont) may result in binding to blood constituents, thereby affecting their extraction. The efficacy of the above agents for determining areas of myocardium having a reduced blood supply is highly dependent upon their ability to be extracted.

The purpose of the present study was to determine the extraction of teboroxime and for comparison, ^{201}Tl and ^{99m}Tc-sestamibi, in the isolated perfused rat heart. It was of greater interest to measure the extraction of these agents after their introduction into the circulation in order to identify their likelihood for uptake after the first pass through the myocardial circulation. These results indicated that extraction of ^{99m}Tc-teboroxime exceeded that of the others and that extraction of ^{99m}Tc-teboroxime diminishes with the time in circulation. The latter finding prompted an examination of the metabolism of ^{99m}Tc-teboroxime in blood. These results showed that binding of this compound to plasma proteins and blood cells was, at least in part, responsible for the decrease in extraction as the duration in circulation was increased.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (275–325 g, n = 36) were used for all heart perfusion studies while smaller rats (100–200 g, n = 23) served as blood donors. All animals were permitted free access to water and rat chow. The rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally, Nembutal, Abbott, Chicago, IL) and heparinized (500 IU, Invenex, Chagrin Falls, OH) via the caudal vena cava. Blood donor animals were heparinized (500 IU) by intraperitoneal injection. These animals were divided into two groups, those providing blood for incubation with radioactive material in vitro and those donating blood following injection in vivo of the radiopharmaceutical. Blood for the in vitro studies was obtained by exsanguinating the animal via a cannula inserted into the abdominal aorta. To obtain blood from animals to be injected with radiopharmaceutical, the jugular vein was exposed for injection of the agent and the femoral artery was cannulated for withdrawal of blood. Body temperature of these animals was maintained at 37°C and was monitored by

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inserting a probe (Sensortek, model bat-12, Clifton, NJ) into the colon 3–4 cm from the anus.

Heart Perfusion

Hearts were perfused retrogradely as described previously (*2,3*) in the isolated state at 37°C with Krebs-Henseleit buffer equilibrated with O_2:CO_2 (95:5) in the single-pass mode. The perfusate contained [in m*M*] NaCl [118], KCl [4.7], $CaCl_2$ [1.8], Na_2EDTA [0.5], KH_2PO_4 [1.2], $MgSO_4$ [1.2], $NaHCO_3$ [25], glucose [11], pyruvate [0.2], and 12 IU/liter of insulin. Perfusion pressure was maintained at 72 cm H_2O and the hearts were paced continuously at 5 Hz.

For determination of oxygen consumption, a cannula was placed in the right ventricle via the pulmonary artery. A pump (Cole-Parmer #7013) removed a small fraction, 1 ml/min, of the coronary effluent, and its oxygen concentration was monitored continuously by an in-line Clark-type electrode. The influent oxygen concentration was maintained at 956 μM. Coronary flow was measured by collecting the effluent from the right and left pulmonary arteries in a 10-ml graduated cylinder. Oxygen consumption was calculated from the product of the influent-effluent oxygen concentration difference and the coronary flow.

For studies in which animals were administered the radiopharmaceutical in vivo, 0.5 ml of blood was withdrawn from the donor animal at 1, 5, 15, 30 and 60 min postinjection of either teboroxime, sestamibi or ^{201}Tl. An equal volume of saline was infused into the animal via the femoral artery after each sampling of blood. An aliquot of the blood (0.25 ml) was immediately injected into a sidearm of the heart perfusion cannula. The remainder was used for analysis of the fractional distribution of radioactive material bound to blood components described below. Each donor animal received between 10 and 40 mCi in 0.1–0.35 ml of the respective vehicle.

Experiments in which blood was incubated with teboroxime in vitro were divided into three groups: whole blood, plasma, and erythrocytes (RBCs). In all cases, temperature was maintained at 37°C with a Dubnoff Metabolic Shaker Bath (Precision Scientific Inc., Chicago, IL). Teboroxime was added to obtain a final activity of 0.1 mCi per ml of blood or plasma. At designated intervals (refer to Figure legends and Table 1), an aliquot (0.1–0.15 ml) of either whole blood, plasma or resuspended RBCs was injected into the isolated perfused heart. For experiments using plasma, the cells were separated from whole blood by low-speed centrifugation (1,000 rpm for 5 min) and the plasma supernatant was used for incubation with teboroxime. For experiments using only RBCs, whole blood was incubated with teboroxime at 37°C. The cells were then separated at designated times by centrifugation and washed (3×) with ice cold saline to remove any remaining plasma and resuspended in saline (hematocrit = 45%).

Teboroxime (^{99m}Tc-Cl(CDO)$_3$MeB; SQ 30217, Bristol-Myers Squibb, NJ) was prepared in saline as described previously (4). Radiochemical purity of teboroxime was always greater than 92% as determined by HPLC (4). Technetium-99m-OH(CDO)$_3$MeB was prepared by alkalization (using chloride-free phosphate buffer, pH = 9.4) and heating (70° C for 20 min) of a reconstituted teboroxime kit. Radiochemical purity of the latter was 89%. Technetium-99m-(sestamibi)$_6^+$ was prepared and suspended in saline according to the procedures described previously (*5,6*). Radiochemical purity was greater than 97% as measured by HPLC using 65% acetonitrile/35% 0.1 *M* citric acid, pH 2.3, at a flow rate of 1.5 ml/min to elute the complex (retention time = 3.7 min). Thallium-201 was provided (Squibb Diagnostics) in saline.

Radioactivity in the perfused heart was detected by a collimated NaI crystal positioned 3–4 cm from the right ventricle and perpendicular to the vertical axis of the heart. The photocurrent was sent to a multichannel analyzer (TN-7200, Tracor Northern, Middleton, WI) in multiscalar mode with a dwell time of 500 msec/channel. The latter instrument digitized the signal and provided real time monitoring of the experiment. The digitized data (counts) were transferred via serial communication to a microcomputer (Dell Computer Corp. 286, Austin, TX). Extraction was calculated by using the "B/A" method described by Raichle and coworkers (7). The data were analyzed using a program developed with commercially available software (Excel, Microsoft Corp., Redmond, WA). This program subtracted the data obtained prior to injection, i.e., background or the monoexponential clearance of the previous injection, from the data acquired during the injection. After obtaining the peak or maximal level of radioactivity (A), this program extrapolated a value (B) from the monoexponential clearance for the injection of interest. The latter was synchronized in time with the "A" value. This method was chosen specifically because it has the advantage of providing direct on-line measurements of radioactivity in the heart and assumes that no metabolism of the experimental compounds takes place on the first pass through the heart.

Blood Fractionation Analysis

Whole blood and plasma samples were analyzed for the fraction of teboroxime bound to cells and proteins by internal surface reversed-phase chromatography. Rapid on-line separation of small hydrophobic compounds from blood cells and plasma proteins was performed using a 75-micron GFF (glycine-phenylalanine-phenylalanine) glass bead Internal Surface Reverse Phase (ISRP) column and a C_8 reversed-phase analytical column (*8*) interfaced with an on-line radioactivity detector. For the first 3 min, the ISRP column (4.6 × 50 mm; 75 micron; a gift from Regis Chemical Co., Morton Grove, IL) was eluted with 0.1 M Na citrate (pH 7.0) at 1 ml/min. Flow was then diverted from the ISRP in the C_8 reverse-phase column (Nucleosil; 5 micron, 4.6 × 250 mm, Alltech, Deerfield, IL) and the mobile phase was changed to acetonitrile: 0.1 *M* Na citrate (72:28) for 17 min. Cells and proteins were eluted in the void volume (0.6 min), whereas teboroxime had a retention time of 16.6 (chloro) and 16.9 (hydroxide) min, respectively.

Data Presentation

Unless stated otherwise, the data represent means ± s.e.m. Statistical analyses evaluating the differences in extraction among means obtained at 5, 15, and 60 min for whole blood, plasma and RBCs were performed by analysis of variance followed by a simple effects analysis and Tukey's α-HSD post-hoc analysis (*9*). The limits of confidence were established at the 0.05 level.

RESULTS

Extraction of Teboroxime in the Isolated Perfused Rat Heart

In order to ascertain that the "B/A" method was an appropriate one for measuring extraction in the isolated perfused rat heart, the effect of radiochemical purity on single-pass extraction was determined. Serial injections of pertechnetate, a possible impurity in the preparation of

teboroxime, and pertechnetate plus teboroxime were made into the perfused heart. The contribution of teboroxime in the injectate was made progressively larger with each injection. It can be seen in Figure 1 that the first injection, which was pertechnetate only, resulted in nominal extraction, less than 5%. As the amount of teboroxime in the injectate was increased, extraction rose in a step-wise manner. For each injection the values of extraction matched closely the amount of teboroxime in the injectate. In the absence of added pertechnetate, extraction of teboroxime was 100%. In separate experiments, a single injection of teboroxime resulted in values of extraction equal to 96% ± 1% (n = 5). These values were not altered upon subsequent injections into the same hearts. Moreover, extraction of teboroxime was not affected by the amount of time that elapsed between preparation of the kit and injection, at least up to 24 hr post-preparation (data not shown). The results above indicate that teboroxime was highly extracted in the isolated perfused rat heart and that the "B/A" method was useful for detecting relative changes in extraction.

Technetium-99m-OH(CDO)$_3$MeB (RCP = 89%), a metabolite of teboroxime, was prepared and its extraction determined. Extraction of this compound was 43% ± 4% (n = 3). When teboroxime was injected into a heart that had received previously ^{99m}TcOH(CDO)$_3$MeB, extraction of the latter was 91% (n = 1). These results indicate that the principal degradation product (^{99m}TcOH(CDO)$_3$MeB) of teboroxime is well extracted but to a lesser extent than its parent compound, ^{99m}Tc-Cl(CDO)$_3$MeB.

The extraction of sestamibi and ^{201}Tl was also measured for comparison to that of teboroxime to determine whether the high level of extraction of teboroxime was peculiar to this compound in the isolated perfused rat heart. Extraction of sestamibi (RCP > 95%) was 15% ± 1% (n = 3) and of ^{201}Tl was 30% ± 5% (n = 2). These values were two- to three-fold less than those of teboroxime (for comparison, see Fig. 2). It should be noted that the levels of coronary flow were similar for all hearts, suggesting that the differences in single-pass extraction, assuming other parameters such as surface area remained constant, were a function of the molecular properties of each respective agent.

Effect of Time in Circulation on Extraction of Teboroxime

It was of interest to determine whether the extraction of teboroxime, measured in vitro, was altered by its duration in circulation, in vivo. After administration of teboroxime to a blood donor animal, an aliquot of arterial blood was withdrawn at 1, 5, 15, 30, and 60 min postinjection. This blood was then injected immediately into the aortic cannula of the isolated perfused rat heart. Figure 3 shows that the level of extraction after 1 min in circulation was comparable to that above, measured in the absence of blood, i.e., nearly 100%. Thereafter, extraction of teboroxime declined progressively. After 5 min in circulation, extraction of teboroxime had diminished by more than 40% from initial levels and by 60 min, extraction had declined to about 20%.

Similar experiments in which either sestamibi or ^{201}Tl was substituted for teboroxime showed that extraction of the former radiopharmaceuticals was not altered throughout the 60-min evaluation period. Moreover, these values were not affected by the presence of blood. The levels of extraction remained at about 22% and 30% for sestamibi and ^{201}Tl, respectively.

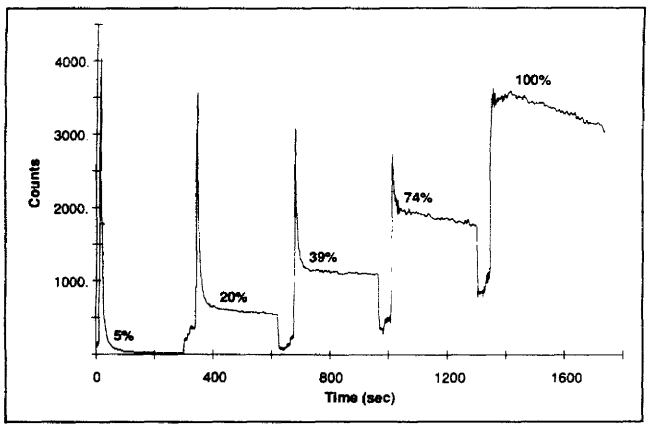

FIGURE 1. Effect of radiochemical purity on extraction of teboroxime. Serial bolus injections of solutions containing teboroxime, either 0%, 17%, 37%, 61%, or 92% ^{99m}Tc-Cl(CDO)$_3$MeB, respectively, balanced with [^{99m}Tc]pertechnetate, were made into the aortic cannula of the perfused rat hearts. In this representative example of four hearts, the data are produced from a continuous scan in which single exponential clearance of the previous injection was subtracted from each succeeding injection. Values of extraction are shown adjacent to each peak. Hearts were perfused as described in Methods.

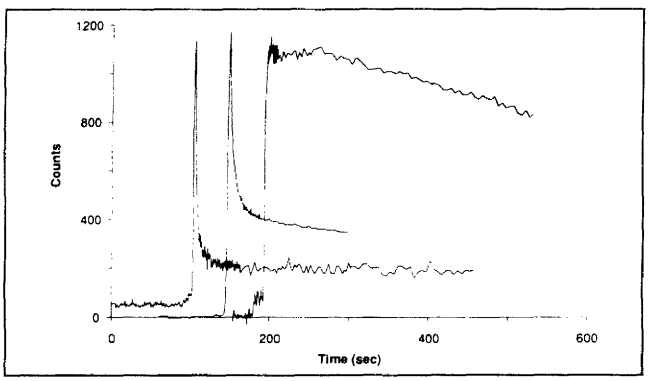

FIGURE 2. Comparison of extraction of teboroxime, ^{201}Tl, and sestamibi in the isolated perfused rat hearts. Hearts received bolus injections of either teboroxime, ^{201}Tl, or sestamibi. The data for teboroxime (third trace from left) was taken from the last injection of Figure 1 (extraction=100%) but is typical of extraction that results without prior exposure of the heart to this compound. Extraction of ^{201}Tl (second trace from left) was 36% and that of sestamibi (first trace from left) was 16%. This figure is a composite of three individual experiments using each respective agent, thus, the scales of the axes have been adjusted to accommodate all three experiments.

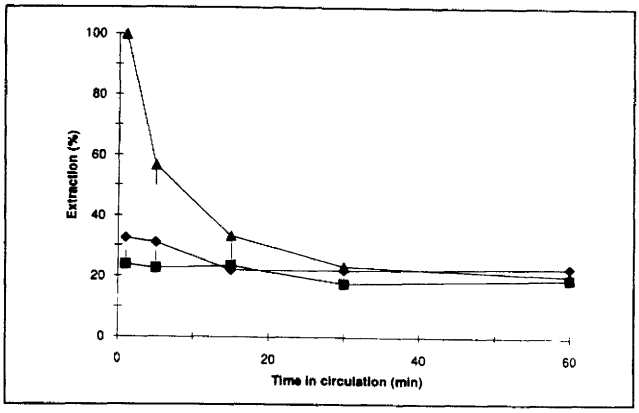

FIGURE 3. Effect of time in circulation on extraction of teboroxime, ^{201}Tl, and sestamibi in the isolated perfused rat heart. Values represent mean ± s.e.m. Animals received a bolus injection of either ^{99m}Tc-teboroxime (n = 4, ▲), ^{99m}Tc-sestamibi (n = 4, ■), or ^{201}Tl (n = 2, no s.e.m., ♦). At 1, 5, 15, 30, and 60 min, heparinized blood (0.25 ml) was withdrawn from the femoral artery and injected immediately into the aortic cannula of the perfused heart.

Figure 4 shows that myocardial oxygen consumption and coronary flow in the perfused hearts remained unaltered during the experimental period despite repeated injections of blood. The levels of coronary flow remained at about 10 ml/min/g wet wt in the teboroxime group and about 8 ml/min/g wet wt in the sestamibi group. Moreover, there were no differences in the levels of oxygen consumption in hearts used for data acquisition for teboroxime or sestamibi. In both cases, oxygen consumption was stable at about 4 μmol/min/g wet wt. The data from hearts injected with blood containing ^{201}Tl are not shown for clarity of presentation, but were similar to those for

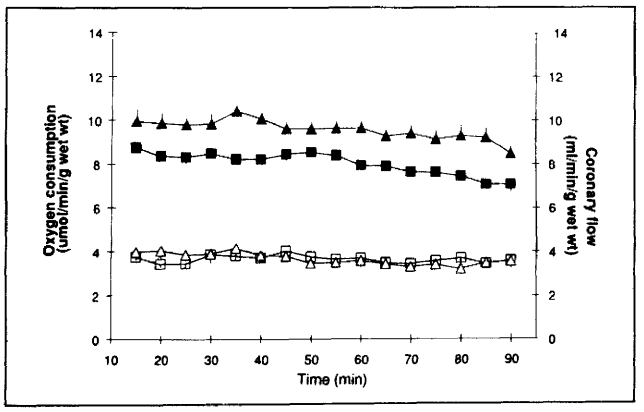

FIGURE 4. Stability of oxygen consumption and coronary flow in the perfused heart after receiving multiple injections of whole blood. The hearts were permitted to stabilize to the isolated state for 25 min before receiving the first bolus of blood (0.25 ml) containing either ^{99m}Tc-teboroxime (n = 4, ▲) or ^{99m}Tc-sestamibi (n = 4, ■). The darkened triangles and squares correspond to values of coronary flow (right axis) and the open triangles and squares correspond to values of oxygen consumption (left axis). All values represent mean ± s.e.m. (the latter is often obscured due to very low levels of variance).

teboroxime and sestamibi, i.e., oxygen consumption was about 3.6 μmol/min/g wet wt and flow was about 7.8 ml/min/g wet wt throughout the experiments.

Metabolism and Extraction of Teboroxime

The results presented above indicated that, unlike sestamibi and ^{201}Tl, teboroxime underwent some process that altered the level of extraction after the first minutes of its administration in vivo. One possible explanation for this change was that plasma levels of ^{99m}Tc-Cl(CDO)$_3$MEB gradually decreased by conversion to ^{99m}TcOH-(CDO)$_3$MEB. Alternatively, as the duration of time that teboroxime was in circulation lengthened, the amount of binding between teboroxime and plasma proteins or blood cells became greater. Thus, in experiments similar to those above, myocardial extraction and metabolism of teboroxime in blood were determined in the same sets of samples.

It can be seen in Table 1 that administration of teboroxime in vivo resulted in a time-dependent decrease in the level of free teboroxime in arterial blood. For example, at 5 min postinjection, free teboroxime was 34% and by 60 min postinjection the level had declined to only 2%. At 5 min postinjection, most of this free fraction was in the form of ^{99m}Tc-Cl(CDO)$_3$MEB. By 60 min, the level of free ^{99m}Tc-Cl(CDO)$_3$MEB was nearly zero. The level of extraction, however, was markedly greater than the amount of free teboroxime (Cl plus OH) at all measured times. Moreover, Figure 5 shows that at 60 min about 20% of the teboroxime in circulation was extracted despite the lack of availability of the free compound.

Further experiments were conducted to determine whether the bound material that was extracted by the perfused heart in the nominal absence of free teboroxime arose from binding either on cells or proteins in blood. In these experiments, teboroxime was incubated in vitro with either whole blood, plasma, or with erythrocytes isolated from labeled blood. In the latter case, the plasma was discarded and the washed erythrocytes were resuspended in saline to approximate a hematocrit of 45%. At designated times, an aliquot of the incubated material was injected into the perfused rat heart and extraction was measured. Simultaneously, HPLC analysis was performed to determine the percent of teboroxime that was free. As compared to the administration of teboroxime in vivo, incubation of teboroxime with whole blood in vitro resulted in a large percent of the compound remaining free in the blood throughout the 60-min experimental period. For example, after 5 min of incubation, 94% of the compound was free and, after 60 min, this value decreased to only 65% (Table 1). These differences between the in vivo and in vitro conditions were likely due, at least in part, to the lack of clearance mechanisms available in the latter experiments. Within the free fraction of the teboroxime mixed with blood in vitro, the percentage of ^{99m}Tc-Cl-(CDO)$_3$MEB decreased progressively from 77% to 17% as the level of ^{99m}TcOH(CDO)$_3$MEB rose from 16% to 46% between 5 and 60 min, respectively. Similar findings re-

TABLE 1
Time Dependence of Extraction and Metabolism of Teboroxime

Condition	Time (min)											
	5				15				60			
	E	Free	Cl^-	OH^-	E	Free	Cl^-	OH^-	E	Free	Cl^-	OH^-
Blood, in vivo, (n = 4)	44 ± 7	34 ± 4	25 ± 2	9 ± 2	28 ± 5	13 ± 2	7 ± 1	6 ± 2	19 ± 3	2 ± 1	0.4 ± 0.4	2 ± 1
Blood, in vitro, (n = 3)	87 ± 2	94 ± 1	77 ± 1	16 ± 1	79 ± 2	89 ± 2	59 ± 2	28 ± 1	61 ± 6	65 ± 4	17 ± 1	46 ± 2
Plasma, in vitro (n = 3)	98 ± 1	94 ± 1	89 ± 3	6 ± 4	87 ± 3	78 ± 5	51 ± 12	28 ± 7	60 ± 2	64 ± 2	7 ± 2	57 ± 1
RBCs, in vitro (n = 3)	72 ± 3	98 ± 2	62 ± 2	35 ± 0.3	53 ± 3	91 ± 4	42 ± 2	48 ± 4	24 ± 3	56 ± 2	9 ± 1	46 ± 1

Values represent mean ± s.e.m. and are expressed as a percent of total radioactivity. E = extraction, Free = unbound fraction of total radioactivity, Cl^- = free ^{99m}Tc-Cl(CDO)$_3$MeB, and OH^- = free ^{99m}TcOH(CDO)$_3$MeB, RBCs = red blood cells. Temperature of the animals and the incubation bath were maintained at 37°C for both in vivo and in vitro experiments.

sulted when teboroxime was incubated with plasma. In the latter case, the level of free teboroxime decreased from 94% to 64% between 5 and 60 min of incubation as free ^{99m}TcOH(CDO)$_3$MEB increased from 6% to 57%. For both whole blood and plasma, the level of teboroxime extraction closely approximated the amount of free compound and, in both cases, a good correlation was found between these two parameters (r = 0.88 and 0.92 for whole blood and plasma, respectively; Fig. 6A). Moreover, for both conditions, extrapolation of these data to zero percent free teboroxime indicated that a portion of the bound teboroxime would have been extracted.

When teboroxime was incubated with blood and the erythrocytes isolated, four salient findings resulted from injection of these cells into the perfused heart. First, although the level of total free teboroxime decreased from 98% to 56% between 5 and 60 min of incubation, free ^{99m}TcOH(CDO)$_3$MEB remained relatively constant, at about 40% (Table 1). Second, the percent of free teboroxime exceeded, by far, that of extraction (Table 1 and Fig. 6A). For example, at 60 min of incubation, 56% of the teboroxime was free but only 24% was extracted, more than a two-fold difference. It should also be noted that these levels of extraction, at all time points, were significantly less ($p < 0.01$) than their counterparts obtained with whole blood or plasma. Lastly, Figure 6B shows that extrapolation of the regression equation resulting from these data produced a negative value for the y-intercept, −27. On the other hand, the y-intercept generated from the data on free ^{99m}Tc-Cl(CDO)$_3$MEB and extraction provided a positive value, 16. The data above suggested that TEBO bound to erythrocytes was extracted less than that bound to plasma proteins and was most likely extracted as the chloro form.

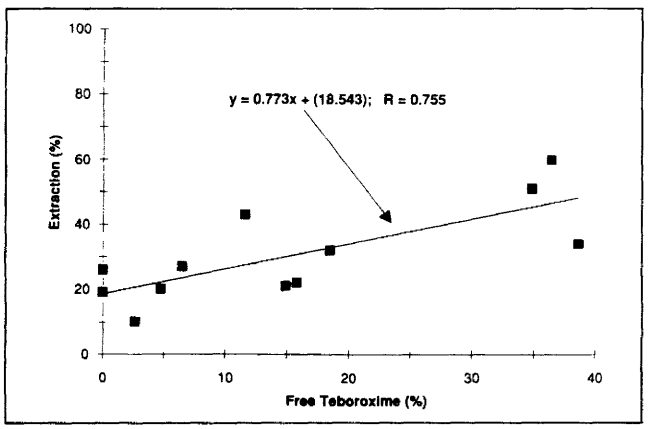

FIGURE 5. Effect of whole blood plus free teboroxime, in vivo, on extraction of teboroxime in the isolated perfused heart. Aliquots (0.50 ml) of whole blood were withdrawn from the femoral artery of rats at 5, 15, and 60 min postinjection of teboroxime. Individual values are plotted for the three intervals of time. As time progressed, the measured amount of free teboroxime decreased. The sample was injected partially (0.25 ml) into the aortic cannula of the perfused heart and the remainder was analyzed by HPLC for free and bound fractions of teboroxime.

DISCUSSION

The purpose of this study was two-fold. First, to determine the single-pass extraction of the myocardial perfusion agent, teboroxime, and for comparison, two other radiopharmaceuticals available currently, ^{201}Tl and sestamibi. Second, to determine whether extraction, after the first pass through the coronary circulation, was affected by the duration these agents spent in circulation in vivo. It should be noted that this evaluation was carried out using the non-recirculating Langendorff rat heart preparation. The non-recirculating Langendorff preparation is an appropriate one for studies of single-pass extraction because it maintains perfusion pressure constant, thereby allowing the tone of the coronary vasculature to adjust to the prevailing rate of cellular respiration as occurs in vivo. Changes in perfusion pressure have been shown to affect the amount of surface area available for extraction (*10*).

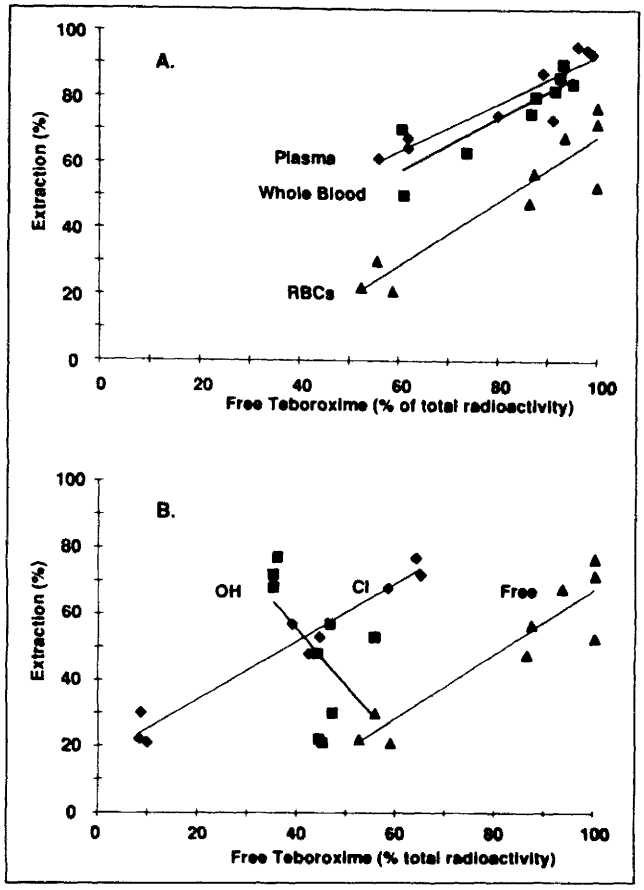

FIGURE 6. Effect of free teboroxime plus blood or its constituents, in vitro, on extraction of teboroxime in the isolated perfused heart. In (A), extraction of teboroxime is plotted as a function of the free fraction of total radioactivity in plasma (♦), whole blood (■), and red blood cells (RBCs, ▲). In (B), the data obtained using RBCs are separated into the Cl^- (▲) and OH^- (■) forms of teboroxime as determined by HPLC. Total free (sum of Cl^- and OH^-) is provided as a reference. For both (A) and (B), the percentage of free radioactivity or that in the OH^- form, was altered by the time of incubation, 5, 15, or 60 min at 37°C. In each case the independent values (n = 3 hearts for each condition) are plotted with the respective regression line.

By pacing the heart, cardiac metabolism, which is responsive to changes in perfusion pressure (*11*), is also unchanged for considerable periods of time during the experiments (*2,3*, and this report). The influence of these extraneous variables was diminished as much as possible and therefore the chemical and molecular properties of the respective radiopharmaceuticals determined their extraction characteristics. Using this preparation, the main findings of the present investigation were: (1) single-pass extraction of teboroxime was high and greater than that of ^{201}Tl or sestamibi; (2) only the extraction of teboroxime was affected by its duration in circulation, decreasing rapidly and progressively as time was increased; and (3) the latter finding was due, at least in part, to a time-dependent increase in the affinity of teboroxime for blood cells and plasma proteins.

Single-pass extraction of teboroxime was shown to be very high in the present study. By substituting the chloro with a hydroxyl group on the technetium atom of teboroxime, extraction decreased by about 50%. This small change in molecular structure decreases the lipophilicity of teboroxime measured as a partition coefficient, i.e., log P of 4.6 (chloro) to 3.96 (hydroxide) without substantially altering its charge or size (*1*). These results suggest that the lipophilic nature of teboroxime serves as a primary mechanism for its extraction. Teboroxime is a large compound, as compared to ^{201}Tl with a molecular weight of 584 and molecular dimensions of 11.6 × 8.1 × 9.3 μm. It is conceivable that the lipophilic nature of this compound promotes its transcapillary transport by partitioning within the lipid domains of the plasma membrane of capillary endothelial cells. Because the permeability coefficient of any diffusing substance is inversely related to the square root of its molecular weight (Fick's Law), the molecular dimensions of teboroxime may impede its diffusion through the lipid bilayer of cell membranes and possibly through interendothelial junctions. It has been suggested from studies on the interaction of BATO compounds, which includes teboroxime, with liposomes that BATOs may be hydrogen bonded to membranes through their oximes of the BATO or nonspecifically adsorbed to the hydrophilic headgroups of the membranes (*12*). On the other hand, it is clear from the present results that teboroxime has an affinity for proteins. The plasma membrane of many cells types contains numerous proteins. For example, erythrocyte ghosts have been reported to be primarily composed of protein (*13*). Thus, proteins in cell membranes could serve as binding sites for teboroxime and provide a possible mechanism for its extraction. The rapid clearance of teboroxime from the heart, i.e., within a few minutes of its extraction (*4*), supports both of these hypotheses regarding the weak interaction of teboroxime with membranes and their proteins.

Extraction of teboroxime was also found to be markedly greater than that of either ^{201}Tl or sestamibi, i.e., nearly 100% compared to 30% and less than 15%, respectively. This trend compares favorably to that obtained previously using a preparation of the isolated perfused rabbit heart (*14,15*). In the latter investigations, a range of values of coronary flow (0.3 to 3.2 ml/min/g wet wt) was used to evaluate extraction. These results also showed that extraction of teboroxime was greater than that of ^{201}Tl (*15*) and extraction of ^{201}Tl was found to exceed that of sestamibi (*14*). Although the relative findings were similar to those found in the present study, the absolute values of extraction were markedly different. For example, at a normal flow value for the blood perfused rabbit heart, 1 ml/min/g wet wt, extraction of teboroxime was about 75% and at a higher flow, 2.5 ml/min/g wet wt, extraction was decreased to nearly 50% (*15*). Several differences existed between the experimental methods used in the investigations by Leppo and coworkers and the present one, which

might explain the divergent values for extraction. In the former study, the hearts were perfused with blood via a perfusion pump to maintain levels of flow constant and extraction was measured using indicator-dilution techniques. Exposure of teboroxime to blood could have lowered extraction via binding to blood cells and protein. On the other hand, the "B/A" method, which was used to measure extraction in the present study, has been criticized for its sensitivity (*16*). This method was used originally by Raichle and coworkers (*7*) to determine extraction of isotope using an in vivo paradigm. In this case, recirculation of radiopharmaceutical would interfere with the accuracy of the "B" value. To avoid this type of problem, the present study used a single-pass mode for perfusing the coronary circulation. Moreover, the results shown in Figure 1 indicate that the "B/A" method was sufficiently sensitive to detect changes in radiochemical purity, at least for teboroxime, and served as an effective means of evaluating extraction. Clearly, other methodological aspects such as species differences might have contributed to the variation in the values of extraction provided by the studies of Leppo and coworkers and the present investigation. Despite these methodological differences, similar findings resulted for experimental conditions that were established to mimic the first pass through the coronary circulation.

The present study also examined events that followed the first pass by sampling arterial blood from animals previously administered either of the three radiopharmaceuticals and injecting this blood into the perfused heart. These experiments were designed to establish whether the agents were in any way modified in circulation, thereby affecting the likelihood of each for continued extraction after the first pass through the coronary circulation. It has been reported, for example, that ^{201}Tl activity continues to rise for up to 40 min postinjection in normally perfused myocardium and may be prolonged in ischemic areas despite very low blood activity (*17*). This type of phenomenon could, at least in part, contribute to the "filling in" of a defect obtained in delayed scintigraphic images which had been observed in previous images taken during an exercise protocol. Our results indicated that the level of extraction of ^{201}Tl and sestamibi measured at 60 min postinjection was essentially the same as that for the first pass. Thus, for these two radiopharmaceuticals, extraction would be expected to continue, dependent upon the blood activity and level of coronary flow. By contrast, extraction of teboroxime declined exponentially after one minute in circulation. The level of extraction continued to decline until 30 min postinjection, at which time, no further change occurred. By this time, extraction was about one-fifth of that for the first pass. These results suggest that after initial passes through the coronary circulation continued uptake of teboroxime becomes progressively unlikely. Although the present experiments did not evaluate extraction in ischemic hearts, the changes which brought about altered levels of extraction in normally perfused ones were due to factors external to the myocardium and, therefore, these results further suggest that delayed uptake would not be likely in ischemic areas of the heart.

The decline in extraction of teboroxime appeared to be due largely to a time-dependent increase in the affinity for binding of teboroxime to blood constituents, i.e., erythrocytes and proteins. When teboroxime was administered in vivo, less than 35% of the total teboroxime in circulation was free at 5 min postinjection and this value decreased to about 2% at 60 min. If extraction was dependent solely on the free levels of teboroxime, the level of extraction should be equivalent to the amount of teboroxime free in circulation. For example, if measured free teboroxime equalled 20%, then complete extraction of this radioactivity would also equal 20%. Extraction of teboroxime contained in arterial blood was greater, however, than the percentage of teboroxime free in circulation at all times postinjection. This was most evident in Figure 5, which showed that about 20% of the teboroxime in the aliquot of blood was extracted even though nearly all of the teboroxime was bound. Although the exposure of teboroxime to blood promotes its conversion from the chloro to the hydroxide form, as demonstrated by the experiments conducted in vitro, this change would not account totally for the temporal decrease in extraction. Thus, our results suggest that interactions within the vascular lumen serve to dissociate teboroxime from the blood constituents in exchange for binding to myocardial tissue. The portion of teboroxime that was extracted may have been either loosely bound to plasma proteins, blood cells or both. It was not possible, however, to determine from the experiments in which teboroxime was administered in vivo whether this extractable material was derived from blood cells or plasma proteins.

When teboroxime was incubated in vitro with whole blood or its primary constituents, the amount of teboroxime that remained free was consistently greater than the levels obtained from the general circulation. This difference was most likely due to lack of clearance mechanisms for the in vitro experiments that existed in vivo. Much of the free teboroxime was likely cleared from the circulation via the liver (*4*). For the in vitro cases using whole blood or plasma, the percent of teboroxime measured as free was closely linked to the amount of extraction. For example, at 60 min of incubation of teboroxime with plasma, the level of free compound was 64% and extraction equalled 60%. On the other hand, when the labeled erythrocyte suspension served as the injectate, the level of extraction was about half of the measured amount of free teboroxime and the extraction of teboroxime from this suspension was significantly less than that obtained with plasma or whole blood. These differences resulted despite the finding that the percentage of free teboroxime was similar at all time points for the three in vitro conditions. The latter findings indicate that the binding affinity of teboroxime was greater for blood cells than for plasma proteins. It would appear

therefore that the small percentage of extraction which resulted in vivo despite an apparent lack of available or free teboroxime in circulation (Fig. 5) arises primarily from that fraction bound to plasma proteins.

SUMMARY

The use of myocardial perfusion agents for the detection of areas of reduced blood flow using SPECT or planar technology is dependent, at least in part, upon the single-pass extraction of the respective probe compound. To this end, teboroxime, as shown by our results, provides a high level of extraction in vitro as compared to ^{201}Tl or sestamibi. Because teboroxime clears rapidly from the heart (*4*), teboroxime cannot be labeled as a "molecular microsphere" (*18*). The clearance of teboroxime from the blood and heart (*4*), however, offers the clinician the advantage of obtaining stress/rest scintigraphic images within a relatively short time frame. Moreover, the blood cell and plasma-protein binding characteristics of teboroxime shown in the present study would likely suppress the continued extraction of teboroxime from the first injection during acquisition of delayed images.

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