Radionuclide Analysis of Drug-Induced Blood-Pool Changes in Liver and Other Organs

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Although it is possible to repopulate the animal liver with transplanted hepatocytes, the success of such transplants depends, in part, on the number of transplanted cells that enter the hepatic sinusoids. Pharmacologic alteration of hepatic vascular tone, and hence blood volume, can increase the number of cells that are successfully transplanted. Although analysis of changes in vascular beds is helpful for developing strategies for cell transplantation, convenient methods to analyze such changes are lacking. The objective of this study was to determine whether ^{99m}Tc-labeled red blood cells could be used to reveal pharmacologically induced blood pool changes in various organs. **Methods:** F344 rats were injected with syngeneic labeled red blood cells and subjected to blood pool analysis with γ camera imaging. Animals were treated with phenylephrine, phentolamine, labetalol, and nitroglycerine. To correlate hepatic blood pool changes with structural alterations at the vascular level, microspheres were injected into the portal circulation of these animals. **Results:** Phenylephrine significantly increased cardiac and pulmonary blood pools, findings in agreement with its α-adrenergic effects. Phentolamine increased the hepatic, splenic, and pulmonary blood pools, whereas labetalol increased only the pulmonary blood pool. Nitroglycerine increased both hepatic and splenic blood pools. Prior administration of phentolamine, labetalol, and nitroglycerine prevented the phenylephrine-induced changes. When microspheres were injected into the portal circulation after nitroglycerine administration, they penetrated more distal locations in the liver lobule. **Conclusion:** These data indicate that it is possible, using radionuclide methods, to noninvasively show pharmacologically induced hemodynamic changes. This finding is potentially useful for studying hepatic physiology and may also have applications for cell therapy.

Key Words: ^{99m}Tc; liver; vascular; drug; microsphere

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The ability to repopulate the liver has important implications for the development of novel therapies for disorders such as acute liver failure, metabolic deficiency states, and genetic diseases (*1*). Recent work has shown that the liver of animals can be repopulated with transplanted hepatocytes (*2–5*). The function of transplanted cells is most optimally preserved in the liver itself compared with ectopic organs (*6*). Deposition of cells in the hepatic sinusoids leads to permanent cell engraftment and survival (*2,7*). Hepatocyte deposition in sinusoids is critical for engraftment because transplanted cells can enter and integrate in liver plates after disruptions in the sinusoidal endothelium, whereas cells retained in proximal portal spaces are destroyed by a phagocytic reaction (*7*). Size–structure relationships between transplanted cells and hepatic sinusoids determine the cell fraction that enters liver sinusoids (*2*). Altering the tone of the hepatic vascular bed and hence changing the hepatic blood volume would permit consideration of novel strategies for improving entry of the cells into distal sinusoids. However, simple methods of assessing such alterations are lacking. Although indicator dilution techniques provide useful information about hepatic blood volume, these methods are cumbersome and require complex mathematic modeling for analysis (*8*). Moreover, application of these methods in the intact animal is generally difficult. Simple, effective, and reproducible methods to analyze changes in hepatic blood volume are desirable.

We hypothesized that analysis of organ-specific blood pools (volumes) would permit demonstration of changes after pharmacologic interventions. To test our hypothesis, we conducted studies in F344 rats, in which syngeneic ^{99m}Tc-labeled red blood cells (RBCs) were injected intravenously. In view of the relatively small magnitude of the resting tone in vascular beds with low-flow states such as the portal circulation (*9*), we used a provocative test to elucidate whether the vascular tone had in fact been altered. Because our major interest concerned vasodilatory mechanisms, we began our studies with phenylephrine, an α-adrenergic agonist (*10*). Administration of a vasoconstrictor after treatment with a vasodilator would confirm the efficacy of vasodilatory treatments. Such a system would permit testing of the effects of various drugs on the liver sinusoidal and other vascular beds. Finally, we correlated hepatic sinusoidal changes predicted by the radionuclide method with histologic analysis.

MATERIALS AND METHODS

Animals

Male F344 rats weighing 150–180 g were obtained commercially (Taconic Farms, Germantown, NY). The animals were

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housed under 14-h light/10-h dark cycles with unrestricted access to pelleted food and water. All animal procedures were performed under pentobarbital anesthesia. Whole blood was collected from donor F344 rats by cardiac puncture. To administer RBCs and various drugs, the ventral tail vein was catheterized with a 21-gauge butterfly needle and flushed with normal saline in a heparinized syringe. RBCs were injected rapidly over 5–10 s in a 0.5-mL volume followed by flushing of the catheter with 0.5 mL normal saline. To inject microspheres into a tributary of the superior mesenteric vein, animals were subjected to a midline laparotomy. The microspheres, injected after initiation of nitroglycerine infusion, were suspended in 1 mL normal saline and injected rapidly over 10 s. After securing hemostasis, the skin was closed with surgical sutures and the animals were monitored until recovery from anesthesia.

Experimental groups were composed of 3 or 4 animals, which were killed at the end of each experiment. Animal use was in accordance with the *Guide for the Care and Use of Laboratory Animals* (*11*) and institutional guidelines. The institutional animal care and use committees approved the protocols.

Radiolabeling, Imaging, and Data Analysis

RBCs were collected in heparin and labeled in vitro using a commercially available kit (UltraTag RBC kit; Mallinckrodt, Inc., St. Louis, MO) according to the manufacturer's instructions. For this purpose, 3 mL whole blood in a 1-mL mixture of acid, citrate, and dextrose were incubated with 555 MBq ^{99m}Tc in 1 mL normal saline for 30 min at room temperature. The tagging efficiency exceeded 98% in all cases.

Each rat was administered 37–111 MBq RBCs within 1 h of labeling, and animals were allowed 30 min for equilibration before use. A γ camera interfaced with a computer and equipped with a low-energy, high-resolution, parallel-hole collimator was used for image acquisition. Energy discrimination was accomplished with a 20% window centered on the 140-keV photopeak of ^{99m}Tc. Dorsal images were acquired at the rate of 1 frame/s for 15 min using a 128 × 128 × 16 matrix. Each acquisition consisted of a 5-min baseline segment, another 5-min segment after injection of drug 1, and a final 5-min segment after injection of drug 2. Time–activity curves were generated over the liver, heart, spleen, kidneys, and lungs. A minimum of the 3 highest or lowest points in time–activity curves, depending on organ-specific responses in each segment, was analyzed in all animals and compared for differences.

Drug Testing

Phentolamine (Regitine; CIBA Pharmaceutical Co., Summit, NJ), an α-adrenergic antagonist; nitroglycerine, a directly acting vasodilator; phenylephrine (Sanofi Winthrop Pharmaceuticals, Inc., New York, NY), an α-adrenergic agonist; and labetalol (Trandate; Allen & Hanburys, Inc., Research Triangle Park, NC), an antagonist of both α- and β-adrenergic receptors, were used. The working dilutions of the drugs were prepared in normal saline. Nitroglycerine was infused into the tail vein using a Harvard syringe infusion pump (Harvard Apparatus, Inc., Holliston, MA), whereas all other drugs were injected manually as a bolus. Phenylephrine was administered at 20 μg/kg, typically 4 μg/0.4 mL normal saline. Phentolamine was given at 0.25 mg/kg, typically 50 μg/0.1 mL normal saline. Labetalol was given at 3 mg/kg, typically 625 μg/0.125 mL normal saline. Nitroglycerin was infused at 6 μg/h in normal saline.

Injection of Latex Microspheres and Biodistribution Analysis

To determine whether changes in hepatic vascular tone were reflected in the distribution of microsphere beads, studies were conducted with microspheres with a mean size of 15.5 ± 0.1 μm. Beads (2×10^6) were injected into the portal vein through a tributary of the superior mesenteric vein. Two groups of 3 rats each were studied. The control group received beads alone, whereas the test group received a nitroglycerine infusion (6 μg/h) for 10 min through the tail vein before injection of microspheres.

Histologic Analysis

Hepatic samples were obtained and frozen to −70°C in methylbutane using OCT resin (Miles Inc., Elkhart, IN). Cryostat sections of 5-μm thickness were prepared and fixed in absolute ethanol and then stained with hematoxylin and eosin. The distribution of microspheres was determined by morphometric analysis of at least 50 consecutive portal areas. The number of beads in each portal area was determined, and the proportion of beads distributed within and outside of portal areas was determined.

Statistical Analysis

The data are expressed as mean ± SD, and the significance of differences was analyzed with the t test, the Mann-Whitney rank sum test, or the χ^2 test as appropriate. Statistical analysis was with the SigmaStat software (Jandel Scientific, San Rafael, CA). $P < 0.05$ was considered significant.

RESULTS

Establishment of a Provocative Test

Phenylephrine administration was associated with prompt hemodynamic alterations, resulting in a significant (25% ± 13%, $P < 0.05$, Mann-Whitney rank sum test) increase in the cardiac blood pool virtually instantaneously. The blood pool returned to baseline over approximately 10 min, in accordance with predictions based on the half-life of the drug. In control animals that received an equal volume of normal saline alone, no changes in the cardiac blood pool were observed (Fig. 1).

Phenylephrine also significantly increased the pulmonary blood pool (15% ± 14%, $P < 0.05$, Mann-Whitney rank sum test). The hepatic blood pool increased, although this increase was relatively less pronounced. There was a trend toward decreased renal blood pool, although this did not reach statistical significance. No change in the splenic blood pool was found (Fig. 2).

Effects of Vasodilatory Drugs on Blood Pools

We next tested whether the hepatic blood pool could be increased with phentolamine, labetalol, and nitroglycerine. In response to phentolamine, hepatic, splenic, and pulmonary blood pools increased significantly. The increase in pulmonary blood pool was particularly prominent. However, no changes in cardiac or renal blood pools were found. The effects of nitroglycerine on the hepatic and splenic blood pools were similar to those of phentolamine. The increase in the pulmonary blood pool after phentolamine administration

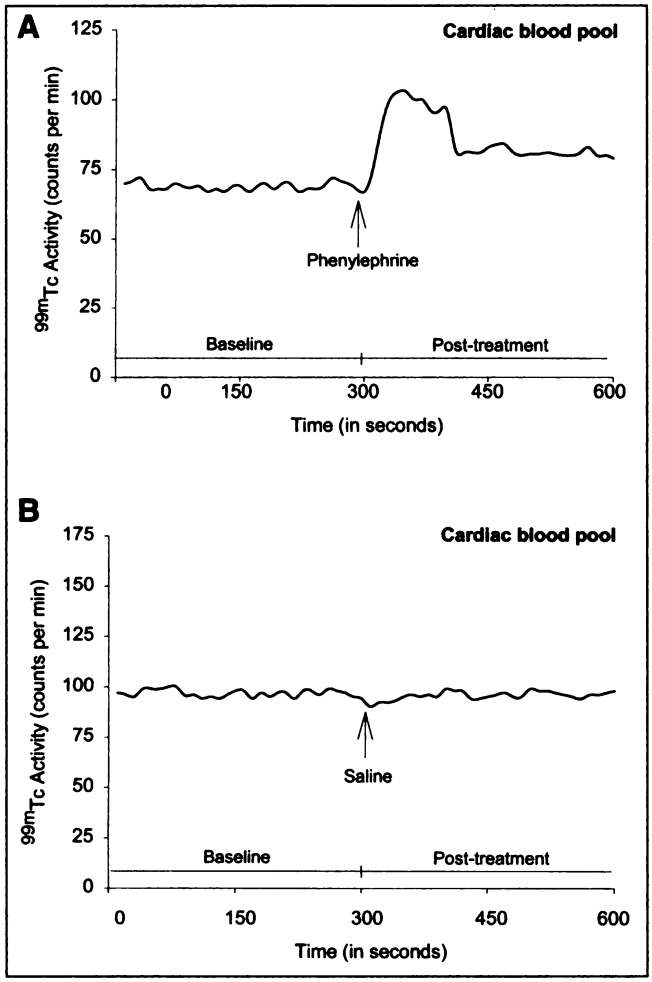

FIGURE 1. (A) Time–activity curve from representative animal shows effect of phenylephrine on cardiac blood pool. After 5-min baseline period, 4 μg phenylephrine was administered as intravenous bolus, which resulted in instantaneous increase in cardiac blood pool with trend toward resolution shortly thereafter. (B) Time–activity curve from control animal shows that injection of saline alone did not produce any change in cardiac blood pool.

was greater than the increase produced by nitroglycerine, but this was not statistically significant. Labetalol increased pulmonary blood pool but had no significant effect on other organ blood pools, including that of the liver (Fig. 3).

Blood pool changes observed after phenylephrine alone were not observed in animals pretreated with phentolamine, nitroglycerine, or labetalol (Fig. 4). There were no significant changes in hepatic, cardiac, splenic, renal, or pulmonary blood pools in animals given phenylephrine after administration of these other agents.

Effect of Vasodilation on Structure–Function Relationships in Hepatic Sinusoids

Transplanted cells enter the liver lobules through the portal vein, with their distribution in the liver lobule being regulated by the disparity between the size of the cell and the size of vascular spaces in the liver (*2*). If the increased blood pool was associated with alteration of the size of the hepatic sinusoids by vasodilatory mechanisms, it should be possible to observe this at the morphologic level. If the dimensions of hepatic sinusoids increased after the administration of vasodilators, injected particles would penetrate into more distal areas of the liver lobule.

The microspheres were injected into 2 groups of animals, 1 of which was treated with nitroglycerine infusion immediately before injection of microspheres. Histologic analysis showed that, although microspheres were distributed in portal spaces in all recipients, in the nitroglycerine-treated animals, microspheres were distributed more distally in the liver lobule. Morphometric analysis showed that the number of portal areas containing the microspheres increased from 60% in control animals to 74% in nitroglycerine-treated animals ($P < 0.01$, χ^2 test with Yates' correction). In control animals, 55% ± 39% of the microspheres were inside portal areas and 45% ± 39% of the microspheres were distal to the portal areas. In contrast, in animals pretreated with nitroglycerine, this pattern was significantly altered, with 12% ± 25% of the microspheres inside portal areas and 88% ± 25% of the microspheres distal to the portal areas ($P < 0.01$, Mann-Whitney rank sum test) (Fig. 5).

DISCUSSION

These data indicate that radionuclide blood pool analysis may be a useful noninvasive method for showing vascular changes in relatively inaccessible organs, such as the liver. The use of phenylephrine as a provocative test was helpful in documenting pharmacologic effects in animals. As an α-agonist, phenylephrine induces arteriolar and venous vasoconstriction, with major effects on cutaneous, skeletal muscle, renal, and splanchnic blood vessels and less prominent effects on the coronary and the pulmonary circulations (*12*).

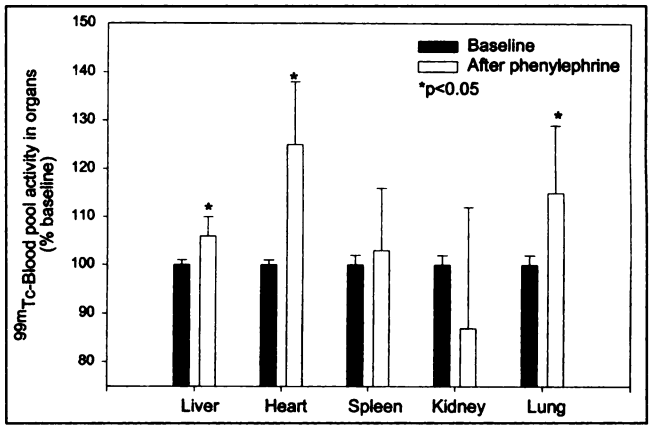

FIGURE 2. Alterations in organ-specific blood pools in response to phenylephrine. Data show profile of blood pool changes in various organs before and after phenylephrine treatment. Data were normalized for individual animals and expressed as percentage of blood pool activity derived from 5-min baseline period in each animal. A significant increase in cardiac, pulmonary, and hepatic blood pool activity was found after phenylephrine treatment. Changes in splenic and renal blood pools did not reach statistical significance.

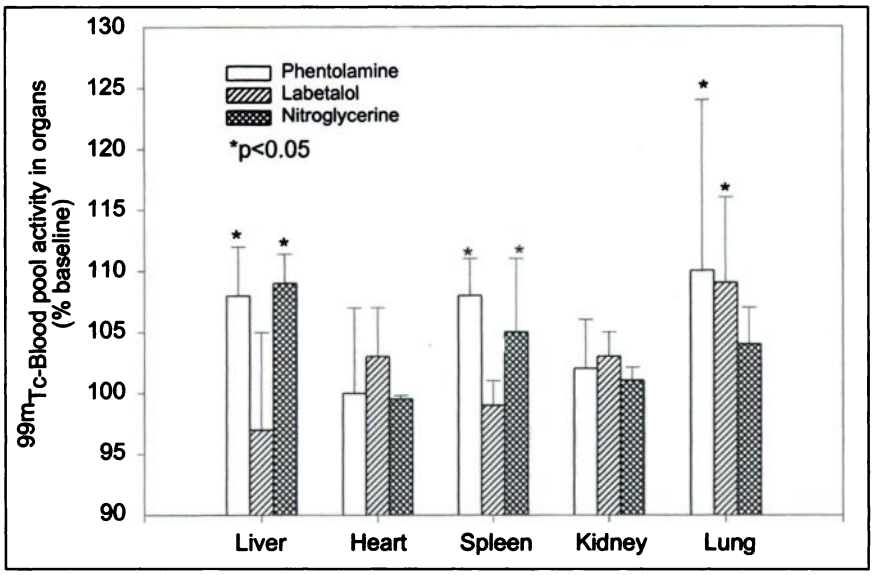

FIGURE 3. Effect of vasodilators on organ-specific blood pools. In groups of 4 animals each, baseline blood pools were measured followed by administration of phentolamine, labetalol, or nitroglycerine. Data were normalized for individual animals and are expressed as percentage of baseline blood-pool activity per animal. Phentolamine and nitroglycerine exerted similar increases in hepatic and splenic blood pools; labetalol-induced increases were of smaller magnitude. Phentolamine and labetalol increased pulmonary blood pools, whereas nitroglycerine was less effective in changing pulmonary blood pools.

The scintigraphically identified changes in the cardiac blood pool parallel the physiologic effects of this drug. The lack of scintigraphic changes in control animals injected with an equal volume of normal saline confirms that the changes observed were pharmacologically, not volumetrically, induced.

The lack of change in cardiac, hepatic, and splenic blood pools after labetalol treatment, which is in contrast to the effects of phentolamine and nitroglycerine, may be associated with the weaker α-blocking activity of labetalol compared with that of phentolamine (*13*). In addition, labetalol has greater β1-blocking activity leading to more prominent cardiac effects, such as negative chronotropic and inotropic activity, and lesser β2-blocking activity causing limited vasodilatation. Similarly, both phentolamine and labetalol increased the pulmonary blood pool in our studies, whereas nitroglycerine did not, possibly in keeping with the more pronounced venodilatory activity of the latter (*12*). Again, these radionuclide-detected changes were what would be expected in light of the increased hepatic blood flow and increased pulmonary capillary pressure induced by α-adrenergic blockade with prazosin in patients with portal hypertension (*14*).

Although regulation of large-vessel reactivity—e.g., the main portal vein—can be analyzed by noninvasive modalities such as Doppler sonography (*15*), analysis of hepatic sinusoidal bed changes in the intact animal is difficult. Previous studies relied on multiple indicator dilution methods along with correction for blood volume by additional tracers (*8*). Such methods require complex mathematic modeling and do not lend themselves to convenient, systematic study. In contrast, the simplicity of radionuclide blood

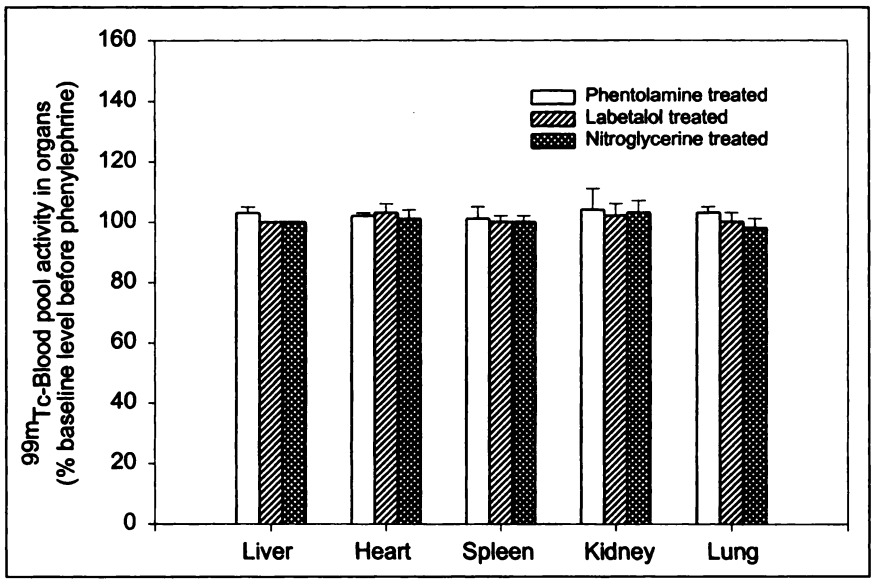

FIGURE 4. No effect of phenylephrine administered to animals pretreated with phentolamine, labetalol, or nitroglycerine was found. Data were normalized for individual animals and expressed as percentage of blood-pool activity per animal after treatment with first drug.

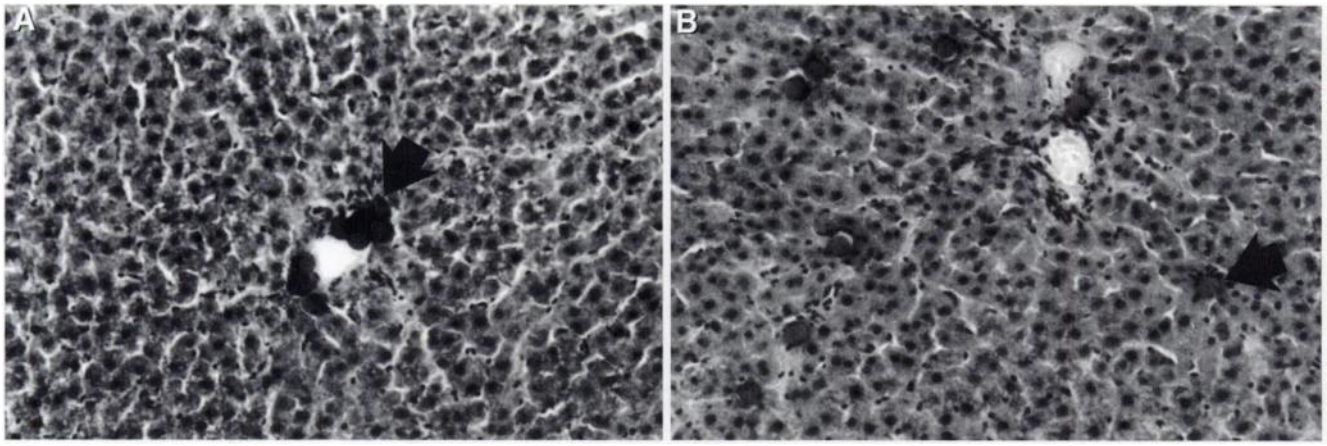

FIGURE 5. Evidence for hepatic sinusoidal dilatation after treatment with vasodilators. (A) Distribution of microspheres in liver of control rat. Injected microspheres (arrow) are confined to portal area. (B) After nitroglycerine administration, microspheres (arrow) can be identified both inside and outside of portal areas, indicating dilatation of hepatic sinusoids resulting in more distal penetration of microspheres into dilated sinusoids.

pool analysis is advantageous. The ability to analyze pharmacologic responses in the hepatic vascular bed is similar to what has been reported for the regulation of the penile vasculature (*16,17*). We found that both phentolamine and nitroglycerine dilated the hepatic sinusoidal bed along with abrogation of systemic α-adrenergic responsiveness. The hepatic sinusoidal bed is supplied with blood from both portal vein and hepatic artery with a complex vascular network at the confluence of portal, hepatic arterial, and sinusoidal confluence, including anastomotic portosystemic channels (*18*). Thus, regulation of portal and hepatic arterial blood flow as well as the tone of this vascular complex could be responsible for determining the hepatic sinusoidal blood pool. Analysis of the distribution of microspheres in the liver sinusoids was instructive in dissecting these mechanisms. Had there been a change only in blood flow, and not in the dimensions of the hepatic sinusoidal bed after vasodilatory treatment, there would have been no change in the distribution of microspheres. The movement of microspheres into more distal locations of the liver lobule after nitroglycerine administration confirms that the sinusoidal bed was in fact altered and that the nitroglycerine-induced increase in the hepatic blood pool was caused by sinusoidal dilatation and not simply altered blood flow–volume relationships in this organ. This documentation is especially significant because even modest changes in the hepatic blood pool were associated with a major effect on size–structure relationships in the liver lobule.

CONCLUSION

The data presented indicate that it is possible to noninvasively show pharmacologically induced hemodynamic changes with radionuclide methods. The approaches outlined may facilitate analysis of the mechanisms that regulate hepatic vascular tone. These techniques are potentially useful in basic studies concerning hepatic physiology and pharmacology, including studies of portal hypertension. In addition, the ability to noninvasively monitor alterations in the tone of the hepatic sinusoidal bed to decrease the initial loss of transplanted cells can provide novel strategies for cell transplantation in the liver, which will have an impact on translational activity in the areas of cell and gene therapy.

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