Kinetics of a Putative Hypoxic Tracer, ^{99m}Tc-HL91, in Normoxic, Hypoxic, Ischemic, and Stunned Myocardium

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^{99m}Tc-4,9-diaza-3,3,10,10-tetramethyldodecan-2,11-dione dioxime (HL91) was developed as a putative hypoxic reagent. This study focused on the myocardial kinetics of ^{99m}Tc-HL91 in various oxygen levels and perfusion states. **Methods:** The time–activity curve of ^{99m}Tc-HL91 was measured in isolated perfused rat heart after the bolus infusion. **Results:** ^{99m}Tc-HL91 was cleared quickly from normoxic hearts, and retention at 30 min after injection was 0.18 ± 0.02 percentage injected dose per gram of wet weight (mean ± SE; n = 6). When the concentration of oxygen bubbling through the perfusate was reduced from 100% to 50%, 20%, 5%, and 0%, retention of ^{99m}Tc-HL91 increased to 0.47 ± 0.03 (n = 5), 0.48 ± 0.03 (n = 5), 0.71 ± 0.01 (n = 5), and 0.70 ± 0.02 (n = 5), respectively ($P < 0.05$). Compartment analysis revealed that the trapping mechanism, which was dependent on tissue oxygen concentration, determined the retention rate. Although not retained in stunned myocardium (0.17 ± 0.02, n = 5; P = not significant), ^{99m}Tc-HL91 was significantly retained when injected before ischemia (1.06 ± 0.06, n = 5; $P < 0.05$). **Conclusion:** These results indicate that retention of ^{99m}Tc-HL91 correlates well with oxygen level in the perfusate, suggesting that the agent may be a useful marker of the severity of myocardial hypoxia.

Key Words: radioisotopes; hypoxia; ischemia; myocardial stunning

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Decreased tissue oxygen concentration is a component of many diseases, and myocardial hypoxia is often observed in persistent low-flow states, such as hibernating myocardium. Detection of hypoxic tissue provides important information for subsequent clinical intervention. Several compounds, including ^{18}F-labeled misonidazole (*1,2*), iodovinylmisonidazole (*3*), and ^{99m}Tc-labeled nitroimidazole (*4,5*), have been developed as putative hypoxic reagents. We and others have reported that a ^{99m}Tc-labeled nitroimidazole is selectively trapped in hypoxic but viable myocardium, and the possibility of imaging hypoxic tissues has been proposed (*4,6,7*). However, the high hepatic uptake of these tracers made their clinical use questionable (*8*). Recently, a ^{99m}Tc-labeled compound without a 2-nitroimidazole moiety, 4,9-diaza-3,3,10,10-tetramethyldodecan-2,11-dione dioxime (HL91), has been developed and shown low hepatic uptake (*9*). ^{99m}Tc-HL91 has also been shown to detect tumor hypoxia (*10,11*). Furthermore, ^{99m}Tc-HL91 showed increased myocardial uptake in hypoxic and low-flow ischemic models (*12–14*). However, the kinetics have not been fully investigated, and the mechanism for retention is not clear. This study characterized the kinetics of ^{99m}Tc-HL91 in normoxic, hypoxic, ischemic, and stunned myocardium.

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MATERIALS AND METHODS

The experimental model has been described (*4,15*). Briefly, male Sprague-Dawley rats (300–600 g body weight) were anesthetized with intraperitoneal thiopental sodium (38 mg/kg) and were given heparin. After rapid excision of the heart, the aorta was cannulated and retrogradely perfused with a solution of the following composition: NaCl, 123 mmol/L; KCl, 5 mmol/L; $MgSO_4$, 1 mmol/L; *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid, 5 mmol/L; $CaCl_2$, 1.5 mmol/L; sodium acetate, 5 mmol/L; and glucose, 6 mmol/L. The pH was adjusted to 7.4 at 37°C with NaOH, and 100% O_2 was bubbled through the solution. The heart rate was maintained at 300 bpm by right ventricular pacing. A latex balloon tied to the end of a polyethylene tube was passed into the left ventricle through the mitral valve and connected to a pressure transducer. The coronary flow rate was controlled by a peristaltic pump and kept constant (10 mL/min) throughout the experiment except during global ischemia. The research protocol was approved by the Animal Care and Use Committee of the institution (Nihon Medi-Physics Co., Ltd.), and animal experiments were performed according to the guidelines of the American Physiological Society.

Measurement of Myocardial Radioactivity

The measurement of myocardial time and activity has been described (*4*). Briefly, after stabilization, the hearts were set in a glass chamber. ^{99m}Tc-HL91 (Nycomed Amersham, Buckinghamshire, UK), 111 MBq/mL, was injected during 4 s through the tubing into the aortic root of the perfused hearts. The time–activity curve of ^{99m}Tc-HL91 uptake in the myocardium was measured with a 2.54 × 2.54 cm (1-in. × 1-in.) NaI(Tl) scintillation detector (Steffi; Raytest, Straubenhardt, Germany) positioned 2 cm from the

heart and fitted with an 8-mm-thick lead collimator that was 6 cm in length. Counting rate data were recorded using a D-2500 Chromato Integrator (Hitachi, Tokyo, Japan). The effluent from the heart was not recirculated. At the end of each experiment, the radioactive contents of the heart and perfusate collected from the effluent were measured by a single-channel analyzer with a 5.08 × 5.08 cm (2-in. × 2-in.) NaI(Tl) scintillator (Ohyo Koken Kogyo, Tokyo, Japan); the uptake ratio of an agent by the heart was expressed as percentage injected dose (%ID). ^{99m}Tc-HL91 was prepared from the kit as indicated. The radiochemical purity was determined (*16*) and was always greater than 95% before use.

Experimental Protocols

After stabilization of the preparation, the following protocols were performed (Fig. 1).

Normoxic Protocol. After 30 min of perfusion, ^{99m}Tc-HL91 was injected into the heart during normoxic perfusion. The activity in the myocardium was measured for 30 min after the injection. These data served as the normoxic control.

Hypoxic Protocol. After 30 min of stabilization, the oxygen concentration bubbling through the perfusate was switched from 100% to 0%, 5%, 20%, or 50%. Hearts reached a new steady state within 10 min after switching of the perfusate. Ten minutes after the switching, ^{99m}Tc-HL91 was injected. Change in developed pressure was measured 20 min after injection.

Ischemia and Stunning Protocols. After 30 min of stabilization, the hearts were subjected to 15 min of global ischemia at 37°C and reperfused. To generate global ischemia, the pump was stopped and the perfusion line was cross-clamped. ^{99m}Tc-HL91 was injected before or after the ischemia. Contractile function was measured 20 min after the reperfusion.

In the protocol for stunned myocardium, ^{99m}Tc-HL91 was injected 30 s after the reperfusion. In the ischemic protocol, ^{99m}Tc-HL91 was injected 30 s before global ischemia. The activity of the hearts was measured from the onset of ischemia to the end of the experiments.

Compartment Analysis

A 3-compartment model (Fig. 2) was used to analyze the data in normoxic and hypoxic experiments. Compartments 1, 2, and 3 represent the extracellular space, the space of intracellular and free ^{99m}Tc-HL91, and the space of ^{99m}Tc-HL91 trapped in myocytes, respectively. Q_1, Q_2, and Q_3 represent the radioactivity of each compartment. The rate constant, k(i,j), indicates the radioactivity from compartment j to compartment i (per minute). The rate constants were determined by fitting the time–activity curve in each experiment to the theoretic equation (*17*).

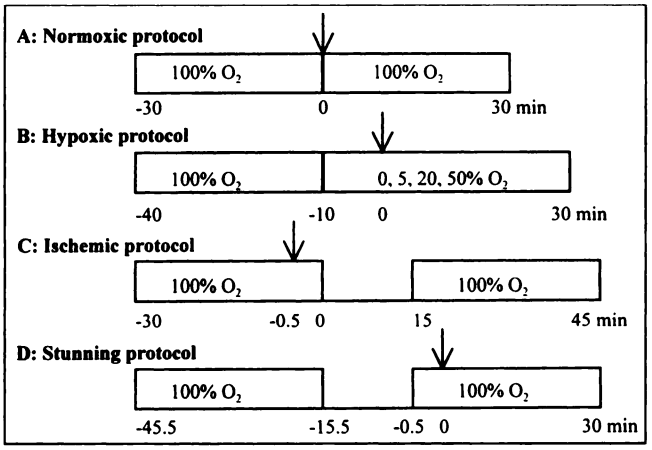

FIGURE 1. Experimental protocols. Arrow indicates injection of ^{99m}Tc-HL91. (A) Normoxic perfusion. (B) Injection 10 min after switching to hypoxia. (C) Injection 30 s before global ischemia. (D) Injection 30 s after start of reperfusion after 15-min global ischemia (stunned heart).

Statistical Analysis

Data are presented as the mean ± SE. Statistical analysis was performed using the paired and unpaired *t* test or ANOVA with the Scheffé test where appropriate. $P < 0.05$ for the null hypothesis was considered significant.

RESULTS

Kinetics of ^{99m}Tc-HL91 in Normoxic Hearts

Left ventricular developed pressure in the normoxic control group was not changed throughout the experiments (Fig. 3). Figure 4A shows the time–activity curve of ^{99m}Tc-HL91 in normoxic hearts. The counts in the myocardium were normalized to the maximum value at 0.1 min after the injection. ^{99m}Tc-HL91 was quickly washed out, and the retention in the myocardium at 30 min after the injection was 0.18 ± 0.02 %ID/g of wet weight (n = 6; Fig. 5).

Kinetics of ^{99m}Tc-HL91 in Hypoxic Hearts

When a perfusate was switched to a hypoxic perfusate, the heart rapidly reduced its contractility and reached a new steady state ($P < 0.05$; Fig. 3). Figure 4B shows the time–activity curve of ^{99m}Tc-HL91 in hypoxic myocardium. Retention at 30 min after injection was 0.70 ± 0.02 (n = 5), 0.71 ± 0.01 (n = 5), 0.48 ± 0.03 (n = 5), and 0.47 ± 0.03 (n = 5) %ID/g of wet weight in the hypoxic myocardium perfused with 0%, 5%, 20%, and 50% oxygen levels, respectively. Retention was higher in all hypoxic hearts than in normoxic control hearts ($P < 0.001$; Fig. 5). Furthermore, retention was inversely related to the level of oxygen buffering the perfusate ($r = -0.94$, $P < 0.0001$; Fig. 6).

Kinetics of ^{99m}Tc-HL91 in Ischemic and Stunned Myocardium

In stunned myocardium, the recovery of developed pressure was not complete (Fig. 3). Figures 4C and D show time–activity curves of ^{99m}Tc-HL91 in hearts briefly subjected to ischemia and reperfused (stunned myocardium). When injected before ischemia, ^{99m}Tc-HL91 was trapped during ischemia, and washout after the reperfusion was lower than with other protocols (Fig. 4C). Retention was higher (1.06 ± 0.06 %ID/g of wet weight, n = 5) compared with normoxic control hearts ($P < 0.001$). In contrast, the behavior of ^{99m}Tc-HL91 in stunned myocardium was similar to that in the control hearts (Fig. 4D). Retention in stunned myocardium was 0.17 ± 0.02 %ID/g of wet weight (n = 5) and was not different from that in control myocardium (Fig. 5).

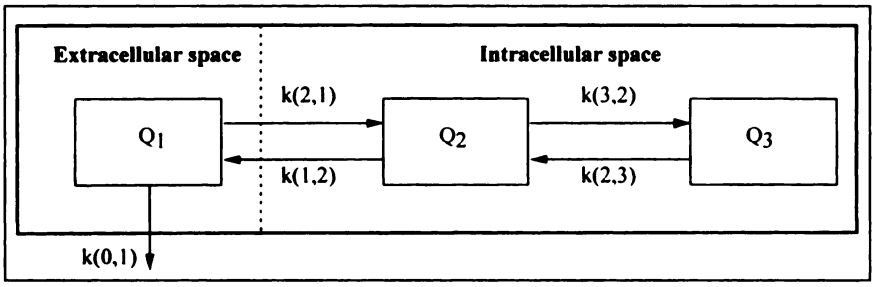

FIGURE 2. Three-compartment model used for analysis of ^{99m}Tc-HL91 kinetics. Compartment 1 represents extracellular space. Compartment 2 represents space for intracellular and free ^{99m}Tc-HL91, and compartment 3 represents space for ^{99m}Tc-HL91 trapped in myocytes. k and Q represent rate constant and activity, respectively.

Rate Constants for the Kinetics in Hypoxic Hearts

The initial compartment analysis revealed that k(2,3) was smaller than k(3,2) and did not differ according to oxygen levels ($r = -0.07$, $P = 0.74$). At 100% O_2, k(2,3) = 0.01 ± 0.001/min and k(3,2) = 0.05 ± 0.01/min ($P < 0.0001$); at 50% O_2, k(2,3) = 0.01 ± 0.001/min and k(3,2) = 0.10 ± 0.01/min ($P < 0.0001$); at 20% O_2, k(2,3) = 0.01 ± 0.002/min and k(3,2) = 0.12 ± 0.01/min ($P < 0.0001$); and at 5% O_2, k(2,3) = 0.01 ± 0.002/min and k(3,2) = 0.16 ± 0.01/min ($P < 0.0001$). Thus, k(2,3) was fixed to zero, and we analyzed the kinetics using the model assuming irreversible trapping of ^{99m}Tc-HL91.

Table 1 summarizes the rate constants obtained from the kinetics in hypoxic hearts. The values of k(0,1), k(1,2), and k(2,1) were not significantly different among the oxygen levels. In contrast, k(3,2) was inversely related to the oxygen concentration ($r = -0.77$, $P < 0.0001$). This result suggests that the kinetics of ^{99m}Tc-HL91 are influenced mainly by the trapping rate of ^{99m}Tc-HL91 by myocytes.

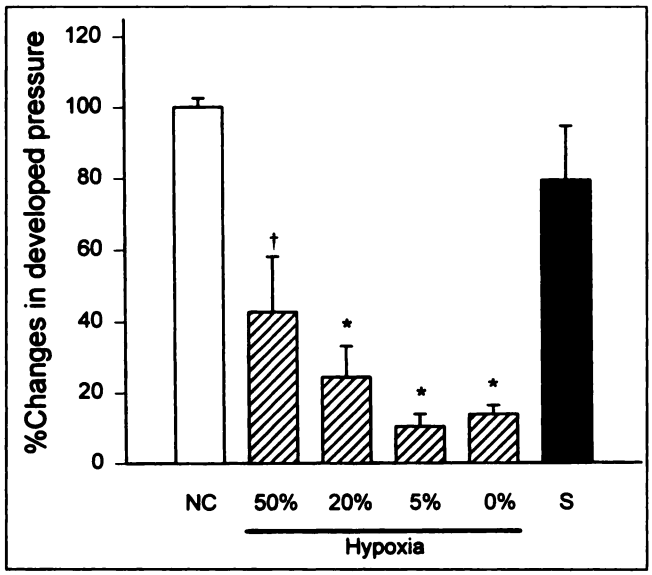

FIGURE 3. Changes in developed pressure in each experimental protocol. Percentage change in developed pressure 20 min after injection of ^{99m}Tc-HL91 was normalized by each control value. Developed pressure was defined as difference between left ventricular peak pressure and end-diastolic pressure. *$P <$ 0.01 versus control. †$P < 0.05$ versus control. NC = normoxic control; S = stunned hearts.

DISCUSSION

We and others have shown that ^{99m}Tc-HL91 can detect myocardial ischemia or viability in in vivo experiments (*12–14,18*). Okada et al. (*19*) showed that ^{99m}Tc-HL91 is not taken up or retained in nonviable and irreversibly injured myocardium. The possibility to estimate myocardial viability has been expected. However, the detailed kinetics of ^{99m}Tc-HL91 in graded hypoxic or stunned myocardium are still unclear. In this study, we analyzed retention kinetics by applying a bolus infusion rather than a steady-state infusion because the bolus was suitable for separating the wash-in and washout parts from myocardial uptake. Our finding that retention of ^{99m}Tc-HL91 was higher in hypoxic hearts is consistent with the findings of Okada et al. (*12,19*) and are inversely related to the level of oxygen bubbling through the perfusate. These results indicate that ^{99m}Tc-HL91 can be used to measure hypoxic levels in myocardium.

In contrast to most other putative hypoxic tissue imaging agents, ^{99m}Tc-HL91 does not have a nitroimidazole functionality. We previously compared the kinetics of BMS181321 (^{99m}Tc-nitroimidazole) with hexamethyl propyleneamine oxime (HMPAO) and PAO-6-Me (a non-nitroimidazole analog of BMS181321) (*4*). Retention of these tracers was higher in hypoxic myocardium than in normoxic control myocardium, and the kinetics were similar. The retention of ^{99m}Tc-HL91 was about 10 times smaller than that of BMS181321 and HMPAO (*4*). However, the hypoxic-to-normal ratio of the myocardial radioactivity at 0% oxygen was similar between ^{99m}Tc-HL91 (3.64) and BMS181321 (4.32) and was higher than that of HMPAO (*4*). The ^{99m}Tc-HL91 retention kinetics, which were inversely related to the level of oxygen bubbling through the perfusate, were similar to those of BMS181321 (*5,20*), although tissue oxygen concentration was not measured because no method has been established for this whole-heart preparation. In stunned myocardium, myocardial necrosis or exacerbated energy metabolism are not observed (*21,22*). Thus, the similarity in kinetics between stunned hearts and normoxic control hearts when ^{99m}Tc-HL91 was injected after reperfusion is not surprising.

In contrast, retention in ischemic hearts was higher than that in normoxic control hearts when ^{99m}Tc-HL91 was injected before ischemia. These kinetics were similar to

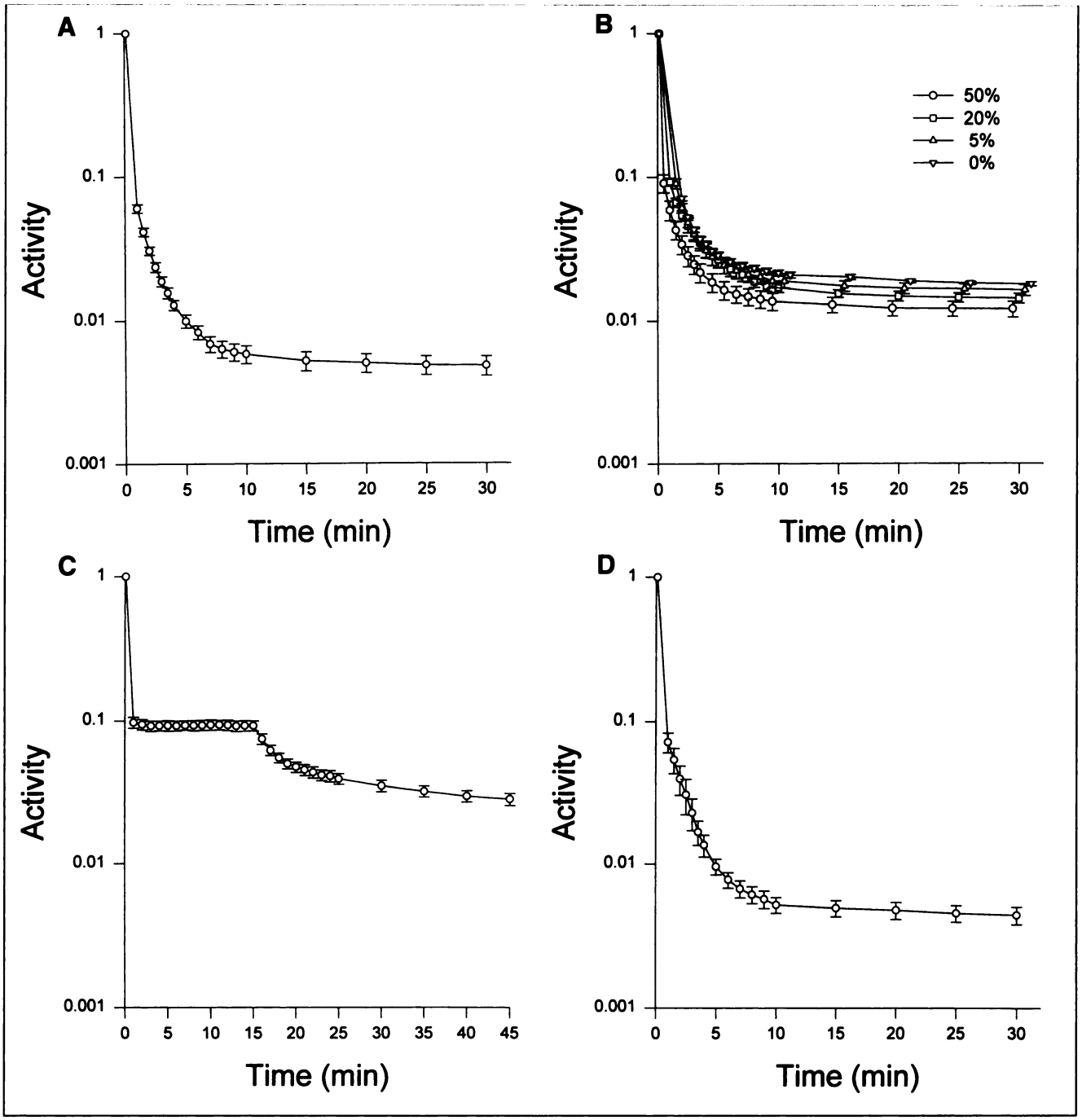

FIGURE 4. Time–activity curves of ^{99m}Tc-HL91 for normoxic hearts (A), hypoxic hearts (B), ischemic hearts (C), and stunned myocardium (D). ^{99m}Tc-HL91 was injected after reperfusion. Radioactivities were normalized by individual peak values, and values represent mean ± SE.

those of BMS181321. The washout kinetics of ^{99m}Tc-HL91 and BMS181321 after reperfusion were also similar. In contrast, HMPAO showed elevated myocardial radioactivity after reperfusion because HMPAO was metabolized during ischemia (*23*). Although the retention kinetics of BMS181321 and ^{99m}Tc-HL91 were similar, the difference between BMS181321 and PAO-6-Me indicates that the retention mechanism of BMS181321 was determined mainly by nitroimidazole metabolism (*4,24*). In this study, we analyzed the kinetics of ^{99m}Tc-HL91 using the 3-compartment model; the results suggested the existence of an intracellular trapping mechanism dependent on the level of oxygen bubbling through the perfusate, although ^{99m}Tc-HL91 does not have nitroimidazole functionality. The kinetic analysis suggests that the trapping of ^{99m}Tc-HL91 dependent on tissue oxygen concentration may be mediated by a mechanism other than metabolism, although no additional data support this hypothesis. Because the existence of chemically equivalent states

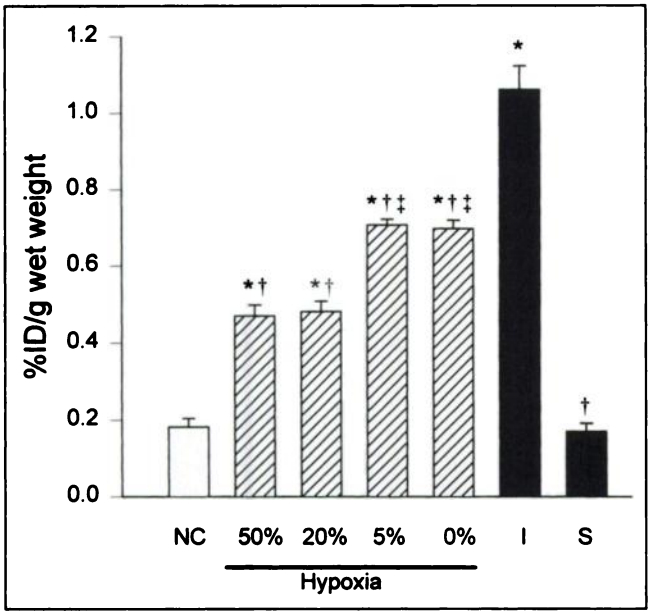

FIGURE 5. Myocardial retention of ^{99m}Tc-HL91 at end of each experiment. *$P < 0.001$ versus normoxic control. †$P < 0.05$ versus ischemia. ‡$P < 0.05$ versus hypoxia with 50% oxygen concentration. NC = normoxic control; I = injection of ^{99m}Tc-HL91 before ischemia; S = injection of ^{99m}Tc-HL91 after reperfusion.

of ^{99m}Tc-HL91, i.e., lipophilic and hydrophilic (*25*), has been reported, functional alterations may contribute to the trapping mechanism. Further study is required to identify the trapping mechanism.

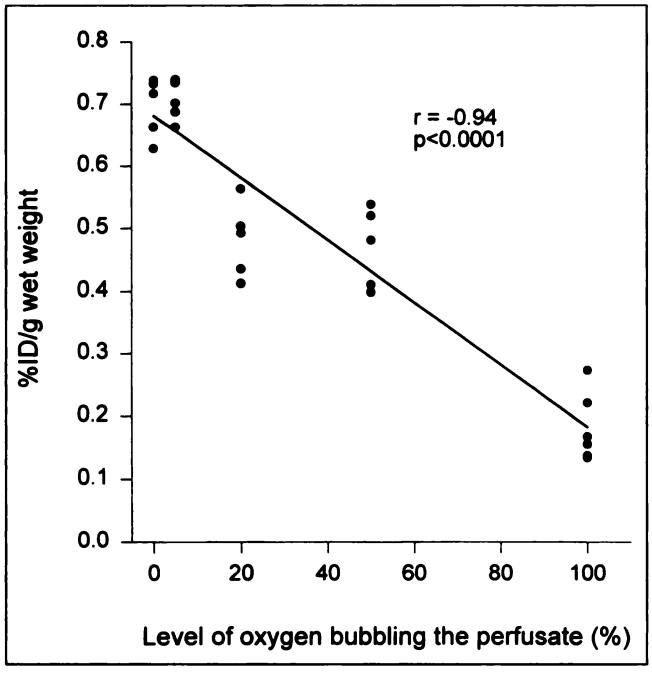

FIGURE 6. Relationship between myocardial retention of ^{99m}Tc-HL91 and level of oxygen bubbling through perfusate. Mean value in each group was 0.18 for 100% (n = 6), 0.48 for 50% (n = 5), 0.47 for 20% (n = 5), 0.71 for 5% (n = 5), and 0.70 for 0% (n = 5).

TABLE 1
Rate Constants in Different Hypoxic States

State	Rate constant (per minute) k(0,1)	k(1,2)	k(2,1)	k(3,2)
Normoxic control	8.47 ± 0.81	0.55 ± 0.01	0.33 ± 0.03	0.04 ± 0.004
Hypoxic				
50%	5.72 ± 0.38	0.60 ± 0.04	0.44 ± 0.11	0.07 ± 0.01*†
20%	7.33 ± 0.65	0.41 ± 0.04	0.44 ± 0.03	0.06 ± 0.01†
5%	6.03 ± 0.70	0.51 ± 0.07	0.39 ± 0.05	0.09 ± 0.01*
0%	6.76 ± 0.65	0.42 ± 0.08	0.33 ± 0.05	0.12 ± 0.01*

*$P < 0.05$ vs. normoxic control.
†$P < 0.05$ vs. hypoxia with 0% oxygen concentration.

CONCLUSION

Our results indicate that, at 30 min after injection, ^{99m}Tc-HL91 shows significantly higher retention in hypoxic myocardium than in control myocardium. Furthermore, retention of ^{99m}Tc-HL91 is inversely related to the level of oxygen bubbling through the perfusate, suggesting that ^{99m}Tc-HL91 can measure the severity of hypoxia. Compartment analysis suggests that the kinetics are influenced mainly by the trapping rate of ^{99m}Tc-HL91 by myocytes.

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REFERENCES

1. Martin GV, Caldwell JH, Graham MM, et al. Non-invasive detection of hypoxic myocardium using fluorine-18-fluoromisonidazole and positron emission tomography. *J Nucl Med.* 1992;33:2202–2208.
2. Shelton ME, Dence CS, Hwang D-R, Welch MJ. Myocardial kinetics of fluorine-18 misonidazole: a maker of hypoxic myocardium. *J Nucl Med.* 1989;30:351–358.
3. Martin GV, Biskupiak JE, Caldwell JH, Rasey JS, Kroll KA. Characterization of iodovinylmisonidazole as a marker for myocardial hypoxia. *J Nucl Med.* 1993;34:918–924.
4. Kusuoka H, Hashimoto K, Fukuchi K, Nishimura T. Kinetics of a putative hypoxic tissue marker, technetium-99m-nitroimidazole (BMS181321), in normoxic, hypoxic, ischemic and stunned myocardium. *J Nucl Med.* 1994;35:1371–1376.
5. Ng CK, Sinusas AJ, Zaret B, Soufer R. Kinetic analysis of technetium-99m-labeled nitroimidazole (BMS-181321) as a tracer of myocardial hypoxia. *Circulation.* 1995;92:1261–1268.
6. Fukuchi K, Kusuoka H, Watanabe Y, Fujiwara T, Nishimura T. Ischemic and reperfused myocardium detected with technetium-99m-nitroimidazole. *J Nucl Med.* 1996;37:761–766.
7. Rumsey WL, Kuczynski B, Patel B, et al. SPECT imaging of ischaemic myocardium using a techenetium-99m-nitroimidazole ligand. *J Nucl Med.* 1995;36:1445–1450.
8. Shi CQ, Sinusas AJ, Dione DP, et al. Technetium-99m nitroimidazole (BMS181321): a positive imaging agent for detecting myocardial ischemia. *J Nucl Med.* 1995;36:1078–1086.
9. Archer CM, Edwards B, Kelly JD, King AC, Burke JF, Riley ALM. Technetium labelled agents for imaging tissue hypoxia in vivo. In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium and Rhenium in Chemistry and Nuclear Medicine.* Padova, Italy: SGE Ditoriali; 1995:535–539.
10. Yutani K, Kusuoka H, Fukuchi K, Tatsumi M, Nishimura T. Applicability of ^{99m}Tc-HL91, a putative hypoxic tracer, to detection of tumor hypoxia. *J Nucl Med.* 1999;40:854–861.

11. Tatsumi M, Yutani K, Kusuoka H, Nishimura T. Technetium-99m HL91 uptake as a tumor hypoxia marker: relationship to tumor blood flow. *Eur J Nucl Med.* 1999;26:91–94.
12. Okada RD, Johnson G III, Nguyen KN, Edwards B, Archer CM, Kelly JD. ^{99m}Tc-HL91: effects of low flow and hypoxia on a new ischemia-avid myocardial imaging agent. *Circulation.* 1997;95:1892–1899.
13. Johnson G III, Nguyen KN, Liu Z, Okada RD. Technetium 99m-HL-91: a potential new marker of myocardial viability assessed by nuclear imaging early after reperfusion. *J Nucl Cardiol.* 1998;5:285–294.
14. Okada RD, Johnson G III, Nguyen KN, et al. ^{99m}Tc-HL91: "Hot spot" detection of ischemic myocardium in vivo by gamma camera imaging. *Circulation.* 1998;97: 2557–2566.
15. Yamamichi Y, Kusuoka H, Morishita K, et al. Metabolism of iodine-123-BMIPP in perfused rat hearts. *J Nucl Med.* 1995;36:1043–1050.
16. Okada RD, Nguyen KN, Lauinger M, Allton IL, Johnson G III. Technetium 99m-Q12 kinetics in perfused rat myocardium: effects of hypoxia and low flow. *Am Heart J.* 1996;132:108–115.
17. Godfrey K. Compartmental models and their application. London, UK: Academic Press; 1983.
18. Fukuchi K, Kusuoka H, Yutani K, Hasegawa S, Nishimura T. Assessment of reperfused myocardium using a new ischemia-avid imaging agent, technetium-99m HL91: comparison with myocardial glucose uptake. *Eur J Nucl Med.* 1998;25:361–366.
19. Okada RD, Johnson G III, Nguyen KN, Carlson LR, Beju D. HL-91-technetium-99m: a new marker of viability in ischemic myocardium. *J Nucl Cardiol.* 1999;6:306–315.
20. Rumsey WL, Patel B, Linder KE. Effect of graded hypoxia on retention of technetium-99m-nitroheterocycle in perfused rat heart. *J Nucl Med.* 1995;36:632–636.
21. Braunwald E, Kloner RA. The stunned myocardium: prolonged, postischemic ventricular dysfunction. *Circulation.* 1982;66:1146–1149.
22. Kusuoka H, Porterfield JK, Weisman HF, Weisfeldt ML, Marban E. Pathophysiology and pathogenesis of stunned myocardium: depressed Ca^{2+} activation of contraction as a consequence of reperfusion-induced cellular calcium overload in ferret hearts. *J Clin Invest.* 1987;79:950–961.
23. Neirinckx RD, Canning LR, Piper IM, et al. Technetium-99m d,l-NMPAO: a new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. *J Nucl Med.* 1987;28:191–202.
24. Nunn A, Linder K, Strauss HW. Nitroimidazoles and imaging hypoxia. *Eur J Nucl Med.* 1995;22:265–280.
25. Brauers G, Archer CM, Burke JF. The chemical characterization of the tumor imaging agent ^{99m}Tc-HL91 [abstract]. *Eur J Nucl Med.* 1997;24:943.