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Estimation of the area at risk in myocardial infarction of rats by means of I-123 β-methyliodophenyl pentadecanoic acid imaging

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Clinical investigations have suggested that the defects in SPECT images of a free fatty acid analog, I-123 β-methyliodophenyl pentadecanoic acid (BMIPP) may indicate the ischemic risk area. To elucidate whether I-123 BMIPP can indicate the area at risk of ischemia, *ex-vivo* autoradiography was performed in rats whose left coronary artery was occluded for 60 min and then reperfused. I-123 BMIPP was injected at the acute stage (n = 10), or the subacute stage (7 days after reperfusion; n = 9). Infarction and the area at risk were identified by triphenyl tetrazolium chloride staining and injection of methylene blue during religation just before sacrifice, respectively. The BMIPP uptake in the risk area was significantly lower than that in the remote area at the acute (risk, 53.7 ± 23.3% of the uptake at right ventricle, mean ± SD; remote, 109.3 ± 11.8%; $p < 0.01$) and subacute (risk, 52.5 ± 11.5%; remote, 97.9 ± 14.3%; $p < 0.01$) stages. In addition, the area with reduced uptake of I-123 BMIPP showed a significant correlation with the area at risk both at the acute ($r = 0.98$, $p < 0.01$) and subacute ($r = 0.92$, $p < 0.01$) stages. In conclusion, the area at risk can be evaluated by I-123 BMIPP both at the acute and subacute stages.

Key words: BMIPP, area at risk, myocardial infarction, reperfusion

INTRODUCTION

IN THE CLINICAL SETTING, the area at risk is an important factor in the evaluation of the effect of interventions during the acute phase of myocardial infarction. Current methods for evaluation of the area at risk include SPECT imaging with Tc-99m-hexakis-2-methoxyisobutyl isonitrile (MIBI) or Tc-99m-tetrofosmin injected before reperfusion therapy[1–5] and myocardial contrast echocardiography using intracoronary artery injections of contrast agent.[6] But these methods are not easy to perform, since the radioactive tracer must be injected before reperfusion, or a contrast agent must be injected intracoronarily. Clinical investigations in patients with acute myocardial infarction suggest that an abnormal SPECT image obtained with the free fatty acid analog, iodine-123-beta-methyliodophenyl pentadecanoic acid (I-123 BMIPP), after revascularization may indicate the area at risk at the subacute stage.[7–11] Since SPECT examination at the subacute stage has little risk, this method would be practical in combination with perfusion SPECT imaging with thallium-201 or Tc-99m-labeled myocardial agents.

This study aimed to elucidate whether BMIPP images accurately indicate the area at risk at the acute and subacute stages of myocardial infarction.

MATERIALS AND METHODS

The method for the ischemia-reperfusion model of rats was previously reported.[12] Briefly, male Wister rats (7–8 weeks old) after overnight fasting were anesthetized with sodium pentobarbital injected intraperitoneally (50 mg/kg, Abbott, North Chicago, USA), intubated by a cannula after cervical tracheostomy, and ventilated with room air.

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The heart was exposed by parasternostomy and pericardiotomy. Figure 1 shows the protocol of this study. The left coronary artery was ligated and reperfused after 60 min. For the assessment of acute response (Fig. 1-A, n = 10), I-123 BMIPP (Nihon Medi-Physics, Hyogo, Japan; 18.5 MBq) was injected through the femoral vein 50 min after reperfusion (i.e., 110 min after the ligation). The rats were sacrificed by intravenous injection of saturated KCl 70 min after reperfusion (130 min after the ligation). Just before the sacrifice, 2% methylene blue dye (0.5–1.0 m*l*; Sigma Chemical Co., Missouri, USA) was injected intravenously after religation of the left coronary artery to evaluate the area at risk of perfusion.[13–15] The area at risk of ischemia was defined according to visual tracing of the area which was not stained by the dye.

For the chronic model (Fig. 1-B, n = 9), fed rats were anesthetized with sodium pentobarbital, intubated, ventilated and kept anesthetized with enflulen (Dynabott Co., Tokyo, Japan). The left coronary artery was occluded for 60 min, then reperfused as in the acute study. The chest was closed, and the rats were detubated. One week later, these rats in overnight fasting were anesthetized with sodium pentobarbital, intubated, and ventilated. The chest was re-opened and I-123 BMIPP (18.5 MBq) was injected through the femoral vein. Twenty min after the injection of BMIPP, the left coronary artery was religated, the dye was injected intravenously, and the rat was sacrificed with KCl.

In both acute and subacute studies, the heart was excised and sliced into three portions (about 3-mm thickness) parallel to the atrioventricular groove. The middle portion was frozen with dry ice and sliced into 5 μm thickness with a cryostat (JUNG CM 3000, Leica, Nussloch, Germany) at −18°C. At the acute stage the lower portion was used for triphenyl tetrazolium chloride staining (TTC; 2% saline solution: Sigma Chemical Co., Missouri, USA) to assess the myocardial infarction area.[16] At the subacute stage Hematoxylin-Eosin (H-E) staining was performed on the frozen slice to assess myocardial viability by means of the histological findings. On the slice TTC stained or the frozen slice H-E stained, the ratio of the infarction area to the left ventricular area was calculated by tracing each area with a digitizer.

Ex-Vivo *Autoradiography with I-123 BMIPP*

The frozen sliced sections were placed on clean glass slides and air-dried. As soon as they were arranged on thick paper and covered with polyethylene vinyl, they were exposed to an imaging plate (BAS cassette 2025, Fuji Photo Film Co., Kanagawa, Japan) for 1 hour to detect the distribution of I-123 BMIPP.

The autoradiographic images were analyzed by means of a biomedical imaging analysis system (BAS 5000 Mac; Fuji Photo Film Co., Kanagawa, Japan). The risk area and the remote area were defined visually by dye staining. The area showing reduced BMIPP uptake was defined as the area with less than 70% of the maximum uptake in the left ventricle. The area showing reduced BMIPP uptake and the area at risk were measured and normalized by the area of the whole heart. To quantify the myocardial uptake of BMIPP, regions of interest (ROI's) were traced on the risk area and the remote area of the left ventricle separated by dye staining, and the right ventricle. The uptake in each ROI was normalized by that traced on the right ventricle, and expressed as % uptake.

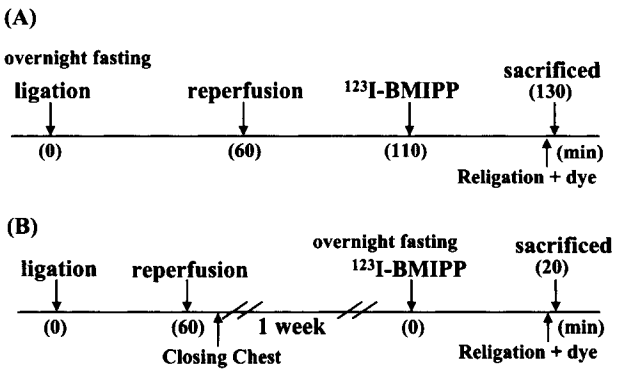

Fig. 1 Schematic representation of experimental protocols at the acute stage (A) and at the subacute stage (B).

Statistical Analysis

Data are expressed as the mean ± SD. Correlation was assessed by Pearson's correlation coefficient and Fisher's method. Differences between the two groups were assessed by paired t-test. A p value less than 0.01 was considered significant. The estimation of the risk area by BMIPP imaging was evaluated with the Bland-Altman plot.[17]

RESULTS

BMIPP Images at the Acute Stage

Figure 2 shows typical images of TTC staining, dye staining, and autoradiography with I-123 BMIPP obtained at the acute stage. The infarction area indicated by TTC staining (Fig. 2A) was a part of the area at risk (Fig. 2B). The area showing reduced uptake of BMIPP (Fig. 2C) was consistent with the area at risk.

The % uptake of BMIPP in the risk area (53.7 ± 23.3%) was significantly lower than that in the remote area (109.3 ± 11.8%; $p < 0.01$; Fig. 3A). As shown in Figure 3B, the % area of reduced BMIPP uptake (to the whole heart area) was significantly correlated with % risk area shown by the dye ($r = 0.98$, $p < 0.01$). Furthermore, the area of reduced BMIPP uptake was also consistent with the area at risk on each regional segment. The area at risk was therefore easily distinguished from the normal area in BMIPP images. Infarct size determined by TTC staining was 22.2 ± 12.8% of the whole heart area and was smaller than the area at risk (47.1 ± 15.3%, $p < 0.01$). The infarction area was 44.0 ± 23.5% of the area at risk.

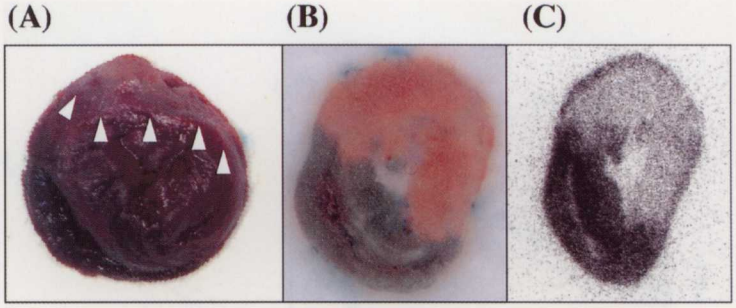

Fig. 2 TTC staining (A), dye staining (B), and autoradiography with I-123 BMIPP (C) of heart obtained at the acute stage of myocardial infarction. Infarction area (arrow heads; A) indicated by TTC staining was a part of the area at risk (red area; B) which was not stained by dye. The area showing reduced uptake of BMIPP (C) was consistent with the area at risk by dye (B).

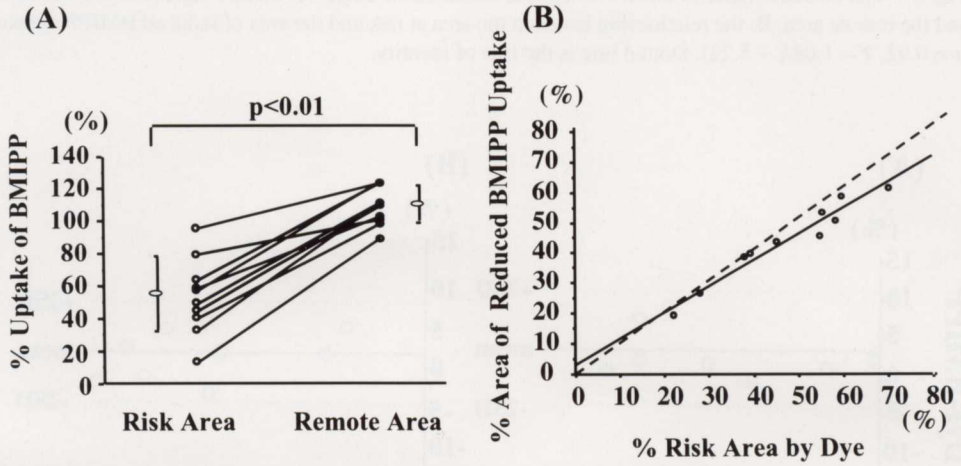

Fig. 3 The characteristics of BMIPP images at the acute stage. A: BMIPP uptake in the risk area and the remote area. B: the relationship between the area at risk and the area of reduced BMIPP uptake ($r = 0.98$, $Y = 0.85X + 3.57$). Dotted line is the line of identity.

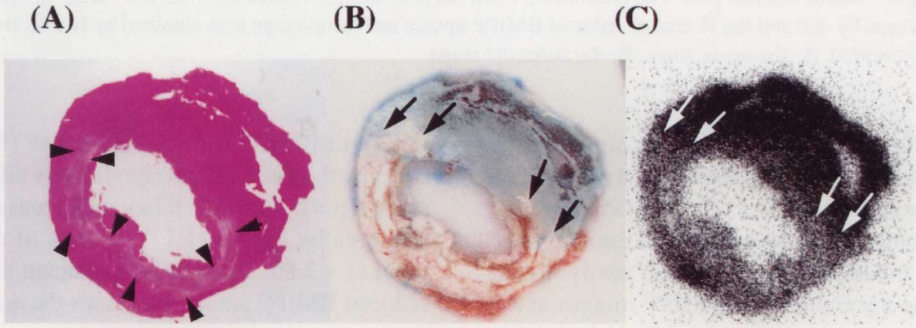

Fig. 4 Hematoxylin-Eosin staining (A), dye staining (B), and autoradiography with I-123 BMIPP (C) of heart obtained at the subacute stage. Infarction area (arrow heads; A) occupied by inflammatory cells and fibrosis was a part of the area at risk (black arrows, red area; B) which was not stained by dye. The area of reduced BMIPP uptake (white arrows; C) was consistent with the area at risk by dye (B).

BMIPP Images at the Subacute Stage

At the subacute stage, the infarction area occupied by inflammatory cells and fibrosis in H-E staining specimen (Fig. 4A) was a part of the area at risk (Fig. 4B). The area of reduced BMIPP uptake (Fig. 4C) was consistent with the area at risk indicated by the dye (Fig. 4B), similar to the acute stage. The % uptake of BMIPP in the risk area ($52.5 \pm 11.5\%$) was significantly lower than that in the remote area ($97.9 \pm 14.3\%$; $p < 0.01$; Fig. 5A). As shown in Figure 5B, the area of reduced BMIPP uptake (as a

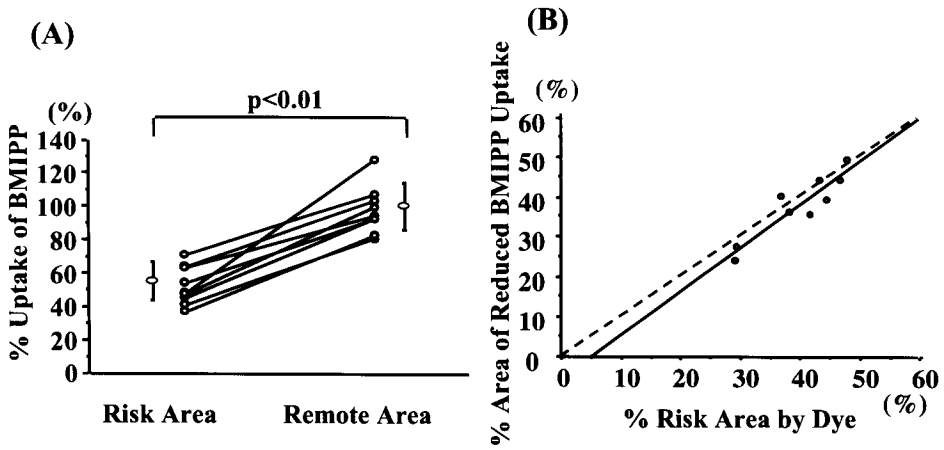

Fig. 5 The characteristics of BMIPP images at the subacute stage. A: BMIPP uptake in the risk area and the remote area. B: the relationship between the area at risk and the area of reduced BMIPP uptake ($r = 0.92$, $Y = 1.08X - 5.22$). Dotted line is the line of identity.

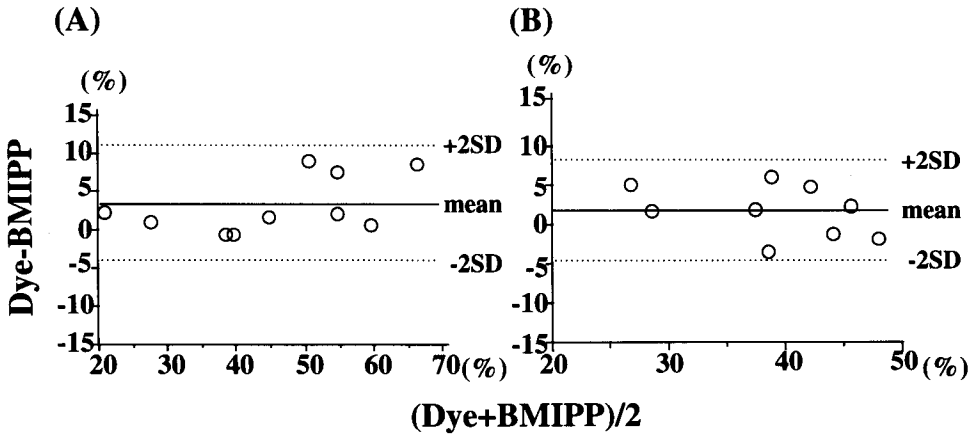

Fig. 6 Bland-Altman plot. The relation between the absolute difference between the values of the % risk area by dye and the % area of reduced BMIPP uptake and the average area obtained by two % areas is presented. A: the acute stage. B: the subacute stage.

percentage of the whole heart area) was significantly correlated with % risk area indicated by the dye ($r = 0.92$, $p < 0.01$). Furthermore, the area of reduced BMIPP uptake was also consistent with the area at risk on each regional segment. Thus, the area at risk was also easily distinguished from the normal area in BMIPP images at the subacute stage. Infarct size determined by H-E staining was $16.8 \pm 9.4\%$ of the whole heart area and was smaller than the area at risk ($39.9 \pm 7.0\%$, $p < 0.01$). The infarction area was $42.2 \pm 24.1\%$ of the area at risk.

The Estimation of the Area at Risk by BMIPP Imaging

The Bland-Altman plot[17] (Fig. 6) indicates that no relevant trend in the areas at risk determined by means of the dye and estimated by BMIPP exists over a wide area range at the acute (Panel A) and the subacute (Panel B) stages. Furthermore, the relationship between the area of reduced uptake of BMIPP and the area at risk (% risk area indicated by the dye) was not significantly different in the two stages ($p = 0.71$). This relationship was expressed as (% risk area by dye) = (1.14) (% area of reduced BMIPP uptake) − 2.86. These results indicate that the region of reduced BMIPP uptake illustrates the area at risk both at the acute and subacute stages.

DISCUSSION

We have showed that the area with reduced I-123 BMIPP uptake indicates the area at risk both at the acute and subacute stages of acute myocardial infarction with reperfusion. These results suggest that BMIPP imaging can be applied to estimate the area at risk in patients with acute myocardial infarction for up to at least 1 week after the onset.

Estimation of the Area at Risk

Estimation of the area at risk is important in predicting the outcome in patients with acute myocardial infarction,[18] and in assessing the efficacy of reperfusion therapy such as thrombolysis or coronary angioplasty,[2–6] and infarct-limiting agents such as potassium channel opener.[19] In previous studies, wall motion estimated by left ventriculography at the acute stage,[20] myocardial contrast echocardiograms with intracoronary injection,[6] and nuclear images with Tl-201,[21] Tc-99m macroaggregated albumin,[18] Tc-99m MIBI,[1–4] and Tc-99m tetrofosmin[5] have been proposed as indices of the area at risk, but all of these methods have some limitations in clinical application. Left ventriculography and myocardial contrast echocardiography are invasive and difficult to use for repetitive assessment because catheterization is required. In addition, the wall motion abnormality in left ventriculography tends to cause overestimation of the area at risk,[7] and neither method is suitable for quantitative analysis. In contrast, nuclear imaging methods are non-invasive and suitable for quantitative analysis. Nevertheless, imaging with Tl-201 must be done before intervention because of hyperwashout of this tracer,[21] and imaging with macroaggregated albumin may deteriorate myocardial infarction.[18] Recently, imaging with Tc-99m MIBI or tetrofosmin has been suggested as an improved method for estimating the area at risk.[1–5] Because of slow washout of these tracers, they may be injected intravenously before intervention, and images can be acquired after intervention when the patient is in stable condition, but images are often needed on the day of intervention when the condition of the patient might be unstable. In the present study, our results indicate that imaging with BMIPP is useful for this purpose and is safe because this method can be used at a stable stage, such as a few days after the onset.

Mechanisms for the Uptake of BMIPP

BMIPP is a radioiodinated fatty acid analog, into which a methyl group has been introduced in the β-3 position in the fatty acid chain to inhibit direct β oxidation and prolong its residence time.[22] BMIPP is therefore mainly trapped in the triglyceride fraction, but is first partially metabolized by β oxidation, then transported into mitochondria, and metabolized by β oxidation.[23] BMIPP follows the enzymatic activation of fatty acids to acyl-coenzyme A, which is common to triglyceride synthesis and to β oxidation. BMIPP accumulation is positively correlated with the intracellular concentration of adenosine triphosphate (ATP), which is required in this activation process.[24] Myocardium, which can uptake and retain BMIPP, must therefore have the capacity to produce ATP, otherwise BMIPP would be washed out from the myocardium soon after the injection. Severe ischemia reduces the capacity for BMIPP trapping, and after reperfusion this capacity continues to decrease for a while. In a canine model, uptake of BMIPP decreased in the area supplied by the coronary artery which was occluded and reperfused as well as in the area supplied by the permanently occluded artery at the acute stage.[25] On the clinical situation, it was reported that BMIPP uptake was decreased in stunned myocardium after reperfusion therapy at the subacute stage (4–10 days after the onset).[8] It is therefore speculated that myocardium reperfused after ischemia fails to retain BMIPP until myocardial ATP levels recover to pre-ischemic levels. These mechanisms may explain why the uptake of BMIPP decreased in the reperfused area not only at the acute stage but also at the subacute stage (1-week after reperfusion) in a rat model.

Limitations

Since dye staining was used as the standard method for the area at risk, we could assess the area of the damaged myocardium but not the severity of the damage to the myocardium. For more accurate evaluation, it may be need to assess the area at risk by means of perfusion tracer or microsphere, but the thickness of the frozen slice was only 5 μm, so that it might be thin enough to assess visually by means of the dye the severity.

Because we could not assess perfusion during reperfusion, we did not confirm the existence of a discrepancy between BMIPP and perfusion tracer such as Tl-201. Nevertheless, as myocardial viability could be assessed by TTC staining or histological findings, we guessed that almost all cases have incomplete infarction and have some residual myocardium. It is thought that in those cases myocardial perfusion recovers earlier than fatty acid metabolism.

Our data show that the area of reduced BMIPP uptake is the area at risk, but in this study the rat model with 60-min complete occlusion and reperfusion of a coronary artery was used. Although this condition produced severe ischemia to cause infarction, we confirmed that there was viable myocardium with mismatch between dye and TTC or H-E staining. If the occlusion time was shorter or ischemia was milder, the area of reduced BMIPP uptake might underestimate the area at risk. In addition, we assessed this method only at two time points, in the acute and subacute phases. It may be necessary to clarify the time course of the relationship between the area of altered BMIPP uptake and the risk area.

In conclusion, our results indicate that the area at risk can be evaluated by BMIPP images both at the acute stage and the subacute stage.

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REFERENCES

1. De Coster PM, Wijns W, Cauwe F, Robert A, Beckers C, Melin JA. Area-at-risk determination by technetium-99m-hexakis-2-methoxyisobutyl isonitrile in experimental reperfused myocardial infarction. *Circulation* 82: 2152–2162, 1990.
2. Sinusas AJ, Trautman KA, Bergin JD, Watson DD, Ruiz M, Smith WH, et al. Quantification of area at risk during coronary occlusion and degree of myocardial salvage after reperfusion with technetium-99m methoxyisobutyl isonitrile. *Circulation* 82: 1424–1437, 1990.
3. Gibbons RJ, Verani MS, Behrenbeck T, Pellikka PA, O'Connor MK, Mahmarian JJ, et al. Feasibility of tomographic ^{99m}Tc-hexakis-2-methoxy-2-methylpropyl-isonitrile imaging for the assessment of myocardial area at risk and the effect of treatment in acute myocardial infarction. *Circulation* 80: 1277–1286, 1989.
4. Christian TF, Schwartz RS, Gibbons RJ. Determinants of infarct size in reperfusion therapy for acute myocardial infarction. *Circulation* 86: 81–90, 1992.
5. Matsuo H, Watanabe S, Nishida Y, Matsubara T, Kano M, Sugiyama A, et al. Assessment of area at risk and efficacy of treatment in patients with acute coronary syndrome using ^{99m}Tc tetrofosmin imaging in humans. *Ann Nucl Med* 7: 231–238, 1993.
6. Ito H, Tomooka T, Sakai N, Higashino Y, Fujii K, Katoh O, et al. Time course of functional improvement in stunned myocardium in risk area in patients with reperfused anterior infarction. *Circulation* 87: 355–362, 1993.
7. Furutani Y, Shiigi T, Nakamura Y, Nakamura H, Harada M, Yamamoto T, et al. Quantification of area at risk in acute myocardial infarction by tomographic imaging. *J Nucl Med* 38: 1875–1882, 1997.
8. Franken PR, De Geeter F, Dendale P, Demoor D, Block P, Bossuyt A. Abnormal free fatty acid uptake in subacute myocardial infarction after coronary thrombolysis: correlation with wall motion and inotropic reserve. *J Nucl Med* 35: 1758–1765, 1994.
9. Nishimura T, Uehara T, Strauss HW. Radionuclide assessment of stunned myocardium by alterations in perfusion, metabolism and function. *Jpn Circ J* 55: 913–918, 1991.
10. Tsubokawa A, Lee JD, Shimizu H, Nakano A, Uzui H, Takeuchi M, et al. Recovery of perfusion, glucose utilization and fatty acid utilization in stunned myocardium. *J Nucl Med* 38: 1835–1837, 1997.
11. Tamaki N, Kawamoto M, Yonekura Y, Fujibayashi Y, Takahashi N, Konishi J, et al. Regional metabolic abnormality in relation to perfusion and wall motion in patients with myocardial infarction: assessment with emission tomography using an iodinated branched fatty acid analog. *J Nucl Med* 33: 659–667, 1992.
12. Fukuchi K, Kusuoka H, Watanabe Y, Fujiwara T, Nishimura T. Ischemic and reperfused myocardium detected with technetium-99m-nitroimidazole. *J Nucl Med* 37: 761–766, 1996.
13. Darsee JR, Kloner RA. The no reflow phenomenon: a time-limiting factor for reperfusion after coronary occlusion? *Am J Cardiol* 46: 800–806, 1980.
14. Iwasaki T, Suzuki T, Tateno M, Sasaki Y, Oshima S, Imai S. Dual-tracer autoradiography with thallium-201 and iodine-125-metaiodobenzylguanidine in experimental myocardial infarction of rat. *J Nucl Med* 37: 680–684, 1996.
15. Yamashita N, Hoshida S, Taniguchi N, Kuzuya T, Hori M, Sakamoto T, et al. Whole-body hyperthermia provides biphasic cardioprotection against ischemia/reperfusion injury in the rat. *Circulation* 98: 1414–1421, 1998.
16. Fishbein MC, Meerbaum S, Rit J, Lando U, Kanmatsuse K, Mercier JC, et al. Early phase acute myocardial infarct size quantification: validation of the triphenyl tetrazolium chloride tissue enzyme staining technique. *Am Heart J* 101: 593–600, 1981.
17. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1: 307–310, 1986.
18. Feiring AJ, Johnson MR, Kioschos JM, Kirchner PT, Marcus ML, White CW. The importance of the determination of the myocardial area at risk in the evaluation of the outcome of acute myocardial infarction in patients. *Circulation* 75: 980–987, 1987.
19. Mizumura T, Nithipatikom K, Gross GJ. Bimakalim, an ATP-sensitive potassium channel opener, mimics the effects of ischemic preconditioning to reduce infarct size, adenosine release, and neutrophil function in dogs. *Circulation* 92: 1236–1245, 1995.
20. Ahrens PJ, Sheehan FH, vom Dahl J, Uebis R. Extension of hypokinesia into angiographically perfused myocardium in patients with acute infarction. *J Am Coll Cardiol* 22: 1010–1015, 1993.
21. Pohost GM, Okada RD, O'Keefe DD, Gewirtz H, Beller G, Strauss HW, et al. Thallium redistribution in dogs with severe coronary artery stenosis of fixed caliber. *Circ Res* 48: 439–446, 1981.
22. Knapp FF Jr, Kropp J. Iodine-123-labelled fatty acids for myocardial single-photon emission tomography: current status and future perspectives. *Eur J Nucl Med* 22: 361–381, 1995.
23. Knapp FF Jr, Ambrose KR, Goodman MM. New radioiodinated methyl-branched fatty acids for cardiac studies. *Eur J Nucl Med* 12: S39–S44, 1986.
24. Fujibayashi Y, Yonekura Y, Takemura Y, Wada K, Matsumoto K, Tamaki N, et al. Myocardial accumulation of iodinated beta-methyl-branched fatty acid analogue, iodine-125-15-(p-iodophenyl)-3-(R,S)methylpentadecanoic acid (BMIPP), in relation to ATP concentration. *J Nucl Med* 31: 1818–1822, 1990.
25. Nohara R, Okuda K, Ogino M, Hosokawa R, Tamaki N, Konishi J, et al. Evaluation of myocardial viability with iodine-123-BMIPP in a canine model. *J Nucl Med* 37: 1403–1407, 1996.