botic effects may have also affected the present results.

This J-LIT subanalysis is the first report of a correlation between the incidence of cerebral infarction and lipid concentrations in a large number of Japanese hypercholesterolemic patients. Our results show that improving the serum lipid concentrations is also important for reducing the incidence of cerebral infarction.

Study Limitation

Although we refer to this study as J-LIT, in reality it was a cohort and observational study rather than an interventional study.

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Alteration in Erythropoietin-Induced Cardioprotective Signaling by Postinfarct Ventricular Remodeling

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ABSTRACT

Postinfarct remodeling impairs mechanisms of ischemic preconditioning. We examined whether myocardial response to activation of the erythropoietin (EPO) receptor is modified by postinfarct remodeling. Four weeks after induction of myocardial infarction (MI) by coronary ligation in post-MI group (post-MI) or a sham operation in sham group (sham), rat hearts were isolated and subjected to 25-min global ischemia/2-h reperfusion. Infarct size was expressed as a percentage of risk area (i.e., left ventricle) from which scarred infarct was excluded (%I/R). The heart weight was 15% larger in post-MI, but there was no intergroup difference in plasma EPO levels or myocardial EPO receptor levels. EPO infusion (5 U/ml) significantly reduced %I/R from 59.9 ± 4.1 to 36.2 ± 4.2 in sham and from 58.1 ± 5.0 to 35.2 ± 4.0 in post-MI. This EPO-induced protection was sensitive to a phosphatidylinositol 3-kinase (PI3K) inhibitor, 2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one (LY294002), in sham. However, neither LY294002 nor wortmannin inhibited the EPO-induced protection in post-MI. Phosphorylation of Janus kinase 2 by EPO was attenuated and phosphorylation of Akt was not detected in post-MI. A guanylyl cyclase inhibitor, 1*H*-[1,2,4]oxadiazole[4,3-*a*]quinoxalin-1-one, and a mitochondrial ATP-sensitive K^+ channel (mitoK$_{ATP}$ channel) blocker, 5-hydroxydecanoate, inhibited EPO-induced protection in both sham and post-MI. Suppressor of cytokine signaling (SOCS)-1 protein level was higher by 50% in post-MI than in sham, although SOCS-3 levels were similar. These findings suggest that postinfarct remodeling disrupts cellular signaling from the EPO receptor to PI3K, presumably by increased SOCS-1. However, in the remodeled myocardium, lack of PI3K/Akt activation by the EPO receptor seems to be compensated by a mechanism upstream of the guanylyl cyclase-mitoK$_{ATP}$ channel pathway to achieve EPO-induced protection.

Erythropoietin (EPO) is an erythropoietic hormone that is produced primarily in juxtatubular cells of the kidney in response to hypoxia, and administration of recombinant human EPO has been used as a standard therapy for renal anemia for a decade. Recently, broad cytoprotective effects of EPO in various tissues have received attention (Maiese et al., 2005). In studies using rat and rabbit hearts, recombinant human EPO as well as derivatives of EPO administered before or at the time of ischemia suppressed myocardial necrosis and apoptosis, ventricular dysfunction, and ventricular remodeling after ischemia/reperfusion (IR) (Calvillo et al., 2003; Moon et al., 2003; Parsa et al., 2003; Lipsic et al., 2004; Wright et al., 2004; Fiordaliso et al., 2005). Furthermore, these studies consistently showed that the phosphatidylinositol 3-kinase (PI3K)-Akt pathway plays a crucial role in the EPO-induced cardioprotection (Parsa et al., 2003; Cai and Semenza, 2004; Bullard et al., 2005; Hanlon et al., 2005; Rafiee et al., 2005), although involvement of mitogen-activated protein kinase, protein kinase C (PKC), and mitochondrial ATP-sensitive K^+ channel (mitoK$_{ATP}$ channel) has also been suggested (Shi et al., 2004; Bullard et al., 2005; Hanlon et al., 2005; Rafiee et al., 2005). However, because normal

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ABBREVIATIONS: EPO, erythropoietin; IR, ischemia/reperfusion; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PC, ischemic preconditioning; mitoK$_{ATP}$ channel, mitochondrial ATP-sensitive K^+ channel; MI, myocardial infarction; LY294002, 2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one; ODQ, 1*H*-[1,2,4]oxadiazole[4,3-*a*]quinoxalin-1-one; 5-HD, 5-hydroxydecanoate; SOCS, suppressor of cytokine signaling; JAK, Janus kinase; eNOS, endothelial nitric-oxide synthase; LVDP, left ventricular developed pressure; bpm, beats per minute; %I/R, infarct size as a percentage of risk area; ERK, extracellular signal-regulated kinase; TNF-α, tumor necrosis factor α; PKG, protein kinase G; HR, heart rate.

myocardium was used in all these previous studies to assess EPO-induced alteration in response to I/R injury, it remains unknown whether EPO can afford cardioprotection to diseased hearts.

In the present study, we assessed whether EPO protects the myocardium in postinfarct hearts with ventricular remodeling. Myocardial infarction provokes remodeling of the heart over a period of several weeks or months, resulting in hypertrophy and interstitial fibrosis in the noninfarcted regions and dilation of the left ventricle. Intracellular signaling pathways responsible for the postinfarct remodeling potentially interact with intracellular signaling that induces cytoprotection. In fact, our previous studies have demonstrated that postinfarct ventricular remodeling made the myocardium refractory to ischemic preconditioning (PC) by disrupting signal transduction from the G-protein-coupled receptor to PKC-ϵ (Miki et al., 2000, 2003). Because there are overlaps in signaling pathways provoked by PC and activated EPO receptors (Fisher, 2003; Smith et al., 2003; Yellon and Downey, 2003), it is possible that signals relevant to the remodeling process may also interfere with the mechanism of EPO-induced protection. Results of the present study suggest that postinfarct remodeling impairs the signaling pathway from activated EPO receptors to the PI3K-Akt pathway, but that a compensatory mechanism upstream of guanylyl cyclase maintains the infarct size-limiting effect of EPO.

Materials and Methods

This study was conducted in accordance with The Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (National Institutes of Health publication 85-23, revised 1996) and was approved by the Animal Use Committee of Sapporo Medical University.

Preparation of Myocardial Infarction and Isolated Heart Perfusion

Male Sprague-Dawley rats (8–10 weeks old) were anesthetized with a mixture of ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.). The rats were intubated with an endotracheal tube and ventilated using a Harvard respirator (model 683; Harvard Apparatus Inc., South Natick, MA) with supplemented oxygen. After a left thoracotomy, a 5.0 silk thread was passed around a marginal branch of the left coronary artery. Rats were then divided into two groups: sham and post-MI groups. In the sham group, the coronary artery was not ligated. In the post-MI group, the coronary branch was permanently ligated to induce myocardial infarction. The surgical wounds were repaired, and 10 mg of ampicillin and 10 mg of cloxacillin were injected intramuscularly for prophylaxis of infection, and the rats were then returned to their cages for recovery. Four weeks (protocol 1) or 2 weeks (protocol 2) after surgery, each rat was brought into the laboratory and reanesthetized with sodium pentobarbital (80 mg/kg i.p.), and the heart was quickly excised for isolated heart preparation. In 28 randomly selected rats (13 rats in the sham group and 15 rats in the 4-week post-MI group), systemic blood pressure and pulse rate were measured in a conscious state using the tail-cuff method (BP-98A; Softran, Tokyo, Japan), and blood samples were collected from the tail vein before isolation of the heart. Plasma EPO level was determined by a radioimmunoassay (Recombigen EPO kit; Mitsubishi Kagaku Iatron, Tokyo, Japan). The excised heart was perfused at a pressure of 75 mm Hg with noncirculating Krebs-Henseleit buffer (118.5 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 24.8 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and 10 mM glucose). The buffer was gassed with 95% O_2, 5% CO_2, and the temperature of the perfusate was maintained at 37°C. A fluid-filled latex balloon with a polyethylene-50 tube was inserted into the left ventricle and was connected to an SCK-580 transducer (Nihon-Kohden, Tokyo, Japan). Coronary flow was measured by timed collection of perfusate dripping from the heart.

Experimental Protocol and Measurement of Infarct Size

Protocol 1. In this protocol, hearts isolated 4 weeks after surgery were examined. After a 20-min stabilization period, all hearts were subjected to 25-min global ischemia and 2-h reperfusion. Before global ischemia, each heart in the sham group was subjected to one of eight treatments: no pretreatment (control); infusion of 5 U/ml recombinant human EPO; 5 μM LY294002, a PI3K inhibitor; EPO plus LY294002; 2 μM ODQ, a guanylyl cyclase inhibitor; EPO plus ODQ; 100 μM 5-HD, a blocker of mitoK_{ATP} channel; or EPO plus 5-HD. In the post-MI group, each heart was subjected to no pretreatment (control); EPO; EPO plus LY294002; EPO plus 100 nM wortmannin, another PI3K inhibitor; EPO plus ODQ; or EPO plus 5-HD. EPO and each inhibitor were infused for 15 min commencing 15 min before the global ischemia.

Protocol 2. Because results of protocol 1 showed that EPO-induced protective mechanisms were altered at 4 weeks after infarction (see *Results*), we examined whether such alteration in myocardial response to EPO develops during the earlier period after infarction. In this protocol, rat hearts were isolated at 2 weeks after coronary ligation and subjected to no pretreatment (control), infusion of EPO, or EPO plus LY294002. Doses of EPO and LY294002 and timings of infusion of these agents were the same as those in protocol 1. All hearts in this protocol were used for infarct size experiments.

After 2 h of reperfusion, hearts were weighed, frozen, and cut into 1.5-mm-thick sections from apex to base. Infarcts in the heart slices were visualized by tetrazolium staining as reported previously (Miki et al., 2000, 2003; Tanno et al., 2000). Fresh infarcts, scar areas because of coronary ligation, and outlines of the ventricle were traced on a clear acetate sheet. The scarred infarct because of coronary ligation was transmural and clearly distinguished from other areas of the left ventricle (Fig. 1), and the scar area was excluded from infarct size measurement.

Immunoblot Assays

In this series of experiments, possible differences in cellular signaling after stimulation of the EPO receptor between post-MI hearts and sham-operated hearts were examined. Left ventricular biopsy samples (0.2~0.3 g) were taken from the isolated perfused hearts using sharp ophthalmology scissors at baseline, at 15 min after EPO infusion or after 25-min ischemia/5-min reperfusion. In the post-MI

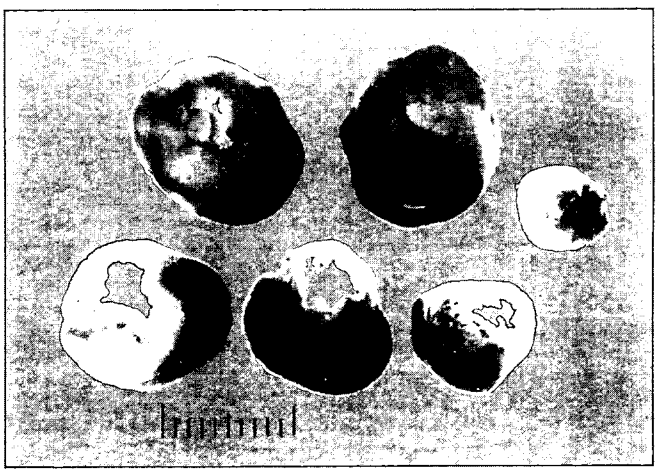

Fig. 1. Photograph of slices of a control heart (i.e., untreated heart) in the post-MI group after 25-min global ischemia/2-h reperfusion. Infarcted areas are visualized by tetrazolium staining.

TABLE 1
Hematological and hemodynamic parameters at 4 weeks after surgery (in situ)
Data are means ± S.E.M.

	WBC	RBC	Platelets	EPO	BW	HW	HR	MBP
	μl	$\times 10^4/\mu l$		mU/ml	g		bpm	$mm\ Hg$
Sham	7908 ± 342	840 ± 16	70.1 ± 2.2	14.5 ± 1.1	456.7 ± 9.5	1.56 ± 0.03	347 ± 10	114 ± 2
Post-MI	7820 ± 436	856 ± 17	72.9 ± 4.4	14.4 ± 0.5	450.7 ± 4.2	1.79 ± 0.03*	338 ± 9	112 ± 2

WBC, white blood cells; RBC, red blood cells; BW, body weight; HW, heart weight; MBP, mean blood pressure.
*$P < 0.05$ versus sham.

group, tissues for immunoblotting were taken from the lateral~posterior wall because scarred infarct was located in the anterior wall~septum of the ventricle. Thus, tissues were sampled from the same regions in the sham group. We carefully excluded border zone tissues (i.e., apparently viable tissues within 1 mm next to scarred infarct) from tissue samples in the post-MI group. Because scarred regions were clearly distinguished from viable myocardium at 4 weeks after the coronary ligation, it was easy to obtain tissue samples exclusively from the noninfarcted areas in rat hearts. The tissues were frozen immediately after sampling in liquid nitrogen and stored at −80°C until biochemical analysis. Frozen heart samples were homogenized in ice-cold buffer containing 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 μg/ml leupeptin, 50 μg/ml phenylmethylsulfonyl fluoride, and a protease inhibitor cocktail tablet (Complete; Roche Applied Science, Penzberg, Germany). The homogenate was centrifuged at 13,000*g* for 15 min to obtain the supernatant. Protein concentration was determined using a Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA). Equal amounts of protein were analyzed by immunoblot assays with the use of antibodies that recognize the following: EPO receptor, suppressor of cytokine signaling (SOCS)-1 and SOCS-3 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), phosphorylated JAK2 (Tyr1007/1008) and total JAK2, phosphorylated Akt (Ser473) and total Akt, and phosphorylated eNOS (Ser1177) and total eNOS (Cell Signaling Technology, Danvers, MA). After detection of phosphorylated proteins, PVDF membranes were stripped from their bound antibodies using a Re-blot Western blot recycling kit (Chemicon, Temecula, CA). The membranes were then blotted with antibodies to total JAK2, Akt, or eNOS. Levels of proteins in immunoblots were determined by densitometric analysis using SigmaGel (SPSS Inc., Chicago, IL). Equal loading of protein onto each lane in the gel was confirmed later from comparable levels of vinculin detected by reblotting with an anti-vinculin antibody (Sigma-Aldrich, St. Louis, MO) and/or comparable densitometric levels of 116-kDa bands (which correspond to vinculin) in the gels stained with Coomassie Brilliant Blue.

Chemicals

LY294002 was purchased from Calbiochem (Darmstadt, Germany), and ODQ, 5-HD, and wortmannin were obtained from Sigma-Aldrich. Recombinant human EPO was kindly provided by Chugai Pharmaceutical Co. (Tokyo, Japan).

Statistics

All data are presented as means ± S.E.M. One-way analysis of variance combined with the Student-Newman-Keuls post hoc test was used to test for differences in heart weight, plasma EPO level, and infarct size between treatment groups. Repeated measures analysis of variance was used to test for differences in cardiac functions in any given group. These statistical analyses were performed by using SigmaStat (SPSS Inc.). The difference was considered significant if the P value was less than 0.05.

Results

Mortality and Complete Blood Counts

In total, 150 rats were initially entered into this experiment. One rat in the sham group and 19 rats in the post-MI group died in their cages after surgery, probably because of atelectasis in the lungs and/or heart failure. Therefore, 130 surviving rats, 59 in the sham group and 71 in the post-MI group, contributed to the following analysis.

There were no significant differences in the numbers of white blood cells, red blood cells, and platelets between the sham and post-MI groups (Table 1). Plasma EPO level in the sham group was 14.5 ± 1.1 mU/ml, and the level was not changed at 4 weeks after MI. Mean blood pressures and heart rates in situ before isolation of the hearts at 4 weeks after surgery were also similar in the two groups. Heart weight, however, was significantly larger in the post-MI group than in the sham group, indicating ventricular remodeling after MI (Table 1).

Data on Cardiac Functions

Protocol 1. Table 2 summarizes parameters of cardiac functions in the sham group. There were no significant dif-

TABLE 2
Summary of data on cardiac functions in isolated sham hearts
Treatment is 1 min before global ischemia; reperfusion is 120 min after reperfusion.
Data are means ± S.E.M.

Sham	Baseline	Treatment	Reperfusion
HR (bpm)			
Control	292 ± 10	291 ± 10	287 ± 7
EPO	277 ± 11	272 ± 10	272 ± 19
LY294002	272 ± 5	269 ± 7	278 ± 7
EPO + LY294002	283 ± 8	272 ± 9	288 ± 2
ODQ	292 ± 16	292 ± 27	281 ± 16
EPO + ODQ	295 ± 16	291 ± 15	269 ± 14
5-HD	285 ± 13	286 ± 14	277 ± 9
EPO + 5-HD	269 ± 14	273 ± 13	267 ± 12
LVDP (mm Hg)			
Control	126 ± 4	126 ± 4	49 ± 4*
EPO	126 ± 5	122 ± 4	66 ± 7*
LY294002	134 ± 17	132 ± 16	45 ± 9*
EPO + LY294002	126 ± 15	121 ± 14	33 ± 7*
ODQ	127 ± 18	113 ± 11	40 ± 5*
EPO + ODQ	128 ± 6	127 ± 5	39 ± 12*
5-HD	128 ± 11	123 ± 9	42 ± 8*
EPO + 5-HD	140 ± 7	140 ± 9	32 ± 10*
CF (ml/min)			
Control	17.5 ± 0.8	17.2 ± 0.8	7.9 ± 0.5*
EPO	19.2 ± 0.6	19.2 ± 0.8	10.6 ± 0.7*
LY294002	22.0 ± 0.7†	23.0 ± 0.7†	10.8 ± 0.5*
EPO + LY294002	18.0 ± 0.4	17.5 ± 0.7	9.0 ± 0.6*
ODQ	16.3 ± 0.5	15.3 ± 0.8	7.5 ± 0.5*
EPO + ODQ	19.2 ± 1.0	19.0 ± 0.8	9.8 ± 0.5*
5-HD	19.0 ± 0.4	18.0 ± 0.8	8.8 ± 1.2*
EPO + 5-HD	20.4 ± 1.2†	19.8 ± 1.1	10.4 ± 1.0*

CF, coronary flow.
*$P < 0.05$ versus baseline; †$P < 0.05$ versus control.

TABLE 3
Summary of data on cardiac functions in isolated post-MI hearts
Treatment is 1 min before global ischemia; reperfusion is 120 min after reperfusion. Data are means ± S.E.M.

Post-MI	Baseline	Treatment	Reperfusion
HR (bpm)			
Control	279 ± 11	278 ± 12	272 ± 10
EPO	282 ± 7	278 ± 13	252 ± 10*
EPO + LY294002	279 ± 13	271 ± 14	264 ± 12
EPO + WM	272 ± 5	244 ± 6*	257 ± 9*
EPO + ODQ	285 ± 8	278 ± 9	287 ± 12
EPO + 5-HD	280 ± 8	279 ± 12	250 ± 8*
LVDP (mm Hg)			
Control	92 ± 11	91 ± 10	37 ± 8*
EPO	106 ± 9	101 ± 10	49 ± 8*
EPO + LY294002	101 ± 10	105 ± 11	40 ± 4*
EPO + WM	99 ± 6	102 ± 4	45 ± 8*
EPO + ODQ	95 ± 4	87 ± 1	35 ± 10*
EPO + 5-HD	100 ± 12	101 ± 13	38 ± 8*
CF (ml/min)			
Control	20.9 ± 1.2	20.7 ± 1.1	11.8 ± 1.6*
EPO	18.5 ± 1.2	18.2 ± 1.2	9.6 ± 0.9*
EPO + LY294002	19.7 ± 0.8	20.7 ± 0.9	11.3 ± 1.8*
EPO + WM	19.2 ± 1.0	18.3 ± 1.1	10.3 ± 1.1*
EPO + ODQ	19.0 ± 0.3	18.4 ± 0.9	9.6 ± 0.7*
EPO + 5-HD	16.7 ± 0.7	16.2 ± 0.8	8.3 ± 0.6*

CF, coronary flow; WM, wortmannin.
*$P < 0.05$ versus baseline.

ferences in baseline heart rate and left ventricular developed pressure (LVDP) among the pretreatment groups, although coronary flow levels in hearts in the LY294002 and EPO + 5-HD groups were slightly higher than that in the control group. Treatment with EPO and/or each blocker did not alter the hemodynamic parameters before the 25-min global ischemia. EPO treatment tended to improve LVDP after reperfusion, although the difference between LVDPs in the control and EPO-treated groups did not reach statistical significance. Coronary flow after reperfusion was decreased in all groups without significant intergroup differences.

In the post-MI hearts, baseline heart rate, LVDP, and coronary flow were comparable in pretreatment groups, although LVDP in the post-MI hearts was slightly lower than that in the sham-operated hearts (Table 3). LVDP and coronary flow after reperfusion were decreased in all pretreatment groups as in the sham group.

Protocol 2. Under baseline conditions, heart rate, LVDP, and coronary flow levels were 278 ± 7 bpm, 103 ± 7 mm Hg, and 17.7 ± 0.5 ml/min, respectively, in post-MI hearts entered this protocol. Alterations of these parameters after ischemia/reperfusion were similar to those observed in post-MI hearts in protocol 1 (data not shown).

Infarct Size Data

Protocol 1. As shown in Table 4, risk area sizes were comparable in the hearts in the sham and post-MI groups that were subjected to pretreatments. In the sham group, pretreatment with EPO significantly reduced infarct size as a percentage of risk area (%I/R) from 59.9 ± 4.1% in controls to 36.2 ± 4.2%. This EPO-induced protection was blocked by LY294002, ODQ, or 5-HD, although these blockers by themselves did not modify infarct size. In the post-MI group, there was no significant difference in the sizes of scarred infarct, ranging from 12 to 19% of the left ventricular mass. These sizes of scarred infarcts seem to be small but actually are underestimates of infarct sizes at the acute phase, because both thinning of scarred infarct and hypertrophy of noninfarct myocardium during the period of 4 weeks after infarction should have reduced volume percentages of the infarcted region. In the post-MI group, size of infarct after 25-min ischemia with no pretreatment (%I/R in control = 58.1 ± 5.0%) was comparable with that in the sham group. EPO limited infarct size (%I/R = 35.2 ± 4.0%) as in the sham group, and this infarct size-limiting effect of EPO was insensitive to PI3K inhibitors; neither LY294002 nor wortmannin abolished the EPO-induced infarct size limitation. ODQ and 5-HD abolished the EPO-induced protection in the post-MI hearts as in the sham-operated hearts. After completion of this protocol, we additionally used three post-MI hearts to

TABLE 4
Infarct size data
Data are means ± S.E.M.

	n	Risk	Infarct	I/R	Scar/LV
		cm^3		%	
Protocol 1 (4 wk)					
Sham					
Control	8	0.43 ± 0.02	0.25 ± 0.01	59.9 ± 4.1	
EPO	7	0.42 ± 0.03	0.15 ± 0.02*	36.2 ± 4.2*	
LY294002	4	0.46 ± 0.02	0.26 ± 0.02	57.1 ± 6.2	
EPO + LY294002	6	0.43 ± 0.01	0.26 ± 0.02	60.8 ± 3.4	
ODQ	4	0.42 ± 0.01	0.29 ± 0.02	68.5 ± 4.4	
EPO + ODQ	5	0.39 ± 0.03	0.24 ± 0.04	60.2 ± 7.3	
5-HD	5	0.41 ± 0.02	0.26 ± 0.02	64.0 ± 2.8	
EPO + 5-HD	6	0.45 ± 0.01	0.27 ± 0.03	61.0 ± 6.8	
Post-MI					
Control	8	0.39 ± 0.03	0.23 ± 0.03	58.1 ± 5.0	15.2 ± 1.7
EPO	6	0.36 ± 0.02	0.13 ± 0.02	35.2 ± 4.0*	17.2 ± 2.7
EPO + LY294002	6	0.36 ± 0.02	0.15 ± 0.03	39.2 ± 6.5*	17.8 ± 2.6
EPO + WM	6	0.39 ± 0.02	0.14 ± 0.02	37.2 ± 5.1*	18.3 ± 2.0
EPO + ODQ	5	0.39 ± 0.02	0.23 ± 0.02	59.2 ± 4.9	18.7 ± 2.4
EPO + 5-HD	6	0.37 ± 0.01	0.23 ± 0.02	60.2 ± 5.0	12.1 ± 2.9
Protocol 2 (2 wk)					
Post-MI					
Control	4	0.36 ± 0.02	0.23 ± 0.01	65.0 ± 5.8	15.5 ± 2.7
EPO	4	0.32 ± 0.01	0.13 ± 0.02*	39.8 ± 4.5*	16.1 ± 2.0
EPO + LY294002	4	0.33 ± 0.01	0.14 ± 0.01*	41.6 ± 2.6*	12.2 ± 1.1

Scar/LV, scar area as a percentage of left ventricle; WM, wortmannin.
*$P < 0.05$ versus control in each group.

assess effects of LY294002 alone on infarct size. In this LY294002-treated post-MI group, %I/R was 61.6 ± 4.1%, which was similar to the %I/R level in the post-MI controls.

Using separate groups of rats, we examined the effect of PC on infarct size in the post-MI group to confirm that postinfarct ventricular remodeling impairs the PC mechanism in rat hearts as we reported for rabbit hearts (Miki et al., 2000, 2003). As expected, PC with two cycles of 5-min ischemia/5-min reperfusion failed to limit infarct size (%I/R = 53.1 ± 5.2%; n = 3) in the post-MI hearts.

Protocol 2. Heart weight was 1.74 ± 0.03 g in the rats 2 weeks after MI, which was only slightly smaller than the heart weight at 4 weeks after MI (protocol 1), suggesting that substantial remodeling had occurred during a 2-week postinfarct period in the rats. EPO significantly reduced %I/R from 65.0 ± 5.8 to 39.8 ± 4.5%. This EPO-induced protection was not blocked by LY294002 (Table 4).

Immunoblot Assays

EPO receptor protein was detected in the rat myocardium (Fig. 2), and its level was not altered by postinfarct ventricular remodeling or acute I/R. There was no significant difference in levels of total JAK2, total Akt, and total eNOS in the study groups; thus, levels of phospho-JAK2, phospho-Akt, and phospho-eNOS were normalized by their total protein levels. EPO increased the level of phospho-JAK2 in sham hearts, but the increase in phosphorylation levels of JAK2 by EPO was significantly attenuated in post-MI hearts (Fig. 3). The baseline level of phospho-Akt was not different in the sham-operated and post-MI hearts. As shown in Fig. 4A, EPO significantly increased the level of phospho-Akt in sham hearts, and this increase was completely blocked by coinfusion of LY294002. The level of phospho-Akt was elevated after I/R compared with the baseline level in the sham group, and this Akt phosphorylation was not enhanced by pretreatment with EPO (Fig. 4B). However, such phosphorylation of Akt by EPO was not observed in the post-MI hearts. EPO tended to increase phospho-eNOS levels both in the sham and MI groups, and this change was diminished by LY294002 in the sham group but not in the MI group (Fig. 5A). There was no significant difference between phospho-eNOS levels after I/R in EPO-treated and untreated hearts (Fig. 5B). SOCS-1 and SOCS-3 levels were higher by 54 and 12%, respectively, in the post-MI group than in the sham group, although only the difference in SOCS-1 levels was statistically significant (Fig. 6). I/R with or without EPO pretreatment did not modify the levels of SOCS-1 and SOCS-3.

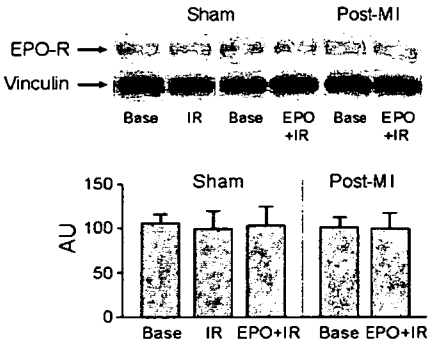

Fig. 2. EPO receptor (EPO-R) protein levels after IR with or without EPO treatment in sham-operated hearts (Sham) and hearts 4 weeks after myocardial infarction (Post-MI). Representative Western blots and summarized data are presented for each group. Values are expressed as arbitrary units (AU) and as means ± S.E.M. n = 3~6. Blots for vinculin are loading controls.

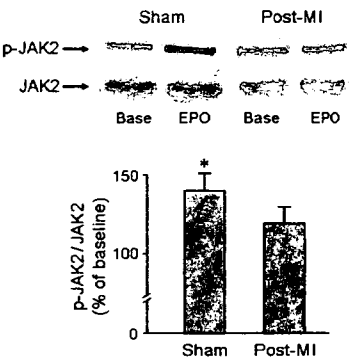

Fig. 3. Phosphorylation of JAK2 after EPO infusion (5 U/ml). Representative Western blots and summarized data are presented for each treatment group. Values are expressed as ratios of phospho-JAK2 protein to total JAK2 protein and as means ± S.E.M. *, $P < 0.05$ versus baseline. n = 5.

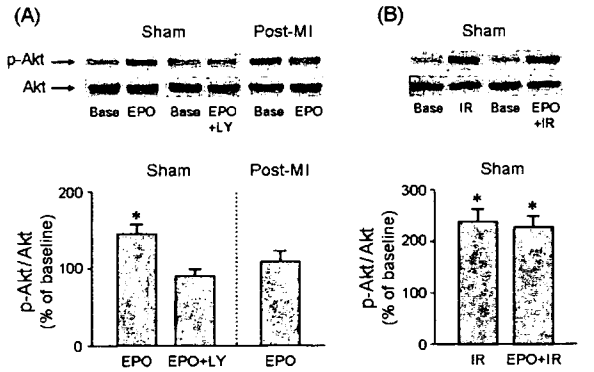

Fig. 4. Phosphorylation of Akt. A, effects of EPO infusion (5 U/ml) and infusion of EPO + LY294002 (5 μM). B, effects of IR with or without EPO. Representative Western blots and summarized data are presented for each treatment group. Values are expressed as ratios of phospho-Akt protein to total Akt protein and as means ± S.E.M. *, $P < 0.05$ versus baseline. n = 3~4.

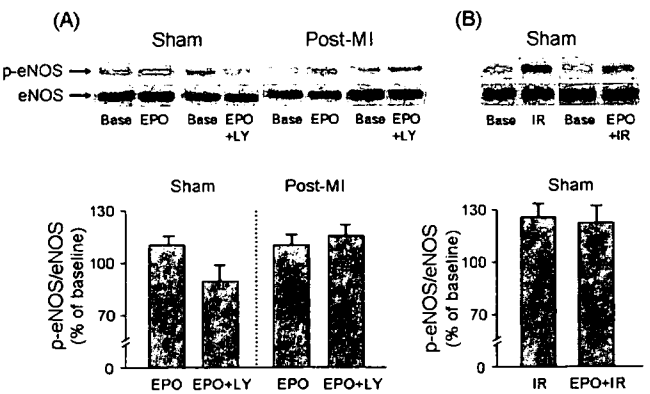

Fig. 5. Phosphorylation of eNOS. A, effects of EPO infusion (5 U/ml) and infusion of EPO + LY294002 (5 μM). B, effects of IR with or without EPO. Representative Western blots and summarized data are presented for each treatment group. Values are expressed as ratios of phospho-eNOS protein to total eNOS protein and as means ± S.E.M. n = 3~5.

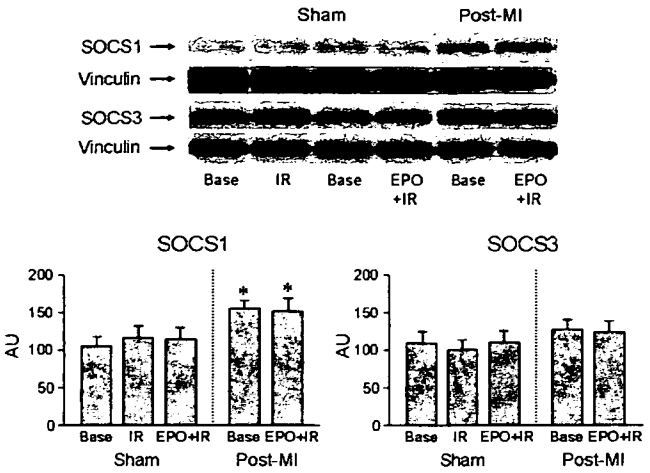

Fig. 6. SOCS-1 and SOCS-3 protein levels after IR with or without EPO treatment in sham-operated hearts (Sham) and hearts 4 weeks after myocardial infarction (Post-MI). Representative Western blots and summarized data are presented for each group. Values are expressed as arbitrary units (AU) and as means ± S.E.M. *, $P < 0.05$ versus sham. n = 3~6. Blots for vinculin are loading controls.

Discussion

In contrast to PC, EPO protected the myocardium in post-MI hearts from myocardial necrosis after I/R, and this EPO-induced protection was equivalent to that in the normal myocardium. However, it was shown that the mechanism by which EPO induced infarct size limitation in the post-MI remodeled hearts was different from that in normal hearts; activation of PI3K and Akt, important signaling steps in protection of sham-operated hearts, was not detected in the myocardium of remodeled hearts. On the other hand, guanylyl cyclase and mitoK$_{ATP}$ channel, which function downstream of Akt (Krieg et al., 2004, 2005; Oldenburg et al., 2004; Costa et al., 2005), were thought to participate in EPO-induced protection in both the normal myocardium and remodeled myocardium.

EPO has been shown to activate a number of cell survival pathways, including those mediated by JAK-signal transducer and activator of transcription, PI3K-Akt, ERK1/2, and PKC (Parsa et al., 2003; Smith et al., 2003; Cai and Semenza, 2004; Shi et al., 2004; Bullard et al., 2005; Hanlon et al., 2005; Rafiee et al., 2005). Although the role of each of these signaling molecules in EPO-induced protection has not been completely clarified, contribution of the PI3K-Akt pathway to the myocardial protection is supported by a number of earlier findings (Parsa et al., 2003; Cai and Semenza, 2004; Bullard et al., 2005; Hanlon et al., 2005; Rafiee et al., 2005). For example, in a study by Parsa et al. (2003), EPO administration resulted in activation of JAK1, signal transducer and activator of transcription 3, Akt, and ERK1/2 in H9c2 cells 15 to 60 min after administration, and EPO-induced reduction in anoxic cell death was eliminated by a PI3K inhibitor but not by an ERK inhibitor. Consistent with these previous findings, 15-min infusion of EPO induced Akt phosphorylation and cardioprotection, both of which were sensitive to LY294002, a PI3K inhibitor, in normal (i.e., sham-operated) hearts in this study (Fig. 4; Table 4). However, such EPO-induced Akt activation was not detected in the myocardium remodeled 4 weeks after infarction (Fig. 4), although EPO protected the myocardium from infarction. Furthermore, neither LY294002 nor wortmannin inhibited the EPO-induced protection in post-MI hearts. Failure of LY294002 to abolish the infarct size-limiting effect of EPO was observed also in the hearts 2 weeks after infarction, suggesting that response of Akt to EPO receptor activation is impaired at the early phase of postinfarct ventricular remodeling.

The mechanism by which myocardial PI3K/Akt lost response to EPO receptor stimulation after development of postinfarct remodeling remains unclear. However, down-regulation of EPO receptor expression can be excluded from possible mechanisms, because the level of the EPO receptor protein was not reduced in the myocardium remodeled 4 weeks after infarction (Fig. 2). One explanation is that up-regulated SOCS proteins suppressed signaling from activated EPO receptors to PI3K. SOCS is a family consisting of eight proteins that function as suppressors of cytokine signaling, and SOCS-1 and SOCS-3 have been shown to negatively regulate JAK activation by EPO and other cytokines (Jegalian and Wu, 2002; Tan and Rabkin, 2005). Indeed, activation of JAK2 by EPO was significantly attenuated in the remodeled myocardium (Fig. 3), in which SOCS-1 level was significantly elevated by 50% (Fig. 6). The level of SOCS-3, which is known to be up-regulated by angiotensin II (Calegari et al., 2003, 2005), was slightly higher in the myocardium after 4 weeks of remodeling than in the control, but this difference was not statistically significant. The reason for SOCS-1 selective up-regulation in the present model of postinfarct remodeling remains to be investigated. Activation of the TNF-α receptor might have been involved in the SOCS-1 up-regulation, because the serum level of TNF-α and myocardial TNF-α mRNA level were shown to be elevated in rats after myocardial infarction (Ono et al., 1998; Berthonneche et al., 2004; Schulz et al., 2004; Tan and Rabkin, 2005).

Despite lack of Akt activation, EPO afforded cardioprotection against infarction to postinfarct hearts, and the extent of cardioprotection was equivalent to that in the normal myocardium. These results suggest that there is a mechanism in postinfarct hearts to compensate the lack of signal input from the activated PI3K-Akt pathway, sending signals to downstream mediators of cell protection. As an important mechanism of cell protective signals distal to Akt, opening of the mitoK$_{ATP}$ channel by protein kinase G (PKG) has been suggested by a series of studies on mechanisms of PC against infarction (Krieg et al., 2004, 2005; Oldenburg et al., 2004; Costa et al., 2005). Krieg et al. (2004) showed that an Akt inhibitor and transfection of dominant negative Akt abolished NO-mediated activation of the mitoK$_{ATP}$ channel in response to PC triggered by bradykinin receptors. A recent study by Costa et al. (2005) demonstrated opening of the mitoK$_{ATP}$ channel in isolated mitochondria by addition of exogenous active PKG. Furthermore, it has been reported that EPO was capable of stimulating NO production in the rat hippocampus and endothelial cells (Beleslin-Cokic et al., 2004; Yamamoto et al., 2004). Unfortunately, we could not demonstrate significant elevation of phospho-eNOS level by EPO, although there was a trend for increase by 10% in the present study. However, a larger difference may have been detected at later time points as it was in a study by Bullard et al. (2005), who observed a 2-fold increase in phospho-eNOS induced by EPO in rat hearts 15 min after reperfusion, and

the possibility of involvement of other NO-producing mechanisms also cannot be excluded. Nevertheless, we found for the first time that ODQ, a guanylyl cyclase inhibitor, abolished cardioprotection afforded by EPO similarly in both sham-operated and postinfarct hearts. Furthermore, this effect of ODQ was mimicked by 5-HD, a mitoK$_{ATP}$ channel blocker. Together with the results obtained by using PI3K inhibitors, these findings provide pharmacological evidence supporting the notion that PKG-mediated opening of the mitoK$_{ATP}$ channel is downstream of PI3K-Akt in EPO-induced cardioprotection and that compensation for the lack of Akt activation in postinfarct hearts is induced upstream of guanylyl cyclase.

It has been reported that EPO induced intracellular translocation of PKC-ϵ in buffer-perfused rabbit and rat hearts and that the infarct size-limiting effect of EPO was blocked by chelerythrine, a selective PKC inhibitor, indicating contribution of PKC to EPO-induced protection (Shi et al., 2004; Hanlon et al., 2005; Rafiee et al., 2005). PKC-ϵ is thought to elicit opening of the mitoK$_{ATP}$ channel, leading to cardioprotection (Sato et al., 1998; Yellon and Downey, 2003). However, our previous studies (Miki et al., 2000, 2003) have shown that PC fails to elicit PKC-ϵ translocation and enhancement of anti-infarct tolerance in rabbit hearts remodeled after infarction. We confirmed lack of myocardial response to PC in postinfarct rat hearts also using infarct size as an endpoint. Therefore, it is unlikely that PKC is involved in maintenance of myocardial response to EPO in postinfarct hearts.

In the present study, EPO was infused before ischemia and responses of signaling molecules during the pretreatment period were correlated with infarct tolerance of the myocardium. No significant difference was detected in the level of phospho-Akt upon reperfusion between the control group and EPO-pretreated group, although infarct size was significantly smaller in the EPO-treated group. However, efficacy of EPO for suppressing lethal reperfusion injury has been examined recently in several studies (Lipsic et al., 2004; Bullard et al., 2005; Hanlon et al., 2005). Administration of EPO at the time of reperfusion resulted in infarct size limitation to an extent similar to that achieved by pretreatment with EPO in those studies (Lipsic et al., 2004; Hanlon et al., 2005). Furthermore, the importance of activation of signaling pathways at the time of reperfusion, including the PI3K-Akt pathway, for myocardial salvage has been suggested in cardioprotection afforded by preconditioning (Hausenloy and Yellon, 2004). These findings suggest the possibility of clinical use of EPO for patients with acute myocardial infarction as an adjunct therapy to coronary reperfusion. Furthermore, a study by Namiuchi et al. (2005) showed that serum EPO level was an independent predictor for cumulative creatine kinase release in patients with first acute myocardial infarction, suggesting a cardioprotective role of endogenous EPO. In the present rat preparation, the role of endogenous EPO in protection against ischemic injury could not be assessed because plasma EPO level was not significantly changed 4 weeks after infarction. The effects of EPO administration at the time of reperfusion on myocardial necrosis and cell signaling in hearts remodeled after infarction warrant further investigation.

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Early Positive Biomarker in Relation to Myocardial Necrosis and Impaired Fatty Acid Metabolism in Patients Presenting With Acute Chest Pain at an Emergency Room

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Background Measurement of circulating biomarkers has enabled early diagnosis and risk assessment of acute coronary syndrome. This study sought diagnostic values of the first single-point data of biomarkers obtained soon after patient arrival by comparing with scintigraphically quantified myocardial injury in patients presenting with acute chest pain at an emergency room.

Methods and Results Serial blood samples were taken soon after arrival in an emergency department in 74 patients with suspected acute coronary syndrome to quantify blood levels of troponin-T (TnT), heart-type fatty acid-binding protein (H-FABP), myocardial-bound creatine kinase (CK-MB), and myoglobin. Myocardial perfusion and metabolic defects were scintigraphically quantified. The first single-point data had high positive predictive values for detecting the defects (80–100%) but low negative predictive values (15–41%). CK-MB and TnT had higher specificities (73–100%) but significantly lower positive rates (22–27%) than the others (61–68%), resulting in greater sensitivities of H-FABP and myoglobin (75–80%) than those of CK-MB and TnT (29–35%). Among biomarkers, TnT peak concentrations most closely correlated with scintigraphic abnormalities.

Conclusion H-FABP can contribute to early detection of myocardial injury and TnT is most likely to correlate with injured myocardial mass. The differential features of biomarkers are complementary in patients with acute chest pain presenting at an emergency room. (*Circ J* 2006; **70:** 419–425)

Key Words: Acute coronary syndrome; Biomarker; BMIPP; Heart-type fatty acid-binding protein; Myocardial infarction; Single-photon emission computed tomography; Troponin-T

Early diagnosis and prognostification of patients with acute chest pain is crucial at an emergency department because there is considerable variability in the patterns of acute chest symptoms mimicking or suggestive of acute coronary syndrome. Advances in techniques for the measurement of circulating troponin levels have enabled early diagnosis and risk assessment of acute coronary syndrome in patients presenting with acute chest pain[1–7] In addition to conventional blood markers, heart-type fatty acid-binding protein (H-FABP) has recently emerged as a new circulating cardiac biomarker. H-FABP is a low-molecular-weight (15–16 kDa) cytosolic protein, is highly specific for cardiomyocytes and plays an important role in intracellular transport of fatty acids for β-oxidation in mitochondria[8–12] Recent investigations have shown that H-FABP enables early and precise diagnosis and risk assessment of acute coronary syndrome in patients presenting with acute chest pain compared to other biomarkers[13,14] Cardiac biomarkers, however, have advantages and disadvantages due to different time-dependent features for the evaluation and management of patients with suspected acute coronary syndrome[13] Although it is necessary to identify peak values of biomarkers for precise quantification of the injured myocardial mass, it would be clinically useful to know how precisely the first single-point data obtained soon after patient arrival at an emergency room can predict the severity of myocardial injury, and to determine differences between the characteristics of biomarkers.

This study was designed to clarify whether the first single-point data of biomarkers in patients with suspected acute coronary syndrome can predict the severity of the injured myocardium quantified by 2 kinds of scintigraphic techniques using perfusion and metabolic tracers, by comparing with the peak values of biomarkers. The present study cohort consisted of consecutive patients presenting with acute chest pain suggestive of acute coronary syndrome at an emergency room and in whom troponin-T (TnT), H-FABP, myocardial-bound creatine kinase (CK-MB) and myoglobin were quantitatively evaluated.

Methods

Patient Population

We enrolled 74 consecutive patients presenting with acute chest pain suggestive of acute coronary syndrome who were admitted to the following registered medical centers in Hokkaido, Japan in this study: Sapporo Medical University Hospital, Sapporo; Oh-ji General Hospital, Tomakomai; Municipal Kushiro General Hospital, Kushiro; Hakodate Goryokaku General Hospital, Hakodate; Sapporo

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Table 1 Patient Backgrounds and Final Diagnoses

Characteristic	
Patient number	*n=74*
Age, years	*64±1*
Gender (M/F)	*46/28*
Final diagnosis	
Acute myocardial infarction	*n=41*
Unstable angina	*n=13*
Effort angina	*n=6*
Spastic angina	*n=4*
Heart failure	*n=5*
Pulmonary embolism	*n=1*
*Non-cardiac disease**	*n=4*
Left ventricular ejection fraction, %	*52±2*
Onset-to-imaging interval, days	
Perfusion SPECT (n=68)	*29±6*
BMIPP SPECT (n=60)	*23±5*

Values are shown as a mean±SD.
**Non-cardiac diseases consisted of neuralgia, neurally mediated syncope, and gastric ulcer.*
SPECT, single-photon emission computed tomography; BMIPP, β-methyl iodophenyl pentadecanoic acid.

Social Insurance General Hospital, Sapporo; Shin-nittetsu Muroran General Hospital, Muroran; Sapporo Junkanki Clinic, Sapporo; and Teine Keijinkai Hospital, Sapporo. Entry criteria were: (1) the presence of acute anterior, precordial, or left-sided chest pain suggestive of acute coronary syndrome based on careful history taking; (2) a clinical need to measure conventional biomarkers for the detection of myocardial injury; (3) the first blood sample for measurements of TnT, H-FABP, CK-MB and myoglobin having been obtained within 30 min after patient arrival at the emergency room; (4) no history of previous myocardial infarction or coronary revascularization; and (5) informed consent having been obtained for entry in the present study. Forty-six patients were men and 28 were women. The mean age of the subjects was 63.7±1.4 years (Table 1).

Final clinical diagnoses were established before discharge. The diagnosis of coronary artery disease was made by clinical history in conjunction with other clinical features, electrocardiographic changes and serum biomarkers as follows: (1) acute myocardial infarction (AMI) established by typical clinical symptoms (severe chest pain lasting for 30 min or more), ECG changes (ST-segment elevation or depression in 2 or more leads of the standard 12-lead electrocardiogram for over 30 min) and positive serum biomarkers (elevated troponin or CK-MB during the first 2 days after onset of chest pain); (2) unstable angina based on the criteria of Brawnwald et al[15] using clinical symptoms (new-onset, prolonged, worsening and/or rest angina), no ST-segment elevation and negative serum biomarkers after onset of chest pain; and (3) other types of angina established by clinical symptoms (rest or effort-induced angina), evidence of reversible ischemia on an electrocardiographic and/or scintigraphic stress test, negative serum biomarkers and an angiographic finding of coronary luminal narrowing of 75% or more or coronary spasm when necessary. The diagnosis of heart failure was made by clinical symptoms (easy fatigability, exertional dyspnea, palpitation and orthopnea) and signs (jugular vein dilatation, moist rales, S3 gallop and peripheral edema), pulmonary congestion or cardiomegaly on a chest radiograph, and impaired cardiac function with a let ventricular ejection fraction of 40% or less.

Study Protocol and Measurement of Biomarkers

The first blood sampling was performed within 30 min after patient arrival at an emergency room, and then serial samplings were performed at 6-h intervals for 48 h when indicated. All samples collected for the present study were delivered to a core laboratory in Tokyo, where biomarkers were measured using methods previously reported![13] Briefly, serum levels of H-FABP, myoglobin and TnT, and serum CK-MB activity were measured by a 2-step direct sandwich-ELISA method with monoclonal mouse anti-human H-FABP antibodies, a radioimmunoassay (Myoglobin Kit [Daiichi] III, Daiichi Radiosiotope Co, Tokyo, Japan), an electrochemiluminescence immunoassay (EcLusys troponin T 3 stat, Roche Diagnostics Co, Tokyo, Japan), and an immunoinhibition assay (Merk Auto CK-MB, Kanto Chemical Co, Tokyo, Japan), respectively. According to the manufacturers' manuals, the cut-off levels of serum myoglobin and TnT concentrations and serum CK-MB activity were 60 ng/ml, 0.1 ng/ml and 25 U/L, respectively. The lower limit of H-FABP detection was 1.25 ng/ml, and a cut-off level to define a significant elevation was set at 6.2 ng/ml![10,13]

During convalescence (nearly 4 weeks) after admission, myocardial perfusion imaging using ^{99m}Tc-tetrofosmin or ^{99m}Tc-sestamibi (600–740 MBq) and myocardial fatty acid imaging using ^{123}I-β-methyl-p-iodophenyl-pentadecanoic acid (BMIPP) (111 MBq) were performed at rest, separately within an interval of a few days. Following planner imaging, single-photon emission computed tomography (SPECT) data were collected at 5-degree increments for 30 s per increment during a 180- or 360-degree rotation using a rotating gamma camera equipped with a high-resolution, parallel-hole collimator, and were stored in a 64×64 word matrix nuclear medicine computer system. Following reconstruction of transverse tomograms by a filtered back projection algorithm, short-axis, vertical long-axis, and horizontal long-axis tomograms were obtained. Myocardial uptake of perfusion tracer and BMIPP was scored visually by 2 nuclear cardiologists with semi-quantitative visual analysis using a 5-point, 20-segment model (Fig 1): 0=normal, 1=slight reduction, 2=moderate reduction, 3=severe reduction, and 4=absence. Summed rest score was calculated in both SPECT images. Abnormality was defined as when there was at least 1 segment with a score of 3 or more

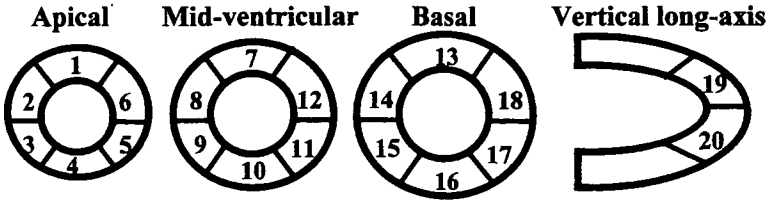

Fig 1. Segmental analysis of perfusion and β-methyl-p-iodophenyl-pentadecanoic acid tomograms using a 5-point, 20-segment model: 0=normal, 1=slight reduction, 2=moderate reduction, 3=severe reduction, 4=absence.

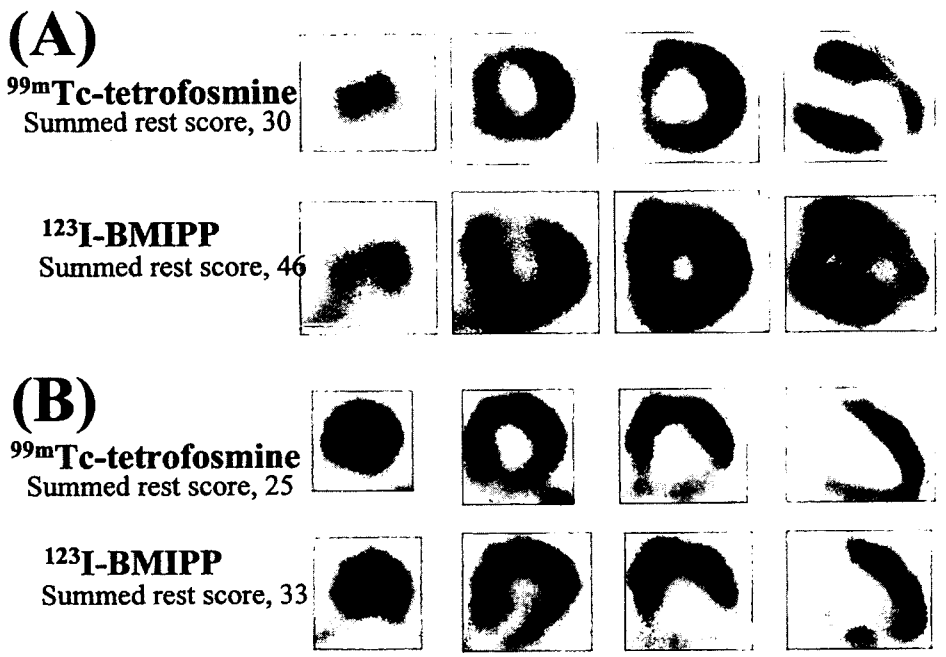

Fig 2. Resting myocardial perfusion and β-methyl-p-iodophenyl-pentadecanoic acid (BMIPP) tomograms of patients with acute myocardial infarction. (A) A 58-year-old male with anterior wall infarction whose initial heart-type fatty acid-binding protein (H-FABP), troponin-T (TnT), myocardial-bound creatine kinase (CK-MB) and myoglobin levels were 400 ng/ml, 21.9 ng/ml, 230.4 IU/L and 2,300 ng/ml, respectively; (B) A 59-year-old male with inferior wall infarction whose initial H-FABP, TnT, CK-MB and myoglobin levels were 22 ng/ml, 0.17 ng/ml, 7.3 IU/L and 97 ng/ml, respectively.

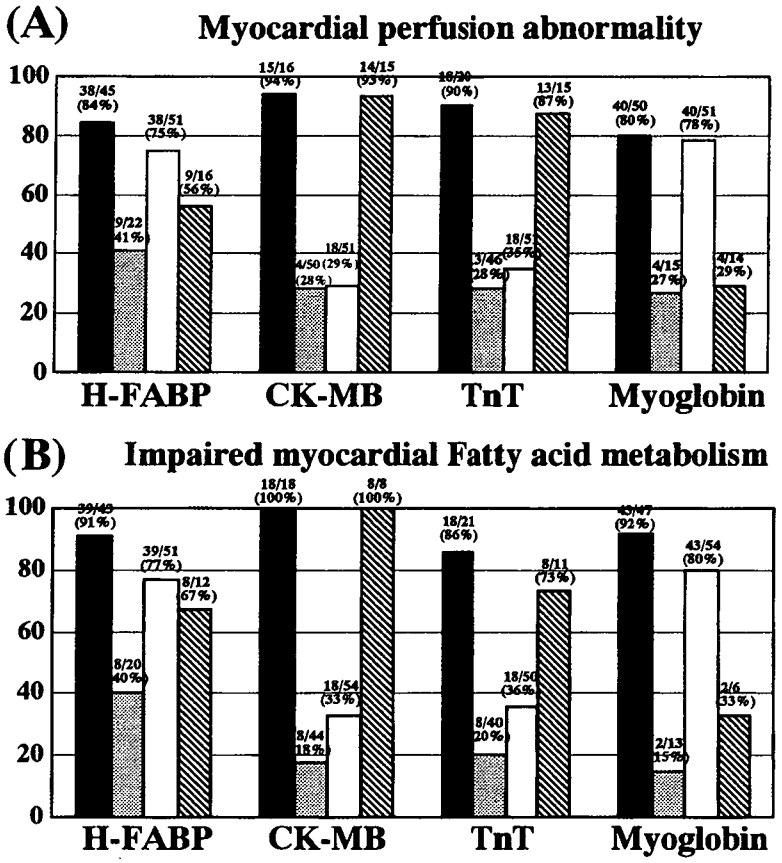

Fig 3. Comparison of positive (closed columns) and negative (dotted columns) predictive values, sensitivities (open columns) and specificities (slashed columns) of the first single-point data of each biomarker for the detection of abnormality of myocardial perfusion (A) and β-methyl-p-iodophenyl-pentadecanoic acid uptake (B). H-FABP, heart-type fatty acid-binding protein; CK-MB, myocardial-bound creatine kinase; TnT, troponin-T.

or when there were 2 segments with a score of 2 or more.

Statistics

Statistical values are shown as mean±1 standard deviation. Comparison of mean values in 2 groups was made using the unpaired Student's t-test, and prevalence was compared using the chi-squared test. Correlations among integrated tracer defect (summed scores) and peak values of biomarkers were analyzed by using linear regression analysis with standard errors of the estimate and a 95% confidence interval, and by using Spearman's rank correlation test because of non-parametric features of scintigraphic scores. A p-value less than 0.05 was considered to be statistically significant. These analyses were performed

Table 2 Correlation Coefficients of the First Sampling Data of Biochemical Markers With Scintigraphic Scores on Perfusion and BMIPP Imagings

	Perfusion			*BMIPP*		
	Linear regression		*Spearman's*	*Linear regression*		*Spearman's*
	Coefficient	*p value*	*p value*	*Coefficient*	*p value*	*p value*
H-FABP	0.143	0.3433	0.0002	0.305	0.0190	0.0001
CK-MB	0.291	0.0498	0.0015	0.265	0.0424	0.0007
Troponin-T	0.324	0.0282	0.0002	0.328	0.0111	0.0003
Myoglobin	0.332	0.0242	0.0002	0.309	0.0174	0.0011

BMIPP, β-methyl iodophenyl pentadecanoic acid; H-FABP, heart-type fatty acid binding protein; CK-MB, creatine kinase-MB.

Table 3 Correlation Coefficients of Peak Values of Biochemical Markers With Scintigraphic Scores on Perfusion and BMIPP Imagings

	Perfusion			*BMIPP*		
	Linear regression		*Spearman's*	*Linear regression*		*Spearman's*
	Coefficient	*p value*	*p value*	*Coefficient*	*p value*	*p value*
H-FABP	0.296	0.0459	0.0005	0.326	0.0350	0.0009
CK-MB	0.438	0.0023	0.0012	0.459	0.0023	0.0009
Troponin-T	0.503	0.0004	<0.0001	0.538	0.0002	<0.0001
Myoglobin	0.450	0.0017	0.0004	0.417	0.0060	0.0046

Abbreviations see in Table 2.

Table 4 Correlations Between Peak Values of Biochemical Markers and Scintigraphic Scores When Blood Sampling Started Within 24 h From Onset

	Perfusion			*BMIPP*		
	Linear regression		*Spearman's*	*Linear regression*		*Spearman's*
	Coefficient	*p value*	*p value*	*Coefficient*	*p value*	*p value*
H-FABP	0.414	0.0102	0.0001	0.421	0.0140	0.0023
CK-MB	0.597	<0.001	<0.0001	0.574	0.0003	0.0012
Troponin-T	0.626	<0.001	<0.0001	0.626	<0.001	<0.0001
Myoglobin	0.641	<0.001	<0.0001	0.565	0.0005	0.0023

Abbreviations see in Table 2.

using a computer software program, the SPSS statistical program package (SPSS version 11.0, SPSS Inc, Chicago, IL, USA).

Results

Table 1 shows the final diagnoses of the 74 patients: 41 patients had AMI, 13 had unstable angina pectoris, 3 had effort angina pectoris, 3 had effort and rest angina pectoris, 4 had spastic angina, and 5 had heart failure. The remaining 5 had non-cardiac diseases; 1 patient had pulmonary embolism and 4 patients had atypical chest pain without definitive cardiac disease. Coronary angiography was performed in 69 of 74 patients. Thirty-two of 41 patients with AMI underwent percutaneous transluminal coronary angioplasty (PTCA) at an acute stage. In 66 (89.2%) of 74 patients, coronary arteries were patent at the first blood sampling.

Mean left ventricular ejection fraction was 52±2%. Perfusion imaging was performed in 68 patients with an onset-to-perfusion SPECT imaging interval of 29±6 days and BMIPP imaging in 60 patients with an onset-to-SPECT imaging interval of 23±5days. The remaining patients, however, did not undergo scintigraphic imaging because of the patients' inconvenience or early discharge. The median period between onset of chest pain and first blood sampling was 5.8 h, and first blood samples in 85% of the patients were obtained within 72 h. The mean interval between onset of chest pain and first blood sampling of each diagnosis were as follows: patients with AMI: 35.1±144.0 h; patients with unstable angina pectoris: 32.6±46.7 h; patients with effort angina pectoris: 855±1,357 h; patients with spastic angina: 75.8±51.0 h; patients with non-cardiac disease: 5.3±4.5 h. The mean interval of AMI and non-AMI were 35.1±144.0 vs 206±661 h, respectively (p=0.118). Fig 2 shows typical SPECT images of myocardial perfusion and fatty acid metabolism in 2 patients with AMI who had elevated first and peak TnT levels.

Diagnostic values of the first single-point data of biomarkers for the detection of myocardial perfusion or BMIPP abnormalities are shown in Fig 3. Each marker had excellent positive predictive values for the detection of myocardial perfusion abnormality (80–94%) and impaired myocardial fatty acid metabolism (86–100%), but low negative predictive values (15–41%). CK-MB and TnT had higher specificities (73–100%) but significantly (p<0.001) lower positive rates (16/74, 22% for CK-MB and 20/74, 27% for TnT) than did H-FABP (45/74, 61%) and myoglobin (50/74, 68%), resulting in greater sensitivities of H-FABP and myoglobin (75–80%) than those of CK-MB and TnT (29–35%). A discrepancy between positive biomarkers and negative scintigraphic abnormality was observed in nearly 10–15% of the patients. Out of 13 patients

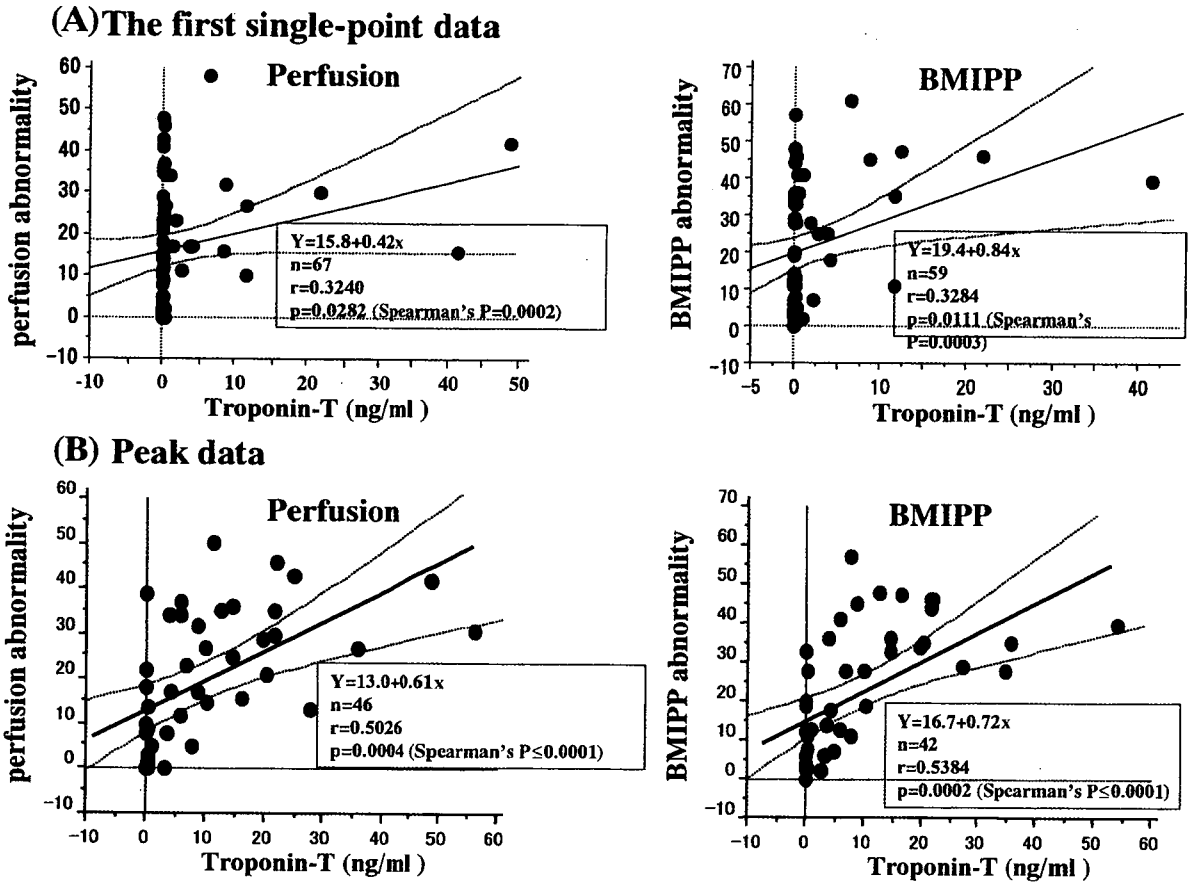

Fig 4. Correlations between troponin-T levels at the first single point and abnormalities (summed rest scores) of myocardial perfusion (A) and β-methyl-p-iodophenyl-pentadecanoic acid (BMIPP) uptake (B). Dotted lines indicate a 95% confidence interval.

with unstable angina but no ST-segment elevation infarction, 4 (31%) patients had positive H-FABP, 8 (62%) had positive myoglobin, 2 (15%) had TnT and all had positive CK-MB, while perfusion defect and metabolic damage were observed in 5 and 7 patients, respectively. In the present study, 41 patients arrived at the hospital within 12 h. The accuracy of H-FABP, CK-MB, TnT, and myoglobin for perfusion SPECT were 78.4, 48.6, 62.2, and 78.4%, respectively, for patients presenting within 0–12 h from onset; 75.9, 55.2, 65.5, and 72.4%, respectively, for those presenting within 2–12 h from onset; and 64.7, 58.8, 64.7, and 58.8%, respectively, for those presenting within 4–12 h from onset. H-FABP and myoglobin had higher sensitivity and specificity in early phase than CK-MB or TnT.

Tables 2 and 3 show results of linear regression analysis and Spearman's rank correlation test using the first single-point or peak data of biochemical markers and abnormalities on myocardial perfusion or BMIPP images. Although the first single-point data of CK-MB, TnT and myoglobin significantly correlated with summed rest scores of perfusion and BMIPP images, peak values of H-FABP, CK-MB, TnT and myoglobin had closer correlations with scintigraphic abnormalities. Overall, the first single-point and peak values of TnT level more closely correlated with extents of perfusion and BMIPP abnormalities than did those of H-FABP or CK-MB (Tables 2 and 3): y=13.0+0.61x, r=0.503, p=0.0004 for myocardial perfusion and y=16.7+0.72x, r=0.538, p=0.0002 for BMIPP (Fig 4). When only patients in whom the first blood sampling had been performed within 24 h after onset of acute chest pain were considered for this analysis, better correlations between peak values of biomarkers and defect scores of myocardial perfusion or BMIPP images were found (Table 4); correlation coefficients were 0.414 and 0.421 for H-FABP, 0.597 and 0.574 for CK-MB, 0.626 and 0.626 for TnT, and 0.641 and 0.565 for myoglobin, respectively. Biomarker levels in 8 patients with AMI who did not undergo PTCA were significantly lower than in patients in whom the coronary artery was patent. In the 8 patients, the interval from onset of chest pain to first blood sampling was longer than in other patients.

Discussion

Biomarkers for the Detection of Myocardial Injury

The results of the present study revealed that biomarkers have high positive predictive values (80–100%) but low negative predictive values (15–41%) for the detection of scintigraphic defects of myocardial perfusion and fatty acid uptake by using the first single-point data on arrival. CK-MB and TnT, however, had lower positive rates (22% for CK-MB and 27% for TnT) than did H-FABP (61%) and myoglobin (68%), resulting in greater sensitivities of H-FABP and myoglobin (75–80%) than those of CK-MB and TnT (29–35%). Among the biomarkers, therefore, H-FABP had better overall diagnostic values for the detection of myocardial injury, including high positive predictive

values (84–91%), high sensitivities (75–77%) and better negative predictive values (nearly 40%). Myoglobin is less specific than is H-FABP or TnT. Troponin is established as a biomarker and is widely used but less sensitive at a very acute phase of myocardial injury (within 6 h from the onset of symptoms)[1,2] Our recent study showed that H-FABP has greater diagnostic value in patients with suspected acute coronary syndrome than does TnT, CK-MB or myoglobin within 6 h following onset of acute chest pain rather than at a later stage[13] This is because H-FABP is a small soluble protein that is abundantly present in the cytoplasm and can be rapidly released into circulation shortly after the onset of sarcolemmal injury.

Low negative predictive values of the 4 biomarkers indicate high false-negative results of first single data, suggesting the necessity of serial measurements of biomarkers for better diagnosis of myocardial injury when negative results are obtained. Conversely, positive biomarkers and negative scintigraphic abnormality was observed in patients with unstable angina but with no ST-segment elevation infarction. Minimal myocardial injury or micronecorosis can be identified by biomarkers but not by an imaging technique because of limited SPECT spatial resolution. Delayed contrast-enhanced magnetic resonance may be a promising imaging tool to identify subendocardial infarction or a small infarct area because of a high spatial resolution. On the other hand, positive predictive values were reduced by false-positive results due to a non-ischemic or non-cardiac cause of elevation in biomarkers observed in patients with acute myocarditis, acute heart failure or impaired renal function[10,13]

Biomarkers for the Prediction of Injured Myocardial Mass

Scintigraphic techniques have contributed to the quantification of injured myocardial mass. A resting myocardial perfusion defect during convalescence reflects myocardial necrosis that is closely related to left ventricular ejection fraction and mortality rate[15–20] Although patients with biomarker-positive but SPECT-negative myocardial injury have preserved left ventricular systolic function, their long-term prognosis has not been determined. In this study, myocardial metabolic injury was also evaluated by fatty acid imaging with BMIPP because myocardial fatty acid metabolism is more susceptible to ischemia and could be a better marker of myocardial injury than myocardial perfusion abnormality[21–24] Cardiac fatty acid metabolism is impaired even in the myocardium that survives after an acute ischemic event, and impaired BMIPP uptake is closely related to intracellular high-energy phosphate depletion due to ischemia[22] and to regional wall motion abnormality[23,24] The present study showed that the first single-point troponin data on arrival can reflect injured myocardial mass, although the accuracy is limited and peak values of biomarkers more closely correlated with scintigraphically assessed injured mass than did the first single-point data. This is because the accuracy depends not only on timing of patient arrival at an emergency room after onset of chest pain, but also on the process by which biomarkers are released into the blood following acute ischemic injury. Overall, TnT had better correlations with myocardial perfusion and metabolic defect sizes among the biomarkers. Measurement of troponin level enables prediction of the risk of mortality in patients with acute coronary syndrome[1–7] Recent studies have shown that an early or sustained elevation in H-FABP level in patients presenting with chest pain at an emergency department could indicate unfavorable clinical outcomes[13,14] The present study results indicate that elevated biomarkers at an early stage of chest symptoms, particularly TnT, are useful for the prediction of the severity of ischemia-induced metabolic injury and myocardial necrosis as determinants of patient prognosis. This is because viable myocardium in an area at risk assessed by cardiac fatty acid imaging or stress perfusion imaging is responsible for future cardiac events as well as infarct size[25,26]

Study Limitations

The false-negative results of the first single-point data on arrival indicate limitations in precise diagnosis and prediction of myocardial injury determined at convalescence. The significant but limited correlations between biomarkers and injured myocardial mass assessed by scintigraphic imaging are due to differences in large variations of timings of patient arrival, time-dependent sensitivities of biomarkers, types of myocardial injury and different process by which biomarkers are released into the circulation. Large variations of blood sampling interval are inevitable in this prospective study because the onset-to-arrival interval is dependent on patient condition and accessibility to the hospital. A large scale study using a number of patients with acute chest pain could overcome the limitations to clarify more precisely the difference in diagnostic efficacies among biomarkers of the first data obtained on patient arrival[27] Myocardial necrosis and metabolic impairment were assessed during convalescence of acute coronary syndrome because perfusion abnormality and metabolic derangement are changeable during the first week but stabilize 2 to 4 weeks later. Quantitative data of H-FABP were obtained 24 h after blood sampling in this study. A rapid panel test for H-FABP measurement using a whole-blood sample, which was recently developed, can provide a qualitative result within 15 min[27] as well as that for troponin[28] Therefore, combined use of a rapid qualitative assessment and a quantitative measurement of biomarkers could contribute to early decision-making in an emergent situation and to early prediction of the severity of ischemia-induced myocardial injury. Finally, more recently noted biomarkers, such as brain natriuretic peptide, C-reactive protein and myeloperoxidase, remain to be compared to reveal early diagnostic and prognostic values in patients with suspected acute coronary syndrome.

Conclusions

Despite limited ability, the first single-point data of H-FABP on arrival at an emergency unit are useful for early detection of myocardial injury among the biomarkers currently available. Quantitative serial assessment of serum TnT level is most likely to contribute to the prediction of the size of the injured myocardium in patients presenting with acute chest pain suggestive of acute coronary syndrome. Thus, differential features among biomarkers are likely to be complementary, and suggest that the combined assessment of these markers can contribute to precise risk stratification in patients with acute chest pain in an emergency room.

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Impact of Abnormal Glucose Tolerance, Hypertension and Other Risk Factors on Coronary Artery Disease

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Background The degree to which abnormal glucose tolerance contributes to the development of coronary artery disease (CAD) has not been clarified in Japanese. The relationship between abnormal glucose tolerance and severity of coronary artery stenosis, as well as the contributions of hypertension, diabetes and other risk factors for CAD to recurrence of the disease, were investigated in the present study.

Methods and Results The subjects were 474 consecutive patients (mean age: 63.8±11.3 years) with suspected CAD who were admitted to Sapporo Medical University Hospital during April 1, 1997 to March 31, 2004. The coronary index and stenosis score were higher in subjects with diabetes mellitus (DM) and in subjects with impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) than in subjects with normal glucose tolerance (NGT). Ischemic episodes recurred during the observation period (mean 2.5 years) in 61 of 341 patients diagnosed as having CAD. In the follow-up subjects, systolic blood pressure (SBP) was significantly higher in the recurrence group than in the non-recurrence group, and SBP was a significant variable in logistic regression analysis after adjustment for age, gender, hemoglobin A1c, total cholesterol, body mass index, smoking history, family history and stenosis score. The relative risk of recurrence became 1.7-fold higher with a rise in SBP of 10 mmHg (95% confidence interval: 1.252–2.250). Analysis of the relationship between glucose tolerance and recurrence showed that the rate of recurrence was higher in patients with IFG+IGT+DM than in those with NGT.

Conclusions CAD progresses not only in patients with DM but also in those with IGT. The rate of recurrence of ischemic episodes increases in individuals with IGT or DM, and suggesting that hypertension is a risk factor for recurrence of ischemic episodes. Management of glucose tolerance and blood pressure is therefore important for prevention of CAD in Japanese. (*Circ J* 2007; **71:** 20–25)

Key Words: Coronary artery disease; Impaired glucose tolerance; Recurrence

There is concern about the reported dramatic increases in recent years in the incidences of diabetes mellitus (DM) and impaired glucose tolerance (IGT) in Japanese[1–3] and it is known that coronary artery disease (CAD) is severe in diabetic patients and they have a poor prognosis[4–7]

There have also been reports on the contribution of abnormal glucose tolerance, including impaired fasting glucose (IFG) and IGT, to the development of CAD[8–11] but there have been only a few reports on the impact of glucose intolerance on severity of coronary artery stenosis determined by coronary angiography[12,13] Therefore, using patients admitted to Sapporo Medical University Hospital as subjects, we investigated the relationship between abnormal glucose tolerance and severity of coronary artery stenosis in a cross-sectional study, and we also investigated the contributions of insulin resistance (IR) and various risk factors for CAD, including hypertension (HT), DM, IGT, hyperlipidemia and obesity, to recurrence of ischemic episodes in a longitudinal study.

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Methods

Study Population

Relationship Between Abnormal Glucose Tolerance and Severity of Coronary Artery Stenosis (Cross-Sectional Study) The subjects in the cross-sectional study were 474 consecutive patients (mean age: 63.8±11.3 years) who were admitted to Sapporo Medical University Hospital for investigation of chest pain and suspected CAD during the period from April 1, 1997 to March 31, 2004 and who were subsequently diagnosed as having CAD, based on findings from coronary angiography, nuclear medical examinations, and exercise and 12-lead electrocardiogram. Patients who had complications such as heart valve disease, pulmonary thromboembolism, aortic dissection, myocarditis, and pericarditis were excluded from the subjects. Of the 474 patients, 224 had acute coronary syndrome, including acute myocardial infarction and unstable angina, 148 had effort angina pectoris, 64 had silent myocardial ischemia, and 38 had vasospastic angina pectoris.

Relationships Between Risk Factors and Recurrence of Ischemic Episodes (Longitudinal Study) Of the subjects who had been diagnosed as having CAD during their first admission and who had been discharged after successful revascularization and chest pain control had been achieved by percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG) and drug therapy, the recurrence group was defined as those who had chest pain be-

Table 1 Comparison of the Characteristics of the NGT, IFG + IGT, and DM Groups

	NGT (n=237)	IFG + IGT (n=65)	DM (n=172)	p value
Age (years)	63.6±11.7	66.6±9.6	64.9±10.6	NS
F/M	69/168	17/48	43/129	NS
FPG (mg/dl)	92.6±11.0	103.1±12.7	124.2±33.0	<0.001
Hemoglobin A1c (%)	5.1±0.4	5.3±0.4	6.6±1.3	<0.001
SBP (mmHg)	120.7±15.0	120.9±15.1	126.5±16.3	0.001
DBP (mmHg)	69.4±8.9	71.2±9.0	71.4±8.9	NS
TC (mg/dl)	183.6±26.6	183.9±33.5	182.2±34.7	NS
TG (mg/dl)	132.2±55.7	135.0±63.4	147.1±92.7	NS
HDL-C (mg/dl)	44.8±14.3	43.1±15.3	42.4±12.5	NS
LDL-C (mg/dl)	113.3±24.0	113.6±27.0	112.0±30.4	NS
BMI (kg/m^2)	23.9±3.1	24.4±3.2	24.9±4.1	0.02
Smoking (%)	55.6	61.8	56.7	NS
Family history (%)	15.7	34.4	21.5	0.004
Drinking (%)	38.6	42.9	40.2	NS
Drug therapy (%)				
ACEI or ARB	57.2	51.5	63.2	NS
β-blocker	43.2	53.0	56.7	0.022
Ca-antagonist	44.5	43.9	45.6	NS
Diuretics	8.1	16.7	12.9	NS
HMG-CoA	44.5	57.6	48.5	NS

Smoking, family history, drinking, drug therapy are shown by percentage of positive for these factors.
Other factors are shown by mean ± SD.
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; HMG-CoA, HMG-CoA reductase inhibitor.
See text for other abbreviations.

cause of ischemia during their second admission and for whom results of exercise tolerance tests, nuclear medical examinations and imaging examinations carried out regularly on an out-patient basis confirmed ischemia.

In order to compare the data for patients with recurrence of myocardial ischemia and patients without recurrence, we carried out a longitudinal study using 341 subjects (mean age: 63.2±12.0 years) selected from those in the cross-sectional study for whom follow-up was possible. There was no difference between items evaluated in the subjects during admission in the cross-sectional study and the follow-up subjects.

Study Protocol

Cross-Sectional Study Coronary angiography was performed on 380 of the 474 subjects in the cross-sectional study and based on the results stenosis was scored according to the American Heart Association functional classification:[14] 5 points for 99–100% stenosis, 4 for 90%, 3 for 75%, 2 for 50%, 1 for 25% and 0 for 0%. The maximum coronary stenosis point in each case in the left anterior descending artery, left circumflex artery, or right coronary artery was defined as the coronary index;[14] and the stenosis score[14] was calculated as the sum of the stenosis points of each branch.

The following items were measured in the early morning after overnight fasting during admission: body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG), hemoglobin (Hb) A1c, total cholesterol (TC), triglyceride (TG), and high-density lipoprotein-cholesterol (HDL-C). Low-density lipoprotein-cholesterol (LDL-C) was calculated by Friedewald's formula![15]

Subjects who showed symptoms of DM or who had a casual plasma glucose (PG) level ≥200 mg/dl, FPG ≥126 mg/dl or 2h-PG during a 75-g oral glucose tolerance test (OGTT) ≥200 mg/dl were diagnosed as having DM according to the ADA criteria![16] Subjects who had 2h-PG ≥140 mg/dl and 2h-PG <200 mg/dl were diagnosed as having IGT, and those who had FPG ≥110 mg/dl and FPG <126 mg/dl were diagnosed as having IFG.

Subjects with a family history of CAD were defined as those with any family member within 2 generations having CAD. Subjects with a smoking habit were defined as current smokers who smoked 1 or more cigarettes per day. Subjects with a drinking habit were defined as all of those who drank other than those who drank only on special occasions.

Data obtained at the time of discharge were used for analysis of the relationship between glucose tolerance and severity of coronary artery stenosis.

Longitudinal Study The endpoint of the longitudinal study was recurrence of ischemic episodes during the observation period, which was 2.4 years.

Items evaluated during admission in this series of study were the same as those in the cross-sectional study. Relationships between recurrence of ischemic episodes and coronary risk factors, including HT, abnormal glucose tolerance, hyperlipidemia and obesity, were analysed. Because the number of follow-up subjects with IGT (n=25) was small, we divided the follow-up subjects into normal glucose tolerance (NGT) and IFG+IGT+DM groups for comparison of the rates of recurrence.

Eighty-five patients underwent 75-g OGTT, and those who had an insulin concentration 2 h after glucose loading (120 min IRI) ≥64 mU/L were defined as the IR group![17] We compared the rates of ischemic episodes recurrence in the IR group and non-insulin resistance (NR) group to determine whether IR is associated with recurrence of ischemic episodes.

Statistical Analysis

Data are expressed as means ± standard deviation. Differences between mean values in 2 groups were examined by the unpaired t-test. One-way ANOVA was used to examine differences between mean values in the 3 groups (NGT,

Table 2 Comparison of the Angiographic Characteristics of the NGT, IFG+IGT, and DM Groups

	NGT (n=190)	IFG+IGT (n=53)	DM (n=137)	p value
No. of diseased vessels	1.39±1.01	1.77±0.81	2.22±0.78*	<0.001
Coronary index	4.01±1.67	4.77±0.53*	4.73±0.52*	<0.001
Stenosis score	6.96±4.05	8.73±3.52*	10.18±3.01*	<0.001

* vs NGT $p<0.05$.
See text for abbreviations.

Table 3 Clinical Characteristic of the Groups With Recurrence of Ischemic Episodes and Without

	Recurrence (−) (n=280)	Recurrence (+) (n=61)	p value
Age (years)	64.1±11.5	64.0±10.6	NS
N (F/M)	72/208	16/45	NS
FPG (mg/dl)	107.8±29.7	102.1±22.5	NS
Hemoglobin A1c (%)	5.8±1.3	5.7±0.8	NS
SBP (mmHg)	120.1±17.0	131.5±5.0	<0.001
DBP (mmHg)	70.0±9.3	72.0±8.2	NS
TC (mg/dl)	185.9±31.5	177.0±27.1	0.039
TG (mg/dl)	140.5±78.6	134.1±57.2	NS
HDL-C (mg/dl)	42.6±12.9	45.7±16.0	NS
LDL-C (mg/dl)	114.8±28.2	110.0±27.0	NS
BMI (kg/m^2)	24.1±3.5	25.2±3.5	0.022
Smoking (%)	59.6	56.1	NS
Family history (%)	19.4	16.4	NS
Drinking (%)	37.0	36.2	NS
Drug therapy (%)			
ACEI or ARB	63.3	59.3	NS
β-blocker	47.7	71.2	0.001
Ca-antagonist	37.7	39.0	NS
Diuretics	10.7	13.6	NS
HMG-CoA	47.7	52.5	NS

See Table 1 for abbreviations.
Values are mean±SD. Recurrence; ischemic episodes recurrence.
Smoking, family history, drinking, drug therapy are shown by percentage of positive for these factors.

IFG+IGT, and DM), and multiple logistic regression analysis was used to examine relations between ischemic episode recurrence and other risk factors. A p-value of less than 0.05 in these analyses was considered significant. All statistical analyses were performed using SPSS version 12.0J (Chicago, IL, USA).

Results

Relationship Between Abnormal Glucose Tolerance and Severity of Coronary Artery Stenosis (Cross-Sectional Study)

Table 1 shows a comparison of data in the NGT, IFG+IGT and DM groups. The percentage of subjects with a family history of CAD was significantly higher in the IFG+IGT group than in the other 2 groups.

Table 2 shows a comparison of the coronary artery lesions in the NGT, IFG+IGT and DM groups. Both the IFG+IGT and DM groups showed higher values than the NGT group for number of diseased vessels (NGT vs IFG+IGT vs DM: 1.39±1.01 vs 1.77±0.81 vs 2.22±0.78; $p<0.001$), coronary index (4.01±1.67 vs 4.77±0.53 vs 4.73±0.52; $p<0.001$) and stenosis score (6.96±4.05 vs 8.73±3.52 vs 10.18±3.01; $p<0.001$), indicating that CAD progresses not only in those with frank DM but also at the stage of IFG+IGT.

Table 4 Multiple Logistic Regression Analysis for Ischemic Episodes Recurrence

	Odds ratio	p value	95%CI
SBP	1.678	0.001	1.252–2.250
Age	0.983	0.399	0.946–1.023
Sex	0.910	0.852	0.339–2.441
TC	0.994	0.464	0.977–1.011
BMI	1.134	0.037	1.008–1.276
Hemoglobin A1c	0.737	0.122	0.501–1.085
Family history	0.606	0.383	0.197–1.866
Stenosis score	1.103	0.166	0.960–1.267
Smoking	0.882	0.780	0.366–2.128

Independent variables: ischemic episodes recurrence.
Dependent variables: SBP, Age, Sex, TC, BMI, Hemoglobin A1c, stenosis score, smoking, family history.
SBP, rise in SBP of 10 mmHg.
CI; confidence interval. See text for other abbreviations.

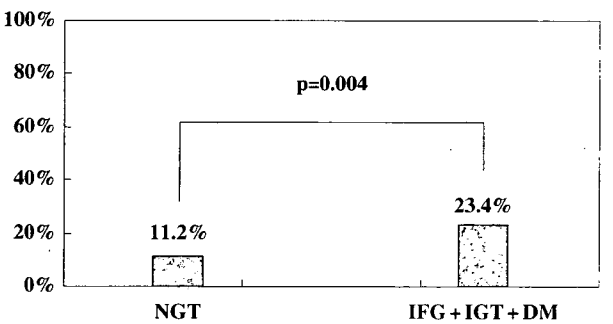

Fig 1. Comparison of ischemic episodes recurrence rate between normal glucose tolerance (NGT), impaired fasting glucose (IFG)+impaired glucose tolerance (IGT)+diabetes mellitus (DM) groups.

Relationships Between Risk Factors and Recurrence of Ischemic Episodes (Longitudinal Study)

Ischemic episodes recurred in 61 of the 341 subjects during the observation period. Table 3 shows a comparison of risk factors in subjects with recurrence of ischemia and those without the recurrence. SBP and BMI were significantly higher in the recurrence group than in the non-recurrence group.

Table 4 shows the results of multiple logistic regression analysis using ischemic episode recurrence as an independent variable. When adjustments were made for age, gender, HbA1c, TC, BMI, smoking history, family history and stenosis score, SBP became a significant variable for recurrence, and the relative risk of recurrent ischemia was 1.7-fold higher with a rise in SBP of 10 mmHg. The rates of ischemic episode recurrence for the NGT and IFG+IGT+DM groups are shown in Fig 1. The rate of recurrence was higher in the IFG+IGT+DM group (23.4%) than in the NGT group (11.2%) ($p=0.004$). When we defined IR according to the plasma insulin level 2 h after glucose loading

as stated before, the rate of recurrence of ischemia in the IR group was higher than that in the NR group (Fig 2) (NR vs IR: 4.8% vs 40.0%, p=0.009). Although not shown in the table, the HOMA-IR value, an index of IR, was higher, though not significantly, in the recurrence group than in the non-recurrence group (recurrence group vs non-recurrence group: 2.1 vs 1.7, p=0.372).

Discussion

Relationship Between Abnormal Glucose Tolerance and Severity of Coronary Artery Stenosis (Cross-Sectional Study)

The subjects with DM in this study also showed stenosis in many coronary artery branches and severe stenosis. Diabetes is often complicated with CAD, and it has been shown that diabetes also has a significant untoward effect on the prognosis and severity of CAD. Our findings agree with the results of a study by Dortimer et al showing that many patients with diabetes had stenosis in 3 branches of the coronary artery;[8] and the results of a study by Kip et al showing extensive diffuse coronary arteriosclerosis in diabetics with CAD.[9]

The results of the present study indicate that not only subjects with diabetes but also those with abnormal glucose tolerance have more severe stenosis and stenosis in more vessels than subjects with NGT. However, the association of abnormal glucose tolerance with severity of CAD is not fully characterized.[12,13] The study by Kataoka et al showed a significant correlation between IGT and severity of CAD;[12] and our results agree with their finding. However, the study by Horimoto et al suggested that there was no significant difference between the severity of angiographic coronary atherosclerosis in subjects with NGT and IGT.[13] This discrepancy may be related to the small numbers of subjects, and differences in the characteristics of the subjects entered in the study protocols. Nevertheless, further investigation of this issue is needed.

It has been shown that individuals with abnormal glucose tolerance have IR in addition to a high PG level. Currently, whether hyperglycemia in IGT patients or IR are responsible for the IGT-CAD association has been a subject of investigation. Recently, Satoh et al[20] and Takezako et al[21] have reported that IR rather than hyperglycemia is associated with the severity of CAD. In our study, we examined the relationships between number of diseased vessels, 2h-PG and 120 min IRI using subjects other than those with DM. We found that subjects with multiple vessel disease had higher values for both 2h-PG (patients with multiple vessel disease vs patients with 1 diseased vessel or less: 139.3±27.1 mg/dl vs 116.5±21.2 mg/dl; p<0.001) and 120 min IRI (92.3±58.7 mU/L vs 67.6±39.1 mU/L; p=0.05) compared with subjects with 1 diseased vessel or less. This suggests that both postprandial hyperglycemia and hyperinsulinemia are involved in the development of CAD in patients with IGT.

IR results in compensatory hyperinsulinemia, which may give rise to an accumulation of risk factors for CAD, provoking a direct mechanism for the progression of arteriosclerosis. It has, in fact, been reported that subjects with IR have a high incidence of cardiovascular disease[22] and show progression of CAD;[23,24] and our results also suggest that IR is involved in the progression of CAD. We previously reported that CAD in IR is characterized by stenosis of multiple coronary artery branches and peripheral lesions[24]

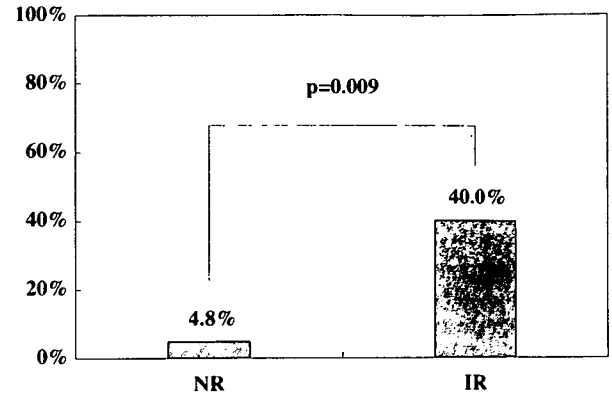

Fig 2. Comparison of ischemic episodes recurrence rate between non-insulin resistance (NR) and insulin resistance (IR).

Not only the accumulation of risk factors for CAD but also a reduction in blood flow in the coronary artery occur in a state of IR;[25] and it is possible that these factors, acting either alone or synergistically, cause progression of CAD.

Relationships Between Risk Factors and Recurrence of Ischemic Episodes (Longitudinal Study)

There was no difference between FPG or lipid levels in the recurrence group and non-recurrence group, but SBP and BMI were significantly higher in the recurrence group. We found in the present study that SBP contributed significantly to recurrence of ischemic episodes, even when adjustments were made for age, gender, HbA1c, TC, BMI, smoking history, family history and stenosis score, and that the relative risk of recurrence was 1.7-fold higher with a rise in SBP of 10 mmHg. Although there have been only a few studies on the relationship between blood pressure and recurrence of ischemic episodes, Xiao-Fei Zhang et al[26] found that higher SBP increases the mortality from cardiovascular disease in Chinese subjects, and our findings agree with their report. In the present study, there was no significant difference between the percentages of subjects receiving hypotensive drugs in the non-recurrence group (90.0%) and recurrence group (93.2%). There was also no significant difference between the 2 groups in the percentages of subjects taking an angiotensin-converting enzyme inhibitor, an angiotensin-receptor blocker, a Ca^{2+} antagonist or a diuretic drug, suggesting that high blood pressure has a direct effect on recurrence of ischemic episodes.

It has been reported that obesity is involved in the onset of CAD[27,28] and in the present study BMI was found to be significantly higher in the recurrence group, which is consistent with the recently proposed concept of metabolic syndrome in which abdominal obesity is an important risk factor for cardiovascular disease.

The rate of recurrence of ischemic episodes was higher in subjects with IFG+IGT+DM than in subjects with NGT. However, no significant difference was found between the levels of FPG or HbA1c or the frequencies of use of hypoglycemic drugs or insulin in the recurrence and non-recurrence groups. On the other hand, analysis of the effect of IR, assessed by the insulin concentration 2 h after glucose loading,[17] showed that the rate of recurrence was significantly higher in the IR group than in the NR group. These results suggest that IR rather than hyperglycemia increases the risk of recurrence of ischemic episodes, which agrees with an earlier study[29] showing association of IR with coro-