Involvement of Cytokines in the Mechanism of Whole-Body Hyperthermia-Induced Cardioprotection

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Background—Hyperthermia increases cardiac tolerance to ischemia/reperfusion injury and activates manganese superoxide dismutase (Mn-SOD), an intrinsic radical scavenger, in myocardium in a biphasic manner. Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) induced a biphasic cardioprotection that corresponded to the activation of Mn-SOD. However, a direct association between Mn-SOD activation in myocardium and the acquisition of tolerance to ischemia/reperfusion injury induced by hyperthermia and the involvement of the cytokines in the signal transduction pathway for the hyperthermia-induced cardioprotection have not yet been elucidated.

Methods and Results—Hyperthermia was induced in anesthetized rats by placement in a temperature-controlled water bath. At 0.5 and 72 hours after hyperthermia, ischemia was induced by occlusion of the left coronary artery for 20 minutes, followed by reperfusion for 48 hours. Inhibition of the increases in Mn-SOD content and activity 72 hours after hyperthermia by the administration of antisense oligodeoxynucleotides to Mn-SOD abolished the expected decrease in myocardial infarct size. The simultaneous administration of neutralizing antibodies to TNF-α and IL-1β before hyperthermia abolished the biphasic cardioprotection and increase in Mn-SOD activity.

Conclusions—The increase in Mn-SOD activity mediated through the production of TNF-α and IL-1β by whole-body hyperthermia is important in the acquisition of early- and late-phase cardioprotection against ischemia/reperfusion injury in rats. (Circulation. 2000;102:452-457.)

Key Words: enzymes • hormones • interleukins • hyperthermia • genes

Cardiac resistance to ischemia/reperfusion injury is increased by exposure to sublethal stress, such as a brief period of ischemia, exercise, or whole-body hyperthermia in a biphasic manner.1–5 Tolerance after exposure to whole-body hyperthermia is manifested both soon after and 24 to 72 hours after hyperthermia.4 We reported that the time course of manganese superoxide dismutase (Mn-SOD) activation in myocardium corresponded to that of the biphasic cardioprotective effects after hyperthermia, and an increase in the content of Mn-SOD appeared to be responsible for the activation of the enzyme at the late phase.4 Direct proof that the activation and induction of this protein led to the acquisition of tolerance to ischemia/reperfusion injury, however, has not yet been presented.

Tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) are known as potent inducers of Mn-SOD.5–6 We and others reported that the administration of TNF-α and IL-1β induced cardioprotection against ischemia/reperfusion injury 24 to 48 hours after the treatment.3–7–9 We also reported that the time course of the cytokine-induced cardioprotection exhibited a biphasic pattern similar to that for the activation of Mn-SOD.3 Moreover, the production of TNF-α and IL-1β during exercise plays a pivotal role in exercise-induced cardioprotection through the activation and induction of Mn-SOD.3 Neta et al10 reported that radioprotection of lipopolysaccharide depended on induction of TNF and IL-1, because blocking the activities of these 2 cytokines completely abolished the radioprotective effect of lipopolysaccharide.

In the present study, we attempted to demonstrate a direct association between the acquisition of tolerance to ischemia/reperfusion injury and Mn-SOD activation in myocardium induced by whole-body hyperthermia, using a rat model of occlusion-reperfusion in the left coronary artery (LCA). We also examined whether the cytokines were involved in the mechanism of hyperthermia-induced cardioprotection.

Methods
This study was carried out under the supervision of the Animal Research Committee in accordance with the Guideline on Animal Experiments of Osaka University and the Japanese Government Animal Protection and Management Law (No. 105).

Animals and Experimental Protocol
Male Wistar rats (300 to 350 g) were maintained on a 12-hour dark/light cycle, housed at 23±1.5°C (45±15% relative humidity),
and allowed access to water and rat chow ad libitum. After the induction of light anesthesia with sodium pentobarbital 5 to 10 mg/kg IP, whole-body hyperthermia was induced by placing the rats in a constant-temperature water bath as described previously.11 During whole-body hyperthermia, the animal was supported by a wire apparatus to prevent the aspiration of water and to facilitate the measurement of rectal temperature. Hyperthermia was maintained at 42±0.2°C for 15 minutes (Figure 1). Rats in the sham-treated control group were placed in a water bath maintained at 36.5±0.2°C. Rats were allowed to recover at room temperature for defined intervals before measurements were performed (Table 1).

Infarction Protocol

The surgical procedures of occlusion-reperfusion by LCA occlusion in rats were described previously.4 At the end of the recovery intervals, rats were anesthetized with sodium pentobarbital (25 mg/kg IP), intubated, and ventilated with a small-animal respirator at a rate of 60 to 70 cycles/min and a tidal volume of 1 mL/100 g body wt. The left femoral artery was cannulated with polyethylene tubing for the continuous measurement of arterial blood pressure with a pressure transducer. The heart rate, incidence of arrhythmias, and ST-segment changes were monitored. Hemodynamic variables were recorded continuously. After a 10-minute period of stabilization, measurement of arterial pressure was initiated and the LCA was ligated. After 20 minutes of coronary occlusion, the snare was released. The surgical wounds were repaired 60 minutes after reperfusion, and the rats were returned to individual cages to recover. Rats were reanesthetized with sodium pentobarbital (25 mg/kg IP) 48 hours after reperfusion and were intubated and ventilated with the respirator. After the heart was exposed and the LCA was reoccluded, Evans blue dye (2%) was injected via the femoral vein to estimate the area perfused by the occluded artery (ischemic region). Rats were killed by an overdose of sodium pentobarbital. The left ventricle was then cut into 6 pieces perpendicular to the apex-base axis. These specimens were incubated with 1% trifluoroleurotional chloride at 37°C to stain the noninfarct region. The ischemic, infarcted, and nonischemic areas of tissue were separated with scissors and weighed.11,12 The area at risk and the infarct size were defined as the ratios of the mass of the ischemic region to the left ventricular mass and the mass of the infarct region to that of the ischemic region, respectively, and were expressed as percentages. This procedure of infarct size measurement was performed in a blinded fashion.

Arrhythmias were monitored by ECG. Ventricular fibrillation (VF) was defined according to the criteria of the Lambeth Conventions.13 If VF occurred and did not resolve spontaneously within 3 seconds, manual cardioversion was attempted by gentle flicking of the nonischemic region of the heart. Rats in which VF continued for >6 seconds or cardioversion had to be performed >3 times were excluded from infarct size analysis.

Myocardial Tissue Sampling

To obtain tissue samples for measurements of Mn-SOD content and activity, rats were killed by an overdose of sodium pentobarbital. The myocardial tissue was rinsed in PBS, and then blood in the left atria and right coronary arteries was washed out with an adequate volume of PBS from the ascending aorta retrogradely. Both atria and the right ventricle were removed, and left ventricular myocardial samples were rapidly frozen by immersion in liquid nitrogen and stored at −80°C until use.4

Measurement of Activity and Content of Mn-SOD

Myocardial levels of Mn-SOD activity and content were determined in rats euthanized after recovery intervals of 0.5 and 72 hours after water-bath treatment and in untreated control rats. Mn-SOD activity of the myocardial samples was determined by the nitroblue tetrazolium method.4,14 Mn-SOD content in rats of the untreated control, sham-treated control, and hyperthermia groups was measured by an ELISA, as reported previously.3,4,14 The activity and content of Mn-SOD were expressed relative to the protein concentration in the supernatant determined by the method of Lowry.

Administration of the Reagents

The phosphorothioated oligodeoxynucleotides were purchased from Bex. A 22-mer phosphorothioated derivative of antisense oligodeoxynucleotides (ASODN, CAGCCGCCCCACAAACATTGT) to Mn-SOD, sense oligodeoxynucleotides (SODN, CAAATGTTGTTGCGCGCCGCGTG) to Mn-SOD, or scrambled oligonucleotide (TTCAGTGAGACCGCTTCTTG) was injected just after whole-body hyperthermia at a dose of 10 mg/kg IP.3 Anti-murine TNF-α antibody (0.5 mL IP) and/or anti-murine IL-1β antibody (0.5 mg IP) was infused 30 minutes before whole-body hyperthermia. Polyclonal rabbit anti-murine TNF-α antibody and monoclonal hamster anti-murine IL-1β antibody were obtained from Genzyme. Both antibodies cross-react with rat cytokines.3,5,16

Materials

Chemicals were purchased from Sigma Immunochemicals and Wako Fine Chemicals.

Statistics

Data are expressed as mean±SEM. The significance of the differences between groups was assessed by 1-way ANOVA with Bonferroni’s post hoc test for multiple comparisons. A level of P<0.05 was considered statistically significant.

Results

Exclusion Because of VF or Death

A total of 6 rats developed serious VF during occlusion (1 in the untreated control group, 2 in the sham-treated control group, 2 in the hyperthermia group treated with ASODN, and 1 in the hyperthermia group pretreated with TNF-α and IL-1β antibodies) and were excluded from the evaluation of myocardial infarct size. Six rats died prematurely (probably because of arrhythmia or heart failure) during the 48-hour reperfusion period (1 in the untreated control group, 2 in the sham-treated control group, 1 in the hyperthermia group, 1 in the hyperthermia group treated with ASODN, and 1 in the hyperthermia group pretreated with TNF-α and IL-1β antibodies).
Hemodynamic Data and Rectal Temperature

No significant differences were observed in the rate-pressure product or in the rectal temperature during the infarct protocol among the groups before ischemia, at the end of the ischemic period, or 0.5 hour after reperfusion (data not shown).

Direct Relationship Between Cardioprotection and Induction of Mn-SOD

We examined the relationship between the acquisition of tolerance to ischemia/reperfusion and the induction of Mn-SOD in the myocardium 72 hours after whole-body hyperthermia. We manipulated the level of expression of Mn-SOD using ASODN that corresponded to the initiation site of Mn-SOD translation. This reagent was administered intraperitoneally to rats immediately after whole-body hyperthermia. There were no significant differences in myocardial Mn-SOD activity and content between the untreated control group and the sham-treated control group with 72-hour recovery (Figure 2). As we previously reported, Mn-SOD activity and content in the hyperthermia group increased at the 72-hour recovery interval (Figures 2 and 5). The administration of ASODN completely inhibited the increases in Mn-SOD activity and content 72 hours after hyperthermia (Figure 2). However, SODN or scrambled ODN did not attenuate the increases in Mn-SOD activity and content induced by hyperthermia (Figure 2).

The size of the area at risk expressed as a percentage of left ventricular area did not differ significantly among the groups (Figures 3 and 4). There was no significant difference in the size of the myocardial infarction between the sham-treated control group with 72 hours of recovery and the untreated control rats (Figures 3 and 4). The induction of whole-body hyperthermia reduced the size of myocardial infarction in rats 72 hours after hyperthermia (Figures 3 and 4), in agreement with our previous report. As shown in Figure 3, the expected decrease in infarct size was abolished in rats treated with ASODN to Mn-SOD, in which the induction of Mn-SOD was specifically inhibited. SODN, which did not attenuate the induction of Mn-SOD in myocardium after hyperthermia, did not abolish the protective effect of whole-body hyperthermia. Administration of the scrambled ODN had no effect on infarct size as seen with SODN.

Involvement of Cytokines in Hyperthermia-Induced Cardioprotection

We reported that TNF-α and IL-1β are involved in exercise-induced cardioprotection. To examine whether these cytokines contribute to the hyperthermia-induced cardioprotec-
tation, we administered neutralizing antibodies to these cytokines intraperitoneally 30 minutes before hyperthermia. There were no significant differences in infarct size among sham-treated control groups (0.5 and 72 hours after treatment) and the untreated control group (Figure 4). Administration of an antibody to TNF-α did not influence infarct size 0.5 or 72 hours after hyperthermia (Figure 4). The administration of an antibody to IL-1β also did not alter the size of the myocardial infarct 0.5 or 72 hours after hyperthermia. However, simultaneous administration of the antibodies to TNF-α and IL-1β abolished the protection against ischemic damage 0.5 and 72 hours after hyperthermia.

In myocardium from sham-treated control groups, Mn-SOD activity was unchanged 0.5 and 72 hours after treatment (Figure 5). Mn-SOD activity in the hyperthermia group increased at the 0.5- and 72-hour recovery intervals compared with that in the corresponding sham-treated control groups (Figure 5). The activity of the cytosolic isof orm of SOD (Cu,Zn-SOD) did not change after hyperthermia (data not shown). Antibody to TNF-α or IL-1β had no effect on the increase in Mn-SOD activity induced by hyperthermia (Figure 5). The simultaneous administration of the antibodies to these cytokines eliminated the increase in Mn-SOD activity 0.5 and 72 hours after hyperthermia (Figure 5) and abolished the increase in Mn-SOD content 72 hours after hyperthermia (data not shown).

**Discussion**

We previously reported that exposure to hyperthermia led to a recovery interval–dependent, biphasic reduction in the size of the myocardial infarction as determined after 48 hours of reperfusion. The time course of the increase in myocardial Mn-SOD activity corresponded to that of the cardioprotection, whereas an increase in the content of Mn-SOD corresponded only to the late-phase effect. In this study, we found that (1) the expected decrease in infarct size at the early phase induced by hyperthermia was abolished in rats treated with neutralizing antibodies to TNF-α and IL-1β, in which the increase in Mn-SOD activity was inhibited; and (2) manipulations including the administration of ASODN to Mn-SOD and neutralizing antibodies to TNF-α and IL-1β, which inhibited the induction of Mn-SOD at the late phase, abolished the delayed protection against ischemia/reperfusion injury induced by hyperthermia. Taken together, these results indicated that Mn-SOD plays a central role in the protective effect of whole-body hyperthermia in both the early and late phases in rats. A mechanism for the activation of Mn-SOD at the early phase after hyperthermia, in which there was no difference in Mn-SOD at the protein level between hyperthermia and sham-treated control groups, remains to be elucidated. The increase in Mn-SOD activity disappeared by 3 hours after hyperthermia, suggesting that a rapid inactivation should follow the activation of Mn-SOD. The inhibition of Mn-SOD induction at the late phase by the administration of ASODN abolished the hyperthermia-induced cardioprotect...
tion. This result indicated that at the late phase, the induction of Mn-SOD leads to an increase in its enzyme activity, resulting in the acquisition of cardioprotection against ischaemia/reperfusion injury.

In this study, neutralizing antibodies to TNF-α and IL-1β, which inhibited the increase in Mn-SOD activity at the early and late phases and the induction of Mn-SOD at the late phase, abolished the biphasic cardioprotection against ischaemia/reperfusion injury induced by whole-body hyperthermia. Because there is some redundancy in the effects of TNF-α and IL-1β, blocking of TNF-α or IL-1β by its antibody did not exhibit any effect in our system. We reported that the administration of TNF-α and IL-1β induces biphasic cardioprotection and Mn-SOD activation in rats. These results suggest that both TNF-α and IL-1β are involved in hyperthermia-induced cardioprotection via the increase in Mn-SOD activity. We reported that reactive oxygen species, which are produced during hyperthermia, induce an increase in Mn-SOD activity, resulting in biphasic hyperthermia-induced cardioprotection. The production of reactive oxygen species leads to increases in TNF-α and IL-1β in myocardium. Therefore, these data indicate that the production of TNF-α and IL-1β, probably via the generation of reactive oxygen species during hyperthermia, is important in the increase in Mn-SOD activity after heat stress.

TNF-α and IL-1 cause rapid activation and nuclear translocation of the transcription factor nuclear factor (NF)-κB, which strongly correlates with the induction of Mn-SOD. It was recently reported that a cis-acting TNF-α- or IL-1β-responsive element was identified for the Mn-SOD gene in mouse, and NF-κB binds to the element. The transcription factor NF-κB is subject to redox regulation. NF-κB might play a role in the cytokine-mediated Mn-SOD induction at the late phase. A mechanism of Mn-SOD activation by TNF-α and IL-1β at the early phase, however, remains to be elucidated.

We reported that exercise induced a biphasic cardioprotection with the activation of Mn-SOD. Combined administration of the antibodies to TNF-α and IL-1β abolished the biphasic cardioprotection induced by exercise. We also reported that reactive oxygen species produced during exercise are involved in the production of TNF-α and IL-1β and the biphasic activation of Mn-SOD. Brief sublethal ischemic or anoxic insults also have been shown to increase Mn-SOD activity and to induce cardioprotection or myocyte protection in a biphasic manner. Mn-SOD is directly associated with the delayed protection of the myocyte against hypoxia-reoxygenation injury in an in vitro model. The acquisition of cardioprotection by sublethal stress, such as whole-body hyperthermia, exercise, or ischemia, may involve a common mechanism that functions through an induction and activation of Mn-SOD via the production of reactive oxygen species and cytokines.

Conclusions

Whole-body hyperthermia induced a biphasic increase in Mn-SOD activity and biphasic cardioprotection in rats. The administration of ASODN to Mn-SOD, which inhibited the induction of Mn-SOD at the late phase, abolished hyperthermia-induced delayed cardioprotection against ischemia/reperfusion injury. The neutralizing antibodies to TNF-α and IL-1β, in which the increase in Mn-SOD activity was inhibited, abolished the expected decrease in infarct size induced by hyperthermia. These results suggest that TNF-α and IL-1β are involved in hyperthermia-induced cardioprotection via the activation and induction of Mn-SOD.

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References


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