Myocardial Infarct Size—Limiting Effect of Ischemic Preconditioning Was Not Attenuated by Oxygen Free-Radical Scavengers in the Rabbit

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Background. The limiting effect of ischemic preconditioning on infarct size has been reported in canine hearts, which contain considerable amounts of xanthine oxidase, a free radical-producing enzyme. Furthermore, a recent study suggested that free radicals generated during preconditioning may contribute to the cardioprotective effect of preconditioning. The present study examined 1) whether preconditioning limits infarct size in rabbits, which, like humans, lack myocardial xanthine oxidase and 2) whether the cardioprotective effect of PC is mediated by free radicals.

Methods and Results. A branch of the circumflex coronary artery in rabbits was occluded for 30 minutes and then reperfused for 72 hours. Myocardial infarct size and area at risk were determined by histology and fluorescent particles, respectively. Five groups were studied: an untreated control group, a preconditioned group (PC group), a high-dose superoxide dismutase (SOD)—treated preconditioned group (high-dose SOD-PC group), a low-dose SOD—treated preconditioned group (low-dose SOD-PC group), and a SOD-plus-catalase—treated preconditioned group (SOD/CAT-PC group). Preconditioning was performed with four episodes of 5 minutes of ischemia and 5 minutes of reperfusion. The free radical scavengers (30,000 units/kg SOD for high-dose SOD-PC group, 15,000 units/kg SOD for low-dose SOD-PC group, and 30,000 units/kg SOD plus 55,000 units/kg catalase for SOD/CAT-PC group) were infused intravenously over 60 minutes starting 20 minutes before preconditioning. Infarct size as the percentage of area at risk was 45.1±3.5% (mean±SEM) in the control group (n=11), 13.3±3.0% in the PC group (n=12), 9.7±1.8% in the high-dose SOD-PC group (n=8), 11.9±2.2% in the low-dose SOD-PC group (n=6), and 9.6±2.3% in the SOD/CAT-PC group (n=6) (p<0.05 versus control for the last four values). The differences in infarct size as the percent of area at risk among the PC, high-dose SOD-PC, low-dose SOD-PC, and SOD/CAT-PC groups were not significant.

Conclusion. Ischemic preconditioning delays ischemic myocardial necrosis regardless of myocardial xanthine oxidase content. Free radicals are unlikely to have a major role in the mechanism of the preconditioning in rabbits. (Circulation 1991;83:1015–1022)

The finding that single or repeated episodes of ischemia/reperfusion paradoxically protects myocardium from injury caused by subsequent longer ischemia is called “ischemic preconditioning” and has received much interest.1-4 The preconditioning has been shown to limit myocardial infarct size1 and suppress both ischemia-5 and reperfusion-induced arrhythmias.2 Recent studies revealed several important metabolic features of preconditioned myocardium: Tissue acidosis during the ischemia was attenuated, the depletion of myocardial ATP level during ischemia was slowed, and accumulation of toxic catabolites, such as lactate, during ischemia was less in the preconditioned myocardium.4,6 Although how those metabolic changes are induced by preconditioning is unknown, Murry et al7 recently proposed an interesting hypothesis that oxygen free radicals generated during the preconditioning procedure may contribute to its cardioprotec-
tive effects. That hypothesis was based on their findings that administration of superoxide dismutase (SOD) and catalase partially blocked the infarct size-limiting effect of preconditioning in a canine model. However, this hypothesis has not been critically tested in other models of myocardial infarction.

Another important issue regarding ischemic preconditioning is whether its cardioprotective effect is a phenomenon that occurs in many species. The beneficial effects of preconditioning were first reported in the dog and then in rat hearts, both of which contain considerable amounts of xanthine oxidase, a source of oxygen free radicals. If oxygen free radicals play an important role in the mechanism of preconditioning, myocardial xanthine oxidase could be the source of free radicals, and the cardioprotective effect of preconditioning may be much less in species deficient of myocardial xanthine oxidase, such as the rabbit and pig. This issue is clinically important because the human heart also lacks myocardial xanthine oxidase.

In the present study, we asked two questions. First, does preconditioning limit myocardial infarct size in the rabbit, a species that lacks myocardial xanthine oxidase? Second, do oxygen free radicals during preconditioning contribute to the cardioprotective effect of preconditioning? To answer these questions, we examined the effects of preconditioning on myocardial infarct size in a rabbit ischemia/reperfusion model. In addition, we analyzed whether the effect of preconditioning on infarct size is modified by administration of oxygen free-radical scavengers.

Methods

This study conformed to the guidelines of Sapporo Medical College on animal use and was conducted in accordance with the position of the American Heart Association on research animal use.

Surgical Preparation

The surgical preparation was essentially the same as in our previous studies. Male rabbits (Japanese White) weighing 2.2–2.9 kg were anesthetized with intravenous sodium pentobarbital (40 mg/kg). The rabbit was intubated through tracheostomy and mechanically ventilated with room air and supplemental oxygen. The respirator (model 683, Harvard Apparatus, North Billerica, Mass.) and oxygen supplement were adjusted to maintain arterial PO2 and pH in the ranges of 85–140 mm Hg and 7.35–7.50, respectively. Catheters were inserted into the carotid artery and jugular vein. The arterial catheter was connected to a Nihon-Kohden SCK-580 pressure transducer to measure systemic blood pressure. Precordial electrocardiography was monitored using bipolar leads across the chest. A left thoracotomy was performed, and the heart was exposed. Silk thread (4-0) was passed around a branch of the left circumflex artery with a taper needle, and the ends of the silk thread were threaded through a small vinyl tube. The coronary branch was occluded by pulling the snare, which was then fixed by clamping the tube with a mosquito clamp. Myocardial ischemia was confirmed by the ST segment elevation of the electrocardiogram and regional cyanosis of the myocardial surface.

The coronary artery was occluded for 30 minutes and then reperfused by releasing the snare. Reperfusion was confirmed by color change (cyanosis to hyperemia) over the ventricular surface, and ST elevation was gradually reduced after reperfusion. The vinyl tube was removed, and the ends of the silk thread were tied together to make a loop and then left in the thorax. The surgical wounds were repaired, and the rabbit was returned to its cage for recovery.

These surgical procedures were performed under sterile conditions, and a combination of 50 mg ampicillin and 50 mg cloxacillin was injected intramuscularly for the prophylaxis of infection. Seventy-two hours after the surgery, the rabbit was heparinized with 2,000 units heparin i.v. and then killed by pentobarbital overdose. The heart was removed for postmortem analysis.

Postmortem Studies

The heart was mounted onto a Langendorff apparatus, and the coronary arteries were perfused with saline at 80 mm Hg to wash out the remaining blood. The coronary branch was reoccluded by ligating the silk tie left around the branch. Fluorescent particles (3–30 μm in diameter, Duke Scientific Co., Palo Alto, Calif.) were injected into the perfusion line to subsequently determine the area at risk (AAR). The heart was then removed from the Langendorff apparatus, weighed, fixed in 20% buffered formalin for 24 hours, and then stored in 10% formalin for at least 3 days to ensure good fixation.

The heart was sectioned into 3-mm slices from the apex to the base using a tissue slicer, and each slice was embedded in paraffin. The slice containing the coronary tie was excluded from the analysis because it had localized necrosis of the myocardium that was obviously due to compression by the ligature. Two 10-μm sections were cut from each paraffin block for light microscopy; one of the two was stained with hematoxylin and eosin, and the other was stained with Mallory’s connective tissue stain modified by Heidenhain. The field of the occluded coronary artery (i.e., AAR) was observed by illuminating the stained slide preparation with ultraviolet light and then was traced onto the slide. The slide preparation was mounted onto a projector and enlarged sevenfold. The infarct and AAR were traced onto paper, and the areas were determined by a cut-and-weigh technique. The tracing of the AAR and the infarct were cut and collected, respectively, for each animal and weighed by using an electronic balance (model AEL-200, Shimadzu, Kyoto, Japan). A pilot study showed that there was a very good linear correlation between the areas of paper (25 mm² to 65 cm²) and their weights in the range of 0.0015–0.4306 g (n=8, r=1.00, p<0.01), within which range the paper weights of the infarct...
tracings in the present study were included, which supported the validity of the cut-and-weigh method. As described in the previous study, heart slices shrank during the process of fixation and embedding in paraffin. To calculate the original ischemic zone and infarct volumes, we divided the areas that were obtained by tracing by 0.72414 and then multiplied by the sample thickness (3 mm).

Experimental Groups

Rabbits were assigned into five groups as shown in Figure 1: control group, preconditioned group (PC group), high-dose SOD-treated preconditioned group (high-dose SOD-PC group), low-dose SOD-treated preconditioned group (low-dose SOD-PC group), and SOD-plus-catalase–treated preconditioned group (SOD/CAT-PC group). All rabbits were subjected to a 30-minute coronary occlusion and a 72-hour reperfusion. The preconditioning was performed with four episodes of 5 minutes of ischemia and 5 minutes of reperfusion. Free radical scavengers (30,000 units/kg SOD for the high-dose SOD-PC group, 15,000 units/kg SOD for the low-dose SOD-PC group, and a combination of 30,000 units/kg SOD and 55,000 units/kg catalase for the SOD/CAT-PC group) were dissolved in saline and infused into the jugular vein catheter using a syringe pump (model A-99, Razel Scientific Inc., Stamford, Conn.) at a rate of 3.18 ml/hr for 60 minutes beginning 20 minutes before preconditioning (Figure 1). The SOD and catalase used in this study were human recombinant zinc-copper SOD (Pharmacia AB, Uppsala, Sweden) and bovine liver catalase (Sigma Chemical Co., St. Louis), respectively.

Statistical Analysis

All values are given as mean±SEM. Difference in survival rate was tested by χ² test. Multiple comparisons of the experimental groups were performed by one-way analysis of variance with a Bonferroni test. Linear regression was obtained by the least-squares method, and the difference between the regression lines was tested by the analysis of covariance.

Results

Mortality

Fifty-nine rabbits were entered into the present study: 15 in the control group, 15 in the PC group, nine in the high-dose SOD-PC group, nine in the low-dose SOD-PC group, and 11 in the SOD/CAT-PC group. The mortality rates of the rabbits in
Each group died of ventricular fibrillation during coronary occlusion (three in the control group and one in the PC group). In the SOD/CAT-PC group alone, two cases of lethal ventricular fibrillation at reperfusion were noted. Ten rabbits died on the first or second postoperative day, probably because of arrhythmias or heart failure: one in the control group, two in the PC group, one in the high-dose SOD-PC group, three in the low-dose SOD-PC group, and three in the SOD/CAT-PC group. Although the mortality rates of the low-dose SOD-PC and SOD/CAT-PC groups appeared higher than those of the other groups, the difference did not reach a statistically significant level. The surviving 43 rabbits contributed to the following analyses.

**Hemodynamic Parameters**

Table 2 summarizes systemic blood pressure, heart rate, and rate-pressure products before and after coronary occlusion and reperfusion. Heart rates after coronary occlusion in the high-dose SOD-PC and low-dose SOD-PC groups were slightly lower than that of the control group (245±7 and 248±8 versus 280±8 beats/min), but the differences were not statistically significant. Systolic and diastolic blood press-

### Table 1. Mortality for the Experimental Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Rabbits (n)</th>
<th>Reper Vf</th>
<th>Late death</th>
<th>Survived</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>11</td>
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<tr>
<td>PC</td>
<td>15</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>12</td>
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<tr>
<td>High-dose SOD-PC</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Low-dose SOD-PC</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>SOD/CAT-PC</td>
<td>11</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

Occl Vf, ventricular fibrillation during coronary occlusion; Reper Vf, ventricular fibrillation upon reperfusion; Late death, death on the first or second postoperative day; PC, preconditioned group; high-dose SOD-PC, high-dose superoxide dismutase–infused preconditioned group; low-dose SOD-PC, low-dose SOD-infused preconditioned group; SOD/CAT-PC, SOD-plus-catalase–infused preconditioned group.

### Table 2. Hemodynamic Parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>BPC</th>
<th>Pre O</th>
<th>Occlusion</th>
<th>Pre R</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>...</td>
<td>278±8</td>
<td>280±8</td>
<td>275±8</td>
<td>274±7</td>
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<td>PC</td>
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<td>265±10</td>
<td>257±9</td>
<td>253±10</td>
<td>249±11</td>
</tr>
<tr>
<td>High-dose SOD-PC</td>
<td>264±7</td>
<td>256±5</td>
<td>245±7</td>
<td>244±8</td>
<td>245±7</td>
</tr>
<tr>
<td>Low-dose SOD-PC</td>
<td>244±14</td>
<td>250±9</td>
<td>248±8</td>
<td>250±7</td>
<td>250±7</td>
</tr>
<tr>
<td>SOD/CAT-PC</td>
<td>274±9</td>
<td>269±10</td>
<td>266±8</td>
<td>273±8</td>
<td>268±9</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>...</td>
<td>112±3</td>
<td>109±4</td>
<td>104±3</td>
<td>103±4</td>
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<td>104±3</td>
<td>103±4</td>
<td>99±4</td>
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<tr>
<td>High-dose SOD-PC</td>
<td>115±3</td>
<td>109±5</td>
<td>108±5</td>
<td>107±5</td>
<td>104±5</td>
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<tr>
<td>Low-dose SOD-PC</td>
<td>110±4</td>
<td>107±4</td>
<td>95±3</td>
<td>94±5</td>
<td>95±6</td>
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<tr>
<td>SOD/CAT-PC</td>
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<td>113±3</td>
<td>108±3</td>
<td>103±1</td>
<td>102±2</td>
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<tr>
<td>Diastolic blood pressure (mm Hg)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>...</td>
<td>90±2</td>
<td>87±4</td>
<td>85±3</td>
<td>83±3</td>
</tr>
<tr>
<td>PC</td>
<td>95±3</td>
<td>89±3</td>
<td>86±3</td>
<td>84±4</td>
<td>76±5</td>
</tr>
<tr>
<td>High-dose SOD-PC</td>
<td>93±3</td>
<td>87±5</td>
<td>87±5</td>
<td>86±5</td>
<td>82±6</td>
</tr>
<tr>
<td>Low-dose SOD-PC</td>
<td>91±4</td>
<td>88±4</td>
<td>77±3</td>
<td>75±6</td>
<td>78±6</td>
</tr>
<tr>
<td>SOD/CAT-PC</td>
<td>97±3</td>
<td>88±3</td>
<td>84±3</td>
<td>78±2</td>
<td>73±3</td>
</tr>
<tr>
<td>Rate-pressure product (/10²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>...</td>
<td>312±15</td>
<td>306±18</td>
<td>286±14</td>
<td>283±18</td>
</tr>
<tr>
<td>PC</td>
<td>296±18</td>
<td>274±14</td>
<td>268±13</td>
<td>262±16</td>
<td>248±16</td>
</tr>
<tr>
<td>High-dose SOD-PC</td>
<td>305±14</td>
<td>268±13</td>
<td>266±17</td>
<td>261±18</td>
<td>256±16</td>
</tr>
<tr>
<td>Low-dose SOD-PC</td>
<td>269±23</td>
<td>270±19</td>
<td>236±14</td>
<td>236±20</td>
<td>241±21</td>
</tr>
<tr>
<td>SOD/CAT-PC</td>
<td>324±11</td>
<td>306±20</td>
<td>287±17</td>
<td>282±10</td>
<td>274±15</td>
</tr>
</tbody>
</table>

BPC, before preconditioning; Pre O, immediately before 30-minute occlusion; Occlusion, 2 minutes after coronary occlusion; Pre R, immediately before reperfusion; Reperfusion, 2 minutes after reperfusion; PC, preconditioned group; high-dose SOD-PC, high-dose superoxide dismutase–infused preconditioned group; low-dose SOD-PC, low-dose SOD-infused preconditioned group; SOD/CAT-PC, SOD-plus-catalase–infused preconditioned group.
TABLE 3. Infarct Size Data for the Experimental Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Heart weight (g)</th>
<th>AAR (cm³)</th>
<th>AAR (% heart wt)</th>
<th>Infarct (cm³)</th>
<th>Infarct (% heart wt)</th>
<th>Infarct/AAR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11</td>
<td>7.2±0.2</td>
<td>0.69±0.10</td>
<td>10.4±1.5</td>
<td>0.32±0.06</td>
<td>4.7±0.8</td>
<td>45.1±3.5</td>
</tr>
<tr>
<td>PC</td>
<td>12</td>
<td>7.7±0.2</td>
<td>0.71±0.07</td>
<td>9.7±0.9</td>
<td>0.09±0.02</td>
<td>1.3±0.2*</td>
<td>13.3±3.0*</td>
</tr>
<tr>
<td>High-dose SOD-PC</td>
<td>8</td>
<td>7.5±0.3</td>
<td>0.69±0.13</td>
<td>9.9±2.1</td>
<td>0.07±0.02</td>
<td>1.1±0.4*</td>
<td>9.7±1.8*</td>
</tr>
<tr>
<td>Low-dose SOD-PC</td>
<td>6</td>
<td>7.2±0.3</td>
<td>0.60±0.11</td>
<td>8.7±1.5</td>
<td>0.08±0.03</td>
<td>1.1±0.3*</td>
<td>11.9±2.2*</td>
</tr>
<tr>
<td>SOD/CAT-PC</td>
<td>6</td>
<td>8.4±0.2*</td>
<td>0.70±0.11</td>
<td>8.9±1.4</td>
<td>0.07±0.02</td>
<td>0.9±0.3*</td>
<td>9.6±2.3*</td>
</tr>
</tbody>
</table>

AAR, area at risk; Infarct/AAR, infarct size as the percent of AAR; PC, preconditioned group; high-dose SOD-PC, high-dose superoxide dismutase–infused preconditioned group; low-dose SOD-PC, low-dose SOD-infused preconditioned group; SOD/CAT-PC, SOD-plus-catalase–infused preconditioned group.

*p<0.05 versus control by analysis of variance with Bonferroni’s test.

pressures and rate–pressure products, an index of myo-
cardial oxygen consumption, were comparable
among all experimental groups.

Sizes of Area at Risk and Infarct

Heart weight, size of AAR, and infarct size are
given in Table 3. The sizes of AAR, a baseline
determinant of infarct size, averaged 0.60±0.11–
0.71±0.07 cm³ in the five experimental groups and
were comparable. Infarct size as a percent of AAR
(%I/AAR) was 45.1±3.5% in the control group. The
PC group had significantly smaller %I/AAR (13.3±3.0%)
than the control group, indicating marked
infarct size limitation by the preconditioning.
Furthermore, %I/AAR was also limited in the high-
and low-dose SOD-PC groups and the SOD/CAT-PC
group (%I/AAR: 9.7±1.8% in the high-dose
SOD-PC group, 11.9±2.2% in the low-dose SOD-PC
group, and 9.6±2.3% in the SOD/CAT-PC group).
Although there was a slight trend for smaller %I/
AAR in the SOD- and SOD/CAT-treated PC groups
compared with the PC group, the differences were
not statistically significant. These findings suggest
that the cardioprotective effect of the precondition-
ing was not attenuated by treatment with SOD or
SOD plus catalase.

Figure 2 presents the relation between the size of
AAR and absolute infarct size. The absolute size of
infarct was closely correlated with the size of AAR in
all groups (control group: y=0.537x–0.055, r=0.93;
PC group: y=0.123x+0.004, r=0.50; high-dose
SOD-PC group: y=0.121x–0.012, r=0.64; low-dose
SOD-PC group: y=0.176x–0.029, r=0.79; SOD/
CAT-PC group: y=0.114x–0.010, r=0.57 (all,
p<0.05). Furthermore, as shown in Figure 2A, the
slope of the regression line in the PC group was
significantly less steep than that in the control group
(p<0.05), whereas the intercept was not different.
These findings indicate that the ischemic precondition-
ing limited infarct size in the rabbit, regardless of
the size of AAR. The slopes of the regression lines in
the high- and low-dose SOD-PC groups and the
SOD/CAT-PC group were also markedly less than in
the control group (Figures 2B–2D). The regression
lines of those preconditioned groups were not statis-
tically different from that of the PC group, which
suggests that the preconditioning effect was not atten-
uated by the administration of free radical scavengers.

Histology

The myocardial infarct of the preconditioned ani-
mal was generally patchy in contrast with more
confluent infarcts in the control animals. However,
cellular components in the infarcts (i.e., macro-
phages, fibroblasts, interstitial hemorrhage, and
necrotic myocytes) were similar in both control and
preconditioned animals.

Discussion

The present study revealed that ischemic precon-
ditioning limits myocardial infarct size in the rabbit, a
species with poor native coronary collaterals and
little myocardial xanthine oxidase. The cardioprotective
effect of preconditioning was not attenuated by
SOD or SOD plus catalase, which suggests that
oxygen free radicals are unlikely to contribute to the
increased ischemic tolerance of the preconditioned
myocardium in the rabbit.

The efficacy of some interventions on myocardial
ischemic injury depends on the species. For example,
verapamil or allopurinol reportedly limits myocardial
infarct size in the dog heart,17–19 but both agents failed
to modify the infarct size in the rabbit.5,20 The delay of
ischemic myocardial necrosis by preconditioning was
first found in the canine heart by Murry et al1 and
recently examined in the pig by Schott et al.21 Unfortu-
nately, Schott et al21 used nitro-blue tetrazolium
staining, which potentially underestimates infarcts
under some interventions.22,23 to identify the necrosis.
Nevertheless, the tetrazolium-negative zone in the
AAR of the pig heart after 60 minutes of ischemia was
limited to approximately 20% of the control value by
ischemic preconditioning, suggesting substantial
myocardial salvage. The present study showed that myocar-
dial protection by preconditioning exists in the rabbit
heart as well. These findings clearly indicate that retar-
dation of ischemic myocardial cell death by ischemic
preconditioning is not an isolated phenomenon in a
particular species.

On the other hand, there are some noticeable
differences in the hearts of the dog, pig, and rabbit.
The dog heart has well-developed coronary collar-
eterals24–26 in contrast with the rabbit and pig hearts,
both of which have very poor native collaterals,16,24,27 The level of myocardial xanthine oxidase, a source of free radicals during reperfusion, is high in the dog8,12 but undetectable in the rabbit9,11 and pig.12 The activity of extracellular SOD, a native free radical scavenger, in the rabbit is approximately 10-fold and 50-fold of that in the pig and dog, respectively.28 Despite those species differences, ischemic preconditioning increased the myocardial tolerance to ischemia in all of those species. Accordingly, the extents of coronary collateral development, myocardial xanthine oxidase, and extracellular SOD activity may not be important in preconditioning the myocardium.

The myocardial salvage observed in the SOD and SOD/CAT-PC groups (Figure 2) may be attributable to either preconditioning or the free radical scavengers. However, in our previous studies, which assessed the effects of SOD and SOD plus catalase on myocardial infarct size, the free radical scavengers did not limit infarct size in the unpreconditioned rabbit heart.14,15 Accordingly, the significant myocardial salvage in the SOD-PC and SOD/CAT-PC groups is most likely induced by ischemic preconditioning, not by administration of SOD or SOD plus catalase.

Our primary objective in the present study was to test whether oxygen free radicals play a major role in the mechanism of preconditioning. Because Omar et al29 recently reported a parabolic dose–response relation for SOD, we set up two different doses of SOD to scavenge oxygen free radicals. In an attempt to achieve a plasma SOD level similar to that in the study by Murry et al7 using the dog model, 30,000 units/kg SOD, which is twice their dose, was first selected because the plasma half-life of exogenous SOD in the rabbit is approximately half of that in the dog (1022 versus 22 minutes30). This dose of SOD with or without coadministration of catalase before and during preconditioning failed to attenuate the infarct-limiting effect of preconditioning in the present study (Figure 2). We also tested the lower dose of SOD used in their study (i.e., 15,000 units/kg), but the results were similar to the effect of the high dose of SOD (Figure 2). These results did not support, at

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**FIGURE 2.** Scatterplots of relation between the size of area at risk and the infarct size. Panel A: Control group versus preconditioned (PC) group. Panel B: Control group versus high-dose superoxide dismutase (SOD)-PC group. Panel C: Control group versus low-dose SOD-PC group. Panel D: Control group versus SOD/CAT-PC group. There were close linear correlations between size of area at risk and infarct size in all five groups. Slopes of regression lines were significantly smaller in the four preconditioned groups than in the control group, whereas the intercepts did not differ among the groups. PC group, preconditioned group; high-dose SOD-PC group, high-dose SOD-treated preconditioned group; low-dose SOD-PC group, low-dose SOD-treated preconditioned group; SOD/CAT-PC group, SOD-plus-catalase–treated preconditioned group.
least in the rabbit heart, the hypothesis that oxygen free radicals during preconditioning render the myocardium more resistant to a subsequent long, sustained ischemia.

The present findings are in contrast to those of Murry et al.,7 who reported attenuation of the protective effect of preconditioning by administration of SOD plus catalase. In Murry et al’s study, ischemic preconditioning caused %I/AAR after 40 minutes of ischemia to decrease from its control value of 27.3±4.7% to 5.2±1.4%. However, in dogs that received SOD plus catalase during preconditioning, %I/AAR was 13.7±3.1%, which is significantly larger than that in the group with preconditioning alone.7 The reason for the discrepancy between values from the study by Murry et al7 and our study is not clear, but the few differences that exist between the two studies might be responsible. First, we are using a different species. Although ischemic preconditioning was markedly protective in both species, they may not completely share the same mechanisms. It is possible for there to be both free radical–dependent and –independent components in the mechanism of preconditioning; the former component is important in the dog heart, whereas the latter is dominant in the rabbit.

Second, the severity of ischemia during the preconditioning was probably not the same in the two studies. Although the protocol of preconditioning was the same (i.e., four episodes of 5 minutes of ischemia and 5 minutes of reperfusion), metabolic change during the preconditioning would have been more severe in the rabbit than in the dog because the collateral blood flow is at an almost negligible level in the rabbit heart16,27 in contrast with the canine heart, which has a considerable level of collateral flow.25,26 This difference in collateral blood flow also explains that infarct size after 30 minutes of ischemia in the rabbit was larger than that after 40 minutes of ischemia in the dog (%I/AAR: 45.1±3.5% versus 27.3±4.7%, respectively). The possibility of the role of free radicals in the preconditioning differing depending on the severity of ischemia during preconditioning procedure appears unlikely but cannot be ruled out.

An alternative mechanism that has been proposed for preconditioning relates to the calcium channel. In the dog, administration of verapamil limited myocardial infarct size when the myocardial ischemia lasted 40 minutes (%I/AAR: 34±8% versus 8±3%), but its protective effect was not detected when the ischemia was extended to 3 hours.17 This relation between ischemia duration and the effect of verapamil on infarct size is very similar to that of ischemic preconditioning. In the same canine ischemia/reperfusion model, preconditioning limited infarct size after 40 minutes of ischemia to 19% of control value but failed to modify the size of infarct resulting from 3 hours of ischemia.1 In our previous rabbit ischemia/reperfusion model, verapamil failed to salvage myocardium,20 but preconditioning markedly reduced the infarct size in the present study. Accordingly, at least in the rabbit, it is unlikely that preconditioning and verapamil share a common mechanism of cardioprotection.

The mechanism underlying ischemic preconditioning is still undefined. In a recent study, Murry et al4 showed that the rate of ATP depletion during sustained ischemia was slowed in the preconditioned myocardium and that the rates of glycogen breakdown and anaerobic glycolysis were also reduced. Calculated ATP use during ischemia was less in the preconditioned myocardium,4 explaining the reduced ATP depletion rate. Whether a similar change in ATP metabolism occurs in the preconditioned rabbit heart is unknown; if that is the case, what leads to the suppression of ATP use remains to be elucidated.

In conclusion, preconditioning of myocardium with brief ischemia and reflow delays ischemic myocardial cell death during subsequent long, sustained ischemia in the rabbit. In contrast with an earlier finding in a dog model, SOD with catalase or SOD alone did not attenuate the infarct size–limiting effect of preconditioning in the rabbit, which indicates that the ischemic preconditioning in this species is unlikely to be attributed to oxygen free-radical generation. These findings suggest that ischemic preconditioning is also cardioprotective in the xanthine oxidase–deficient heart, but its major mechanism might be different from that in the xanthine oxidase–rich canine heart.

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