THE PROTECTIVE EFFECT OF PRECONDITIONING ON REPERFUSION-INDUCED ARRHYTHMIA IS LOST BY TREATMENT WITH SUPEROXIDE DISMUTASE

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The purpose of this study was to investigate the relation between free radicals and the effect of preconditioning on reperfusion arrhythmias using the isolated working rat heart model.

Rats were divided into 3 groups: control, preconditioned without superoxide dismutase (SOD) and preconditioned with SOD (n=8 each group).

The incidence of reperfusion-induced ventricular fibrillation (Vf) in the preconditioned without SOD group was reduced from 100% to 13% ($p < 0.005$ vs control). This protective effect of preconditioning was not observed in the SOD-treated group (incidence of Vf increased from 13% to 75%, $p < 0.025$).

The protective effect of preconditioning on reperfusion arrhythmias is related to the presence of oxygen free radicals which occur during the reperfusion period of the preconditioning phase.
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THE increasing use of percutaneous transluminal coronary angioplasty (PTCA) and related procedures has heightened interest in the consequences of reperfusion of the myocardium after moderately short periods of ischemia. This interest has been further stimulated by the suggestion that reperfusion of ischemic tissue, caused by the spontaneous relief of coronary spasm, may cause reperfusion-induced arrhythmias and sudden cardiac death^{1,2}. Brief episodes of ischemia, such as in unstable angina or variant angina, might also be expected to exert profound and long-lasting effects on myocardial metabolism and function³. Such episodes of ischemia alter the myocardium's response to a subsequent coronary occlusion,

a phenomenon which is referred to as "preconditioning".

Many investigators have reported on the protective effect of preconditioning on reperfusion arrhythmias^{3–8} but the mechanism of this effect is still unclear. Furthermore, there have been only a few investigations of the relation between oxygen free radicals and the effect of preconditioning^{9,10}.

The purpose of this study was to assess, 1) whether preconditioning has a protective effect in the isolated rat heart, and 2) whether oxygen-derived free radicals are related to the preconditioning effect.

METHODS

Animals

Male Sprague-Dawley rats (270–350 g) were used in all of the studies. All of the ex-

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Fig.1. Experimental protocol

Hearts were perfused in the Neely-Morgan working heart mode. In the preconditioned with scavenger group, superoxide dismutase was administered during all 3 of the reperfusion periods.

Reperfusion-Induced Ventricular Arrhythmia

Fig.2. ECG of reperfusion-induced arrhythmia

Fibrillation occurred 20 sec after the onset of reperfusion.

periments were conducted according to the Guidelines for Animal Experiments at Yamanashi Medical College.

Perfusion Technique

Animals were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal).

The hearts were rapidly excised and perfused using the Langendorff technique with modified Krebs-Henseleit bicarbonate solution at pH 7.4 and 37 °C, containing 11 mM glucose equilibrated with a gas mixture of 95% O₂ and 5% CO₂. The left atrium was cannulated and both caval veins were ligated. Five

Fig. 3. Incidence of reperfusion-induced ventricular fibrillation

SOD: superoxide dismutase

* $p < 0.005$, ** $p < 0.025$

minutes after Langendorff perfusion, the heart was perfused in the working heart mode¹¹ for 10 min as control perfusion.

Whole heart ischemia was induced using a one-way aortic valve with ventricular pacing of 300 beats/min.

Experimental Protocol (Fig. 1)

Rats were divided into 3 groups.

Control group ($n=8$): After a 21 min control phase, 15 min of whole heart ischemia was followed by 15 min of reperfusion.

Preconditioned without superoxide dismutase (SOD) group ($n=8$): Five minutes of ischemia and 2 min of reperfusion were repeated 3 times in the preconditioning phase. The heart was then subjected to 15 min of ischemia and 15 min of reperfusion, as in the control group.

Preconditioned with SOD group ($n=8$): In the preconditioning phase, SOD (from bovine erythrocyte, Sigma) 50,000 U/L, was administered during all 3 of the reperfusion periods.

Measurement of Rhythm Disturbances

Epicardial electrocardiogram (ECG) was recorded via 3 silver electrodes attached directly to the heart surface throughout each experiment. We estimated the incidence of ventricular fibrillation (Vf) during the reperfusion phase for 15 min. We used the Lambeth conventions as the criteria for Vf or VT (ventricular tachycardia).

A typical recording of reperfusion-induced arrhythmia is shown in Fig. 2.

Tests of Statistical Significance

Fisher's exact probability test was used to evaluate the incidence of Vf. A p value of

less than 0.05 was considered to be significant.

RESULTS

The Incidence of Reperfusion-induced Ventricular Fibrillation (Vf) (Fig. 3)

The incidence of Vf in the control, preconditioned without SOD and preconditioned with SOD groups were 100% (8/8 hearts), 13% (1/8 hearts) and 75% (6/8 hearts), respectively. The incidence of Vf was significantly reduced in the preconditioned without SOD group ($p < 0.005$ vs control), but this protective effect was lost after the addition of SOD ($p < 0.025$ vs preconditioned without SOD).

The incidence of sinus recovery by fibrillated hearts was 0% (0/8 hearts) in the control, 0% (0/1 hearts) in preconditioned without SOD and 50% (3/6 hearts) in preconditioned with SOD.

All of the cases in the 3 groups are shown in Fig. 4.

DISCUSSION

Radical scavengers have a protective effect on reperfusion arrhythmias by reducing free radicals.¹²⁻¹⁵ We previously demonstrated this protective effect of SOD (50,000 U/L) in the working rat heart model.¹² However, in the present study, SOD (also 50,000 U/L) in the preconditioning phase did not protect the heart in the reperfusion phase. Similar findings have been previously reported by Murry et al⁹ and Omar et al.¹⁰ This finding raises the question of whether this phenomenon is related to the mechanism of the preconditioning effect.

Xanthine oxidase is a key enzyme in the genesis of oxygen-derived free radicals. It uses hypoxanthine or xanthine as a substrate and O_2 as a cofactor to produce superoxide radical and uric acid.^{16,17} Recently, Terada et al¹⁸ reported autoinactivation of xanthine oxidase: i.e., xanthine oxidase suffers autoinactivation during catalysis by superoxide radical or hydrogen peroxide. Preconditioning may produce oxygen free radicals and thus inactivate xanthine oxidase in the heart. Therefore, a preconditioned heart would show a low incidence of reperfusion arrhythmias because of fewer free radicals.

Fig.4. All of the hearts in the reperfusion phase
Hearts which fibrillated during the ischemic phase were excluded from this study.
SOD: superoxide dismutase

An alternate mechanism for the preconditioning effect may be related to metabolic changes. Preconditioning slows the rate of high energy phosphate utilization⁴ which leads to reduced hypoxanthine and xanthine production during ischemia, which in turn may limit free radical production during reperfusion via the xanthine oxidase pathway^{16,17}. However, this theory does not explain our present result that the preconditioning effect was lost by the addition of a radical scavenger.

In the present study, isolated rat heart, which is not influenced by the autonomic nervous system, was examined and the effect of preconditioning was observed. Thus, it is unlikely in this study that preconditioning produced a "sympathectomized" myocardium which could withstand the effects of ischemia better than an ischemic myocardium with a normal catecholamine content¹⁹. An increase in collateral blood flow²⁰ could also be ruled out under the present ex-

perimental conditions?

Other factors, such as reduced potential for anaerobic glycolysis²¹, induction of protective proteins²², reduced regional hydrogen ion production²³, or large depletion of intracellular potassium²⁴, were not assessed in our study. However, these factors may also be important for understanding the mechanism of the preconditioning effect.

We previously examined the incidence of arrhythmias at various ischemic time points (5, 10, 15, 20 min). A bell-shaped curve of reperfusion arrhythmias (peak incidence at 15 min) was observed in both the control and preconditioned hearts. Therefore, we selected 15 min of ischemia for this study.

In a preliminary study, 5 min of non-ischemic perfusion and 2 min of SOD perfusion were repeated in the "preconditioning phase". The incidence of reperfusion-Vf was 100% (5/5 hearts). Therefore, the possibility that SOD may have had non-specific effects was ruled out.

We previously reported that the incidence of reperfusion-induced ventricular fibrillation was similar in the preconditioned group and the preconditioned with scavenger group!¹² These previous findings appear to contradict those in the present study. However, the method of preconditioning (i.e., number and duration of ischemia and reperfusion periods) differed widely between the 2 studies. The degree of ischemia and the damage to cardiac cells may have been more severe in the previous study. These differences should be clarified in a future study.

Although our observations were limited to the rat heart and did not exactly correspond to clinical ischemia, our results might add a new perspective to the understanding of several clinicopathological conditions, including sudden death associated with conditions such as unstable or variant angina.

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