Effects of Diabetes on Lipid Peroxidation and Scavenger Activity in Ischemia/Reperfusion Injury

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SUMMARY

This study examined the effects of ischemia/reperfusion on cardiac function and diabetes-associated lipid peroxidation and scavenger induction in isolated working hearts. Cardiac function in diabetic rats was depressed in proportion to the duration of diabetes. Plasma levels of malondialdehyde (MDA) were increased significantly after diabetes of 1-week duration (1wD). MDA levels in the myocardium were increased significantly after 4-weeks (4wD) and tissue superoxide dismutase (SOD) activity was also elevated significantly in 8-week diabetic rats (8wD) as compared to control values. In both controls and diabetics, tissue MDA concentrations increased significantly during the first 3 min of reperfusion following 20 min of ischemia, compared with preischemia levels. At the end of reperfusion, tissue MDA levels decreased in controls and 1wD hearts, but remained elevated in the 4wD and 8wD cases. The SOD activity in 8wD hearts decreased significantly after 20 min of global ischemia followed by 30 min reperfusion, compared with preischemia levels. By contrast, there were no significant differences between preischemic and after-reperfusion data for parameters of cardiac function in 8wD rats. Thus, lipid peroxidation appears to have little influence on cardiac function during ischemia/reperfusion in rats with chronic diabetes. (*Jpn Heart J* **34**: 591–599, 1993)

Key Words:

Diabetes Ischemic/reperfused rat heart Malondialdehyde
Superoxide dismutase Cardiac function

CARDIOVASCULAR disorders, especially acute myocardial ischemia, are frequently associated with diabetes. Oxygen radical formation has been found to induce acute myocardial damage,^{1,2)} and some reports suggest that free radical-mediated reactions may also be involved in the pathogenesis of microangiopathy in diabetes.^{3,4)} However, the role of oxygen radical formation in acute myocardial damage in diabetic hearts has not been investigated.

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Received for publication February 26, 1993.

Accepted April 9, 1993.

This study examined changes in the concentration of malondialdehyde (MDA), an index of lipid peroxidation, and the activity of superoxide dismutase (SOD), responsible for free radical scavenging, under various conditions in working diabetic rat heart preparations. We also assessed cardiac function before ischemia and during ischemia/reperfusion to determine relationships with tissue levels of MDA and SOD.

MATERIALS AND METHODS

Animals

Diabetes was induced in male Wistar rats by an intravenous injection of streptozotocin (Sigma Chemical Co., St. Louis, MO, USA) at a dose of 50 mg/kg. Diabetic rats were injected with streptozotocin at 15 weeks (1-week diabetic rats), at 12 weeks (4-week diabetic rats), or at 8 weeks (8-week diabetic rats) of age. Rats with a serum glucose concentration below 300 mg/dl were excluded. Control rats were allowed to develop normally and received a single tail-vein injection of an equal volume of citrate buffer. All animals were sacrificed at 16 weeks of age.

Perfusion methods

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). Thirty seconds later, hearts were excised quickly and placed in a cold perfusion medium until contractions ceased. Each heart was then perfused through the aorta on a noncirculating Langendorff apparatus, operating at a constant pressure (75 cm of water) with a nonprotein Krebs-Henseleit bicarbonate buffer (37°C, pH 7.4) oxygenated with 95%O₂-5%CO₂. Perfusate containing (in mmol/l) 118.0 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25.0 NaHCO₃, 0.5 Na₂EDTA, and 5.5 glucose was filtered through a membrane with a 5- μ m pore size before use. The hearts were then switched to a working-heart apparatus which has been described previously⁵⁾ and allowed to stabilize for 15 min in this recirculating system. The height of the left atrial reservoir was set to maintain a pressure of 10 cm H₂O and the aortic column to maintain a pressure of 75 cm H₂O at all times. Only preparations displaying a sinus rate of at least 200 beats/min, a coronary flow of at least 9 ml/min, and the absence of arrhythmias 5 min before ischemia were used in this study.

Global ischemia was induced by cross-clamping the aortic perfusion tube and left atrial cannula for 20 min at 37°C. The preparation was then reperfused with the same oxygenated buffer. If ventricular fibrillation occurred during the reperfusion state, the heart was excluded from the data analysis.

Physiological measurements

Electrocardiograms were recorded via two silver electrodes attached to the ventricular apex and the aortic cannula. Coronary flow was measured by collecting the effluent and aortic output was measured with an electromagnetic flowmeter (Nihon Kohden, Tokyo, Japan). A polyethylene catheter was placed into the left ventricle via the left atrium to measure the left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP). Heart rate and left ventricular pressure were recorded with a transducer and polygraph system (Nihon Kohden). Individual hearts were frozen in liquid nitrogen and metabolic parameters measured before ischemia, after the first 3 min of reperfusion, or 30 min after reperfusion.

Metabolic measurements

The concentration of MDA, a lipid peroxidation product, was measured in heart homogenates by the thiobarbituric acid test.⁶⁾ Optical density was determined at 532 nm. SOD activity was determined according to the luminescence method using a Cypridina luciferin analog, CLA [2-methyl-6-phenyl-3, 7 dihydroimidazo (1, 2a) pyradin 3-one] (Sigma Chemical Co.).⁷⁾ The standard reaction mixture contained 6×10^{-7} M CLA, 5×10^{-5} M hypoxanthine, 65 units of xanthine oxidase (XOD), SOD (0–20 ng/ml), and 50 mM Tris-HCl buffer containing 0.1 mM EDTA at a pH of 7.8, in a total volume of 3.0 ml. Chemiluminescence measurement was initiated by adding CLA to the reaction mixture without XOD; XOD was added to the mixture 2 min later. Chemiluminescence was then measured with a luminescence reader (BLR-102, Aloka, Tokyo, Japan) at 25°C.

Statistical analysis

The results are presented as mean $\pm$ SE. Statistical analysis was carried out by analysis of variance with Scheffe's test for multiple comparisons. A value of $p < 0.05$ was considered significant.

RESULTS

Plasma MDA content

All three groups of diabetic rats showed significantly higher levels of plasma MDA than the control group. There were no significant differences between the diabetic groups (Fig. 1).

Tissue MDA content

The myocardial MDA content was significantly higher in the 4-week and 8-

Fig. 1. Plasma MDA (malondialdehyde) content. Each bar represents the mean $\pm$ SE of data for 10 rats. 1wD=1-week diabetic rats; 4wD=4-week diabetic rats; 8wD=8-week diabetic rats. * p <0.01 compared with the control group.

Fig. 2. Effects of diabetes and ischemia/reperfusion on myocardial MDA (malondialdehyde) content. Hearts were subjected to 20 min of global ischemia followed by 30 min of reperfusion. The MDA content was determined before ischemia and 3 min and 30 min after reperfusion. Each bar represents the mean $\pm$ SE of data for 5 to 7 hearts. 1wD=1-week diabetic rats; 4wD=4-week diabetic rats; 8wD=8-week diabetic rats. * p <0.05 compared with the control group. † p <0.05, †† p <0.01 compared with preischemic values.

week diabetic groups, even in the preischemic state, compared with the control group and 1-week diabetic group (Fig. 2). Three minutes after reperfusion a marked increase in MDA levels was observed in all four groups.

Thirty minutes after beginning reperfusion, the MDA levels in the control and 1-week diabetic groups had returned to 98% and 95% of the preischemic levels, respectively. However, the MDA levels did not decrease during

reperfusion in the 4-week and 8-week groups (112% and 143% of preischemic levels, respectively) (Fig. 2).

SOD activity in cardiac muscle

There were no significant differences in total SOD activity in cardiac muscle among the control group and 1-week and 4-week diabetic groups. In the 8 week diabetic group, SOD activity was significantly higher in the preischemic state compared with the other groups. In addition, this group displayed a significant decrease 30 min after reperfusion compared with the preischemic state (Fig. 3).

Fig. 3. Effects of diabetes and ischemia/reperfusion on myocardial SOD (super-oxide dismutase) activity. Hearts were subjected to 20 min of global ischemia followed by 30 min of reperfusion. The SOD activity was determined before ischemia and 3 min and 30 min after reperfusion. Each bar represents the mean $\pm$ SE of data for 5 to 7 hearts. 1wD=1-week diabetic rats; 4wD=4-week diabetic rats; 8wD=8-week diabetic rats. * $p<0.05$ compared with the control group. † $p<0.05$ compared with preischemic values.

Hemodynamic measurements

Heart rate: Before ischemias, the heart rates of preparations from the 1-week diabetic group did not differ significantly from controls (Table I). The heart rate was significantly depressed from control values in the 4-week diabetic group, and the heart rate in the 8-week diabetic group was significantly lower than in all three other groups. After 30 min of reperfusion, the heart rate was lower than preischemic values in control, 1-week and 4-week diabetic groups.

LVSP: There was no significant difference in LVSP between the control group and the 1-week diabetic group (Table I). In the preischemic state, LVSP was significantly lower in the 4-week and 8-week diabetic groups than in the

Table I. Hemodynamic Data

	Control (n=8)		1wD (n=7)		4wD (n=6)		8wD (n=6)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
HR (beats/min)	298±31	272±38*	272±14	229±26*	261±27†	228±20*	220±20†‡§	209±20
LVSP (mmHg)	110±6	105±5	114±4	106±9	91±6†‡	92±7	86±11†‡	85±4
LVEDP (mmHg)	1.7±1.5	4.3±2.1*	2.1±1.1	4.4±0.9*	4.5±1.1†	4.4±1.1	4.8±1.8‡	5.8±1.8
+ dp/dt (mmHg/s)	3240±390	3400±280	2940±270	2850±270	2700±260	2650±310	2400±420†	2450±90
- dp/dt (mmHg/s)	3050±260	2650±360	2780±180	2330±240	2050±190†‡	2130±220	1730±220†‡	1650±170

1wD=1-week diabetic rats; 4wD=4-week diabetic rats; 8wD=8-week diabetic rats; Pre=before ischemia; Post=after 30 min of reperfusion; HR=heart rate; LVSP=left ventricular systolic pressure; LVEDP=left ventricular end-diastolic pressure; +dp/dt=maximum rate of left ventricular pressure rise; -dp/dt=maximum rate of left ventricular pressure fall.

* $p < 0.05$ compared with preischemic values.

† $p < 0.05$, †† $p < 0.01$ compared with the control group.

‡ $p < 0.05$, ‡‡ $p < 0.01$ compared with the 1wD group.

§ $p < 0.05$ compared with the 4wD group.

control group. LVSP did not deteriorate during ischemia/reperfusion.

LVEDP: In the preischemic preparations, LVEDP was similar in the 1-week diabetic and control groups (Table I). The 4-week and 8-week diabetic groups demonstrated significantly higher LVEDP values. After reperfusion, LVEDP increased significantly in the control and 1-week diabetic groups.

Maximum rate of left ventricular pressure change: The maximum rate of left ventricular pressure rise (+dp/dt) was significantly lower in the 8-week diabetic group compared with the control group. The maximum rate of the left ventricular pressure fall (-dp/dt) was significantly lower in the 4-week and 8-week diabetic groups.

In 8-week diabetic rats, indices of cardiac function showed no deterioration during ischemia/reperfusion.

DISCUSSION

We evaluated the influence of diabetes on lipid peroxidation and scavenger induction and the effects of ischemia/reperfusion on lipid peroxidation, scavenger action, and cardiac function in isolated hearts. Plasma and myocardial levels of MDA, an indicator of lipid peroxidation, were elevated in the diabetic groups, and myocardial SOD activity was significantly elevated in 8-week diabetic rats. In hearts from both the control and diabetic animals, the tissue MDA concentration was elevated significantly from preischemic values during the first 3 min of

reperfusion. Although it remained high in the 4-week and 8-week diabetic groups, no correlation with functional deterioration was observed.

Free radical-mediated oxidative damage has been implicated in diabetic tissue injury, including microangiopathy and metabolic disorders,³⁾ and it also contributes to atherosclerosis, especially in diabetic patients. When radical formation occurs in cell membranes, there may be a progressive disturbance of structural organization, which has a negative importance on cardiac function. To date, however, information on tissue levels of antioxidant enzymes and lipid peroxide in diabetic animals has been incomplete and equivocal. Biological tissues contain a variety of antioxidants, including dismutase, catalase, glutathione peroxidase, thiol compounds, and vitamins, which can protect the cell and its membranes against peroxidative deterioration.⁸⁾⁻¹²⁾ In normal cells, SOD (which inhibits cellular damage by neutralizing superoxide radicals to H_2O_2 ,^{13),14)}) is activated by low oxygen tension and excess production of superoxide radicals. Although antioxidant enzymes like SOD play important roles as scavengers of free radicals, their activity can be gradually exhausted.^{15),16)} Matkovics et al reported a decreased SOD activity in rat hearts with streptozotocin-induced diabetes of 2 months duration.¹⁷⁾ Lammi-Keefe et al also reported a slight decrease in SOD activity in rat hearts 80 days after streptozotocin injection.¹⁸⁾ On the other hand, Asayama et al found both CuZnSOD and MnSOD levels to be unchanged in rat hearts 2 weeks after streptozotocin injection.¹⁹⁾ The discrepancies between these studies may be partly the result of variations in the methodology used for measuring SOD activity. The present study suggests that these changes are time-dependent because SOD activity increased from 4 to 8 weeks as a consequence of elevated lipid peroxidation.

The involvement of oxygen-derived free radicals in the pathogenesis of myocardial ischemia/reperfusion injury is well established.²⁰⁾⁻²³⁾ Our data showed that the 8-week diabetic rats had high tissue MDA levels in the preischemic state and that MDA formation increased during reperfusion, despite an increased SOD activity. Increases in glycosylated proteins,^{15),24)} alterations of phospholipids,²⁵⁾ membrane damage¹⁹⁾ and depleted vitamin E²⁶⁾ are believed to contribute to this increase in lipid peroxidation. In addition, the marked free radical formation during ischemia/reperfusion exceeded the scavenging ability of SOD, judged by its significant decrease after reperfusion.

Deterioration of cardiac function in the streptozotocin-induced diabetic rats corresponded to duration of diabetes, with the poorest cardiac function occurring in the 8-week diabetic group. However, this group also showed no change in relevant parameters between the preischemic and ischemia/reperfusion states. Functional abnormalities, such as decreases in heart rate, LVSP, and $\pm dp/dt$, have been observed in acute and chronic diabetic rat hearts.²⁷⁾⁻³⁰⁾ Diabetic ani-

mals have also been reported to be more susceptible to ischemic stress than non-diabetic animals.^{25),31)} However, our data indicated that the observed increase in MDA formation did not have a significant influence on heart function.

In conclusion, our results demonstrate that cardiac function in diabetic rats deteriorated during chronic diabetes, and that the deterioration was associated with lipid peroxidation. However, no changes in cardiac function-related parameters were found before ischemia and after reperfusion, suggesting that the diabetes-associated lipid peroxidation had little effect during ischemia/reperfusion. By contrast, parameters of cardiac function in nondiabetics decreased after reperfusion. Thus, the depressed cardiac function of diabetic rat hearts is unresponsive to ischemia/reperfusion.

ACKNOWLEDGMENT

The authors gratefully acknowledge the expert secretarial and technical assistance of Miss Yoshie Mano.

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