Effects of nicorandil preconditioning on membrane dystrophin

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Abstract

Objective: Dystrophin is an integral membrane protein that stabilizes the sarcolemmal membrane integrity, and its loss may be involved in the mechanism of ischemia and reperfusion injury. It has been reported that ischemic preconditioning is related to the preservation of membrane dystrophin during ischemia and reperfusion. Preconditioning with nicorandil, a mitochondrial KATP channel opener, may attenuate the injury by preventing a disturbance in the level of this membrane-associated protein. Methods: The isolated rat hearts were subjected to 60 min of cardioplegic arrest, followed by 60 min of reperfusion. The hearts were divided into the following three groups according to the drugs given before cardioplegic arrest. The control group received saline intravenously 30 min before heart isolation. The nicorandil group received nicorandil (0.3 mg/kg) intravenously 30 min before isolation. The 5-HD group received 5-hydroxydecanoate (1 mg/kg) intravenously, a mitochondrial KATP channel blocker, 5 min before nicorandil administration. Cardiac function, myocardial metabolism, dystrophin distribution and protein levels of dystrophin were assessed before and after cardioplegic arrest. Results: The nicorandil group showed significantly better cardiac function and a significant reduction in creatine kinase release during reperfusion. After 60 min of cardioplegic arrest, dystrophin, which was distributed predominantly in the sarcolemmal membrane before ischemia, was translocated to the costameric cytoskeleton in all groups. During reperfusion, the level of membrane dystrophin remained decreased in the majority of cardiomyocytes in the control and 5-HD groups, whereas it was restored to nearly the baseline level in the nicorandil group. The immunoblot analysis supported this result. Conclusions: Depletion of sarcolemmal membrane dystrophin occurred during cardioplegic arrest and reperfusion. Nicorandil preconditioning may attenuate ischemia and reperfusion injury by maintaining the membrane structural integrity.

Keywords: Myocardial preconditioning; Mitochondrial K-ATP channel; Ischemia—reperfusion injury; Cardioplegia

1. Introduction

Despite the advances in myocardial protection techniques, myocardial ischemia/reperfusion injury is still a major problem in patients undergoing cardiac operations. Such injuries can contribute significantly to postoperative complications. To avoid this problem, numerous cardioprotective techniques, such as pretreatment and additives, have been studied. Among them, an endogenous protective mechanism that transient myocardial ischemia makes the heart more resistant to subsequent prolonged ischemia [1] has received much attention. Extensive investigations have been performed to clarify this phenomenon of so-called ischemic preconditioning, and it has been confirmed that the cardioprotective effect can be pharmacologically reproduced by mitochondrial ATP-sensitive K+ (mitoKATP) channel openers. The mitoKATP channel plays an important role during the preconditioning phase, ischemia phase and reperfusion phase for cardioprotection [2]. Pain et al. [3] showed that mitoKATP channel opening triggers the preconditioned state, and this protective effect persisted despite wash-out of mitoKATP channel opener. Therefore, it is considered that single administration of a mitoKATP channel opener to an intact heart before cardioplegic arrest may exert a cardioprotective effect throughout cardiac surgical procedures.

It has been well recognized that acute ischemic injury is pathophysiologically characterized by cell swelling and disturbance of the membrane-associated structural proteins [4]. Distinctive groups of structural proteins, such as the dystrophin-associated protein complex, the vinculin—integrin link and the spectrin-based submembranous cytoskeleton, that link the extracellular and intracellular milieus and confer structural stability to the cell membrane are present in cardiac myocytes [5]. Among these structural proteins, dystrophin is reported to be the most sensitive to ischemic insults and to be a useful index of ischemic cellular damage [6]. Dystrophin is believed to function in the mechanical stabilization of the sarcolemmal membrane and to link...
cytoskeletal actin and talin to transmembrane proteins and via laminin receptors to the basement membrane and extracellular matrix [7]. A recent study in which an association between ischemic heart disease and dystrophin was examined demonstrated that a decrease in dystrophin may play an important role in the pathophysiology of chronic heart failure following acute myocardial infarction [8].

We therefore hypothesized that dystrophin depletion also occurs during cardioplegic arrest and reperfusion, and that this disturbance plays an important role in the occurrence of ischemia/reperfusion injury. We also hypothesized that administration of a mitoK<sub>ATP</sub> channel opener before cardioplegic arrest would mimic the cardioprotective effect of ischemic preconditioning and attenuate the disturbance of the dystrophin-associated protein complex.

2. Materials and methods

All animals in this study received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health (NIH publication 85-23, revised 1985), and this study was approved by the Institution Committee for Experimental Research in our University.

2.1. Blood-perfused isolated rat heart preparation

The blood-perfused isolated rat heart preparation was a modification of the paracorporeal rat heart preparation used by Walters and colleagues [9]. A large male Wistar rat weighing 550—600 g was anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The rat was placed in a supine position on a heating plate (HP-4530; Iuch, Osaka, Japan) which was maintained at 38 °C. The rat was ventilated and oxygenated with a ventilator (SAR-830/A; CWE Inc., Ardmore, PA) in order to maintain arterial PO2 (200 mmHg and CO2 within 30—40 mmHg. Teflon catheters (Angiocath, 24-gauge; Becton Dickinson Infusion Therapy System Inc., UT) were positioned in the left femoral vein (for the administration of fluids and the return of blood collected from the isolated perfused heart) and the left femoral artery (for supplying arterial blood to the isolated heart). The heart was excised rapidly and was perfused in the Langendorff mode. The interval between isolation of the heart and initiation of coronary perfusion was less than 30 s in all experiments. After a 20 min equilibration period, the isolated heart was arrested with warm blood cardioplegia (37 °C, 3 ml/min, 2 min). The cardioplegic solution was prepared by mixing four parts of oxygenated blood to one part of crystalloid solution (K+ 40.0 mequiv./l, Mg2+ 30.0 mequiv./l, Na+ 9.4 mequiv./l, Cl— 40.0 mequiv./l, glucose 76.2 g/l) by means of a syringe pump (KDS 100; KD Scientific, Holliston, MA). The final concentrations of the blood cardioplegia were; approximately K+ 13 mequiv./l, Mg2+ 6.0 mequiv./l, Na+ 103 mequiv./l, Cl— 73 mequiv./l, and glucose 125 g/l. The second and third dose (37 °C, 3 ml/min, 1 min) of cardioplegia were given at 20 min intervals. The last dose was given immediately before reperfusion. Coronary effluents during cardioplegia were collected and discarded. After 60 min of cardioplegic arrest, 60 min reperfusion was begun with 37 °C oxygenated blood from the support rat. The experimental protocols are summarized in Fig. 1.

The heart donor rats were assigned to three groups (n = 6 per group). The first group consisted of control hearts (control group) that received intravenous administration of 0.5 ml saline 30 min before the heart isolation. The second group consisted of pharmacologically preconditioned hearts (nicorandil group) that received intravenous administration of 0.5 ml saline containing 0.3 mg/kg of nicorandil (generously supplied by Chugai Pharmaceutical Co., Tokyo, Japan) 30 min before heart isolation. The third group consisted of the potassium channel blocked hearts (5-HD group) that were given 0.5 ml saline containing 1 mg/kg of 5-hydroxydecanoate (Sigma, St. Louis, MO), a selective blocker of mitoK<sub>ATP</sub> channels, intravenously 5 min before nicorandil administration. In the third group, nicorandil was also administered at the same dose as for the nicorandil group. These drugs were administered systemically through a teflon catheter positioned in the femoral vein. After anticoagulation with heparin (1000 IU/kg, IV), the heart was excised rapidly and was perfused in the Langendorff mode. The interval between isolation of the heart and initiation of coronary perfusion was less than 30 s in all experiments. After a 20 min equilibration period, the isolated heart was arrested with warm blood cardioplegia (37 °C, 3 ml/min, 2 min). The cardioplegic solution was prepared by mixing four parts of oxygenated blood to one part of crystalloid solution (K+ 40.0 mequiv./l, Mg2+ 30.0 mequiv./l, Na+ 9.4 mequiv./l, Cl— 40.0 mequiv./l, glucose 76.2 g/l) by means of a syringe pump (KDS 100; KD Scientific, Holliston, MA). The final concentrations of the blood cardioplegia were; approximately K+ 13 mequiv./l, Mg2+ 6.0 mequiv./l, Na+ 103 mequiv./l, Cl— 73 mequiv./l, and glucose 125 g/l. The second and third dose (37 °C, 3 ml/min, 1 min) of cardioplegia were given at 20 min intervals. The last dose was given immediately before reperfusion. Coronary effluents during cardioplegia were collected and discarded. After 60 min of cardioplegic arrest, 60 min reperfusion was begun with 37 °C oxygenated blood from the support rat. The experimental protocols are summarized in Fig. 1.
2.3. Functional study

Bipolar pacing electrodes were placed on the right ventricle and the hearts were paced at 300 beats/min. A latex balloon, inserted into the left ventricular cavity through an incision in the left atrium appendage, was connected by a short polyvinyl tube to a pressure transducer (UK4006SB; Baxter, Tokyo, Japan), and the signal was amplified, converted digitally and displayed on a computer screen using a Mac LAB system (AD Instruments, NSW, Australia). The balloon was large enough that no measurable pressure was generated by the balloon itself over the range of left ventricular volumes used during the experiment. Left ventricular function was evaluated during isovolumic contraction by inflation of the intraventricular balloon; the balloon was inflated so that the end-diastolic pressure (LVEDP) was 1–3 mmHg in a 20 min equilibration period. During cardioplegic arrest period, the balloon was deflated and the hearts were not paced. During reperfusion, the hearts were paced at 300 beats/min and the balloon was re-inflated with the same balloon volume as the baseline value. Left ventricular developed pressure (LVDP) was calculated as the difference between the peak-systolic pressure and LVEDP. LVDP, LVEDP, positive maximum rate of left ventricular pressure rise (d/dt, mmHg/s) and negative maximum d/dt (−d/dt, mmHg/s) were measured and recorded (Mac LAB system) at 5 min before cardioplegic arrest (Base), and at 5 min (R5), 15 min (R15), 30 min (R30), 45 min (R45) and 60 min (R60) of reperfusion. The coronary blood flow was measured by timed collection of the coronary sinus blood.

2.4. Blood sample study

Blood samples were obtained and assayed for the determination of creatine kinase (CK). Coronary sinus blood samples and coronary arterial blood samples were collected at Base, R5, R15 and R60. The samples were immediately centrifuged at 4°C at 3000 rpm for 15 min and were stored at −80°C until analysis. CK levels were measured with an Ultraviolet Spectrophotometer (Mitsubishi Kajaku Bio-Clinical Laboratories, Inc., Tokyo, Japan). CK release was calculated as coronary flow multiplied by the difference between the coronary arterial and coronary sinus content.

2.5. Immunofluorescence microscopy

For immunohistochemical and immunoblot study, an additional 36 rat hearts were used. The hearts (n = 12 per group) were cut into transverse sections at Base, I60, R15 and R60 (n = 3 in each phase). Sections of the mid-left ventricle (LV) were mounted on stubs with O.C.T. compound (Sakura Finetechncial Co., Tokyo, Japan) and rapidly frozen by hexane cooled to −80°C. The sections of apical LV used for the immunoblot study were rapidly frozen under liquid nitrogen. Heart slices were cryosectioned at 5 μm onto glass slides, incubated for 5 min in −20°C acetone, rinsed with PBS, and blocked with 10% normal rabbit serum. The sections were incubated for 1 h at room temperature with mouse monoclonal anti-dystrophin antibodies (MANDRA-1; Sigma) at a dilution of 1:100 and washed with PBS. They were then incubated for 1 h at room temperature with anti-mouse Alexa-Fluor 488-linked antibodies (Molecular Probes, Eugene, OR) at a dilution of 1:500 and washed with PBS. To investigate whether ischemia and reperfusion influence other structural protein, vinculin, a membrane-associated component, double staining was performed. After the staining of dystrophin, the sections were then incubated for 1 h at room temperature with mouse monoclonal anti-vinculin antibodies (Sigma) at a dilution of 1:200 and washed with PBS. They were then incubated for 1 h at room temperature with anti-mouse Alexa-Fluor 555-linked antibodies (Molecular Probes) at a dilution of 1:500 and washed with PBS. Just prior to mounting the samples, a Prolong Antifade Kit (Molecular Probes) was used to minimize fading of the fluorescent dye. The fluorescence staining was visualized using a confocal laser microscope (ECLIPSE E600W; Nikon Instech Co., Kanagawa, Japan) at an excitation wavelength of 488 nm for Alexa-Fluor 488 and 543 nm for Alexa-Fluor 555. The photographic images were scanned into Photoshop 7.0 and the contrast was digitally enhanced to an equal degree to highlight the details of the myocardium.

2.6. Tissue sample preparation and immunoblot of dystrophin protein level

The frozen apical LV tissues (n = 3 in each phase) were homogenized in 1 ml of lysis buffer (sucrose 303 mM, sodium pyrophosphate 20 mM, sodium phosphate 20 mM, MgCl2 1 mM, EDTA 0.5 mM, EGTA 1 mM, sodium orthovanadate 1 mM, PMSF 0.25 mM, benzamidine 2.5 mM, antipain 20 μg/ml, leupeptin 20 μg/ml, chymostatin 5 μg/ml, and pepstatin 5 μg/ml). The homogenates were centrifuged at 10000 × g for 15 min, and the resulting supernatant was centrifuged at 100,000 × g for 60 min. The pellet was designated the membrane fraction and was sonicated in 200 μl of 1% sodium dodecyl sulfate. The protein concentration was determined using a BCA protein assay kit (Pierce, Rockford, IL). Equal amounts of total protein (50 μg/lane) were separated by a 4–12% Bis-Tris Criterion gel (Bio-Rad Laboratories, Hercules, CA). Separated proteins were transferred to a nitrocellulose membrane (Schlei-cher & Schuell, Keene, NH). Membranes were immunoblotted with anti-dystrophin antibodies (MAN- DRA-1) at room temperature for 2 h. Antibody binding was revealed by incubation with anti-mouse Alexa-Fluor 680-linked antibodies (1:5000; Molecular Probes) for 30 min and the Odyssey Infrared Imaging System (LI-COR, Lincoln, NE). Fluorescence at infrared wavelengths was detected by the Odyssey Imager, and quantified using Odyssey software. For each experiment, values obtained at I60, R15 and R60 were calculated as a percentage of the baseline value.

2.7. Statistical analysis

Statistical analysis was performed with Stat View J-5.0 software (SAS Institute, Cary, NC) and Prism4 software (GraphPad Software, San Diego, CA). All data are expressed as the mean ± the standard deviation or the standard error of the mean. One- or two-way repeated-measure analysis of variance (ANOVA) was used to test the effect of group and time. When analysis of variance indicated a significant effect of group or time (p < 0.05), the differences were specified with Scheffe’s test for comparison of cardiac
function, CK and coronary flow. Bonferroni post hoc test was also used for comparison of quantitative immunoblot data. Significance was assumed at a probability level of less than 0.05.

3. Results

3.1. Cardiac function

There were no significant differences in the baseline cardiac function parameters among the three groups. LVDP was best preserved in the nicorandil group during reperfusion (Fig. 2). Significant increases in LVEDP was observed in all groups at the initial phase of reperfusion; however, the extent of this change was smallest in the nicorandil group, and the recovery was more prompt in this group. The degrees of deterioration in cardiac function parameters in the 5-HD group were quite similar to those in the control group.

3.2. Coronary flow and CK release

Coronary flow was significantly lower in the 5-HD group at 15 and 60 min of reperfusion than in the nicorandil group (Fig. 2). The level of CK release at 15 min of reperfusion in the nicorandil group was significantly lower than those in the control and 5-HD groups (Fig. 2).

3.3. Dystrophin and vinculin localization

Immunofluorescence confocal laser microscopy showed that dystrophin localized predominantly at the sarcolemmal membrane of myocytes, and there was slight immunostaining of dystrophin in the cytoplasmic region at baseline in all groups (Fig. 3A, C, and E).

The staining pattern of the membrane dystrophin altered after 60 min of cardioplegic arrest. The immunofluorescence intensity of dystrophin in the membrane decreased slightly; however, its intensity in the cytoplasmic region increased.

Fig. 2. Cardiac function, coronary flow and CK release. The LVDP was significantly ($p < 0.001$) greater and the LVEDP was significantly ($p < 0.04$) lower in the nicorandil group than those in the control and the 5-HD groups during reperfusion. The coronary flow was significantly ($p < 0.04$) greater in the nicorandil group during reperfusion. The CK release was significantly ($p < 0.01$) less in the nicorandil group than those in the control and 5-HD groups during reperfusion ($n = 6$ in each group). LVDP = left ventricular developed pressure; LVEDP = left ventricular end-diastolic pressure; $+\frac{dp}{dt}$ = positive maximum rate of left ventricular pressure rise; $-\frac{dp}{dt}$ = negative maximum rate of left ventricular pressure rise; CK = creatine kinase. $p < 0.05$ versus control and 5-HD group; $p < 0.05$ versus control group; $p < 0.05$ versus 5-HD group. Cardiac function data are shown as mean $\pm$ SEM; Coronary flow and CK release data are shown as mean $\pm$ SD.
reciprocally after 60 min of cardioplegic arrest in comparison with the baseline value (Fig. 3B). The longitudinal section revealed a regular costameric pattern in the cytoplasmic region. Dystrophin distribution after 60 min of cardioplegic arrest in the nicorandil and 5-HD groups was similar to that in the control group (Fig. 3D and F).

At 15 min of reperfusion, however, the membrane dystrophin markedly decreased in the control group and 5-HD group (Fig. 4A and E) whereas it was well maintained in the nicorandil group (Fig. 4C).

At 60 min of reperfusion, membrane dystrophin was decreased markedly in the majority of cardiomyocytes in the control group and 5-HD group (Fig. 4B and F). In contrast, it was restored to nearly the baseline level in the nicorandil group (Fig. 4D).

Similar to dystrophin, vinculin was distributed throughout the sarcomemal membrane of myocytes, whereas it was few in the cytoplasmic region at baseline in all groups (Fig. 5A). At 60 min of reperfusion, the sarcomemal membrane vinculin was preserved as the baseline levels in all groups where that of dystrophin decreased in the control group and 5-HD group (Fig. 5B and D).

3.4. Immunoblot analysis of dystrophin

Immunoblot analysis demonstrated that the total dystrophin content decreased at 60 min of reperfusion compared with the baseline value in the control group and 5-HD group ($p < 0.01$), whereas it was well maintained in the nicorandil group during reperfusion (Fig. 6A).

Although a slight decrease was observed in dystrophin content in the membrane fraction at 60 min of ischemia in all groups, it was not statistically significant. At 15 and 60 min of reperfusion, however, the membrane dystrophin content decreased significantly in the control and 5-HD groups ($p < 0.01$), whereas it was restored in the nicorandil group (Fig. 6B). These results of the immunoblot analysis lent support to those of the immunohistochemical study.

4. Discussion

Nicorandil has been used clinically for the treatment of ischemic heart disease because it has the pharmacological properties of both an ATP-dependent potassium channel
ischemia and reperfusion injury are considered to be hyperpolarizing factor-mediated relaxation in coronary hyperkalemic solution may restore the endothelium-derived function. In this study, it was reported that the addition of nicorandil to cardioplegic solution conferred improved myocardial protection completely abolished by 5-HD administration. Therefore, administration of nicorandil before sustained ischemia with cardioplegia conferred improved myocardial protection mediated by its preconditioning effects through an activation of mitochondrial ATP-sensitive channel. The present study also demonstrated that the preconditioning effects of nicorandil on dystrophin distribution during ischemia and reperfusion were completely abolished by 5-HD pretreatment. It is therefore speculated that the ability of nicorandil to preserve membrane dystrophin is related to its ability to act as a mitochondrial ATP channel opener.

Vinculin is also a cytoskeletal protein, located at the costameric junction and fascia adherence junction of intercalated disk, and it plays a role in the linkage between the cytoskeleton and the plasma membrane. It has been demonstrated that its progressive loss occurred during ischemia and reperfusion injury [18, 19]. The transition from the reversible to the irreversible phase of cellular ischemic damage is followed by a cytoskeletal injury, which is characterized by a sarcolemmal membrane disruption with accompanying formation of subsarcolemmal blebs. It has been shown that sarcolemmal fragility is attributed to some extent to loss of the structural proteins, such as vinculin, talin, and paxillin [18, 19]. Dystrophin is also identified as an integral membrane protein in the cytoskeleton that forms a link between the sarcolemma and the costameric junction [5, 7]. This cytoskeletal protein is believed to maintain the sarcolemmal membrane integrity and stability. Inherent mutation of the dystrophin gene is responsible for Duchenne-and Becker-type muscular dystrophy, and for X-linked dilated cardiomyopathy, an absence or reduction of dystrophin causes degradation of both skeletal and cardiac muscles because of a markedly increased susceptibility to contraction-associated membrane rupture and osmotic stress [20]. Several reports have revealed that alterations in a dystrophin-related protein complex occurred during ischemia and reperfusion despite the absence of genetic defect [6, 8, 21]. A recent report has demonstrated that dystrophin, rather than the structural proteins mentioned above, was more susceptible to ischemic insults, and its reduction or absence indicated severe or irreversible ischemic injury [6]. The degree of loss of dystrophin also coincided with suppression of transcription and translation [6]. Therefore, it has been postulated that dystrophin can be a useful marker of cellular ischemic injury, and its preservation is a cause of reduced ischemia/reperfusion injury by maintaining the sarcolemmal membrane integrity.

Kyoji and associates [22] have shown that dystrophin distributed exclusively in the sarcolemmal membrane was translocated to the myofibrils during 30 min of ischemia and then lost during reperfusion in buffer-perfused rat cardiomocytes. They also revealed that the loss of sarcolemmal dystrophin was responsible for contractile force-induced reperfusion injury. In the present study, we also observed translocation of dystrophin to the costameric cytoskeleton after 60 min of cardioplegic arrest irrespective of the presence or absence of nicorandil preconditioning. During reperfusion, however, membrane dystrophin was significantly restored in the hearts that received nicorandil preconditioning, whereas it remained depleted in the hearts without preconditioning. The results of our study are consistent with those of a study reported by Kido and associates [23] in which ischemic preconditioning introduced by three cycles of 5-min ischemia was used instead of pharmacological preconditioning. The present study also demonstrated that the preconditioning effects of nicorandil on dystrophin distribution during ischemia and reperfusion were completely abolished by 5-HD pretreatment. It is therefore speculated that the ability of nicorandil to preserve membrane dystrophin is related to its ability to act as a mitochondrial ATP channel opener.
immunofluorescence staining demonstrated that the vinculin distribution was well preserved after 60 min of cardioplegic arrest and reperfusion, whereas dystrophin was markedly depleted. As shown by Steenbergen and colleagues [19], longer periods of ischemia, i.e., 120 min or longer, may require to produce loss and translocation of vinculin. In other words, dystrophin is more sensitive to ischemic insult than vinculin.

The mechanisms of membrane dystrophin loss and restoration during ischemia and reperfusion observed in the present and previous studies [22,23] remain to be elucidated. Ganote and colleagues [18], however, postulated that because cytoskeletal assembly is maintained by protein phosphorylation, severe ATP depletion may reduce the level of phosphorylation of cytoskeletal structural or regulatory proteins, and lead to disassembly of cytoskeletal elements. A recent study by Rodriguez et al. [6] also showed significant morphological changes of mitochondria and functional deterioration of nuclear protein synthesis at both the transcriptional and translational levels in myocytes with depleted dystrophin. Accordingly, mitochondrial injury and subsequent ATP depletion during ischemia and reperfusion may explain the dystrophin loss.

There is considerable evidence that preconditioning with a K$_{ATP}$ channel opener preserves mitochondrial oxidative phosphorylation and leads to a reduction in the rate of ATP loss during ischemia and reperfusion [2,15,24]. Because dystrophin is a phosphoprotein and is phosphorylated in vivo within the COOH-terminal at both serine and threonine residues, oxidative phosphorylation activity of mitochondria is believed to be essential for the restoration of dystrophin [25]. It is therefore conceivable that the preservation of mitochondrial function and attenuation of ATP depletion induced by nicorandil preconditioning are associated with the restoration of sarcosomal dystrophin during reperfusion. In the present study, however, mitochondrial function and ATP levels were not examined. Therefore, further studies are required to elucidate the association between nicorandil preconditioning effects and restoration of cytoskeletal elements. Moreover, the present study was intended to investigate the effects of nicorandil preconditioning through an activation of K$_{ATP}$ channel, the effects of nitrates on dystrophin after ischemia/reperfusion were not studied. Further studies are also required to elucidate the association between nitrate-like properties and restoration of cytoskeletal elements.

In conclusion, depletion of sarcosomal membrane dystrophin and its translocation to myofibrils occurred during cardioplegic arrest and reperfusion. Administration of nicorandil before cardioplegic arrest preserved cardiac function via activation of the mitochondrial K$_{ATP}$ channel. This pretreatment also facilitated the restoration of membrane dystrophin during reperfusion. The results indicated that nicorandil preconditioning may attenuate ischemia and reperfusion injury by maintaining the cytoskeletal structural integrity.

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