—Review—

Pathogenesis and Protection of Ischemia and Reperfusion Injury in Myocardium

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Abstract

The important factors that influence the progress of ischemic cardiac lesion are blood flow condition and abnormal cardiac metabolism. Myocardial ischemia is promoted by either an increase in oxygen demand or a shortage of oxygen supply. The Na\textsuperscript{+}-Ca\textsuperscript{2+} ion exchange mechanism is very important for myocardial contraction and cell damage. Na\textsuperscript{+} - K\textsuperscript{+} ATPase and Ca\textsuperscript{2+} ATPase are enzyme histochemically localized in subsarcolemmal cisterns, sarcolemmal reticulum and capillary endothelium, and keep myocardial function. These ATPases are impaired by anoxia, superoxides and free radicals. The reduction of O\textsubscript{2} results in the production of superoxides as well as hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). H\textsubscript{2}O\textsubscript{2} is highly diffusible and induces cell damage. H\textsubscript{2}O\textsubscript{2} appears to affect not only lipids but also intramembranous proteins embedded in the cell membrane. The hydroxyl radical (OH\textsuperscript{•}) also participates in lipid hyperoxidation. In the pathogenesis of ischemic and/or reperfused heart disease, ischemia induces rapid or gradual changes in all membrane systems and causes reversible or irreversible injury including necrotic and apoptotic cell death. Advanced glycation end products (AGEs) accumulation induced by diabetic conditioning is an etiologic factor inducing cardiomyopathy. The AGEs protein affects cell changes such as increased number, transformation, functional disturbance and cytokine elimination. In coronary arteries, the migration of smooth muscle cells caused by the taking up of AGEs proteins through the receptor (RAGE), and cytokine discharge are suggested. AGEs accumulation may induce diabetic macroangiopathy through RAGE, and the increase in the level of RAGE expression by endothelial cells could be a reason that diabetes mellitus accelerates atherosclerosis. On the other hand, we also reported that hyperglycemia was a promoting factor of ischemic heart injury in diabetic animals. Ischemic preconditioning is a useful phenomenon that limits myocardial damage. We focused on protein kinase C (PKC), mitogen-activated protein kinase (MAPK) and mitochondrial ATP-dependent potassium (mitoK\textsubscript{ATP}) channel as mediator or end which effector are necessary for adaptation. The opening of the mitoK\textsubscript{ATP} channel induces the depolarization of mitochondria, reducing Ca\textsuperscript{2+} overload during reperfusion. The regeneration of myocardial cells is confirmed using embryonic stem cells. Myocardial cells that exhibit self-pulsation are generated from mesenchymal stem cells in...
mesodermal tissues of the bone marrow. (J Nippon Med Sch 2003; 70: 384–392)

**Key words:** ischemic cardiomyopathy, ATPase depletion, hydrogen peroxide, AGEs, preconditioning

**Introduction**

An ischemic condition caused by decreased coronary blood flow is one of the most important factors inducing heart failure. Thus, myocardial ischemia is diagnosed by either an increase in oxygen demand or a shortage of oxygen supply. In particular, it can be said that the functional stenosis of the artery is caused by coronary sclerosis and vascular contraction. On the other hand, reperfusion is the only therapy through as percutaneous transluminal coronary angioplasty or coronary artery bypass surgery capable of restoring myocardial blood flow to the ischemic myocardium. However, cardiac dysfunction including arrhythmias, stunning, microvascular damage and cell death can be induced by reperfusion injury. The important factors that influence the progress of ischemic cardiac lesions are ischemia, blood flow condition and abnormal metabolism. The major mechanisms for the injury are affected by such factors as free radicals and calcium overload. In this communication, we investigate the occurrence of cell injury in ischemic heart disease and how to prevent this pathological condition.

**I. Structure and Function of the Myocardium**

The function of cardiac cells is basically maintained by a membrane complex, which is integrated by structural components such as sarcolemma, sarcoplasmic reticulum and mitochondria. The sarcolemmal membrane formed by T tubules permits close communication among the sarcoplasmic reticulum, mitochondria and other cell components.

Ultrastructural observations have revealed that atrial cells tend to have very few T tubules and that the Golgi complex and specific dense granules in the atria are well developed compared to those in the ventricles. Histologically, Na⁺-K⁺ATPase is localized in subsarcolemmal cisterns, the inner side of the plasma membrane, T-tubule, free and junctional sarcoplasmic reticulum, and capillary endothelium (Fig. 1). This enzyme is also a receptor for digitalis. Moreover, the extent of action of Na⁺-K⁺ATPase on the plasma membrane and T-tubules decreases with ouabain or potassium deletion. However, the reaction products in mitochondria, sarcoplasmic reticula (free and junctional) and subsarcolemmal cisterns are unaffected by ouabain or potassium deletion. On the other hand, calmodulin is located in the plasma membrane and plasmalemmal vesicles as well as in the cytoplasm of vascular endothelial and cardiac cells. Calmodulin may also play an important role in the regulation of Na⁺-K⁺ATPase⁷. The activity of acid phosphatase in the atria is higher than that in the ventricles since the Golgi complex and subsarcolemmal cistern are more highly developed in the atria, and specific granules in the atria exhibit acid phosphatase activity. The activity of lysosomal enzymes such as acid phosphatase has been proved to be irregular in the cytoplasm, suggesting the leakage of this hydrolytic enzyme⁸. Alkaline phosphatase is located in the endothelium of blood vessels and the plasma membrane of cardiac cells. And glucose-6-phosphatase is distributed in the sarcoplasmic reticulum, the nuclear membrane and the Golgi complex. However, acid and alkaline phosphatases are not found in the peroxisome of cardiac cells. Lipid
droplets in rat cardiac cells are surrounded by a ring of dense granular reaction products of enzymes, such as acid and alkaline phosphatases, or 3-3'-diaminobenzidine (DAB) reaction products.

II. Pathogenesis of Ischemic Heart Disease

1. Ischemic and reperfusion injury in the myocardium

Cardiomyocytes are affected by hypoxic/anoxic conditions. Ischemia induces rapid or gradual changes in all membrane systems and causes reversible or irreversible injury. From experimental studies, tumidity changes are observed in the cells surrounding the ischemic area of the endocardium 30 minutes after coronary ligation, and one hour later what extends to the cardiomyocyte area. Irreversible myocardial damage such as contraction band necrosis and myocyteolysis becomes prominent after prolonged coronary occlusion. Such irreversible myocardial damage immunohistochemically reveals the diminution of creatine phosphokinase (CPK) and myoglobin staining under ischemic conditions. After 20 and 40 minutes of ischemia followed by 60 minutes of reperfusion, irreversible myocardial damage in dog hearts is observed when regional myocardial blood flow before reperfusion is less than 30% of that before occlusion. When 60 minutes of ischemia is followed by 60 minutes of reperfusion, irreversible myocardial damage is observed. The development of collateral blood flow may prevent early irreversible morphologic changes in the myocardium, especially within 40 minutes of ischemia. Experimentally, anoxic damage is initially localized in the cytomembrane during various types of reversible and irreversible changes.

Morphologically, at 60 minutes after coronary occlusion, edematous changes expand into the myofibrilar areas. Nuclear changes including chromatin clumping and vascular endothelial swelling are observed in the endocardial layer of the ischemic zone. Takashi et al reported that apoptosis is induced in rat cardiac cells. At this level, the extent of induction of both Bel-2 and Fas increases in the cardiac cells, and he also suggests that apoptosis may be caused by the decrease in pH of the cardiac cells. The reason for the expansion of the ischemic lesion towards the epicardium is that the volume of blood in the inner area is relatively smaller than that in the epicardium, and both mural tension and oxygen demand are also increased. Ultrastructurally, mild glycogen depletion and mitochondrial swelling are encountered in the endocardial layer of the ischemic zone after 15 minutes of occlusion of the coronary artery. At 30 minutes, slight dilatation of the sarcoplasmic reticulum and intercalated disc occurs. At 1 hour after coronary artery ligation, mitochondrial swelling occurs with the loss of matrix density and disorganization of cristae, clumping of nuclear chromatin, disappearance of glycogen granules in the cardiac cells and vascular endothelial swelling with partial disruption of the plasma membrane at a higher extent in the endocardium than in the epicardium. In dog cardiomyocytes, myofibrils separate and electron dense deposits are localized in the mitochondria. The ultrastructural changes in the mitochondria and sarcoplasmic reticulum in early ischemia are confirmed to be irreversible morphological changes in the endocardium and epicardium of the ischemic myocardium. Na⁺-K⁺ATPase is faintly located on the inner side of the plasma membrane and in the pinocytotic vesicles of endothelial cells. Clumping and dispersion in the glycocalyx of endothelial cells are observed in the ischemic heart, which may prove the functional disturbance of the plasma membrane. Potential and functional defects with reduced activity of Na⁺-K⁺ATPase occur within 1 hour of coronary artery ligation (Fig. 2). Membrane dysfunc-
tion due to these molecular changes has been proved by membrane permeability as well as the intracytoplasmic localization of horseradish peroxidase (HRP) as a tracer. On the other hand, Ca$^{++}$ ATPase localization is detected in the plasma membrane and endoplasmic reticulum, and it is restrained by quercetine. Excess contraction is recognized when Ca$^{++}$ penetration accelerates due to the changes in the membrane system caused by the hypoxicity of Ca$^{++}$ ATPase. Also, a decrease in mitochondrial ATP production due to Ca$^{++}$ deposition accelerates cell malfunction. As regards Ca$^{++}$, the following phenomena occur: 1) Ca$^{++}$ pump inhibition, 2) Ca$^{++}$ inflow from the Ca$^{++}$ channel, 3) Ca$^{++}$ inflow through the Na$^+$/Ca$^{++}$ exchange mechanism, and 4) Defluxion from the endoplasmic reticulum. The above-mentioned factors cause malfunction in the plasmalemmal and intracellular Ca$^{++}$ densities. Thus, Ca$^{++}$ overload results in the formation of free radicals, which in turn induce Ca$^{++}$ overload and thus ischemia-reperfusion injury occurs. As to the protective effect of a calcium antagonist, it improves vascular perfusion in the ischemic myocardium, and protects metabolic disturbances such as a decrease in enzyme consumption caused by myocardial damage.

As mentioned above, clumping and dispersion of the glyocalyx in endothelial cells are observed in ischemic hearts and this shows the functional disturbance of is chemic cells. Apoptosis is absent in early ischemia after 20 minutes of coronary ligation with or without reperfusion when sufficient ATP is present, and appears only after 30 minutes of coronary ligation and reperfusion. Apoptotic cells lose membrane integrity accompanied by a decrease in the extent of glyocalyx and cell swelling contrary to the normal characteristics of apoptotic cells. Lumican is localized in collagen fibers and fibroblasts of fibrotic lesions. A few myocardial cells close to the ischemic lesion express lumican mRNA. Lumican is considered to play an important role in the fibrillogenesis of the ischemic and reperfused rat heart. The degree of cAMP-dependent phosphorylation by endogenous and exogenous protein kinases is the same in the sarcoplasmic reticulum between controls and ischemic areas.

2. Roles of superoxide in ischemia-reperfusion injury

Reactive oxygen-derived radicals and metabolites are known to play important roles in the pathogenesis of ischemia/reperfusion and anoxia/reoxygenation injury. Free radicals are induced by the reperfusion blood flow in addition to the lack of oxygen ($\text{O}_2$) supply to the ischemic cell. Oxygen molecules ($\text{O}_2$) introduced by reperfusion are deoxidized individually, and produce superoxiansions and hydrogen...
oxide (H₂O₂). H₂O₂ plays a major role in the injury. O₂⁻ does not appear to damage the heart directly, although it is important as a precursor of H₂O₂ and other radical species including the hydroxyl radical (Fig. 3). A decrease in the degree of cytochemical staining by Na⁺-K⁺-ATPase is associated with an increase in the cell membrane permeability. H₂O₂ appears to affect not only lipids but also intramembranous proteins embedded in the cell membrane²⁵. The hydroxyl radical (OH) also participates in lipid hyperoxidation. Free radicals affect lipid, sugar, protein and DNA formation and also induce the loss of essential roles. They are also associated with the genesis and harmful factors of many diseases. The reduction of O₂ results in the production of superoxides as well as hydrogen peroxide (H₂O₂). H₂O₂ is highly diffusible and is potentially capable of inducing damage to biological tissues. Metabolic inhibitors, namely iodoacetate, acetate and carbonyl cyanide m-chlorophenyl hydrazone, induce ATPase depletion within five minutes, but do not affect membrane permeability or increase bleb formation and lipid peroxidation²⁷. These factors suggest that the damage caused by H₂O₂ is primarily attributable to lipid peroxidation which is not affected by ATPase loss.

III. Role of Diabetes Mellitus as Promoting Factor

Chronic hyperglycemia due to long-term diabetes mellitus is the primary etiologic factor in the pathogenesis of ischemic cardiomyopathy (Fig. 4). A group of patients with acute myocardial infarction (AMI) with non-insulin-dependent diabetes mellitus (NIDDM) showed an atherosclerotic lesion with decreased number of smooth muscle cells, and increased number of macrophages and TUNEL-positive cells associated with increased extent of the localization of tenascin and TGF-β1 compared with the coronary arteries of the controls. In AMI with NIDDM, increased TGF-β1 activity may induce apoptosis in an atherosclerotic lesion contributing to the development of acute myocardial infarction²⁸. After hyperglycemia, Millard-reaction is recognized and glucose aldehyde responds to the amino acid of proteins. Amadoryn rearranged product is advanced glycation endproducts (AGEs). The AGEs protein affects cell changes in the following ways: increased number, transformation, functional disturbance and cytokine elimination. In diabetic patients, granular and circled autofluorescent structures are observed in the foamy cells in this atherosclerotic lesion. Also some specificities are found in the fluorescent wave length, and the amount of fluorescent substances increase in the atherosclerotic lesions of patients with diabetes mellitus²⁹. In coronary arteries, the migration of smooth muscle cells caused by the taking up of AGEs proteins through the receptor (RAGE), and cytokine discharge are suggested. AGEs accumulation may induce diabetic macroangiopathy through
RAGE, and the increase in the level of RAGE expression by endothelial cells could be a reason that diabetes mellitus accelerates atherosclerosis\(^5\). Immunohistochemically, AGEs are observed in the cytoplasm of vascular and heart cells, and ultrastructurally, the reaction products have been demonstrated in the endoplasmic reticulum and lysosomes of cardiomyocytes and vascular cells in STZ-treated models\(^9\). Therefore, glycosylation and cross-linking of proteins in cells, in addition to structural alterations, could lead to enzyme inactivation and functional disorders. Thus, AGEs accumulation in cardiomyocytes is an etiologic factor other than coronary artery disease in the pathogenesis of cardiomyopathy\(^5\). It has been thought that AGEs are formed mainly in long-lived extracellular proteins such as collagen and laminin. The glycation of intracellular proteins may result in the prominent structural and functional changes leading to cellular damage and/or dysfunction of cardiomyocytes\(^10,11\). Therefore, we thought it necessary to clarify the alteration of the expression level of extracellular signal-regulated kinase (ERK) 1/2, a mitogen-activated protein kinase (MAPK) that is important in the function of the heart, in addition to ultrastructural examination of cardiomyocytes after ischemia-reperfusion\(^12\). Naito\(^9\) demonstrated that under long-term hyperglycemia, the expression level of phosphorylated ERK 1/2 decreases in cardiomyocytes compared with that under short-term hyperglycemia and in control groups (Fig. 5). We also confirmed the increase in the degree of ultrastructural alteration in cardiomyocytes under long-term hyperglycemia compared with that under short-term hyperglycemia\(^12\).

**IV. Protection Against Ischemia and Reperfusion Injury**

Ischemic preconditioning, a phenomenon in which brief episodes of ischemia and reperfusion before a prolonged ischemic event limits myocardial cellular damage, was first described by Murry et al.\(^12\). The protection lasts approximately 2 hours and reappears after 24 hours and is called the delayed phase of cardioprotection\(^12\). The late phase of preconditioning is triggered by factors such as adenosine, opioids, radicals and nitric oxide. These results suggest that protein kinases may regulate numerous biological processes, including the regulation of contraction and ion transport. Since these initial observations, several studies have been performed to determine the mechanism underlying this remarkable cardioprotective effect using preconditioning-like drugs. Both classical preconditioning and delayed preconditioning are initiated during brief ischemia by the administration of activators, especially adenosine\(^20\). There is evidence that the activation of kinases such as protein kinase C (PKC) and MAPK is necessary for adaptation\(^14,20\). Although the distal downstream effectors of both types of precondition-
Fig. 6 Potential preconditioning signaling mechanisms
mitoK<sub>ATP</sub> channel: mitochondrial K<sub>ATP</sub> channel, 5-HD: 5-hydroxydecanoic acid, PKC: protein kinase C, ERK 1/2: extracellular signal-regulated kinase 1/2 and DAG: diacylglycerol.

... have not been clarified, there is pharmacological evidence indicating an important synergistic role of the mitochondrial ATP-dependent potassium (mitoK<sub>ATP</sub>) channel with the end effector of preconditioning<sup>22</sup>. Adenosine metabolism can be manipulated in the formation of O<sub>2</sub> during reperfusion, and it has an important effect on the cardiac recovery of the ischemic myocardium. The generation of O<sub>2</sub> is related only to inosine release during initial reperfusion<sup>23</sup>. Some studies have recently demonstrated that an opener of the mitoK<sub>ATP</sub> channel, diazoxide, induces preconditioning through the activation of PKC and the mitoK<sub>ATP</sub> channel against Ca<sup>2+</sup> overload and ischemic injury in the rat heart<sup>24</sup>. On the other hand, 5-hydroxydecanoic acid (5-HD), which is a mitoK<sub>ATP</sub> channel inhibitor, reinforces the myocardial cell damage and increases the size of myocardial infarction. Thus, several studies have reported that the mitoK<sub>ATP</sub> channel is the end effector of preconditioning and PKC activity is important in mitoK<sub>ATP</sub> channel-mediated protection. Moreover, there is evidence that the activation of the mitoK<sub>ATP</sub> channel also leads to delayed preconditioning against lethal ischemic injury via the PKC signaling pathway. PKC appears to be one of the intracellular mediators responsible for protection. The complex stimulus of transient ischemia appears to provide global myocardial protection against ischemic injury<sup>22</sup> (Fig. 6).

As prolonged ischemia is known to cause cytoskeletal disruption, ERK 1/2 can contribute to the protective action of ischemic preconditioning by maintaining the actin cytoskeleton<sup>22</sup>. The identity of the end effector of protection is being studied extensively and the mitoK<sub>ATP</sub> channel is a strong candidate. There may be a connection between the actin cytoskeleton and the mitoK<sub>ATP</sub> channel. The role of mitoK<sub>ATP</sub> channel in regulating Ca<sup>2+</sup> homeostasis may be pivotal in cardioprotection. The opening of the mitoK<sub>ATP</sub> channel causes the depolarization of mitochondria, thus reducing Ca<sup>2+</sup> overload during reperfusion. Also the opening of the mitoK<sub>ATP</sub> channel by diazoxide supports Takashi’s findings on reduced Ca<sup>2+</sup> accumulation by mitochondria in the preconditioning heart<sup>24</sup>. Thus, one of the beneficial effects of mitoK<sub>ATP</sub> channel activation could be reduced Ca<sup>2+</sup> overload in mitochondria and increased ATP content, both being the major parameters of cell viability. The administration of this mitoK<sub>ATP</sub> channel-opening medicament also maintains a relatively high quiescent potential. On the other hand, it seems that...
Fig. 7 The origin of stem cells and regenerative versatility in adult tissue

sthenia of the Na⁺-Ca⁺⁺ ion exchange mechanism decreases Ca⁺⁺ level, delays myocardial contraction and inhibits cell damage. Both permanent ischemia and reperfusion induce the expression of heat shock protein 72 mRNA in the ischemic myocardium from 30 minutes to 24 hours after the onset of ischemia and reperfusion⁶. The overexpression of heat shock protein 72 in the myocardium reduces the infarct size and improves the contractile function following sublethal ischemia.

V. Clinical Correspondence

It seems possible to prevent ischemic reperfusion injury by the administration of some medicament before infarct development. In particular, the administration of growth factors promotes the development of collateral vascular distribution, therefore, it can delay the development of ischemic myocardial cell damage. It can be of great help in maintaining the cardiac function as an organism’s self-protection mechanism against ischemic cardiomyopathy (i.e., myocardial infarction and angina pectoris). In the future, it is necessary to elucidate the mechanism of ischemic preconditioning by medicament administration. Also, the clinical application of these procedures can be considered. The regulation of endogenous antioxidant enzymes is expected to be of great help in preventing oxidation damage after reperfusion.

It has been well established that the cardiomyocytes lost due to ischemia are not replaced. Recently, the regeneration of myocardial cells was confirmed using embryonic stem cells. Myocardial cells that exhibit self-pulsation are generated from mesenchymal stem cells of mesodermal tissues of the bone marrow. From these results, the reactivity of cardiac function performing stem cell transplantation may occur. The introduction of regenerative therapy is greatly looked forward to (Fig. 7). From the oxygen demand point of view, the major factors are the contraction of the cardiac cells, and the increase in heart rate and oxygen demand following the reinforcement of ventricular wall tension. Considering the above study, the important factors that influence the progression of ischemic cardiac lesion are the duration of ischemic period, blood distribution and abnormal metabolism in the heart.

References

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