Oxygenated Perfluorochemicals Improve Cell Survival during Reoxygenation by Pacifying Mitochondrial Activity

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ABSTRACT

Perfluorochemicals (PFCs) are known to provide a unique tool for controlled uptake and delivery of oxygen. We have characterized the effects of incremental oxygen delivery on cell viability of human ischemic cardiomyocytes using chemically inert PFCs as oxygen carrier. We have found that cell viability after prolonged ischemia depends on the dose of oxygen supplementation by oxygenated (ox) PFCs during reoxygenation. Although reoxygenation with the transient addition of oxPFCs in high concentrations (2250 μM O₂ in 0.4 μM PFCs) results in decreased cell viability compared with normoxic reoxygenation, cell survival increases by 30 ± 4% after reoxygenation with moderate oxPFC concentrations (750 μM O₂ in 0.1 μM PFCs). Immunoblot analysis revealed that oxPFC-supplemented reoxygenation causes marked (16-fold) deactivation of death-associated protein kinase (DAPK) signaling an increase in mitochondrial membrane potential and a decreased steady-state level of superoxide by 19 ± 3%. Reoxygenation with oxPFCs is further responsible for a 2-fold activation of AMP-activated protein kinase (AMPK) signaling an inadequate ATP supply by oxidative phosphorylation during reoxygenation. Addition of oxPFCs stabilizes both hypoxia-inducible factor (HIF) 1-α and 2-α during reoxygenation. Overall, these results indicate that moderate doses of oxPFCs can improve cell survival during reoxygenation, causing deactivation of DAPK, up-regulation of AMPK, and HIF1-α and 2-α stabilization. These effects of oxPFCs are dose-dependent, and they lead to a stabilization of the mitochondrial membrane potential, decreased steady-state levels of superoxide, and pacification of mitochondrial activity.

The only proven way of salvaging ischemic tissue from cell death and impaired function today is early reperfusion. An end-link between reperfusion and cell viability is obviously cellular respiration in the mitochondrion. Mitochondria determine the energy metabolism of cardiomyocytes, because they produce more than 90% of the energy for the heart by oxidative phosphorylation (Barth et al., 1992). Oxygen, the final electron acceptor in oxidative phosphorylation, maintains ATP synthesis in the inner membrane of the mitochondrion. In contrast, oxidative phosphorylation is a central site of reactive oxygen species production in the heart (Cai and Jones, 1998; Chandel et al., 2000; Yellow and Hausenloy, 2007) that may induce irreversible cellular damage and cell death (Davies, 1995). Therefore, mitochondria have been recognized as central integration site at the crossroads of either cell survival or cell death (Murphy, 2004). Alterations of oxidative phosphorylation in the mitochondrion have a key function in cardiomyocyte survival after ischemia/reperfusion. Accordingly, opposite effects of oxygen delivery during reperfusion and increased ATP availability have been reported, including increased cell viability but also increased apoptosis (Weiss et al., 2003). These data raise the question of how the concept of normoxic reperfusion can be improved considering the fact that it triggers apoptosis.

Proapoptotic death-associated protein kinase (DAPK) is activated early during apoptosis (Schumacher et al., 2002a), and its inhibition is protective after ischemia of the brain (Velentza et al., 2003). It has been previously shown that DAPK is activated in human neuroblastoma cells by mitochondrial respiratory chain inhibitors that induce a decrease in the mitochondrial membrane potential and an increased steady-state level of superoxide (Shang et al., 2005). However, it is unclear, whether oxygen can influence DAPK expression by changing the mitochondrial membrane potential or by increasing superoxide production.

Antiapoptotic AMP-activated protein kinase (AMPK) serves as an indicator of ATP availability in cells, detecting changes in the AMP-ATP ratio and signaling an inadequate ATP supply by oxidative phosphorylation (Hardie et al., 1998; Baron et al., 2005). AMPK initiates a metabolic stress response that protects human cardiomyocytes against cell death (Spector et al., 2007).

ABBREVIATIONS: DAPK, death-associated protein kinase; AMPK, AMP-activated protein kinase; HIF, hypoxia-inducible factor; PFC, perfluorochemical; ox, oxygenated; p, phosphorylated; BSA, bovine serum albumin; PBS, phosphate-buffered saline; LDH, lactate dehydrogenase; MnSOD, manganese superoxide dismutase; ΔΨm, mitochondrial membrane potential.
but it has never been shown whether AMPK activity is affected by an gradual increase in oxygen concentration.

HIF1-α and 2-α are both known to be expressed in ischemic cardiomyocytes (Jürgensen et al., 2004), and stabilization of HIF1-α is known to be a cardiac survival factor during hypoxia (Lee et al., 2000). However, it is unclear whether factors other than NO production by inducible nitric-oxide synthase mimic a hypoxic response under normoxia and could stabilize HIF during reoxygenation (Sandau et al., 2001).

Chemically inert perfluorochemicals (PFCs) provide a unique tool for controlled delivery of oxygen (Rafikova et al., 2004). Perfluorochemicals possess a high oxygen-dissolving capacity that follows Henry’s law, leading to oxygen solubility that is directly proportional to oxygen partial pressure. As a result, oxygen can be extracted rapidly and extensively from PFCs (Riess, 2005). Determining the conditions under which oxygen delivery by PFCs improves survival of ischemic cells and thereby preventing apoptosis in the surviving ischemic cells could result in an optimized regime of oxygen delivery during reperfusion.

Therefore, the aim of this study was to characterize the effects of delivering different dosing regimes of oxygenated (ox) PFCs with regard to changes in cell viability, oxidative phosphorylation, mitochondrial membrane potential, and superoxide production, and in particular to evaluate of the role of DAPK, AMPK, and HIF1-α and 2-α during reoxygenation.

Materials and Methods

Materials. Acridine orange was from Difco-BD Biosciences ( Sparks, MD). Albumin bovine fraction V ( BSA), bisbenzimide Hoechst 33342, collagenase VII, diethyldithiocarbamate, HEPES, lucigenin, phenylmethylsulfonyl fluoride, protease inhibitor cocktail, and Triton X-100 were purchased from Sigma-Aldrich (Steinheim, Germany). Enliten ATP assay system was from Promega (Madison, WI). KH2PO4 was bought from Fluka (Buchs, Switzerland), and tetra-butylammonium bisulfate was obtained from Merck (Darmstadt, Germany). MitoTracker Red 7513 (chloromethyl-X-rhodamine) was used by an gradual increase in oxygen concentration.

Model of Hypoxia and Reoxygenation. Ischemia was mimicked by exposing cardiomyocytes (seeding density 3000 cells/cm²) to a low-oxygen atmosphere (pO2 < 40 mm Hg). Cardiomyocytes showed the most pronounced decrease of cell viability after increasing the period of hypoxia to 12 h. Hypoxia was maintained within the cell culture incubator by continuous infusion with a mixture of 95% N2/5% CO2 in an airtight chamber for a period of 12 h before reox-

Volumetric Dosing of Perfluorooctyl Bromide. Oxygen solubility of 50 vol.% means that 1 volume of oxygen is dissolved in 2 volumes of perfluorooctyl bromide when 100% perfluorooctyl bro-

Measurement of Lactate Dehydrogenase. Lactate dehydrogene-

Cell Viability Staining. Cell viability was analyzed by fluorescein staining as reported by Foglieni et al. (2001). In brief, we used a triple dye combination of acridine orange, Hoechst 33342, and propidium iodide that visualizes all cell viability states simultane-

Culture of Human Cardiomyocytes. Monolayer cultures of human cardiomyocytes were prepared by modifying the method of Merante et al. (1998). In brief, heart tissue from the right atrium was obtained from male Caucasian patients with an average age of 76 years undergoing cardiovascular bypass surgery. The tissue was carefully dissected and further softened by digestion with 0.1% collagenase VII. Tissue specimens were washed in PBS and cultured in smooth muscle growth medium (PromoCell). After outgrowth of cells, cardiomyocyte colonies were transferred to new culture dishes. Cultures exhibiting >95% purity, as assessed by visual monitoring for rod-shaped cells and by fluorescent monoclonal antibody staining for troponin I during the first two passages, were used for subsequent studies.
Mitochondrial Membrane Potential Visualization. Mitochondrial potential was assessed by the fluorescent dye MitoTracker Red 7513 (reduced chloromethyl-X-rosamine) as described previously by Gurevich et al. (2001). In brief, cardiomyocytes were incubated in cell medium supplemented with 0.5 μM chloromethyl-X-rosamine for 45 min within the cell culture incubator. Cells were fixed in 3.7% formalin for 15 min and in ice-cold acetone for 5 min. Before and after each staining step, cells were washed intensely with PBS. Axiosvion 3.1 software and KS 300 3.0 software, both obtained from Carl Zeiss, were used for quantification.

Western Blot Analysis. Cardiomyocytes were washed and scraped off in ice-cold PBS supplemented with 1% protease inhibitor cocktail and 1 mM phenylmethylsulfonyl fluoride. The cells were centrifuged for 10 min at 22,000 g, and then they were lysed using the MEM-PER eukaryotic membrane protein extraction kit from Pierce Chemical (Rockford, IL), according to the manufacturer’s instructions. The lysate was centrifuged for 10 min at 10,000g at 4°C. From the clear supernatants, protein concentration was determined by the Bio-Rad protein assay kit (Bio-Rad, Munich, Germany) using BSA as control. Protein aliquots were boiled for 3 min in 2× loading buffer and separated by electrophoresis on 7.5% SDS-polyacrylamide gel electrophoresis. Proteins were blotted onto polyvinylidene fluoride membranes (Millipore, Schwalbach, Germany), and then they were immunoblotted overnight with the indicated primary antibody followed by secondary antibody conjugated with alkaline phosphatase. The CDP-Star Reagent from New England Biolabs (Beverly, MA) was used for detection.

Seroxidation Chemiluminescence. Seroxidation production was analyzed by lucigenin chemiluminescence. In brief, after incubating cardiomyocytes with 50 mM diethyldithiocarbamate for 10 min (Omar et al., 1991), cells were analyzed in HEPES buffer containing lucigenin that emits light on reduction of superoxide (Pagano et al., 1995). Chemiluminescence was determined after a 2-s delay for 20 min, integrated over a 30-s period, and repeated every 2 min using a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA).

Immunohistochemistry. Consecutively, cardiomyocytes were incubated in 1% formalin at 4°C overnight, in 0.2% Triton X-100 for 5 min, blocked in 5% BSA for 1 h, and then stained in 5% BSA with the indicated primary antibody (1:25) for 1 h and fluorescence-labeled secondary antibody (1:200) for 4 h. Before and after each staining step, cells were washed with PBS. After fluorescent staining, cardiomyocytes were visualized with an Axioplan 2 imaging fluorescence microscopy under a fluorescein isothiocyanate filter from Carl Zeiss. Axiosvion 3.1 software and KS 300 3.0 software, both from Carl Zeiss, were used for quantification.

ATP Determination. ATP was measured by the luciferin/luciferase method with a chemiluminescence kit (Promega) following the manufacturer’s instructions. Chemiluminescence was determined in a TD-20/20 luminometer (Turner Designs), and data were analyzed in Excel (Microsoft, Redmond, WA). Alternatively, ATP was extracted from cardiomyocytes after reoxygenation treatment, separated by high-performance liquid chromatography, and quantified as described by Matoba et al. (1999).

Statistical Analysis. Data are normalized for the amount of protein or number of cells engaged. All experiments were repeated at least three times. Data are given as percentage from normoxic value, which was set at 100%, and they are presented as mean ± S.E. Comparisons were made using unpaired Student’s t test or one-way analysis of variance when appropriate. Results were considered to be significantly different when P < 0.05.

Results

Dosing Regime of oxPFCs Determines Cell Survival during Reoxygenation. PFCs possess chemical and physical properties that arise directly from their electronic structure (Fig. 1). The electronically dense fluorine atoms cause the very high oxygen-dissolving capacity of PFCs. By comparing the activity of LDH after exposing ischemic cardiomyocytes to reoxygenation with transient addition of PFCs with and without oxygen in moderate concentrations of 0.1 μM and in high concentrations of 0.4 μM and to normoxic reoxygenation (250 μM O2), we investigated the potential toxicity of PFCs with a standard marker of cytotoxicity. Figure 2 shows that transient addition of PFCs without oxygen did not induce changes in LDH release of cardiomyocytes compared with normoxic reoxygenation (250 μM O2). However, ischemic cells released significantly less LDH (~15%; P < 0.05) after reoxygenation with oxygenated PFCs in moderate concentrations (750 μM O2 in 0.1 μM PFCs). After exposing ischemic cardiomyocytes to reoxygenation with oxygenated PFCs in moderate concentrations (750 μM O2 in 0.1 μM PFCs) and in high concentrations (2250 μM O2 in 0.4 μM PFCs) and to normoxic reoxygenation (250 μM O2), we investigated the effect of supplemental oxygen delivery on cell viability during reoxygenation (Fig. 3). Ischemic cells show a significant increase in cell viability (~30% at 1 and 3 h and ~20% at 6 h; P < 0.05), when O2 concentrations were elevated transiently to 750 μM O2 dissolved in 0.1 μM PFCs (Fig. 4). No further increase was seen.
when hypoxic cardiomyocytes were reoxygenated with a higher \( \text{O}_2 \) concentration of 2250 \( \mu \text{M} \text{O}_2 \) dissolved in 0.4 \( \mu \text{M} \) PFCs (Fig. 5). On the contrary, ischemic cardiomyocytes reoxygenated with 2250 \( \mu \text{M} \text{O}_2 \) in 0.4 \( \mu \text{M} \) PFCs showed a significant decrease in cell viability (\( \sim 40\% \) at 3 h and \( \sim 100\% \) at 6 h; \( P < 0.05 \)).

**Deactivation of DAPK by Moderate Doses of oxPFCs during Reoxygenation.** An increase in mitochondrial membrane potential after reoxygenation with addition of moderate concentrations of oxPFCs (750 \( \mu \text{M} \text{O}_2 \) in 0.1 \( \mu \text{M} \) PFCs) compared with normoxic reoxygenation seemed to be mediated by phosphorylation, and, consequently, by deactivation of DAPK. As shown in Fig. 6A, phosphorylated DAPK (pDAPK) was found almost at background level at normoxia (250 \( \mu \text{M} \text{O}_2 \)) and maximal after addition of 750 \( \mu \text{M} \text{O}_2 \) in a moderate dosage of oxPFCs (\( P < 0.05 \)). In contrast to this finding, DAPK phosphorylation did not increase significantly, when ischemic cardiomyocytes were exposed to 2250 \( \mu \text{M} \text{O}_2 \) by a high dosage of oxPFCs (Fig. 6B).

**Stabilization of Mitochondrial Membrane Potential after oxPFC-Supplemented Reoxygenation.** After normoxic reoxygenation (250 \( \mu \text{M} \text{O}_2 \)), a loss of mitochondrial membrane potential occurred as redistribution of the fluorochrome chloromethyl-X-rosamine from mitochondria to cytosol (Fig. 7A). When cardiomyocytes were exposed to oxPFCs in moderate concentrations (750 \( \mu \text{M} \text{O}_2 \) in 0.1 \( \mu \text{M} \) PFCs), mitochondrial membrane potential stabilized and cardiomyocytes displayed a mitochondrion-selective fluorochrome staining (Fig. 6B). An increase in mitochondrial membrane potential as well as a diminished loss of mitochondrial membrane potential was observed, when \( \text{O}_2 \) concentrations were elevated to 750 \( \mu \text{M} \text{O}_2 \) by 0.1 \( \mu \text{M} \) oxPFCs (Fig. 7C). Six hours after \( \text{O}_2 \) concentrations were moderately elevated, the stabilization of the mitochondrial membrane potential was significant (\( \sim 40\%; P < 0.01 \)) compared with normoxic reoxygenation.

**Dosing Regime of oxPFCs Affects Superoxide Production of Hypoxic Cardiomyocytes after Reoxygenation.** The role of reoxygenation on superoxide production in hypoxic cardiomyocytes was investigated by measuring superoxide chemiluminescence after cardiomyocytes were ex-
posed to oxPFCs in moderate concentrations (750 μM O₂ in 0.1 μM PFCs), to 2250 μM O₂ by a high dosage of oxPFCs or to normoxia (250 μM O₂). Figure 8A shows an immediate decrease in superoxide production, when O₂ concentrations were elevated transiently to 750 μM O₂. This decrease in superoxide production became significant after 3 h (≈15% at 3 h and ≈20% at 6 h; P < 0.05) compared with normoxic reoxygenation. This decrease disappeared when hypoxic cardiomyocytes were reoxygenated with 2250 μM O₂ in 0.4 μM PFCs (Fig. 8B). Hypoxic cardiomyocytes reoxygenated with a concentration of 2250 μM O₂ showed a cumulative increase in superoxide production that became significant at 6 h (≈50%; P < 0.05).

**Mitochondrial Manganese Superoxide Dismutase Expression Decreases after Reoxygenation with Addition of oxPFCs in Moderate Concentrations.** After reoxygenation applying 750 μM O₂ in 0.1 μM PFCs, mitochondrial MnSOD expression decreased significantly in comparison with normoxic reoxygenation (≈26%; P < 0.05). When ischemic cardiomyocytes were reoxygenated with hyperoxic oxygen concentrations of 2250 μM O₂, MnSOD expression was increased (≈154%; P < 0.05; Fig. 9) compared with normoxic reoxygenation.

**Activation of AMPK after Moderate Doses of oxPFCs during Reoxygenation.** The contribution of ATP generation on myocardial cell survival was tested by measuring AMPK activation, after exposing hypoxic cardiomyocytes transiently to oxPFCs in moderate concentrations (750 μM O₂ in 0.1 μM PFCs), and in toxic concentrations (2250 μM O₂ in 0.4 μM PFCs), or entirely to normoxic reoxygenation. Figure 10A shows that AMPK activation increased significantly (≈2-fold; P < 0.01) after treatment of ischemic cardiomyocytes with oxPFCs in moderate concentrations (750 μM O₂). However, oxPFCs (0.4 μM) delivering oxygen in the toxic concentration of 2250 μM O₂ did not increase but decreased
cardiomyocytes after transient exposure to moderate concentrations of oxPFCs delivering 750 μM O₂.

**Moderate Doses of oxPFCs during Reoxygenation Stabilize HIF1-α and HIF2-α.** We investigated whether a transient treatment of ischemic cardiomyocytes with hyperoxia induced a change in HIF1-α and HIF2-α stabilization compared with normoxic reoxygenation (250 μM O₂). One hour after reoxygenation with a brief hyperoxia of 750 μM O₂, cardiomyocytes showed a weak HIF1-α expression signal (data not shown) that became prominent 3 h after reoxygenation with 750 μM O₂. Three hours after the oxygen concentration was elevated transiently to 750 μM O₂, ischemic cardiomyocytes showed a significant increase in HIF1-α stabilization (~45%; P < 0.05), as shown in Fig. 11A. This increase in HIF1-α stabilization disappeared 6 h after reoxygenation with 750 μM O₂, when the HIF1-α signal was not visible any longer. A particularly intense signal was obtained for HIF2-α after applying 750 μM O₂ (~130%; P < 0.05), as shown in Fig. 11B. However, when ischemic cardiomyocytes were reoxygenated with the higher oxygen concentration of 2250 μM O₂, HIF1-α stabilization was not detectable at any time (data not shown).

**Discussion**

In the late 1980s, administration of oxygen using a perfluorochemical carrier was reported to result in a significant and sustained reduction in myocardial infarct size when delivered via the intracoronary route (Forman et al., 1991), but further studies using different administration routes and carrier doses resulted in conflicting data on infarct reduction (Wall et al., 1994). Recent studies using intracoronary oxygen perfusion with an aqueous oxygen solution have been reported to reduce infarct size and to improve myocardial contractility (Glazier, 2005). Although promising, reperfusion strategies that supplement oxygen require fine-tuning to gain the desired cardioprotective effect and to limit toxic side effects.
effects. So far, it has never been specifically addressed how reoxygenation regimes differing in oxygen concentrations influence cell viability in ischemic cardiomyocytes.

We have found that the cell viability of human cardiomyocytes after prolonged ischemia depends on the regime of reoxygenation. Although reoxygenation with oxPFCs in high concentrations results in decreased cell viability compared with reoxygenation at normoxic conditions, reoxygenation with oxPFCs in moderate concentrations improves cell survival significantly by reducing necrosis and apoptosis. Our experiments show that the regenerative effect of oxPFC is a dose-dependent oxygen effect, whereas addition of PFCs alone had no effect on cell survival.

Proapoptotic DAPK has been suggested to serve as a sensor of mitochondrial membrane potential because mitochondrial respiratory chain inhibitors cause DAPK activation by decreasing mitochondrial membrane potential and increasing steady-state levels of superoxide (Shang et al., 2005). Furthermore, DAPK plays an important role during the recovery phase after brain injury (Schumacher et al., 2002b), and it has been shown that a selective DAPK inhibitor is neuroprotective in a model of brain ischemia (Shamloo et al., 2005). We show that an increase in mitochondrial membrane potential after reoxygenation with moderate doses of oxPFCs may cause deactivation of DAPK, but normoxic reoxygenation does not alter DAPK, although this result is methodologically limited by a change of cellular milieu by the fluorescent dye chloromethyl-X-rosamine. Toxic doses of oxPFCs lead to an increase in apoptosis and reduced viability of cardiomyocytes, and they do not deactivate DAPK. Thus, the dosing regime of oxygen is an important modulator of cell viability during reoxygenation.

In addition, our experiments show that the superoxide production of human cardiomyocytes after hypoxia depends on the regime of reoxygenation. Reoxygenation with toxic doses of oxPFCs results in a cumulative increase in superoxide production compared with normoxic reoxygenation. However, moderate doses of oxPFCs lead to a decrease in steady-state levels of superoxide. Our results underscore the “oxygen paradox,” the mitochondrial electron-transport chain of higher eukaryotic aerobic organisms generates superoxide that seems to be responsible for oxygen toxicity (Davies, 1985).

MnSOD is the principal antioxidant enzyme located in the mitochondrial matrix (Muller et al., 2004) that detoxifies mitochondrial superoxide into hydrogen peroxide. We show that MnSOD expression decreases after reoxygenation with oxPFCs in moderate concentrations. As mitochondrial superoxide is formed during oxidative phosphorlation from the mitochondrial electron transport chain (Babior et al., 1973), the decrease in MnSOD expression implies a decrease in oxidative phosphorylation and superoxide production, underscoring the pacifying effect of moderate doses of oxPFCs on mitochondrial activity.

AMPK adjusts the cardiac energy metabolism to ischemia (Xing et al., 2003), and it prevents postischemic cell death (Russell et al., 2004). We show that AMPK activation increases after reoxygenation of ischemic cardiomyocytes using moderate doses of oxPFCs compared with normoxic reoxygenation. In addition, we observed a decrease in cellular ATP content with moderate doses of oxPFCs. Our findings corroborate previous findings that AMPK serves as an indicator of ATP availability in cells, detecting changes in the AMP:ATP ratio and signaling an inadequate ATP supply through phosphorylation (Hardie et al., 1998; Baron et al., 2005). Reoxygenation with high doses of oxPFCs does not increase but rather decreases AMPK activation, indicating that toxic oxygen doses of oxPFCs may unbalance the protective capacity of antiapoptotic AMPK, leading eventually to necrosis and apoptosis.

We have demonstrated a dose-dependent effect of oxygen on stabilization of HIF1-α and HIF2-α in ischemic cardiomyocytes during reoxygenation. We were able to show a regenerative effect of moderate doses of oxPFCs that is lost when the dose of oxPFC is further increased. Although reoxygenation with oxygen in moderate concentrations (750 μM O₂) increases HIF1-α and HIF2-α stabilization, compared with reoxygenation at normoxic conditions, reoxygenation with oxygen in higher concentrations (2250 μM O₂) has no effect on HIF stabilization.

In conclusion, we report for the first time that treatment of ischemic cardiomyocytes with oxPFCs in moderate concentrations results in improved cell viability by stabilization of the mitochondrial membrane potential and reducing steady-state levels of superoxide in an in vitro model of ischemia and reperfusion. The gain in mitochondrial membrane potential is consistent with the pattern of increased AMPK expression after reoxygenation with moderate doses of oxPFCs, which signals a reduced rate of oxidative phosphorylation. DAPK senses an increase in mitochondrial membrane potential mediated by reduced steady-state levels of superoxide and becomes deactivated. We propose that increased viability of ischemic cardiomyocytes results from a pacifying effect of moderate doses of oxPFCs on the mitochondrial activity, superoxide steady-state levels and the stabilizing effect on HIF1-α and HIF2-α during reoxygenation. We propose that HIF1-α and HIF2-α stabilization could mediate a protective effect on ischemic cardiomyocytes during reperfusion. Accordingly, reperfusion strategies may offer advantages over conventional normoxic reperfusion when applying optimal oxygen concentrations to the ischemic myocardium.

References


References


Jürgensen JS, Rosenberger C, Wiesner MS, Warnecke C, Horstrup JH, Grafe M,

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