Pharmacological cardiac protection

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Abstract

Pharmacological agents administered right at the onset of reperfusion, have the potential to reduce the impact of ischemia reperfusion injury (IRI), and have been proposed as possible strategies to induce cardioprotection (CP) in patients presenting with acute coronary syndromes. Treatment with these agents, mimicking preconditioning, can be instituted at the time of reperfusion obtained by successful coronary angioplasty. In the last two decades, growing body of evidences have elucidated the mechanisms involved in IRI and CP, while several animal and clinical studies have tested different drugs with putative role in CP. In this article we describe first the pathway involved in IRI and in CP; subsequently, while focusing on adenosine, we briefly review other pharmacological agents proposed to induce cardioprotection.

Keywords: ischemia-reperfusion injury; cardioprotection; preconditioning; postconditioning; mitochondrial permeability transition pore; adenosine

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Main Clinical Article

Introduction

Coronary artery disease accounts for more than 50% of cardiovascular events, and has a significant impact on the global economics as a leading cause of disability and loss of productivity. Acute myocardial infarctions, as well as procedures such as percutaneous coronary interventions (PCI) or cardiac surgeries are associated with ischemia and subsequent ischemia–reperfusion injury (IRI). A systematic study by Reimer et al. [1] confirmed that necrosis progressed in a "wavefront" pattern related to the duration of ischemia, which firmly established the concept that myocardium could be salvaged by initiating reperfusion as early as possible. Jennings et al [2] subsequently recognized and first introduced the concept of IRI. They described significant morphological alterations that appear after the onset of reperfusion, including cardiomyocytes swelling, mitochondrial clarification, amorphous/flocculent densities representing calcium phosphate deposits, hypercontracture, and loss of sarcomere organization. The existence of lethal reperfusion injury is strongly supported by evidence of reduced infarct size achieved by interventions when applied at the onset of reperfusion. Lethal reperfusion injury occurs only after severe ischemic injury and does not take place after fully reversible ischemic episodes. Reperfusion initiates a cascade of events within the first minutes after restoration of flow, and causes injury in a relatively short time (Fig. 1). The mechanisms of IRI have been under intense investigation for several decades, and have been reviewed elsewhere in this issue. Numerous agents and mechanical interventions have demonstrated significant infarct size reduction in experimental studies. Decreasing the burden of ischemia–reperfusion

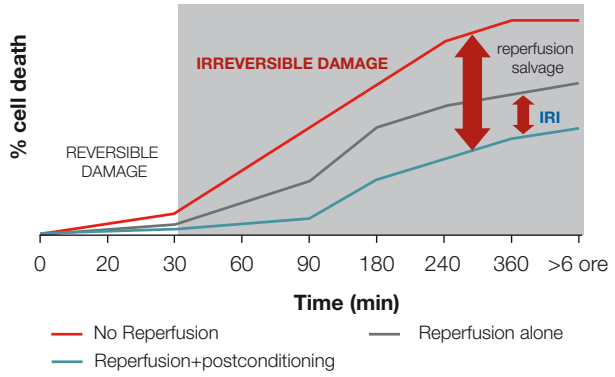

Fig. 1 *The concept of lethal reperfusion injury. During ischemia, irreversible cell injury leading to cell death occurs within the ischemic risk zone in a time-dependent manner. In the absence of reperfusion, ischemic injury would progressively, eventually accounting for near total cell death (red line). Reperfusion halts the process of ischemic cell death but in its early stages imposes injury that results in further cell death, beyond that due to the ischemic period: this is lethal reperfusion injury. The net result, however, is that the reperfused tissue sustains less cell death than would occur in ischemic tissue without reperfusion. Hence, targeting cell death due to reperfusion has the potential to maximize cell salvage. Postconditioning applied at the onset of reperfusion limits the extent of reperfusion injury and maximizes reperfusion salvage. The beneficial effects of reperfusion and of adjunctive cardioprotective strategies decrease with longer ischemic time.*

injury is a focus of continued research, and it seems that conditioning the heart may become a promising approach that could translate into clinical practice in the near future.

Ischemia reperfusion injury and the role of mPTP

Mitochondria play a crucial role in cell life and death. They provide cells with ATP produced via oxidative phosphorylation under physiological conditions, and initiate cell death through both apoptosis and necrosis in response to severe stress. Oxidative stress accompanied by calcium overload and ATP depletion induces the formation of pathological, non-specific mitochondrial permeability transition pores (mPTP) in the inner mitochondrial membrane. Opening of the mPTP with a high conductance results in matrix swelling ultimately inducing rupture of the mitochondrial outer membrane and releasing pro-apoptotic proteins into the cytoplasm. Cardiac cells undergoing ischemia followed by reperfusion possess exactly the same conditions mentioned above to induce mPTP opening. Due to its critical role in cell death, inhibition of mPTP opening has been accepted as a promising therapeutic approach to protect the heart against IRI. Indeed emerging studies suggest that cardioprotection (CP) elicited by both ischemic preconditioning and postconditioning may be mediated through the modulation of the mPTP, whose opening uncouple oxidative phosphorylation and induce mitochondrial swelling. Both ischemic preconditioning and postconditioning, and pharmacological preconditioning have been demonstrated to confer cardioprotection in part through the inhibition of the mPTP.

Cardioprotection

The concept of cardioprotection refers to a variety of pharmacological agents or non-pharmacological strategies that applied before or after prolonged ischemia have been shown to reduce the negative impact of ischemia and attenuate the effect of reperfusion injury on the ischemic myocardium. Short episodes of ischemia before the onset of sustained ischemia produce ischemic preconditioning (IPreC). Intermittent reperfusion with repetitive episodes of recurrent ischemia is termed ischemic postconditioning (IPostC). Transient ischemia in a remote organ, which prevents ischemia-reperfusion injury in the heart at distance, is known as remote ischemic conditioning (RPostC). Administration of pharmacological agents able to activate those signaling pathways thought to be involved in cardioprotection is known as pharmacological conditioning. These interventions involve a complex and incompletely understood network of molecular triggering and signaling pathways. Agonist that have shown to provide CP in vivo and in vitro models (Fig. 2) include adenosine, opioids, nitric oxide, bradykinin, tumor necrosis factor-alfa, brain natriuretic peptides, and interleukin-6. Putative signaling pathways include opening of sarcolemmal and/or mitochondrial adenosine triphosphate-dependent potassium channels and activation of prosurvival kinases (Akt and ERK-1/2), protein kinase C and G, hypoxia-inducible factor-1, endothelial nitric oxide synthase, and the recently described survivor activating factor enhancement (SAFE) pathway. Blockade of the mPTP is considered a final common pathway. Pharmacological pre and postconditioning may be achieved by administration of agents that activate these cytoprotective pathways (Fig. 3). This review will discuss briefly on pharmacological agents able to induce cardioprotection, focusing most on the role of adenosine.

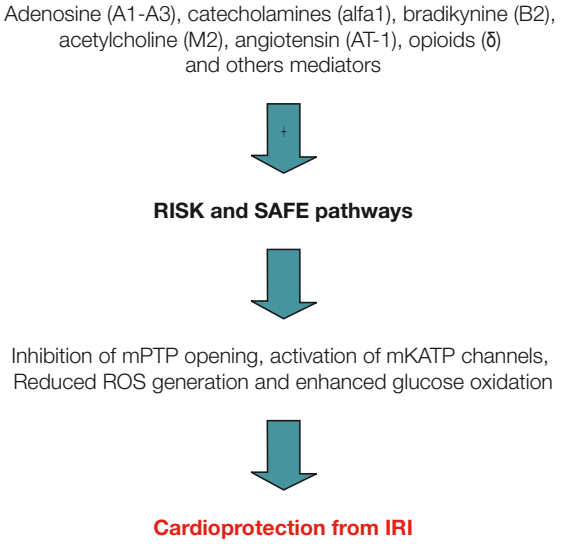

Fig. 2 *Triggers, mediators, and end-effectors proposed to play a role in the mechanism of pre- and post-conditioning.* catechol. *catecholamine;* MAP *mitogen-activated protein.*

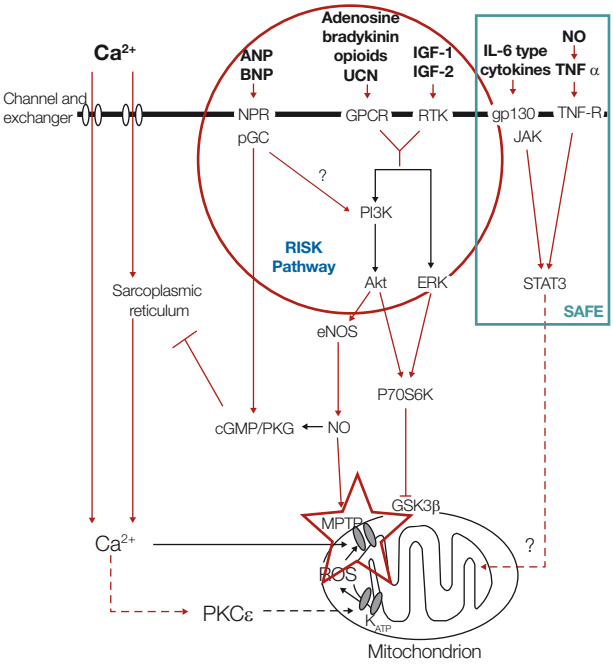

Fig. 3 *Proposed mechanisms and effectors involved in cardioprotection. Adenosine, bradykinin, opioid peptides, and other autacoids natriuretic peptides (ANP and BNP), peptide growth factors (IGF-1 and FGF-2), and TNF-alpha could play a role in induce conditioning (pre and/or postconditioning). After binding to cell surface receptors, these autacoids promote the activation of kinase signaling pathways. Evidence from some models implicates the activation of PI3K/Akt and p42/p44 ERKs. This pathway, known as the RISK pathway, is proposed to result in inhibition of mPTP opening at reperfusion, via distal components of the cascade which include NO and inhibition of GSK3β. Furthermore, it has been proposed that the activation of an intramitochondrial pool of PKC1 might cause opening of the mitochondrial KATP channel (mitoKATP), resulting in a slight increase in reactive oxygen species (ROS) formation which eventually causes mPTP inhibition. An alternative pathway, the so-called SAFE pathway, has been proposed to play a role in ischemic postconditioning. The major components of the SAFE pathway are TNF-a, the kinase JAK, which phosphorylates the transcription factor STAT3. It is proposed that after translocation to the nucleus, STAT3 controls the transcription of factors that confer cardioprotection. Also a mitochondrial localization of STAT3 has been suggested; however, both actions of STAT3 need to be finally proven.* eNOS *endothelial nitric oxide synthase;* GPCR *G-protein coupled receptor;* GSK3β *glycogen synthase kinase-3β;* MPTP *mitochondrial permeability transition pore;* ERK, *p42/p44 extracellular regulated kinase;* NPR *natriuretic peptide receptor;* pGC *particulate guanylyl cyclase;* PKG *cGMP-dependent protein kinase;* RTK *receptor tyrosine kinase;* SR *sarcoplasmic reticulum;* TNF-R *TNF receptor. A question mark (?) indicates that the link between those two pathways is not completely understood. Adapted from [30].*

Adenosine in preconditioning and post conditioning

A number of locally acting mediators of cardioprotection, acting in an autocrine–paracrine fashion, have been identified. The best-characterized autacoid that can trigger CP by receptor-mediated mechanisms is adenosine. Downey et al. [3] opened the door to the investigation on mechanisms of preconditioning with a report showing that protection by ischemic preconditioning was abolished by the inhibition of the adenosine receptor before sustained ischemia in vivo. This suggested that adenosine was a trigger of ischemic preconditioning. However, they later reported a conflicting result in vitro. In this study, preischemic treatment with either adenosine or selective adenosine-A1 receptor agonist, 2-chloro-N6-cyclopentyladenosine, exerted minimal protection [4], implying that adenosine was not a candidate for pharmacological preconditioning. This inconsistency was overcome by later reports from other groups showing successful pharmacological preconditioning with selective adenosine-A1 receptor agonists, 2-chloro- N6 cyclopentyladenosine or R-phenylisopropyladenosine, strongly supporting a major role for adenosine receptor activation in ischemic preconditioning [5,6]. In conjunction with other receptors, various membrane adenosine receptors are thought to play an important role in the transduction of extracellular signals, leading to protection by preconditioning, lasting 1–3 hr. Adenosine A1- and A3-receptors mediate inhibition of adenylate cyclase via a guanine nucleotide binding inhibitory protein (Gi/o). A2-receptors couple to a comparable stimulatory protein (Gs). Adenosine receptors are found in many tissues, including the heart. A1-receptors are located

on cardiomyocytes and vascular smooth muscle cells, A2-receptors on endothelial and vascular smooth muscle cells, and A3-receptors on ventricular myocytes. Ischemic preconditioning by endogenous adenosine takes place through A1- and A3-receptors. A2A/B-receptor activation results in vasodilation. Signals from different receptors converge at PKC, reaching a threshold activation of the kinase necessary to induce protection. Tyrosine and mitogen-activated protein kinases may play a role in addition to PKC. However, this hypothesis has been questioned by some studies and reports that have failed to show that preconditioning-induced cardioprotection was associated with an increase in the intramyocardial level of adenosine. Possible reasons for these observations might be due to the sensitivity of the method used for adenosine measurement and, the extremely short biological half-life of adenosine. Conversely, the adenosine hypothesis has been supported by the observation that cardioprotection could be restored by the addition of exogenous adenosine or by extending its biological half-life by either a coadministration with dipyridamole to delay clearance through intracellular uptake [7] or a sustained targeting release using a liposomal envelope [8].

Given the role of adenosine to induce preconditioning, it is logical to assume that administration of adenosine at the time of reperfusion will have a beneficial effect on protecting the heart from IRI. Adenosine has provided to have anti-inflammatory and anti-platelet effects. It is thought that part of the beneficial effect of adenosine involves opening ATP-sensitive potassium channels (mK_{ATP}), whose opening can protect the mitochondria from Ca2+ overload and prevent cytochrome c loss [9,10]. It replenishes high-energy phosphate stores in endothelial cells and myocytes. Adenosine inhibits cytokine release from mononuclear cells, oxygen free radical formation, and neutrophils activity and accumulation. It reduces cardiomyocytes apoptosis, improves microvascular function and causes preconditioning responses [11]. The efficacy of adenosine as pharmacological postconditioning drug was clearly documented by Marzilli et al. [12]. In this study, 54 patients with acute myocardial infarction, referred for primary PCI (pPCI) within 3 hour from symptoms onset, were enrolled and received either intracoronary injection of adenosine (n=27, 4 mg of adenosine/2 ml of saline solution) or placebo (n=27, saline solution) at the time of pPCI. No deaths occurred in the adenosine group while five patients in the saline group died in hospital (p=0.02). The composite end point of recurrent angina, nonfatal MI, heart failure, and cardiac death was present in 5 patients in the adenosine group and in 13 patients in the control group (p=0.03). This proof of concept clinical trial clearly demonstrated that intracoronary administration of high dose of adenosine was well tolerated by patients and effective in inducing cardioprotection. However, three larger randomized trials have reported limited benefits from adenosine in acute myocardial infarction. The Acute Myocardial Infarction Study of Adenosine (AMISTAD) was performed in 236 patients with anterior and inferior infarctions [13]. Patients received thrombolytic therapy plus continuous intravenous adenosine infusion (70 μg/kg per min) together with PCI had a smaller infarct size and a better functional recovery than those without adenosine infusion, especially in the instances of anterior wall infarction and adenosine infusion. The Attenuation by Adenosine of Cardiac Complications (ATTACC) study was a prospective, large-scale, randomized, placebo-controlled study using a low dose of adenosine (10 μg/kg per min) infused intravenously for six hours in patients undergoing thrombolysis. At the six-month follow-up of 292 patients with anterior infarcts, the adenosine group showed a trend for less all-cause mortality (8.4% versus 15.3%; p=0.07) and cardiovascular mortality (8.4% versus 14.6%; p=0.08). In a post hoc analysis of a subgroup with anterior infarcts and severely depressed left ventricle function, the six-month death rate was significantly lower in the adenosine group (2.0% versus 12.1%; p=0.007) [14]. However, the Acute Myocardial Infarction Study of Adenosine II (AMISTAD-II) specifically designed to investigate the role of adenosine in 2,118 patients with anterior STEMI undergoing thrombolysis or pPCI, found no difference in the primary end point of new congestive heart failure, rehospitalization for CHF, or death from any cause within 6 months. Two doses of adenosine were tested: 50 and 70 μg/kg per min [15]. Despite the lack of difference between the two groups in terms of major clinical endpoints, the infarct size tended to decrease in a dose-dependent manner with a marked reduction in infarct size observed in the high-dose group. In a post hoc analysis, among patients receiving reperfusion therapy within three hours of symptoms, adenosine reduced one-month

and six-month mortality rates significantly: 5.2% versus 9.2% (p=0.014) and 7.3% versus 11.2% (p=0.03), respectively [16].

Statins

Among recent studies using the cardioprotective signaling of postconditioning, the use of 3-hydroxy-3-methylglutaryl-CoA inhibitors (statins) is reported as a readily available, safe, and hopeful option to date. The immediate use of statins either before the onset of ischemia [17] or around the reperfusion period [18], reduced infarct size regardless of hyperlipidemia. This result was also shown in humans [19]; in addition, as shown in the MIRACL trial, administration of statins reduced adverse outcomes when used as late as 24 h after reperfusion. This evidence strongly suggests that cardioprotection induced by statins goes beyond lipid lowering and involves the signaling cascades of postconditioning, such as Akt activation and oxidative stress reduction [20].

Volatile anesthetics

Although the exact signaling pathway is not yet fully understood, the beneficial effect of volatile anesthetic on the heart seems to be mediated by adenosine A1 receptors, G proteins, reactive oxygen species, protein kinase C, and KATP channels [21,22]. The cardioprotective effects of sevoflurane were studied in human volunteers as well as in patients with ischemic heart disease. It seems that a relatively low dose of sevoflurane is sufficient to provide protective effect [23,24]. Sevoflurane administration during cardiac surgery was found to significantly reduce the incidence of myocardial infarction, intensive care unit and hospital stay, time on mechanical ventilation, in-hospital mortality and long term cardiac events [25,26]. The data regarding sedation with sevoflurane in patients not undergoing surgery are limited. In a small study involving patients undergoing elective PCI, low-dose sevoflurane before PCI, had no effect on myocardial damage after PCI [27].

Cyclosporine A

Another emerging target is the use of the mPTP inhibitor cyclosporine A. The role of cyclosporine A was first investigated by Pioer et al [28] which documented cardioprotection in patients presenting with acute myocardial infarction. Patients were randomized to receive intravenous bole of cyclosporine A or normal saline immediately before undergoing pPCI. Administration of cyclosporine was associated with a reduction in infarct size of approximately 40%, measured in terms of CK release, although a not significant reduction in troponin I levels. MRI image on day 5 after infarction confirmed the decrease in infarct size in the cyclosporine group. The findings of protection by cyclosporine are now expanded in endothelial function upon reperfusion in humans [29] but seem still immature and need further confirmation in large-scale clinical trials to establish evidence-based medicine.

Conclusions

Clinical trials on cardioprotective strategies and drugs have given inconsistent results. These may be related to 1) not applying the intervention at the beginning of reperfusion, 2) delaying its application beyond the brief window within which salvage is achievable. Any strategy used to induce cardioprotection is time-sensitive; it can exert beneficial effect only if applied when there are still viable cells that can be salvaged during reperfusion. Therefore, the most striking difference among animal and humans studies is that pharmacological conditioning in animals is applied within few minutes after reperfusion and that, most importantly, reperfusion is initiated 30 minutes to 1 hour after the onset of ischemia. This important concept is well manifested in the study from Marzilli et al. in which they have shown a beneficial effect of adenosine as a pharmacological intervention to induce postconditioning, by using i.c. infusion of adenosine at the onset of reperfusion, including only those patients in which the ischemic time was less than 3 hours.

The controversial results reported about mechanisms involved in cardioprotection can be further explained by the differences between protocols applied to induce ischemia-reperfusion, and by the choice of animal models. Although one of the goals of experimental studies using animals is translational to humans, species differences remain a critical issue that needs to be taken into account. Importantly animal used to study the effect of ischemia-reperfusion had no coexisting comorbidities, and no pharmacological treatment that could attenuate or enhance the expected results from the intervention used to induce cardioprotection. However, animal study remains of

particular importance in order to verify new hypothesis and test the effectiveness of the interventional strategy. •

References

1. Reimer KA, Lowe JE, Rasmussen MM et al (1977) The wavefront phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs. Circulation 56(5):786–794
2. Jennings RB, Sommers HM, Smyth GA et al (1960) Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. Arch Pathol 70:68–78
3. Liu GS, Thornton J, Van Winkle DM et al (1991) Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. Circulation 84 (1):350–356
4. Ganote CE, Armstrong S, Downey JM (1993) Adenosine and A1 selective agonists offer minimal protection against ischaemic injury to isolated rat cardiomyocytes. Cardiovasc Res 27 (9):1670–1676
5. Miura T, Liu Y, Kita H et al (2000) Roles of mitochondrial ATP-sensitive K channels and PKC in anti-infarct tolerance afforded by adenosine A1 receptor activation. J Am Coll Cardiol 35 (1):238–245
6. Kitakaze M, Takashima S, Funaya H et al (1997) Temporary acidosis during reperfusion limits myocardial infarct size in dogs. Am J Physiol 272(5 Pt 2):H2071–2078
7. Auchampach JA, Gross GJ (1993) Adenosine A1 receptors, KATP channels, and ischemic preconditioning in dogs. Am J Physiol 264(5 Pt 2):H1327–1336
8. Takahama H, Minamino T, Asanuma H et al (2009) Prolonged targeting of ischemic/reperfused myocardium by liposomal adenosine augments cardioprotection in rats. J Am Coll Cardiol 53(8):709–717
9. Garlid KD, Paucek P, Yarov-Yarovoy V et al (1997) Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K+ channels. Possible mechanism of cardioprotection. Circ Res 81(6):1072–1082
10. Korge P, Honda HM, Weiss JN (2002) Protection of cardiac mitochondria by diazoxide and protein kinase C: implications for ischemic preconditioning. Proc Natl Acad Sci U S A 99 (5):3312–3317
11. Stone GW (2008) Angioplasty strategies in ST-segment-elevation myocardial infarction: part II: intervention after fibrinolytic therapy, integrated treatment recommendations, and future directions. Circulation 118(5):552–566
12. Marzilli M, Orsini E, Marraccini P et al (2000) Beneficial effects of intracoronary adenosine as an adjunct to primary angioplasty in acute myocardial infarction. Circulation 101 (18):2154–2159
13. Mahaffey KW, Puma JA, Barbagelata NA et al (1999) Adenosine as an adjunct to thrombolytic therapy for acute myocardial infarction: results of a multicenter, randomized, placebo-controlled trial: the Acute Myocardial Infarction STudy of ADenosine (AMISTAD) trial. J Am Coll Cardiol 34(6):1711–1720
14. Quintana M, Hjemdahl P, Sollevi A et al (2003) Left ventricular function and cardiovascular events following adjuvant therapy with adenosine in acute myocardial infarction treated with thrombolysis, results of the ATTenuation by Adenosine of Cardiac Complications (ATTACC) study. Eur J Clin Pharmacol 59(1):1–9
15. Ross AM, Gibbons RJ, Stone GW et al (2005) A randomized, double-blinded, placebo-controlled multicenter trial of adenosine as an adjunct to reperfusion in the treatment of acute myocardial infarction (AMISTAD-II). J Am Coll Cardiol 45 (11):1775–1780
16. Kloner RA, Forman MB, Gibbons RJ et al (2006) Impact of time to therapy and reperfusion modality on the efficacy of adenosine in acute myocardial infarction: the AMISTAD-2 trial. Eur Heart J 27(20):2400–2405
17. Iliodromitis EK, Andreadou I, Prokovas E et al (2011) Simvastatin in contrast to postconditioning reduces infarct size in hyperlipidemic rabbits: possible role of oxidative/nitrosative stress attenuation. Basic Res Cardiol 105(2):193–203
18. Sanada S, Asanuma H, Minamino T et al (2004) Optimal windows of statin use for immediate infarct limitation: 5′-nucleotidase as another downstream molecule of phosphatidylinositol 3-kinase. Circulation 110(15):2143–2149
19. Merla R, Daher IN, Ye Y et al (2007) Pretreatment with statins may reduce cardiovascular morbidity and mortality after elective surgery and percutaneous coronary intervention: clinical evidence and possible underlying mechanisms. Am Heart J 154(2):391–402
20. Merla R, Ye Y, Lin Y et al (2007) The central role of adenosine in statin-induced ERK1/2, Akt, and eNOS phosphorylation. Am J Physiol Heart Circ Physiol 293(3):H1918–1928
21. Riess ML, Kevin LG, McCormick J et al (2005) Anesthetic preconditioning: the role of free radicals in sevoflurane-induced attenuation of mitochondrial electron transport in Guinea pig isolated hearts. Anesth Analg 100(1):46–53
22. Riess ML, Stowe DF, Warltier DC. Cardiac pharmacological preconditioning with volatile anesthetics: from bench to bedside? Am J Physiol Heart Circ Physiol 286(5):H1603–1607
23. Lucchinetti E, Ambrosio S, Aguirre J et al (2007) Sevoflurane inhalation at sedative concentrations provides endothelial protection against ischemia-reperfusion injury in humans. Anesthesiology 106(2):262–268
24. Landoni G, Fochi O, Tritapepe L et al (2009) Cardiac protection by volatile anesthetics. A review. Minerva Anestesiol 75 (5):269–273
25. Symons JA, Myles PS (2006) Myocardial protection with volatile anaesthetic agents during coronary artery bypass surgery: a meta-analysis. Br J Anaesth 97(2):127–136
26. Jakobsen CJ, Berg H, Hindsholm KB et al (2007) The influence of propofol versus sevoflurane anesthesia on outcome in 10,535 cardiac surgical procedures. J Cardiothorac Vasc Anesth 21(5):664–671
27. Landoni G, Zangrillo A, Fochi O et al (2008) Cardiac protection with volatile anesthetics in stenting procedures. J Cardiothorac Vasc Anesth 22(4):543–547
28. Piot C, Croisille P, Staat P et al (2008) Effect of cyclosporine on reperfusion injury in acute myocardial infarction. N Engl J Med 359(5):473–481
29. Okorie MI, Bhavsar DD, Ridout D et al (2011) Postconditioning protects against human endothelial ischaemia-reperfusion injury via subtype-specific KATP channel activation and is mimicked by inhibition of the mitochondrial permeability transition pore. Eur Heart J 32(10):1266–1274
30. Michel Ovize, Gary F. Baxter, Fabio Di Lisa, Péter Ferdinandy, David Garcia-Dorado, Derek J. Hausenloy, Gerd Heusch, Jakob Vinten-Johansen, Derek M. Yellon, and Rainer Schulz (2010) Postconditioning and protection from reperfusion injury: where do we stand? Cardiovasc Res 87:406–423