Review

CARDIOPROTECTION BY VOLATILE ANESTHETICS: FROM BENCH TO BEDSIDE AND BACK

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Abstract

Ischemia and reperfusion (I/R) injury of the heart can be ameliorated by volatile anesthetics (VAs). Application of VAs prior to the ischemic event triggers endogenous cardioprotective program that persists even after anesthetic removal, and it is called anesthetic-induced preconditioning (APC) (1). VAs can also reduce infarct size if applied during the reperfusion period (anesthetic postconditioning), where they can also exert protection by the direct effects on cardiac cells, specifically on the mitochondrial function. In this review, authors will summarize the fundamental concepts related to cardioprotection by VAs and implementation of research based on animal models to clinical practice. We will specifically address the role of human stem cell-based models in studying normal cardioprotective pathways, and more importantly, in studying certain diseases, such as diabetes mellitus that negatively affect APC.

Discovering APC using animal models

Studies from basic science laboratories and clinical departments improved our understanding of biological processes that underlie damage and death of cardiomyocytes caused by I/R, and more importantly, offered promising therapeutic strategies, such as APC for ameliorating this injury. However, most of our knowledge pertaining to molecular mechanisms behind APC is based on animal studies. Before the notion of APC had been investigated, the potential cardioprotection by halothane was discovered by Hoka, Bosnjak and Kampine in 1987 (2). Using the Langendorff preparation of isolated guinea pig hearts, the authors found that the calcium accumulation by the ischemic area of the heart is significantly reduced in the presence of 1% halothane. This was the first study to suggest that volatile anesthetic may be beneficial during the I/R injury. This was followed by studies in 1988 when Warltier et al. showed that the dogs anesthetized with isoflurane or halothane exhibit a better recovery of ventricular function caused by brief coronary artery occlusion (3). It is believed that APC elicits innate pro-survival pathways that are naturally triggered by brief ischemic periods, which precede prolonged ischemia. In 1986, Murry et al. described for the first time that four repeated occlusions of coronary artery each lasting 5 min reduced infarct size in dogs caused by prolonged I/R (4). The fact that the specific pre-treatment can reduce the damage from prolonged I/R and observations that reperfusion itself causes substantial cell damage led to conclusion that the injury to cardiomyocytes results from processes where cells take an active role in generating noxious stimuli. A large number of pathways that lead to necrotic and/or apoptotic cell death have been discovered. However, two pathophysiological events emerged as principal mediators of injury: excessive production of reactive oxygen species (ROS) occurring at the early reperfusion and intracellular calcium overload, which was detected during both ischemia and reperfusion (2, 5). The preconditioning of isolated rat cardiomyocytes with isoflurane was found to reduce both cytosolic and mitochondrial calcium overload (6, 7), as well as to attenuate excessive ROS production in isolated guinea pig hearts (8). The role of ROS in APC is seemingly paradoxical since VAs also trigger preconditioning by generating a small burst of mitochondrial ROS (9). However, the amount of triggering ROS is much less than the amount of ROS produced during reperfusion, and it may be one of the common stimuli between preconditioning with VAs and brief ischemic episodes, as discussed later.

Upon anesthetic application to isolated cardiomyocytes or entire hearts, multiple signaling pathways occur that transmit the signal from the plasma membrane surface receptors via network of cytosolic kinases down to end-effectors of protection. Similarly to ischemic preconditioning, APC signaling begins with the activation of G protein coupled receptor superfamily by primary messengers like adenosine, opioids, and endothelin that are released during the preconditioning stimulus (10). Signal is further transduced and multiplied by activation of kinases and phosphatases, like protein kinase C-ε, protein tyrosine kinase, mitogen-activated protein kinases, and protein kinase B (Akt) (11). Lastly, the activation of end-effectors of protection, opening of mitochondrial and sarcolemmal ATP-sensitive potassium (KATP) channels, or the transcription of genes attenuates both intracellular calcium buildup and massive ROS production (5). The role of mitochondria as the key organelles in cardioprotection by APC is described in more detail in the companion

review by Camara et al. For a detailed description of signaling cascade of APC, please see review by Zaugg et al. (10).

As mentioned earlier, very interesting molecules that participate in the signaling cascade of preconditioning are ROS. Apart from causing oxidative stress, ROS participate in many signaling pathways and regulate important functions and biomolecules including cell cycle (12), cell proliferation (13), apoptosis (14), metalloproteinase function (15), oxygen sensing (16), protein kinases (15, 17), phosphatases (18), and transcription factors (19). The importance of ROS for APC is reflected in studies which demonstrate that application of ROS scavengers during the triggering phase of preconditioning with desflurane and sevoflurane abrogates protection of isolated rat cardiomyocytes (9). Moreover, exogenous hydrogen peroxide can induce preconditioning in isolated chicken cardiomyocytes (20, 21). Our recent work indicated that volatile anesthetics directly and moderately enhance mitochondrial ROS production by inducing specific changes to electron fluxes along electron transport chain. These could be additional mechanisms by which VAs trigger preconditioning since ROS can activate many mediators/effectors of pro-survival signaling, such as protein kinase C (22), mitochondrial KATP channels (23), hypoxia-inducible factor-1α (24), mitogen-activated protein kinases (25), and transcription factors, such as activator protein-1 and nuclear factor-κB (26).

Diabetes mellitus and preconditioning

Clinical observations suggest inability to elicit cardioprotection by preconditioning in patients with diabetes mellitus (27, 28). Although this is contradicted by several basic science studies (29, 30), the majority of reports using animal models confirm clinical observations that preconditioning-induced cardioprotection is lost in the presence of diabetes (30-38).

As a complex metabolic disease with genetic and environmental component, diabetes mellitus could interfere with APC on several levels. APC-induced modification of mitochondrial bioenergetics plays a central role in the survival of cardiomyocytes, as elaborated in the companion review. Changes in the mitochondrial function found in diabetic myocardium could be one of the important underlying factors of disrupted preconditioning pathways (39). As mentioned earlier, a slight increase in ROS production during anesthetic application is a signal that triggers pro-survival pathways. However, consistent high levels of oxidative stress caused by excessive ROS production in diabetic myocardium can mask the signaling effect of ROS produced during anesthetic exposure. One of the possible mechanisms by which oxidative stress is elevated in diabetic myocardium is caused by hyperglycemia, one of the disorders in diabetes mellitus. Increased oxidation of glucose by mitochondria causes a buildup of mitochondrial substrates that pushes more electron donors (NADH and FADH2) into the mitochondrial electron transport chain, increasing the voltage gradient across the inner mitochondrial membrane (40). This in turn hampers the electron transfer through respiratory complex III causing the electrons to back up to ubiquinone and molecular oxygen, resulting in a formation of superoxide radical, i.e. ROS. Oxidative stress causes oxidation of numerous proteins important for preconditioning and for normal cellular function like mitochondrial enzymes and/or ion channels (40). In fact, it was shown that ROS production at baseline was elevated in diabetic hearts (41), and that preconditioning stimuli failed to enhance ROS generation in isolated mitochondria (39). Interestingly, hyperglycemia itself, in the absence of other disorders connected with diabetes, can prevent preconditioning (42).

The opening of cardiac mitochondrial KATP channels is in a close relationship with the generation of signaling ROS during anesthetic application, but it is also recognized as one of the most important end-effectors of cardioprotection. On the molecular level, the existence of dysfunctional mitoKATP and/or sarcolemmal KATP channels, as another end-effector of cardioprotection, have been closely correlated with impaired preconditioning in diabetes (30, 37, 39, 43, 44). It has been proposed that the opening of mitochondrial KATP channels exerts cardioprotection by inducing mitochondrial depolarization and thereby attenuating voltage-driven mitochondrial Ca2+ accumulation that initiates cellular death pathways. However, possibly dysfunctional mitochondrial KATP channels in diabetic myocardium would lead to impaired mitochondrial depolarization (39, 45). In diabetic myocardium, an altered expression of connexin 43, that is located in plasma and mitochondrial membranes, could also be a contributing factor of disrupted signaling via ROS (41, 46).

A linkage association study in humans showed a significant association between mutations or polymorphisms in genes coding KATP channel subunits and diabetes mellitus type II (47, 48). This indicates an important role of KATP channels in the etiology of diabetes mellitus, as they play an important role in the process of insulin secretion in the pancreas. However, a concomitant dysfunction of cardiovascular KATP channels could be an underlying factor of the inefficient preconditioning. Therapeutic approaches in the treatment of diabetic patients that involve sulfonylurea hypoglycemic agents can interfere with the beneficial effects of VAs on cardiac resistance to ischemic insult by blocking cardiovascular KATP channels (49). However, some of the agents from this group, such as glime-

pride, that are more selective for pancreatic KATP channels were shown not to interfere with cardioprotective strategies (50, 51). Some suggest that oral hypoglycemic agents should therefore be discontinued 24 to 48 h before elective surgery to preserve cardioprotective effects of VAs (52). Perioperative use of insulin could be a promising alternative to avoid the negative effects of KATP inhibition by sulfonylurea agents (53). Moreover, insulin itself may have cardioprotective properties since it can activate some of the pro-survival signal transduction pathways, namely phosphatidylinositol 3-kinase/Akt pathways (37, 39, 54).

In contrast, several studies showed an increased ischemic tolerance of diabetic myocardium (55-57). This suggests that diabetic myocardium is already in a preconditioned state triggered by oxidative stress, and other preconditioning stimuli like VAs cannot exert additional protection. An overstimulation of preconditioning pathways by chronic hyperglycemia, as a cause of oxidative stress, could possibly modify pro-survival pathways and/or cause an increase in the threshold for preconditioning stimulus (54). One of the interesting candidates involved in impaired preconditioning signal transduction is glycogen synthase kinase-3β, an enzyme that is normally phosphorylated and inactivated by preconditioning. Inactivation of glycogen synthase kinase-3β results in inhibition of mitochondrial permeability transition pore which is a key event in the death of cardiomyocytes. However, studies suggest that glycogen synthase kinase-3β is already activated in diabetes mellitus (58, 59).

Endothelial cell dysfunction is another disorder reported in diabetic patients that is characterized by impaired production or decreased bioavailability of nitric oxide. As nitric oxide plays a crucial role in normal vasodilatation, this may impair coronary circulation and exacerbate ischemic incidents. Moreover, nitric oxide, synthesized by endothelial isoform of nitric oxide synthase, is also involved in signal transduction pathways of preconditioning (60, 61). Hyperglycemia impairs the function of endothelial nitric oxide synthase through tetrahydrobiopterin and heat shock protein 90-dependent pathways (62, 63).

Altogether, it seems that multiple mechanisms underlie the inability to precondition patients with diabetes mellitus. Thus, it will require increased research efforts: First to understand pathophysiological processes in diabetic myocardium that negatively interfere with preconditioning mechanisms, and then, based on these findings, to implement novel therapeutic strategies that will restore APC-induced cardioprotection.

APC in isolated human myocardium

Although mammalian organisms share many similarities in respect to cardiac (patho)physiology, there is a need to validate data obtained from animals using human models. The most commonly used model of human myocardium is atrial appendage obtained from cardiac surgeries, which is used to generate atrial trabeculae for contractility measurements or to obtain isolated cardiomyocytes or mitochondria for biochemical measurements. Studies using human cardiac tissue in most part verified findings obtained in animal models. By measuring the recovery of contractile force in isolated human atrial trabeculae, Hanouz et al. found that APC with desflurane and sevoflurane improved contractility following hypoxic stress, which was abrogated when ROS were scavenged during triggering phase of APC (64). Moreover, Mio et al. demonstrated that isoflurane preconditioning protects cardiomyocytes and mitochondria isolated from right atrial appendages and confirmed the crucial role of sarcolemmal KATP channels (65). The same study also showed that cardioprotective potency of APC is attenuated in myocardium obtained from patients older than 60 years, which is in agreement with clinical observations that the older population is more resistant to APC. Several other studies documented involvement of other APC mediators in human myocardium: sarcolemmal KATP channels and adenosine A-1 receptors in isoflurane preconditioning (66); mitochondrial KATP channels and adenosine A-1 receptors, α and β adrenoceptors in desflurane preconditioning (67); and mitochondrial and sarcolemmal KATP channels and adenosine A-1 receptors in sevoflurane preconditioning (68).

However, these studies have several shortcomings. The supply of human heart muscle to research community and the amount of tissue are very limited. Moreover, patients are usually polymedicated, which complicates the interpretation of experimental outcomes. Due to the limited amount of cardiac tissue and poor ability to culture these cells, the studies are also limited in the number and type of experiments that can be performed. A promising alternative emerged with cardiomyocytes derived in vitro from various types of stem cells that could resolve many of the methodological difficulties inherent to human myocardium obtained from patients.

Clinical Cardioprotection

The classical preconditioning protocols used in animal experiments are generally not applicable for the clinical studies. Moreover, since most of the clinical studies of anesthetic preconditioning were performed during coronary artery bypass surgeries, the procedures used during operation are likely to influence (69) or even abrogate the anesthetic preconditioning. Highly variable results from the clinical studies are also attributed to different protocols used (70, 71); the pres-

ence of various drugs; high glucose level; influences of different diseases (especially diabetes and coronary artery disease); the role of age, gender, and so on. Nevertheless, despite these obstacles, anesthetic cardioprotection appears to be one of the clinically useful cardioprotective strategies with potentially beneficial effects on patient's outcome. Belhomme et al. were the first to publish clinical study using a preconditioning protocol with isoflurane prior to aortic cross-clamping (72). They reported less postoperative creatine kinese-MB and troponin release in the study group, however, the differences did not reach statistical significance. The only circumstantial evidence for the occurrence of anesthetic preconditioning in this study was in the form of activation of protein kinese C which represents one of the major steps in the signaling transduction involved in both ischemic and anesthetic preconditioning. Subsequent clinical evidence suggested that the effect of volatile anesthetics are most prominent when our anesthetics are present during the entire surgical procedure (73). They demonstrated that sevoflurane preserves post bypass cardiac function and reduced cellular damage as compared with propofol-based protocol during the coronary bypass surgery. These cardioprotective effects were subsequently confirmed by other studies (73-76), and also observed in high-risk elderly patients (77), aortic valve replacement procedure (78), and also in off-pump surgery (79). Over the last 12 years at least two dozen clinical studies have used some form of anesthetic preconditioning protocols and most (about 3/4) have been able to associate volatile anesthetic preconditioning with some beneficial cardiac effects. The rest of the clinical studies found no significant cardioprotective properties of volatile anesthetics when compared to total intravenous anesthesia used during cardiopulmonary bypass surgery (72, 80-83).

As pointed out, the cardioprotective effects of volatile anesthetics compared with intravenous anesthetics in the clinical studies are highly variable. The results, nevertheless, point to the likelihood that the use of volatile anesthetics regiment may protect the heart during the coronary artery bypass surgeries. Even more difficult is the question whether this cardioprotective phenomenon is ultimately associated with improved postoperative morbidity and clinical recovery in patients with coronary artery disease. So far all of the studies were underpowered and unable to address this issue directly. There are, however, some studies suggesting a shorter hospital stay, decrease atrial fibrillation, and lower incidence of adverse cardiac events one year after coronary artery bypass graft surgery (75, 84, 85). Subsequent retrospective study in over 10,000 patients found no difference in postoperative mortality and infarction rate, although the study found a lower cardiac-related mortality after sevoflurane anesthesia in patients without recent angina or myocardial infarction (86). Similar postoperative issues were also examined in meta-analysis studies (87-89). The new volatile anesthetics desflurane and sevoflurane were shown to reduce postoperative mortality, incidence of myocardial infarction, and significant advantages in form of troponin release, cardiac index, need for inotropic support, ventilation time, and the overall intensive care unit and hospital stay.

The American College of Cardiology and the American Heart Association Guidelines now recommend that volatile anesthetic agents should be used during non-cardiac surgery for the maintenance of general anesthesia in patients at risk for myocardial ischemia (90). Despite these guidelines, the clinical trials examining the benefits of volatile anesthetics during non-cardiac surgery are anything but conclusive (91). For instance, the incidence of postoperative cardiac events and troponin levels did not differ between volatile anesthetics and intravenous anesthetic regiments in patients undergoing peripheral arterial surgeries (92). In a more recent study examining the patients undergoing aortic surgery, no differences were found in the incidence of elevated troponin levels (93). In summary, while not unanimous, there is strong support for the clinical benefits, including reduced morbidity, of volatile anesthetics in patients undergoing heart surgery. Further studies are warranted, not only to confirm that the choice of anesthetics can improve the patients outcome following cardiac surgery, but also high risk, non-cardiac surgery to provide a definitive evidence of anesthetic induced preconditioning. Because of the relative safety of surgery conducted today and the various confounding factors this might be a very difficult task to accomplish.

Human stem cell-derived cardiomyocytes as an experimental model for APC

Human embryonic stem cells (hESCs) are pluripotent cell lines derived from an early embryo. These cells possess the capacity for both self-renewal and differentiation into derivatives of all three embryonic germ layers, which makes them capable of creating virtually any cell type existing in the human body, including cardiomyocytes (94, 95). Therefore, hESCs are seen as a potential therapeutic tool for replacement therapy of various human diseases, and that aspect of hESCs research has been extensively studied. However, before clinical application of hESCs becomes reality a number of issues must be resolved that include successful and specific differentiation into particular cell type, made of administration, avoidance of tumor development and immune rejection (96). On the other hand, existing models of various types of cells differ-

entiated from hESCs can be of immediate value as an attractive research tool for studying early human development, for studying different (patho)physiological processes, for drug screening and toxicity testing, and for genetic manipulation (97-100).

Besides hESCs, other types of cells have also been extensively studied toward finding an ideal candidate for deriving cardiomyocytes: bone marrow-derived and circulating progenitor cells, skeletal myoblasts, hematopoietic stem cells, mesenchymal stem cells derived from adipose tissue, and a number of distinct resident cardiac stem cells (101, 102). Cell types from tissues other than cardiac, like bone marrow stem cells or skeletal myoblasts, showed very low potential for transdifferentiation; and early claims that they were able to transdifferentiate have been refuted (101, 102). Resident cardiac stem cells, although exhibiting a number of promising characteristics like self-renewal, multipotency, expression of a number of transcription factors like Nkx2.5, GATA-4 and MEF2C, which are positive early in the myocyte lineage, have one big disadvantage. These cells represent less than 1% of the total number of cells in the adult heart; methods of their isolation are very strenuous and an additional effort must be invested into their development (102).

Up to date, the most promising source of derived cardiomyocytes are induced pluripotent stem cells (iPSCs). iPSCs technology uses defined transcription factors to reprogram somatic cells into pluripotent cells (103, 104). The first report of iPSCs generation was published in 2006 by Takahashi and Yamanaka (105). They showed the induction of iPSCs from mouse embryonic and adult fibroblasts by retroviral delivery of four defined factors: Oct4, Sox2, Klf4, and c-Myc. Many protocols for iPSCs generation use retroviruses or lentiviruses, which, by integrating into the genome, enable the expression of reprogramming genes. To avoid unwanted effects of viral integration into genome, like reactivation of c-Myc transgene which leads to tumor formation, protocols free of viral reprogramming factors have been developed (103). Recently, iPSCs were developed from a Parkinson's disease patient by using a Cre-lox recombination method, and fibroblasts were reprogrammed into iPSCs by piggyBac transposition (106, 107). Furthermore, Kim et al. reported the generation of iPSCs without the use of genetic material (108). Major advantages of iPSC lines over hESCs are the possibilities to induce patient/disease-specific iPSCs. Also, ethical concerns about derivation of hESCs from embryos are circumvented. There are several studies which evaluate the cardiac potential of the iPSC lines in comparison to the extensively investigated hESCs (109). Most of these studies suggest that iPSC lines are a viable alternative to hESCs, but some differences between iPSCs and hESCs were also identified and remain to be investigated.

The cardiac research field is one of the areas in which new model systems are particularly needed because present models are not sufficient for either basic (patho)physiological and pharmacological studies or genetic manipulation (98-100). Cardiomyocytes derived from hESCs and iPSCs have distinctive advantages over the primary isolated cells or the cell lines that are available for any of the purposes mentioned above. Primary isolated ventricular and atrial cardiomyocytes from various animals or humans have been used as major models in the cardiac research area. However, a major obstacle in using primary isolated cardiomyocytes is the impossibility of their culturing, resulting in hampered genetic manipulation procedures with these cells. On the other hand, isolation methods of these cells are so extensively developed that their morphology and function are almost intact upon isolation, and that is a major advantage in using animal models. However, in respect to genetic, developmental, metabolic, and biochemical characteristics, animals are different from human beings. For studying any of the aforementioned processes the ideal model would include the use of human cells. In the case of using primary human cardiomyocyte cultures the research is also hampered by more difficult procedures of isolation than in animal models and there are numerous problems with obtaining the tissue and the fact that the tissue might be affected by various diseases and treatments. It is almost impossible to conduct any studies that would involve manipulation with gene expression, such as gene knockouts or gene over-expression experiments using human atrial appendages. Also, it is difficult to conduct experiments that have longer protocols or require chronic exposures to drugs.

Considering all of the aforementioned problems with the research model systems currently used, both hESCs- and iPSCs-derived cardiomyocytes are promising to become a major tool in the cardiac research field.

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