

Mechanisms of cardioprotection by isoflurane against I/R injury

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1. ABSTRACT

Myocardial infarction is responsible for most cardiovascular mortality as well as the pathogenesis of myocardial damage during and after infarction. Efforts are underway to modulate the development of ischemia–reperfusion (I–R) injury. Recently, the protective effect of isoflurane against myocardial I–R injury emerged as a possibility. Nitric oxide, nitric oxide synthases, factors related to energy metabolism, adenosine triphosphate-sensitive potassium channels, phosphatidylinositol-3-kinase and hypoxia-inducible factor1-alpha have been shown to participate in the mechanisms of cardioprotection elicited by isoflurane against I–R injury. In this review, we focus on the mechanisms of cardioprotection offered by isoflurane against I–R injury.

2. INTRODUCTION

The World Health Organization has reported that cardiovascular diseases (CVD) are the leading cause of death worldwide, and that ≈ 23.6 million people will die from CVDs by 2030, mainly from myocardial infarction (1). Cardioprotection by ischemic pre-conditioning (IPC) was first described by Murry *et al.* (2). In 1988, Warltier and colleagues demonstrated that pretreatment with isoflurane improved left ventricular systolic function after occlusion of the left anterior descending coronary artery (LAD) lasting 15 min (3). Until now, the protective effect of isoflurane against myocardial ischemia–reperfusion (I–R) injury has been reported in several studies. Kehl *et al.* (4) found that low concentrations of isoflurane were sufficient to precondition the myocardium against

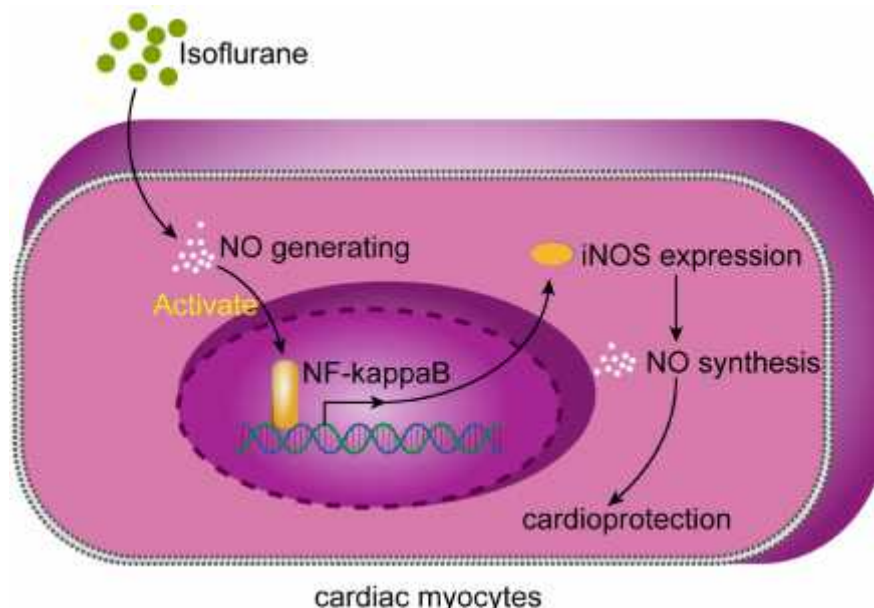


Figure 1. Cardioprotection by isoflurane through iNOS and NO. Isoflurane exposure can lead to NO generation, which triggers downstream activation of NF- κ B. Activated NF- κ B up-regulates the expression of iNOS and NO synthesis in the myocardium. This action mediates isoflurane pre-conditioning-induced delayed cardioprotection.

infarction. They speculated that high concentrations of isoflurane may have greater efficacy to protect the myocardium during low coronary collateral blood flow. Emulsified isoflurane can enhance the cardioprotection of St Thomas cardioplegic solution in a model of isolated heart I-R injury in rats (5). In a model of cardiopulmonary bypass in dogs, researchers found that alternative use of isoflurane and propofol conferred superior cardioprotection against post-ischemic injury and dysfunction of the myocardium (6).

However, a recent study reported that, during isoflurane inhalation (first pre-conditioning stimulus), remote ischemic pre-conditioning (second pre-conditioning stimulus) did not provide benefit to the myocardium of patients undergoing on-pump coronary artery bypass grafting (7). Several studies have found that isoflurane plays a part in the myocardial protection against I-R injury. Hence, investigating the underlying mechanism of this effect may be beneficial for patients with I-R injury. Thus, we investigated the mechanisms of myocardial protection by isoflurane against I-R injury.

3. NITRIC OXIDE SYNTHASES (NOS) IN THE MECHANISM OF ISOFLURANE-INDUCED CARDIOPROTECTION

NOSs are a family of enzymes that catalyze the production of nitric oxide (NO). The roles of NOS and NO in the regulation of cardiac function are complex, controversial and probably multifactorial (8). The cardioprotective effect of isoflurane against I-R injury has been found to be through NO (9, 10), and to cause an increasing antioxidative effect in the myocardium (9). There has been an increased understanding of the role of NOSs in the mechanism of cardioprotection by isoflurane, especially of

inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS).

3.1. iNOS

Induction of the high-output iNOS usually occurs in an oxidative environment, so high levels of NO can react with superoxide, leading to peroxynitrite formation and cell toxicity (11). A significant role for iNOS in the early phase of cardiac ischemia was not found in Langendorff preparations from iNOS^{-/-} mice (12), but overexpression and activation of iNOS was found to be indispensable in isoflurane inhalation, thereby inducing a second “window” of pre-conditioning after 24–72 h in the rat heart (13). Conversely, isoflurane exposure can lead to NO generation, which triggers downstream activation of nuclear factor kappa B (NF- κ B). This results in the subsequent upregulation of myocardial iNOS expression and NO synthesis that mediate anesthetic-induced pre-conditioning (APC)-induced delayed cardioprotection (14) (Figure 1). Thus, a positive feedback in the cardioprotection of NO and iNOS is “switched on” by isoflurane.

3.2. eNOS

In an *in-vivo* study, eNOS was shown to mediate the protective effects of isoflurane against infarction during early reperfusion (15). A further study showed that isoflurane post-conditioning protects mouse hearts from reperfusion injury by preventing opening of mitochondrial permeability transition pores (mPTPs) through an eNOS-dependent mechanism (16). That study also found that NO functioned as a trigger and mediator of the cardioprotection produced by isoflurane post-conditioning (16). Those results suggest a positive role of eNOS and NO in isoflurane-induced cardioprotection. Based on those results, we speculate that eNOS and NO may also form a positive

feedback mechanism in the cardioprotection by isoflurane. Though delayed myocardial pre-conditioning by isoflurane is mediated by eNOS in male rabbits, and eNOS can mediate a female sex-induced reduction in infarct size, remote exposure to isoflurane (1.0 MAC) before I-R does not produce additional cardioprotection *in vivo* (17).

Taken together, the reports described above suggest that NO and iNOS or eNOS may form a positive feedback mechanism in isoflurane-induced cardioprotection. In this positive feedback mechanism, isoflurane may be the initiator. Conversely, the sex of the patient may influence the effect of isoflurane in the cardioprotection with NOSs. Therefore, the sex of the patient should be considered in future studies.

4. ENERGY METABOLISM IN THE MECHANISM OF ISOFLURANE-INDUCED CARDIOPROTECTION

The cardioprotection elicited by isoflurane involves energy metabolism. Factors related to energy metabolism, including protein kinase C (PKC), adenosine receptors, cyclo-oxygenase (COX)-2, and electron transport chain complex III, were found to participate in isoflurane-induced cardioprotection.

4.1. PKC

In 1999, Toller *et al.* (18) first proposed that the role of PKC during isoflurane-induced recovery of the stunned myocardium cannot be excluded. Later, the role of PKC in the mechanism of isoflurane-induced cardioprotection attracted considerable attention. Isoflurane was shown to delay the decrease in levels of adenosine triphosphate (ATP) in the myocardium during ischemia and improve the recovery of mechanical function and energy state 60 min after ischemia, and PKC was required for this to occur (19). The isoenzyme PKCepsilon (PKCε) can prime the sarcolemmal adenosine triphosphate-sensitive potassium (sarc K(ATP)) channel to open in the presence of isoflurane (20). Although isoflurane pre-conditioning resulted in a reduction in infarct size at all concentrations tested, the protection was mediated by phosphorylation and translocation of the PKCε only at 0.4 MAC (21). Isoflurane-induced pre-conditioning induces the delay of mPTP opening through PKCε-mediated inhibition of mPTP opening, but not through the PKC-delta (PKCδ) isozyme, suggesting a connection between the cytosolic and mitochondrial components of the cardioprotection provided by isoflurane (22).

4.2. Adenosine receptors

Isoflurane-induced cardioprotection in stunned myocardium is partially mediated by adenosine type 1 receptor activation accompanying by decreases in endogenous adenosine release (23). Roscoe *et al.* later reported that adenosine A1 receptors could mediate the beneficial effects of anoxia and isoflurane in human myocardium (24). These findings demonstrate a mediating role of adenosine receptors in isoflurane-induced cardioprotection.

4.3. Other factors

Besides PKC and adenosine receptors, COX-2 is an important mediator of isoflurane-induced delayed pre-conditioning (25). Reactive oxygen species (ROS) generated from mitochondrial electron transport chain complex III were also found to mediate isoflurane pre-conditioning (26). Furthermore, the trigger in the cardioprotection of isoflurane has been found. In the adult rat, ROS and nitrogen species can trigger the delayed cardioprotection elicited by isoflurane (27). During post-conditioning-induced cardioprotection, a novel phosphorylation site was detected in adenine nucleotide translocator-1 (ANT1) at residue Tyr(194), which suggested that phosphorylation of mitochondrial proteins may have a critical role in cardioprotection (28).

5. ADENOSINE TRIPHOSPHATE-SENSITIVE POTASSIUM (K(ATP)) CHANNELS IN THE MECHANISM OF ISOFLURANE-INDUCED CARDIOPROTECTION

5.1. Evidence of K(ATP) channels involved in the mechanism of isoflurane-induced cardioprotection

A K(ATP) channel is a type of potassium channel that is gated by ATP. Isoflurane can directly pre-condition the myocardium against infarction *via* activation of K(ATP) channels in the absence of hemodynamic effects and exhibit the acute memory of pre-conditioning *in vivo* (29). K(ATP) channels were found to mediate the beneficial effects of isoflurane in the human myocardium (24, 30, 31). Patel *et al.* were the first to demonstrate that isoflurane and opioids work in conjunction to confer protection against myocardial infarction through potentiation of the opening of cardiac K(ATP) channels (32). In fact, mitochondrial K(ATP) (mito K(ATP)) and sarc K(ATP) channels can contribute to isoflurane-induced delayed cardioprotection (33, 34).

5.2. Effect of K(ATP) channels involved in the mechanism of isoflurane-induced cardioprotection

The K(ATP) channel acts as a mediator when trifluoroacetic acid mediates isoflurane-induced cardioprotection during myocardial I-R (35). One *in-vivo* study showed that combined administration of isoflurane and morphine enhanced protection against myocardial infarction to a greater extent than either drug alone, and that mito K(ATP) channels and opioid receptors mediated this beneficial effect (36). After that study, researchers began to focus on the mito K(ATP) channel in isoflurane-induced cardioprotection.

Activation of the mito K(ATP) channel was found to be the essential trigger of pre-conditioning with isoflurane, as well as being a crucial mediator of isoflurane-induced cardioprotection (37). However, enhanced cardioprotection conferred by combined pre-conditioning is mediated through mito K(ATP) channel-independent and -dependent mechanisms (37). Through a K(ATP) channel-independent mechanism, isoflurane can inhibit neutrophil-endothelium interactions and the inflammatory response *in vitro*, which is involved in the cardioprotection elicited by isoflurane *in vivo* (38). In a mito K(ATP) channel-

dependent mechanism, mPTP inhibition enhances isoflurane-induced post-conditioning (39). In addition, opening of mito K(ATP) channels followed by superoxide (O_2^-) signaling induces post-ischemic augmentation of production of manganese superoxide dismutase and preservation of the activities of mitochondrial respiratory enzymes. This leads to an attenuated cardiac O_2^- surge and restores ATP production during reperfusion, and underlies isoflurane-induced cardioprotection (40).

Marinovic *et al.* (41) found that whereas the mito K(ATP) channel has a dual role as a trigger and an effector, the sarc K(ATP) channel acted as an effector of pre-conditioning in isoflurane-induced protection against I-R injury. From those observations, we found that the mito K(ATP) channel can act as a trigger, mediator and effector in isoflurane-induced cardioprotection, whereas the sarc K(ATP) channel acts as an effector.

5.3. Factors influencing the role of K(ATP) channels in the mechanism of isoflurane-induced cardioprotection

The protein tyrosine kinase (PTK)–protein tyrosine phosphatase signaling pathway may be one of the regulators of the cardiac sarc K(ATP) channel. It may play a part in modulating its responsiveness to isoflurane, but the relative importance of this modulation for cardioprotection by volatile anesthetics remains to be established (42). PKC ϵ primes the sarc K(ATP) channel to open in the presence of isoflurane, whereas PKC δ is significantly less effective in modulating the isoflurane effect on this channel (20). Moreover, age influences the effect of isoflurane-induced pre-conditioning. Mio *et al.* (43) found that anesthetic-induced pre-conditioning with isoflurane decreases stress-induced cell death and preserves mitochondrial respiratory function to a greater degree in mid-aged than in old-aged myocytes; however, the activity of the sarc K(ATP) channel is not differentially affected by isoflurane. Therefore, the effectiveness of APC in humans may decrease with advancing age because (at least in part) of altered the mitochondrial function of myocardial cells.

6. SIGNALING PATHWAYS IN THE MECHANISM OF ISOFLURANE-INDUCED CARDIOPROTECTION

6.1. Phosphatidylinositol-3-kinase (PI3K)

Isoflurane acts during early reperfusion after prolonged ischemia to salvage the myocardium from infarction and reduces the threshold of ischemic post-conditioning by activating PI3K (44). Isoflurane-induced post-conditioning is enhanced by morphine *via* activation of PI3K *in vivo*, as well as by opioid receptors (45). Through modulation of PI3K/Akt and eNOS signaling, hyperglycemia inhibits isoflurane-induced post-conditioning in the rabbit heart (46).

6.2. Hypoxia-inducible factor1-alpha (HIF1- α)

The hypoxia-inducible factor (HIF) signaling cascade mediates the effects of hypoxia (i.e., a state of low oxygen concentration) on the cell. Extracellular signal-regulated kinases (Erk) trigger isoflurane pre-conditioning concomitant with upregulation of HIF1- α , as well as

expression of vascular endothelial growth factor, in rats (47). Isoflurane stimulates endothelial cells to produce HIF1- α , which may contribute to protection of the myocardium during I-R injury (10).

7. Other factors involved in the mechanism of isoflurane-induced cardioprotection

Erk1/2 and 70-kD ribosomal protein s6 kinase (p70s6K) mediate the protective effects of isoflurane against infarction during early reperfusion *in vivo* (15). Inhibition of the apoptotic protein p53 lowers the threshold of isoflurane-induced cardioprotection during early reperfusion *in vivo* (48).

A study using a cardiopulmonary bypass model in dogs showed that alternative use of isoflurane and propofol conferred superior cardioprotection against post-ischemic injury and dysfunction of the myocardium, and that this protection was probably mediated by attenuation of cardiac oxidative damage (6). In hyperglycemia, excessive quantities of ROS generated during hyperglycemia impair isoflurane-induced pre-conditioning in dogs (49).

Isoflurane can act as an upstream regulator in I-R injury. I-R-induced depression of cardiac performance is associated with a down-regulation of the major Ca^{2+} -cycling proteins in the sarcoplasmic reticulum. APC with isoflurane was shown to prevent I-R-related degradation of the Ca^{2+} -release channels (RyR2) and Ca^{2+} -adenosine triphosphatase (SERCA2a) in the sarcoplasmic reticulum, and this was independent of its activation of K(ATP) channels (50). Isoflurane can inhibit p38MAPK activity during myocardial I-R and modulate expression of the cytokine tumor necrosis factor (TNF)- α , which may be one of the molecular mechanisms of isoflurane-delayed pre-conditioning on cardioprotection (51). Furthermore, isoflurane has been shown to induce O-linked -N-acetylglucosamine (O-GlcNAc) modification of mitochondrial voltage-dependent anion channels. This modification has been shown to inhibit the opening of the mPTP, and to confer resistance to I-R stress (52).

At the mitochondrial level, the effect of isoflurane mediate (at least in part): (i) enhance the net rate of state-2 calcium (Ca^{2+}) uptake by inhibiting the Na^+/Ca^{2+} exchanger (NCE), independent of changes in mitochondrial membrane potential and matrix volume, and (ii) the decrease in rates of state-3 electron transfer and adenosine diphosphate (ADP) phosphorylation by inhibiting complex I. These direct effects of isoflurane to increase mitochondrial free Ca^{2+} , while depressing NCE activity and oxidative phosphorylation, could underlie the mechanisms by which isoflurane provides cardioprotection against I-R injury at the mitochondrial level (53).

8. CONCLUSION

An increasing number of studies have demonstrated the protective effect of isoflurane against myocardial I-R injury. In the mechanism of

cardioprotection, isoflurane can act as a trigger or a mediator. Most of the current studies are based on animal models; further studies are needed to draw clear conclusions in humans.

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Abbreviations: I/R: ischemia/reperfusion; ATP: adenosine triphosphate-sensitive potassium; PI3K: Phosphatidylinositol-3-kinase; HIF 1- α : Hypoxia-

inducible factor-1 α ; CVD: cardiovascular diseases; IPC: ischaemic preconditioning; LAD: left anterior descending coronary artery; NOSs: Nitric oxide synthases; iNOS: inducible NOS; eNOS: endothelial NOS; mPTP: mitochondrial permeability transition pore; PKC: protein kinase C; COX-2: cyclooxygenase-2; PKC ϵ : PKC-epsilon isozyme; ANT1: adenine nucleotide translocator-1; Erk1/2: Extracellular signal-related kinases; O-GlcNAc: O-linked β -N-acetylglucosamine

Key Words: ischemia and reperfusion; HIF 1- α ; inducible NOS, Review

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