Pretreatment by Hyperoxia - A Tool to Reduce Ischaemia-Reperfusion Injury in the Myocardium

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Abstract: Atherosclerosis leads to narrowing and occlusion of coronary arteries, resulting in inadequate oxygen supply for maintenance of normal oxidative metabolism. To avoid profound ischaemia and subsequent necrosis of cardiomyocytes, blood flow has to be restored by means of thrombolysis, percutaneous coronary intervention, or surgical revascularisation. Besides restoring oxygen supply to the cells, introduction of molecular oxygen to the ischaemic tissue results in a spectrum of unfavourable events, termed altogether as reperfusion injury. Exposure to hyperoxia for a limited time before ischaemia induces a low-grade oxidative stress and evokes an (ischaemic) preconditioning-like effect in the myocardium, which protects the heart from subsequent injury. This review addresses the effects of pretreatment by hyperoxia both in experimental and clinical setting.

Key Words: Hyperoxia, preconditioning, myocardium, hyperbaric oxygenation, ischaemia-reperfusion injury, oxidative stress, reactive oxygen species

INTRODUCTION

Early reperfusion after atherosclerotic occlusion of a coronary artery is an absolute prerequisite for survival of the ischaemic myocardium. Even if this is achieved, an adverse complex of events, instead of expectedly good myocardial performance, can develop. Since the first reports of reperfusion accelerated necrosis in experimental [1] and clinical setting [2], reversible postsichaemic myocardial dysfunction (myocardial stunning), reperfusion arrhythmias, necrosis of cardiomyocytes, endothelial and microvascular dysfunction including the no-reflow phenomenon have been defined as the early manifestations of the ischaemia-reperfusion (IR) injury of the heart [3-5]. Experimental studies indicate that myocardial reperfusion injury accounts for up to 50% of the final size of the infarction [6]. Besides, ischaemia and reperfusion can induce apoptosis [7, 8] and a link has been established between apoptosis and heart failure [9]. Autophagy after ischaemia and reperfusion has been described recently - it can promote survival by degrading damaged mitochondria, or enhance cell death when activated excessively [10].

After first description of the reperfusion injury, research in the field of myocardial protection has grown extensively. A large number of studies have explored the cellular mechanisms and protective strategies from IR injury of the heart. In this article, we review recent advances in understanding of how a clinically applicable strategy - pretreatment by hyperoxia - may contribute to the reduction of IR injury in the myocardium.

MYOCARDIAL PRECONDITIONING

In 1986 a landmark study demonstrating a new concept of myocardial protection - ischaemic preconditioning (IPC) - was published by Murry et al. [11]. IPC refers to the phenomenon of inducing tolerance against the IR injury by controlled brief periods of ischaemia and reperfusion prior to a sustained ischaemic insult. It is characterised by an early phase of protection manifesting from the first minutes and lasting for few hours after the preconditioning stimulus (i.e. the first window of protection or classic preconditioning). It is associated with post-translational modification (phosphorylation and translocation) of the involved signalling proteins. A longer delayed phase evinces 24 - 72 hours after the initial preconditioning stimulus (second window of protection) as it requires the synthesis of new proteins. A thorough overview discussing mechanisms of the preconditioning phenomena has been published recently [12].

The first clinical study investigating the effect of IPC during coronary artery bypass grafting (CABG) was published in 1993, where a significant 76% increase in the ATP concentration in myocardial biopsies from the IPC group following the ischaemic insult was found [13]. Followed by years of extensive research, it is recognised nowadays, that IPC is one of the most powerful manifestations of endogenous adaptation against the ischaemic injury in all species and tissues tested. In numerous clinical studies it has been shown to reduce size of myocardial infarction and improve cardiac function [14-16], attenuate the release of markers of myocardial necrosis [14, 17, 18] and interleukin-6 (IL-6)
[19], reduce the incidence of postoperative ventricular arrhythmias [20], and attenuate endothelial dysfunction and systemic neutrophil activation [21].

In the clinical practice IPC can be achieved by intermittent aortic cross-clamping. The necessity to repeatedly cross-clamp the aorta and the resulting risk of atheroembolism limits its clinical applicability [22]. Besides, in patients with coronary artery disease, an extra ischaemic load imposed as a preconditioning stimulus may further endanger the already diseased myocardium.

To overcome these problems, a large body of studies has tried to identify triggers, mediators and end-effectors to develop pharmacological agents to mimic this powerful phenomenon. But so far none of the numerous agents proven to mimic preconditioning in experimental studies has been implemented for routine clinical use. The only exceptions may be volatile anaesthetics [23], and opioids [24, 25], although their indication as “preconditioning drugs” not as “anaesthetic drugs” is still very limited in the everyday clinical practice.

RATIONAL FOR HYPEROXIA INDUCED CARDIOPROTECTION. EXPERIMENTAL STUDIES

Oxygen is routinely administered during the perioperative period, with the inspired concentrations varying from 30% to 100%. From one side, this is essential to ensure adequate tissue oxygenation. On the other side, introduction of molecular oxygen may cause cellular injury due to formation of reactive oxygen species (ROS).

ROS play an important role in a wide variety of cellular processes including upregulation and activation of myocardial antioxidant enzymes [26-28]. Hearts stimulated in vivo with transient ischaemia, hyperthermia, or inflammatory mediators have increased antioxidant enzyme activity and tolerance to subsequent IR injury [29-31]. This in turn may further imply that activation of the endogenous antioxidant defense system could promote tolerance to ischaemia in the myocardial cells.

A role of ROS in preconditioning was first suggested by Murry et al. who demonstrated that administration of oxygen radical scavengers during reperfusion blocked the beneficial effect of the IPC on the infarct size [32]. Later, ROS were shown to be involved in triggering IPC [33-35]. Brief repeated ischaemic insults alter myocardial antioxidant activity not only immediately after, but also 24 hours after sublethal ischaemia [36], indicating that antioxidant enzymes may belong into effector proteins of intracellular signalling pathways activated by IPC [27, 37].

Other studies have shown that the beneficial effects of IPC can be prevented by exogenous antioxidants during both the preconditioning episode [38] as well as during reperfusion after a sustained ischaemic insult [39,40]. Furthermore, exposure of isolated hearts to non-toxic doses of ROS - in the absence of ischaemia - may reproduce the beneficial effects of IPC [41]. It has also been demonstrated that low doses of exogenous ROS protect myocardial contractile function against subsequent myocardial injury [34, 42, 43].

Hyperoxia is known to generate oxidative stress [44-46] and induce antioxidant activity in the heart [47, 48]. Stock et al. postulated that chick embryo adapts to hyperoxia to escape additional free radical damage, perhaps by increasing the capacity of its antioxidant defenses to compensate for a potential increase in the rate of free radical generation [49]. Preconditioning with normobaric hyperoxia increases antioxidant enzyme activity of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase [50]. Thus, pretreatment with an oxidative stimulus to enhance the antioxidant defence may offer protection against IR injury. Hyperoxia generates ROS and induces lipid peroxidation in cell cultures and isolated organs [51-54]. Although prolonged exposure to high oxygen fractions is injurious, a short exposure inducing generation of low amounts of ROS, that is insufficient to induce cell injury, can elicit a preconditioning-like response (Fig. 1). The concept of hyperoxia-induced cardioprotection is indirectly supported by several studies demonstrating that moderate oxidative stress can activate endogenous adaptive responses in the heart [27, 32, 34, 41]. Due to biological variability and individual differences in antioxidant systems capacity, however, it is difficult to draw a clear line between excessive and moderate ROS production. Therefore individuals with impaired antioxidant systems may have increased damage from hyperoxic exposure.

The studies on rats and mice have revealed that in vivo normobaric exposure to suprernal oxygen fraction in inspired gas mixture lasting 30 minutes for mice and 60 minutes for rats before sustained ischaemia protects the heart against subsequent injury [44, 46, 55-60]. Hyperoxia improves recovery of the postischaemic contractile function, reduces infarct size both in normal and atherosclerotic hearts [44, 55, 57] and reduces the incidence of IR induced arrhythmias [59, 61]. There is a dose-dependent relationship, the minimal concentration to evoke protective effect in the rat heart is 80%, the most efficient >95% with the exposition time of 60 minutes [56]. Similar to ischaemic preconditioning, hyperoxia evokes immediate and delayed phases of protection [56, 57, 59], with the second window of hyperoxic preconditioning may last for more than 48 h and be even prolonged by intermittent pre-exposure to the same environment [62]. Furthermore, hyperoxia modifies vasomotor response of isolated aortic rings [57].

The protective effect of hyperoxia is mediated through nuclear factor kappa-B [58], tumour necrosis factor receptor 1- [63] and mitogen activated protein kinases- and nitric oxide- [64] dependent mechanisms. It has been suggested that hyperoxia induces low-grade oxidative stress which leads to myocyte mitochondrial KATP channel opening [46]. Emerging evidence suggests that after injury-producing stresses, ROS are largely responsible for the mitochondrial permeability transition pore induction. When cell protection signalling pathways are activated, the integral membrane protein Bcl-2 family members relay the signal onto a target at or in close proximity to the pore [65].

REACTIVE OXYGEN SPECIES AND NORMOBARIC HYPEROXIA IN CLINICAL STUDIES

Similar to experimental studies, generation of ROS has been evidenced during cardiac surgery. Occurrence of oxida-
tive stress during reperfusion has been confirmed by release of oxidised glutathione [66-69], impaired (i.e. more oxidised) glutathione redox ratio [70], or by direct measurement by electron spin-trapped spectroscopy [71]. The release of oxidised glutathione from the heart after cardioplegia has been related to cardiodepressive effects in some [66, 67, 71], but not in all studies [68-70].

Although there are plenty of experimental data showing the advantageous effects of hyperoxia on IR injury of the heart, only one preliminary study investigating the effect of hyperoxia during cardiac surgery has been published [69]. Patients undergoing elective CABG were ventilated before cardioplegia with either 40% or >96% oxygen for approximately 120 min and normoxic ventilation was implemented thereafter. In the 1st postoperative morning hyperoxia-pretreated patients showed neither lower values of troponin I and creatine kinase-MB isoenzyme levels nor improvement of parameters of cardiac function compared to normoxic controls. The only potentially beneficial effect observed was decreased myocardial release and systemic levels of IL-6. As levels of IL-6 during reperfusion have been shown to correlate with the severity of injury - left ventricular wall motion abnormalities [72] and dysfunction [73], negative inotropic effect [74], development of postoperative fever [75] and poor prognosis [76], a marked reduction of IL-6 concentrations in the coronary sinus blood after declamping the aorta could be considered as a sign of reduced injury. However, as no protective effect of hyperoxia upon myocardial infarction or function was observed, the exact importance of this finding remains to be elucidated. Besides, data suggest that the inflammatory response during open-heart surgery depends partly on the genetic background of the individual [77].

So far only one inspiratory concentration of oxygen with a delimited exposure time has been tested in a clinical setting [69]. It cannot be excluded that an average exposure of 2 hours is 'on the borderline' between the induction of protection and tissue injury, especially when keeping in mind the additional oxidative burst possibly caused by the institution of cardiopulmonary bypass. Oxidative stress is evident within minutes after the restoration of blood flow to the ischaemic myocardium, and precedes the activation of the inflammatory response [78]. The rapid up-regulation and production of pro-inflammatory cytokines represents an intrinsic or innate stress response against myocardial injury [79]. During CABG, reperfusion of the ischaemic heart, general response to surgical trauma and blood contact with the CPB circuit may account for the increased inflammatory response [80, 81].

One concern in association with the hyperoxic pretreatment could be increased oxidative stress after the intervention [82]. Study by Karu et al. did not reveal a difference between hyperoxia and control group in the glutathione redox ratio, a sensitive index of oxidative stress [69]. Even though increased release of oxidised glutathione was evident in the 1st minute of reperfusion in the hyperoxia group, no

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**Fig. (1).** Schematic diagram showing pathophysiologic implications of reactive oxygen species and endogenous antioxidants in hyperoxia-induced oxidative stress.
aggravation of oxidative stress was detected at later time-points [69].

Not only the pretreatment by hyperoxia, but clinical studies of myocardial preconditioning in general, have not shown as unequivocally beneficial results as the experimental setting. Both benefits [14, 17, 83] and no superiority [84, 85] over or in addition to conventional myocardial protection in terms of cardiac function and metabolic / necrosis markers have been described. This is a common problem of clinical studies in the cardiac surgery, where modern cardioprotective techniques are applied also to control patients, and thereby the degree of injury is often insufficient to demonstrate the additive effect of a studied intervention. These divergent results have led to the hypothesis that in the setting of coronary artery bypass surgery, the additional protection conferred by preconditioning may only be demonstrable where a potential for suboptimal myocardial protection increases the risk of perioperative infarction [86].

**HYPERBARIC OXYGEN AS A TRIGGER FOR MYOCARDIAL PRECONDITIONING**

One of the earliest studies to examine the effects of hyperbaric oxygenation (HBO) on IR injury was conducted by Sterling et al. [87] in 1993. In a study on open-chest rabbit model of regional ischaemia, the authors exposed animals either to normobaric (FiO₂ 100%, 1 atmosphere absolute (ATA)) or hyperbaric hyperoxia (2.5 ATA). The animals exposed to HBO during ischaemia only, reperfusion only, or ischaemia and reperfusion had significantly smaller infarcts compared to controls, indicating protection induced by HBO. The protecting mechanisms of HBO include expressing of Bcl-2 [45] or stimulation of endogenous NO production [88]. Cabigas et al. demonstrated that under conditions of combined hyperoxia and hyperbaria, the heart increases production of NO from endothelial nitric oxide synthase (NOS3). The latter is activated by association with heat shock protein-90 and responses to changes in oxygen availability - an adaptive response offering protection against subsequent ischaemic insult [89]. Pretreatment with HBO enhances enzymatic activity and gene expression of catalase, and significantly reduces infarct size in a rat heart. A catalase inhibitor abolishes the infarct-limiting effect, suggesting that HBO-induced tolerance against IR injury is due to catalase induction [90]. Other studies have demonstrated that repeated episodes of HBO before coronary artery ligation improve the function of the rat heart and accelerate angiogenesis as demonstrated by increase in capillary density, vascular endothelial growth factor protein levels and cell proliferation [91].

Aparci et al. showed in a clinical study that repetitive HBO therapy improved the myocardial diastolic function of diabetic patients as indicated by improved relaxation capability of left ventricular myocardium and the diastolic filling dynamics of the right ventricle [92]. After myocardial infarction HBO offers adjunctive effect after thrombolysis. HBO treated patients showed attenuated rise in creatine phosphokinase levels and more rapid resolution of pain and ST-segment changes [93]. Hyperbaric hyperoxia also showed a favourable effect on left ventricular systolic function and the remodeling process [94].

**SYSTEMIC EFFECTS OF HYPEROXIA**

The lungs serve as a primary target organ for hyperoxia. Among clinicians hyperoxic lung injury is one of the best known side-effects of long-term oxygen administration. Studies in humans have described the first signs of toxicity only after 12-16 hours of oxygen administration [95]. In a recent study in severe brain trauma patients breathing of 100% oxygen for 3 hours, the intervention did not result in increases of IL-6 and IL-8 bronchial alveolar fluid [96]. Besides, the intermittent exposure to hyperoxia has been shown to increase tolerance against lung injury [97]. The growing body of experimental and clinical evidence shows that mechanical ventilation, especially usage of high tidal volumes, appears to be more deleterious to the lung than hyperoxia per se [98-100]. One of the effects, that clinicians have to be aware of while exploiting 100% oxygen, is the formation of absorption atelectases [101, 102]. Still, reducing the inspired concentration of oxygen to 80% does not cause more atelectases than breathing of 30% oxygen [103].

In clinical studies, beneficial systemic effects of hyperoxia have been described. Administration of 80% oxygen has been reported to reduce the wound infection rate in patients undergoing colorectal resection [104], but poorly treated postoperative pain abolishes this effect [105]. Two recent meta-analyses addressed this effect in a more complex manner. Brar et al. did not find significant reduction of surgical site infection but conferred a mortality benefit [106], whereas Al-Niaimi et al. showed beneficial effect of supplemental oxygen administration [107]. Breathing 100% oxygen preserves antimicrobial function of alveolar macrophages after surgery [108]. Initial reports describing the effect of hyperoxia on postoperative nausea and vomiting showed very promising results [109, 110], but later reports could not find such benefits [111, 112].

Other favourable effects of normobaric hyperoxia include extension of the reperfusion window in focal cerebral ischaemia and reduction of neurological deficit after stroke [113, 114], improvement of liver transplant function and survival [115], improvement of renal function after IR injury in a rat model [116, 117]. In zymosan-induced sterile inflammation mice model, treatment with 100% oxygen decreased the levels of serum pro-inflammatory cytokines, increased the level of serum anti-inflammatory cytokine, up-regulated the activities of serum and tissue antioxidant enzymes and improved the 14-day survival rate whereas ROS scavenger pretreatment partly abolished the protective effects of 100% oxygen treatment [118], improved organ function and attenuated tissue apoptosis during early hyperdynamic porcine septic shock [119].

In clinical studies exposure to hyperoxia after traumatic brain injury has been described as a very promising treatment model [120].

**CARDIOVASCULAR EFFECTS OF HYPEROXIA**

Breathing of hyperoxic gas mixture has several well-established effects upon the cardiovascular system, namely reduction of cardiac index, stroke index, heart rate, and left ventricular diastolic relaxation with concomitant rise in systemic vascular resistant index and left ventricular filling
pressures. The effects are reversed when FiO₂ is reduced back to normal, and they occur similarly in healthy volunteers [121], anaesthetised or awake patients [122], after CABG [122, 123], or in patients with congestive heart failure [124]. In the coronary circulation, breathing 100% oxygen significantly reduces coronary blood flow, increases coronary vascular resistance and reduces myocardial oxygen consumption [125].

In experimental animals, increasing of PaO₂ during an acute low-flow myocardial ischaemia, in contrast, improved both function and flow distribution to the ischaemic myocardium, and decreased glycolytic metabolism in the ischaemic zone [126]. Besides, hyperoxic ventilation increased tolerance to acute normovolemic anaemia by creating readily usable plasmatic oxygen reserve [127] and reduced 6-hour mortality after haemorrhagic shock [128].

CONCLUSIONS

Experimental studies have demonstrated that exposure to hyperoxia induces a preconditioning-like effect in the heart, which is triggered by low-grade oxidative stress and a concomitant increase in antioxidant enzyme activity. Exposure of experimental animals to hyperoxia, normobaric or hyperbaric, protects the heart from subsequent IR injury. This activation of endogenous adaptive processes results in a "readiness" to cope with the oxidative stress and reduction of deleterious effects of myocardial ischaemia and following reperfusion.

In humans pre-treatment by hyperoxia results in reduced inflammatory response associated with cardiac surgery. Whether this manoeuvre reduces the extent of myocardial IR injury in humans, for example after cardiac surgery, as well as defining the optimal dosage in terms of both inspired oxygen fraction and exposure time, remains to be elucidated in future studies.

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