

Cardio-Protective Effects of Oxygen: New Applications of an Old Therapeutic Tool

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ABSTRACT

Myocardial infarction is one of the major causes of mortality in western countries imposing huge annual costs. Ischemia-reperfusion injury in myocardial infarction is one of the major damages occurring during thrombolytic therapy which increases infarct size. In this regard, introduction of treatment strategies capable of reducing the infarction-induced damages could play an important role in reducing the mortality rate. Pre-conditioning phenomenon is one of the defence mechanisms which can reduce ischemic reperfusion injury resulting in reduction of infarct size and fatal ventricular arrhythmias. Oxygen pre-treatment either in normobaric or hyperbaric conditions are among the strategies capable to induce cardio protection in various models. Reactive oxygen species are involved in the mechanism of this protective phenomenon. In this review article, both animal and human studies in this field are discussed.

keywords: Preconditioning; Hyperoxia; Myocardium; Oxygen

INTRODUCTION

The preconditioning phenomenon

The ischemic preconditioning phenomenon in the heart was originally introduced by Murray et al in 1986. After some short term ischemia-reperfusion periods in the heart of dogs, the heart was more resistant against the injury induced by subsequent more prolonged ischemia followed by reperfusion and this lead to reduction of the infarct size. The early phase begins immediately and lasts for 1-2 hours, whereas the delayed phase will be activated 12-24 hours later and lasts for 3- 4 days. The early phase is only protective against the infarction while the delayed one protects against both infarction and stunning. Numerous factors involve in the ischemic preconditioning mechanism including adenosine, bradykinin, opioids, nitric oxide, vasopressin and Reactive Oxygen Species (ROS). As a trigger, they initiate various intracellular pathways that ultimately lead to protection of cell against damage. The cardio protective effects of ischemic preconditioning have been proved in various species such as feline, canine, sheep, baboon, mouse, rat, rabbit

and human. Mitochondria play the most important intracellular role in preconditioning phenomenon, so that the opening of KATP channels in mitochondria triggers the protective effects of preconditioning. Some agents induce the protective effects by activating these channels, for example some Factors that inhibit the opening of this channel can reverse the protective effects of preconditioning. The focus of this paper is on the role of ROS in inducing the preconditioning [1].

Reactive oxygen species (ROS) and preconditioning

Mitochondria are an important source of free oxygen radicals. Superoxide is produced in several parts of the electron transport chain and a large part of it is converted by Superoxide Dismutase (SOD) into to H_2O_2 in the mitochondria and cytosol matrices. H_2O_2 is then converted into water and oxygen by glutathione peroxidase and catalase. Under physiological conditions, 1 to 4% of the body's oxygen is converted into free radicals and this level can increase with hyperoxia exposure. Studies on isolated myocytes have shown that free radicals increase during anoxia (ischemia), but the amount of free

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radicals during this phase is very low. During the reoxygenation (reperfusion) phase, a large volume of free radicals is produced. It has been suggested that the increase of free radicals at the beginning of the reperfusion phase would cause extensive oxidative damage to the cell. ROS production during reperfusion phase also can lead to stunning. Although some studies have shown that administration of antioxidants such as SOD and catalase can reduce the infarct size, several other studies did not report these cardio protective effects [2]. On the other hand, the combinational use of antioxidants such as SOD, catalase and N-2-mercaptopyrionyl glycine (MPG, as a free radical scavenger), at the onset of the ischemia phase in pigs showed that these compounds could prevent the delayed phase of preconditioning against stunning. In another study, coronary infusion of free radicals in rabbits simulated cardio protective effect of preconditioning. Free radicals have a paradoxical role in cellular damage, in a way that reperfusion after coronary artery occlusion can produce large amounts of free radicals causing damage to the myocardium while low release of free radicals from mitochondria during short ischemic periods can induce preconditioning. Pre-treatment with free radical scavengers abolish the cardio protective effects of preconditioning. Also, diazoxide, as one of the activators of KATP channels in mitochondria, can produce free radicals and induce preconditioning. The use of antioxidants inhibits the protective effects of diazoxide and preconditioning [3]. Therefore, these studies suggest the important role of free radicals in creating protective effects of preconditioning. About the mechanism of hyperoxia-induced preconditioning, some studies have suggested the role of nitric oxide in reducing the ischemia-reperfusion injury in the myocardium. It has been shown that hyperbaric oxygen increases the expression of inducible nitric oxide synthase (iNOS) resulting in an increase in nitric oxide levels. Also, in rats lacking the iNOS gene, hyperoxia is not able to produce beneficial protective effects in the heart. In another study, use of L-NAME (N omega-nitro-L-arginine methyl ester)-a non-specific nitric oxide synthase inhibitor- prior to hyperoxia blocked the beneficial effects of hyperoxia in reducing ischemia reperfusion injury. Additionally, KATP channels are the other important mediator in protecting from IRI, which are activated by increasing the nitric oxide levels. Therefore, nitric oxide can be considered as one of the main mediators in induction of hyperoxia protective effects. Other proposed mechanism involves NFkB (a translation factor up-regulating the intracellular protective enzymes) activation by free radicals. This factor activates during ischemia at a low level and its activity increase significantly in reperfusion phase. The protective role of this factor in ischemic preconditioning has been proposed in several studies. This factor is induced by normobaric hyperoxia. Administration of NFkB inhibitors before hyperoxia leads to abolish hyperoxia beneficial effects. This factor also increases the expression of inducible nitric oxide synthase (iNOS) which triggers delayed preconditioning phase. However, activating of NFkB in ischemia-reperfusion injury without previous adaptation to ischemia can be harmful; so that inhibition of this factor in this phase can reduce the infarct size and improve cardiac function. Another studies reported that inhibition of activation of NFkB reduced infarct size in reperfused rat heart. Thus, this factor has a paradox effect on ischemia-reperfusion of

the myocardium, and to discuss about the role of NFkB we must to view all of these studies alongside. Another proposed mechanism is that hyperoxia reduces IR injury by inhibiting MPTP opening and cytochrome c release. In addition, hyperoxia prevents mitochondrial NAD (+) depletion, an indicator of MPTP opening. In addition, short episodes of hyperoxia could induce changes in the expression of some genes in the heart. The role of normobaric or hyperbaric hyperoxia in inducing preconditioning against ischemia reperfusion injury in both basic and clinical studies will be explained in the next sections (Figure1).



Figure 1: Normal LV size with mild LV systolic dysfunction , LVEF : 45% , Normal RV size with moderate RV dysfunction ,Severe LA and RA enlargement ,At least moderate TR , sPAP: 70 mmHg.

Normobaric hyperoxia in experimental studies

Applicability of ischemic preconditioning in clinical conditions has both technical and ethical problems due to complete disruption of the blood flow, risk of thrombosis, etc. Therefore, the use of clinical applicable drugs for pharmacological preconditioning induction with the same beneficial protective effects on the heart has been considered. One of these pharmacological approaches is hyperoxic pretreatment which has been described to reduce the ischemia-reperfusion injury in various organs including the heart, kidney, brain, liver and the spinal cord. Several studies have examined the effects of hyperoxia on reducing ischemia-reperfusion injury in rat heart. Tahepold et al. assessed various concentrations of hyperoxia with different durations in the rat and mouse heart. In rats Sixty minutes of hyperoxia with concentrations of $\geq 95\%$, 80% and 60%, but not 40%, could improve post-ischemic contractile function, as was evidenced by reducing LVEDP. The coronary blood flow was also increased. Talking about the duration of hyperoxia ($\geq 95\%$) in these two species; in rats, hyperoxic. Pretreatment for 60 or 180 min was able to induce protective effects. In rats, hyperoxia could induce both early and delayed phases of preconditioning in the heart but In the mouse, 15 to 30 minutes of hyperoxia (Fio2 95%) induced protective effects. However, in mouse only the initial protective effect of preconditioning was stimulated, and these finding shows that the mechanisms of initial and latent phase of pre-conditioning are different. The difference in stimulating the protective effects in

different periods of time in both species could be due to the fact that the mouse heart rate is more than the rat and its oxygen consumption relative to the body size is much higher. Therefore, in comparison with rats, hyperoxia can stimulate intracellular pathways faster than mice (Figure 2).



Figure 2: Small LV cavity (ED V index: 32.8 ml/m²), LV mass: 52 g Normal LV wall thickness, RVH, Cardiac t2*: 40 ms No myocardial inflammation, Normal pericardial thickness Late gad enhancement at LV attachment to ventricular septum.

In another study, Tähepold et al investigated the role of hyperoxia in reducing ischemia-reperfusion injury in isolated rat hearts. The researchers applied hyperoxia (>95% O₂) for 60 to 180 minutes and evaluated the initial and delayed phases of preconditioning. The results of this study showed that there were no significant differences between measured myocardial antioxidant enzymes activities as well as myocardial eNOS and HSP 72 expressions, but the infarct size, incidence of arrhythmias and the LVEDP level decreased significantly after hyperoxia. Furthermore, these investigators demonstrated in a separate experiment that pre-exposure to 60 min of hyperoxia (>95% O₂) increases the relaxation of isolated aortic rings in response to acetylcholine (Ach) and sodium nitroprusside (SNP). Studied the delayed effects of hyperoxic preconditioning. Infarct size, release of CK and LDH, and reperfusion-induced arrhythmias such as PVC, VT and VF were investigated. In this study, 95% concentration of oxygen was administered in different durations, 24 hours prior to the ischemia-reperfusion. The most reduction in the infarct size and CK and LDH levels was observed in 120 and 180 minutes durations. Also, significant decrease in the incidence of arrhythmias was observed after oxygen pretreatment. Oxygen pretreatment for 120 minutes was considered as the optimum duration for induction of the late phase of preconditioning and different concentrations of oxygen were tested in this duration. It was evidenced that, the protective effects are not only dependent on the duration of hyperoxia but also to the concentration of it: the infarct size, levels of CK and LDH, and incidence of reperfusion-induced arrhythmias such as PVC and VF, were dramatically reduced with concentration of 95% but not concentrations of 80% [4]. In another study, hyperoxia (95% O₂) for 60 and 180 minutes reduced reperfusion-induced ventricular arrhythmias such as PVC, VT and VF, and also

decreased the infarct size. Cardiac function was also improved. It seems that, there is a relationship between the infarct size and the incidence of arrhythmia. Therefore, one of the strategies in reducing arrhythmias could be infarct size reduction. In this study, the greatest protective effect occurred after 180 min of hyperoxia. Also, in a study by Baharvand et al, 120 minutes of daily oxygen (95%) pretreatment was applied for 1, 2 or 3 consecutive days prior to IR injury in a rat model of regional heart ischemia and was compared to a single 120 minutes of oxygen pre-exposure [5]. The IR injury was induced 24, 48 or 72 after oxygen exposure. In the groups of 24 and 48 hours, hyperoxia was able to significantly reduce infarct size, CK level and ventricular arrhythmias. And the delayed protective window resulted from a single 120 min exposure to oxygen lasts for about 48 hours and not for 72 hours. based on these results the duration of delayed protective window can be long with administration of oxygen for consecutive days. The authors have not clear explanation for this results. Also, the anti-arrhythmic effects of oxygen pretreatment were enhanced by intermittent exposure. A similar improvement or prolongation of protective effects of oxygen pretreatment by intermittent oxygen administration also has been shown in other tissues and against other injuries [6].

Examined the effects of 95% hyperoxia for 60 and 180 min immediately or 24 hours prior to ischemia-reperfusion in rats. They concluded that the infarct size and the release of the cardiac injury biomarkers such as CK and LDH decreased both in early and delayed phases. In this study, there was no significant difference in the incidence of ventricular arrhythmias VT and VF, but the durations of these arrhythmias were less in oxygen pretreated groups than the control group. In another study pretreatment with 95% oxygen for 60 and 180 minutes was performed in both healthy and diabetic rats. It was evidenced that hearts of diabetic rats are less susceptible to ischemia-induced arrhythmias and infarction compared to normal rats. Hyperoxic preconditioning had highly protective effects in hearts of normal rats but failed to provide additional cardio-protective effects in diabetic rats. In another study, the effects of hyperoxia and steroids alone and combination were evaluated in rats. In the first group, dexamethasone was administered prior to ischemia reperfusion; while in the second group, hyperoxia at concentration 95% was administered for one hour immediately; finally, in the combined group dexamethasone and hyperoxia were administered similar to protocol of the first two groups. The results revealed that the infarct size decreased in all groups as compared to the control group. But there was no difference between the combined group and the two other groups. Also, the LVEDP decreased in all groups compared to the control group, but it was more profound in the combined group. Protective effects against cardiac arrhythmia were seen in all three intervention groups and no difference was seen between them. Also, endothelium-dependent relaxation was increased in the combined group compared to the control group used 120 minutes of hyperoxia at a concentration of 95% immediately and 24 hours before ischemia and showed that the infarct size and the ventricular arrhythmias were reduced both in early and delayed pre-treatment groups with hyperoxia. Hyperoxia immediately

preceding ischemia had the most cardioprotective effect. Also, the Bcl2/Bax ratio was increased and reduced the apoptosis [7].

In addition, the infarct area did not increase with increasing reperfusion time, but apoptosis increased with increasing reperfusion time. In a study in morphine-dependent mice, normobaric hyperoxia decreased creatine kinase release and increased mechanical function of the heart but there was no significant difference in the incidence of ventricular arrhythmia [8].

There is a theory that administration of oxygen in the reperfusion phase due to exacerbation of release of free radicals will increase the infarct size. In a study conducted by Mariero and colleagues, hyperoxia during the last 10 min of ischemia and the first 10 min of reperfusion did not increase the infarct size. Also, this theory was not proved in another study on rabbits [9].

Normobaric hyperoxia in clinical setting

The first clinical trial on the protective role of hyperoxia in CABG surgery was investigated by in which a pre-treatment with oxygen for 130 minutes immediately before cardioplegia did not show protective effects. The possible reason for these negative results could be the lack of enough time between the hyperoxia and start of cardiac surgery which is necessary to induce preconditioning pathway. In the second study by the same researchers, pretreatment with normobaric hyperoxia for 60 minutes with suitable interval between hyperoxia and initiation of cardiac surgery did not show protective effects either. The increase in troponin levels was the same in both groups. However, the ratios of IL10/IL6 and IL10/TNF α were increased in hyperoxia group and showed that hyperoxia can have anti-inflammatory effects. In addition Karu et al reported in other study that pre exposure to hyperoxia (Fio₂ .0.4 or 1.0) before cardiopulmonary bypass could improve myocardium contractility in patients who undergoing CABG surgery but release of cardiac biomarkers had no significant difference between the two groups [10].

In a clinical study that conducted on patients undergoing coronary angioplasty, pretreatment with normobaric hyperoxia (twice a day: 12 and 2 hours before angioplasty) failed to prevent the increase in cardiac biomarkers in the oxygen group. However, chest pain in the hyperoxia group was lower than that of the control group. In this study, the balloon inflation time during angioplasty was very short (20 seconds) to induce chest pain in all patients. Therefore, we had to be cautious for concluding that oxygen administration led to the reduction of chest pain in this study with small sample size (32 patients). Based on these human studies, there was no difference in the release of troponin and creatine kinase enzyme in the normobaric hyperoxia compared to control groups and these results are in contrast to animal studies which showed cardiac protection after hyperoxic pretreatment. Why Hyperoxia did not show protective effects in human heart is still unclear. In the interpretation of these results, four theories arise: First, the oxygen administration protocol used in these studies is likely to be insufficient to stimulate intracellular protective pathways of preconditioning in the human heart. Therefore, the oxygen

protocol should be changed in further studies in order to achieve effective results, for example administration of oxygen twice or three time a day with sufficient duration and concentration may lead to better results. The difference in the number of periods and duration of hyperoxia for induction of preconditioning has been shown also in animal studies. The Second theory for explanation of these negative results is that, unlike the mouse and rat hearts, normobaric hyperoxia is not capable to induce protective effects of preconditioning in the human heart. However, more clinical studies are needed to prove this hypothesis. In addition to these two theories the other proposed reason is that CABG surgery is associated with a major oxidative stress and IR injury on the heart and hyperoxic preconditioning like effect is not sufficient to protect heart from this great injury completely. The last theory that was proposed is a weak theory that interpret these results with this reason that nowadays with improve protective technics of cardioplegia arrest in CABG surgery the IRI is minimal. Thus the protective pathway of preconditioning in the heart does not active. But all of studies showed that the CABG surgery despite use of cardioplegia has a major IRI in the heart.

The most common type of administered oxygen is normobaric oxygen as it is easy to use, can be easily accepted by the patients and does not require complicated apparatus. However, the important issue in the pretreatment with hyperoxia is the type of oxygen, the times and periods of hyperoxia and the interval between the administration of oxygen and the onset of ischemia-reperfusion injury. Thus further clinical studies are need and researchers should answer these questions in future clinical studies. In a summary of all animal and human studies on the role of hyperoxia in inducing preconditioning in myocardium are listed.

Clinical application of hyperoxia: harmful or beneficial in acute coronary syndrome?

Oxygen has been used as a medicine in many pulmonary and cardiac diseases, but oxygenation is a double-edged sword as it has advantages as well as side effects for cardiovascular system. Oxygen therapy reduces heart rate and cardiac index in healthy people; while it increases SVR and MAP. Also, the administration of oxygen with concentration 100% in patients with heart failure (function class 3) may lead to a decrease in stroke volume and cardiac output, while increasing the SVR and PCWP. Also, the role of hyperoxia in coronary blood flow has been studied extensively which showed that the coronary blood flow was decreased in both normal and CAD patients. Also, the coronary vascular resistance was increased. Clinical studies have indicated that administration of oxygen in patients with myocardial infarction, with saturation above 90%, does not have beneficial effects and can reduce cardiac output and increase the SVR. Therefore, oxygen should be prescribed only in hypoxic patients.

In a randomized controlled trial conducted by the authors investigated the effect of oxygen therapy in patients with suspected myocardial infarction and showed that the oxygen therapy had no significant beneficial effect on long-term clinical outcome in the test group, as compared to the control group.

According to these findings, one can conclude that oxygen may not render a magic potion for patients with acute myocardial infarction. On the other hand, it sounds that exposure to oxygen for short time with low concentration may not be harmful during acute myocardial infarction. The possible explanation for these results is that the ROS which generated due to oxygen therapy may be superimposed with oxygen free radicals that released during reperfusion phase of myocardial infarction and this collusion leading to myocardial damage. This theory is in contrast to the old theory that oxygen therapy can increase myocardial oxygenation during ischemia due to coronary artery occlusion resulting in smaller infarct size. It seems that sum of these two theory can justify the results of this trial. Besides the results of mentioned study, interestingly, however, hyperoxia can induce preconditioning in the heart, leading to myocardial protection in acute myocardial infarction based on this phenomenon that investigated during two decades, brief episodes of hyperoxia with induces low grade oxidative stress can mimic preconditioning like effect in myocardium. Several experimental studies have demonstrated cardiac preconditioning effect of hyperoxia in rats in terms of reduced infarct size and cardiac arrhythmia due to reperfusion during myocardial infarction in these studies both early and delayed effects of preconditioning confirmed in rat and mouse heart and also different oxygen concentration and exposure time was tested to reached an optimal protocol of oxygen therapy to inducing preconditioning in myocardium. In contrast some clinical trials have rejected beneficial effect of hyperoxia preconditioning on human heart. These clinical trials were performed in patients who undergoing Coronary Artery Bypass Grafting (CABG) and Percutaneous Transluminal Coronary Angioplasty (PTCA). In these studies, the cardiac troponin levels were measured as a hallmark of cardiac injury, but there was no significant difference between release of cardiac troponin in both oxygen and control groups. There are two points to consider in this regard. Firstly, in contrast to rats, humans may experience no preconditioning effect on their hearts upon applying hyperoxia, and secondly, in clinical practice, it is impossible to use oxygen to induce preconditioning because the exact timing of myocardial infarction is unknown, and we cannot administer oxygen at any time prior to the occurrence of myocardial infarction for inducing preconditioning. Thus hyperoxia preconditioning is not applied treatment for acute myocardial infarction. The above discussion arises an important question: is hyperoxia preconditioning reaching a dead end in the field of clinical cardiology? Formulating an exact answer to this question is challenging; clinical trials performed so far have not shown any myocardial protection effect, but the hyperoxia protocols are somewhat limited, supporting the hypothesis that some preconditioning effect may be induced on human heart at some other oxygen concentration and exposure time. If this hypothesis will confirm in future studies, the oxygen can be used in some selective procedures in clinical settings, such as coronary angioplasty, coronary artery bypass surgery and heart transplantation, because we can use of oxygen before to these cardiac procedures. Thus future clinical trials are necessary to explain this ambiguous topic in the field of cardiology (Figure 3).



Figure 3: moderate to severe swelling of submandibular gland after using contrast material during angioplasty.

RESULT AND DISCUSSION

Primary restrictive non hypertrophied cardiomyopathy is the least common type of cardiomyopathy, owing to its rarity diagnosis is challenging .the our case was diagnosed based on careful assessment. Essential feature of this patient was restrictive filling pattern ,based on above data we exclude Amyloidosis, Sarcoidosis, hemochromatosis (3 major causes of RCM) and other rare cause of RCM for instance Idiopathic Diabetic CMP, Scleroderma, Myofibrillar myopathies, Pseudoxanthoma elasticum,Sarcomeric protein disorder, Werners syndrome (appearance of premature aging).for exact diagnosis We had planned to do Right heart catheterization and endomyocardial biopsy, but the patient refused and we lost her in short period due to SCD. However in spite of the biopsy failed and controversy in its exact definition, By excluding other causes of RCM the primary cardiomyopathy was proposed but based on radiological findings We introduced this term for this presentation, the LVEF was difference between echocardiography and cardiac MRI findings and the reason that justify this difference is that in CMR the LVEF is measured by volume and because of taking diuretic and consequently reduced preload, this difference was likely, two reasons justify the reduced LVEF, one of them is pulmonary hypertension and the other one is advanced disease. According to this definition there were two special points in this case .one of them is the extra cardiac involvement was absent and the other one is that she had no family history. However these two points had no role in diagnosis because these are not diagnostic criteria. Based on this case report the authors suggest that cardiologists should be considered this diagnostic term for this presentation.

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