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Minireview

Oxygen and oxygenation in stem-cell therapy for myocardial infarction

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ABSTRACT

Myocardial infarction (MI) is caused by deprivation of oxygen and nutrients to the cardiac tissue due to blockade of coronary artery. It is a major contributor to chronic heart disease, a leading cause of mortality in the modern world. Oxygen is required to meet the constant energy demands for heart contractility, and also plays an important role in the regulation of heart function. However, reoxygenation of the ischemic myocardium upon restoration of blood flow may lead to further injury. Controlled oxygen delivery during reperfusion has been advocated to prevent this consequence. Monitoring the myocardial oxygen concentration would play a vital role in understanding the pathological changes in the ischemic heart following myocardial infarction. During the last two decades, several new techniques have become available to monitor myocardial oxygen concentration *in vivo*. Electron paramagnetic resonance (EPR) oximetry would appear to be the most promising and reliable of these techniques. EPR utilizes crystalline probes which yield a single sharp line, the width of which is highly sensitive to oxygen tension. Decreased oxygen tension results in a sharpening of the EPR spectrum, while an increase results in widening. In our recent studies, we have used EPR oximetry as a valuable tool to monitor myocardial oxygenation for several applications like ischemia–reperfusion injury, stem-cell therapy and hyperbaric oxygen therapy. The results obtained from these studies have demonstrated the importance of tissue oxygen in the application of stem-cell therapy to treat ischemic heart tissues. These results have been summarized in this review article.

Contents

Introduction

Oxygen is hailed as "…the elixir of life — a wonder tonic, a cure for ageing, a beauty treatment and potent medical therapy" (Lane 2002). Oxygen plays a principal role in aerobic respiration. In aerobic organisms, cellular respiration involves enzyme-catalyzed oxidation of fuel substrates, primarily by oxygen, to yield the energy required for biological processes. The physiological homeostasis of these organisms is strictly maintained by optimal cellular and tissue oxygenation status through complex oxygen-sensing mechanisms, signaling cascades, and transport processes. In the event of fluctuating oxygen levels leading to either an increase (hyperoxia) or decrease (hypoxia) in cellular oxygen, the organism faces a crisis involving depletion of energy reserves, altered cell-signaling cascades, oxidative reactions/events, and cell death or tissue damage. Particularly, hypoxia can lead to serious disorders such as ischemic stroke or myocardial infarction (MI). For example, during myocardial ischemia which occurs when a region of the heart is deprived

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of blood supply due to a blockage of a coronary artery, the region becomes oxygen-limited, leading to myocardial cell death. If left untreated, MI will lead to congestive heart failure, which remains a major cause of morbidity and mortality in the world.

Despite the recent advances in reperfusion strategies and medical treatment, MI and its consequences remain a serious clinical concern. The prolonged imbalance between the supply and demand for oxygen and nutrients by the myocardial tissue leads to a sub-acute or chronic state of myocardial ischemia, resulting in a new state of equilibrium. Chronic ischemia contributes to poor quality of life in many patients suffering from coronary heart disease (Thom et al. 2006b) and is an increasingly important aspect of clinical practice. In chronically ischemic myocardium, early revascularization may interrupt a vicious cycle of cardiomyocyte degradation that would otherwise lead to progressive fibrosis and/or contractile dysfunction.

Stem-cell therapy for treating myocardial infarction

The use of cell therapy to promote myocardial repair has gained profound scientific and public interest. The first study on cell therapy using intracoronary mononuclear bone-marrow cells (mBMC) after MI in humans was reported in 2002 (Strauer et al. 2002). Even though several randomized, controlled trials are using the same method of cell delivery (Martin-Rendon et al. 2008a; Martin-Rendon et al. 2008b), the primary endpoint has generally been associated with an increase in left-ventricular (LV) ejection fraction (EF) after 3 to 6 months. Feasibility of this treatment has been confirmed, and it appears to be safe in the short term. However, the efficacy of this approach varies between studies. The possible explanations for these differences could be related to limited study samples, selection of measurement techniques and disparity in timing of applied treatment. Moreover, the number of cells used for treatment along with the selection of placebo treatment or protocols for cell processing and expansion could greatly contribute to differences in clinical outcome of performed studies (Arnesen et al. 2007; Egeland and Brinchmann 2007a,b; Seeger et al. 2007; van Beem et al. 2008).

Studies have shown that the grafting of skeletal myoblasts, fetal cardiomyocytes, or embryonic or bone-marrow-derived stem cells into myocardial scar tissue has resulted in marginal improvements in cardiac function and in the attennuation of abnormal cardiac remodeling (Agbulut et al. 2004; Chiu et al. 1995; Ghostine et al. 2002; Kamihata et al. 2001; Orlic et al. 2001a,b; Pagani et al. 2003; Retuerto et al. 2004; Tse et al. 2003). The reason for such modest improvements was attributed to the limited survival of the transplanted cells in the infarcted myocardium (McConnell et al. 2005). The hypovascular nature of the infarcted tissue may severely compromise the availability of oxygen, nutrients, and growth factors essential for the survival, engraftment and differentiation of the transplanted cells. The local hypoxic environment in the infarcted myocardium might be the main impediment to the survival of the transplanted cells. However, it is not clear whether the oxygen concentration in the ischemic myocardium (infarcted area) is altered by strategies leading to regional angiogenesis and/or by cell transplantation. It is also unknown whether there is a direct relationship between local oxygen concentration and transplanted cell survival.

Role of oxygen in stem-cell therapy

Oxygen tension plays an important role in the growth of stem cells in culture and significantly influences their expansion and differentiation (Ezashi et al. 2005; Ma et al. 2009; Salim et al. 2004; Wang et al. 2005). In response to acute hypoxia, cardiomyocytes have been shown to exhibit adaptations that may facilitate cell survival and develop tolerance to subsequent acute severe hypoxia (Silverman et al. 1997). However, the levels and roles of oxygen concentration at the sites of transplantation in the heart have not been investigated. This is primarily due to the significant technical challenges in obtaining reliable and repeated measurements of oxygen tension in a functional, beating organ during and after stem-cell therapy.

A number of methods are available to obtain measurements of tissue oxygen concentration *in vivo* (Heidt et al. 2009; Mik et al. 2009; Swartz and Clarkson 1998; Vogt et al. 2009). Of these, electron paramagnetic resonance (EPR) oximetry would appear to be the most promising to obtain reliable, accurate, and repeated measurements over time (Ahmad and Kuppusamy 2010). Our group used EPR oximetry to measure myocardial pO_2 in rodent (murine and rat) hearts under a variety of pathophysiological and therapeutic conditions, including ischemia, reperfusion, pharmacological intervention, and cell therapy. The results have shown significant variations and importance of oxygen tension during cardiac damage and repair.

Most recent studies on stem-cell therapy for MI utilizing mesenchymal stem cells (MSCs) suggest that understanding the importance of the tissue micro-environment and how it may be manipulated is critical to realize the effective therapeutic potential of these cells (Chen et al. 2007; Djouad et al. 2007). The most recent study from our laboratory had shown that oxygen concentration is one of the vital components within the micro-environment (Chacko et al. 2009). Oxygen plays a significant role in the control and regulation of many physiological, metabolic, and signaling pathways involved in cellular engraftment and host-tissue regeneration (Chacko et al. 2009). It is also known that low oxygen tension is involved in holding the stem cells in a quiescent state in which they retain their plasticity (D'Ippolito et al. 2006).

A study by Rochefort et al. (2006) has shown that MSCs are regularly observed in the circulating blood of rats and that the circulating pool of MSCs is consistently and substantially increased (15-fold) in animals exposed to chronic hypoxia. In several *in vitro* studies, low oxygen concentrations have been shown to stimulate the differentiation of MSCs into an adipocyte-like phenotype (Fink et al. 2004; Lennon et al. 2001; Ren et al. 2006). Other researchers have reported suppressive effects of reduced oxygen tension on MSC plasticity (Fehrer et al. 2007; Potier et al. 2007). In this article we have summarized our findings that demonstrate the dynamics of oxygen concentration during pathophysiological changes leading to MI, as well as modification of regional cardiac tissue oxygenation during cell therapy.

EPR oximetry for monitoring myocardial oxygenation

Although several methods are used to measure oxygen concentration, a suitable technique for noninvasive, repeated, and reliable measurements of oxygen in the same tissue over time was previously not available. The methods which are commonly available to measure tissue oxygen concentration include: Clarke electrode, NADH fluorescence, phosphorescence-quenching, myoglobin saturation, and ^1H NMR. EPR spectroscopy, a technique commonly used for direct detection of free radicals and paramagnetic species, has recently been adapted to reliably and accurately quantify the concentration of molecular oxygen in viable cells and tissues (Ahmad and Kuppusamy 2010; Swartz and Clarkson 1998). EPR oximetry refers to the measurement of the partial pressure of oxygen (pO_2) by EPR spectroscopy. The principle of EPR oximetry is based on the paramagnetic characteristics of molecular oxygen, which in its ground state has two unpaired electrons, and undergoes spin exchange interaction with the paramagnetic EPR spin probe. This process is sensitive to oxygen content, with the relaxation rate of the spin probe increasing as a function of oxygen content (concentration/pressure). This increased spin–spin relaxation rate results in increased line broadening that is directly proportional to the oxygen content (Pandian et al. 2003).

EPR oximetry requires the implantation of an oxygen-sensing paramagnetic spin probe into the tissue of interest. The particulate probes sense and report pO_2 in the tissue *milieu*. The crystalline form of lithium octa-*n*-butoxy-substituted naphthalocyanine radical (LiNc–BuO) is a particulate oximetry spin probe that we have recently synthesized and validated for *in vivo* oximetry (Chacko et al. 2009; Pandian et al. 2003; Wisel et al. 2007). The LiNc–BuO crystals are composed of stacks of neutral radicals of lithiated naphthalocyanine macrocycles (Pandian et al. 2006). The EPR spectra of these particulates are characterized by a single and very narrow absorption peak due to the strong exchange coupling between the unpaired electrons within the molecular stack. The peak-to-peak linewidth of the EPR spectrum obtained is used to calculate pO_2 using a standard calibration curve (Pandian et al. 2003). The probe, in the form of submicron-sized (270 ± 120 nm) crystals (hereafter referred to as OxySpin), can be internalized in cells without compromising their function (Chacko et al. 2009; Khan et al. 2007; Wisel et al. 2007). Unique advantages of OxySpin probes are that they are retained in cells/tissues for substantially long periods of time, and do not alter the differentiation capability of the stem cells (Chacko et al. 2009), thus enabling continuous monitoring of myocardial pO_2 *in vivo* for weeks after stem-cell transplantation in a mouse heart. Fig. 1 shows some typical EPR spectra obtained from murine hearts receiving OxySpin-labeled stem-cell transplants. The results demonstrated that high quality EPR spectral data could be obtained from beating mouse hearts after several days or weeks after transplantation.

Despite being a unique and powerful method for noninvasive monitoring of oxygen concentration in the heart, EPR oximetry has certain limitations. A major limitation is that the method, at present, is applicable only to measurements in the hearts of small animals such as mice and rats. In larger animals, motional (contractile/respiratory) artifacts and loss of sensitivity due to increased depth from the chest wall are major concerns. Further advances in the EPR instrumentation would be required to perform the measurements in large animals, including humans.

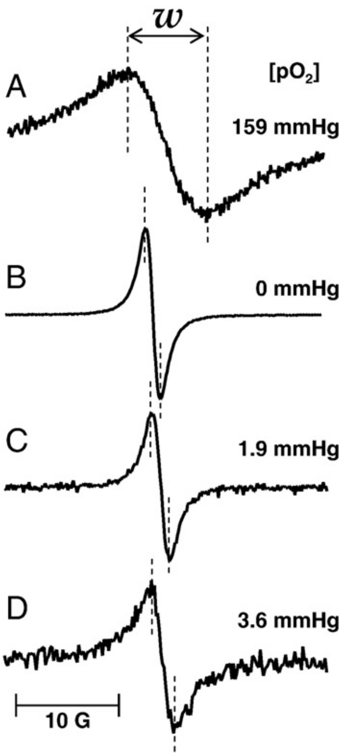

Fig. 1. Representative EPR spectra obtained from OxySpin in the heart. EPR spectra of a suspension of OxySpin in PBS equilibrated with room air (A) and 100% nitrogen (B). EPR spectra obtained from an infarcted mouse heart, *in vivo*, 1 day (C) or 2 weeks (D) after implantation of OxySpin-labeled skeletal myoblast (SM) cells. The peak–peak width (w) of the spectrum is a measure of oxygen concentration. The measured pO_2 values are noted on the respective spectrum.

Myocardial oxygenation under conditions of prolonged ischemia (PI) and stem-cell treatment

The myocardial pO_2 measurements were performed using an *in vivo* EPR spectrometer (Magnettech; Berlin, Germany) equipped with automatic coupling and tuning controls for measurements in beating hearts. Microcrystals of LiNc–BuO were used as a probe for EPR oximetry. Mice and rats, under inhalation anesthesia (air mixed with 1.5–2% isoflurane), were implanted with OxySpin probes in the left-ventricular, mid-myocardium. We have previously reported that the implantation of OxySpin probe is non-toxic to the heart and long-term myocardial pO_2 measurements could be performed in the same animal for up to 3 months (Fig. 2) and possibly longer (Khan et al. 2009a). Hence, EPR oximetry has several significant advantages over other techniques for *in vivo* applications.

In our earlier reports, we have shown that the ischemic region of a mouse heart was significantly hypoxic for 4 weeks after the induction of myocardial ischemia by permanent LAD ligation (Khan et al. 2007). Furthermore, there was no significant change in tissue pO_2 during the development of LV remodeling. Transplantation of skeletal myoblast (SM) cells in the ischemic region resulted in a significant increase in pO_2 compared to untreated ischemic tissue (Fig. 3). Therefore, it is inferred that the transplanted SM cells may be responsible for the augmented myocardial pO_2 in the infarcted heart. Overall, we observed an increase of ~2 mmHg, which corresponds to an increase of ~2.5 μM of steady-state concentration of oxygen at the site of stem-cell therapy. Thus the magnitude of increase in oxygenation, *albeit* small, may still be sufficient for the survival of cells in the infarcted region (Khan et al. 2007). Therefore, it is imperative to investigate newer strategies that would enhance myocardial oxygenation and to study whether it improves the survival and engraftment of transplanted stem cells.

Myocardial oxygenation under conditions of ischemia–reperfusion

The most common cause of MI is narrowing of the epicardial blood vessels due to atheromatous plaques. Plaque ruptures, with subsequent exposure of the basement membrane, result in platelet aggregation, thrombus formation, fibrin accumulation, and hemorrhage into the plaque, followed by varying degrees of vasospasm. This can result in partial or complete occlusion of the vessel and subsequent myocardial ischemia. Total occlusion of the vessel for more than 4–6 h results in irreversible myocardial necrosis, but reperfusion within this period can salvage the myocardium and reduce morbidity and mortality. Monitoring the myocardial tissue oxygenation may play a vital role in

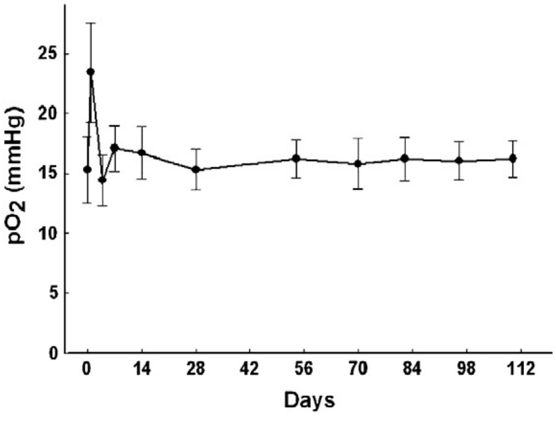

Fig. 2. Long-term monitoring of myocardial pO_2 values in beating hearts. The measurements were performed repeatedly for 16 weeks using *in vivo* EPR oximetry in murine hearts implanted with microcrystals of LiNc–BuO (OxySpin) probe in the mid-ventricular region without LAD coronary artery ligation. Data represent mean ± SD; ($n = 6$). The results show the feasibility of pO_2 measurements for more than 3 months in the beating hearts of mice.

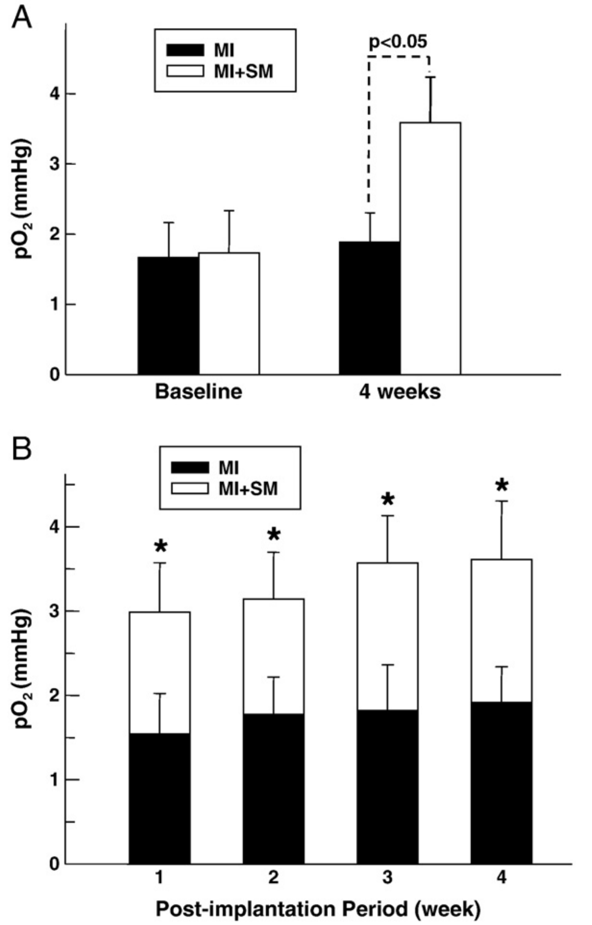

Fig. 3. Myocardial pO_2 in the infarcted heart at the site of cell transplantation. Myocardial pO_2 values were measured repeatedly for 4 weeks using *in vivo* EPR oximetry in murine hearts transplanted with OxySpin-labeled SM cells. (A) Tissue pO_2 at 4 weeks after treatment with SM cells (MI + SM) was significantly higher when compared to untreated (MI) hearts. (B) The time-course values of myocardial pO_2 measured from infarcted hearts (MI), and infarcted hearts treated with SM cells (MI + SM) are shown. Values are expressed as mean ± SD; ($n = 7$). $^*p < 0.05$ versus MI group.

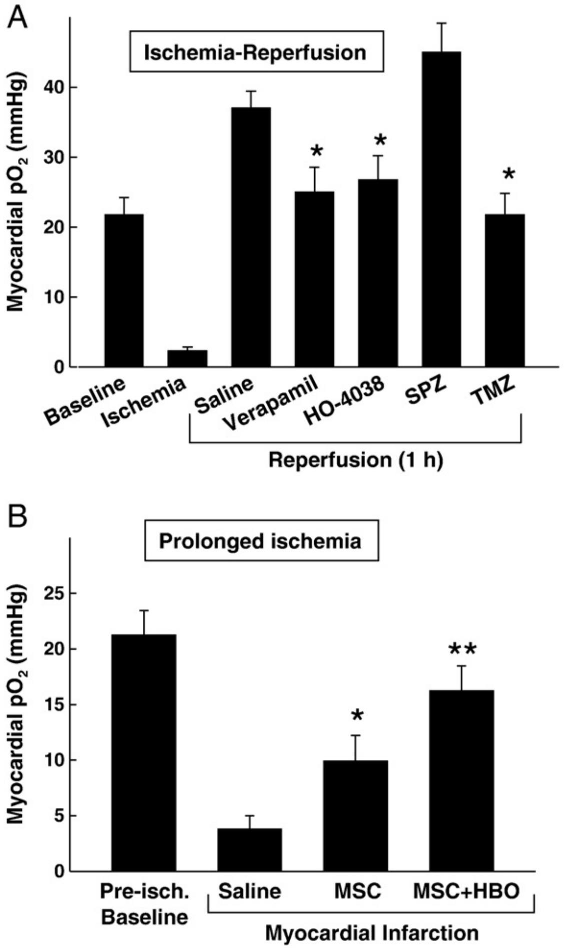

Fig. 4. Values of pO_2 in rat hearts under conditions of ischemia–reperfusion and stem-cell therapy for myocardial infarction. (A) Effect of pretreatment of rats with verapamil (VER), HO-4038, SPZ, and TMZ on myocardial pO_2 during regional ischemia followed by reperfusion. Myocardial tissue pO_2 values were measured by EPR oximetry at 30 min of ischemia, followed by at 1 h of reperfusion. $^*p < 0.05$ versus saline group; $n = 6$. (B) Measurement of myocardial pO_2 in rat hearts transplanted with mesenchymal stem cells (MSC) at 2 weeks. The results show an increase in myocardial oxygenation levels in hearts treated with MSC and further enhancement in pO_2 was observed in hearts treated in conjunction with HBO and MSCs The "baseline" data were obtained from hearts before induction of ischemia. Data represent mean ± SD; $n = 6$. $^*p < 0.05$ versus Saline group. $^{**}p < 0.05$ versus MSC group.

understanding the pathophysiology of oxygen changes that occur as a result of ischemia–reperfusion (I/R) injury in the heart, and it may serve as an important indicator to monitor the pathophysiology of several cardiovascular drugs that are administered following an acute myocardial infarction.

Recently, we have published several reports on pharmacological pre- and post-conditioning of rat hearts using various treatment remedies like verapamil (VER), HO-4038 (a verapamil derivative), sulfaphenazole (SPZ) and trimetazidine (TMZ) (Khan et al. 2010; Khan et al. 2009b; Mohan et al. 2009). In all groups of hearts, the baseline myocardial pO_2 was about 20 mmHg, which dropped to 2–4 mmHg upon induction of regional ischemia by ligation of the left-anterior-descending (LAD) coronary artery (Fig. 4A). After 30 min of ligation, blood flow was restored (reperfusion) by release of the ligation. Immediately upon reperfusion, there was a substantial hyperoxygenation in the untreated (saline only) I/R control group (Fig. 4A). The myocardial oxygenation remained significantly elevated even after 48 h of reperfusion (Khan et al. 2010). The hyperoxygenation may be due to contractile "stunning", which is a reversible loss of contractlity known to occur immediately upon reperfusion and last for several hours to days (Bolli and Marban 1999). During this time the myocardium receives adequate oxygen supply, but it does not fully utilize the oxygen because of depressed contractility (myocardial work) which is not immediately restored to preischemic levels. This condition may lead to a paradoxical hyperoxia due to the lack of oxygen utilization. Ambrosio et al. have observed an overshoot of cardiac phosphocreatine concentration in rabbit hearts upon post-ischemic reflow (Ambrosio et al. 1987). This overshoot effect was attributed to a decrease in phosphocreatine utilization leading to an imbalance between supply and rate of utilization of the high energy phosphate metabolic reserve in the "stunned" heart (Ambrosio et al. 1987). Similarly, in yet another study the marked hyperoxygenation was attributed to decreased oxygen consumption due to NO-mediated inhibition of mitochondrial respiration (Zhao et al. 2005).

In studies conducted by our laboratory, this reperfusion-induced hyperoxygenation was significantly attenuated in verapamil, HO-4038, and TMZ-treated groups when compared to untreated I/R controls (Fig. 4A). The reduction in hyperoxygenation observed in the treated groups may be attributed to increase in oxygen demand due to improved recovery of cardiac function, as much as 3-fold increase in RPP (Rate-Pressure Product) in the HO-4038 group compared to control (Mohan et al. 2009). On the other hand, pretreatment of rats with SPZ showed a substantial hyperoxygenation at 1-h reperfusion (Fig. 4A), which was attributed to enhanced NO levels that may promote increased blood flow upon reperfusion (Khan et al. 2009b). The marked hyperoxygenation could also occur as a result of decreased oxygen consumption due to NO-mediated inhibition of mitochondrial respiration (Khan et al. 2009b). In a recent study we observed that TMZ ameliorated the I/R-induced oxygen overshoot (Khan et al. 2010). The absence of hyperoxygenation (Fig. 4A) at reperfusion in the TMZ-treated hearts could be associated with an increased recovery of contractility and attenuation of myocardial injury. Also, the hyperoxygenation at reperfusion might indicate a decrease in oxygen consumption by the injured tissue.

Effect of post-administration of hyperoxygenation on stem-cell treatment for myocardial infarction

Hyperbaric oxygenation (HBO) is a safe, clinically-viable treatment that has been used as a primary therapy in patients with decompression sickness, arterial gas embolism and carbon monoxide poisoning (Tibbles and Edelsberg 1996). It is also used as an adjuvant therapy to promote wound healing (Thackham et al. 2008), and for the treatment of various conditions, including ischemic injury (Yogaratnam et al. 2006). HBO involves inhalation of 100% oxygen under greater-than-one atmospheric absolute (ATA) pressure. Such doses of oxygen have a number of beneficial biochemical, cellular, and physiologic effects (Yogaratnam et al. 2007). HBO, administered at the onset of reperfusion in an open-chest rabbit model of myocardial ischemia–reperfusion injury, showed a significant reduction in infarct size (Sterling et al. 1993). More recent studies have also shown that HBO attenuates myocardial injury *via* nitric oxide signaling (Yogaratnam et al. 2007), improves cardiac function in patients with acute myocardial infarction (Dekleva et al. 2004), and helps mobilization of stem cells by stimulating nitric oxide synthesis (Thom et al. 2006a) and enhancing CXCR4 and VEGFR-2 in humans (Thom et al. 2006a). However, until recently, the efficacy of HBO as an adjuvant to cell therapy had not yet been studied.

We used periods of HBO exposure in combination with myocardial stem-cell therapy to treat rat hearts subjected to MI. HBO exposure (100% O_2, 2 ATA, 90 min daily for 2 was weeks) started three days after cell transplantation to allow the animals recover from surgical trauma. Administration of HBO, in conjunction with MSCs, increased tissue oxygenation in the infarct heart as measured by EPR spectroscopy (Khan et al. 2009a). *In vivo* myocardial pO_2 measurements were obtained at baseline and at the end of 2 weeks in all groups, including the rats that were receiving stem-cell therapy applied to the infarct heart. The mean baseline pO_2 in healthy hearts prior to simulated MI was 21.25 ± 2.0 mmHg (Fig. 4B). The myocardial pO_2 in hearts receiving MSC transplants was significantly improved when compared to untreated MI hearts (9.8 ± 2.3 versus 3.8 ± 1.2 mmHg, respectively). An additional group given adjuvant HBO therapy after receiving MSC transplants to the MI heart had further significant ($p<0.05$) enhancement in myocardial oxygenation when compared to the MSC-alone treated group (16.2 ± 2.2 versus 9.8 ± 2.3, respectively) (Fig. 4B). The pO_2 data indicated that HBO therapy further increased myocardial oxygenation values to near-normal in infarct rat hearts treated with transplanted stem cells. Most importantly, this study showed that HBO treatment lead to increased stem-cell engraftment and improved cardiac function (Khan et al. 2009a).

EPR spectroscopy is a unique technique by which we can monitor non-invasively the myocardial oxygenation in the infarct heart. Our lab has previously published long-term monitoring of myocardial oxygenation, for up to 3 months, following OxySpin implantation in a healthy mouse heart (Khan et al. 2008). As mentioned earlier in our previous reports with skeletal myoblast transplantation (Khan et al. 2007), there was a marginal improvement in myocardial oxygenation. However, the combined treatment of stem cells along with hyperbaric oxygen (HBO) therapy showed a significant enhancement in myocardial oxygenation and functional recovery in the infarct heart (Khan et al. 2009a). The oximetry studies clearly demonstrated the role of oxygen in myocardial infarction and its importance in the treatment of cardiac repair by stem-cell therapy.

Overall, we have demonstrated the use of EPR oximetry for noninvasive monitoring of local tissue oxygenation in hearts treated with stem cells. The results clearly show that tissue oxygenation is an important factor for the survival and engraftment of stem cells in the heart.

Conflict of interest statement

The authors do not have any conflict of interest.

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