



A post-surgical adjunctive hypoxic therapy for myocardial infarction: Initiate endogenous cardiomyocyte proliferation in adults

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ABSTRACT

Myocardial infarction (MI) is a major threat to health worldwide, but today's methods for recovering heart function are limited, which is due largely to the deficient proliferative capacity of adult cardiomyocytes in the human body. To successfully overwhelm this deficiency, we propose a promising hypoxic therapy and highlight its unique role in directly eliciting endogenous myocardial regeneration *in vivo*. In the hypothesis, sufficient oxygen could be a restrictive factor of myocardial growth, whereas a moderate hypoxia might stimulate cardiomyocyte proliferation and enhance myocardial regeneration, heart weight and cardiac function recovery. The potential involvements of the hypoxia inducible factor-1 (HIF-1) and its downstream oncogenic signalings were hypothesized and evaluated in detail. Notably, the hypoxic treatment may initiate spontaneous proliferation of pre-existing cardiomyocytes in adult human body, which cannot (or hardly) be achieved by current MI-therapeutic approaches such as cardiovascular drugs, cardiac surgeries and aerobic exercise. The hypoxic therapy will lead to lower surgical risks compared with tissue regeneration *in vitro* and putative cardiac transplantation. With optimized moderately-low oxygen concentration, available therapeutic frequency and cycles, and controllable side effects, the hypoxic therapy will be a non-invasive, non-surgical, low-cost and low-risk approach to promoting myocardial regeneration *in vivo* and recovering cardiac function for MI patients who have large-area myocardial necrosis, in addition to other current MI-therapeutic methodologies.

Backgrounds

Many individuals are facing the risks of atherosclerosis, cardiac ischemia and myocardial infarction (MI). As we know, blood lipids, cholesterol, platelet aggregation, radiation, smoking and drinking are some risk factors that may lead to MI [1–3]. Recently, a lot of recent studies developed prophylactic drugs [3], immunological and microRNA biomarkers [1,4], vasoactive medicines [5], gene therapy [6], carbon monoxide-releasing molecules [7], nitric oxide regulators and Pin1 inhibitors [8–10], etc., putting forward the mechanisms, prevention and treatment of MI.

However, MI still represents the major cause of cardiovascular morbidity and mortality, despite all our efforts to develop novel

medicines and interventional procedures [11–14]. Some key issues are still uncertain and make it difficult to cure MI. Most importantly, although coronary artery stenting and bypass-grafting surgeries are of great significance for the treatment of MI, many patients are substantially troubled by insufficient cardiac function recovery after surgery in clinic.

Interestingly, recent studies gradually show that the heart can adapt to stresses including ischemic stress and reperfusion, oxidative stress and continuous high-intensity exercise, and these oxygen-related stresses influence the *in-vivo* expression of some genes such as thioredoxin-1 (Trx1) [15–19]. As a result, brief and repeated ischemic episodes enhance myocardial tolerance to subsequent ischemic events, which to some extent reflects the putative genetic plasticity of

Abbreviations: MI, myocardial infarction; HIF-1, hypoxia inducible factor-1; Trx1, thioredoxin-1

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cardiomyocytes in response to environmental stimuli [15,16,19,20]. In view of these noteworthy cardiac physiological phenomenon, we herein intend to focus on another key oxygen-regulated molecule, the hypoxia inducible factor-1 (HIF-1), and highlight a non-surgical and low-risk therapeutic approach potentially improving cardiac function after MI and relevant surgeries.

The hypothesis

MI patients need to recover cardiac function efficiently during convalescence, unfortunately however, the self-renewal of cardiomyocytes in adults is too deficient to improve heart function. Thus, we hypothesize:

1. A hypoxic circumstance might stimulate the *in-vivo* proliferation of adult cardiomyocytes, which is usually inhibited by sufficient oxygen;
2. The underlying molecular mechanism may be attributed to the hypoxia-induced activation of HIF-1 and subsequent HIF-1-induced downstream oncogenic signalings;
3. A moderate hypoxic treatment might be valuable for cardiac function recovery of MI patients with myocardial necrosis (e.g. Type 1 MI, Type 4a MI, MI with ST-segment elevation and/or new Q waves), especially being used as an adjuvant method in addition to current standard approaches in order to prevent heart failure and cardiac death.

Evaluation of the hypothesis

Does oxygen have a dual role in the heart?

On the one hand, the first key issue is to underline whether oxygen has double effects in heart tissues. What we all know is the fact that adequate oxygen is essential for the health of cardiomyocytes. Oppositely, inadequate oxygen, hypoxia, and myocardial ischemia are detrimental to cardiac tissues, so MI will occur shortly after oxygen supply stops. Curiously however, could insufficient oxygen bring any clinical benefit to the cardiovascular system?

On the other hand, as an innovative therapeutic strategy, cardiac tissue regeneration *in vitro* has been suggested by many studies, aiming to transplant vessels and tissues (even an artificial heart) into MI patients [21–23]. For example, we demonstrated the potential of three-dimensional bio-printing to fabricate cardiovascular tissues *in vitro* [21], and another study highlighted a series of novel scaffolds for myocardial regeneration *in vitro* [24].

Hence, an interesting question arises: Is there a reasonable link between oxygen concentration and myocardial regeneration *in vivo*? In the next, we will list the recent relevant literature and provide clues about why hypoxia might promote endogenous and spontaneous heart function recovery after MI.

Hypoxia potentially stimulates endogenous cardiomyocyte proliferation in vivo

The following are recent studies published from 2017 to 2018, and they cooperatively indicated that a moderate hypoxia has the potential to inspire spontaneous myocardial renewal in animal bodies.

1. In a recent article of *Nature*, Nakada et al. discovered that the hypoxia of ~7% oxygen for 2 weeks is an effective way of improving cardiomyocyte proliferation, myocardial repair, heart weight and systolic function in the mice with heart injury [25]. The mild hypoxia of 10% oxygen was less effective than 7% oxygen, but excessively low oxygen would increase the mortality of animals [25].
2. In the neonatal period after a fetus leaves the hypoxic uterine environment, increased atmospheric oxygen induced cell cycle arrest and inhibited proliferation of valve interstitial cells, but the experiments shown by Amofa et al. verified that chronic hypoxia up-regulates the extracellular glycosaminoglycan matrix in both chicken embryo aortic valves and adult murine heart valves, supporting the stimulative and indispensable role of hypoxia (but not adequate oxygen) in cardiac cell proliferation and heart tissue development [26].
3. According to the studies on neonatal mouse hearts shown by Lalowski et al., the glycolysis pathway that manifests hypoxia was very active in 1-day-old neonatal mice, so strikingly these mice possessed a very strong capability to repair heart injury [27]. But later the hypoxia-based glycolysis metabolism gradually changes to oxidative phosphorylation, which manifests sufficient oxygen utilization, in about 7 days after birth, and then the regenerative capacity of mouse hearts disappeared gradually [27]. More interestingly, they also demonstrated that the *in-vitro* hypoxic culture retains the proliferative ability of the cardiomyocytes isolated from fetal mice [27]. These findings pinpoint that hypoxia may be such an important, necessary and even mandatory physiological condition for heart regeneration in injured animals.
4. As indirect evidence, a recent study of *PNAS* by Ferrari et al. showed that hypoxia (~11% oxygen) improves the neuronal regeneration and prolongs the life-span of some neurodegenerative mice [28].

Taken together, these descriptions primarily support the proliferation-stimulating role of hypoxia in the heart tissues of neonatal and adult animals, predictably putting forward a potential hypoxic treatment for heart regeneration of cardiac patients.

HIF-1-induced oncogenic signalings may act as cardiomyocyte-proliferative effectors

The mechanisms underlying hypoxia’s novel function have not been clarified evidently. As illustrated in Fig. 1, up-regulated HIF-1 might play a key role in these processes [25]. HIF-1 consists of a stable HIF-1β

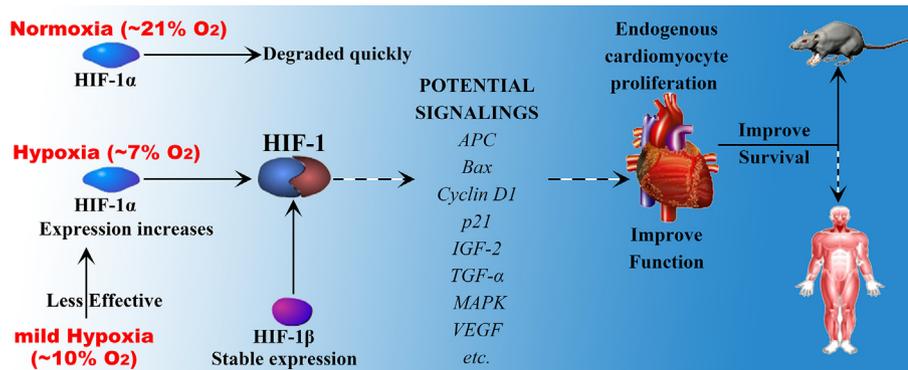


Fig. 1. Hypoxia, HIF-1, and the related proliferative signalings may elicit a non-surgical, low-cost, and convenient hypoxic therapeutic approach to curing myocardial infarction. Hypoxia-induced HIF-1 activates kinds of oncogenic and proliferative signaling molecules, and they may promote proliferation of pre-existing cardiomyocytes in mice and possibly in humans.

subunit and an unstable HIF-1 α subunit, and HIF-1 α degrades very fast under normoxia (21% oxygen) but increases significantly under hypoxia.

To our knowledge, HIF-1 is overexpressed in many human tumors and can activate various genes related to cell proliferation, including *APC*, *Bax*, *Cyclin D1*, *p21*, *IGF-2*, *TGF- α* , *MAPK*, *VEGF*, etc. (Fig. 1), and in theory these molecules may stimulate cardiomyocyte proliferation and promote heart tissue growth under hypoxic circumstances [29–33]. By contrast, normal oxygen concentration (~21% oxygen) suppresses the level of HIF-1 α and results in a lower production of HIF-1, hypothetically hindering cardiomyocyte renewal in adults [25,29].

In accordance with the hypothesis, an article of *Nature Communications* in 2018 proved that a high-intensity running exercise can increase cardiomyocyte regeneration in adult mice [34], which may be partially attributed to temporary hypoxia, glycolysis pathway, and moderate activation of HIF-1-related signalings during the intensive movement periods [17,34]. Moreover, also in 2018, Nguyen et al. found that HIF-1 and its down-stream substrates cyclin D1, ERKs and Akt (some of them were included in Fig. 1) enable quiescent articular cartilage cells to re-enter cell cycle and re-proliferate, but the HIF-1 inhibitor acriflavine attenuated these effects substantially [30].

The above descriptions, directly or indirectly, support the hypothetical roles of HIF-1 and HIF-1-regulated oncogenic pathways in cardiomyocyte re-proliferation very well, enlightening that HIF-1 may play a key part in the predicted hypoxic treatment for MI.

Clinical significances of the hypoxic therapy versus other therapeutic strategies

Generally speaking, the hypoxic therapy is an unique strategy different from other methods, and its potential superiorities over other relevant therapeutic approaches may involve the following respects:

Hypoxic therapy VS. cardiovascular drugs

A lot of vasoactive and antithrombotic drugs are useful for decreasing the risk of coronary atherosclerosis, but some drugs are expensive and are associated with side effects, bleeding, organ injury and even increased mortality [35–39]. Besides, current cardiovascular drugs cannot effectively reverse cardiomyocyte loss and cardiomyopathy defects [6]. Relatively, the hypoxic therapy seems to be a convenient and economic method which directly promotes cardiomyocyte re-proliferation *in vivo* and subsequently improves myocardial function.

Hypoxic therapy VS. cardiovascular surgeries

Coronary stenting, bypassing and angioplasty surgeries are frequently applied to acute MI patients, but later the cardiac deficiency necessitates effective post-surgical adjunctive approaches. Several adjuvants such as esmolol, interleukin-18 and glutathione sodium salt were recently evaluated to improve the outcomes of cardiac surgeries by reducing cardiac troponin release and by regulating endothelial function [40–44]. For similar purposes, although the hypoxic therapy cannot substitute cardiac surgeries, but it could be a new clinical-assisting approach for postoperative cardiac function recovery. In fact, these methodologies can compensate each other because they target and repair different functional aspects of the cardiovascular system.

Hypoxic therapy VS. cardiac tissue regeneration in vitro

To date, a series of *in-vitro* studies have made substantial progression towards cardiac regeneration, including ventricle-remodeling tissue regeneration, stem cell-based cardiac regeneration, three-dimensional cardiac printing with myocardial cells, and so on [21–23,45–48]. However, these methods are still facing additional technical difficulties, noteworthy high economic costs, and potential life-threatening risk of organ transplantation. In our opinion, it is very attractive that the hypoxic therapy may provoke myocardial regeneration and renewal directly *in vivo* with less technical problems,

minor financial charges and almost no risk of organ transplantation.

Hypoxic therapy VS. aerobic exercise

Owing to oxygen's positive effects, aerobic exercise shows some beneficial efficacy for MI patients. For instance, aerobic exercise shields the transportation mechanism of mitochondria, constrains the apoptosis of myocardial cells, and prevents myocardial fibrosis [49–51]. Nevertheless, it seems likely that aerobic exercise protects myocardial tissues from continuous deterioration, but it cannot further ameliorate heart function by adding new myocardial cells, which in turn is expected to be the positive function of the hypoxic therapy that we are discussing here. Therefore, keeping a balance between aerobic exercise and hypoxic therapy is worthy of future exploration, in order to bring their clinical benefits into full play and make full use of their specific superiorities, respectively.

Testing the hypothesis

In the above discussions, the hypoxic treatment appears promising as a therapeutic strategy for recovery of function of post-infarct myocardial areas by eliciting proliferation of viable cardiomyocytes. Hypoxia might have a considerable therapeutic potential as a simple and effective treatment of left ventricular dysfunction, but there are several evident challenges in translating hypoxia, as a treatment option, into the clinical settings. Accordingly, the following dilemmas deserve to be further resolved step by step.

Cellular testing

To better understand the impact of hypoxia on myocyte viability, the hypoxic protocol should be precisely designed and tested to identify a series of critical characteristics (intensity, duration, frequency, etc.). The cardiomyocyte-culturing parameters, for instance, 5%–12% oxygen for 2 to 15 days, should be effective to up-regulate the HIF-1 signaling pathways in hypoxic episodes. The up-regulation of HIF-1 and down-stream genes should be determined by means of ELISA, Western blotting, real-time quantitative PCR, etc., and re-activated myocyte proliferation would be confirmed by cellular experimental assays.

Animal testing

The cardio-protective and therapeutic effects of hypoxia on myocardial infarction are attractive, but clinical translation may be difficult due to inadequate ischemic/reperfusion animal models to simulate human patients. Thus, available animal models, not only mice and rats but also monkeys and pigs, are emergently needed to establish the intensity, duration and frequency of hypoxic protocols. In animals, potential damage of repeated intermittent hypoxia (e.g. 5%–12% oxygen for three days every week) to the whole-body organs should be carefully examined and systematically evaluated, in order to ensure a minimal hypoxic tolerance degree for simulated long-term treatment.

Clinical trials

To further verify the hypoxic hypothesis in case-control clinical trials, the main concern regards the selection of post-infarct population that might benefit from hypoxia. In theory, the hypoxic treatment may provoke considerable clinical benefits for two clear-cut clinical situations. **First, Patients with old not revascularized MI.** They have large areas of chronic dysfunctional (stunned and/or hibernating) myocardium where lie viable and necrotic cells [13,14]. **Second, patients with timely reperfused acute MI.** Reoxygenation of ischemic myocardium may provoke myocardial cell death and endothelial dysfunction, which is mainly driven by heightened oxidative status that overwhelms endogenous antioxidant activity thus increasing infarct size [13,15–17,19].

According to the latest Fourth Universal Definition of Myocardial Infarction (2018) endorsed by European Society of Cardiology (ESC), American College of Cardiology (ACC), American Heart Association (AHA) and World Heart Federation (WHF), the criteria for myocardial injury, acute MI (Types 1, 2 and 3) and coronary procedure-related MI (Types 4 and 5) were updated especially relating to coronary atherosclerosis, oxygen demand and supply imbalance, myocardial injury, myocardial infarction, sudden cardiac death, therapeutic procedures and prognostic outcomes [13,14]. Amongst, myocyte necrosis is often associated with Type 1 MI caused by atherothrombotic coronary artery disease, Type 4a MI with macroscopically large necrotic areas, and MI with prolonged new convex ST-segment elevation and/or new Q waves [13,14]. Timely reperfusion therapy prevents continuous myocardial cell death but doesn't reverse and regenerate necrotic cardiomyocytes induced in the occlusion periods. Therefore, the hypoxic treatment will serve for a long-term and gradual recovery of cardiac function against large-area myocardial necrosis aiming to prevent or delay heart failure and cardiac death.

Especially, for patients who have severer coronary atherosclerosis with prolonged ischaemia, coronary stenting and bypassing surgeries are often necessary and even mandatory; however, numerous myocyte necrosis from subendocardium to subepicardium might have occurred before surgeries as diagnosed by abnormal cardiac biomarkers and ultrastructural changes such as relaxed myofibrils and sarcolemmal disruption. These patients seem to recover a normal oxygen supply in the heart after surgeries, so self-proliferation of cardiocytes will be impossible under normoxia condition as described above, which thus necessitates an effective hypoxic treatment.

Moreover, considering of patient's personalized responses to hypoxia and precision medication, oxygen concentration should be reduced slowly for each MI patient, and extensive experimental assays should be carried out to monitor patient's physiological changes, including changes in serum levels of HIF-1 and relevant genes, improvements of electrocardiogram and echocardiography, enhancements of cardiac function scores, etc. Frankly, only a moderate effective hypoxia is suggested, but excessive hypoxia should be avoided in order to guarantee therapeutic efficacy as well as minimize side effects, with the aim of prolonging basic life activity but not expecting a completely new heart.

Conclusions

In summary, it seems too exaggerated to assert that a hypoxic therapy can replace all of the cardio-therapeutic strategies, but it is reasonable to conclude that the hypoxic therapy could be an alternative post-surgical adjunctive approach for MI patients in addition to other available strategies. Interestingly enough, the hypoxic therapy is highly expected to recover heart function for its complementary and unique effects on arousing endogenous proliferation of the existing adult cardiomyocytes *in vivo*.

According to the recent opinions on MI [7,25], therapeutic approaches to increasing spontaneous cardiomyocyte proliferation are of particular importance for heart function recovery in the future. Hence, the proposed novel hypoxic approach to directly inducing endogenous cardiomyocyte proliferation will have no cardiac surgical risk and may be an effective, convenient, and economic treatment for patients with coronary atherosclerosis, MI, heart failure, etc.

Herein lies several more problems. As a putative treatment for cardiac repair, the hypoxic therapy should find effective ways of solving some highly crucial issues in the future, such as the optimized moderately-low oxygen concentration for human beings, the hypoxia treatment frequency and cycles, and particularly the need to avoid tumorigenesis, with the aim of achieving expected therapeutic benefits as well as controlling acceptable side effects. These undefined parameters necessitate future determination in randomized clinical trials, but actually the hypotheses have gained an updated understanding of probable

novel approaches to ameliorating cardiac prognosis of adult MI patients.

Conflict of interest statement

None.

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