Hypoxia is essential for bone-tendon junction healing: the molecular biological evidence

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Abstract Bone-tendon junction (BTJ) injury is difficult to cure due to its special anatomical structure. Most methods applied for BTJ injury treatment cannot lead to the perfect restoration of the fibrocartilage zone and perfect vascular regeneration, which are two important facets of BTJ reconstruction. Based on current research, hypoxia, which has been discovered to induce chondrogenesis and angiogenesis *in vivo*, plays an essential role in the tissue repair process. Consequently, it is reasonable to confirm that a hypoxic environment is the prerequisite condition to obtain physiological healing of BTJ injury. In this paper, the potential relationship between hypoxia and BTJ healing is discussed. Moreover, an operation model and possible drug application to obtain hypoxic conditions are delineated.

Introduction

The injury of the bone-tendon junction (BTJ) is not uncommon in clinical practice, yet its repair is both slower and poorer than that taking place at bone-to-bone or tendon-to-tendon healing [29]. Although many methods have been tried for therapy of the BTJ, it is difficult to obtain perfect restoration of this special anatomical structure [13]. A poorly reconstructed fibrocartilage zone and less vascular regeneration are two factors that result in inferior BTJ healing [23, 31]. Moreover, in the process of BTJ reconstruction, a large amount of fibrous tissue occurs between the tendon and bone, which can hardly differentiate into fibrocartilage and contributes to inferior mechanical capacity of the BTJ healing interface [12].

Hypoxia accounts for chondrogenesis, osteogenesis and angiogenesis [26], all of which are essential to the reconstruction of the BTJ. According to a review of the literature and a discussion of our previous investigations [18, 29, 30], the relationship between BTJ healing and hypoxia is described from the perspective of molecular and cellular biology.

The BTJ is enclosed by a hypoxic environment physiologically

The BTJ is a unique structure that connects the bone and muscle. Understanding of the physiological status and the normal anatomy of the BTJ will be helpful for us to explore new therapeutic approaches for BTJ complex healing. Studies on the blood supply of the Achilles tendon report very few arterioles within this specific region, implying that the BTJ is naturally surrounded by a hypoxic environment [1]. Research on the posterior tibial tendon [21] and tibialis anterior tendon [22] also displayed similar results. There-

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fore, the BTJ complex is a region lacking blood supply and adapted to the hypoxic condition physiologically.

Hypoxia is essential for chondrogenesis and vascular regeneration

The chondrocyte is the major cell type in the fibrocartilage zone of the BTJ [4]. Consequently, chondrogenesis is the essential process to restore this unique anatomical structure. Physiologically, the chondrocyte is enclosed in an avascular and hypoxic environment and is able to sense the concentration of oxygen and glucose in the extracellular matrix and respond appropriately by adjusting cellular metabolism [25]. Therefore, chondrocytes have the capacity to survive under conditions of limited nutrients and low oxygen tensions [19]. Moreover, studies on chondrogenesis show that precursor cells are facilitated in deeper hypoxic zones of cartilage repair tissue and stimulated by growth factors to enhance hypoxia-inducible factor-1 α (HIF-1 α) activity [11]. Furthermore, enhanced collagen type II mRNA was found after hypoxic administration [32]. Similar results were obtained by Lafont et al. who emphasised that hypoxia had a broader beneficial effect on the chondrocyte phenotype than had previously been described, and they described a new pathway to activate the SOX9 transcription factor independent of HIF-2 α , i.e. another new chondrogenesis-modulating protein [15]. Research on the fibrocartilage tissue in intervertebral discs strengthens the opinion that the hypoxic condition plays a pivotal role in the fibrocartilage formation process [5]. The above in vivo and in vitro studies indicate that hypoxia is indispensable for chondrogenesis.

Furthermore, hypoxia has been found to promote the formation of blood vessels and the formation of a vascular system in embryos [17]. The hypoxia in wounds not only promotes the formation of blood vessels, but also the migration of keratinocytes and the restoration of the epithelium [3].

The key role of HIF

Both chondrogenesis and angiogenesis result from the activation of HIF, which is the main factor that modulates the effects of hypoxia on the cell [6]. The α subunit of HIF-1 is a target for prolyl hydroxylation by HIF prolyl hydroxylase, which makes HIF-1 α a target for degradation by an E3 ubiquitin ligase, leading to quick degradation by the proteasome. While the above condition only occurs in oxygenated conditions, in hypoxic conditions, oxygen is used as a cosubstrate and the HIF prolyl hydroxylase is inhibited [24]. Hypoxia also results in a build up of

succinate, due to inhibition of the electron transport chain in the mitochondria. The build up of succinate further inhibits HIF prolyl hydroxylase action. When stabilised by hypoxic conditions, HIF-1 will upregulate several genes to promote survival in low-oxygen conditions, among which the vascular endothelial growth factor (VEGF) gene, which may promote angiogenesis, is included. HIF-1 acts by binding to HIF-responsive elements (HREs) in promoters that contain the sequence NCGTG [16]. Muscle A kinase-anchoring protein (mAKAP) organised E3 ubiquitin ligases were found to affect the stability and positioning of HIF-1 inside its action site in the nucleus. Furthermore, the relationship between HIF and nuclear factor κ B (NF- κ B) has been discovered. Small interfering RNA (siRNA) studies for individual NF- κ B members revealed differential effects on HIF-1 α mRNA levels, indicating that NF- κ B can regulate basal HIF-1 α expression [8]. Finally, it was shown that when endogenous NF- κ B is induced by tumour necrosis factor α (TNF α) treatment, HIF-1 α levels also change in an NF- κ B-dependent manner [28].

The expected model

We believe that haematoma is an excellent mode of hypoxia [9]. Pathologically, the erythrocytes in the haematoma are destroyed and no oxygen can be transferred into this region, which makes it an excellent medium to produce a hypoxic condition. Furthermore, research shows that many cytokines in the haematoma such as transforming growth factor (TGF- β) and platelet-derived growth factor (PDGF) are useful for chondrogenesis [2, 10] as well as migration of bone mesenchymal stem cells (BMSCs) [27, 33]. With regard to the role of mesenchymal stem cells (MSCs) which is the main cellular resource for chondrogenesis in the haematoma: A recent study showed that MSCs survived under a hypoxic environment [20]. After exposure to hypoxic or ischaemic conditions, MSCs remained alive and retained the ability to differentiate into chondrocytes. Hence, the haematoma cannot only provide a hypoxic environment but also can maintain the multipotential differentiation ability of MSCs. A haematoma should be created via injection of bone marrow blood which consists of BMSCs and inducible cytokines. The BMSCs in the haematoma as well as those recruited by inducible cytokines which may migrate through the tunnel made by internal fixation will contribute to obtaining better BTJ reconstruction.

The expected drug therapy

The HIF-targeted drug intervention is another therapeutic strategy. Recently several drugs have been developed

which act as selective HIF prolyl hydroxylase inhibitors [7]. By inhibiting HIF prolyl hydroxylase, the activity of HIF-1 α in the bloodstream is prolonged, leading to the increase in endogenous production of erythropoietin which may even enhance chondrogenesis and vascular formation [14]. The application of such drugs in the healing process of the BTJ may obtain better reconstruction and mechanical rehabilitation.

The prospect

In summary, the hypoxia-targeted new therapeutic concept may shed light on future studies of BTJ healing. Operation innovation and the application of HIF-targeted drugs are expected to maintain a hypoxic environment, and may well verify the advantages of hypoxia-targeted therapy. Based on the above review and analysis, it is reasonable to believe that hypoxia-induced chondrogenesis and angiogenesis may lead to better reconstruction of the BTJ.

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