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Can we enhance fracture vascularity: What is the evidence?☆

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ABSTRACT

Angiogenesis is a vital component of bone healing. The formation of the new blood vessels at the fracture site restores the hypoxia and nutrient deprivation found at the early stages after fracture whilst at a later stage facilitates osteogenesis by the activity of the osteoprogenitor cells. Emerging evidence suggests that there are certain molecules and gene therapies that could promote new blood vessel formation and as a consequence enhance the local bone healing response. This article summarizes the current in vivo evidence on therapeutic approaches aiming at the augmentation of the angiogenic signalling during bone repair.

Introduction

Bone is a dynamic tissue undergoing continues regeneration, i.e. osteoclastogenesis and osteogenesis, a feature responsible for the scarless healing after a fracture [1–3]. In this process, the formation of new blood vessels is vital and interdependent to bone formation facilitating primarily oxygen and nutrients delivery [4]. The availability of oxygen is essential for aerobic metabolism and the function of most cells but also for the activity of molecules like hydroxylases and cyclooxygenases, which play a vital role on collagen synthesis and the expression of growth factors [5–9]. Similarly, deprivation of nutrients results in poor cellular function, cell apoptosis and reduced regenerative capacity [10]. Still, the role of blood vessels is more complex than fulfilling solely bioenergetic functions as it has been shown for instance that blocking of the vascular invasion results in absence of ossification [11]. Equally, by blocking the osteogenesis, seen in the genetically modified animal models i.e. Runx2$^{-/-}$, a complete absence of vascular invasion is noted [12]. It has been also hypothesized that osteoprogenitor cells could use the vascular network as a scaffold to invade surrounding space during ossification [12]. This is further strengthened by the fact that osteogenesis follows vascularization [13]. It is still to be defined however, whether osteoprogenitor cells are in control of this process by pulling vessels to the surrounding soft tissue or whether interactions with other cells types like endothelial cells and osteoclasts are responsible for these observations.

Overall, two distinct forms of new blood vessel formation exist the so called angiogenesis and vasculogenesis, both complementing each other in the restoration of mature circulation [14,15]. Angiogenesis occurs by intussusceptive microvascular growth where vessel sprouting occurs from an existing vessel [14,16]. In this process, resident endothelial cells adjacent to a mature vascular network are positioned in such a way to form a tubular structure which is reinforced by the presence and actions of other cell types like pericytes, fibroblasts and/or smooth muscle cells [14]. Vasculogenesis is the de novo formation of a vessel or vascular network [15,16]. It involves the recruitment and differentiation of endothelial cells from mesoderm-derived precursor cells (angioblasts) which are organized to form blood vessels [16]. Although vasculogenesis was thought to occur during embryogenesis, this process is recapitulated in several postnatal stages including bone and wound healing, myocardial or peripheral ischaemia, stroke and retinopathy [15–17].

Following injury, bone mounts a number of events aiming to restore its continuity and function. The initial hyperaemia following disruption of the existing blood vessels together with the vasodilatation, oedema and swelling lead to the formation of the fracture haematoma [1]. Immediately after, hypoxia follows associated with necrosis, intense osteoclast activity and the

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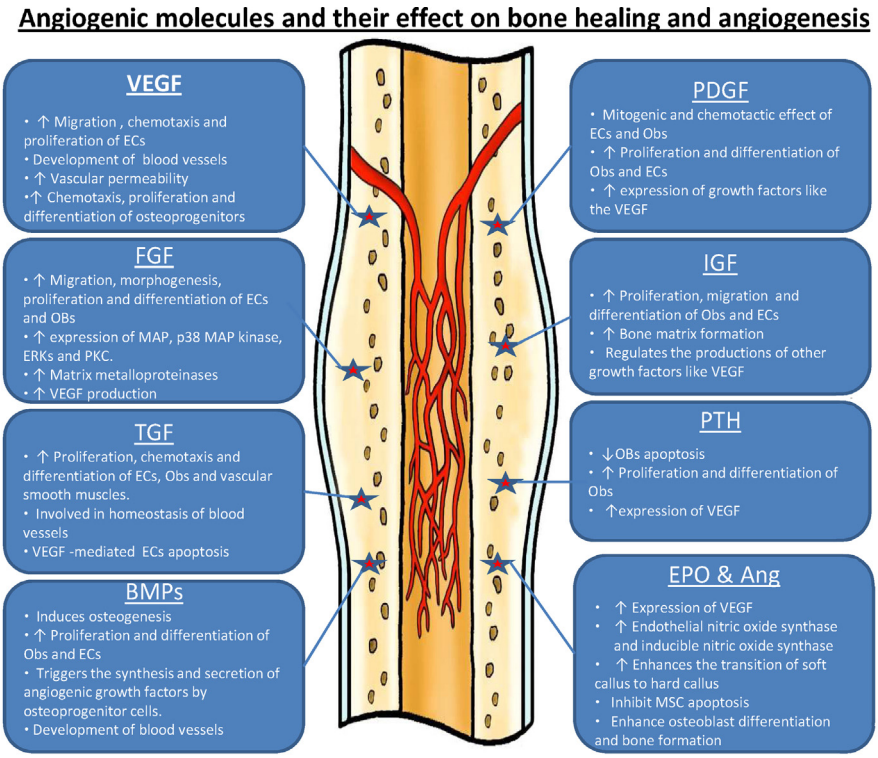

Fig. 1. Angiogenic molecules and their effect on bone healing and angiogenesis [30–77].

formation of granulation tissue [1]. Subsequently, this hypoxic environment recovers mainly due to proliferation of blood vessels around the fracture zone [18]. New blood vessels appear on the second week after injury and mainly derive from the adjacent soft tissues but also the periosteum and medullary canal [18–21]. Later on, soft and hard callus develop which are associated with maturation of blood vessels followed by a final long-lasting remodelling stage [18].

Among the many factors that can influence the bone healing process, adequate blood supply is crucial and the lack of perfusion is closely related to delayed union or non-union [1,22–25]. Animal models of tibial bone healing with prior resection of the femoral artery resulted in delayed healing or non-union with decreased cell proliferation, increased apoptosis and fibrous tissue formation [26,27]. Similarly, the local inhibition of angiogenesis in animal models resulted in bone union arrest and development of atrophic non-union [28]. Clinical studies in patients with concomitant vascular injuries reported an impaired healing rate of 46% which is far higher than the 5–10% non-union rate seen for long bone fractures in general [2,29].

At a cellular level, a number of important molecules have been identified to be implicated in the physiological process of new blood vessel formation (Fig. 1) [30–77]. Vascular endothelial growth factor (VEGF) is a key angiogenic mediator which stimulates new vessel formation and increases blood flow [78]. It exerts a chemotactic and proliferative effect both on osteoblasts and endothelial cells [56,73]. Blocking VEGF signalling results in arrest of intramembranous bone formation and VEGF gene knockout animals die due to deficient endothelial cell development and lack of blood vessels [34,75,79]. Platelet-derived growth factor (PDGF) and Fibroblast Growth Factor (FGF) are involved in the regulation of migration, morphogenesis, proliferation and differentiation of osteoprogenitor cells, osteoblasts and endothelial cells [32,41,45,53,61]. The transforming growth factor beta (TGF-β) superfamily is strongly engaged in developmental angiogenesis but also regulates the vascular integrity [31,65]. TGF-β is involved in the regulation of endothelial cell functions and is required for the development and homeostasis of blood vessels and plays an irreplaceable role on the differentiation of the vascular smooth muscle cells in the blood vessels [31,54,65]. Animal models with deficient TGF-β receptors acquire vasculogenic defects resulting in haemorrhage and vascular leakage [49]. Insulin-like growth factor (IGF) promotes bone matrix formation, regulates the productions of other growth factors including the VEGF and is a potent angiogenic factor that can stimulate endothelial cell proliferation, migration and morphological differentiation via binding to IGF-1R [37,39,57,72]. Parathyroid hormone (PTH) promotes angiogenesis and upregulates the expression of VEGF been able to reverse the hypovascularity produced in radiation induced depleted osseous vascularity animal models [38,48,51,58]. Finally, angiopoietin, thrombin and erythropoietin play an important role in vessel formation, inhibit MSC apoptosis due to nutrient deprivation and enhance osteoblast differentiation and bone formation [50,55,60]. Erythropoietin upregulates the expression of VEGF, endothelial nitric oxide synthase, and inducible nitric oxide synthase [42,46].

Several authors have suggested that an adequate vascularity response enhances and up-regulates the fracture healing process and outcome [18,21]. Such observations, delineate the importance of new blood vessel formation during bone healing and triggers the hypothesis whether early angiogenesis promoting intervention could expedite the bone repair process and outcome. The aim of this systematic review therefore was to evaluate the effectiveness of in vivo interventions of manipulation of the local environment utilizing the above molecules in terms of blood vessel formation (neo-angiogenesis) and bone regeneration.

Materials and methods

We searched Medline for general keywords such as ‘angiogenesis’, ‘vasculogenesis’, ‘bone healing’ isolated or in combination to specific words including ‘fracture healing’, ‘growth factors’