Growth factors for therapeutic angiogenesis in hypercholesterolemic erectile dysfunction

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Abstract

The past decade has seen an explosion of new information on the physiology of penile erection, and pathophysiology of erectile dysfunction (ED). Hypercholesterolemia is a chronic condition that can lead to degeneration in the vasculature bed and can result in ED if the penile vasculature is involved. Angiogenesis is the growth of new blood vessels from preexisting vasculature. Therapeutic angiogenesis seeks to harness the mechanisms of vascular growth to treat disorders of inadequate tissue perfusion, such as coronary artery disease and ED. There have been tremendous changes in the field of therapeutic angiogenesis over the past decade, and there is much promise for the future. Initial preclinical work with cytokine growth factor delivery resulted in a great deal of enthusiasm for the treatment of ischemic heart and/or peripheral vascular disease, though clinical studies have not achieved similar success. With an increased understanding of the complex mechanisms involved in angiogenesis, novel therapies which target multiple different angiogenic pathways are also being developed and tested. The penis is a convenient tissue target for gene therapy because of its external location and accessibility, the ubiquity of endothelial lined spaces, and low level of blood flow, especially in the flaccid state. Therapeutic angiogenesis is an exciting field that continues to evolve. This review will focus on the development of growth factors for hypercholesterolemic ED, the use of various growth factors for ED therapy, their routes of delivery, and the results in animal studies. (Asian J Androl 2008 Jan; 10: 23–27)

Keywords: erectile dysfunction; hypercholesterolemia; angiogenic growth factors; nitric oxide synthase

1 Introduction

The use of phosphodiesterase type 5 (PDE-5) inhibitors has a proven record of safety and efficacy in preclinical and human trials for erectile dysfunction (ED) [1, 2]. However, there are approximately 30% of patients with ED in whom PDE-5 inhibitors are not effective. The clinical efficacy of PDE-5 inhibitors is diminished in “difficult to treat” patient groups [3, 4]. This prompts the development of new approaches, including gene-based therapies for the treatment of ED.

Indeed the widespread use of the PDE inhibitors has resulted in an explosion of new information on the vascular biology that underlies the physiology of erection and the pathophysiology of ED. Endothelial cell mediated venous-occlusion, by vascular smooth muscles in the corpus cavernosum, became recognized as the principle event in normal penile erection [5]. Nitric oxide (NO) has been shown to be the critical mediator of this endothelial/smooth muscle interaction [5]. ED and atherosclerosis share many common risk factors [6]. Hypercholesterolemia is one of the major risk factors for the development of ED; studies have shown that in men there is a 1.32-fold increase in the risk of developing ED for every 1 nmol/L increase in cholesterol [6]. Moreover, there was high prevalence of undiagnosed hypercholesterolaemia and hypertriglyceridaemia in men presenting with ED [7]. The induction of endothelial cell dysfunction in the penile vasculature tissue using a hypercholesterolemic diet is also an established model for evaluating the pathogenesis of ED [8–12].

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Angiogenesis is defined as the growth and proliferation of blood vessels from pre-existing vascular structures. Therapeutic angiogenesis seeks to harness the mechanisms of vascular growth to treat disorders of inadequate tissue perfusion. There have been tremendous changes in the field of therapeutic angiogenesis over the past decade, and there is much promise for the future. Initial preclinical work with cytokine growth factor delivery resulted in a great deal of enthusiasm, even though clinical studies conducted to date have failed to achieve the same level of success [13]. A number of angiogenic growth factors, including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), have been shown to be present in corporal tissue and can also act as protective factors in the setting of any vascular injury, such as ED [14–17]. The penis is a convenient tissue target for gene therapy because of its external location and accessibility, the ubiquity of endothelial lined spaces, and low level of blood flow, especially in the flaccid state [18]. This review will focus on the development of growth factors in hypercholesterolemic models of ED, the use of various growth factors being investigated for ED therapy, their routes of delivery, and the results in animal studies.

2 Angiogenic growth factor families

The most extensively studied angiogenic cytokines are VEGF and bFGF. Both VEGF and bFGF are known to be present in the blood vessel wall and the proper expression of these angiogenic growth factors is required for normal blood vessel growth during embryonic development [13, 16–17, 19–20]. In adult blood vessels, VEGF can act as a survival factor for the micro-vascular endothelium and VEGF can help endothelial cells avoid cell death (apoptosis) when subject to injury [21–23]. Currently, the VEGF family consists mainly of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E. VEGF interacts with three subtypes of VEGF receptors occurring on the cellular membrane known as VEGFR-1, -2, -3, VEGFR-1, which binds VEGF-A, -B, acts as inhibitor of angiogenesis; VEGFR-2, which binds VEGF-A, -C, and -D, plays a critical role in angiogenesis; VEGFR-3, which binds VEGF-C and -D, is important in lymphogenesis [24]. Structurally, the VEGFs are related to the platelet derived growth factor (PDGF) family of growth factors, with intrachain and interchain disulfide bonds between eight cysteine residues in conserved positions. The crystal structure of VEGF-A revealed two monomers that are organized in an anti-parallel fashion to form a dimer, with the receptor-binding sites located at each pole of the dimer [25]. The VEGFs preferentially form homodimers, although VEGF-A and PDGF heterodimers have been identified [26]. Alternative splicing of several of the VEGF family members gives rise to splice variants with different biological activities. The human splice variants are denoted VEGF-A121, VEGF-A145, VEGF-A165, VEGF-A189 and VEGF-A206. The mouse splice variants are one amino-acid residue shorter than the corresponding human splice variants, and they are denoted VEGF-A120 and so forth. The activities of the VEGFA splice variants are dictated by their different abilities to interact with VEGFR co-receptors, such as neuropilins and heparin sulfate proteoglycans (HSPGs). Another splice variant of VEGF-A, known as VEGF165b, has been proposed to negatively regulate VEGFR activity, although whether this splice variant is present in corporal tissue is unknown [27]. The bioactivity of VEGF family members is also regulated by proteolytic processing. This mechanism might enable specific interactions with different types of receptor. For example, in humans, processed VEGF-C and -D bind to VEGFR-2, as well as to VEGFR-3. Furthermore, proteolytic processing of VEGFA splice variants affects their ability to interact with the VEGF co-receptors HSPGs and neuropilins [28]. When VEGF binds to its membrane receptor, the receptor becomes dimerized, it autophosphorylates, and the receptor may then signal through the phosphatidylinositol 3 kinase (PI3K) pathway with activation/phosphorylation of Akt and endothelial nitric oxide synthase (eNOS) [24, 29].

Basic FGF is an 18-kDa protein with a strong affinity for heparin sulfate molecules on the cell surface and in the extra-cellular matrix and bFGF binds to a family of receptors found on multiple cell types [30]. FGF-2 interacts with specific cell surface receptor proteins derived from at least four separate genes (FGFR1–4). A number of splice variants exists for each receptor type resulting in differing ligand binding domains. The splice variants confer specificity in signaling in response to the various FGF family members. FGF-2 has been proposed to have two separate receptor binding sites, which might allow a single FGF-2 to bind to two receptors or to interact with a single receptor in two separate positions [31]. HSPGs can increase the affinity of FGF-2 for its receptors [32] and potentially act as a bridge to facilitate the dimerization of receptors. Indeed, a heparin-binding site on FGFR1 has also been identified [31], providing additional evidence that a ternary complex of FGF-2, HSPG and receptor exist.

Vascular network formation requires several endothelial cell growth factors working together. These factors have a potent angiogenic effect, and their precise coordination is essential for vascular development. Among them, angiopoietins function through the Tie2 receptor, whose signaling is critical to regulate vascular stabilization and remodeling. It has been reported that the angiopoetin/Tie2 signal is involved in survival and migration of endothelial cells and regulates vascular re-
modeling and maintenance of vascular integrity [33]. This remains an area of great interest that is underexplored.

Both VEGF and FGF are present in corporal tissue [34–39]. One of the downstream effects of VEGF includes the phosphorylation and activation of Akt and eNOS, which has been shown to mediate VEGF-induced penile erection by further mediating activation of guanosine 3', 5'-cyclic monophosphate (cGMP) and cyclic GMP-dependent kinase (cGK-1) [25, 40].

3 Changes in expression of angiogenic growth factors in hypercholesterolemic models of ED

Some studies have begun to look at the changes in angiogenic growth factors in the setting of atraumatic vascular injury. Azadzoi [41] and Wang et al. [42] showed that in corporal tissue VEGFR-2 was decreased in rabbits on 0.5% high cholesterol (HC) diet, while VEGF was increased at early timepoints but decreased at late timepoints. Ryu et al. [11] demonstrated that VEGF (including mRNA levels of three VEGF splice variants VEGF120, VEGF164, and VEGF188) and VEGFR-2 were downregulated in rat corporal tissue with a 4% HC diet for 3 months. Byrne et al. [14] and Xie et al. [43] demonstrated decreased VEGF-A (including mRNA levels of 3 VEGF splice variants VEGF121, VEGF165, and VEGF189) in corporal tissue of rabbits on 1% HC diet, which developed at an early timepoint in their study. Thus, differences in cholesterol content, duration of feeding, animal species, and injury models may account for different timing for VEGF decrease. Recent studies in skeletal muscle have shown that changes in the VEGF receptor ligand system can affect injury and repair [44].

4 Changes of NOS expression in hypercholesterolemic models of ED

Both neuronal and eNOS generate NO [45–49]. Activation of the central and peripheral nervous systems leads to NO production from neuronal NOS (nNOS). The initiation of tumescence and reduction in nNOS play an important role in ED [48, 49]. Initial tumescence is produced by appropriately stimulated release of neuronal nitric oxide, which is followed by an increase in eNOS activity that alters intracorporal blood flow for the further promotion of tumescence [49]. Xie et al. [12], Lee et al. [28] and Ryu et al. [11] demonstrated that Akt phosphorylation and eNOS phosphorylation were downregulated in the corporal tissue of hypercholesterolemic animals compared with that of control animals. Xie et al. [12, 29] also report that a reduction of eNOS phosphorylation was at Ser-1177 while no change at Thr 495 in the corporal tissue of hypercholesterolemic rabbit or apolipoprotein E knockout (ApoE−/−) mice. Interestingly total eNOS was higher in the ApoE−/− mice (1.25% HC diet) than that of wild-type C57BL6 mice but not in hypercholesterolemic rabbits (1% HC diet) [12, 29]. Xie et al. [12] and Lee et al. [28] also reported that changes in nNOS occur as early as 4 weeks after the start of the HC diet in hypercholesterolemic mice and rabbits. Azadzoi et al. [42] found that cavernosal nNOS and eNOS protein levels were unaffected at week 4 but were significantly decreased at weeks 8 and 16 after the induction of atherosclerosis by a 0.5% HC diet. Interestingly, they found inducible NOS (iNOS) protein was markedly increased during the course of the induced arterial disease [42]. Understanding the manner in which angiogenic growth factors work in concert with changes in NOS isoforms is an area of intense interest.

5 Therapeutic angiogenesis trials in hypercholesterolemic models of ED

In the setting of vascular injury, angiogenic growth factors can act as survival factors for the microvascular endothelium [14, 17, 21]. Both VEGF protein and VEGF-DNA can be successfully delivered to the rat penis [50]. Henry et al. [14] showed that VEGF had beneficial effects on the structure and function of corporal endothelial and smooth muscle cells in hypercholesterolemic rabbits. Additionally, the effect of systemic (intravenous) VEGF on corporal tissue was superior to the effect of local intra-cavernously injected VEGF on corporal tissue in restoring vasoactivity [14, 15]. When given exogenously VEGF causes an increase in vascular permeability and a breakdown of the integrity of the endothelium [51]. Combined gene transfer of both angiopeitien-1 and ad-VEGF165 has been shown to significantly increase cavernous angiogenesis, eNOS phosphorylation, and cGMP expression compared with that in the groups treated with either therapy alone. Erectile function, as evaluated by electrical stimulation of the cavernous nerve 2 and 8 weeks after treatment, was completely restored in the combined treatment group, whereas intracavernous injection of either ad-Ang1 or ad-VEGF165 alone elicited partial improvement [52]. In a preclinical model of hypercholesterolemic arterial injury, intravenous bFGF was able to protect the arterial endothelium [17]. However, bFGF also has the potential to cause fibrosis and systemic bFGF has been shown to cause drug-related toxicities, including proteinuria and hypotension [53–55]. Dai et al. [56] found that systemic low dose bFGF induces favorable histological changes in the corpus cavernosum of hypercholesterolemic rabbits. In the same pre-clinical model of ED, Xie et al. [15] showed that intracavernosal injection of low dose of bFGF improved vasoactivity and increased nNOS expression. They also observed bFGF induced
increased VEGF expression and eNOS phosphorylation at Ser 1177 [16].

6 Summary and future directions

In preclinical hypercholesterolemic animal models, VEGF, bFGF, and angiopoietin have shown their promising application in improving erectile function or corporal vasoreactivity. Various routes of application may bring different efficacies. Combined application of angiogenic factors may enhance cavernous angiogenesis synergistically and restore on erectile function by reinforcing the endothelium both structurally and functionally [52].

Further studies are needed to study potential differential changes in VEGF family members, and the potential mechanisms for bFGF to induce eNOS phosphorylation and nNOS expression. On the therapeutic side, new combined regimens such as VEGF+ brain-derived neurotrophic factor, have shown efficacy in animals with neurogenic impotence [48], and engineered transcription factors haver peripheral artery disease [57]. These results give us new directions therapeutic angiogenesis in hypercholesterolemic ED.

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