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·*Review*·

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 trea" 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 cell 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-A_{121}, VEGF-A_{145}, VEGF-A_{165}, VEGF-A_{189} and VEGF-A_{206}. The mouse splice variants are one amino-acid residue shorter than the corresponding human splice variants, and they are denoted VEGF-A_{120} 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 $VEGF_{165b}$, 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 angiopoietin/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 $VEGF_{120}$, $VEGF_{164}$, and $VEGF_{188}$) 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 $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$) 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 hyperchoelsterolemic 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 hyepercholesterolemic 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 vasoreactivity [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 ad-angiopoietin-1 and ad-$VEGF_{165}$ 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-$VEGF_{165}$ 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 vasoreactivites 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.

References

1 Abbott D, Comby P, Charuel C, Graepel P, Hanton G, Leblanc B, Lodola A, *et al*. Preclinical safety profile of sildenafil. Int J Impot Res 2004; 16:498–504.
2 Mittleman MA, Glasser DB, Orazem J. Clinical trials of sildenafil citrate (Viagra) demonstrate no increase in risk of myocardial infarction and cardiovascular death compared with placebo. Int J Clin Pract 2003; 57: 597–600.
3 Anderson PC, Gommersall L, Hayne D, Arya M, Patel HR. New phosphodiesterase inhibitors in the treatment of erectile dysfunction. Expert Opin Pharmacother 2004; 5: 2241–9.
4 Cappelleri JC, Rosen RC, Smith MD, Mishra A, Osterloh IH. Diagnostic evaluation of the erectile function domain of the International Index of Erectile Function. Urology 1999; 54: 346–51.
5 Fournier G Jr, Juenemann KP, Lue TF, Tanagho EA. Mechanism of venous occlusion during canine penile erection: an anatomic demonstration. J Urol 1987; 137: 163–7.
6 Wei M, Macera CA, Davis DR, Hornung CA, Nankin HR, Blair SN. Total cholesterol and high-density lipoprotein cholesterol as important predictors of erectile dysfunction [see comments]. Am J Epidemiol 1994; 140: 930–7.
7 Smith NJ, Sak SC, Baldo O, Eardley I. The prevalence of newly diagnosed hyperlipidaemia in men with erectile dysfunction. BJU Int 2007; 100: 357–61.
8 Azadzoi KM, Goldstein I. Erectile dysfunction due to atherosclerotic vascular disease: the development of an animal model. J Urol 1992; 147: 1675–81.
9 Kim JH, Klyachkin ML, Svendsen E, Davies MG, Hagen PO, Carson CC 3rd. Experimental hypercholesterolemia in rabbits induces cavernosal atherosclerosis with endothelial and smooth muscle cell dysfunction. J Urol 1994; 151: 198–205.
10 Nehra A, Azadzoi KM, Moreland RB, Pabby A, Siroky MB, Krane RJ, *et al*. Cavernosal expandability is an erectile tissue mechanical property which predicts trabecular histology in an animal model of vasculogenic erectile dysfunction. J Urol 1998; 159: 2229–36.
11 Ryu JK, Shin HY, Song SU, Oh SM, Piao S, Han JY, *et al*. Downregulation of angiogenic factors and their downstream target molecules affects the deterioration of erectile function in a rat model of hypercholesterolemia. Urology 2006; 67: 1329–34.
12 Xie D, Odronic SI, Wu F, Pippen AM, Donatucci CF, Annex BH. A mouse model of hypercholesterolemia induced erectile dysfunction. J Sex Med 2007; 4 (4 Pt 1): 898–907.
13 Byrne RR, Henry GD, Rao DS, Huynh TT, Pippen AM, Annex BH, *et al*. Vascular endothelial growth factor restores corporal smooth muscle function *in vitro*. J Urol 2001; 165: 1310–5.
14 Henry GD, Byrne R, Hunyh TT, Abraham V, Annex BH, Hagen PO, *et al*. Intracavernosal injections of vascular endothelial growth factor protects endothelial dependent corpora cavernosal smooth muscle relaxation in the hypercholesterolemic rabbit: a preliminary study. Int J Impot Res 2000; 12; 334–9.
15 Xie D, Pippen AM, Odronic SI, Annex BH, Donatucci CF. Intracavernosal basic fibroblast growth factor improves vasoreactivity in the hypercholesterolemic rabbit. J Sex Med 2006; 3: 223–32.
16 Meurice T, Bauters C, Vallet B, Corseaux D, van Belle E, Hamon M, *et al*. bFGF restores endothelium-dependent responses of hypercholesterolemic rabbit thoracic aorta. Am J Physiol 1997; 272: H613–7.
17 Jones WS, Annex BH. Growth factors for therapeutic angiogenesis in peripheral arterial disease. Curr Opin Cardiol 2007; 22: 458–63.
18 Kendirci M, Teloken PE, Champion HC, Hellstrom WJ, Bivalacqua TJ. Gene therapy for erectile dysfunction: fact or fiction? Eur Urol 2006; 50: 1208–22.
19 Huynh TT, Davies MG, Annex BH, Hagen PO. Tissue factor in the pathobiology of vein grafts. In: Vascular Surgery: Proceedings of the Third Congress of the Asian Vascular Society. Wang ZG, editor. International Academic Publishers. 1998; 385–8.
20 Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. Circulation 1999; 99: 1726–32.
21 Folkman J. Clinical applications of research on angiogenesis. N Engl J Med 1995; 333: 1757–63.
22 Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, *et al*. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. J Biol Chem 1998; 273: 30336–43.
23 Kwak HJ, Lee SJ, Lee YH, Ryu CH, Koh KN, Choi HY, *et al*. Angiopoietin-1 inhibits irradiation and mannitol-induced apoptosis in endothelial cells. Circulation 2000; 101: 2317–24.
24 Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signaling–in control of vascular function. Nat Rev Mol Cell Biol 2006; 7: 359–71.
25 Muller YA, Li B, Christinger HW, Wells JA, Cunningham BC, de Vos AM. Vascular endothelial growth factor: crystal structure and functional mapping of the kinase domain receptor binding site. Proc Natl Acad Sci USA 1997; 94: 7192–7.
26 De Falco S, Gigante B, Persico MG. Structure and function of placental growth factor. Trends Cardiovasc Med 2002; 12: 241–6.
27 Woolard J, Wang WY, Bevan HS, Qiu Y, Morbidelli L, Pritchard-Jones RO, *et al*. VEGF165b, an inhibitory vascular endothelial growth factor splice variant: mechanism of action, *in vivo* effect on angiogenesis and endogenous protein expression. Cancer Res 2004; 64: 7822–35.
28 Lee S, Jilani SM, Nikolova GV, Carpizo D, Iruela-Arispe ML. Processing of VEGF-A by matrix metalloproteinases regulates bioavailability and vascular patterning in tumors. J Cell Biol 2005; 169: 681–91.
29 Xie D, Kontos CD, Donatucci CF, Annex BH. Cholesterol feed-

ing reduces vascular endothelial growth factor signaling in rabbit corporal tissues. J Sex Med 2005; 2: 634–40.
30 Nugent MA, Iozzo RV. Fibroblast growth factor-2. Int J Biochem Cell Biol 2000; 32: 115–20.
31 Kan M, Wang F, Xu J, Crabb JW, Hou J, McKeehan WL. An essential heparin-binding domain in the fibroblast growth factor receptor kinase. Science 1993; 259: 1918–21.
32 Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. Cell surface, heparin-like molecules are required for binding basic fibroblast growth factor to its high affinity receptor. Cell 1991; 64: 841–8.
33 Morisada T, Kubota Y, Urano T, Suda T, Oike Y. Angiopoietins and angiopoietin-like proteins in angiogenesis. Endothelium 2006; 13: 71–9.
34 Burchardt T, Burchardt M, Chen MW, Buttyan R, de la Taille A, Shabsigh A, *et al.* Expression of VEGF splice variants 144/145 and 205/206 in adult male tissues. IUBMB Life. 1999; 48: 405–8.
35 Burchardt T, Burchardt M, Chen MW, Buttyan R, de la Taille A, Shabsigh A, *et al.* Expression of messenger ribonucleic acid splice variants for vascular endothelial growth factor in the penis of adult rats and humans. Biol Reprod 1999; 60: 398–404.
36 Te AE, Santarosa RP, Koo HP, Buttyan R, Greene LA, Kaplan SA, *et al*. Neurotrophic factors in the rat penis. J Urol 1994; 152: 2167–72.
37 Haraguchi R, Suzuki K, Murakami R, Sakai M, Kamikawa M, Kengaku M, *et al*. Molecular analysis of external genitalia formation: the role of fibroblast growth factor (Fgf) genes during genital tubercle formation. Development 2000; 127: 2471–9.
38 Ogino Y, Suzuki K, Haraguchi R, Satoh Y, Dolle P, Yamada G. External genitalia formation: role of fibroblast growth factor, retinoic acid signaling, and distal urethral epithelium. Ann N Y Acad Sci 2001; 948: 13–31.
39 Satoh Y, Haraguchi R, Wright TJ, Mansour SL, Partanen J, Hajihosseini MK, *et al*. Regulation of external genitalia development by concerted actions of FGF ligands and FGF receptors. Anat Embryol (Berl) 2004; 208: 479–86.
40 Musicki B, Palese MA, Crone JK, Burnett AL. Phosphorylated endothelial nitric oxide synthase mediates vascular endothelial growth factor-induced penile erection. Biol Reprod 2004; 70: 282–9.
41 Azadzoi KM. Vasculogenic erectile dysfunction: beyond the haemodynamic changes. BJU Int 2006; 97: 11–6.
42 Wang T, Soker S, Atala A, Siroky MB, Azadzoi KM. Alterations in angiogenic growth factors and neuronal nitric oxide synthase expression in chronic cavernosal ischemia. Int J Impot Res 2004; 16: 403–11.
43 Xie D, Thompson MA, Pippen AM, Waters RE, Donatucci CF, Annex BH. Decreases in corporal vascular endothelial growth factor expression precede vasoreactivity changes in cholesterol fed rabbits. J Urol 2005; 173: 1418–22.
44 Hazarika S, Dokun AO, Li Y, Popel AS, Kontos CD, Annex BH. Impaired angiogenesis after hindlimb ischemia in type 2 diabetes mellitus: differential regulation of vascular endothelial growth factor receptor 1 and soluble vascular endothelial growth factor receptor 1. Circ Res 2007; 101: 948–56.
45 Andersson KE, Wagner G. Physiology of penile erection. Physiol Rev 1995; 75: 191–236.
46 Burnett AL, Calvin DC, Silver RI, Peppas DS, Docimo SG. Immunohistochemical description of nitric oxide synthase isoforms in human clitoris. J Urol 1997; 158: 75–8.
47 Toda N, Ayajiki K, Okamura T. Nitric oxide and penile erectile function. Pharmacol Ther 2005; 106: 233–66.
48 Lin CS, Lue TF. Growth factor therapy and neuronal nitric oxide synthase. Int J Impot Res 2004; 16 (Suppl 1): S38–9.
49 Burnett AL. Novel nitric oxide signaling mechanisms regulate the erectile response. Int J Impot Res 2004; 16 (Suppl 1): S15–9.
50 Burchardt M, Burchardt T, Anastasiadis AG, Buttyan R, de la Taille A, Shabsigh A, *et al.* Application of angiogenic factors for therapy of erectile dysfunction: protein and DNA transfer of VEGF 165 into the rat penis. Urology 2005; 66: 665–70.
51 Peters KG. Vascular endothelial growth factor and the angiopoietins–working together to build a better blood vessel. Circ Res 1998; 83: 342–3.
52 Ryu JK, Cho CH, Shin HY, Song SU, Oh SM, Lee M, *et al*. Combined angiopoietin-1 and vascular endothelial growth factor gene transfer restores cavernous angiogenesis and erectile function in a rat model of hypercholesterolemia. Mol Ther 2006; 13: 705–15.
53 Inoue Y, King TE, Barker E, Danilof E, Newman LS. Basic fibroblast growth factor and its receptors in idiopathic pulmonary fibrosis and lymphangioleiomyomatosis. Am J Respir Crit Care Med 2002; 166: 765–73.
54 Mulhall JP, Thom J, Lubrano T, Shankey TV. Basic fibroblast growth factor expression in Peyronie's disease. J Urol 2001; 165: 419–23.
55 Cooper LT Jr, Hiatt WR, Creager MA, Regensteiner JG, Casscells W, Isner JM, *et al*. Proteinuria in a placebo-controlled study of basic fibroblast growth factor for intermittent claudication. Vasc Med 2001; 6: 235–9.
56 Dai Q, Silverstein AD, Davies MG, Hagen PO, Donatucci CF, Annex BH. Systemic basic fibroblast growth factor induces favorable histological changes in the corpus cavernosum of hypercholesterolemic rabbits. J Urol 2003; 170 (2 Pt 1): 664–8.
57 Kontos CD, Annex BH. Engineered transcription factors for therapeutic angiogenesis. Curr Opin Mol Ther 2007; 9: 145–52.