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RATIONALE FOR BASIC FGF IN WOUND HEALING AND REVIEW OF THERAPEUTIC APPLICATIONS

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ABSTRACT

Several scientific groups are evaluating the opportunity to develop basic fibroblast growth factor (bFGF) for ophthalmic and other uses. While a number of clinical studies with growth factors have been conducted in the United States and Europe, most experience of bFGF appears to derive from studies in Japan and China, where products based on bFGF have been marketed for more than a decade, primarily for cutaneous wound healing, but also involving studies in bone regrowth and periodontal procedures; relatively less data are available in the open literature regarding ophthalmological use in humans. In Asia, many cytokines are currently under development, including TGF-β, EGF, and IGF-1 aside from the already marketed Fiblast Spray® (Japan) and the Beifushu series from Essex Biotechnology Ltd (China). A general concern with all growth factor-based therapies is promotion of tumour growth; in animal models, bFGF injected at the tumour inoculation site induce marked tumour growth and lymph node metastasis, correlating with an increase in neovascularization in the host stroma. Increased mortality from malignancies has been reported in patients treated with becaplermin, also involving tumours distant from the site of application. These concerns are likely to be more pronounced in relation to systemic treatment approaches (*i.e.* cardiovascular, bone fractures), even though the permanence of systemically administered FGF is likely to be short in the circulation. Specifically in the case of ophthalmological use, another general concern of growth factor therapy is corneal neovascularisation, which would represent an absolute contraindication of FGF therapy for corneal lesions. Against this background, it may be concluded that bFGF may have therapeutic utility in cutaneous wound healing and plastic surgery, while the ophthalmic utility appears less substantiated by published data, where no specific advantages of bFGF treatment have appeared as compared to current standard of care. The concerns regarding tumour promotion likely represent a significant hurdle, and may preclude systemic use (cardiovascular; bone healing) until large-scale and long-term safety studies have been completed to allow a more stringent risk/benefit analysis.

KEYWORDS: fibroblast growth factor, wound healing, corneal neovascularisation, cytokines, becaplermin.

1. BACKGROUND

Several scientific groups are evaluating the opportunity to develop basic fibroblast growth factor (bFGF) for ophthalmic and other uses. While three presentations with the same active biological ingredient are on the market in China, no data regarding these products were available for review and the ensuing discussion is based on a review of similar products licensed in Japan and US and investigational products from other companies presented in the open literature. While a number of clinical studies with growth factors have been conducted in the United States and Europe, most experience of bFGF appears to derive from studies in Japan and China, where products based on bFGF have been marketed for more than a decade, primarily for cutaneous wound healing, but also involving studies in bone regrowth and periodontal procedures; relatively less data are available in the open literature regarding ophthalmological use in humans.

1.1 Cutaneous Wound Healing

The wound healing process has three phases; inflammation, proliferation and remodelling. In the inflammatory phase, collagen exposed during wound formation activates both the intrinsic and extrinsic clotting pathways, initiating both haemostasis and inflammation. After injury, the cell membranes release vasoconstrictors such as thromboxane A_2 and prostaglandin 2α that aid in limiting haemorrhage. Subsequently, capillary vasodilatation occurs secondary to local histamine release, and inflammatory cells migrate to the wound bed.[1]

The timeline for cell migration in a normal wound healing process is predictable; platelets release platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β) from their α-granules to recruit neutrophils and macrophages. Neutrophil- and

macrophage infiltration is followed by lymphocyte and fibroblast infiltration. New capillaries start forming at the end of the first week following injury. Macrophages are the most important mediators of wound healing and continue to emit growth factors to recruit fibroblasts and trigger the next phase of healing, the proliferative phase, which begins approximately 72h after injury and overlaps with the inflammatory phase. Fibroblast recruitment peaks approximately one week from injury and signals angiogenesis, epithelialization and collagen formation. Epithelialization starts from the basement membrane if this remains intact (as in first-degree burns). If the basement membrane is not intact, epithelialization starts from the wound edges. During this phase, fibroblasts mainly produce Type III collagen, which continues until collagen synthesis and breakdown become equal, triggering the start of the remodelling phase. Increased collagen production and breakdown can continue for 6-12 months after injury. The initial Type III collagen is replaced by Type I collagen until a Type I-to-Type II ratio of 4:1 is reached. Fibroblasts differentiate into myofibroblasts, causing tissue contraction during this phase of wound healing. Collagen reorganizes along lines of tension and crosslinks, giving added strength, which eventually approaches 80% of the strength of uninjured tissue. Vascularity decreases, producing a less hyperaemic and more cosmetically appealing wound as this phase progresses.[1]

The timetable for wound healing is subject to variations caused by both intrinsic and extrinsic factors; chronic wounds can stall in the inflammatory phase because of poor perfusion, poor nutrition or other factors. Healing may become exaggerated, resulting in keloid and hypertrophic scar formation. Excessive Type III collagen formation in the proliferative phase causes an overgrowth of scar tissue in these wounds. Phases can also be blunted as in foetal tissue, which has a decreased inflammatory phase and heals without scars, which has been shown to result from higher levels of TGF-β_3 than in adults[2] and which is thought to antagonise the effects of TGF-β_1 and TGF-β_2 found to be upregulated in keloids and hypertrophic scars.

1.2 Corneal Lesions

Corneal wounds involve an epithelial defect, exposing the corneal stroma to the external environment. Within minutes after a small corneal epithelial injury, cells at the edge of the abrasion begin to migrate centripetally to rapidly cover the defect at a rate of 60-80 μm/h. In larger defects, a delay of 4-5 hours is required for preparatory cellular changes prior to rapid cell movement. Epithelial cells adjacent to the area of the defect flatten, lose their hemidesmosome attachments, and migrate on transient focal contact zones that are formed between cytoplasmic actin filaments and extracellular matrix proteins. Vinculin, a plasma protein, links fibres to talin – a cell membrane protein – which itself is linked to integrin. Contraction of actin fibres pulls the cell body forward. Vinculin, integrin, fibronectin, fibrinogen, and fibrin are formed continuously and cleaved to allow for cell migration. Plasmin is responsible for cleaving fibrinogen and fibrin at these focal contact zones. The basement membrane is also important for epithelial migration, and abnormalities in basement membrane structure, whether due to trauma or dystrophy (*e.g.* basement membrane dystrophy), can lead to persistence of corneal epithelial defects and stromal ulceration.

After 24-30 hours, mitosis begins to restore epithelial cell population. Basal and limbal stem cells contribute to mitosis. A sufficient supply of progenitor stem cells to facilitate epithelial cell proliferation is important for the cornea and a deficiency of limbal stem cells, from either disease or trauma, can preclude adequate epithelial wound healing.[3]

Stromal wound healing occurs *via* stromal keratocyte migration, proliferation and deposition of extracellular matrix molecules, including Type III collagen, adhesion proteins, and glycosaminoglycans. These processes are facilitated by a phenotypic change among quiescent keratocytes to become active myofibroblasts, a task mediated by transforming growth factor-beta (TGF-β). Invasion of monocytes/macrophages is critical in wound healing; however, in the corneal stroma, excessive infiltration of monocytes/macrophages is unfavourable because they secrete matrix metalloproteinases (MMPs) and other proteins counteracting tissue healing. Numerous cytokines and growth factors that are up-regulated in corneal cells further contribute to tissue inflammation. Barely detected in an unwounded cornea, MMPs are strongly induced during wound healing. Metalloproteinases are secreted as proenzymes by neutrophils infiltrating the wound, injured epithelial cells and keratocytes. They are activated by proteolytic cleavage of the N-terminal region in the extracellular compartment. A relatively higher degree of collagenolysis relative to synthesis is thought to result in degradation, progressive corneal thinning, and, hence, ulceration of the corneal stroma. MMPs are induced at the transcriptional level by various cytokines and growth factors, such as interleukin 1 (IL-1), interleukin 6 (IL-6), tumour necrosis factor α (TNF-α), epidermal growth factor (EGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF) and TGF-β.

2. Cytokines Involved in Wound Healing

Growth factors represent the intercellular signalling that orchestrates the sequence of cell migration, division, differentiation, and protein expression during wound healing. The major families of growth factors are expressed to varying degrees by the cells involved in the healing process and are listed in the table below.[5]

Growth Factor	Production Site	Known Effects
Epidermal Growth Factor (EGF)	Platelets, macrophages	Stimulates fibroblasts to secrete collagenase to degrade the matrix during the remodelling phase. Stimulates keratinocyte and fibroblast proliferation. May reduce healing time when applied topically.
Transforming Growth Factor (TGF)	Platelets, macrophages, lymphocytes, hepatocytes	TGF-α: Mitogenic and chemotactic for keratinocytes and fibroblasts TGF-β_1 and TGF-β_2: Promotes angiogenesis, up-regulates collagen production and inhibits degradation, promotes chemo-attraction of inflammatory cells. TGF-β3 (antagonist to TGF-β_1 and TGF-β_2): Has been found in high levels in foetal scarless wound healing and has promoted scarless healing in adults experimentally when TGF-β_1 and TGF-β_2 are suppressed.
Vascular Endothelial Growth Factor (VEGF)	Endothelial cells	Promotes angiogenesis during tissue hypoxia.
Fibroblast Growth Factor (FGF)	Macrophages, mast cells, T-lymphocytes	Promotes angiogenesis, granulation and epithelialization via endothelial cell, fibroblast and keratinocyte migration, respectively.
Platelet-Derived Growth Factor (PDGF)	Platelets, macrophages, and endothelial cells	Attracts macrophages and fibroblasts to zone of injury. Promotes collagen and proteoglycan synthesis.
Interleukins	Macrophages, keratinocytes, endothelial cells, lymphocytes, fibroblasts, osteoblasts, basophils, mast cells	IL-1: Proinflammatory, chemotactic for neutrophils, fibroblasts, and keratinocytes. Activates neutrophils IL-4: Activates fibroblast differentiation. Induces collagen and proteoglycan synthesis. IL-8: Chemotactic for neutrophils and fibroblasts.
Colony-Stimulating Factors	Stromal cells, fibroblasts, endothelial cells, lymphocytes	Granulocyte colony stimulating factor (G-CSF): Stimulates granulocyte proliferation. Granulocyte Macrophage Colony Stimulating Factor (GM-CSF): Stimulates granulocyte and macrophage proliferation.
Keratinocyte growth factor	Fibroblasts	Stimulates keratinocyte migration, differentiation and proliferation.

In the cornea, epithelial cell migration, proliferation and differentiation are influenced by the stromal keratocyte cytokines, KGF and hepatocyte growth factor, HGF. The cornea is not unique with respect to the stromal-epithelial interactions of these two cytokines, which are mediators of similar interactions in the breast, skin and lung. Although the expression profiles of these cytokines lend themselves toward a linear interpretation of their stromal-epithelial interactions, they are modulated further by the effects of other cytokines and truncated receptors of these molecules: both KGF and HGF mRNA production are altered by the fibroblast cytokines, EGF, TGF-α, PDGF and IL-1. In addition, EGF, PDGF, IL-1α, IL-6 and TNFα at low concentrations appear to enhance fibronectin-induced epithelial cell migration.

Platelets are known to be involved in the healing of epithelial and internal wounds. They have storage pools of growth factors, including platelet-derived growth factors, TGF-β, epithelial growth factors, fibroblast growth factors, insulin-like growth factor I, and vascular endothelial growth factors. Autologous platelet-rich plasma has a large quantity of growth factors that have been found to promote the healing of dormant corneal ulcers and to reduce pain and inflammation.[6]

2.1 Basic Fibroblast Growth factor (bFGF)

In 1974, Gospodarowicz[7] reported that a substance in bovine pituitary extracts stimulated fibroblast growth and named it "fibroblast growth factor" (FGF). The complete amino acid sequences of the basic FGF (bFGF) was determined in the 1980s, enabling production of recombinant bFGF (also known as FGF-2 or FGF-β). In 1990, Kaken Pharmaceutical (Tokyo, Japan) began clinical trials of recombinant human bFGF (trafermin INN), and a product named Fiblast Spray® was launched in Japan in June 2001. Its indications include decubitus ulcers and skin ulcers (burn ulcers and leg ulcers). Several reports have also shown its therapeutic effects on periodontal disease and bone fractures. Essex Biotechnology Ltd (Hong Kong, PRC) developed and launched a series of topical bFGF presentations (Beifushu, Beifuxin and Beifuji) for corneal wound healing in China. The same product is being studied by Essex also for topical cutaneous wound healing and other indications (haemorrhagic stroke, bone fractures).

In humans, 22 members of the FGF family have been identified. FGFs most commonly act as mitogens but also have regulatory, morphological, and endocrine effects. Some level of "promiscuity" is evident in the

overlapping receptor specificity and the four FGF receptor subtypes can be activated by more than one growth factor. The functions of FGFs in development include mesoderm induction, antero-posterior patterning[8], limb development, neural induction and neural development[9] and in mature tissues/systems angiogenesis, keratinocyte organization and wound healing processes. One important function of aFGF and bFGF is the promotion of endothelial cell proliferation and the physical organization of endothelial cells into tube-like structures, promoting angiogenesis. Indeed, aFGF and bFGF are more potent angiogenic factors than vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF).[10] During the development of the central nervous system, FGFs play important roles in neural stem cell proliferation, neurogenesis, axon growth, and differentiation. In addition, bFGF appears to be involved in the regulation of synaptic plasticity and processes attributed to learning and memory, at least in the hippocampus.

2.2 bFGF in Treating Cutaneous Wounds

Topical application of bFGF can promote dermal and epidermal repair[11-13]; in normal tissue, bFGF is present in the basement membranes and in the sub-endothelial extracellular matrix of blood vessels, where it stays membrane-bound in the absence of a trigger – upon injury, the action of heparan sulphate-degrading enzymes activates bFGF, which is released into the microenvironment.

2.2.1 Preclinical Studies

bFGF-knockout mice exhibit no prominent phenotypes under normal conditions. However, they have been reported to exhibit delayed healing of full thickness skin wounds compared with wild-type mice. In addition, the knockout mice showed reduced collagen deposition at the wound site and thicker scabs.[14] Kanazawa *et al.*[15] blocked the effect of bFGF on fibroblast proliferation using mitomycin-C and examined bFGF-induced fibroblast migration in wound healing and reported that bFGF promoted fibroblast migration in a dose-dependent manner *via* the PI3-kinase-Rac1-JNK pathway.

Tattini *et al.*[16] studied radiation-impaired wound healing in a rat model following dorsal skin surface irradiation with 2500 cGy and a full-thickness skin incision followed by treatment with either vehicle, TGF-β, bFGF, or TGF-β and bFGF combined. All irradiated animals had significantly lower ultimate tensile strength than the sham-irradiated control group ($p<0.05$). All three growth factor-treated groups had significantly higher tensile strengths than either of the untreated irradiated groups at 14 days after wounding ($p<0.05$). Histological evaluation of the irradiated groups revealed increased cellularity and more organized collagen architecture of all treated groups when compared with the untreated groups.

2.2.2 Clinical Studies

Zhang *et al.*[17] reviewed randomised controlled trials (RCTs) to investigate efficacy and safety of growth factors in the management of partial-thickness burns. Searches were conducted in PubMed and the Cochrane databases using wound healing and scar formation as search parameters. Thirteen studies comprising a total of 1,924 participants with 2,130 wounds (1,131 patients receiving growth factors versus 999 controls) were identified and included, evaluating the effect of fibroblast growth factor (FGF), epidermal growth factor (EGF) and granulocyte macrophage-colony stimulating factor (GM-CSF) on partial-thickness burns. Topical application of these agents significantly reduced healing time by 5.02 (95% confidence interval, 2.62 to 7.42), 3.12 (95% CI, 1.11 to 5.13) and 5.1 (95% CI, 4.02 to 6.18) days, respectively, compared to standard wound care alone. In addition, scar improvement following therapy with FGF and EGF was evident in terms of pigmentation, pliability, height and vascularity. No significant increase in adverse events was observed in patients receiving growth factors.

Literature reports on the anti-scarring effect of bFGF indicate growing interest on the effects of bFGF "to treat wounds that are difficult to cure" and "to cure wounds without scarring", especially after burn injury.[18- 20] Akasaka *et al.* suggested that, in the remodelling phase of wound healing, bFGF might inhibit scar formation by induction of apoptosis in fibroblasts in granulation tissue.[21] Based on these results, the Japanese Society for Burn Injuries recommended bFGF as a drug to treat second degree burns in the 2009 Japanese Clinical Practice Guidelines for Burn Injuries – despite this recommendation, bFGF is not covered by the Japanese national health insurance system as a treatment for non-ulcer wounds. However, bFGF has also been reported to be effective in treating incisional wounds; Ono *et al.* administered bFGF immediately after surgery in patients who had undergone resection of skin tumours and reported that bFGF significantly inhibited scar formation.[22] There are also reports in which bFGF reduced pre-existing scars: Eto *et al.* examined the effects of bFGF on fibroblasts from normal tissue and hypertrophic scar tissue and observed markedly increased MMP-1 and HGF gene expression.[23] They found that when scar tissue was implanted in nude mice, bFGF promoted the resorption of scar tissue, suggesting that bFGF might be useful in the reduction of scar tissue in pre-existing scars.

The use of bFGF has been reported to shorten the healing period after artificial dermis application at the wound site, such as the fingertip. Sugamata *et al.*[24] used artificial dermis and Fiblast Spray® in six cases of fingertip amputation and found that the shapes of the fingertips were satisfactorily reconstructed in all patients.

Kawai *et al.* developed a type of artificial dermis with controlled release of bFGF using a collagen sponge,

which contained bFGF-impregnated gelatine microspheres[25] and demonstrated its effectiveness in an animal model of wound healing[26,27] and are currently conducting a clinical trial to examine the effects of this product in patients with intractable ulcers.

2.2.3 Clinical Safety of bFGF in Cutaneous Use

At the time of approval of the Fiblast Spray®, the safety database contained a total of 729 patients, out of which 11 patients (1.51%) developed adverse effects. These effects were mainly associated with the drug administration site: irritation and pain in seven cases (0.96%), erythema in three cases (0.41%) and pruritus in three cases (0.41%). Administration of drug resulted in changes in clinical laboratory test values in 41 of 729 cases (5.62%) and comprised ALT (GPT) elevation in 15 of 612 cases (2.45%) and AST (GOT) elevation in seven of 611 cases (1.15%). In post-marketing surveillance, 3411 patients reported 125 adverse events (3.66%) at the completion of re-evaluation of safety and efficacy under the Japanese Pharmaceutical Affairs Law. These effects were mainly excessive granulation tissue formation in 35 cases (1.03%) and pain at the administration site in 8 cases (0.23%).

2.3 bFGF in Treating Corneal Abrasions

2.3.1 Preclinical Studies

Several studies of bFGF and corneal healing following experimental lesions in preclinical models have been published; Hoppenreijs *et al.*[28] concluded that bFGF induces a dose-dependent cell migration response in corneal endothelial cells at concentrations from 1 ng/mL. Hecquet *et al.*[29] studied the effects of both acidic and basic FGF on cell proliferation and DNA synthesis in stromal fibroblasts and epithelial cells of rabbit cornea and found that stromal fibroblasts were more sensitive to aFGF than to bFGF in terms of DNA synthesis. No significant difference was seen in the rate of proliferation. Epithelial cells maintained in medium allowing survival are equally sensitive to aFGF and bFGF regardless of the assay used. Scalinci *et al.*[30] tested bFGF eye drops in mice after bilateral photorefractive laser keratectomy but found only marginal benefit of bFGF over standard therapy (tobramycin, diclofenac and dexamethasone eye drops) in terms of time to complete corneal re-epithelialization.

Rieck *et al.*[31] found that bFGF injected into the anterior chamber of rabbit eyes immediately after wounding produced stimulation of endothelial regeneration, while Fredj-Reygrobellet[32] found that acidic FGF was more potent than bFGF in increasing the rate of wound healing of the cornea; similar results were reported by Yan *et al.*[33] Finally, Hu *et al*. found significantly improved healing rates in dog cornea following experimental lesions from 100 ng/mL of bFGF administered thrice daily for up to 7 days.[34]

2.3.2 Clinical Studies

Despite the preclinical data presented above, a search in Clinical Trials. gov returns no hits for controlled human clinical trials with bFGF in treating corneal lesions across Europe or US. Only one report of a clinical trial with bFGF conducted in China[35] was found in PubMed and compared the effect of the Essex Biotechnology bFGF eye drops to 0.3% hyaluronic acid in 30 subjects with moderate corneal abrasions, randomized to receive either treatment. Efficacy results of this trial suggested superimposable effects of the two treatments, and the absence of a control group therefore makes assessment of the results difficult. No differences in safety or tolerability between the two groups were recorded.

2.4 Bone Fractures

Fibroblast growth factor receptor 1 (FGFR1) is one of the FGF receptors. Mutations in the FGFR1 gene cause Pfeiffer syndrome and mutations in the FGFR2 gene cause Apert syndrome, Crouzon syndrome and Jackson-Weiss syndrome. Constitutively active mutations in the FGFR3 gene cause achondroplasia and Type II thanatophoric dysplasia. These findings have suggested a role for bFGF in bone healing and locally administered bFGF stimulated fracture healing in experimental preclinical models.[36] *In vitro* studies have shown that bFGF stimulates proliferation of undifferentiated osteoblast progenitor cells and bone marrow stromal cells. Kawaguchi *et al.*[37] performed a trial in 194 patients to investigate the effectiveness of bFGF on bone union after osteotomy. In this trial, a gelatine hydrogel was used to generate a controlled release of bFGF into knee osteoarthritis patients during upper tibial osteotomy. bFGF was reported to accelerate bone healing in a dose-dependent manner at 16 weeks after surgery and shortened the time until bone union. In terms of safety, there were no differences in the type ad frequency of adverse events in the treated *vs.* placebo groups.

2.5 Periodontal Disease

In canine experimental models, bFGF administration has been shown to stimulate regeneration of periodontal tissue and triggered a Phase II trial in 2001 to investigate the safety and efficacy of bFGF to induce periodontal tissue regeneration.[38] This was a placebo-controlled trial and involved a dose response element and was conducted in 13 institutions in Japan. Radiographic findings showed that local administration of bFGF induced significant new alveolar bone formation in 2- and 3-walled alveolar bone defects. In 2005, a larger Phase II trial involving 25 institutions in Japan showed that bFGF induced significant new alveolar bone formation.[39] Both these trials reported that bFGF was safe and well tolerated.

2.6 Cardiovascular Disease

bFGF has received attention with respect to its angiogenic potential in the cardiovascular area and while therapeutic angiogenesis has a clear role in salvaging chronically ischemic myocardium, more acute treatment

modalities are being sought to increase cardiac resistance to injury (*i.e.* preconditioning) and to protect against secondary injury after an acute ischemic insult. Experimental models have suggested that bFGF may have a cardioprotective role in this setting in enhancing myocardial and peripheral extremity perfusion and function within ischemic regions that are not amenable to traditional modes of revascularization.[40-42]

Takagi *et al.*[43] studied the safety and efficacy of controlled-release bFGF for peripheral artery disease (PAD), compared with autologous bone marrow mononuclear cell implantation (BMCI). PAD patients were divided into a bFGF group (n=10) and BMCI group (n=15). Patients were treated with gelatine hydrogel containing 600 μg bFGF or BMCI. A visual analogue pain scale (VAS), ^{99m}Tc-tetrofosmin scintigraphy, trans-cutaneous oxygen tension, and ankle-brachial index (ABI) were evaluated before and 4 weeks after each treatment and at 2 years. Results suggested that both treatment groups experienced similar and significant clinical benefit in term of VAS and transcutaneous oxygen tension, while scintigraphy and ABI were not changed. Safety was comparable in the two groups.

Kumagai *et al.* investigated the safety and efficacy of a sustained-release system of bFGF using biodegradable gelatine hydrogel in patients with critical limb ischemia (CLI) in a Phase I/II study that analyzed 10 CLI patients following a 200-μg intramuscular injection of bFGF-incorporated gelatine hydrogel microspheres into the ischemic limb. Primary endpoints were safety and transcutaneous oxygen pressure at 4 and 24 weeks after treatment. During follow-up, there was no death or serious procedure-related adverse event. After 24 weeks, the transcutaneous oxygen pressure showed significant ($p<0.01$) improvement as well as the distance walked in 6 min, the Rutherford classification, the rest pain scale and the cyanotic scale also showed significant improvement (all $p<0.05$). Blood levels of bFGF were within the normal range in all patients. A subanalysis of patients with arteriosclerosis obliterans (n = 7) or *thromboangiitis obliterans* (Buerger's disease) (n = 3) revealed that the transcutaneous oxygen pressure had significantly improved in both subgroups.[44]

2.7 Other Uses

Kanazawa *et al.*[45] treated five cases of severe vocal fold sulcus, six cases of vocal cord scars, seven cases of vocal cord paralysis, and 17 cases of vocal cord atrophy using a local injection of bFGF. The injection regimen involved injecting 50 μg of bFGF dissolved in 0.5 mL saline as a single dose into the superficial lamina propria using a 23-gauge needle. Phonological outcomes were evaluated at two or three months following the procedure. The maximum phonation time (MPT), mean airflow rate, pitch range, speech fundamental frequency, jitter, and voice handicap index improved significantly after the bFGF injection. Furthermore, improvement in the MPT was significantly greater in patients with (in increasing order): vocal fold atrophy, scar, and paralysis. The improvement in the MPT among all patients was significantly correlated with age; the MPT improved relatively more in younger patients.

Acharya *et al.*[46] conducted a study to investigate the utility of bFGF in tympanic membrane perforation (TMP) closure in a small cohort of paediatric patients. Response to treatment was monitored with serial otoscopy and audiometric outcomes were determined. TMPs were successfully closed in 7/12 children at the first attempt (58%) and in 10/12 children overall (83%). Hearing improvement was observed in 8/10 successfully treated cases (80%). There were no complications or adverse outcomes and the authors concluded that minimally invasive bFGF treatment is an alternative to conventional myringoplasty in paediatric patients with comparable success and reduced morbidity and cost.

3. DISCUSSION

Currently, cytokines have a limited role in wound healing in clinical practice in Europe and the US, but appear to be more widely used in Asia. The only growth factor product approved for wound healing is a PDGF-BB formulation (Regranex®, becaplermin INN), which has been available in the US since 1997 to treat diabetic foot ulcer[47,50]; this product was available also in Europe from 1999, but was voluntarily withdrawn in 2012. A similar product with the same active ingredient was refused registration in the EU in 2010 for periodontal use on the basis of a weak benefit/risk ratio.

In Asia, many other cytokines are currently under development, including TGF-α, EGF, and IGF-1[47,48] aside from the already marketed Fiblast Spray® (Japan) and the Beifushu series from Essex Biotechnology Ltd (China).

A general concern with all growth factor-based therapies is promotion of tumour growth; in animal models, bFGF injected at the tumour inoculation site induce marked tumour growth and lymph node metastasis, correlating with an increase in neovascularization in the host stroma. Tumour growth, pulmonary metastasis and intensive neovascularization in tumour parenchyma have been observed even after a single administration of bFGF at the inoculation site.[see *e.g.* 49]

Increased mortality from malignancies has been reported in patients treated with becaplermin, also involving tumours distant from the site of application. In post-marketing surveillance studies, 491 (75%) of 651 subjects from two randomized, controlled trials of becaplermin gel 0.01 % were followed for a median of approximately 20 months to identify malignancies diagnosed after the end of the trials. Eight of 291 subjects (3%) from the becaplermin group and two of 200 subjects (1 %) from the standard of care group were diagnosed with cancers during the follow-up period, a relative risk of 2.7 (95% confidence interval 0.6-12.8);

the types of cancers varied and all were remote from the treatment site. As a result, the Regranex® product is associated with a Black Box warning in the US[50], while the EMA requested exclusion of all patients with a pre-existing cancer.[51] These concerns are likely to be more pronounced in relation to systemic treatment approaches (*i.e.* cardiovascular, bone fractures), even though the permanence of systemically administered FGF is likely to be short in the circulation.

Specifically in the case of ophthalmological use, another general concern of growth factor therapy is corneal neovascularisation, which would represent an absolute contraindication of FGF therapy for corneal lesions.

As for all biological products, risks relating to immune reactions from the bFGF itself or relating to contaminants also need to be factored into the risk assessment. Patients treated with becaplermin did not develop neutralizing antibodies, but no other data on immunogenicity are available for review.

Against this background, it may be concluded that bFGF may have therapeutic utility in cutaneous wound healing and plastic surgery, while the ophthalmic utility appears less substantiated by published data, where no specific advantages of bFGF treatment have appeared as compared to current standard of care. The concerns regarding tumour promotion likely represent a significant hurdle, and may preclude systemic use (cardiovascular; bone healing) until large-scale and long-term safety studies have been completed to allow a more stringent risk/benefit analysis.

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