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BECAPLERMIN: A NEW EFFECTIVE AND SAFE ADJUVANT TOPICAL THERAPY IN PATIENTS WITH CHRONIC NEUROPATHIC DIABETIC FOOT ULCER

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BEKAPLERMIN: NOVA I EFIKASNA ADJUVATNA LOKALNA TERAPIJA BOLESNIKA SA HRONIČNIM NEUROPATSKIM DIJABETESNIM ULKUSOM STOPALA

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SAŽETAK

Zarastanje rana je kompleksan i dobro koordinisan biološki process koji protiče kroz tri faze: inflamaciju, proliferaciju i maturaciju odnosno remodelovanje. Hronične rane su rane kod kojih je prekinut normalan proces zarastanja. U hronične rane spada i dijabetesni ulkus stopala koji nastaje zbog periferne neuropatije, lokalne traume i ishemije. U procesu zarastanja rana značajno mesto pripada faktorima rasta. Jedan od najznačajnijih faktora je trombocitni faktor rasta koji je aktivan u svim fazama zarastanja rana. Bekaplermin (0,01%) gel je rekombinantni humani trombocitni faktor rasta. Biološka aktivnost bekaplermina slična je prirodnom humanom trombocitnom faktoru rasta-BB. Stimulacija hemiotakse i proliferacije ćelija koje učestvuju u procesu zarastanja rana su najvažnije zajedničke osobine bekaplermina i prirodnog humanog trombocitnog faktora rasta-BB. Bekaplermin primenjen lokalno jedanput dnevno u toku 20 nedelja ili do potpunog zatvaranja rana ubrzava i skraćuje vreme zarastanja neuropatskog dijabetesnog ulkusa stopala. Uslovi za uspešnu lokalnu primenu bekaplermina su da postoji adekvatna oksigenacija (transkutani parcijalni pritisak kiseonika treba da bude bar 30mmHg ili viši na dorzumu stopala ili na ivicama ulkusa), debridman, kontrola lokalne infekcije i rasterećenje pritiska. Bekaplermin gel, primenjen lokalno, ne izaziva bilo kakva ozbiljna neželjena lokalna ili resorptivna dejstva. Minimalna je sistemska resorpcija, ne deluje kancerogeno, ne izaziva fibrozu na mestu aplikacije i ne pogoršava dijabetesnu neuropatiju.

Ključne reči: zarastanje rana, faktori rasta, bekaplermin, prirodni humani trombocitni faktor rasta, neuropatski dijabetesni ulkus stopala.

Skraćenice: PDGF - Trombocitni faktor rasta, bFGF - Bazični fibroblastni faktor rasta, EGF - Epidermalni faktor rasta, TGF - Transformišući faktor rasta, IGF-1 - Insulinu sličan faktor rasta-1, TNF - Tumor nekrozis faktor, IL-1 - Interleukin-1, IL-6 - Interleukin-6

ABSTRACT

Wound healing is a complex and well coordinated biologic process that involves inflammatory, proliferative and maturation or remodeling phases. A chronic wound results when the normal process of wound healing is interrupted. Diabetic foot ulcer is also a chronic nonhealing wound resulting as a consequence of peripheral neuropathy, local trauma and ischemia. Current research and clinical evidence revealed a fundamental role of growth factors in the biology of wound healing process. Among them one of the most important is the platelet derived growth factor active in the all phases of healing process. Becaplermin (0.01%) is the recombinant human platelet derived growth factor. The biologic activity of becaplermin is similar to native human platelet derived growth factor-BB. Promotion of chemotactic recruitment, as well as the proliferation of cells involved in the wound repair, are the common characteristics of becaplermin and the indigenous human platelet derived growth factor-BB. Becaplermin, topically applied, once daily for 20 weeks or complete healing successfully stimulates the incidence of complete wound closure and decreases the time of complete wound closure of neuropathic diabetic foot ulcer. To ensure that efficacy and healing of diabetic foot ulcers using becaplermin it is essential to ensure adequate peripheral circulation (transcutaneous partial pressure of oxygen pressure at least or more than 30mmHg on the foot dorsum or at the margin of ulcer), sharp debridment, pressure relief and local infection control. Topical becaplermin has no serious local or systemic unwonted effects. The systemic absorption appears to be minimal, it does not produce cancer at the site of application and fibrosis and it does not worsen diabetic neuropathy.

Key words: wound healing, growth factors, becaplermin, indigenous human platelet derived growth factor, neuropathic diabetic foot ulcer.

Abbreviations: PDGF - Platelet derived growth factor, bFGF - Basic fibroblast growth factor, EGF - Epidermal growth factor, TGF - Transforming growth factor, IGF-1 - Insulin like growth factor-1, TNF - Tumor necrosis factor, IL-1 - Interleukin-1, IL-6 - Interleukin-6

Becaplermin (0.01% Regranex® gel) is the recombinant platelet derived growth factor (rh-PDGF-BB). Becaplermin has similar biological activity of endogenous PDGF-BB. Two main actions are: promotion of chemotactic recruitment and proliferation of cells subserving the wound healing process. Growth factors are peptides and represent one subgoup of cytokines.

For better understanding the mechanisms of action of becaplermin first a brief summary of various factors (cytokines, growth factors) subserving the process of wound healing and characteristics of healing cascade, acute and chronic wounds, neuropathic diabetic foot ulcer will be reviewed. Thereafter, in more details, biological activities, mechanisms of action, as well as clinical studies in humans will be described.

CYTOKINES

Specific proteins that are used for cell communications are called „cytokines“. They are synthesized and secreted by macrophages, lymphocytes, endothelial cells, fibroblasts, smooth and sceletal muscle cells. They control and modulate inflammatory and immune processes as well as growth and differentiation of hemopoetic, epithelial and mesenchymal cells. Cytokines have multiple effects on different cell types and, therefore, the actions of two cytokines may overlap. Cytokines and growth factors are secreted in minute amounts from one cell. Thereafter they bind to a cellular receptor of the nearby cell („paracrine“), to its own receptor („autocrine“), via blood stream in areas distant from the original cell („endocrine“) and through direct cell contact („juxtacrine“)(1, 2).

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GROWTH FACTORS

In the broadest sense, the term growth factors is used for substances with chemotactic properties to attract proinflammatory cells and fibroblasts into the wound, to promote cellular proliferation, angiogenesis, production and degradation of the extracellular matrix, and synthesis of cytokines and growth factors by neighboring cells. However, these multiple effects are not stimulant for all cells. For instance, TGF-beta stimulates the mitosis of cells deriving from mesoderm (fibroblasts), while inhibits the mitosis of cells derived from ectoderm (keratinocytes). The most important growth factors for the wound healing are families of PDGF, EGF, FGF, TGF and IGF (2, 3).

The binding of growth factors to cell surface receptors initiates the specific cell transduction pathways. This process produces changes in gene expression what leads to new protein synthesis, changes in cellular activity or proliferation (2, 3). Growth factors promote, generally, wound healing via several mechanisms (2):

- chemotaxic activities attraction of inflammatory cells and fibroblasts into the wound,
- stimulation of cellular proliferation,
- angiogenesis (ingrowth of new blood vessels),
- production and degradation of extracellular matrix, and
- synthesis of cytokines and growth factors by neighboring cells.

WOUND HEALING

Wound healing is a complex biologic process that optimally leads to restoration of tissue integrity and function. The process is very orderly and well coordinated. Generally, the healing process progresses through three characteristic stages: inflammatory phase, proliferative (granulation tissue formation), and repair (scar formation stage) and remodelling stage. Inflammation begins immediately upon tissue injury, thereafter migration of fibroblasts and other cells into the site of injury, as well as the initial scar formation initiate the proliferative stage. Finally after initial scar formation, proliferation and neovascularization cease, the wound enters the terminal remodelling stage (1). The stages of wound healing overlap temporarily and the entire process can last for several months. Usually, the inflammation process lasts several days, the proliferative stage several weeks, whereas the remodelling phase several months (1).

ACUTE WOUNDS AND GROWTH FACTORS

Acute wounds are generally less complex than chronic wounds. The spontaneous healing of acute wounds does not represent a serious problem in clinical practice. However, the rate of spontaneous healing is not the highest rate that can be achieved. For instance, topical application of EGF, TGF-alpha, PDGF and IGF accelerates the rate of epidermal full or partial thickness wound healing in experimental animals approximately for 20% to 30% than vehicle controls, whereas topical EGF accelerates the epithelial wound healing in humans by average of 1.5 day (1). It appears, therefore, that the amounts of peptide growth factors are probably not optimal in spontaneous healing skin wounds. The clinical benefit, therefore, of topical growth factors in healing wounds would be negligible in a small wound, but not in large size wounds.

Interestingly, apart from qualitative differences in growth factors, quantitative changes also exist in fluids of acute and chronic healing wounds. As a rule, contrary to chronic healing wounds, inflammatory cytokines, proteases and growth factors occur in relatively small amounts in spontaneously healing wounds.

CHRONIC WOUNDS AND GROWTH FACTORS

When the normal healing process is interrupt a chronic wound occurs. Chronic skin and soft tissue wounds are classified as diabetic foot ulcer, decubitus ulcer, venous stasis and ischemic ulcer caused by arterial insufficiency. Many different causes underlay the chronic wounds. The most common causes are similar in that each is characterized by one or more persistent stimuli, repeated trauma, ischemia or low grade bacterial contamination (4–7). In contrast to acute wounds, in chronic wounds the levels of inflammatory cytokines are very high, whereas the amounts of growth factors are decreasing. Multiple possible causes, such as the presence of bacteria and their endotoxins, degradation products of platelets and degradation products of extracellular matrix trigger the production of inflammatory cytokines like TNF and interleukins (IL-1, IL-6)(2, 3). The increased production of these inflammatory cytokines leads to a concomitant elevation of production of proteases. The increase of proteases, including matrix metalloproteinases, leads to degradation of growth factors. When the growth factors are drastically reduced the communication between the various cells participating in the process of healing stops and the wound healing is delayed. Therefore, it is not surprising that nonhealing wounds have been described as being „stacked“ in the inflammatory stage. In fact, healing process only after the inflammation is controlled (5, 9, 10).

DIABETIC FOOT ULCERS

Etiology and pathogenesis of chronic wounds appear to be rather heterogeneous and similarity exists in that each is characterized by one or more persistent inflammatory stimuli. Diabetic foot ulcers typically occur when there is excessive and repetitive mechanical stress to a part of the foot (forefoot, heal), because of the lack of sensitivity caused by neuropathy. This leads to ischemia, injury and desintegration of the skin and soft tissue. Infection further complicates the underlying foot ulcer. Degraded tissue fragments and bacteria, as well as their products such as endotoxins (lipofysaccharide) stimulate the synthesis and release of inflammatory cytokines supporting and maintaining further the infection (9, 11).

BECAPLERMIN (RECOMBINANT PLATELET DERIVED GROWTH FACTOR)

Chemistry

PDGF is a dimer consisting of A and/or B chains held together by a disulfide bond. From human platelets AA, BB and AB isomers have been isolated. The most potent isomer is BB (2, 12, 13).

Using recombinant DNA technology a homodimeric protein was produced by inserting the gene for the B chain of human PDGF into the yeast Saccharomyces cerevisiae. The recombinant PDGF-BB (rh-PDGF-BB) of becaplermin has biological activity similar to endogenous PDGF-BB (2, 14).

Receptors

Two cell surface PDGF receptors have been identified to date: alpha- and beta-PDGF receptors. The alpha-PDGF receptor is non-specific and binds to all isoforms of PDGF. On the other hand, beta-PDGF receptor selectively binds only to PDGF-BB isoform. The most common type of receptors is beta PDGF cell surface receptor (14, 15).

Synthesis and release

Platelets, macrophages, endothelial cell, fibroblasts, keratinocytes, smooth and skeletal muscle cells and astrocytes synthtize and release PDGF (10, 13).

Mechanism of action

After binding of PDGF to its receptor dimerization leads to tyrosine autophosphorylation, intracellular transduction, induction of early response genes (c-myc, c-fos) and the range of cellular responses, such as new protein synthesis, changes in cellular activity and proliferation (1, 9, 16).

Biological activities

Biological activities of becaplermin are similar to that of endogenous PDGF-BB promoting the chemotaxic recruitment and proliferation of cells subserving the wound-healing cascade (2, 13, 14). The basic effect of PDGF-BB and becaplermin is the promotion of wound healing. PDGF-BB is actively involved in all stages of wound healing process.

The promotion of wound healing process is ascribed (2, 16) to:

- chemotaxic effect (migration of neutrophils, monocytes, fibroblasts and smooth muscle cells into wound),
- stimulation the synthesis of fibroblasts and extracellular matrix, and
- stimulation the proliferation of smooth muscles.

Other (non-dermal) effects include (16):

- antiinflammatory effect,
- smooth muscle hypertrophy of uterus,
- lens growth and transparency and
- central nervous system gliogenesis.

Clinical studies

Almost two decades ago it has been shown that growth factors promote all kinds of wound healing in experimental animals. The stimulation of wound healing in healthy animals is negligible. However, healing occurs in animals when the host defense mechanisms are impaired. Growth factors, including PDGF-BB, ameliorate healing in animals impaired by diabetes (2, 17, 18), malnutrition (19), infection (20), hypoxia (21), chemotherapy agents (22), steroids (23) and irradiation (24). Curiously, PDGF-BB ameliorated the wound breaking strength and accelerated the healing (16). In fact, PDGF-BB markedly increased the intensity of inflammatory phase of the wound healing cascade, characterized by an increased presence of inflammatory cells (neutrophils, monocytes and fibroblasts). At the same time, the production of granulation tissue increased as well (13, 16).

Clinical trials on the efficiacy and safety of PDGF-BB and becaplermin in patients with diabetic foot ulcer began also almost twenty years ago. The earliest clinical studies, although performed on relatively small number of patients, revealed that PDGF-BB improves the healing of chronic wounds being most effective in patients with neuropathic diabetic fool ulcer (7, 25, 26). Later, as well as current clinical studies confirmed the effcacy and safety of PDGF-BB and becaplermin in the treatment of patients with neuropathic diabetic foot ulcer (8, 27–31). Some of these trials were multicentric and meta-analysis was performed to asses the results (28, 32).

Most thoroughly it will be described the most characteristic study. This was a randomized, placebo-controlled, double-blind study (Phase III). The study assessed and compared the efficacy and safety of rh-PDGF-BB gel versus placebo in the local treatment of patients with chronic neuropathic diabetic foot ulcer (27). Becaplermin gel (0.01%) was applied topically once a day in doses of 30 μg/g (132 patients) and 100 μg/g (123 patients). Placebo gel (9127 patients) was also applied once daily. Good ulcer care in all groups was performed as well. Dressings were changed twice daily and consisted of saline-soaked gauze. In comparison to placebo gel (34.6%) becaplermin only in the highest doses of 100 μg/g increased the incidence of complete wound healing by 49.5% ($p=0.007$). At the same time, becaplermin only in the highest doses of 100 μg/g accelerated the time to healing (86 days) compared to placebo treated group (127 days; $p=0.013$). It is concluded that becaplermin gel 100 μg/g when combined with good wound care, significantly increased the number of wounds healed and decreased the time to complete lesion closure. However, it remained unclear why the lower doses of 30 μg/g did not prove as effective as the highest dose of 100 μg/g of rh-PDGF-BB. The safety of topical becaplermin was confirmed.

Interestingly, there is a relationship between ulcus debridment and the effect of becaplermin. Grossly, a lower rate of healing was seen in those studies performing less frequent surgical debridment (33). On the other hand, in the placebo group there was no relationship between

the healing rate and frequency of debridment. A hypothesis has been advanced that debridment removes tissue containing cells that no longer respond to the action of growth factors (33).

Comparative studies also demonstrated that topical becaplermin gel (0.01%; 100 μg/g) and good wound care produced the highest rate of incidence of healing (44.1%) in comparison to topical carboxymethylcellulose gel (35.7%) and good wound care, and patients with only good wound care (22%) by 20 weeks (31). Topical carboxymethylcellulose gel is a chemically modified dressing material designed to „take out" proteases of chronic wound fluid (34). In this connection, it should be also mentioned that topical lipid calf thymic extract accelerates the wound repair in healing impaired patients (35). On the other hand, contrary to rh-PDGF-BB, the growth factor bFGF, essential also for the healing cascade, applied topically (0.25–0.75 μg/cm^3) does not accelerate the healing in patients with chronic neuropathic diabetic foot ulcers (36).

Indications

Chronic neuropathic diabetic foot ulcus extending in the subcutaneous tissue (stage III and IV) with adequate tissue oxygenation (transcutaneous partial pressure of oxygen of 30 mmHg or greater on the foot dorsum or at the margin of the ulcer) and a wound management program (sharp debridment, pressure relief and infection control) at present is the only indication for topical becaplermin.

Unwanted effects

Serious local or systemic effects of topical application of becaplermin gel (0.01%) have not yet been reported. The systemic absorption is negligible. The peptide does not cause cancer, fibrosis and does not worsen the diabetic retinopathy.

Clinical use

Becaplermin is applied topically as a gel (0.01%; 100 μg/g) once a daily to a clean, dried wound in a thin layer for 20 weeks or complete healing. The patient at home can apply the gel. A moist healing environment, debridment of all necrotic debris and callus, control of infection and pressure relief of affected area are essential conditions for the application of becaplermin gel. The thin layer of becaplermin gel has to be covered with a moist gauze dressing. Debridment, a sharp incision with subsequent bleeding, at the site of the wound is a fundamental condition since there is evidence that debridment removes cells no longer responding to the action of growth factors, as well as that platelets from the bleeding surface release PDGF into the wound.

In conclusion, becaplermin (rh-PDGF-BB) is the first commercially available growth factor, a new highly effective and safe adjuvant, topical drug treatment of chronic neuropathic diabetic foot ulcer if used under optimal wound-healing (debridment of all necrotic debris and callus, control of infection and pressure relief of affected area) environment. Serious unwanted effects have not yet been reported from the topical application of becaplermin.

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