Primljen/ Recived on: 19.02.2015.
Revidiran/ Revised on: 03.04.2015
Prihvaćen/ Accepted on: 23.10.2015.

INFORMATIVNI RAD
INFORMATIVE ARTICLE
doi:10.5937/asn1572524S

ULOGA FAKTORA RASTA U ZARASTANJU EKSTRAKCIONE RANE

THE ROLE OF GROWTH FACTORS IN EXTRACTION WOUND HEALING

Simona Stojanović[1], Miloš Tijanić[1,2], Goran Jovanović[1,2], Milan Spasić[2,4], Branislava Stojković[1,3], Milica B.Petrović[4],Tijana Denčić[1,5]

[1] UNIVERZITET U NIŠU, MEDICINSKI FAKULTET NIŠ, SRBIJA
[2] KLINIKA ZA STOMATOLOGIJU, ODELENJE ZA ORALNU HIRURGIJU, NIŠ, SRBIJA
[3] KLINIKA ZA STOMATOLOGIJU, ODELENJE DEČJE I PREVENTIVNE STOMATOLOGIJE, NIŠ,SRBIJA
[4] UNIVERZITET U NIŠU, MEDICINSKI FAKULTET, STUDENT DOKTORSKIH STUDIJA
[5] CENTAR ZA PATOLOGIJU I PATOLOŠKU ANATOMIJU,NIŠ,SRBIJA

[1] UNIVERSITY OF NIŠ, FACULTY OF MEDICINE NIŠ
[2] CLINIC OF DENTISTRY, DEPARTMENT OF ORAL SURGERY, NIŠ, SERBIA
[3] CLINIC OF DENTISTRY DEPARTMENT FOR CHILDREN AND PREVENTIVE DENTISTRY
[4] Ph.D.STUDENT, FACULTY OF MEDICINE ,UNIVERSITY OF NIŠ
[5] CENTER FOR PATHOLOGY AND PATHOLOGICAL ANATOMY, NIŠ, SERBIA

Sažetak

Zarastanje rane je složeni proces koji uključuje hemostazu, inflamaciju, proliferaciju i remodelaciju tkiva. Faktori rasta su prirodni biološki posrednici koji regulišu najznačajnije ćelijske procese uključene u regeneraciju tkiva, kao što su DNA sinteza, angiogeneza, metabolička aktivnost, migracija, hemotaksa, proliferacija, diferencijacija i sinteza matriksa. Najznačajniji faktori rasta koji učestvuju u zarastanju ekstrakcione rane i regeneraciji koštanog tkiva su: trombocitni faktor rasta-PDGF, transformacioni faktor rasta-TGF β ,faktor rasta sličan insulinu-IGF, koštani morfogenetski proteini-BMP-2, BMP – 7 , vaskularni endotelijalni faktor rasta, VEGF, ,fibroblastni faktor rasta-FGF. Faktori rasta pojavljuju se u različitim koncentracijama u različito vreme, pa se na osnovu njihovog prisustva može proceniti starost rane. Osim zarastanja rane faktori rasta mogu se primeniti za bolju oseointegraciju implanata,augmentaciju alveolarnog grebena, alveolita itd. Studije fizioloških procesa u kojima faktori rasta imaju regulatornu ulogu ukazuju da ovi molekuli retko kada svoje aktivnosti vrše u biološkoj izolaciji. Proučavanje interakcije između faktora rasta u alveolarnoj kosti može pružiti objašnjenje o sposobnosti tkiva da zaraste i pod nepovoljnim uslovima, kao što suinfekcija i zračenje

Ključne reči: *faktori rasta, ekstrakciona rana, zarastanje*

Corresponding author:
Simona Stojanović, DDS,
Faculty of Medicine Niš,
Dr Zoran Djindjić Bld 81,
Niš, Srbija
E-mail: tarana.simona@gmail.com

Abstract

Wound healing is a complex process that includes hemostasis, inflammation, proliferation and tissue remodeling. Growth factors are natural biological mediators that regulate crucial cellular processes involved in the tissue repair, such as DNA synthesis, angiogenesis, metabolic activity, migration, chemotaxis, proliferation, differentiation and matrix synthesis. The most important growth factors that play a part in the extraction-wound healing process and bone tissue regeneration are: platelet - derived growth factor-PDGF, transforming growth factor Beta-TGF β ,insulin- like growth factor-IGF, bone morfogenetic protein-BMP-2, BMP– 7 ,vascular endothelial growth factor- VEGF, fibroblast growth factor-FGF. Growth factors appear at various concentrations at different times, so that the wound age may be estimated by their age. Execpt wound healing growth factors may be used in better oseointegration of imlants, alveolar ridge augmentation, alveolit etc. Studies of physiological processes in which growth factors have a regulatory role indicate that these molecules rarely act in biological isolation. The study of the interaction between the growth factors in the alveolar bone can explain tissue ability to heal even under adverse conditions, such as infection and radiation.

Key words: *growth factors, extraction wound, healing*

Uvod

Zarastanje rane je složeni proces koji uključuje hemostazu, inflamaciju, proliferaciju i remodelaciju tkiva. U procesu zarastanja rane učestvuju različiti tipovi ćelija, složena signalizaca događaja i brojni faktori rasta.

Faktori rasta su prirodni biološki posrednici koji regulišu najznačajnije ćelijske procese uključene u regeneraciju tkiva, kao što su DNA sinteza, angiogeneza, metabolička aktivnost, migracija, hemotaksa, proliferacija, diferencijacija i sinteza matriksa. Faktori rasta su ćelijski specifični, što znači da svaki faktor deluje na određenu vrstu ćelija [1] .

Najznačajniji faktori rasta koji učestvuju u zarastanju ekstrakcione rane i regeneraciji koštanog tkiva su:

1. trombocitni faktor rasta – PDGF;
2. transformacioni faktor rasta – TGF β superfamilija;
3. insulinu sličan faktor rasta – IGF;
4. koštani morfogenetski proteini – BMP – 2, BMP – 7 ;
5. vaskularni endotelijalni faktor rasta – VEGF;
6. fibroblastni faktor rasta – FGF;

Polipeptidni faktori rasta ispoljavaju svoje dejstvo vezujući se za target ćelije preko svojih aktivnih krajeva ”dimera” za tirozin-kinazu, transmembranski receptor na površini ćelijske membrane[2]. Aktivirani T-K trans-membranski receptor pokreće energetsku aktivnost ćelije podizanjem intracelularne koncentracije cirkularnog adenozin- monofosfata i dovodi do oslobađanja intracitoplazmatičnih proteina prenosioca signala. Oslobođeni od transmembranskih receptora tirozin-kinaze, proteini prenosioci signala odlaze do nukleusa, gde otključavaju specifičnu genetsku sekvencu za kontrolu ćelijske funkcije i indukciju ekspresije normalne genetske aktivnosti.

Povreda tkiva praćena je inflamatornim odgovorom, aktiviranjem komplemenata i oštećenjem krvnih sudova koje uzrokuje krvaranje. Posle povrede tkiva, pokreće se kaskadni proces koagulacije krvi koji vodi u formiranje fibrinske mreže kao osnove za nakupljanje uobličenih krvnih elemenata. Na mestu povrede tkiva dolazi do adhezije i agregacije trombocita, a njihovom degranulacijom oslobađaju se mnogobrojni glikoproteini i faktori rasta kao što su faktor rasta poreklom iz trombocita (PDGF), epidermalni faktor rasta (EGF), transformišući faktor rasta beta (TGF-β), vaskularni endotelijalni faktor rasta (VEGF) i bazni fibroblastni faktor rasta (bFGF) [3,4].

Mnoštvo faktora rasta oslobođenih tokom hemostaze ima različite uloge, uključujući

Introduction

Wound healing is a complex process that includes hemostasis, inflammation, proliferation and tissue remodeling. The process involves different types of cells, complex signaling events and a number of growth factors.

Growth factors are natural biological mediators that regulate crucial cellular processes involved in the tissue repair, such as DNA synthesis, angiogenesis, metabolic activity, migration, chemotaxis, proliferation, differentiation and matrix synthesis. Growth factors are cell-specific, with each factor regulating particular type of cells[1].

The most important growth factors that play a part in the extraction wound healing process and bone tissue regeneration are:

1. Platelet-derived growth factor (PDGF)
2. Transforming growth factor - TGF-β superfamily
3. Insulin-like growth factors (IGF)
4. Bone morphogenetic proteins - BMP- 2, BMP- 7
5. Vascular Endothelial Growth Factor (VEGF)
6. Fibroblast growth factor (FGF) .

Polypepitide growth factors work by binding to tyrosine kinase[2] - transmembrane receptor on the surface of the target cell membrane - via their active parts called "dimers"[2]. The activated T-K transmembrane receptor triggers the cell activity by raising the intracellular concentration of circular adenosine monophosphate and leads to the release of intracytoplasmic transducer signaling proteins. Transducer signaling proteins, once disengaged from the tyrosine kinase transmembrane receptors, proceed to the nucleus where they unlock specific gene sequences for controlling the cell function and inducing the expression of normal gene activity.

Tissue injury is accompanied by the inflammatory response, complement activation and blood vessels damage that leads to bleeding. This is followed by blood coagulation cascade and fibrin network formation as a structural framework for the blood elements accumulation. Plateletadhesion and aggregation together with their degranulation at the site of injury infuence the releases of numerous glycoproteins and growth factors, such as: platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor

podsticanje i aktivaciju monocita, neutrofila i makrofaga na mestu rane sa pokretanjem faze zapaljenja.Nastaje endotelijalna vaskulogeneza i organogeneza, a fibroblasti se regrutuju na mestu povrede i tako formiraju ekstracelularnu matricu za dalji proces zarastanja i remodelaciju tkiva[5]. Više tipova ćelija neophodnih za zarastanje rane potiče iz raznih depoa, uključujući i koštanu srž i krvne sudove, bazalni sloj epitela, folikul dlake i masno tkivo, koji su bogati matičnim ili progenitornim ćelijama, koje se diferenciraju u određene tipove ćelija, utičući na selekciju signalnih molekula ili faktora rasta tokom zarastanja.

Tokom proliferativne faze fibroblasti infiltriraju ranu i proizvode matriks metaloproteinazu MMP[6] i interraguju sa ekstracelularnim proteinima i na taj način podspešuju granulaciju. Fibroblasti dalje podležu fenotipskoj promeni u miofibroblaste koji poravnavaju ivice rane, generišući kontraktilnu silu i olakšavajući na taj način zatvarnje rane.

Kolagena vlakna granulacionog tkiva deluju kao provizorni matriks na koji se vezuju nedifrentovane osteoprognitorne ćelije koje će se pod dejstvom faktora rasta, pre svega BMP, diferentovati u hondrocite i osteoblaste. Hematopoetske matične ćelije i prekursori endotelnih ćelija vode zajedničko poreklo od matičnih ćelija hemangioblasta i prisutne su u koštanoj srži.

Remodelacija je završna faza procesa zarastanja rane i podrazumeva regresiju krvnih sudova i zamenu granulacionog tkiva ekstracelularnim matriksom i njegovu rekonstrukciju.

1. Trombocitni faktor rasta PDGF

PGDF familija se sastoji od pet različitih disulfidnih dimera sastavljenih od četiri polipeptidna lanca formirajući izoforme PGDF-AA, PGDF-BB, PGDF-AB, PGDF-CC, PGDF-DD [7]. PGDF-BB homodimer biološki je aktivniji od drugih izoformi AA i AB. PGDF AB i BB funkcionišu kao sistemski faktori rasta, dok PGDF AA deluje kao lokalni faktor rasta u kosti.

Izgleda da je to prvi faktor rasta prisutan u rani i ključni posrednik u zarastanju rane. PGDF je glikoprotein koji se prvenstveno oslobađa iz trombocita (njihovih alfa granula) posle povrede i privlači neutrofile i makrofage. Neutrofili čiste ranu od bakterija i debrisa a makrofagi, koji su medijatori zarastanja rane, nastavljaju sa otpuštanjem faktora rasta privlačeći fibroblaste i indukuju sledeću fazu zarastanja [8]. U najvišim koncen-

beta (TGF- β), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)[3,4].

The various growth factors released during hemostasis have different roles, including stimulation and activation of monocytes, neutrophils and macrophages causing inflammation at the site of injury. Endothelial vasculogenesis and organogenesis takes place, and fibroblasts are recruited to the site of injury to form an extracellular matrix for the further healing process and tissue remodeling [5]. Most types of cells necessary for wound healing come from various depots, such as bone marrow, blood vessels, the epithelium basal layer, hair follicle and fatty tissue, that are rich in stem or progenitor cells which can differentiate into specialized cells influencing the selection of signaling molecules or growth factors during the healing process.

During the proliferative phase, fibroblasts infiltrate the wound, produce matrix metalloproteinase MMP[6] and interact with extracellular proteins thus promoting granulation. Fibroblasts further undergo the phenotypic change into miofibroblasts that even out the edge the wound, generating contractile force and facilitating wound closing.

Granulation tissue collagen fibers act as a provisional matrix that binds undifferentiated osteoprogenitor cells that, influenced by growth factors, primarily BMp ones, further differentiate into the cartilage and bone. Hematopoetic stem cells and endothelial cells precursors have their common origin in hemangioblast stem cells and are present in the bone marrow.

The finial phase of the wound healing process-remodeling, involves the regression of blood vessels and the replacement of the granulation tissue with extracellular matrix as well as its reconstruction.

1. Platelet-derived growth factor PDGF

PDGF family consists of five different disulphide dimers composed of four polypeptide chains, forming isoforms of the PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC, PDGF-DD[7]. The PDGF-BB homodimer is biologically more active than the other isoforms AA and AB. PDGF AB and BB operate as system growth factors, while PDGF AA works as a local growth factor in the bone.

tracijama prisutni su na samom početku zaceljenja tkiva, koje dostižu pik trećeg dana [9-11]. Najvažnije specifične aktivnosti PDGF uključuju mitogenezu STEM ćelija, angogenezu i osteogenezu, kao i modulaciju delovanja drugih faktora rasta [12]. Kako rana sazreva, broj trombocita se smanjuje i nivo PGDF pada. Bez obzira na to, PGDF ima aktivno učešće u toku celog procesa zarastanja. Kasnije, u proliferativnoj fazi, podstiče diferencijaciju fibroblasta u miofibroblaste, dovodeći do kontrakcije kolagene matrice i rane [13].

Kada se govori o koncentraciji PGDF u ekstrakcionoj rani, Lalani i sar,[14] koji su ispitivanje radili na modelu zeca, utvrdili su da je u periodu od drugog do četvrtog dana prisutna manja količina ovog faktora rasta u alveolarnoj kosti, a od četvrtog dana do kraja prve nedelje nivo se ne menja značajno. Između prve i druge nedelje postoji porast koncentracije PGDF, koja se opet smanjuje između druge i četvrte nedelje. U periodu između 4. i 8. nedelje, nivo PGDF-a raste šest puta. Manji porast PGDF primećen je i između 8. i 12. nedelje, zatim i opadanje od 12. do 16. nedelje [14].

Uloga u fazi remodulacije ogleda se u podsticanju proizvodnje kolagna i fibroblasta. Kako debridman rane napreduje, povećava se količina PGDF sve do početka formiranja kosti. U toku druge nedelje, PGDF je lokalizovan oko spikula kosti i oko osteocita. Od druge do četvrte nedelje sporo povećanje koštane mase poklapa se sa smanjenjem PGDF. Međutim, kako od četvrte do 8. nedelje povećanje i remodelacija kosti napreduje, količina PGDF se povećava kao rezultat aktivnosti osteoblasta i osteoklasta. Proces remodelacije se usporava od 12. do 16. nedelje i nivo PGDF postepeno opada [14]. Ovaj faktor rasta je izolovan i danas se preporučuje u lečenju koštanih defekata kao visoko prečišćeni rekombinovani humani rh PGDF-BB [15].

2.Transformacioni faktor rasta TGF-β

Transformacioni faktori rasta su peptidi koji utiču na rast i fenotip ćelija sa sposobnošću da pokrenu transformaciju fenotipa ne-neoplastičnih fibroblasta u ćelije nalik malignim [16]. TGF-β je glavni predstavnik ove grupe faktora rasta i naziva se još TGF-β superfamilija. Nju čini više faktora rasta: TGF-β (β1, β2 i β3), activin (A, B i AB), koštani morfogenetski proteini (BMP-i) i inhibini. Kod sisara i ptica su izolovane tri TGF-β izoforme (TGF-β1, -β2 i β3), dok kod nižih kičmenjaka postoje još tri dodatne izoforme [17].

It seems that PGDF is the first growth factor present in the wound and is a key mediator in wound healing. PDGF is a glycoprotein that is primarily released from platelets (their alpha granules) after injury and attracts neutrophils and macrophages. Neutrophils clean the wound from bacteria and debris and macrophages as mediators of wound healing continue with the release of growth factors attracting fibroblasts, and induce the next wound healing phase [8]. It is present in the highest concentration at the very beginning of the tissue healing, which peaked the third day [9-11]. The most important specific activities of PDGF include STEM cells mitogenesis, angigenesis and osteogenesisso as well as the modulation of other grow factors' activities [12]. During wound maturation, the platelet count reduces and the level of PDGF falls. Nevertheless, the PDGF actively participates in all stages of healing. Later, in the proliferative stage, it stimulates the differentiation of fibroblasts into myofibroblasts, leading to a contraction of collagen matrix and extraction wound [13].

As far as PDGF concentration in extraction wound is concerned, Lalani et al.[14] who tested a rabbit model extraction wound, found that in the period from 2 to 4 days, there is a smaller amount of this growth factor in the alveolar bone, and from the 4th day until the end of the 1st week the level does not change significantly. Between the 1st and 2nd week the levels of PDGF increase, which in turn decreases between the 2nd and 4th week. Between the 4th and 8th week, the level of PDGF increases by six times. A small increase in the PDGF was observed between the 8th and 12th week, which then declined between the 12th to 16th week [14].

The remodelation role reflects in stimulating production of collagen and fibroblasts. As wound debridement progress increases, PDGF amount also increases until bone formation start. PDGF is localized around the spicules of bone and around the osteocytes during the second week. From the 2nd to 4th week, a slow increase in the osseus mass coincides with a reduction in PDGF. However, as of the 4th to 8th week bone remodeling progresses, and the amount of PDGF increases as a result of the activities of osteoblasts and osteoclasts. The process of remodeling is slowed down from the 12th to the 16th week and the PDGF-level gradually declines[14]. This growth factor has been isolated, and nowadays it is recommended in the treatment of bone defects as highly purified recombinant human rh PDGF-BB[15].

Transformacioni faktor rasta beta igra važnu ulogu u raznovrsnim ćelijskim funkcijama tokom svih faza zarastanja rane, uključujući produkciju ECM, ekspresiju proteaze, migraciju, hemotaksu, difrencijaciju i proliferaciju različitih tipova ćelija [18]. Sva tri izoformna oblika sintetizovana su i pronađena u trombocitima i makrofagima, a proizvode ih i osteoblasti inkorporirani u mineralizovani koštani matriks. TGF-β1 ima ulogu u inflamatornoj fazi, angiogenezi, formiranju granulacionog tkiva i remodelaciji ECM matriksa, kao i esencijalnu ulogu u reepitelizaciji. TGF-β1 je hemostatski i mitogeni faktor, koji privlači nediferencirane ćelije na mestu povrede i podstiče njihovu proliferaciju i diferencijaciju u fibroblaste, hondroblaste i osteoblaste. Koštane ćelije sintetišu ovaj faktor rasta i skladište ga u inertnom obliku u ECM stvarajući rezerve TGF-β u telu [19].

TGF-β se ispoljava uglavnom u zrelim osteoblastima na površini kosti tokom koštanog razvoja i rasta i u kalusima zaceljujuće frakture. Najvažnije funkcije TGF-β1 i TGF-β2 su hemotaksa i mitogeneza prethodnice osteoblasta, proliferacija i diferencijacija osteoblasta i sposobnost odlaganja oseoblasta na kolageni matriks rane koja zarasta u kosti. TGF-β inhibira formiranje osteoklasta i koštanu resorpciju, omogućavajući formiranje kosti [20].

Kao posledica koštane povrede, latentni oblik TGF-β1 se konvertuje u aktivni oblik u kiseloj sredini. Aktivni oblik TGF-β indukuje konverziju nedifrentovanih mezenhimalnih ćelija u fibroblaste, osteoblaste i hondroblaste. Takođe, stimuliše proizvodnju kolagena i fibronektina i plazminogen aktivirajućeg faktora u koštanim ćelijama. Izgleda da TGF-β1 i TGF-β2 najčešće naizmenično deluju u većini sistema [19]. Najizraženije dejstvo im je u procesima morfogeneze i na mestima epitelizacije.

U studiji koja ispituje vremensku i prostornu zastupljenost faktora rasta tokom zarastanja ekstrakcione rane kod zečeva, porast TGF-β1 je primećen od drugog do četvrtog dana posle ekstrakcije, u najvećem delu prisutnog u ECM, što je i očekivano, jer trombociti i zapaljenske ćelije oslobađaju proteine tokom akutne inflamatorne faze, a onda se koncentracije TGF-β1 smanjuju do kraja prve nedelje[14]. Tada se proteini mogu uglavnom naći na periferiji novoformiranog osteoida, a minimalne količine prisutne su u ECM.

Između prve i druge nedelje, takođe, dolazi do porasta koncentracije TGF-β1. Od druge nedelje, povećanje koncentarcije se nastavlja, ali u manjem stepenu, tako da u četvrtoj nedelji koncentracija dostiže dvostruko

2. Transformation growth factor TGF-β

Transforming growth factors are peptides that have an influence on the growth and phenotype of cells with the ability to initiate the transformation of non-neoplastic fibroblasts phenotype into malignant-like cells[16]. TGF-β is the representative member of this group of growth factors, also called the TGF-β superfamily. It is composed of several growth factors: TGF-β (β 1, β 2 and β 3), activin (A, B and AB), bone morphogenetic proteins (BMP-s) and inhibin. In mammals and birds three TGF-β isoforms were isolated (TGF-β1,-β2 and-β3), while in lower vertebrates there are three additional isoforms[17].

The transformation growth factor beta plays an important role in a variety of cellular functions during all phases of wound healing, including: ECM production, protease expression and migration, chemotaxis, differentiation and proliferation of different cell types [18]. All three isoforms are synthesized and found in platelets and macrophages, and also produced by osteoclasts incorporated in the mineralized bone matrix. TGF-β1 plays a role in the phase of inflammation, angiogenesis, granulation tissue formation, and remodeling of the ECM and has an essential role in the re-epithelization. TGF- β1 is a haemostatic and mitogenic factor, which attracts undifferentiated cells at the site of injury and stimulates their proliferation and differentiation in fibroblasts, chondroblasts and osteoblasts. Bone cells synthesize this growth factor and store it in an inert form in the ECM creating reserves of TGF β in the body [19].

TGF- β is expressed mainly in mature osteoblasts on the surface of the bone during bone development and growth and calluses of the healing fractures. The main functions of TGF-β1 and TGF-β2 are chemotaxis and mitogenesis of precursors of osteoblasts, osteoblast proliferation and differentiation, and the ability to delay osteoblasts on the collagen matrix in the healing wound in the bone. TGF β inhibits the formation of osteoclasts and bone resorption, allowing bone formation [20].

As a result of bone injury, a latent form of TGF-β1 is converted into an active form in the acid environment. The active form of TGF-β induces the conversion of undifferented mesenchymal cells into fibroblasts, osteoblasts and chondroblasts. veći stepen od početne. Od dvaneste nedelje

opet dolazi do pada koncentracije TGF-β1 i TGF-β2. Tako da bi značilo da je TGF-β1 okidač za početak formiranja kosti i da porast njegove koncentracije znači i porast koštane mase. U svim vremenskim razdobljima, koncentracije TGF-β1 su veće u maksili nego u mandibuli [14].

Aktivno formiranje kosti je u periodu između četvrte i dvanaeste nedelje i tada osteoklasti počinju da resorbuju kost. TGF-β1 se otpušta u svom latentnom obliku iz koštanog matriksa i transformiše se u aktivni oblik u kiseloj sredini. TGF-β1 je tada najviše prisutan u ECM i duž periferije remodulujuće kosti. Kako se broj osteoklasta u Haversovim kanalima smanjuje, koncentracija TGF-β1 se stabilizuje i ne raste dalje[14].

3.Faktor rasta sličan insulinu IGF-I i IGF-II

Insulinski faktori rasta su klasa polipetida sa aminokiselinskom sekvencom koja je slična insulinu. IGF-I i IGF-II su najobilniji faktori rasta prisutni u koštanom matriksu.

Jetra je glavni izvor ovih faktora rasta koji se sintetišu i u drugim tkivima iz kojih dospevaju u vanćelijsku tečnost. Fetus i placenta su takođe bogati izvori. Insulinu sličan factor rasta-II je nutritivno regulisan. Veća količina IGF-I i IGF-II ukazuje na prisustvo dovoljno energije i proteina za vreme rasta. Ovi faktori rasta imaju značajne efekte na koštanu homeostazu, sa proanaboličkom i antikataboličkom aktivnošću. Odgovorni su za razvoj i održavanje skeletnomišićnog sistema[21]. IGF-I i IGF-II stimulišu koštane ćelije na autokrin ili parakrin način delovanja putem podizanja DNA sinteze, osteokalcin sinteze i aktivnosti alkalne fosfataze[13]. Zavisno od doze, podstiču usmerenu migraciju proosteoblasta. IGF-I je hemotaktik za fibroblaste, osteoblaste i osteoblast-progenitor ćelije, podstiče stvaranje koštanog matriksa (sintezu kolagenih i nekolagenih proteina). IGF-II ima uticaj na metabolizam kostiju sa izraženim anaboličkim efektima, delujući na mitogenezu osteoblasta.

4. Koštani morfognetski proteni

Jedinstvenu podfamiliju u okviru superfamilije TGF-a formiraju BMP-i. Do sada je otkriveno 15 koštanih morfognetskih proteina koji se dele u podfamilije po sličnosti u njihovoj aminokiselinskoj sekvenci. Njihova

It also stimulates the production of collagen and fibronectin and plasminogen activating factor in bone cells. It seems that TGF-β1 and TGF-β2 usually alternately operate in most systems[19]. Their most pronounced effect is in the process of morphogenesis and epithelization.

In a study that examined the temporal and spatial presentation of growth factors during extraction wound healing in rabbits, TGF- β1 increasing was observed from the 2nd to the 4th day after the extraction, mostly present in the ECM, which was expected as platelets and inflammatory cells release proteins during the acute inflammatory phase; the concentrations of TGF- β1 were reduced by the end of the first week[14]. The proteins can generally be found on the outskirts of the newly formed osteoid, and minimum quantities are present in the ECM.

Between the 1st and the 2nd week, there was an increase in the concentration of TGF-β1. From the second week, the concentration continued to increase but in lower amounts, so that in the 4th week the concentration was twice as high compared to the initial level. From the 12th week, TGF- β1 and TGF- β2 declines were noticed again. It seems as the TGF-β1 is a trigger for the beginning of bone formation and that increase in its concentration means that bone mass is increased too. In all periods, the concentration of TGF-β1 was greater in the maxilla than in mandible [14].

Active bone formation takes place in the period between the 4th and the 12th week, and then the osteoclasts begin to resorb the bone. TGF-β1 is released in its latent form from the bone matrix, and converts into the active form in the acid environment. TGF-β1 is mostly present in the ECM and along the periphery of the remodeling bone. As the number of osteoclasts in the Haversian channels decreases, the concentration of TGF- β1 stabilizes and grows no more[14].

3. Insulin-like growth factor IGF-I and IGF-II

Insulin-like growth factors are a class of polypeptides with the amino-acid sequence that is similar to insulin. IGF I and IGF II are the most abundant growth factors present in the bone matrix.

biološka aktivnost je pokazala sposobnost za ektopično formiranje enhondralne kosti[22]. Korišćenjem tehnike molekularnog kloniranja, 6 članova iz porodice BMP-a svrstano je u posebnu grupu od BMP-2 do BMP-7 (koji se još naziva i osteopontin OP-1).

Faktori rasta BMP su sa jakim osteoinduktivnim potencijalom, a osnovna uloga im je regulacija zarastanja koštanog tkiva. Najizraženije dejstvo im je diferencijacija mezenhimalnih ćelija u osteoblaste i hondroblaste [23,24]. Neke studije [24] ukazuju na to da BMP ne stimulišu zrele osteoblaste, a zreli fibroblasti nisu sposobni da indukuju osteogenu aktivnost posle tretmana BMP-2. Ovo bi značilo da je osteogeni uticaj BMP usmeren isključivo na nezrele i multipotentne ćelije. Mnogobrojni lokalni i sistemski faktori utiču na delovanje BMP tokom formiranja kosti. Lokalni faktori koji pokazuju sinergizam sa BMP su bazni faktor fibroblasta i prostaglandini, dok postoji suprotno delovanje BMP-2 i TGF- β1 na diferencijaciju osteoblasta [25].

U studiji koja ispituje koncentracije faktora rasta na mestu ekstrakcije zuba kod zečeva utvrđeno je da su koncentracije BMP-2 niske odmah posle vađenja zuba, ali rastu progresivno između drugog i četvrtog dana. Zatim sledi minimalni porast vrednosti BMP-2 do četvrte nedelje, da bi se vrednosti BMP-2 dvostruko uvećale između četvrte i 8. nedelje i u istoj meri se povećale od 8. do 12. nedelje. U početku ga najviše ima u ECM, gde stimuliše difrencijaciju mezenhimalnih ćelija, dok je krajem prve nedelje prisutan više u osteoidu. Interesantna je činjenica da sa povećanjem količine BMP-2 dolazi do smanjenja TGF-β1. Inverzan odnos između TGF-β1 i BMP-2 je primer interakcije faktora rasta u zarastanju rane. Porast BMP-2 je povećan od četvrte i 12. nedelje u periodu aktivnog formiranja i remodelacije kosti[14]. Kako se remodelacija kosti smanjuje, tako dolazi do stabilizacije TGF-β1 i BMP-2.

Produkcija BMP-7, koji se u velikoj meri detektuje kod reparacije posle ekstrakcije zuba, najizraženija je u periodu između druge i 8. nedelje posle ekstrakcije, sa progresivnim padom u periodu od 12. do 24. nedelje [26,27]. To bi značilo da je ovaj faktor važan za ranu i intermedijsku fazu zarastanja kosti, tj. za mineralizaciju koštanog matriksa i privremenu sintezu trabekularne kosti. Nasuprot tome, količine

The main source of these growth factors is the liver but they are also synthesized in other tissues, fetus and placenta, being particularly rich sources, from which they reach the extracellular fluid. Insulin-like growth factor IGF-II is nutritionally regulated. The high amount of IGF-I and IGF-II indicates the presence of sufficient amount of energy and proteins during the growth. These growth factors have significant effects on bone mineral homeostasis, with proanabolic and anticatabolic activity. They are responsible for developing and maintaining musculoskeletal system [21]. IGF-I and IGF-II stimulate bone cells in an autocrine or paracrine manner by increasing DNA synthesis, osteocalcin synthesis and alkaline phosphatase activity [13]. Depending on the dose they also stimulate directed pre-osteoblast migration. IGF-I is a chemotactic factor for fibroblasts, osteoblasts and osteoblast-progenitor cells, and it promotes production of bone matrix (i.e. the synthesis of collagen and non-collagen proteins). IGF-II influences the bone metabolism mith marked anabolic eftects,aftechug affecting oteoblast mitogenesis.

4. Bone morphogenetic proteins BMP -2,BMP-7

BMPs form a unique subfamily within the TGF superfamily. Fifteen bone morphognetic proteins that have been discovered so far are divided into subfamilies according to their similarities in terms of amino-acid sequence. Their biological activity has been shown to enable endochondral bone formation[22]. Using molecular cloning technique, six members of BMP family, from BPM-2 to BPM-7, have been classified into a special group (BPM-7 that also known as osteopontin OP-1).

BMPs are growth factors with strong osteoinductive potential, with bone tissue healing regulation as their basic role. They have the strongest effect on differentiation of mesenchymal cells into osteoblasts and hondroblasts [23,24]. Some studies [24] suggest that BMPs do not stimulate mature osteoblasts and that mature fibroblasts are unable to induce osteogenic activity after BMP-treatment. This would mean that BMPs have an osteogenic effect exclusively on immature and multi-potent cells. Many local and systemic factors affect the BMPs mechanism of action during bone formation.

BMP-7 se smanjuju dok se akumulacija osteokalcina u sazrevajućem koštanom matriksu povećava.

5.Vaskularni endotelijalni faktor rasta

Angiogeneza i vaskulogeneza su primarni uslovi za regeneraciju tkiva[28]. Ekspresija VEGF regulisana je hipoksijom i ishemijom tkiva. Povećani nivoi VEGF koji su indukovani hipoksijom čine VEGF vođenu angiogenezu centralnim odgovorom na nizak pritisak kiseonika i daje mu glavnu ulogu u terapeutskoj angiogenezi [29,30]. VEGF se vezuje za receptore endotelnih ćelija, što dovodi do njihovog rasta, proliferacije i migracije. Aktivirane endotelijalne ćelije spajaju se u tubularne strukture (morfogeneza) i dalje se vezuju sa pericitima (sazrevanje). Periciti omogućavaju niz regulatornih signala, uključujući TGF-β1 i druge, što dovodi do mirovanja endotela i opstanaka krvnih sudova nezavisno od dalje stimulacije angiogeneze (stabilizacija). Prostorna lokalizacija angiogenetskih signala u ekstracelularnom matriksu (ECM) ima temeljnu ulogu u osiguravanju odgovarajućeg završetka svih koraka u vaskulogenezi [31].

Vaskularni endotelijalni faktor rasta je važan medijator i u limfangiogenezi i vaskularnoj permeabilnosti. Takođe, stimuliše epitelizaciju i depoziciju kolagena[32]. Neki citokini i faktori rasta kao što su TGF-β1, TGF-β2, KGF, FGF-2, PGDF-BB, EGF deluju na parakrin način i podstiču ekspresiju VEGF, što upućuje na njihovu visoku biološku interakciju [33, 35].

U studiji o zastupljenosti faktora rasta u ekstrakcionoj rani kod zečeva utvrđeno je da nema promene vrednosti VGDF u maksili u periodu do 48 sati do četvrtog dana nakon ekstrakcije. Od četvrtog dana do kraja prve nedelje nivo VEGF raste i ta vrednost se ne menja između prve i druge nedelje, da bi se u periodu između četvrte i 8. nedelje skoro trostruko povećala. U periodu od 12. do 16. nedelje nema promene u količini VEGF[36]. U studiji koja ispituje nivo VEGF u krvi pacova posle vađenja zuba utvrđeno je da je nivo VEGF u venskoj krvi u prva 24 sata posle ekstrakcije povećan, naročito kod mladih pacova. Koncentracije VEGF na mestu ekstrakcije ne razlikuju se značajno kod mladih i odraslih pacova[37].

Local factors that show synergism with BMP are basic fibroblast factors and prostaglandins, while BMP-2 and TGF-â1 have the opposite effect on osteoblasts differentiation [25].

A study that examined the concentration of growth factors at the site of tooth extraction in rabbits showed that concentration of BMP-2 is low immediately after tooth extraction but increase progressively between the second and the fourth day. The BMP-2 values then increase minimally until the week four. In the period between the 4th and the 8th week the values are tripled, however, there were no changes in the period between the 8th and 12th week. In the very beginning, most of BMP-2 is present in the ECM where it stimulates the differentiation of mesenchymal cells, while towards the end of the first week its presence is more prominent in the osteoid part. An interesting fact is that as the concentration of BMP-2 increases, whereas the concentration of TGF-â1 decreases. The inverse relationship between TGF-â1 and BMP-2 is an example of the growth factors interaction in the wound healing process. BMP-2 increases between the 4th and the 12th week during the period of active bone formation and remodelation[14]. As the bone remodeling process weakens, TGF-â1 and BMP-2 concentrations become more stable.

The production of BMP-7 which is largely detected during the reparation process after tooth extraction is most pronounced in the period between the 2th and the 8th week after extraction, with a progressive decline in the period from week 12 to 24[26,27]. This would mean that this particular factor is important in early to inter-medial stage of bone healing. i.e. during the mineralization of the bone matrix and provisional synthesis of trabecular bone. In contrast, the amount of BMP-7 decreases as osteocalcin accumulation increases in maturing bone matrix.

5.Vascular endothelial growth factor

Angiogenesis and vasculogenesis are the primary requirements for tissue regeneration[28]. VEGF expression is regulated by tissue hypoxia and ischemia. Increased levels of VEGF that are induced by hypoxia make VEGF-driven angiogenesis central response to low oxygen pressure and a major factor in the therapeutic angiogenesis[29,30].

6. Fibroblastni faktori rasta

Fibroblasti i fibrociti igraju važnu ulogu u rekonstrukciji tkiva, zamenom starog kolagena tipa III kolagenom tipa I i umrežavanjem kolagenih molekula. Tenzione sile na ivicama rane se povećavaju zahvaljujući generisanom elastinu, završavajući tako proces približavanja ivica rane i formiranje ožiljka[38].

Fibroblastni faktori rasta čine veliku porodicu polipeptida od ukupno 22 člana, koji imaju ključnu ulogu u neurološkim funkcijama, razvoju i metabolizmu[39,40]. FGF proizvode keratinociti, fibroblasti, endotel, glatke mišićne ćelije, hondrociti i mast ćelije. Kiseli fibroblastni faktor rasta (aFGF-1) i bazni fibroblastni faktor rasta (bFGF-2) identifikovani su u fluidu rane, naročito u najranijim fazama zarastanja[41]. Uloga FGF u zarastanju rane je dokazana *in vitro i in vivo* kroz akciju u ranoj aktivaciji makrofaga, povećanju komponenti ekstracelularnog matriksa, povećanju proliferacije i diferencijacije neuroektodermalnih i mezodermalnih derivata, endotelnoj ćelijskoj proliferaciji, adipogenezi, angiogenezi i reepitelizaciji [42,43].

Najveću ulogu u procesu zarastanja rane ima kiseli fibroblastni faktor rasta (aFGF-1) koji utiče na proliferaciju fibroblasta i endotela, promovišući zarastanje rane i angiogenezu. FGF-2 povećava nivo osteokalcina u medijumu, što sugeriše da on moduliše funkciju osteoblastnih ćelija[44]. Faktor rasta keratinocita KGF-1 ili FGF-7, član porodice FGF-a, utiče na bržu reepitelizaciju preko ubrzanja proliferacije i diferencijacije epitelnih ćelija i inhibira apoptozu[45,46]. Osim toga, moćni je mitogen za ćelije vaskularnog endotela i ushodnu regulaciju VEGF. Faktor rasta keratinocita-2 (KGF-2) ili FGF-10 slično deluje, ali i stimuliše formiranje granulacionog tkiva i kolagena.

U studiji koja ispituje prostorne i vremenske koncentracije FGF-2 kod zečeva, u ekstrakcionoj rani nije primećena promena koncentracije FGF-2 u periodu od 48 sati do četvrtog dana posle vađenja zuba u maksili. Od kraja prve do druge nedelje, koncentracija FGF-2 dvostruko raste, a zatim od druge do četvrte nedelje opada. Između četvrte i osme nedelje nastaje značajno uvećanje koncentracije FGF-2, koje se umereno nastavlja od 8. do 12. nedelje. Smanjenje koncentracije FGF-2 zapaža se od 12. do 16. nedelje [36].

VEGF binds to receptors of endothelial cells leading to their growth, proliferation and migration. Activated endothelial cells aggregate into tubular structures (morphogenesis) and further connect with pericytes (maturation). Pericytes provide a variety of regulatory signals, including TGF-β1 and others, which leads to the endothelium standstill and blood vessel survival irrespective of further angiogenesis stimulation (stabilization). The spatial localization of angiogenetic signals in the extracellular matrix (ECM) plays a fundamental role in ensuring an appropriate completion of all steps in vasculogenesis [31].

Vascular endothelial growth factor is an important mediator in lymphangiogenesis and vascular permeability. It also stimulates epithelization and collagen deposition [32]. Certain cytokines and growth factors such as TGF- β1, TGF- β2, KGF, FGF-2, PDGF-BB and EGF act in a paracrine manner and stimulate VEGF expression, suggesting their high biological interaction [33-35].

A study on growth factors presence at the site of the extraction wound in rabbits showed no change in the values of VGDF in the maxilla in the period from 48 hours to the 4th day after extraction. From the 4th day to the end of the 1st week, VEGF levels increase and this value did not change between the 1st and the 2nd week. However, this level almost tripled in the period between week 4 and 8. In the period between weeks the 12th to the 16th week there was no change in the amount of VEGF [36]. Another study assessing the level of VEGF in the blood of rats after tooth extraction has confirmed that the level of VEGF in the venous blood increases in the first 24 hours after extraction, especially in young rats, although VEGF levels at the site of extraction are not significantly different in young and adult rats [37].

6. Fibroblast growth factors

Fibroblasts and fibrocytes play an important role in tissue reconstruction by replacing the old collagen type III with collagen type I and forming a network of collagen molecules. Tension forces on the edges of the wound increase owing to generated elastin, thus completing the process of wound edges approximation and the scar formation [38].

Slične promene koncentracije FGF2 postoje u mandibuli posle vađenja zuba. Od prve do druge nedelje porast koncentracije bio je oko šest puta. Vrednosti FGF2 u periodu od druge do osme nedelje su znatno veće u mandibuli nego u maksili. Koncentracije FGF-2 se nisu menjale u periodu od četvrte do osme nedelje, a dvostruko su se povećale od 8 do 12 [36].

Prečišćeni rekombinantni FGF-2 u optimalnim koncentracijama indukuje proliferaciju prethodnice ćelija pulpe, periodo-ntalnog ligamenta, fibroblasta, mezenhimalnih ćelija i utiče na njihov osteogeni potencijal [47].

Primena faktora rasta u zarastanju ekstrakcione rane

Autologni trombocitni koncentrati: plazma bogata trombocitima (PRP) i fibrin bogat trombocitima (PRF) sadrže visoke nivoe faktora rasta, uključujući tri izomera PGDF-a, transformišući faktor rasta beta, insulinu sličan faktor rasta, epidermalni faktor rasta, vaskularni endotelijalni faktor rasta i zato se već dugo primenjuju u oralnoj i maksilofacijalnoj hirurgiji.

Regeneracija koštanih defkata je eksperimentalno indukovana izolacijom i dugoročnom kulturom autolognih matičnih ćelija. BMP-2 je produkt matičnih ćelija kojih ima u izobilju u masnom tkivu. Upotreba matičnih ćelija masnog tkiva može biti brzo sprovedena, čak u roku od 24 sata, zahvaljujući posebnoj metodi tkivnog inžinjeringa i daje pozitivne rezultate u zarastanju koštanih defekata[48]. Osim zarastanja rane, neki autori predlažu i upotrebu faktora rasta u lečenju alveolita[49], bolju oseointegraciju implanata[50], augmentaciju alveolarnog grebena[51]. Postoje podaci da je postoperativno zarastanje bilo bolje kao i da je postoperativni bol slabiji kod pacijenata tretiranih faktorima rasta bogatim plazmom[52]. Arany i sar[53] navode da na povećanje koncentarcije faktora rasta TGF-β1 utiče tretman ekstrakcione rane laserom male snage, što objašnjava ubrzano zarastanje rane posle zračenja laserom[54]. Munkhdulam i sar [55] su u studiji na psima, utvrdili da su kvalitet i kvantitet kosti bolji posle ubrizgavanja rhBMP-2 u dentoalveolarne defekte.

Fibroblast growth factors make a large polipeptide family of 22 members which have a key role in neurological function, development and metabolism [39,40]. FGF are produced by keratinocytes, fibroblasts, endothelium, smooth muscle cells, chondrocytes and fat cells.Acidic fibroblast growth factor (aFGF-1) and basic fibroblast growth factor (bFGF-2) have been identified in the wound fluid, especially in the early stages of the healing process [41]. The role of FGF in wound healing has been demonstrated *in vitro* and *in vivo* through its action in the early activation of macrophages and increase of the components of the extracellular matrix, as well as in the proliferation and differentiation of neuroectodermal and mesodermal derivatives, endothelial cell proliferation, adipogenesis, angiogenesis and epithelia-lization [42,43].

The acidic fibroblast growth factor (aFGF-1) which influences the proliferation of fibroblasts and endothelial cells, promoting angiogenesis and wound healing has the most important role in the wound healing process. FGF-2, on the other hand, increases the level of osteocalcin in the medium, which suggests that it modulates the function of osteoblastic cells[44]. Keratinocyte growth factor KGF-1 or FGF-7, a member of the FGF family induces faster re-epithelization by accelerating the proliferation and differentiation of epithelial cells and inhibiting apoptosis[45,46]. In addition, it is a potent mitogen for vascular endothelial cells and up-regulation of VEGF. Keratinocyte growth factor-2 (KGF-2) or FGF-10 has a similar mechanism of action, but also it stimulates the granulation tissue and collagen formation.

A study that examined the spatial and temporal concentrations of FGF-2 in rabbit extraction wound showed no changes in the concentration of FGF-2 in the period from [48] hours to four days after tooth extraction in the maxilla. From the end of the 1st to the end of the 2nd week FGF-2 concentration is doubled, and then decreased from the 2nd to 4th week. Between the 4th and the 8th week there was a significant increase in the concentration of FGF-2 which then continued to grow moderately from week 8 to 12. A decrease in the concentration of FGF-2 was observed from the 12th to the 16th week[36].

Efekti BMP-a zavise od koncentracije koja je optimalna za svaku životinjsku vrstu, kao i od nosača sa koga se koncentracije otpuštaju. Uglavnom su ti nosači demineralizovani koštani matriks, bioaktivno staklo, neresorptivni hidroksiapatit ili resorptivni beta kalcium tri fosfat.

Zaključak

Izolovana karakterizacija efekata faktora rasta je praktično nemoguća, jer su njihove aktivnosti plejotropne i međusobno se preklapaju. Studije fizioloških procesa u kojima faktori rasta imaju regulatornu ulogu ukazuju da ovi molekuli retko kada svoje aktivnosti vrše u biološkoj izolaciji. Proučavanje interakcije između faktora rasta u alveolarnoj kosti može pružiti objašnjenje o sposobnosti tkiva da zaraste i pod nepovoljnim uslovima, kao što su infekcija i zračenje. O primeni faktora rasta treba svakako razmišljati u onim slučajevima kod kojih se očekuje otežano i sporo zarastanje rane.

Similar changes in the concentration of FGF2 have been noticed in the mandible after tooth extraction. From the 1st to the 2nd week, an increase in the concentration was approximately six-fold. The values of FGF2 in the period from the 2nd to the 8th week were significantly higher in the mandible than in the maxilla. The concentrations of FGF-2 did not change over a period from the 4th to the 8th week but, they doubled from the 8th to the 12th week[36].

Purified recombinant FGF-2 in optimal concentrations induces the proliferation of pulp cell predecessors, periodontal ligament fibroblasts and mesenchymal cells, while at the same time affecting their osteogenic potential[47].

The use of growth factors in extraction wound healing

Autologous platelet concentrates - platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) contain high levels of growth factors including three PDGF isomers, transforming growth factor beta, insulin-like growth factor, epidermal growth factor and vascular endothelial growth factor, which is why they have been long used in oral and maxillofacial surgery.

Bone defect regeneration was experimentally induced by the isolation and long-term cultivation of autologous stem cells. BMP-2 is the product of stem cells which are abundant in fat tissue. The fat tissue stem cells application can be carried out as promptly as within 24 hours owing to a special method of tissue engineering, and has positive results in healing bone defects[48]. In addition to wound healing, some authors propose the use of growth factors in the treatment of alveolitis[49], implant osseointegration[50] and alveolar ridge augmentation[51]. There is evidence that the postoperative healing was better and postoperative pain lower in patients treated with growth-factors-rich plazma[52]. Arany et al.[53] report the increase in TGF- β1 growth factor concentration after low-level laser treatment of extraction wound, which explains the accelerated wound healing after low-level laser radiation[54]. In a study that used dogs as test subjects, Munkhdulami et al.[55] found that both bone quality and quantity were better after rhBMP-2 was injected into dentoalveolar defects.

The effects of BMP depend on its concentration, which is specific for each animal species, as well as on the carrier the concentrations are released from. These carriers are in most cases demineralized bone matrix, bioactive glass, non-absorbable hydroxyapatite or resorbable beta calcium tree-phosphate.

Conclusion

Isolated characterization of the respective growth factors' effects is practically impossible due to their actions being pleiotropic and mutually overlapping. Studies of physiological processes in which growth factors have a regulatory role indicate that these molecules rarely act in biological isolation.

The study of the interaction between the growth factors in the alveolar bone can explain tissue ability to heal even under adverse conditions, such as infection and radiation. The application of growth factors should certainly be considered in cases where difficult and slow wound healing can be expected.

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