

SKENING ELEKTRONSKO-MIKROSKOPSKA ANALIZA ALVEOLARNE KOSTI IZMENJENE OSTEOPOROZOM INDUKOVANOM KORTIKOSTEROIDNIM TRETMANOM

SEM ANALYSIS OF THE OSTEOPOROTIC ALVEOLAR BONE INDUCED BY CORTICOSTEROID TREATMENT

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Kratak sadržaj

Osteoporoza je veoma često patološko stanje koštanog sistema, nastalo kao posledica brojnih metaboličkih poremećaja, a u nekim slučajevima i posledica nepovoljnog uticaja terapijskih činilaca.

Istraživanje je rađeno na pacovima Wisstar soja, ženskog pola, starosti 6-8 nedelja. Eksperimentalna grupa životinja tretirana je kortikopreparatima. Po isteku tog perioda jedan deo eksperimentalne grupe žrtvovan je, a ostatak je podeljen u dve podgrupe i tretiran terapijom oporavka (Calcijum gluconatom, timusnim ekstraktom, spontanom oporavkom). Kontrolna grupa bila je bez terapije i na normalnom režimu ishrane. Posle 15 nedelja eksperimenta, životinje su žrtvovane, uzimani uzorci koštanog tkiva donje vilice pacova, pripremani i analizirani SEMom.

Skening Elektronskom Mikroskopijom zapaženo je istanjenje trabekula, smanjenje broja trabekula spongiozne kosti i povećanje sržnih prostora, kao posledica delovanja kortikopreparata na alveolarnu kost donje vilice pacova. Po prestanku kortikoterapije i prelasku na terapiju oporavka, spongiozna kost povoljno reaguje, pa se SEMom uočava da su sržni prostori uobičajenih dimenzija, trabekule su varijabilne po veličini, obliku i rasporedu, prisutna je jedna polimorfija. Oporavak sa ekstraktom timusa uočava se kao razrastao površinski matriks, ćelijska gustina je veća i aktivnost ćelija je veća—osteoklastna aktivnost.

Na osnovu dobijenih rezultata može se zaključiti da kortikopreparati posle duže upotrebe mogu dovesti do istanjenja i smanjenja broja trabekula koštanog tkiva, tj. razređenja i resorpcije kosti, dok je terapija oporavka pokazala delimičan uspeh u ispitivanom periodu.

Ključne reči: osteoporoza, alveolarna kost, pacov, SEM.

Uvod

Poslednjih godina milioni ljudi izloženo je brojnim faktorima rizika i destrukcije koštanog

Abstract

Osteoporosis is a very frequent pathological state of the bone system which appears as a result of numerous metabolic disturbances and in some cases of the negative influence of the therapeutic factors.

The research was performed on Wisstar rats, female, 6-8 weeks old. The experimental animal group was treated by cortico-preparations. After that period one part of the experimental group was sacrificed and the rest was divided into two groups: control (without corticosteroid treatment) and experimental group treated by the recovery therapy (Calcium gluconate, Thymus extract, and spontaneous recovery). After 15 weeks of experiment the animals were sacrificed, bone tissue samples of the rats' lower jaw were taken, prepared and SEM analyzed.

SEM showed that trabeculae became thinner, the number of spongius bone trabeculae was reduced and marrow spaces increased as the result of corticopreparation influence on the alveolar bone of the rats' lower jaw. After the cortico-therapy and transition to therapy of spontaneous recovery, spongius bone of mandible positively reacted, and rough surface of bone, trabeculae of various dimensions, forms and localizations and one polymorphia were noticed. These changes represented slow decrease of resorptive process and increased recovery of bone tissue with process of osteogenesis prevailing. As soon as recovery therapy started, spongius bone reacted favorably as it was noticed by SEM. Trabeculae were polymorphic, variable in size, shape and arrangement, normal in appearance and size, marrow spaces were of usual size, and bone surface was unequal.

Using SEM analysis the recovery by thymus extract showed: enlarged surface matrix, cell density and cell activity (osteoclastic activity). According to the achieved results, it can be concluded that corticopreparations after a long use can lead to decalcification and reduction of bone tissue trabecular number, i.e. bone dilution and resorption, while the recovery therapy showed partial success in the examined period.

Key words: SEM, alveolar bone, osteoporosis, rat.

Introduction

Every year millions of people were exposed to the numerous risk factors of bone tissue ex-

tkiva, izazvanih traumama ili patološkim procesima, koji zahtevaju rekonstrukciju i lečenje.¹

Osteoporoza je veoma učestala patološka promena u koštanom sistemu, nastala kao posledica brojnih metaboličkih poremećaja, deficita u ishrani, inaktiviteta, endokrinih poremećaja, oboljenja koštane srži, bolesti vezivnog tkiva, a u nekim slučajevima i nepovoljnog uticaja terapijskih činilaca.² Posebnu pažnju zaslužuje osteoporoza alveolarnih kostiju, nastala zbog ubrzane resorpcije koštano tkiva, ili nedovoljne sinteze kosti, izazvane terapijom kortikopreparatima.^{3,4,5} U nekim istraživanjima Skenung Elektronsko-Mikroskopskom (SEM) analizom, praćene su razlike u strukturi dentina i viličnih kostiju.⁶ Koristeći njihova iskustva, svrha ovog istraživanja je da se utvrdi efekat kortikoterapije, kao i efekat terapije oporavka na eksperimentalnim životinjskim koštanim sistemima, sa posebnim osvrtom na alveolarnu kost korišćenjem SEM analize.

Cilj istraživanja

Cilj ovog istraživanja je na osnovu Skenung Elektronsko-Mikroskopske analize utvrditi kakav efekat ima terapijska primena kortikosteroida, kao i terapija oporavka na koštani sistem eksperimentalnih životinja, sa posebnim osvrtom na viličnu kost.

Materijal i metod rada

Istraživanje je rađeno na pacovima Wistar soja, ženskog pola, starosti 6–8 nedelja, što predstavlja period potpune polne zrelosti i mineralizacije kostiju životinja. Životinje su podeljene u dve grupe:

1. Eksperimentalna grupa (A-16 životinja),
2. Kontrolna grupa (B-10 životinja).

Grupi A je indukovana osteoporoza glikokortikoidima.^{3,7} Životinjama su u toku 12 nedelja intramuskularno aplikovani glikokortikoidi, i to naizmenično, svakog drugog dana Methylprednisolone succinat natrium (Lemod-Solu, Hemofarm, Vršac, Srbija) u dozi od 2 mg/kg telesne mase i Dexamethasone-natrium-fosfat (Dexason, ICN Galenika, Beograd, Srbija) u dozi od 0,2 mg/kg. Glikokortikoidi su aplikovani uvek u isto vreme, od 14–17 sati, jer je tada nivo kortizola u krvi najveći, pa životinje

traction that are provoked by traumas or pathological processes, which demands reconstruction and healing.¹

Osteoporosis is very frequent disease of modern civilization as the pathological changes in bone system are consequences of numerous metabolic disturbances, nutrition deficit, inactivity, endocrine disturbance, bone marrow disease, connective tissue diseases and sometimes as consequences of undesirable effects of therapeutics factors.² There is special interest of alveolar bone osteoporosis after the accelerated bone tissue resorption or inadequate bone synthesis as consequences of cortico-therapy.^{3,4,5} In some researches differences were monitored in dentin structure and jaw bones with Scanning Electronic Microscopic analysis (SEM) analysis.⁶ Using their experience, the purpose of this research was to determine the effects of cortico-therapy, as well as the effect of recovery therapy on experimental animal bone system with special interest in alveolar bone, using SEM.

Aim of research

Using SEM analysis, the aim of this study was to determine the effects of the application of both cortico-therapy and recovery therapy on skeletal system of experimental animals particularly focused on mandibular bone.

Materials and methods

Investigation was done on Wistar rats, female sex, 6–8 weeks old which represents the period of maturity and completed mineralization of bone. Animals were divided in two groups:

1. Experimental group (A-16 animals),
2. Control group (B-10 animals).

Glycocorticoid induced osteoporosis was done in group.³ In 12 weeks time glycocorticoids were applied intramuscularly to the animals every second day. Methylprednisolone succinat natrium (Lemod-Solu, Hemofarm, Vršac, Srbija), in doses of 2mg/kg of body weight and Dexamethasone-natrium-fosfat (Dexason, ICN Galenika, Belgrade, Serbia) in doses of 0,2 mg/kg. Cortico medicaments were applied always in the same time, from 14h–17h, because of the highest level of cortisol in blood, so animals had the minimal stress. After 12 weeks of treatment with glycocorticoids, a part of group B (6 animals) was ready for sacrificing

minimalno reaguju na stres. Posle 12 nedelja glikokortikoidnog tretmana, jedan deo eksperimentalne A grupe (6 životinja), pripremljen je za žrtvovanje aplikacijom Diazepam (Bensedin, ICN Galenika, Beograd, Srbija) i anestezi- ranjem Ketamin hydrochloridom USP (Ketalar, Rotexmedica Gmbh, Trittau, Germany) i nakon toga žrtvovan da bi se SEM-om dijagnostikovala osteoporoza, a ostatak eksperimentalne grupe (A) je podeljen u dve podgrupe i tretiran terapi- jom oporavka.

Grupa A je podeljena u dve podgrupe:

- * A₁ (5 životinja) podgrupa je posle kortiko tretmana bila bez terapije i na normalnom režimu ishrane—spontani oporavak.
- * A₂ (5 životinja) podgrupi je iniciran i.m. timusni ekstrakt Lyophilied total (Thymex-L, Thymoorgon-Gmbh Pharmazie, Germany), u dozi od 20mg/kg telesne mase, i Calcium gluconat (Novartis Consumer Health, Nürnberg, Germany) u dozi od 0,05 ml po životinji dnevno.

Kontrolna grupa B bila je bez terapije, na normalnom dnevno-noćnom ritmu i režimu ishrane.

Nakon 15 nedelja eksperimenta, životinje A i B grupe su žrtvovane, uzimani su uzorci koštanog tkiva donje vilice pacova na potezu od medijalne linije do foramena mentale. Koštani uzorci donje vilice sečeni su u vestibulo-oralnom smeru. Uzorci su fiksirani u 2% gluteraldehidu, zatim stavljani u Millonig pufer. Nakon toga vršena je postfiksacija u osmijumtetraoksidu, zatim su uzorci tretirani alkoholom, sutradan tretirani acetonskim rastvorima, posle toga kritikovani, spaterovani i mikroskopirani na elektronskom mikroskopu marke JOEL (JSM-5300).

Rezultati istraživanja

SEM analizom je zapaženo istanjenje trabekula sa smanjenjem broja trabekula i povećanjem sržnih prostora spongiozne kosti mandibule pacova (slika 1). Ova analiza ukazuje da kost nije produktivno aktivna i nema stvaranja nove kosti .

Po prestanku dejstva kortikoterapije i prelaskom na terapiju spontanog oporavka, spongiozna kost mandibule povoljno reaguje, tako da se SEM analizom uočava neravna površina kosti, trabekule su varijabilne po veličini, ob-

with application of Diazepam (Bensedin, ICN Galenika, Belgrade, Serbia) and anesthetized with Ketamin hydrochlorid USP (Ketalar, Rotexmedica Gmbh, Trittau, Germany). After that they were sacrificed in order to diagnose osteoporosis and the rest of experimental A group was split in 2 subgroups and was treated with recovery therapy.

Group A was split in 2 subgroups:

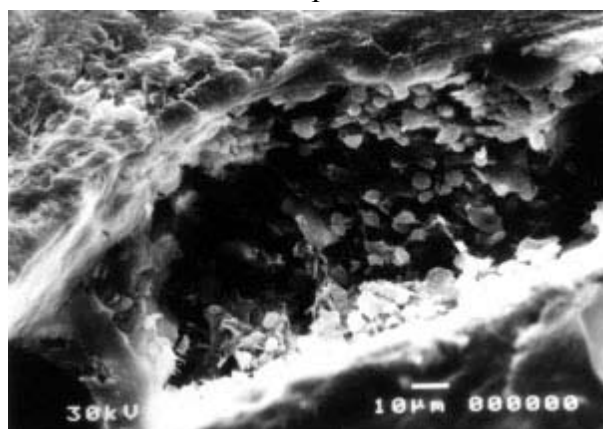
- * A1 (5 animals) subgroup was after cortico-treatment put on regular nutrition regime without any therapy.
- * A2 (5 animals) subgroup received i.m. thymus extract Lyophilied total (Thymex-L), Thymoorgon-Gmbh Pharmazie, in doses of 20mg/ kg of body weight and Calcium gluconat in doses of 0,05 ml daily on each animal..

B control group was without therapy on regular day-night rhythm and regular nutrition regime.

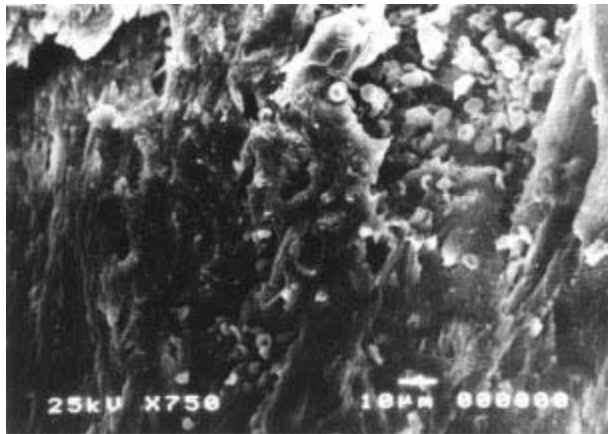
After 15th week of experiment animals of A and B group were sacrificed, samples of lower jaw bone tissue of the rats were taken from medial line till foramen mentale. Lower jaw bone tissue samples were cut in vestibulo-oral direction. Samples were fixed in 2% gluteraldehid and then put in Millonig puffer. After the postfixation had been done in osmiumtetraoxid, they were treated with alcohol and the day later with acetone solution. Then samples were criticized, spattered and observed on electronic microscope Joel (JSM-5300).

Results

SEM showed that trabeculae became thinner, the number of spongioos bone treabeculae reduced and marrow spaces increased as the

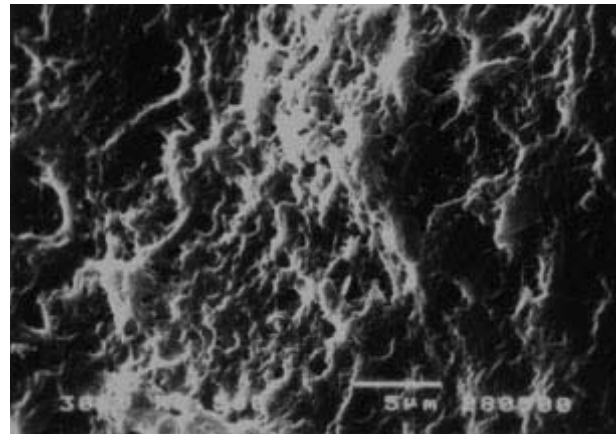


Slika 1. Osteoporoza spongiozne kosti mandibule pacova
Fig. 1 Osteoporosis of spongy bone of mandible of rats



Slika 2. Spontani oporavak trabekularne kosti mandibule pasova

Fig. 2 Spontaneous recovery, trabecular bone of mandible of rats



Slika 3. Oporavak trabekularne kosti pacova dejstvom ekstrakta timusa

Fig.3 Recovery therapy of trabecular bone of rats started favorably of extract *Thymex-L*

liku i rasporedu, uočava se jedna polimorfija (slika 2), što ukazuje na spontani i spori oporavak koštanog tkiva mandibule pacova.

Nakon tretmana oporavka ekstraktom timusa (slika 3), SEM analizom se uočava smanjenje veličine sržnih prostora, povećanje gustine trabekula (u odnosu na spontani oporavak-podgrupa A₁), razrastao površinski matriks, ćelijska gustina je veća, pa je i aktivnost ćelija veća, remodelacija kosti je intenzivnija (naročito posle tretmana oporavka jačom dozom timusnog ekstrakta od 20mg/ kg telesne mase). U pacova podgrupe A₂, po prestanku dejstva kortikoterapije i prelaskom na terapiju oporavka ekstraktom timusa, uočljiv je veći intenzitet osteogeneze i pravilnija struktura kosti u odnosu na ostale ispitivane podgrupe.

Diskusija

Razređenje kosti, tj. osteoporoza, posledica je poremećaja koštanog metaboličkog prometa sa disbalansom između pojačane koštane razgradnje i normalnog (ili nedovoljnog) stvaranja nove kosti.⁵ Čest uzrok ovog disbalansa u koštanom tkivu je primena kortikopreparata uočenih SEM analizom.⁶ Posledice dejstva kortikopreparata uočene SEMom su razređenje i resorpcija alveolarne kosti pacova. Navedene činjenice ukazuju da je terapija kortikopreparati- ma udružena sa morbiditetom koštanog tkiva u obliku razređenja kosti, tj. osteoporoze.^{7,8,9}

Reakcija anabolizma u spongioznoj kosti nakon prestanka kortikoterapije ukazuje da se lagano smanjuju resorptivni procesi, a blago raste oporavak koštanog tkiva sa preovladavanjem procesa osteogeneze kosti, što je saglasno literaturnim podacima.^{10,11,12}

result of corticopreparation influence on rats' lower jaw alveolar bone (Fig. 1).

After the cortico-therapy and transition to therapy of spontaneous recovery, spongy mandibular bone positively reacted, and rough surface of bone, trabeculae of various dimensions, forms and localizations and one polymorpha were noticed by SEM analysis (Fig. 2).

When cortico therapy ended and recovery therapy started, spongy bone reacted favorably as it was noticed by SEM: trabeculae were polymorphic, variable in size, shape and arrangement normal in appearance and size, marrow spaces were of usual size, and bone surface was unequal (Fig. 3).

Discussion

Bone dilution, osteoporosis, is the consequence of bone metabolic disturbances with disbalance between accelerated bone resorption and normal process of creation of new bone.⁵ Very frequent cause of this disbalance in bone tissue is long-term and sometimes inadequately controlled use of cortico medicaments, which was confirmed in many researches.⁶ These changes represented slow decrease of resorptive process and increased recovery of bone tissue with process of osteogenesis prevailing.^{7,8,9}

After recovery treatment with thymus extract, SEM analyses confirmed size reduction of bone marrows, improvement of trabecular density (regarding spontaneous recovery), and proliferation of bone matrixes surface. With

U spongioznoj kosti eksperimentalnih životinja metabolizam je intenzivniji, pa preparati timusa dovode do bržeg oporavka i stimulacije sinteze koštanog tkiva po prestanku delovanja kortikosteroida. Dejstvom preparata timusa došlo je do stimulacije remodelacije kosti, koja se ogleda u povećanju metaboličkih promena u pravcu ubrzane koštane izgradnje.^{7,10,13,14}

Na osnovu dobijenih rezultata može se zaključiti da patogeneza osteoporoze indukovane kortikosteroidima, još uvek nije u potpunosti razjašnjena, ali je poznato da je formiranje kosti smanjeno, a razgradnja, tj. resorpcija kosti nepromenjena ili povećana. Ovu razliku tumačimo gubitkom koštanog tkiva i smanjenjem koštane mase.^{14,15} Terapija oporavka ekstraktom timusa pokazala je izuzetno dobar uspeh u ispitivanom periodu, dok je spontani oporavak dao delimične rezultate.

Kortikopreparati primenjeni kod eksperimentalnih životinja u vreme intenzivnog rasta, razvoja i mineralizacije kostiju, doveli su do izrazitog razređenja alveolarne kosti. Kod izrazite osteoporoze oslabljene su fiziološke osteopoezne funkcije, pa se zato javljaju hipofunkcije osteoblasta. Spongioza je tada reducirana, a korteks izrazito stanjen.^{2,3} Vremenski duže trajanje terapije i veće doze kortikosteroida vode sistemskoj osteoporozi. Sistemska osteoporoza veoma je rasprostranjena metabolička bolest kostiju, koju karakteriše smanjena masa kostiju i promena njihove arhitekture, a sve to vodi povećanoj fragilnosti i riziku od fraktura.^{3,9} Zbog velike učestalosti i rasprostranjenosti, osteoporoza danas dobija mesto jednog od vodećih zdravstvenih problema.^{13,14} Za stomatologiju je od izuzetne važnosti jer zahvata alveolarnu kost. Sistemska osteoporoza neposredno zahvata sve segmente kostiju vilice, a posebno je izražena kod donje vilice, što pokazuju rezultati ove studije, kao i rezultati mnogih drugih istraživanja.^{15,16}

Zaključak

Na osnovu SEM analize može se zaključiti da kortiko-preparati posle duže upotrebe dovode do istanjenja i smanjenja trabekula spongiozne kosti, tj. do razređenja i resorpcije koštanog tkiva uopšte, pa samim tim i tkiva alveolarne kosti. Terapija oporavka, naročito terapija većom dozom ekstrakta timusa, pokazala je izuzetno dobar uspeh u ispitivanom periodu, dok je spontani oporavak dao delimične rezultate.

the increase of cell density, cell activity also increased, and remodeling of the bone was intensified specially, after recovery treatment with thymus extract higher doses, which was in compliance with other researches.^{10,11,12}

In this subgroup there was a greater intensity of osteogenesis and more regular bone structure in rats than in the ones in other examined subgroups. Bone dilution, decreased bone density, is the consequence of bone forming inhibition after effect of corticosteroids, which was confirmed by SEM analyses.^{7,10,13,14}

On the basis of the obtained data it can be concluded that pathogenesis of corticosteroids induced by osteoporosis has not been fully explained, but it is known that bone formation has been reduced and bone resorption has been the same or increased. This difference can be proven with a loss of bone tissue and reduced bone masses.^{14,15} Recovery therapy has been proven as excellent in the examined period.

Corticopreparation used by experimental animals in time of intensive growth and mineralization of bone brought the osteoporosis of alveolar bone. In osteoporosis expression are physical functions and osteopoetic functions and so the hipofunctions osteoblasts is happening. Spongiosis is reduced and cortex thinner.^{2,3} If therapy lasts longer and larger doses corticosteroids are used it will lead to system osteoporosis. Osteoporosis is worldwide metabolic bone disease whose characteristic is smaller bone mass and change in their architecture and leads to fragility and risk of fracture.^{3,9} Because of being worldwide osteoporosis today is one of the biggest problem in the world.^{13,14} Osteoporosis is dentistry from exceptional important because includes alveolar bone. System osteoporosis also includes all segments if jaw bones especilay mandible thath are results of this study as well as many other researches.^{15,16}

Conclusions

Using SEM analysis the recovery by thymus extract showed: enlarged surface matrix, cell density and cell activity. According to the achieved results, it can be concluded that corticopreparations after a long use can lead to decalcification and reduction of bone tissue trabeculae number, i.e. bone dilution and resorption, while the recovery therapy showed partial success in the examined period.

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