ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LV, 27 SECTIO D 2000

Katedra i Klinika Chirurgii Stomatologicznej i Szczękowo-Twarzowej
Akademii Medycznej w Lublinie
Department and Clinic of Dental and Maxillofacial Surgery,
Medical University of Lublin

MANSUR RAHNAMA, TOMASZ TOMASZEWSKI

An assessment of the osteoporosis changes in rat mandible using the scanning electron microscope (SEM)

Ocena zmian osteoporotycznych żuchwy szczura na podstawie techniki elektronowego mikroskopu skanującego (SEM)

At present, in dentistry examinations with the scanning-electron microscope technique (SEM) are carried out very often. This technique has been used to study hard teeth tissues, dental plaque, dental fillings, bond systems and in endodontic treatment (7,8,9,13). The scanning-electron microscope technique has been also of practical importance for evaluation of early bone changes in drug-related osteoporosis. The progress of controlling the process of osteoporosis depends on early detection of bone changes and its dynamics (4,14).

The aim of the experiments based on scanning-electron microscope technique was to evaluate early changes in the bone structure and to determine the dynamics and the degree of rats' mandible demineralization in drug-related osteoporosis.

MATERIAL AND METHODS

Young adult male rats were classified for the experiment after two weeks' period of adaptation to experimental conditions (6). The rats were randomized into 6 groups - 20 rats in each. Each group consisted of 2 subgroups (10 rats in each) according to duration of the experiment. In the first one the research was carried out after 4 weeks of experiment and in the other one after 8 weeks.

The control group consisted of rats which were given 0.9% NaCl twice a day in a dose of 0.5 mg/kg of weight, subgroup K_1 - for 4 weeks and subgroup K_2 - for 8 weeks.

The experimental group H was composed of rats taking Hydrocortisonum hemisuccinatum (Polfa) interperitoneally in a dose of 30 mg/kg of weight, twice a day, subgroup H_1 - for 4 weeks and subgroup H_2 - for 8 weeks.

The rats of the experimental group Ca obtained 10% Calcium Polfa interperitoneally in a dose of 0.01 g/kg of weight and the solution of vitamin A + D_3 (Terpol), with a gastricprobe, moreover 200 i.u. of vitamin A and 100 i.u. of vitamin D_3; subgroup Ca_1 - for 4 weeks, and subgroup Ca_2 - for 8 weeks.

The animals of the experimental group H + Ca received Hydrocortisonum hemisuccinatum with Calcium Polfa 10% in the same doses and for the same period of time as the rats in groups H and Ca. The animals were also given the solution of vitamin A + D_3; subgroups H_1 + Ca_1 - for 4 weeks, and subgroups H_2 + Ca_2 - for 8 weeks.

The animals of the groups M were given 5 i.u./kg of weight salmon calcitonin Miacalcic (Sandoz) once a day; subgroup M_1 - for 4 weeks, and subgroup M_2 - for 8 weeks.

Both Hydrocortisonum hemisuccinatum given interperitoneally and salmon calcitonin Miacalcic were taken by experimental groups H + M in the same dose and for the same period of time as the rats in groups H and M, subgroup H_1 + M_1- for 4 weeks and subgroup H_2 + M_2 – for 8 weeks.

After 4 weeks the rats from subgroups K_1, M_1, Ca_1 H_1 + M_1, H_1 + Ca_1 were anaesthetised with ketamina, decapitated and the mandible bones were prepared. The samples for each experimental group were carefully labelled, and kept separately. After 8 weeks of experiment the remaining rat's mandible bones were prepared in the same way.

Each rat's mandible was cut longitudinally and analysed in a scanning-electron microscope, BS-300 Tesla, with electric tension 24 kV. Properly prepared, the mandible samples were covered with gold in spectron sprinkler CS-100. On the TV screen the examined surfaces were visualized. After surveying each sample of rat mandibles, the selected pictures were photographed with magnification from 70 to 5000x.

RESULTS

After a detailed analysis of the samples and photographs the assessment criteria were established. On the basis of the photographs characteristic of each group of animals, the changes appearing in the structure of mandible of the examined animals were presented.

Normal appearance of the bone in a scanning microscope is demonstrated in the mandible structure of animals from the control group. Microfibrous laminae with thin and smooth surface were arranged regularly (Fig.1).

After the hydrocortisone therapy the structure of bone tissue of mandible of rats from group H_1 and H_2 showed great changes. Significant osteolysis and marks of resorption were observed on the surface of irregular bone laminae of different thickness. The observed picture was characteristic of osteoporosis and osteolysis (Fig 2).

Mandible bones of rats after the calcium therapy (subgroup Ca_1 and Ca_2) resembles a normal structure, however, with an increased amount of calcospherites on the laminae surface. Generally, the thickness of bone structure was observed (Fig 3).

In the group of rats receiving hydrocortisone and calcium (H_1 + Ca_1 and H_2 + Ca_2) changes in bone tissue were slightly different from the picture of bone in previous experimental groups. The resorption was less marked and so was osteoporosis and osteolysis of mandible, compared with the hydrocortisone group. Laminae were similar in thickness as in the experimental group Ca, but in comparison with the control group they were more irregular. On their surface calcospherites were present (Fig. 4).

In the group of rats receiving Miacalcic the bone tissue was more mineralized. Bone laminae were thick and numerous centers of calcification were found on their surface (Fig. 5).

In the experimental hydrocortisone and Miacalcic groups the appearance of bone structure was quite normal, with only locally intensified resorption processes. Thick, regularly arranged laminae had some calcospherites on their surface (Fig. 6).

DISCUSSION

The results obtained from the scanning microscope indicate a precise dependence of administered drugs and mineral elements on the structure of mandibular bones of rats and on the symptoms of osteoporosis due to drugs overdose. A characteristic picture of this disease was observed after administering hydrocortisone to the animals. In the group of animals receiving hydrocortisone for 4 weeks there appeared resorption caverns in the spongy tissue of the mandible and the irregular broken bone laminae of various thickness. The symptoms of osteopenia could be still more clearly observed in the samples of mandibles of animals receiving hydrocortisone for 8 weeks. Apart from irregular bone laminae, clear defects on their surface could be seen. In the group of animals which were given hydrocortisone with calcium the bone laminae were thickened, irregularly arranged and numerous calcospherites could be seen on their surface. Resorption and enlarged Howship cavities indicated advanced form of osteolysis. The bones picture was slightly different in the group of animals which received calcium. As compared with the control group the bone laminae were less regularly arranged, they were thickened and having numerous calcospherites. The picture of mandibular bone in the experimental group of animals receiving hydrocortisone and Miacalcic was similar to the normal one. The bone structure maintained a regular appearance. The bone laminae were thick with a few calcospherites. There were visible enlarged osseous caverns and this accounted for focally occurring resorption processes. It should be then assumed that osteoporosis due to overdosis of drugs depends on the amount of dose and the length of time of receiving the drugs (10). Unfavourable changes in the mandibular bone of the experimental group of animals were present early enough, i.e. especially after 4 weeks of hydrocortisone supply.

In the rats which received calcium we obtained a picture characteristic of hypercalcemia in bone samples.

According to G a l l a g h e r and co-workers (2, 3) a high supply of calcium and vitamin D in the diet may also result in hypercalcemia and hypercalcuria in patients. It may be present in patients with osteoporosis still during the first several weeks of determining the therapeutic dose. It seems that in order to avoid side effects of calcium overdosage, a daily dose should be evaluated in relation to the dose of vitamin D and established respectively according to the individual capabilities of daily absorption and excretion (1).

The bones of masticatory system undergo rebuilding cycles as other bones of the skeleton: resorption caused by osteoclasts and regeneration caused by osteoblasts. In osteoporosis due to drugs overdosis there is an imbalance between the resorption and bone regeneration (11,12). Intensification of resorption is particularly observed in the arrangement and shape of the laminae in the spongy bone which has a faster metabolism than the lamina dura.

CONCLUSIONS

1. Scanning electron microscope studies can show different stages of demineralization of bones.
2. Changes in the ultrastructural picture of the rat bones depend on administered different drugs.
3. 4- and 8-week administration of hydrocortisone in the dose 30 mg/kg of body mass cause advanced osteoporosis and ostolysis in the rat mandible.

REFERENCES

1. B a d u r s k i J. et al.: Osteoporoza. Osteoprint, Białystok 1994.
2. G a l l a g h e r J. C. et al.: Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporosis patients. Effects of age and dietary calcium. J. Clin. Invest., 64, 613, 1963.
3. G a l l a g h e r J. C., R i g g s B.L.: Action of dihydroxy vitamin D_3 on calcium balance and bone turnover and its effect on vertebral fracture rate. Metabolism 39, 30, 1990.
4. H o s z a r s k i K. et al.: Postępy w nieinwazyjnych badaniach densytometrycznych kości. Medycyna 2000, 17-18, 30, 1991.

5. Marowska J.: Przebudowa kości w warunkach fizjologicznych. Medycyna 2000, 17-18, 20, 1991.
6. Mlosek K.: W sprawie diagnostyki radiologicznej osteoporosis przede wszystkim obszaru twarzoczaszki. Pol. Przeg. Rad., 53, 73, 1989.
7. Pawlicka H., Żęcin A.: Ocena bocznej kondensacji gutaperki z użyciem badania w SEM. Czas. Stomat., 48, 644, 1995.
8. Piątowska D., Brauman-Furmanek S: Zastosowanie mikroskopii elektronowej skaningowej do badań stomatologicznych. Czas. Stomat., 32, 1043, 1979.
9. Piątowska D. et al.: Ocena szczelności materiałów do wypełnień kanałowych za pomocą mikroskopu skaningowego. Czas. Stomat., 30, 901, 1977.
10. Rahnama M.: Gęstość kości szczęki i żuchwy szczura w doświadczalnej osteoporozie polekowej. Czas. Stomat., 51, 598, 1998.
11. Rahnama M., Tomaszewski T.: Poziom wapnia w szczęce i żuchwie szczura po doświadczalnym zastosowaniu hydrokortyzonu. Mag. Stomat., 8, 25, 1998.
12. Sieradzki J.: Osteoporozy wtórne. Med. Dypl. Suppl.,11, 17, 1996.
13. Stopa J., Matthews-Brzozowska T.: Ocena ultrastrukturalna połączenia wypełnień z różnych materiałów kompozycyjnych z tkankami zęba. Czas. Stomat., 48, 637, 1995.
14. Walecki J., Wagiel K.: Współczesna metoda diagnostyki osteoporozy. Medycyna 2000, 17-18, 33, 1991.

Otrz.: 2000.07.17

STRESZCZENIE

Na podstawie badań mikroskopowo–elektronowych SEM dokonano oceny struktury kości badanych zwierząt we wtórnej osteoporozie polekowej. Wyniki tych badań potwierdziły zmiany zachodzące w kości żuchwy szczurów pod wpływem działania leków. Po podaniu hydrokortyzonu struktura tkanki kostnej żuchwy szczurów była charakterystyczna dla zaawansowanej osteoporozy i osteolizy. Kości żuchwy szczurów, którym podawano Calcium, przypominały prawidłowy obraz, jednak z większą liczbą kalkosferytów, zaś w grupie zwierząt, która otrzymała Miacalcic, tkanka kostna charakteryzowała się znacznym wysyceniem substancjami mineralnymi i pogrubieniem beleczek kostnych.

EXPLANATION TO FIGURES

Fig 1. The appearance of normal bone laminae. Mag. 5000 x.

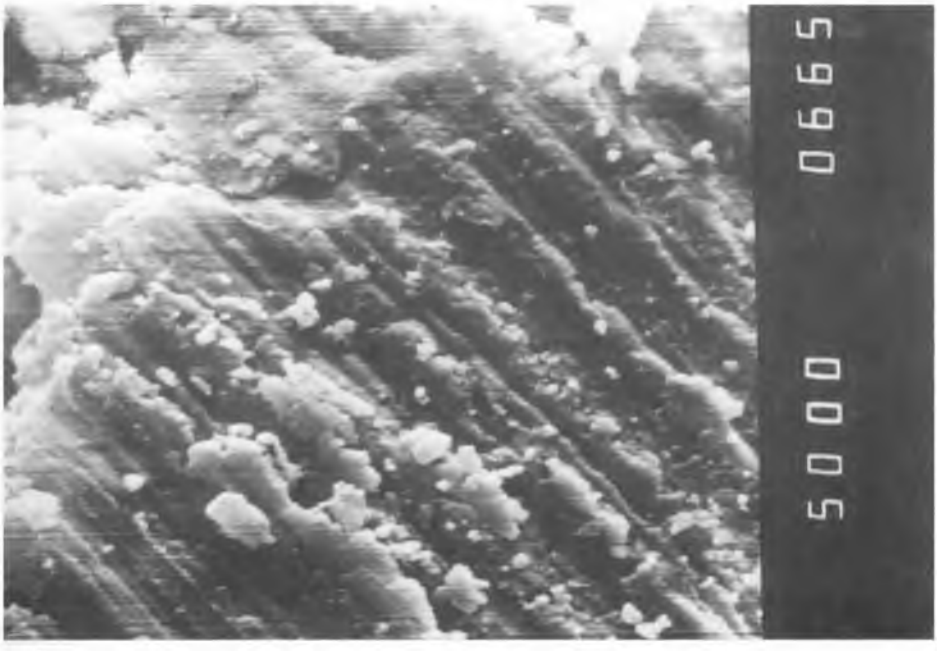
Fig 2. The bone tissue of the rat mandible after 8 weeks hydrocortisone administration. The irregular shape and resorption of bone laminae. Mag. 2000 x.

Fig 3. The picture of hypercalcemia. Accumulation of calcospherites in the rats bone tissue of mandible after 8 weeks of calcium administration. Mag. 5000 x.

Fig 4. The sample of the rat mandible after 8 weeks of hydrocortisone and calcium administration. Irregular bone laminae with small local resoption and calcospherites. Magn. 2000 x.

Fig 5. The rat bone mandible after 8 weeks of Miacalcic administration. Noticeable mineralization of the bone tissue. Mag. 5000 x.

Fig 6. The rat bone mandible after 8 weeks of hydrocortisone and Miacalcic administration. Normal structure of bone tissue with single callosperites and a small focus of osteolysis. Mag. 5000 x.



g. 1

Fig. 3

Fig. 4

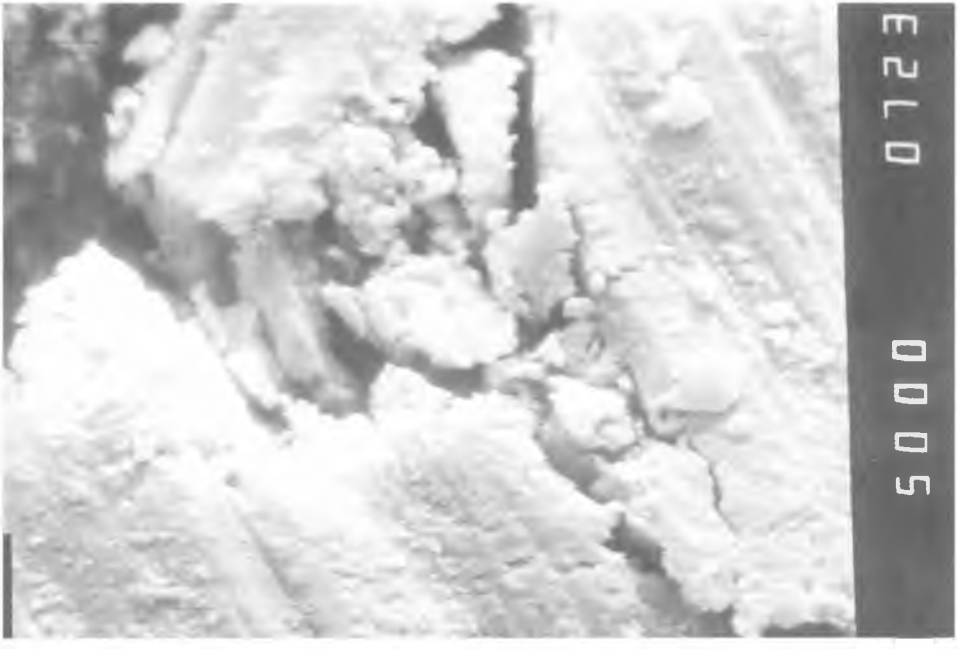


Fig. 5

Fig. 6