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INFLUENCE OF TAMOXIFEN ON THE MANDIBLE BONE AND HARD TEETH TISSUES OF ANIMALS

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Tamoxifen, protactedly used, can cause disadvantageous changes in the bones and in the hard teeth tissues. The aim of this paper was to define to what extent tamoxifen given to the animals influences the structure of the mandible bone and the hard teeth tissues.

Key words: *tamoxifen, changes in the resistance tissues*

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INTRODUCTION

Years of clinical observation, described in literature, show that the changes of dystrophic character in the stomatognathic system are noted to be expressed more strongly among women undergoing hormonal replacement therapy. Pharmacological prophylaxis of metastasis of hormone-dependent tumors resolves into the use of antiestrogen drugs, e.g. tamoxifen. This drug, which has been largely used in recent years, causes morphological changes in bones, e.g. aplasia and fibromatosis in marrow cavity [6]. As opposed to biosphonians, which are the antagonists of endogenous estrogens, tamoxifen blocks estrogen receptor. Moreover, it obstructs the transport of calcium through the cell membranes [7]. During the tamoxifen treatment, the amount of cell-type estrogen receptors decreases. Weak effectiveness of estrogen is probably responsible for the occurance of cell receptors for progestins, which is observed during the tamoxifen treatment [1]. This drug stops mitosis of cells, which have receptors for estradiol [receptor plus: ER (+)]. The susceptibility of the breast cancer to the drug is strictly dependent on the number of ER in the tissue and it increases when the tumor cells also cointain receptors for progestins [2–4]. The direct anti-cancer effect and the possibility of the increase of tumor by the influence on the action of hypothalamo-ovarion system have also been considered [8, 9]. After oral intake, tamoxifen is quickly absorbed from the digestive system, attaining maximum concentration in serum after 4–7 h. Constant therapeutic concentration of the drug in plasma can be reached after the period of saturation lasting for 4 weeks at the 40 mg of the daily drug dosage. Necessary time in order to reach therapeutic effect is approximately 10 months, but the result of treatment can last for many years. Higher efficiency can be observed among elderly women, while among premenopausal women the results are worse [5].

Tamoxifen is the drug whose efficiency in curing breast cancer has been proven. Although if used protractedly it has adverse effects, the benefits of therapy with this drug are higher than the drawbacks. That is why we would like to know the range and the level of these drawbacks. For a doctor, prosthetics bone base of mandible and jaw is of basic significance for the transportation of chewing forces, both – in the case of patients with dentition and those who are toothless, being under traditional treatment and using implant systems.

The aim of this paper was to answer the following questions.

1. To what extent tamoxifen taken by animals influences the level of calcium, magnesium and phosphorus in the mandible bone and hard teeth tissues?
2. In what way does the radiological picture of mandible and teeth change after taking this drug in animals?
3. Does tamoxifen cause morphological changes in the mandible bone and in the hard teeth tissues?

MATERIALS and METHODS

Tests have been carried out on 30 white, full-grown, female Wistar rats from the Toxicology Department of Medical Academy in Poznań. The animals were 12 months old, their initial weight was 280–290 g. During the experiment, the rats were placed in cages – 5 rats in each cage. The animals included in the experiment undergone one-month adaptation period in animal rooms of the Toxicology and Pharmacology Department of Pomeranian Medical University in Szczecin. The temperature in the rooms was 20–22°C and the average humidity was 60%. Those rooms were darkened and illuminated by glow-light that was switching itself off automaticaly every 12 h. The animals were fed with standard laboratory fodder LSM granulatum. Thirty three animals were divided into 3 groups of the same size: group I – control one for groups B1 and B2. Group B1 – tamoxifen was given at the dose of 2 mg/kg for the period of three months; group B2 – tamoxifen was given at the dose of 4 mg/kg also for 3 months. The drug dissolved in small amount of water and mixed with fodder was given to the rats every day. The animals were observed and weighed every week. After this time, the animals were anesthetized with ether. During the dissection, mandible bone together with teeth was skeletonized. Then the tests of mineral composition of the mandible bone and teeth were carried out at the Institute of Chemistry and Biochemistry of our university and the levels of calcium, magnesium and phosphorus were determined. Calcium and magnesium were assessed by the atomic spectometer absorption method, using the Pu 9100X Philips apparatus. Calcium was determined at the wavelength of 422.7 nm, magnesium at 258.2 nm. The measure-

ment was carried out in the oxacetylene flame in the presence of ionizing buffer in the form of 0.5% lanthanum solution. Concentration values were calculated on the basis of standardization curve, with correction for the weighed amount and dilution. Phosphorus was determined spectrometrically with Analco test based on the reaction yielding florid phosphoric-molbydic complex, using the Lambda 40 Perkin-Elmder apparatus. In order to detect pathological changes in mandibles and in hard teeth tissues, the radiodensitometric test in the Digora system was done and the average optical consistency was estimated in the examined groups. X-ray pictures were taken using the X-ray Satalec X mind apparatus. Mandible bone with teeth was photographed in the front-back position, then the pictures were analyzed in the Digora single-color option. In order to estimate mean consistency of teeth hard structures, the enamel saturation in incisors and premolars was measured radiodensitometrically in particular groups. The mean consistency of mandible bone saturation was tested and estimated in 5 reference points on the particular bones. During the dissection, the rats mandible bones with teeth were taken and skeletonized. The segments taken were put in the 4% solution of formalin neutralized by calcium carbonate. Next, at the Department of Pathomorphology histological specimens of the mandible bone and teeth were prepared, which were later tested under the light microscope and compared with the control group. Fragment of the mandible was decalcified in 3% nitric acid during the period of 12 h at room temperature. The tissue segment 4.5 mm thick, was always taken from the same place of the mandible cross-section. Histological specimens were made using the paraffine method, then they were cut into fragments 5 microns thick. The specimens were stained with hematoxicin and eosin. In total, 90 specimens were made and compared. Because not all variables had normal distribution, non-parametric methods were used in statistical estimates. The comparisons of mean values of incoherent tests were made using the U Mann-Whitney test. In the case of mutual comparisons of more than two mean values the Kruskal-Wallis test was employed. The values differing at p 0.05 were considered significant.

RESULTS and DISCUSSION

Mean levels of calcium, phosphorus and magnesium in the mandible bone and in teeth have been presented in Tables 1, 2 and 3.

As the estimates in group B1 show only the level of phosphorus in bone differed significantly from the level in the control group. However, in group B2 (higher dose of tamoxifen) magnesium level in the bone and calcium content in the mandible bone, in the incisors and premolars differed significantly.

In group B2 after tamoxifen treatment at the dose of 4 mg/kg, only the correlation between the phosphorus in bone and the calcium in incisors was found. However, there was a lack of correlation between other elements composing hydroxyapatites.

After analyzing the mean values of optical consistency, no differences were detected between the

Table 1. Control group – average content of elements in mg/g

Element distribution	N	Average	SD	Min	Max
Ca in molar tooth	11	297.4273	19.58749	278.7700	347.3700
Ca in incisive tooth	11	274.4236	24.84937	236.0900	315.1000
Ca in mandible bone	11	275.5509	23.34987	240.8500	316.3900
Mg in molar tooth	11	3.1082	0.38652	2.6900	3.8700
Mg in incisive tooth	11	9.4127	2.58550	3.6600	13.3700
Mg in mandible bone	11	4.5182	0.39544	3.5400	5.0700
P in molar tooth	11	140.3582	9.26529	131.4000	162.4800
P in incisive tooth	11	141.3700	11.05595	129.5500	167.7000
P in mandible bone	11	125.8345	10.74046	113.1600	152.9800

Average – arithmetical means; Min, Max – minimum and maximum values; SD – standard deviation

Table 2. Study group B1 – average content of elements in mg/g

Element distribution	N	Average	SD	Min	Max
Ca in molar tooth	11	316.6155	29.62018	269.4600	383.8100
Ca in incisive tooth	10	284.5610	20.75447	255.6500	322.2600
Ca in mandible bone	11	285.4936	19.55827	253.8900	308.5400
Mg in molar tooth	11	2.8282	0.43079	2.2300	3.6100
Mg in incisive tooth	10	10.0780	1.18704	8.8500	12.1600
Mg in mandible bone	11	4.3273	0.40581	3.8200	5.1100
P in molar tooth	11	151.8136	19.08217	125.4200	180.2900
P in incisive tooth	10	181.9160	60.36291	131.4600	321.4300
P in mandible bone	11	136.1445	13.07723	121.4400	169.3400

Descriptions: see Table 1

Table 3. Study group B2 – average content of elements in mg/g

Element distribution	N	Average	SD	Min	Max
Ca in molar tooth	11	265.4018	18.70431	231.6600	304.6500
Ca in incisive tooth	11	252.0591	17.79567	227.4000	286.8500
Ca in mandible bone	11	249.9364	11.65855	234.9500	266.5400
Mg in molar tooth	11	4.2655	0.69530	3.5100	6.1200
Mg in incisive tooth	11	10.9209	1.17804	8.5900	12.1400
Mg in mandible bone	11	5.1327	0.56821	4.5800	6.4700
P in molar tooth	11	134.1518	12.16840	113.5200	157.2600
P in incisive tooth	11	153.9545	17.32542	133.6500	181.5600
P in mandible bone	11	132.7155	20.38892	112.3900	188.3600

Descriptions: see Table 1

values in the examined groups and in the control group (Fig. 1–3).

Also, in the histological specimens, no morphological changes were observed. Great number of publication is devoted to optimization of the early osteoporosis detection in people with low bone mass in the early stage of the disease. What is emphasized is the significance of the mandible radiographic studies for the early detection of the osteoporotic changes. In the presented work, the mandible and teeth were studied with biochemical, radiodensitometrical and histomorphological methods, in order to estimate the changes caused pharmacologically by tamoxifen in these tissues. The results of the biochemical studies show that tamoxifen, given at the dose of 4 mg/kg, causes distempers in the transport or the exchange of the elements composing hydroxyapatites. Similar estimation of calcium, phosphorus and magnesium levels in the mandible and hard teeth tissues (after tamoxifen) has not been reported so far. However, the histological studies of mandible and teeth in rats did not reveal (after 3 months of tamoxifen treatment) any morphological differences in comparison to the control group. The result of our study differs from the results of other authors [6]. They obtained the increase in the number of empty antrums (necroticly changed osteocits) in the mandible bone after giving tamoxifen at both doses in comparison with the control group. Because the histological specimens of dense bone are never identical, the quantitative estimate of the changes in these specimens cannot be carried out, and the estimate of the number of osteocits changed necrotically is subjec-

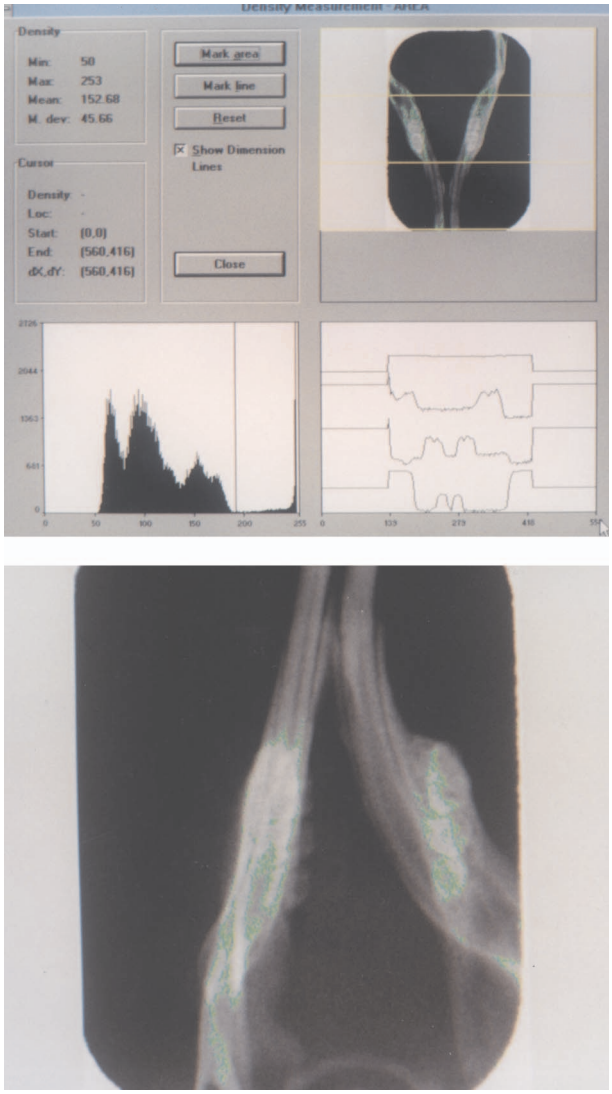

Fig. 1. Photos of mandible with teeth in Digora-system – control group (green color means optical consistency with the same parameters)

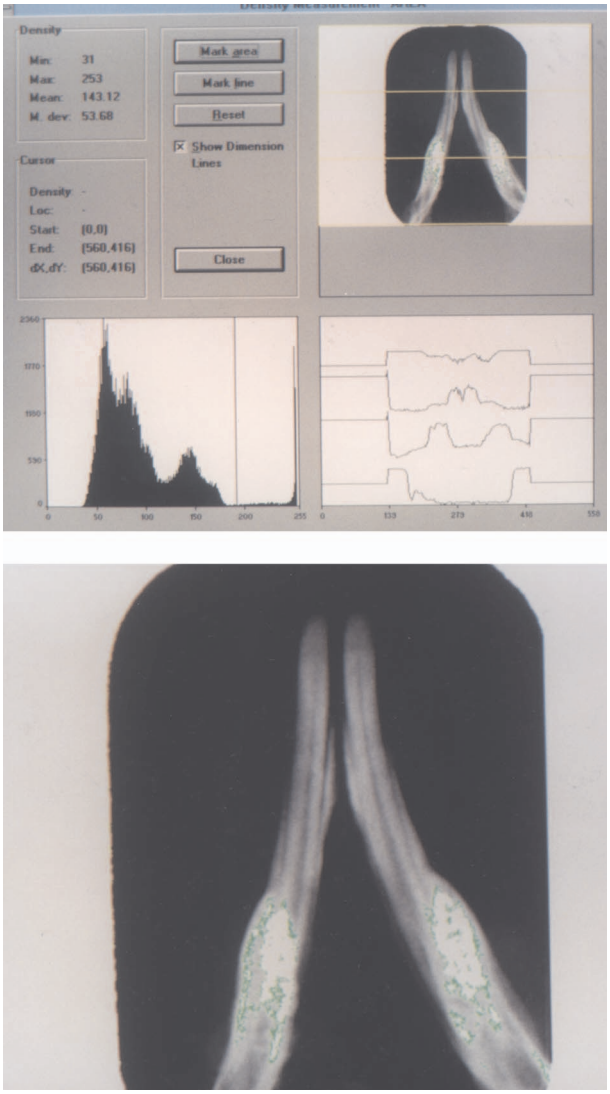

Fig. 2. Photos of mandible with teeth in Digora-system – B1 group

tive and inaccurate. The results of these morphological studies cannot prove the change in the animal bone structure receiving tamoxifen.

Summing up, one can conclude that tamoxifen given to animals during the period of 3 months does not cause any perceptible changes in the radiological and morphological picture of the mandible and teeth. This drug given at higher dose caused only the lack of correlation between the elements composing hydroxyapatites. Tamoxifen given for the period longer would probably change the radiological and the morphological picture. The biochemical changes constituting often the beginning of all the distempers may serve as a proof.

CONCLUSIONS

1. Tamoxifen taken by the animals at the dose of 4 mg/kg probably causes distempers in the transportation and the exchange of elements which are the constituents of hydroxyapatite. It was evidenced by the lack of correlation between calcium, magnesium and phosphorus.
2. No radical changes in the mean values of optical consistency of the mandible bone and teeth have been found.
3. After three-month intake of tamoxifen, no detectable morphological changes in the mandible and in teeth occured in the examined animals.

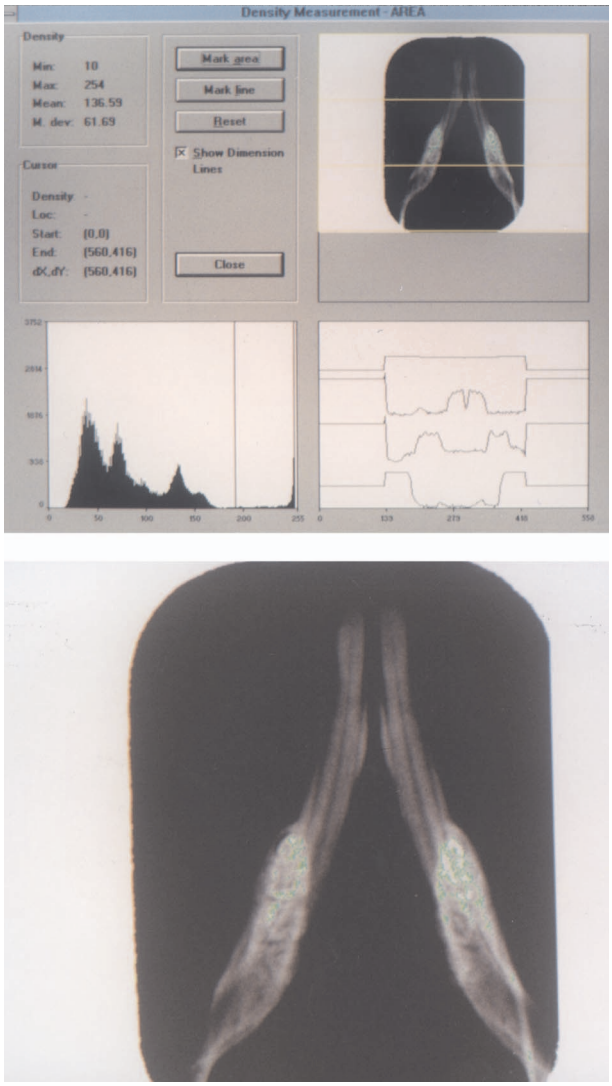

Fig. 3. Photos of mandible with teeth – B2 group

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