RELATIONSHIP BETWEEN QUANTITATIVE CHARACTERISTICS OF THE REGENERATIVE PROCESS OF BONE TISSUE IN MANDIBULAR FRACTURES AND THE LEVEL OF ENDOGENOUS INTOXICATION

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Abstract

Introduction. Fractures of the lower jaw are one of the most common injuries in the head and neck. Bone regeneration is a key step in the fracture healing process and plays an important role in restoring jaw function. However, the presence of endogenous intoxication can complicate the healing process and lead to a decreased efficiency of the regenerative process. In this article, we examine the relationship between the regenerative process of bone tissue in mandibular fractures and the level of endogenous intoxication. We investigate the mechanisms of bone regeneration and study the effect of endogenous intoxication on the healing process. Our research will help better understand the healing processes of mandibular fractures and develop more effective treatments.**The aim of the study** was to establish the effects of chronic endogenous intoxication on both intact bone and the process of reparative osteogenesis in mandibular fractures. **Materials and methods**. Regularities of morphofunctional transformations in bone tissue were studied during spontaneous healing of mandibular fractures in rats against the background of chronic endogenous intoxication after 30, 60 and 90 days. Simulation of chronic endogenous intoxication was performed by repeated administration of tetrachlormethane and bacterial lipopolysaccharide doses. Determination of medium molecular weight substances, as well as their oligopeptide fraction in blood serum were used as integral laboratory and biochemical indicators of ET development. Concentrations of products of free radical oxidation of lipids were also determined in the blood plasma of rats. Monoclonal antibodies to the proliferating cell nuclear antigen PCNA were used to detect proliferative activity in the regenerate and surrounding bone tissue. The morphometric study was performed in accordance with the principles of systemic quantitative analysis. **Results and discussion**. The formation of an osteoregenerate at mandibular fracture in rats against the background of chronic endotoxicosis is accompanied by a decreased growth of the volume fraction of bone tissue and stromal-vascular component which, according to correlation analysis, is associated with the level of medium molecular weight substances and malonic dialdehyde in blood plasma. There is a strong positive correlation between the volume fraction of the regenerate vessels and the level of malonic dialdehyde (r=0.671-0.692). Negative correlations were detected for the numerical density of osteoblasts and osteocytes (r=-0.622) and positive correlations for osteoclasts (r=0.654-0.873). **Conclusions**. The study provided a lot of morphological evidence of the effect of chronic endogenous intoxication on both intact bone and the process of reparative osteogenesis after mandibular fractures.

Keywords: *mandibular fractures, chronic endogenous intoxication, reparative osteogenesis.*

1. INTRODUCTION

In the modern world, one of the significant medical and social problems is represented by traumatisms [1]. Domestic and occupational traumas are of particular importance. In the structure of traumas, fractures of the maxillofacial region account for about 12% of all fractures [2].

In the regulation of reparative regeneration of bone tissue after fractures and combined injuries, the relationship between the nervous, endocrine and immune mechanisms of osteogenesis regulation, which are implemented at tissue level by a whole package of biologically active mediators, is of significant importance [3]. They regulate the growth and differentiation of osteoblastic, osteoclastic, vascular and connective tissue growths, eventually forming a functioning bone [4]. When the regulatory mechanisms of reparative osteogenesis are disrupted, pathological remodeling of bone tissue and formation of structurally and functionally incomplete regenerate occur [5].

One of the factors affecting bone tissue regeneration is the impact of various toxic substances, including endogenous toxic

compounds. Chronic endotoxemia (ET) refers to multicomponent processes, being observed in various background diseases, competing pathology, developing against the background of autonomic and endocrine dysregulation [6]. Because of this, it is of interest to solve the urgent problem of establishing the features of osteoregeneration against the background of chronic ET.

The aim of the study was to establish the effects of chronic endogenous intoxication on both intact bone and the process of reparative osteogenesis in mandibular fractures.

2. MATERIALS AND METHODS

Regularities of morphofunctional transformations in the bone tissue were studied during spontaneous healing of mandibular fractures in rats against the background of chronic endogenous intoxication after 30, 60 and 90 days. Fracture of the mandible was carried out in rats in a typical location, generally accepted for experimental studies [7]. For this purpose, an incision of the soft tissues of the gum on the buccal and lingual sides in the projection and slightly distally to the right incisor of the mandible was made in rats under nembutal anesthesia. The instrument was then placed under the soft tissues, pressed against the exposed periosteum, and a visual diastasis of 1 mm was achieved by breaking and biting with rocking movements in the sagittal plane. After that, mechanical restoration of the anatomical congruence of the alveolar process and mandible was performed. The operation was completed by microspinning the incisor and the alveolar process to the homonymous adjacent structures on the left side.

Chronic endogenous intoxication was simulated by repeated administration of doses of tetrachloromethane (TCHM) and bacterial lipopolysaccharide (LPS). S.Thyphi LPS (Sigma, USA), well known for its toxicological characteristics, was used. General scheme of the model: animals received 0.5 ml/kg of TCM on an empty stomach 5 times a week [8]. On day 6, LPS was added intraperitoneally, in a dose of 0.2 mg/kg body weight. On day 7, no manipulations were performed. As integral laboratory and biochemical indicators of ET development, we used the determination of medium molecular weight substances (MMWS) at 210-320 nm on a Shimadzu double-beam spectrophotometer, as well as their oligopeptide fraction (at 584 nm) in blood serum [9]. Concentrations of products of free radical oxidation of lipids were also determined in blood plasma of rats. Concentration of malonic dialdehyde (MDA) by reaction with thiobarbituric acid was determined according to a modification based on extraction of stained reaction products into the hydrophobic phase [10]. To detect proliferative activity in the regenerate and surrounding bone tissue, we used monoclonal antibodies to the proliferating cell nuclear antigen (PCNA, DakoCytomation, Denmark). Monoclonal antibodies to vimentin (clone V9) and osteonectin (clone 15G12) (Novocastra, UK) were used to determine connective and bone tissue components [11]. Visualization was performed using an indirect immunoperoxidase method (Novocastra, UK) with high-temperature and enzymatic demasking of antigens [12].

Special attention was paid to the correlations between biochemical indices of endogenous ET intoxication and quantitative indices of bone regeneration [13]. The morphometric study was performed in accordance with the principles of systemic quantitative analysis [14,15]. In the regenerate, quantitative methods were used to determine the volume fractions (Units, μm/μm) of the connective tissue, vessels and bone beams, as well as the numerical density of cellular elements (1/μm). The results of the study on regenerates were supplemented using the method of radial morphometry [16].

Mathematical data processing was performed directly from the EXEL 10.0 general data matrix (Microsoft, USA) using STATGRAPH 5.1 (Microsoft, USA). This included determining the mean, standard deviation, mode, and representativeness error. Then, the reliability of differences between the samples was assessed using regularities accepted for biomedical research (sample size, nature of distribution, and nonparametric criteria). Correlation analysis was performed by the simple paired Spearman correlation method.

3. RESULTS

The content of MMWS and their oligopeptide fraction in blood plasma in the simulation of chronic endogenous intoxication was increased at all terms of the experiment. On the 30th day, it exceeded the analogous values in animals of control group by 1.44 times ($p<0.05$) and 2.06 times ($p<0.01$), respectively, and on the 90-th day - by 2.06 and 2.63 times ($p<0.01$) already. The concentration of malonic dialdehyde (MDA), as a reflection of the total lipid peroxidation activity, increased by approximately 1.5-fold and remained stable throughout the experiment (Table 1).

Table 1. Biochemical markers of endotoxemia in rats during chronic endogenous intoxication (M±m)

Indicator	Control group	Chronic endogenous intoxication		
		30 days	60 days	90 days
MMWS, units.	0.18±0.02	0.26±0.03*	0.32±0.04*	0.37±0.05*
Oligopeptides, mg/l	124.5±9.3	249.1±20.7*	307.0±25.1*	325.6±27.5*
MDA, mmol/l	5.61±0.32	7.16±0.51*	7.43±0.59*	8.02±0.60*
Note: *- significant differences compared to the control group				

Simulation of a fracture against the background of chronic endogenous intoxication revealed an increase in MMWS and their oligopeptide fraction, which occurred similarly to the main series: 1.22 – 1.35-fold by day 14, 1.44 – 1.56-fold by day 30 (all $p<0.05$).

From the 30th day of the experiment, a relatively high increase in the level of malonic dialdehyde was detected, which exceeded that of the control group rats almost twofold. These signs indicated an additional contribution of the fracture itself and its regeneration processes to the formation of the pool of endogenous toxic compounds (Table 2).

Table 2. Biochemical markers of endotoxemia in rats simulating fracture against the background of chronic endogenous intoxication (M±m)

Indicator	ET without fracture	Chronic endogenous intoxication		
		30 days	60 days	90 days
MMWS, units.	0.26±0.03	0.28±0.03	0.35±0,04*	0.40±0.05*
Oligopeptides, mg/l	249.1 ±0.7	292.5±22.8	329.4±28,0*	340.2±29.2*
MDA, mmol/l	7.16±0.51	8.32±0.62	10.89±0.73*	11.17±1.02*
Note: *- significant differences compared to ET without fracture				

Next, we investigated the relationships between these three relatively independent quantitative indices characterizing the development of chronic endogenous intoxication and the indices of regenerate morphometry by means of correlation analysis. Among the morphometry indices, only those formed by direct measurement or counting, that is, not related to each other by mathematical formulas, were selected. In the randomly selected morphological material of control animals and at 3 terms of the process, at least 10 fully formed matrices for correlation analysis appeared in each group.

The results of correlation analysis revealed a close correlation between the indices of endogenous intoxication and bone tissue morphometry, as well as tissue regenerates after mandibular fractures (Table 3).

Thus, the decrease in the volume fraction of bone beams in chronic endogenous intoxication depended on the level of MMWS and MDA in blood plasma (r=-0.810 and -0.635, respectively). Similar relationships were detected for vascular volume fraction (VF) (between -0.729 and -0.671), but not for connective tissue VF. As to the numerical density (ND) of osteoblasts, osteocytes, fibroblasts, and fibrocytes, no conclusive correlations were obtained, some of them being multidirectional for individual indicators of endogenous intoxication. However, strong positive correlations (r from 0.702 and 0.873) were revealed for the numerical density of osteoclasts.

Table 3. Matrix of correlations between markers of chronic ET and indices of bone tissue morphometry in rats (r)

Morphometric indices bone tissue	ET markers		
	MMWS	Oligopeptides	MDA
VF bone beams	-0.810*	-0.612	-0.635
VF connective tissue	0.355	0.348	-0.236
Volume fraction of vessels	0.729*	0.721*	0.671*
ND osteoblasts and osteocytes	-0.425	-0.304	-0.622
ND osteoclasts	0.873*	0.711*	0.702*
ND fibroblasts and fibrocytes	-0.294	0.157	0.252
PCNA-positive osteoblasts	0.352	0.494	0.722*
Note: * - strong reliable correlations (>0.669)			

The correlation analysis of ET markers and parameters of regenerate morphometry after mandibular fractures revealed somewhat different relationships (Table 4).

The data obtained reflects the known notion of a significant contribution of systemic and local activation of lipid peroxidation in the initiation of tissue damage, in response to endogenous intoxication, and the involvement of these factors in the disorders of reparative osteogenesis after bone fractures.

Table 4. Matrix of correlations between markers of chronic ET and indices of morphometry of tissue regenerates after mandibular fractures in rats (r)

Morphometry indices regenerates	ET markers		
	MMWS	Oligopeptides	MDA
VF bone beams	-0,538	-0,474	-0,701*
VF connective tissue	0,711*	0,697	0,714
Volume fraction of vessels	0,238	-0,149	0,692*
ND osteoblasts and osteocytes	-0,425	-0,304	-0,622
ND osteoclasts	0,654	0,582	0,693
ND fibroblasts and fibrocytes	-0,276	-0,102	0,272
PCNA-positive osteoblasts	0,352	0,394	0,482
Note: * - strong reliable correlations (>0.669)			

The decrease in bone tissue VF gain as a result of fracture healing in this series was less dependent on plasma levels of MMWS and oligopeptides (r=-0.538 and -0.474, respectively), but more dependent on MDA levels (r=0.701). There was also a correlation with strong positive correlations for connective tissue VF (r between 0.697 and 0.771). There was a strong positive correlation between the level of MDA (but not with MMWS s and oligopeptides) and the volume fraction of the regenerate vessels (0.692). Negative correlations were found for the numerical density of osteoblasts and osteocytes, and positive correlations for osteoclasts, but not as strong as in the series without fracture modeling. The correlations with the percentage of PCNA-positive cells were not so strong, not exceeding 0.482 modulo.

4. DISCUSSION

Formation of an osteoregenerate at mandibular fracture in rats against the background of chronic endotoxicosis is accompanied by a decreased growth of the volume fraction of bone tissue and stromal-vascular component which, according to correlation analysis, is associated with the level of medium molecular weight substances and malonic dialdehyde in blood plasma [17,18]. There is a strong positive correlation between the volume fraction of the regenerate vessels and the level of malonic dialdehyde (r =0.671-0.692). Negative correlations were detected for the numerical density of osteoblasts and osteocytes (r=-0.622), and positive correlations for osteoclasts (r=0.654-0.873).

The developed quantitative criteria for evaluation of bone regenerate (volume fraction of connective tissue, vessels and bone beams, numerical density of osteoblasts and osteoclasts) and X-ray densitometry study allowed us to give an accurate characteristic of osteoregeneration processes against chronic endotoxicosis, which can serve as a basis for the development of targeted prevention of the detected violations of reparative osteogenesis.

5. CONCLUSIONS

Our study provided a lot of morphological evidence on the effect of chronic endogenous intoxication on both intact bone and the process of reparative osteogenesis after mandibular fractures. The patho- and morphogenesis of such disorders is certainly of a complex nature, including the impact of toxic compounds directly on the vascular endothelium, osteoblasts and osteoclasts, causing structural restructuring of bone tissue with the predominance of diffuse compensated osteoporosis.

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