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Changes in Long Tubular Bones of Rats in Experimental Osteoporosis

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Abstract: After gastric resection, most patients experience serious digestive disorders, a decrease in body weight [1] and the appearance of severe clinical manifestations of a number of diseases [10]. The gastrointestinal tract (GIT), as one of the subsystems of bone mineral metabolism, takes an active part in maintaining the age-related bone mass constant [4]. In diseases of the gastrointestinal tract, the absorption of minerals and vitamins (including Ca and vitamin D) is impaired. This leads to a decrease in the calcium content in bone tissue, a decrease in bone mineral density (BMD) – osteoporosis [6]. In our study, the experimental condition of osteoporosis was created by removing a certain part of the stomach by simulating the method of gastric resection by the Billroth-II method.

Purpose of the study: creation of an experimental state of osteoporosis by gastric resection in experimental rats by the Billroth-II method. Study of the morphometric state of bone tissue in long tubular bones and the stomach wall.

Material and methods: 27 white male rats weighing 170-200 g from the Vistar line were selected. Of these, 12 rats were divided as a control group and 15 as an experimental group. The rats of the experimental group underwent gastric resection using the Billroth-II method, and three months later morphometric changes in the long tubular bones and the stomach wall were studied.

Conclusion: removal of a certain part of the stomach in experimental animals in the form of resection according to Billrot-II led to a significant morphological change in its wall, osteomorphometric parameters and architectonics of the long tubular bone..

Keywords: experimental osteoporosis, gastric resection, bone tissue, morphometry.

Introduction. Experimental modeling of pathological processes plays an important role in the study of the mechanisms of development of various diseases [1, 11]. To date, experimental osteoporosis in experimental animals (primates, dogs, pigs, mice, rats) is induced by various methods: ovariectomy [7], castration [9], hypokinesia and hypodynamia [8, 14], immobilization, administration of high concentrations of thyroidin, PTH, glucocorticoids and others [2, 12]. In our case, experimental osteoporosis was obtained by removing a certain part of the stomach, simulating gastric resection according to the Billroth-II method.

The gastrointestinal tract (GIT), as one of the subsystems of bone mineral metabolism, takes an active part in maintaining the age-related bone mass constant [4]. In diseases of the gastrointestinal tract, the absorption of minerals and vitamins (including Ca and vitamin D) is impaired. This leads to a decrease in the calcium content in bone tissue, a decrease in bone mineral density (BMD) – osteoporosis [6].

In the clinic, often, gastric resection in humans leads to significant nutritional disorders, which are manifested by weight deficit [1] and is accompanied by severe clinical manifestations of a number of diseases [10].

The preservation of the integrity of the stomach during its surgical treatment contributes to the preservation of the naturally inherent cycle of digestive physiology. In practical medicine, there are numerous publications on the effect of various surgical interventions in the stomach on the state of bone tissue, but the results are contradictory.

The aim of the study was to study the morphofunctional state of bone tissue after experimental osteoporosis according to the Billroth-II method in experimental animals.

Material and methods: For the experiment, 27 outbred white male rats, weighing 170-200 g, were selected. The first group consisted of 12 rats of the control group, the second - the experimental group - of 15 rats, in which the stomach was resected according to the Billroth-II method.

Both groups of rats were kept in the usual conditions of the vivarium and on the same diet. Before the start of the experiment (PNE), on days 30, 60 and 90, the body weight of the animals of both groups was measured. The animals were taken out of the experiment after 90 days by decapitation under ether anesthesia in accordance with the «rules for working with experimental animals». The femur was freed from the soft tissues, weighed on an analytical balance - VRL-200, and osteometric measurements were performed with a caliper with an accuracy of 0.05 mm. Blocks were prepared from the walls of the stomach, as well as from parts of the femur, according to the rules for the preparation of histological preparations.

Serial sections were made from soft tissues with a thickness of 2-3, hard ones - 7-10 microns, the preparations were stained with hematoxylin, eosin and according to Van Gieson.

The results were processed using Microsoft Excel XP, and the arithmetic mean values (M) and their errors (m) were calculated.

Results and discussion. Analyzes of determining the live weight of animals for 3 months showed that the weight of the operated animals, starting from 1 month, significantly decreased until the end of the experiment. In comparison with the control group, they ate little, their wool lost its natural shine, and were inactive (Table №1).

In the clinic, often gastric resection in humans leads to significant nutritional disorders, which are manifested by weight deficit [1] and is accompanied by severe clinical manifestations of a number of diseases [6,18].

Table №1. Live weight of animals during the experiment (in grams), (M ±m)

Timing of determining the weight of	Group of animals	
	Control, n = 12	Billroth-II, n = 15
Before the beginning of the experiment	174,80 ± 2,85	181,80 ± 1,05*
30 day	202,00 ± 3,54	197,40 ± 1,71**
60 day	212,60 ± 3,81	199,40 ± 2,76**
90 day	228,00 ± 3,61	201,20 ± 3,28**
Note: * - p < 0,05; ** - p < 0,001 validity from the control group		

Atrophic changes were revealed on the stomach wall of the operated animals, which was confirmed by morphometric changes. The thickness of the stomach wall in intact and experimental animals was, respectively: mucous membrane- (610,30 ± 8,70) и (500,40 ± 6,30) (decreased by 18%); submucosal layer - (78,50 ± 1,80) and (71,50 ± 1,20) (8,9 %); muscular layer - (627,90 ± 8,70) and (530,40 ± 7,20) (15,5 %), serous membrane - (23,40 ± 0,66) and (21,20 ± 0,51) mkm (9,4 %).At the same time, the data of the operated animals were significantly reduced in comparison with the control group in all cases (p <0,001).

Gastric resection according to Billroth-II leads to anatomical changes in the gastroduodenal zone [16,17]. Such a change in humans often leads to a disease of the operated stomach: dumping syndrome; deficiency of hydrochloric acid and pepsin, which are very necessary for the breakdown of calcium salts; accelerated passage of food, which leads to malabsorption disorder of absorption of calcium and vitamin *D.* Ultimately, all these processes eventually lead to the demineralization of bone tissue, osteopenia and osteoporosis develop [3,21]. Chronic calcium and vitamin D deficiency or malabsorption after gastrectomy can lead to bone loss [13, 15,19]. One of the problem areas, in terms of injury, in osteoporosis is the femur. Therefore, in the example of this bone, its morphometric parameters of both groups were given, respectively:bone mass-(563,83 ± 4,22) and (506,95 ± 4,53) mg; bone length-(35,98 ± 0,26) and (32,38 ± 0,28); proximal pineal gland width- (7,55 ± 0,05) and (6,78 ± 0,06); distal pineal gland width - (6,48 ± 0,04) and (5,84 ± 0,05); mid-diaphysis - (3,64 ± 0,02) and (3,27 ± 0,02); and anteroposterior diaphysis size - (3,54 ± 0,02) and (3,18 ± 0,02) mm. In all cases, the indices of the operated animals were significantly reduced in comparison with the data of intact animals (p <0,001).

We also studied the morphometric parameters of the femoral metaphysis in intact and operated animals. At the same time, it was revealed that the volumetric density of the primary spongiosis of the femoral metaphysis was: (46,8 ± 1,2) and (38,1 ± 1,2) (decreased by 18,6 %); secondary spongiosa - (30,3 ± 0,9) and (17,50 ± 0,51) (42,5 %); the relative volume of osteoblasts was (28,80 ± 0,81) and (16,70 ± 0,48) (42 %); and the volume of osteoclasts-(2,10 ± 0,06) and (3,70 ± 0,12) % (increased by76,2 %). It should be noted that in the control and experimental groups of animals, the length of the trabeculae of the primary spongiosis of the femoral metaphysis was, respectively: - (453,60 ± 6,0) и (306,50 ± 10,2) (32,4 %), and the thickness of the plate(206,50 ± 6,06) и (187,20 ± 6,30) мкм (9,3 %). The given digital values were statistically significantly different (p <0,001) among themselves.

Conclusion. Thus, after resection of the stomach by the Billroth-II method in experimental animals, there is a violation of the digestive process, as evidenced by a decrease in body weight and histomorphological changes in the stomach wall. The experimental data obtained by us and their comparison with a number of clinical literature data allow us to conclude that resection according to Billroth-II causes a number of changes that, ultimately, lead to morphofunctional deviations in the structure of the bone tissue of experimental animals, that is, cause changes in osteomorphometric parameters and femur architectonics.

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