Correction of homeostatic mechanisms of humoral regulation of bone remodeling processes in piglets with pathology of vitamin-mineral metabolism

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Abstract. The aim of the research was to study the correction of ossification processes and the mechanisms of humoral regulation of this process in the pathology of vitamin-mineral metabolism against the background of an immunosuppressive state in piglets. As a result of the experiment, it was found that proposed pharmacological correction scheme contributes to an increase in the level of total calcium (3.48±0.29 mmol/l), ionized calcium (1.65±0.07 mmol/l), ion-exchange calcium (2.52±0.05 mmol/l), and protein-bound calcium (0.54±0.06 mmol/l), 2,3-diphosphoglycerate (1.43±0.02 mmol/l), vitamin A (2.63± 0.09 μg/l), citric acid (2.90±0.16 μg/l), a significant decrease in alkaline phosphatase activity (2.02±0.04 mmol/l). Optimization of the structural and functional organization of the thyroid and parathyroid glands was noted, activation of hematopoiesis and bone tissue remodeling was observed in bone tissue, which suggests a pronounced reparative and osteoprotective effect of the proposed pharmacocorrection scheme.

1. Introduction

One of the most important environmental challenges of our time is the formation of a healthy animal population in order to maintain the health of the population. However, modern technologies for large-scale intensification of pig breeding include livestock concentration over a limited area, widespread use of antimicrobial and biological preparations, which violates the evolutionary relationship between animals and the environment [1, 2, 3]. Such isolation of animals from natural external factors of biogeocenosis leads to a qualitatively new habitat and is one of the prerequisites for the development of vitamin-mineral metabolism disorders, accompanied by a violation of bone remodeling processes against the background of an immunosuppressive state [4, 5].

Bone tissue is active and constantly renewed. In the postnatal period, the main cellular activity of bone tissue is aimed at remodeling, which results in the renewal of the bone matrix with the ability to participate in the regulation of mineral metabolism. Bone tissue remodeling is a multilevel process that is rigidly supported and regulated by numerous factors and components, including the endocrine system [6, 7]. Humoral regulation is the most important link in the chain of metabolic reactions that provide homeostasis of the body. The homeostatic mechanisms of humoral regulation of calcium and
phosphorus in the body are a complex evolutionarily early system of metabolic process correlation, one of the leading places in which belongs to bone tissue [8, 9, 10, 11]. Parathyroid hormone, calcitonin and calcitriol are the leading osteotropic hormones that realize humoral regulation of calcium metabolism and affect osteoclasts, intestinal enterocytes and renal tubule epithelium [12, 13, 14]. Changes in these mechanisms lead to a violation in the transport of calcium from bone tissue into the intercellular fluid, and a change in the secretion of calcium-trophic hormones [15, 16, 17].

Parathyroid hormone and vitamin D are the most important hormonal products of vertebrate evolution that regulate the extracellular homeokinesis of calcium and phosphorus in the body; in addition, there is a high role in the regulation of mineral metabolism and calcitonin as an antagonist of parathyroid hormone [18, 19]. Thus, the issues of environmentally safe pathogenetically adequate pharmacocorrection of vitamin-mineral metabolism against the background of an immunosuppressive state in piglets, aimed at optimizing the immune and hormonal status, are an urgent and promising area in modern veterinary medicine, based on the concept of "environmentally safe animal husbandry".

The aim of the present studies was to develop an environmentally safe pathogenetically grounded scheme for the complex pharmacocorrection of homeostatic mechanisms of humoral regulation of bone remodeling processes in piglets in the pathology of vitamin-mineral metabolism against the background of an immunosuppressive state. To achieve this goal, the following tasks were set: to study the biochemical status of animals, the structural organization of bone tissue, parathyroid and thyroid glands in piglets before and after the experiment.

2. Materials and Methods

The studies were carried out at the Department of Therapy and Propaedeutics of the Don State Agrarian University; at the Department of Biology and General Pathology of the Don State Technical University; on the basis of the pathological morphology department of the All-Russian Scientific Research Veterinary Institute of Pathology, Pharmacology and Therapy of the Russian Academy of Agricultural Sciences (Voronezh). Scientific and production experiments, approbation and production tests were carried out in pig farms in the Veselovsky district of the Rostov Region.

The experiment was carried out in two stages. At the first stage, experimental and control groups of animals were formed on the principle of pairs of analogues. In each group, there were 20 piglets of 45 days of age with signs of impaired vitamin-mineral metabolism against the background of a secondary immunosuppressive state. Clinical examination of animals was carried out according to generally accepted methods, blood samples were taken and biochemical studies were carried out. Biochemical studies were performed on an Idexx vet lab station vet Test 8008 biochemistry analyzer. Serum calcitonin level was determined by the immunoluminescent method, and parathyroid hormone by the radioimmune method. Blood was taken for biochemical studies three times before the start of the experiment, on the 15th and 30th day of complex pharmacocorrection.

In order to carry out morphological studies, 6 piglets were killed, samples of the terminal sections of the ribs, thyroid and parathyroid glands were taken before and after the experiment. These samples were fixed in a 10% solution of neutral formalin for 2–3 days, compacted in paraffin, paraffin sections were prepared with a thickness of 5–7 μm on a microtome, and then stained with hematoxylin-eosin by Van Gieson technique. Before the histological processing of bone tissue, it was calcified in a solution of nitric acid, then it was fixed in a 10–12% solution of neutral formalin and Carnoy fluid, poured into paraffin according to the generally accepted method, and serial sections of 7-9 μm thick were prepared from paraffin blocks. To study the general morphological structure of bone tissue, sections were stained with hematoxylin-eosin.

To animals of both groups were applied bentonite clay inside at a dose of 0.1 g per kilogram of body weight with food 1 time per day, for 30 days; intramuscular nitamine 1.0 ml per animal on the 1st day, 11th day and 21st day. The duration of the complex pharmacocorrection course was 30 days.

Piglets of the experimental group were injected ligol intramuscularly in a volume of 0.1 ml per animal on the 1st day, 0.5 ml on the 6th day, 1.0 ml on the 11th day.
3. Results
As a result of biochemical blood tests prior to the experiment, the following was detected in animals of both groups: hypocalcemia (total calcium - 2.73±0.82 mmol/l and 2.67±0.90 mmol/l), hypophosphatemia (1.08±0.07 mmol/l and 1.12±0.07 mmol/l) and a slight decrease in the amount of 2,3-diphosphoglycerate (1.26±0.09 mmol/l and 1.28±0.07 mmol/l) (Table 1). In this case, a decrease in most calcium fractions, and especially ionized (0.82±0.08 mmol/l and 0.84±0.06 mmol/l), was observed, which is the physiologically most important and strictly supported by the combined effect of parathyroid hormone, calcitonin and calcitriol.

Table 1. The level of homeostatic mechanisms of bone remodeling processes in piglets with pathology of vitamin-mineral metabolism against the background of an immunosuppressive state.

| Indicators                     | Experienced          | Control            | Clinically healthy |
|-------------------------------|----------------------|--------------------|--------------------|
| Total calcium, mmol/l         | 2.73±0.82*           | 2.67±0.90*         | 3.25±0.32          |
| Ionized calcium, mmol/l       | 0.82±0.08**          | 0.84±0.06**        | 1.50±0.05          |
| Non-protein calcium, mmol/l   | 1.47±0.08*           | 1.42±0.05*         | 1.26±0.03          |
| Calcium ion exchange, mmol/l  | 2.22±0.05*           | 2.25±0.07*         | 2.73±0.04          |
| Protein-bound calcium, mmol/l | 0.40±0.05            | 0.42±0.03          | 0.52±0.06          |
| Inorganic phosphorus, mmol/l  | 1.08±0.07*           | 1.12±0.07*         | 1.26±0.02          |
| Total phosphorus, mmol/l      | 1.95±0.08*           | 1.97±0.09*         | 2.25±0.12          |
| Alkaline phosphatase, mmol/l  | 5.6±0.13**           | 5.1±0.15**         | 2.02±0.03          |
| 2,3-diphosphoglycerate, mmol/l| 1.26±0.09*           | 1.28±0.07*         | 1.48±0.01          |
| Parathyroid hormone, pmol/l   | 17.21±2.86**         | 17.10±2.69**       | 6.7±0.55           |
| Calcitonin, ng/l              | 28.1±3.19            | 27.9±3.24          | 27.0±0.84          |
| Citric acid, μg/l             | 1.43±0.40**          | 1.42±0.39**        | 2.81±0.14          |
| Vitamin A, μg/l               | 1.54±0.59**          | 1.55±0.68**        | 2.53±0.16          |

Note: * - P<0.05; ** - P<0.01; *** - P<0.001

In sick animals, an increase in acidosis was observed due to hypophosphatemia, which was manifested by an increase in alkaline phosphatase activity to 5.6±0.13 mol/l in the experimental group and to 5.1±0.15 mol/l in the control group (Table 1).

The level of osteotropic hormones in sick animals was characterized by an increase in parathyroid hormone to 17.21±2.86 pmol/l in the experimental group and up to 17.10±2.69 pmol/l in the control group, which caused morphological rearrangements of bone and cartilage tissue and led to imbalance between the processes of osteosynthesis and osteodegeneration.

In sick animals, a decrease in the quantitative indicator of citric acid (1.43±0.40 μg/l and 1.42±0.39 μg/l) and vitamin A (1.54±0.59 μg/l and 1.55±0.68 μg/l).

Prior to the experiment, the structural organization of the thyroid gland was characterized by progressive dystrophy, which was accompanied by vacuolization of the colloid or its dissolution in small follicles (Figure 1 a, 1 b). In single follicles, a change in structure and desquamation was revealed, which led to a violation of architectonics. Some thyrocytes were filled with a homogeneous oxyphilic mass, occupying a significant part of the cytoplasm. In thyrocytes, hyperchromia of the nuclei and vacuolization of the cytoplasm were observed (Figure 1 c).
Figure 1. The structural organization of the thyroid gland in piglets in the pathology of vitamin-mineral metabolism against the background of an immunosuppressive state: a - follicle filling with a homogeneous oxyphilic colloid, staining with hematoxylin and eosin, 7x10 magnification; b - follicle formation by flat or cubic thyrocytes, hematoxylin and eosin staining, 7x10 magnification; c - hyperchromia of nuclei and vacuolization of thyrocyte cytoplasm, staining with hematoxylin and eosin, 7x40 magnification.

The parathyroid gland of sick animals was hypertrophied. An increase in the number of main and oxyphilic cells was noted, which indicated hypersecretion of the organ (Figure 2 a, 2 b, 2 c).

Figure 2. The structural organization of the parathyroid gland in piglets in the pathology of vitamin-mineral metabolism against the background of an immunosuppressive state: a - the formation of the glandular structure by the main cells with a light cytoplasm, staining with hematoxylin and eosin, 7x10 magnification; b - the formation of a “lobed” glandular structure, stained with hematoxylin and eosin, 7x10 magnification; c - hypertrophy and dystrophy of the main cells of the gland, staining with hematoxylin and eosin, 7x40 magnification.

Histological examination of bone tissue samples showed insufficient endochondral ossification with excessive formation of cartilaginous tissue, increased formation of osteoid tissue from the endoost and periosteum, as well as delayed deposition of calcium phosphate in the costal bones. The epiphyseal part of the bone marrow cavity of the ribs was expanded. An uneven increase in periosteal stratification was recorded in the area of parallel bone platelets of the rib and islets of bone marrow hematopoiesis (Figure 3 a). In the ribs, focal proliferation of cartilage tissue (Figure 3 b) was carried out. In the area of hypertrophied cartilaginous tissue of the rib, areas of pronounced thinning of the compact layer of bone tissue were observed (Figure 3 c). Focal expansion of the periosteum with proliferation of cambial cells was observed at the sites of resorption of the external insertion plates of bone tissue.
Figure 3. Structural organization of the rib in piglets in the pathology of vitamin-mineral metabolism against the background of an immunosuppressive state: a) periosteal layering in the area of parallel bone plates of the rib and islets of bone marrow hematopoiesis, stained with hematoxylin and eosin, 7x10 magnification; b) foci of proliferation of cartilaginous tissue in the region of the rickets of the rosary rib, hematoxylin and eosin staining, 7x10 magnification; c) thinning of the bone plate of the rib, staining with hematoxylin and eosin, 7x3.2 magnification.

Chondrocyte growth in the end regions of the ribs with diffuse implantation of single blood capillaries was noted in cartilage tissue (Figure 4 a). At the places of its transition to bone, an increase in the thickness of the chondroblast proliferation layer was revealed (Figure 4 b). Dystrophy of hematopoietic myeloid germ cells was recorded in the bone marrow cavity of the ribs (Figure 4 c).

Figure 4. The structural organization of the ribs in piglets in the pathology of vitamin-mineral metabolism against the background of an immunosuppressive state: a - micromorphology of the blood capillary, staining with hematoxylin and eosin, 7x10 magnification; b - cross section of a rickets rosary, stained with hematoxylin and eosin, 7x10 magnification; c - cell dystrophy of myeloid hematopoiesis, staining with hematoxylin and eosin, 7x40 magnification.

After the experiment, the pigments of the experimental group showed an increase in the level of total calcium to 3.48±0.29 mmol/l, and in the control group this indicator was 6.23% less (Table 2). The fractional composition of serum calcium was characterized by an increase in ionized calcium (1.65±0.07 mmol/l and 1.60±0.06 mmol/l), ion-exchange calcium (2.52±0.05 mmol/l and 2.48±0.06 mmol/l) and protein-bound calcium (0.54±0.06 mmol/l and 0.52±0.08 mmol/l). The non-protein fraction of calcium decreased to 1.23±0.04 mmol/l in the experimental group and to 1.27±0.03 mmol/l in the control group. There was also a significant decrease in alkaline phosphatase activity to 2.02±0.04 mmol/l in the experimental group and to 2.05±0.07 mmol/l in the control group.
Table 2. Dynamics of indicators of bone remodeling in piglets with complex pharmacocorrection of the pathology of vitamin-mineral metabolism against the background of an immunosuppressive state.

| Indicators                     | Experienced Groups of animals | Control Groups of animals |
|--------------------------------|-------------------------------|---------------------------|
|                                | On the 15th day | On the 30th day | On the 15th day | On the 30th day |
| Total calcium, mmol/l          | 3.05±0.26        | 3.48±0.29*        | 2.97±0.32        | 3.26±0.15*      |
| Ionized calcium, mmol/l        | 1.31±0.05        | 1.65±0.07*        | 1.27±0.08        | 1.60±0.06*      |
| Non-protein calcium, mmol/l    | 1.31±0.05        | 1.23±0.04*        | 1.35±0.03        | 1.27±0.03*      |
| Calcium ion exchange, mmol/l   | 2.37±0.04        | 2.52±0.05*        | 2.32±0.06        | 2.48±0.06*      |
| Protein-bound calcium, mmol/l  | 0.49±0.04        | 0.54±0.06*        | 0.46±0.05        | 0.52±0.08*      |
| Inorganic phosphorus, mmol/l   | 1.22±0.03        | 1.36±0.01         | 1.20±0.04        | 1.31±0.02       |
| Total phosphorus, mmol/l       | 2.12±0.02        | 2.26±0.15*        | 2.08±0.02        | 2.24±0.12*      |
| Alkaline phosphatase, mmol/l   | 3.49±0.03        | 2.02±0.04*        | 3.52±0.05        | 2.05±0.07*      |
| 2,3-diphosphoglycerate, mmol/l | 1.33±0.08        | 1.43±0.02*        | 1.31±0.09        | 1.40±0.04*      |
| Parathyroid hormone, pmol/l    | 10.26±0.3        | 6.77±0.5**        | 10.42±0.7        | 6.85±0.6**      |
| Calcitoning, ng/l              | 27.52±0.9        | 27.09±0.8         | 27.50±0.6        | 27.13±0.7       |
| Citric acid, μg/l              | 2.12±0.14        | 2.90±0.16**       | 2.08±0.10        | 2.86±0.17**     |
| Vitamin A, μg/l                | 2.19±0.11        | 2.63±0.09**       | 2.16±0.13        | 2.59±0.10**     |

Note: * - P<0.05; ** - P<0.01; *** - P<0.001

The optimization of parathyroid secretion was observed, while the amount of parathyroid hormone in the experimental group was 6.77±0.5 pmol/l, and in the control group it was 6.85±0.6 pmol/l (Table 2).

In animals of both groups normalization of vitamin metabolism was noted, but the indices of the experimental group were more pronounced. So, the level of 2,3-diphosphoglycerate in animals of the experimental group increased by 11.88%, and the control - by 8.57% (Table 2), the value of vitamin A increased to 2.63±0.09 μg/l and 2.59±0.10 μg/l, respectively. A significant increase in citric acid (2.90±0.16 μg/l and 2.86±0.17 μg/l) was also revealed in animals of both groups.

After the experiment, the structural organization of the thyroid gland was characterized by the presence of follicles of various sizes, the basement of which was lined with epithelial cells with round or oval nuclei (Figure 5 a). The bulk of the organ was made up of large follicles that were filled with a homogeneous colloid with marginal vacuolization or enlightenment (Figure 5b), while the small follicles were lined with cubic or cylindrical epithelium.

Figure 5. Structural organization of the thyroid gland in piglets after complex pharmacocorrection of vitamin-mineral metabolism disorders against the background of an immunodeficiency state: a - thyroid follicles of various sizes, stained with hematoxylin and eosin, 7x10 magnification; b - various degrees of functional activity of the thyroid follicles, stained with hematoxylin and eosin, 7x40 magnification

After the experiment, a decrease in the number of hypertrophied main and oxyphilic cells was revealed in the histological structure of the parathyroid gland in piglets, which was associated with the
normalization of the secretory activity of the gland. The glandular structure of the organ was characterized by the presence of secretory cells (Figure 6 a), the development of interlobular connective tissue was noted, and the formation of “lobulation” was observed inside the large parathyroid glands (Figure 6 b).

After the experiment, the histological structure of the rib in piglets was characterized by activation of hematopoiesis processes in the terminal sections, which was manifested by the presence of myeloid and lymphoid germ cells at different stages of maturation and single megakaryocytes (Figure 7 a). In the bone plates, the formation of the brain cavity of the ribs with foci of myeloid hematopoiesis in the bone marrow was noted (Figure 7 b). Under the periosteum of the rib, there was a differentiation of the cartilage into bone, in the form of bone plates of various thicknesses (Figure 7 c).

4. Discussion

As a result of studies in sick animals, hypocalcemia was revealed, manifested primarily by a decrease in ionized calcium (0.82±0.08 mmol/l and 0.84±0.06 mmol/l), which is a biologically active fraction of this element. Due to the fact that calcium ions are in a constant equimolar ratio with phosphate anions, this led to the release of valency for hydrogen ions in the extracellular fluid and damage to the body's phosphate buffer system. The result of this was the development of hypophosphatemia (1.08±0.07 mmol/l and 1.12±0.07 mmol/l) in sick animals, the development of which reduces the intensity of oxidative processes in the body, which causes the accumulation of unoxidized products of interstitial metabolism in tissues and, leads to an increase in acidosis. The body aligns the acid-base
balance by removing acidic products, in particular acidic phosphates, which further enhances hypophosphatemia. An increase in the activity of alkaline phosphatase (5.6±10.13 mol/l and 5.1±0.15 mol/l) is one of the markers of impaired metabolic rate in bone tissue. An increase in the activity of alkaline phosphatase along with hypokalemia caused an increase in bone mineralization processes, which was manifested by insufficient enchondral ossification with excessive formation of cartilaginous tissue, enhanced formation of osteoid tissue from the endosteum and periosteum, as well as delayed deposition of calcium phosphate in the costal bones.

Vitamin deficiency was also one of the leading etiopathogenetic mechanisms for the development of the pathology of vitamin-mineral metabolism, which was manifested by a slight decrease in the amount of 2,3-diphosphoglycerate (1.26±0.09 mmol/l and 1.28±0.07 mmol/l) and vitamin A (1.54±0.59 μg/l and 1.55±0.68 μg/l) in experimental animals.

In addition, one of the most important homeostatic mechanisms that tightly regulates the level of calcium in the extracellular fluid is the secretion of parathyroid hormone, which is strictly regulated by the concentration of ionized calcium in the blood serum. Thus, the parathyroid gland is involved in the pathological process already at the beginning of the development of the pathology of vitamin-mineral metabolism, which leads to increased synthesis of hormones (17.21±2.86 pmol/l and 17.10±2.69 pmol/l) and how a consequence of organ hypertrophy, and determines the development of pathology from the bone-cartilage tissue. The structural organization of the thyroid gland in the pathology of vitamin-mineral metabolism was characterized by progressive dystrophy. A change in the endocrine status in piglets with a violation of the vitamin-mineral entails an imbalance between the processes of osteosynthesis and osteodegeneration, which is the initial cause of morphological rearrangements of bone and cartilage tissue.

After a course of pharmacocorrection in animals of the experimental group, an increase in the level of total calcium (3.48±0.29 mmol/l), ionized calcium (1.65±0.07 mmol/l), and ion-exchange calcium (2.52± 0.05 mmol/l) and protein-bound calcium (0.54±0.06 mmol/l), 2,3-diphosphoglycerate (1.43±0.02 mmol/l), vitamin A (2.63±0.09 μg/l), citric acid (2.90±0.16 μg/l), as well as a significant decrease in alkaline phosphatase activity (2.02±0.04 mmol/l). Optimization of the structural and functional organization of the thyroid and parathyroid glands was noted, activation of hematopoiesis and bone remodeling was observed in bone tissue. The obtained results give grounds to assert a pronounced reparative and osteoprotective effect of the proposed pharmacocorrection scheme due to an adequate combination of bentonite clay, which regulates the composition and concentration of digestive tract electrolytes, and through them - mineral metabolism and acid-base balance in animals, a complex multivitamin drug - nitamine and ligfola, which has pronounced antioxidant, immunomodulatory and adaptogenic properties.

5. Conclusions
Thus, the ecologically safe pathogenetically adequate pharmacocorrection scheme of the vitamin-mineral homeostasis violation against the background of the immunosuppressive state in piglets that we offer gives a pronounced therapeutic effect due to the normalization of bone remodeling processes, metabolic processes, as well as hormonal regulation mechanisms. Consequently, the use of bentonite clay and nitamine in combination with ligfol makes it possible to correct the mechanisms of humoral regulation of bone remodeling processes through the effect on calcium-phosphorus and vitamin-mineral metabolism, while normalizing the homeostatic mechanisms of the body's immune response.

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