Ways to develop and improve a pharmacologically active compound based on a polymer complex for the prevention and treatment of microelementosis in fur farming

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Abstract. The analysis of iron-containing products made in Russia for veterinarian use against iron deficiency anaemia in fur farming was carried out. The absence of hydroxide-polymaltose iron complexes on the pharmaceutical market has been shown and their promise as the main components for oral preparations has been substantiated. In this context, the Collective of the Department of Physiology, Pharmacology and Toxicology, FSBEI HE MGAVMiB-MVA named after K.I. Skryabin, in collaboration with the research and production company A-BIO conducted research to develop and study the effect of pharmacologically active compounds based on a polymer (iron-hydroxide polymantose) complex for treatment and prevention of microelemetosis in the fur animals.

1 Introduction

Key factor, decreasing the growth and development of fur animals of various breeds, is the disbalance in metabolism of trace elements in the body, which leads to impaired hair state and hair quality [1, 2].

Microelementosis (iron deficiency anemia, hypothyrosis, hypocobaltosis, hypocuprosis, etc.) in fur animals, as well as in other animal species, is a very prevalent non-infectious disease, which causes significant economic damage. In most cases, the disease is caused by animal nutrition and management. Causes of iron deficiency anemia are similar [1, 3, 4].

The main factor that affects growth, development, reproductive ability, hair and pelt quality is feeding. Complete (balanced in nutrients and energy) feeds allow you to achieve genetic potential of animal in productivity, and it is the basis for prevention and treatment of metabolic disorders.

In fur animals feeding, the wastes of slaughterhouses and fishing industry are widely used. However, the use of certain species of fish (blue whiting, cod fish waste, polar cod) as feed components makes this trace mineral inaccessible for animals, as they contain trimethylamine oxide (TMAO) that binds iron ions. This can lead to metabolic disorders of iron, including iron deficiency anaemia, decreased productivity and impaired hair state and hair quality in fur animals [2, 5, 6].

However, excess iron is also an unfavorable factor. Being a metal with variable valency, iron has a prooxidant effect, that is, in a number of biochemical reactions (Fenton, Haber – Weiss, Osipov) it leads to the formation of reactive oxygen species and has a toxic effect on the functions of the liver, cardiovascular system, disrupts hormonal status, and causes immune system dysfunction.

Side reactions are associated with the fact that iron is a metal carrier, accelerating the catalysis of the formation of free radicals and reactive oxygen species. So, when Fe (II) salts are added to serum in vitro, peroxidation of LDL and VLDL occurs, and the products of peroxidation accumulate, which increases the chemiluminescence of blood plasma. At the same time, substances that damage the biomembranes and vascular endothelium accumulate, these include diene conjugates, malondialdehyde.

Ferric iron is an integral part of ferritin and transferrin structure. Therefore, products containing ferrous salts gastrointestinal mucosa are oxidized with the formation of free radicals, which are the cause of oxidative stress observed in animals. [2, 6, 7].

At the same time, the activation of free-radical oxidation processes and a decrease in the body's antioxidant defence occurs in many diseases, including iron deficiency anaemia and other microelementoses, which explains the contraindication of the use of iron-containing products for animals with hypovitaminosis E.

With hypoxia and ischemia, the formation of a low-activity superoxide radical anion enhances, as it is a weak oxidizing agent and is not able to directly initiate lipid peroxidation reaction. In this regard, there is evidence of important role in induction of lipid peroxidation processes for more aggressive hydroxyl and hydroperoxyl radicals, whose formation is sharply enhanced with the participation of transition metal ions, including iron, while iron-containing products are used to prevent and treat iron deficiency anaemia [1–4, 8, 9].

Replenishment of iron deficiency not only activates free radical oxidation, but also initiates hepatic

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expression of hepcidin, which, in turn, interferes with the absorption of iron. So, the more iron is introduced into the body the worse it is absorbed.

The immunobiological reactivity of animal body with iron deficiency decreases due to a decrease in the myeloperoxidase activity of polymorphic neutrophils, thymus hypofunction, a decrease in phagocytic activity and a slowdown in the movement of macrophages, interleukin-2 is produced in small quantities by lymphocytes. Changes are reversible, recovery occurs with adequate ferrotherapy.

Immunodeficiency occurs, most commonly, three weeks after birth. To increase nonspecific immunity and immune reactivity, the use of iron-containing drugs, immunomodulators is required. Animals with anemia are retarded and have lower reproductive rates.

Thus, in modern conditions, in the prevention and treatment of iron deficiency anaemia, it becomes necessary to correct changes in free radical processes caused by ferrotherapy, on the one hand, and hypoxia, on the other hand, while stimulating iron uptake that does not activate hepcidin expression.

This point is especially important in the development and use of modern drugs for treatment and prevention of iron deficiency anaemia, because iron containing products are the basis for the successful treatment of iron deficiency anaemia [1, 2, 10, 11].

Due to the fact that it is not an increase in formation of reactive oxygen species, that leads to anaemia, but mostly a weakening of the antioxidant defence of the body, special attention should be paid to new complex products, containing not only iron, but also such important trace elements involved in iron metabolism, hematopoiesis and the body's antioxidant defence system as selenium, iodine, copper and cobalt.

Thus, the problem of ferrotherapy requires further study, development and study of pharmacological properties and indications for the use of modern complex iron containing products.

Currently, iron containing products for enteral and parenteral administration are used for treatment and prevention of iron deficiency anaemia.

Ferrodrugs can be divided into inorganic and organic by chemical nature, and in relation to the dissociation of iron in water – into ionic and nonionic. Organic compounds are divided into proteins, fats and carbohydrates. As a result, we get ferro-carbohydrates (dextran and iron dextrin), ferro-proteins (compounds with methionine and albumin). The presence of iron-lipid complexes has not been reliably established. Depending on the purpose, the pharmaceutical forms of the ferrodrugs may be aqueous solutions for injection, liquid and solid dosage forms for oral administration.

Products for parenteral administration contain ferric iron in the form of a complex with dextran, sucrose or sodium gluconate; the second is ferrous or ferric iron, which can be monocomponent or combined. Existing iron-containing preparations can be systematized depending on the method of absorption of iron from preparations: including Fe^{2+} (ionic, salt compounds of iron); including Fe^{3+} (non-ionic iron compounds). Iron, most commonly, is in bivalent form when absorbed from ionic compounds, while the pH value of gastric juice severely limits the activity of utilization of iron products in trivalent form [1, 3, 10, 12].

Divalent iron compounds enter the mucous cells of the intestinal mucosa, mainly through passive diffusion through the gastrointestinal tract, and then into the bloodstream.

In mucous cells of the gastrointestinal tract, compounds containing Fe^{2+} are oxidized by ferroxidase-1, iron from Fe (II) is converted to Fe (III). The compound Fe (III) with transferrin and ferritin forms a reserve of iron used in iron-dependent reactions (metalloenzymes, production of IL-2) or for binding iron-containing molecules [1, 3–5, 9, 12].

All of the above indicates the complexity of selecting an iron-containing product for ferrotherapy. Therefore, at the current level of development of science, it is important to study the effect of iron preparations for enteral and parenteral administration in various chemical forms (salt, chelate, dextran complex, hydroxide-polymaltose complex) on peroxide oxidation processes lipids, in combination with antioxidants (selenium) and microelements affecting the absorption of iron (copper, cobalt, iodine).

2 Materials and methods

A methodological approach to achieving the goal and solving the tasks was a systematic study of objects of study, analysis and generalization of the results. The subject of the study is the pharmacological effects of complex iron-containing drugs used for prophylactic and therapeutic purposes in laboratory and farm animals.

To study the current state of the pharmaceutical market, the data of the State Register of Medicinal Products for Veterinary Use of the Russian Federation were analyzed. Statistical methods were used.

3 Results

We have conducted an analysis of drugs for veterinary use containing iron in the Russian Federation. In accordance with the Federal Law of the Russian Federation “On the Circulation of Medicines” dated April 12, 2010 No. 61 and by order of the Ministry of Agriculture of the Russian Federation dated April 1, 2005 No. 48 “On the Approval of the Rules for State Registration of Medicinal Products for Animals and Feed Additives,” as of 01. 01. 2020, 2154 medicinal products for animals were registered and entered into the State Register for use in veterinary medicine in the prescribed manner. This includes 614 biological products (vaccines, serums, etc.) and 1540 other (chemical-pharmaceutical) drugs.

Among the pharmaceuticals, 0.9 % are iron-containing.

In a further analysis, the percent of domestic and foreign manufacturers of iron-containing drugs was clarified. As a result, it can be said that domestic manufacturers occupy about 60 % of the market for iron-

containing drugs, and 40 % account for foreign manufacturers.

Among regional manufacturers, represented by the largest number of registered iron preparations, are A-BIO LLC, Moscow Region, Pushchino; Nita-Farm CJSC, Saratov Region, Saratov; LLC Vetbiokhim, Stavropol Territory, Stavropol. Among foreign manufacturers in terms of the number of iron-containing preparations, the leader is FE "VIK – Animal Health", the Republic of Belarus and "Pharmacosmos A / S", Denmark.

It is a common fact that the effectiveness and safety of iron preparations is influenced by the simultaneous use of substances that promote the absorption of iron. Therefore, the next step characterizing the market of iron-containing drugs for veterinary use was the characterization of the number of complex preparations and single preparations of iron.

As a result, it turned out that most of the iron-containing preparations presented on the veterinary pharmaceutical market are complex and contain, in addition to iron, other trace elements (copper, cobalt, selenium, etc.) and vitamins. Complex preparations also account for about 60 % (Figure 1).

About half (55 %) of iron-containing products are iron dextrans. The remaining 45 % are approximately equally divided between mineral salts and chelate complexes. The hydroxide-polymaltose complex of iron is not represented on the pharmaceutical market.

However, when choosing a product, you should take into account not only the chemical nature of the metal and the presence of other substances in the complex, but also the route of utilization of the drug, which also affects the effectiveness and safety of the drug. It was found that the vast majority (90 %) of iron-containing drugs are represented by injection solutions, and only a small proportion of drugs are for oral use.

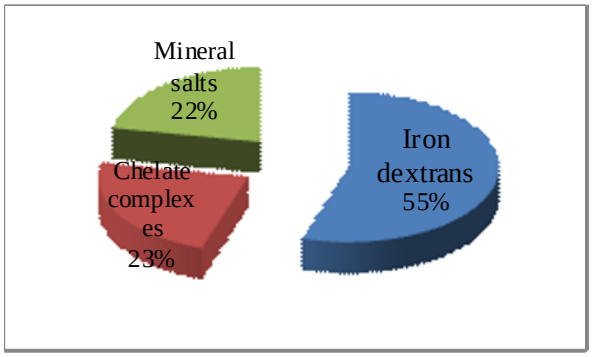

Fig. 1. The ratio of the main forms of iron containing products presented on the domestic pharmaceutical market for veterinary use

The next stage of the analysis of the market of iron-containing products for veterinary use was the characterization of drugs by the species of animals for which they are intended. It was found that 64 % of products are recommended for use by all species of farm animals, as well as fur animals, 18 % of drugs are recommended only for use in swine, 13 % of drugs for use in young cattle, and one drug is recommended for use in poultry.

As can be seen from the presented analysis, the majority of iron-containing products on the market are complex iron-dextran products for injection of domestic production, intended for all species of farm animals. A lack of oral dosage forms of iron can be noted, in addition, hydroxide-polymaltose iron complexes are not currently available on the pharmaceutical market at all.

Iron dextran preparations have proven themselves most positively. In the 50s of the last century, the first iron-dextran preparations were synthesized (iron dextran complex), in which ferric iron is in conjunction with dextran. Dextran is a glucose polymer produced by the microorganisms Leuconostas mesenteroides, as well as other types of microorganisms, when grown on sucrose containing agar.

Commercially available products of this series are usually aqueous solutions of dextran complexes of iron (III) hydroxide and usually contain 5.0-10.0 % iron on a weight basis.

Their attractiveness is, first of all, in the simplicity of the operations of synthesis of iron-dextran compounds, the relatively low cost of the starting reagents and their high therapeutic activity with low toxicity (4th hazard class according to GOST 12.1.007-76) [4].

An analysis of technical solutions allowed us to identify methods for producing an iron-dextran complex by direct synthesis from iron (III) hydroxide and dextran, resulting in mixtures containing 10–24 % of iron from the dry weight of the product (US 3666749, US 2885393).

However, there are some disadvantages of such drugs. For example, the so-called “iron-dextran complexes” are not chelate complexes of any specific composition, but are dextran-stabilized colloidal solutions of iron hydroxide particles of various sizes. In reality, such preparations contain a mixture of the initial unreacted dextran and “low-iron” complexes of dextran (2–4 % iron (III)) and complexes with a very high iron content (up to 45 % of dry weight).

The experimental data of the authors also show that on the basis of low molecular weight dextran, using the simplest method (quick neutralization of a mixture of ferric chloride and dextran with a concentrated solution of sodium carbonate, quick alkalization, heating in a boiling bath, filtering and acidification), it is possible to obtain only mixtures containing iron, not exceeding 18–19 % of the dry weight of the product. Excess iron hydroxide does not bind to dextran when boiled for several hours.

The iron content in aqueous solutions of such products is 5–6 wt. %, It corresponds to 25–30 % of the total concentration of the complex in the solution: such solutions are characterized by a relatively low viscosity. However, an increase in the iron concentration to 7.5–10 wt % requires already a 40–55 % polymer concentration: such solutions turn out to be very viscous and extremely inconvenient in practical use.

In reality, about half of the total weight of the dry product obtained in this way is “ballast” – the initial dextran and its “low-iron” complexes. An increase in the

iron / dextran ratio in such complexes is therefore of paramount importance.

However, a change in the synthesis conditions, such as dextran and the ratio of reagents, cannot significantly improve this parameter. Therefore, in the last 20–25 years, a number of new iron-dextran products have been developed that are free of these drawbacks. All of them can be considered as Fe^3 complexes of "carboxylated" dextrans: it is proved that the introduction of a carboxy group into the glucose fragment of the polysaccharide dextran molecule allows for a sharp increase in the iron / dextran ratio in the reaction product. The introduction of carboxyl groups can be carried out in various ways.

From US Pat. No. 3,151,107, a method is known for producing an iron-containing products with carboxymethylated dextran obtained by treating dextran with sodium chloroacetate; from Patent EP 0150085, there is known a method for producing an iron-containing products with carboxymethylated dextran obtained by treating dextran with sodium chlorite.

However, the need for additional stages of synthesis and, most importantly, purification of such derivatized dextrans significantly increases the cost of carbohydrate raw materials: the preparation of Fe^3 complexes of carboxylated dextrans (in comparison with the complexes of the initial dextran) is, as a rule, noticeably more complex and time-consuming, and their stability and water solubility to a much greater extent depends on the pH of the medium.

A-BIO LLC (Moscow) has developed a method for producing Fe-dextran, devoid of all the above disadvantages (RU 2198665 C1). This is achieved by the fact that the proposed method for producing an iron-containing product, comprising in mixing a soluble iron (III) salt with polysaccharides, subsequent partial neutralization of the resulting mixture before the formation of an iron (III) hydroxide precipitate, alkalization with alkali with stirring, heating, re-filtering the solution, its filtration and separation of the iron-containing complex, characterized in that the separation of the iron-containing complex is carried out by ultrafiltration on membranes with a limit of exclusion 10 kDa or greater.

4 Conclusion

An important point to consider is the method by which trace minerals are utilised in the body. Undoubtedly, their oral administration is more physiological and less laborious, than injecting, which entails significant side effects and labour costs.

The mineral compounds of various trace elements used for oral administration, their chelate compounds (gluconates, aminoates, complexonates) also have a number of disadvantages, in the form of high toxicity, and a small functional role of the ligand, which is only responsible for maintaining the water solubility and bioavailability of the target ion, since the ligand and ion assimilated by the body separately.

In addition, the use of trace elements in this form does not exclude the antagonism between the trace elements. That is, the development of an integrated pharmacologically active system based on biopolymers for the prevention and treatment of microelementosis is an urgent fundamental and practical task of science. Increased bioavailability, reduced toxicity and side effects, increased pharmacological efficacy by eliminating competition, and enhancing synergistic action, perhaps due to the development and optimization of an integrated system based on biopolymers.

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