General principles of conducting preclinical toxicology studies of antiparasitic drugs for veterinary use

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Abstract. Determining the degree of safety of new antiparasitic drugs for veterinary use is based on the results of a whole complex of preclinical study. The quality and effectiveness of preclinical studies are guaranteed by compliance with the rules provided for in normative documents, which are harmonized with international standards. However, due to the exploratory nature of such studies and the variety of methodological approaches used, there are no strict criteria for planning experiments. The choice of methods and tools when planning work on the preclinical evaluation of safety and efficacy of antiparasitic agents should be based on the specifics of their chemical structure and application to the target animals. The paper considers approaches to the regulation of preclinical toxicology studies of antiparasitic agents for veterinary use, taking into account their usually high or medium toxicity, the ability to cause death or impaired functions in living things (both the parasite and the host) and short-term use of the target to animals. The use of such approaches will make it possible to unify the methodology of experiments, to ensure the reliability and reproducibility of the results of preclinical pharmacology studies, and to increase their scientific and practical significance.

1. Introduction
Currently, the range of drugs intended for use in treatment of animals and the prevention of infectious and non-infectious diseases is extensive. However, due to constantly occurring changes in the environment, animals’ immune response to certain pathogens, resistance emergence and development of pathogens to the drugs used the search and implementation of new pharmacological agents in veterinary medicine remains relevant [1]. In full, this should be attributed to antiparasitic therapy.

The introduction into clinical practice of new medicines for animals is possible only if there is experimentally proven in accordance with modern requirements data on their high efficacy and safety. To do this, a certain order of scientific research must be carried out, the most important of which is the preclinical evaluation of the safety based on international standards [2, 3, 4].

2. Regulatory frameworks for preclinical studies
In the Russian Federation, the main law governing the search, development, study in the complex of preclinical and clinical trials of medicines is Federal Law of April 12, 2010 No. 61 “On drug
circulation”, which in article 12 stipulates that preclinical study of a drug for veterinary use is carried out by applying scientific evaluation methods in order to obtain evidence of its safety, quality and efficacy.

The procedure for conducting a preclinical study of a veterinary medicinal product is established by Order of the Ministry of Agriculture of the Russian Federation dated 06.03.2018 No. 101 “On approval of the rules for conducting a preclinical study of a veterinary medicinal product, a clinical trial of a veterinary medicinal product, and bioequivalence studies for a veterinary medicinal product”.

Researchers should take into account the guidelines for good laboratory practice (GLP) developed according to national specifics and approved by Order No.199n of the Ministry of Health of the Russian Federation on April 1, 2016 “On Approving the Rules for Good Laboratory Practice” as guidelines. The proposed rules are harmonized with the GLP Rules of the World Health Organization (WHO) [5] and the Eurasian Economic Union (EAEU) [6], which have supranational regulation and define “good laboratory practice (laboratory practice rules)” as a system of “requirements for organization, planning and conducting preclinical (non-clinical) studies of substances (medicines), reporting results and quality control of these studies.”

All work using laboratory animals should be carried out in accordance with the existing ethical standards for the treatment of animals, based on standard operating procedures that comply with international rules for the protection of vertebrate animals used for research and other scientific purposes [7].

There are other methodological documents that provide separate stages and directions of research on toxicological and pharmacological work. However, at present no universal amount of preclinical pharmacology and toxicology studies has been developed, suitable for drugs and preparations of all pharmacological groups. The research plan is usually determined by the specific test substance.

3. Aspects of preclinical studies of antiparasitic drugs

The purpose of preclinical studies is to obtain information on the safety, pharmacological properties, pharmacokinetics and metabolism of the studied medicinal substance (preparation) [2].

The focus of preclinical studies is the study of the safe use of the new drug, which involves the study of the toxic (damaging) effect of the drug on the tissues and organs of the animal, the dependence of the effect on dose, exposure, severity and reversibility. The data obtained become the basis for determining the parameters for further clinical studies of the drug [8].

In this regard, the development of a plan and the conduct of preclinical studies of antiparasitic drugs have their own characteristics.

Substances with antiparasitic activity are, as a rule, compounds with a high or medium level of toxicity, capable of causing death or impairment of functions in the body of living things (protozoans, helminthes, insects and others). In the process of using such substances simultaneously with exposure to a parasitic organism, a negative, harmful effect on the tissues, organs, and their animal host systems is often observed.

It should be noted that modern complex antiparasitic drugs are largely devoid of such an adverse effect due to the introduction of excipients into their composition. Such excipients help to reduce the concentration or dose of the main toxic active substance while preserving the antiparasitic effect or provide protection for organs, tissues and life support systems of the target animal’s body. However, it is not yet possible to completely protect the macroorganism from the damaging effects of the poison.

In connection with the foregoing, when planning preclinical studies, special attention should be paid to methods and means of toxicological evaluation, including the study of the destructive effect of an antiparasitic substance or a finished preparation on both the parasite and the damaging effect on the body of the target animals.

Another feature of antiparasitic drugs that must be used when planning and conducting preclinical studies is that the treatment regimen for animals, in most cases, does not imply their repeated use. Short-term therapeutic use of antiparasitic drugs can significantly reduce the time taken to study their toxicological effects, in particular in chronic and subchronic experiments.
The guidelines for preclinical studies of drugs [2] give methodological approaches to the preclinical study of the antiprotozoal and anthelmintic activity of medicinal products for medical purposes: the study of animals for helminth infections; isolation of helminth eggs from the feces of invasive animals; cultivation of eggs and larvae of helminthes; infection of laboratory animals with helminthes and the introduction of test drugs, as well as taking into account the anthelmintic efficacy of drugs. Such drugs should have low toxicity with no side effects on the host organism, have high specific effectiveness, be active in relation to different stages of the development of the parasite, and be available. However, the aspects of their possible veterinary use, the effect on the body of the target animal, the processes of excretion and the content of residual quantities in livestock products are not taken into account.

Thus, when planning and conducting preclinical studies of the safety of a new or reproduced antiparasitic drug, pharmacists and pharmacologists should take into account the characteristics of this group of drugs.

4. The choice of methods for preclinical toxicology studies of antiparasitic drugs

As a rule, toxicology studies are carried out in full when examining the substance of the original pharmacological substance, as well as in the case when the dosage form contains excipients (stabilizers, solvents, excipient, etc.), not previously authorized for use in veterinary practice [2, 9, 10, 11].

Preclinical toxicology studies include determining the parameters of acute, subchronic and chronic toxicity, the study of allergenic and local irritant effects, immunotoxicity, embryotoxicity, reproductive toxicity, teratogenicity, mutagenicity, carcinogenicity.

Studies on the determination of general toxic properties are carried out obligatorily for drugs of all groups. Studies include determining the parameters of acute toxicity (the effect of a substance on the animal’s body with single or multiple administration during the day with an interval of 4-6 hours). In this case, significant toxic and lethal doses (LD50, LD100, MTD and others) are determined, allowing classification of the studied substance. The causes of laboratory animal’s death, morphological and functional changes in organs and tissues of animals are evaluated.

In further experiments, the parameters of the subchronic and chronic toxicity of the drug, i.e. force of action upon repeated (multiple) administration into the body of an animal. The duration of the experiments depends on the frequency of the intended use of the drug to the target animals, while assessing the totality of functional and morphological disorders of tissues, organs and systems of experimental animals without causing their death.

When conducting preclinical toxicology studies of antiparasitic agents, it is advisable to determine the coefficient of functional accumulation by the method of “subchronic toxicity” according to Lima (1961).

In experiments to determine the parameters of subchronic and chronic toxicity of drugs, it is customary to use 2-3 types of laboratory mature animals (mice, rats, guinea pigs, rabbits, dogs) of both sexes, obtained from certified nurseries and quarantined for 10-14 days. The choice of animal species should be justified. Rats and mice are widely used in pharmacology and toxicology studies as experimental models for their relatively short lifespan and accessibility [7, 12].

In experiments on males and females are carried out separately; the minimum size of the group is 5-6 individuals for rodents, 3-5 individuals for dogs or rabbits. Groups of animals are formed according to the principle of analogs by the method of continuous sampling, kept in the cells for at least five days before the administration of the test substance, which allows them to get used to laboratory conditions. In the study of chronic toxicity, the most preferred type of rodent is a rat; mainly females are used, as they are more sensitive to the effects of toxicants if there is no data on the greater sensitivity of animals of this sex [13].

Oral and parenteral routes for drug administration are used, depending on the route by which the active substance can enter the body of the target animal. Medicines for local administration are applied or administered in the appropriate area according to the method proposed for clinical trials; medicines for oral administration are administered through a probe, laid on the root of the tongue; inhalation drugs
are studied by placing small laboratory animals in exposure chambers equipped with special exposure devices [4, 8].

The study of the toxicological properties of drugs at the stage of preclinical studies is a complex task, for the solution of which a number of scientific methods are used. The choice of the necessary research methods and tools should be scientifically substantiated, the results obtained are informative.

5. Conclusion

Medicinal substances with antiparasitic activity are considerably toxic substances, therefore, when conducting preclinical studies, special attention should be paid to methods for their toxicological evaluation. A unified methodology for studying the safety of such substances does not exist today, hence, in the absence of a strictly regulated systematic approach to their preclinical studies there is a risk of insufficiently safe preparations being introduced into the practice of veterinary medicine. The development of a unified evidence-based methodology for conducting preclinical studies of the safety of new antiparasitic drugs for veterinary use will allow the creation of highly effective and safe drugs.

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