Development of alternative methods for obtaining, studying the physicochemical and pharmacokinetic properties of beta-adrenergic agonists and their metabolites

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Relevance of the research topic. Beta-adrenergic agonists (β-adrenergic agonists, β-agonists) are long-known and widely used in medical and veterinary purposes therapeutic agents for the treatment of bronchial asthma and other obstructive bronchial diseases, which have a relaxing and antispasmodic effect. On the other hand, such drugs stimulate protein synthesis, have a significant anti-catabolic and fat-burning effect. Despite the fact that β-agonists are not steroid hormones, they are used as alternative compounds to hormonal growth regulators, since they improve the efficiency of feed use and contribute to increased weight gain in cattle, pigs, sheep, and poultry.

As feed additives, β-agonists are used in doses exceeding therapeutic ones by tens of times. Along with this, long-term use ensures the accumulation of these compounds in the body of farm animals. Residual amounts of β-adrenergic agonists in meat products cause serious harm to the health of consumers. Since 1990, many cases of human poisoning by meat and offal containing residual amounts of β-adrenergic agonists have been registered in different countries around the world. This problem is mainly caused by the illegal use of clenbuterol, the most famous representative of this class of compounds. China is one of the countries in which, despite the ban on the use of clenbuterol and its analogues by the Ministry of Agriculture, in effect since 1997, the illegal use of β-agonists as feed additives is not uncommon. In recent years, the country's authorities have tightened controls over the use of β-adrenergic agonists in agriculture, which served as the basis for Chinese scientists to search for ways to synthesize new compounds of this group.

The use of β-agonists as feed additives in the Russian Federation, the Eurasian Economic Community and the European Union is prohibited and strictly regulated in accordance with EU Directives 96/22/EC, 96/23/EC, and the Rosselkhoznadzor Instruction of September 21, 2012. However, in more than twenty countries around the world (USA, Canada, Brazil, Argentina, Chile, etc.), these drugs are registered and used as growth promoters in animal husbandry. Every year, new analogues of known β-adrenergic agonists appear, possessing similar biological activity and, often, higher toxicity. For this reason, there is a need for mandatory control of the content of residual amounts of β-agonists in agricultural products (meat, offal) entering the Russian market from abroad, and for conducting pharmacokinetic studies to assess their impact

on humans and animals. To solve these problems, it is necessary to develop fast and modern analytical methods for determining β-agonists in trace amounts, which, in turn, is possible only with standard samples.

For a more complete assessment of compounds from the point of view of pharmacokinetics, it is necessary to study their metabolic transformations in the body of humans and animals. This aspect plays a major role both in determining residual amounts of β-agonists in agricultural products and in establishing the fact of their prohibited use as feed additives, since in some cases metabolites are identified in biological environments for a longer time than the substance entering the body. To confirm the pathways of biotransformation, standard samples of both target compounds and their metabolites are also necessary.

Thus, the development of methods for the synthesis and production of samples of compounds of the β-agonist group and their metabolites, conducting pharmacokinetic studies, and the development of analytical methods for identifying target compounds and their metabolites in biological environments are **an important and urgent task**.

Objective of the work. Development of methods for the synthesis and production of a number of β-agonists and their metabolites for the determination of xenobiotics in the control of meat products imported into Russia from foreign countries.

To achieve the stated objective, the following **tasks** were solved in the work:

- compounds of the β-agonist group of various subclasses were synthesized by simple methods using available reagents;

- metabolites of β-agonists were synthesized for the first time;

- an analytical technique for determining β-agonists and their metabolites in biological fluids (blood, urine) was developed for conducting pharmacokinetic studies, pharmacokinetic studies were conducted on laboratory animals;

- a selective and sensitive analytical technique for the simultaneous determination of β-agonists and their metabolites in trace amounts in meat products (liver) was developed and the developed technique was tested on offal imported to Russia from a number of countries.

Scientific novelty.

1. Alternative methods for obtaining 14 β-agonists with the traditional 2-amino-1-arylethanol structure and 5 compounds with the 2-amino-2-arylethanol structure (2 compounds not described in the literature) with similar β-agonist activity were developed using

commercially available reagents. A simple one-pot synthesis method was implemented for ractopamine and dobutamine.

2. Methods for synthesizing metabolites of clenbuterol, brombuterol, and vilanterol, which were previously characterized only by liquid chromatography, were developed.

3. A chromatograph mass spectrometric method for the simultaneous determination of β-agonists and their metabolites in urine samples was developed.

4. Pharmacokinetic studies were conducted for brombuterol and 2-(4-amino-3,5-dichlorophenyl)-2-(alkylamino)ethanol on the dynamics of changes in their concentration in the blood of laboratory animals, the excretion profile of target compounds and their metabolites with urine was studied.

5. An analytical method for the simultaneous determination of β-agonists and their metabolites in trace amounts in the liver of farm animals was developed using high-performance liquid chromatography in combination with high-resolution tandem mass spectrometry. This method was tested on samples of cow and pig liver imported to Russia from a number of foreign countries.

Theoretical and practical significance. As a result of the work, 22 compounds of the β-agonist group of various subclasses and 5 metabolites were synthesized using commercially available reagents. The obtained samples can be used as standard samples in the analysis of by-products entering the Russian market for the presence and quantitative determination of trace amounts of β-agonists and their metabolites. The analytical technique developed for these purposes can be used by competent authorities responsible for the safety of food and feed. The pharmacokinetic studies can be used for medical purposes to develop forms of drugs based on β-agonists, optimize the conditions for the use of drugs in clinical practice.

Provisions submitted for defense:

1. Developed alternative methods for the synthesis of a number of β-agonists using available reagents.

2. Developed methods for the synthesis of 5 metabolites of clenbuterol, brombuterol, vilanterol.

3. Pharmacokinetic studies for brombuterol and 2-(4-amino-3,5-dichlorophenyl)-2-(alkylamino)ethanols, including establishing the detection time of target compounds and their metabolites, determining the main pharmacokinetic parameters and assessing the excretion of the studied compounds in the urine.

4. Results on the development of an analytical method for the simultaneous determination of β-agonists and their metabolites in liver samples and on testing this method on samples imported into Russia from abroad.