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BIOLOGICAL ACTIVITY OF FENBENDAZOLE BASED ON SUPRAMOLECULAR DELIVERY SYSTEM WITH DISODIUM SALT OF GLYCYRRHIZIC ACID

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Abstract

Due to the wide spread of animal helminthiasis, it becomes necessary to use innovative antiparasitic drugs. Fenbendazole is widely used all over the world for chemotherapy of helminthiasis, but in some cases, it is effective only in a high dose. This study, for the first time, has shown changes of physicochemical properties, pharmacokinetic parameters and an increase in the anthelmintic efficacy of mechanochemically obtained complexes of fenbendazole with disodium salt of glycyrrhizic acid for targeted delivery. Our research aimed to increase the biological activity of a solid dispersion of fenbendazole with disodium salt of glycyrrhizic acid (SDF with Na_2GA), to evaluate the solubility of SDF compositions with Na_2GA, pharmacokinetic parameters, and anthelmintic efficacy on laboratory models of *Trichinella spiralis* and *Hymenolepis nana* and in field tests on sheep naturally infected with gastrointestinal nematodes and moniesia. SDF with Na_2GA was obtained in one-stage process in LE-101 ball mill (Hungary). The ratio of fenbendazole (Changzhou Yabong Pharmaceuticals Co., Ltd., China) and disodium salt of glycyrrhizic acid (Yuli County Jinxing Licorice Products Co., China) was 1:10. The process continued for 4 hours at 90 rpm. Pharmacokinetic parameters of fenbendazole and its metabolites in sheep were studied by high-performance liquid chromatography-tandem mass spectrometry detection. SDF with Na_2GA and the substance of fenbendazole (FBZ) was administered to two groups of clinically healthy sheep (5 animals each) once per or at the dose of 2 mg/kg of active substance. Blood samples were taken from the jugular vein 0, 1, 2, 4, 6, 8, 12, 24, 33, 48, 72, and 144 hours after administration of SDF with Na_2GA and the basic drug. The absorption rate constant, absorption half-life, clearance of the drug from the blood plasma, maximum drug concentration, time to reach maximum plasma drug concentration following drug administration, elimination half-life, area under the concentration–time curve and mean residence time were calculated. The efficacy of SDF with Na_2GA against *Hymenolepis nana* and *Trichinella spiralis* was studied with 50 white inbred female BALB/c mice weighing 16-18 g. The eggs of *H. nana* were administered intragastrically with a syringe, 200 eggs per animal. On day 13 after infection, SDF with Na_2GA in 1 % starch gel was administered into the stomach of mice of I, II and III groups (10 animals each) at doses of 3.0; 2.0 and 1.0 mg/kg of active substance, respectively. FBZ was the basic drug which was applied at the dose of 2.0 mg/kg (experimental group IV). The animals of the control group received the same volume of the starch gel. *Trichinella spiralis* was isolated by serial passages of the first stage larvae to female rats. Before infection, the mice were kept on a starvation diet for 12 hours, and then 200 larvae were injected into their stomachs using a tuberculin syringe. On day 3 after infection, the mice were divided into four experimental and one control groups (10 animals each). SDF with Na_2GA in 1 % starch gel was administered into the stomachs of mice of experimental groups I, II, and III at doses of 3.0; 2.0 and 1.0 mg/kg of active substance, respectively. The FBZ substance was administered to mice of IV experimental group at the dose of 2 mg/kg. Control group of animals received 1,5 % starch gel at the same dose. The efficacy of the drugs against *H. nana* and *T. spiralis* was determined from necropsy data. The anthelmintic activity of SDF with Na_2GA was also studied on young Stavropol merino sheep in field tests (LTD Agroresurs, Sa-

mara Province, Pestravsky District) in the summer 2016-2017. SDF with Na_2GA was administered per or to the animals of the experimental groups (a single application per or at the doses of 3.0; 2.0 and 1.0 mg/kg of active substance vs. FBZ at the dose of 2.0 mg/kg. The control group of animals did not receive the drugs. Anthelmintic activity of drugs was determined according to the data of necropsy of the intestines of mice and the results of studies of sheep feces samples by the McMaster method before and after administration of the drugs. The data of physicochemical studies have shown an increase in solubility, a decrease in the particle size of the compositions of SDF with Na_2GA, and the formation of irregularly shaped aggregates. The pharmacokinetic parameters indicated a significant increase in the rate of absorption of SDF with Na_2GA and their entry into the blood, a 2,5-fold increase in the maximum concentration of fenbendazole and its metabolites in the blood, as well as a decrease in the rate of drug elimination from the body compared to the FBZ. SDF with Na_2GA (3.0; 2.0 and 1.0 mg/kg) showed 100; 98,05 and 92,74 % activity against *T. spiralis*, 100, 98.67 and 89.04 % against *H. nana*, 100, 95.37 and 92.07 % against *Nematodirus* spp., 100, 95.42 and 90.75 % against gastrointestinal strongylates, and 96.44, 91.61 and 81.12 % against *Moniezia expansa*. The FBZ (2.0 mg/kg) anthelmintic activity was 3.4 times lower than that of the same dose of SDF with Na_2GA upon experimental trichinellosis of mice. Its efficacy was 28.88 % against experimental hymenolepiasis of mice. The FBZ substance showed low efficacy against *Nematodirus* spp. (33.33 %), other gastrointestinal strongylates (39.14 %) and *Moniezia* spp. (17.55 %). These findings allow us to conclude that the development of drugs based on fenbendazole solid dispersion with glycyrrhizic acid disodium salt is promising, and the production technology can be scaled up.

Keywords: fenbendazole, solid dispersion, disodium salt of glycyrrhizic acid, efficacy, pharmacokinetics, helminthiasis

Fenbendazole (Panacur) is a broad-spectrum anthelmintic of the group of benzimidazole carbamates [1, 2]. It has high activity against animal nematodes at a dose of 7.5-10 mg/kg, against *Protostrongylus* spp. at a dose of 15 mg/kg, against *Fasciola hepatica* and *Dicrocoelium lanceatum* at a dose of 100 mg/kg [3], being less effective against *Trichocephalus* spp. and *Strongyloides* spp. [1]. The mechanism of the anthelmintic action of fenbendazole is the destruction of cytoplasmic microtubules in the cells of the parasite. This is accompanied by impaired absorption and transport of glucose, as well as a decrease in the activity of fumarate reductase, which subsequently leads to the death of helminths [2]. At present, fenbendazole is widely used all over the world at the recommended doses [4-7]. According to the Bio-pharmaceutics classification system (BCS) guidance, Food and Drug Administration (FDA, USA) (https://www.fda.gov/), it belongs to the IV class of drugs with low permeability and poor solubility, that is, it has low bioavailability. Therefore, an increase in its water solubility will affect the anthelmintic properties.

Recently, drug delivery systems (DDS) of biologically active molecules have been widely developed in order to improve the solubility of drugs and their bioavailability by increasing absorption, blood concentration and permeability of the drug through biological membranes to the receptors. DDS technologies improve the effectiveness of the drugs, reduce their therapeutic dose and possible side effects. To improve the solubility of drugs, different methods are used, e.g. grinding and changing the shape of the crystal lattice, creating solid dispersions of drugs with fillers, changing the particle size and crystal structure [8-10]. Cyclodextrins, polysaccharides, liposomes, micelles, and nanosized inorganic particles, which form supramolecular systems, are most often used as drug delivery vehicles [11-13].

Mechanochemical modification of solid medicinal substances and excipients is one of the DDS technologies. Under the influence of pressure and shear deformations in mills, the crystal structure of substances can be disordered until complete amorphization followed by polymorphic transitions and chemical reactions with the formation of complexes or micelles with increased solubility [14, 15].

Selyutina et al. [16] found that derivatives of glycyrrhizic acid (GA) are able to integrate into biological membranes of cells, providing lipid motility. Due to its amphiphilicity, GA can form micelles with hydrophobic drug compounds and participate in their transmembrane transfer [17-20].

In our previous work we investigated the effect of mechanochemical technology on the anthelminthic efficacy of a solid dispersion of fenbendazole with polyvinylpyrrolidone and revealed its high therapeutic effect in laboratory models of parasitosis and in farm conditions at a reduced dose. The data of physicochemical studies showed about 3-fold increase in the solubility of the resultant dispersion, a decrease in the particle size to 5-20 microns and amorphization of the fenbendazole substance [21].

Here, we have shown for the first time a change in the physicochemical properties, pharmacokinetic parameters and an increase in the anthelmintic efficacy of fenbendazole obtained by mechanochemical treatment using the disodium salt of glycyrrhizic acid for its targeted delivery

This paper aimed to increase the biological activity of a solid dispersion of fenbendazole with a disodium salt of glycyrrhizic acid, to assess the solubility of compositions of a solid dispersion of fenbendazole (SDF) with Na_2GA, the parameters of pharmacokinetics and anthelmintic efficacy on laboratory models of *Trichinella spiralis* and *Hymenolepis nana* in field conditions on sheep naturally infected with gastrointestinal nematodes and moniesia.

Materials and methods. Animal experiments followed the Guidelines for Experimental (Preclinical) Study of New Pharmacological Substances [22], the Rules of Good Laboratory Practice of the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 199n of 04/01/2016 "On Approval of the Rules of Good Laboratory Practice") and the European Convention for the Protection of Vertebrate Animals used for Experimental or Other Scientific Purposes (ETS 123, Strasbourg, 1986).

A solid dispersion of fenbendazole (methyl 5-(phenylthio)-2-benzimidazole carbamate, 99.0%, molecular weight ~ 299.35) with a disodium salt of glycyrrhizic acid (SDF with Na_2GA) was obtained at the Nesmeyanov Institute of Organoelement Compounds RAS (Moscow) at one-stage mechanochemical process in a LE-101 ball mill (Hungary). The ratio of fenbendazole (Changzhou Yabong Pharmaceuticals Co., Ltd., China) and disodium salt of glycyrrhizic acid (Yuli County Jinxing Licorice Products Co., China) in the experiments was 1:10. The process continued for 4 hours at 90 rpm until particle aggregates with a size of 0.1-10 microns were formed.

The solubility of SDF with Na_2GA in water was assessed in samples with a component ratio of 1:5, 1:10, and 1:20 obtained after 4 hours of mechanochemical treatment, and in the initial fenbendazole substance. Solubility was determined after stirring in a shaker-incubator GFL-3031 (GFL, Germany) at 25 °C and 180 rpm for 3 hours. Then the suspension was centrifuged (5810R, Eppendorf AG, Germany) and the concentration of the drug was estimated in solution by high-performance liquid chromatography (an Agilent 1100, Agilent Technologies, Germany) with a Hypersil C18 column (length 150 mm, diameter 4.6 mm, column temperature 30 °C, diode array detector). Eluent was acetonitrile-acetate buffer, pH 3.4 (1:1), at flow rate of 1 ml/min. Detection was carried out at λ = 290 nm. The volume of an injected sample was 1 μl [23].

The pharmacokinetic parameters of fenbendazole and its metabolites were studied on Stavropol Merino sheep by high-performance liquid chromatography with tandem mass spectrometric detection [24]. Clinically healthy sheep (5 animals each) were assigned into two treatments groups, the group I was once administered SDF with Na_2GA orally, the group II received the substance of fenbendazole (FBZ) at a dose of 2 mg/kg. During the experiment, the animals were kept under the same conditions (Podolsk department of VNIIP — Branch of FSC VIEV RAS). Blood samples were taken from the jugular vein before and 1, 2, 4, 6, 8, 12, 24, 33, 48, 72, and 144 h after ad-

ministration of SDF with Na_2GA and the basic drug (FBZ). Samples were analyzed by high-performance liquid chromatography (an Agilent 1290 with Agilent 6430 mass spectrometric detector, Agilent Technologies, Germany); chromatographic separation on a Kromasil Eternity XT-2.5-C18 column, length 100 mm, 2.1 mm inner diameter, sorbent particle size 2.5 μm, with a Kromasil Eternity guard column 2.1×10 mm (Nouryon, Sweden). Detection of analytes and internal standard was conducted using tandem mass spectrometry in the mode of recording signals of selected ionic reactions for negatively charged ions at an ionization temperature of 350 °C, a gas flow of 10 l/min, and a nebulizer pressure of 40 psi. Absorption rate constant (k_a), absorption half-life ($t_{1/2ka}$), clearance (CL), maximum blood concentration (C_{max}), time of maximum concentration (T_{max}), elimination half-life ($T_{1/2}$), area under the plasma drug concentration-time curve (AUC_{0-t}), and mean residence time (MRT) were calculated based on the blood levels of fenbendazole, fenbendazole sulfoxide and fenbendazole sulfone.

The efficacy of SDF with Na_2GA against *Hymenolepis nana* and *Trichinella spiralis* was tested on 50 female white inbred BALB/c mice (16-18 g) (Stolbovaya Branch of Science and Technology Center for Biological Medicine, FMBA of Russia, Moscow Province). Mice, after a 7-day quarantine, were confined in polycarbonate cages, 10 animals each. The ambient temperature in the vivarium was 20-22 °C with 60-70% humidity, lighting was natural and artificial. Mice were fed a standard feed (OOO Laboratorkorm, Russia) in accordance with the RF feeding standards (Order of the USSR Ministry of Health No. 1179 dated 10.10.1983 "On the approval of standards for feed costs for laboratory animals in health care institutions").

The mice were injected intragastrically with the eggs of *Hymenolepis nana* (200 eggs per animal) using a syringe. To obtain cestode eggs, the worms of *H. nana* were destroyed in a small amount of tap water by repeated pipetting using a syringe with a cannula for oral infection. On day 13 after infection, SDF with Na_2GA in 1% starch gel was administered via direct injection into the stomach of mice of groups I, II, and III (10 animals each) at doses of active ingredient (a.i.) of 3.0, 2.0 and 1.0 mg/kg, respectively. FBZ was a basic drug which was given to 10 mice of the group IV at a dose of 2.0 mg/kg. Ten mice of the control group received starch gel in the same volume. On day 4 after administration of the drugs, all mice were decapitated. Anthelmintic action was determined by counting cestodes in the dissected small intestine of mice and calculating efficacy [25].

Trichinella spiralis was isolated after a serial passage of the I stage larvae in female rats. Infective larvae were isolated by digestion of rat muscle tissue. The tissue was treated for 12 hours in a liquid for digestion (1 liter of saline with 20 ml of concentrated hydrochloric acid and 20 g pepsin) at 37 °C with constant mixing (an RK-1D mechanical shaker, DAIHAN Scientific, South Korea). The suspension was centrifuged for 2 min at 1000 rpm (5810R, Eppendorf AG, Germany). The precipitate was washed with saline (0.9 % NaCl), centrifuged and resuspended in 1.5% gelatin in saline to obtain a stable suspension. A hemocytometer (MiniMed, Russia) was used to calculate the required number of larvae for infection. Before infection, the mice were kept on a starvation diet for 12 hours, then 200 larvae per animal were injected into the stomach with a tuberculin syringe [25]. On day 3 after infection, the mice were assigned into five treatments (10 animals per each group). SDF with Na_2GA in 1% starch gel was directly injected into the stomachs of mice of the groups I, II, and III at doses (a.i.) of 3.0, 2.0 and 1.0 mg/kg; FBZ was administered to mice of the group IV at a dose of 2 mg/kg; control animals received 1.5% starch gel at the same dose.

On day 2 after the drug administration, the animals were decapitated. The efficacy of the anti-nematode agents was determined post mortem. The small intestine of the mice was cut with scissors along its entire length, placed in saline in a Berman apparatus, and kept at 37-39 °C in a thermostat for 2 hours. The sediment was examined under a binocular magnifying glass to count the number of *T. spiralis*. Activity of SDF with Na_2GA, in comparison with the control group of mice, was measured by the average number of detected nematodes and calculating the efficacy.

Anthelmintic action of SDF with Na_2GA was tested in field trials on young Stavropol Merino sheep at the Agroresurs LLC farm (Samara Province, Pestravsky District) where high helminth levels of infection were recorded. The tests were conducted in the summer 2016-2017 at maximum infection of sheep. Of 141 sheep (17-34 kg) involved in the trials 50 animals were naturally infected with *Nematodirus* spp., 52 animals by other gastrointestinal nematodes of *Strongylata* suborder, and 39 animals with *Moniezia expansa*. At each infection, the animals of the groups I, II and III were once received orally SDF with Na_2GA at a dose of 3.0, 2.0 and 1.0 mg/kg, respectively, the animals of the group IV received FBZ at a dose of 2.0 mg/kg, and no drugs were administered to the control animals. Sheep fecal samples were examined by the McMaster method [26] before and 15 days after drug administration. The drug efficacy was calculated from the number of helminth eggs in the feces of test and control sheep [27].

Statistical processing was carried out with the SAS/Stat No. 9.4 SAS System for Windows computer program (https://www.sas.com/en_us/software/sas9.html). The pharmacokinetic parameters were calculated using a one-chamber model (Microsoft Excel PKSolver 2.0) [28]. The mean number of helminths/eggs (*M*) with the standard error of the mean (±SEM), relative standard deviation (RSD) for pharmacokinetic parameters and significance level (p) using the Student's *t*-test were calculated.

Results. The physicochemical properties of anthelmintics with the disodium salt of glycyrrhizic acid as a targeted delivery system were studied in detail on the example of albendazole (ABZ), praziquantel (PZQ) and fenbendazole (FBZ) by Meteleva et al. [19, 29] and Arkhipov et al. [23]. It was shown that solubility of the drug in the system with Na_2GA can increase 300-fold (300 mg/l) for ABZ, 3.5-fold for PZQ, and 71-fold for FBZ. However, in terms of the concentration of medicinal substances (drugs) in aqueous solutions, compositions FBZ/Na_2GA and ABZ/Na_2GA with a mass ratio of components 1:10 were selected, since an increase in the proportion of drugs leads to a decrease in solubility, and Na_2GA "overloads" the mass of the dosage form intended for oral administration [19]. We found that the SDF solubility for SDF with Na_2GA in a ratio of 1:5, 1:10 and 1:20 increased 31.2, 40.6 and 70.9 times, respectively (Table 1).

1. Water solubility of albendazole (ABZ)**, praziquantel** (PZQ)**, fenbendazole** (FBZ) **and their solid dispersions with disodium salt of glycyrrhizic acid** (Na_2GA) (analytical error of ±3 %) [19, 23, 29]

Sample (mass ratio)	Solubility, g/l	Increase in solubility
ABZ	0.001	
ABZ/Na_2GA (1:5)	0.042	42-fold
ABZ/Na_2GA (1:10)	0.200	200-fold
ABZ/Na_2GA (1:20)	0.300	300-fold
PZQ	0.234	
PZQ/Na_2GA (1:5)	0.557	2.38-fold
PZQ/Na_2GA (1:10)	0.687	2.94-fold
PZQ/Na_2GA (1:20)	0.819	3.49-fold
FBZ	0.19	
FBZ/Na_2GA (1:5)	12.1	31.20-fold
FBZ/Na_2GA (1:10)	17.4	40.63-fold
FBZ/Na_2GA (1:20)	34.5	70.96-fold

X-ray phase analysis, thermal analysis, and electron microscopy showed particle fragmentation and formation of aggregates irregular in shape [23, 29]. Meteleva et al. [19], based on phase diagrams of solubility and dynamic ^{1}H NMR spectroscopy, noted the appearance of intermolecular interaction of praziquantel with ~ 80 kDa micelles which are formed in Na_2GA aqueous solution. The assay of PZQ permeability through an artificial membrane using the PAMPA method (parallel artificial membrane permeability assay) and a monolayer of Caco-2 cells showed that the diffusion rate of PZQ molecules with Na_2GA is much higher than that of the initial PZQ [19].

Tests on Stavropol Merino sheep showed a significant difference in the kinetics of fenbendazole used at a dose of 2.0 mg/kg in the forms of the basic drug and a supramolecular complex (Tables 2, 3). Fenbendazole and its sulfoxide and sulfone metabolites began to be detected in blood serum 2 hours after a single oral administration of SDF with Na_2GA and only 4-6 hours after application of FBZ. The concentration of FBZ and its metabolites after the administration of SDF with Na_2GA was 2-3 times higher. The maximum levels of fenbendazole, fenbendazole sulfoxide and fenbendazole sulfone in blood serum were 58.4, 64.0 and 54.0 ng/ml, respectively, in 33 hours after administration of SDF with Na_2GA, and 22.1, 16.6 and 18.6 ng/ml after administration of basic drug FBZ.

The anthelmintic effect of SDF with Na_2GA against experimental trichinellosis of white mice confirmed an increase in the efficacy of the drug form obtained by mechanochemical technology as compared to fenbendazole substance (Table 4). The efficacy against *T. spiralis* increased at increasing the doses of SDF with Na_2GA. Thus, a 100% effect was obtained for SDF with Na_2GA at a dose of 3.0 mg/kg. The SDF with Na_2GA also showed high nematodicidal efficacy (98.05 and 92.74%, respectively) at doses of 2.0 and 1.0 mg/kg. For the FBZ at a dose of 2.0 mg/kg, this indicator was 3.4 times lower than for SDF with Na_2GA at the same dose. On average 107.3±5.6 *T. spiralis* helminths were found in the control group of mice. The cestodocidal activity of SDF with Na_2GA showed 100, 98.67 and 89.04% efficacy of the drug against *H. nana* at 3.0, 2.0 and 1.0 mg/kg, respectively. The efficacy of FBZ was 28.88% at a dose of 2.0 mg/kg. On average 3.74±0.4 *H. nana* helminths were found in the control mice.

2. Pharmacokinetic parameters of fenbendazole (FBZ) **and its metabolites in blood of Stavropol Merino sheep after administration of FBZ and its solid dispersion** (SDF) **with disodium salt of glycyrrhizic acid** (Na_2GA) (a.i. 2.0 mg/kg, model test)

Parameter	Fenbendazole		Fenbendazole sulfoxide		Fenbendazole sulfone	
	M	RSD	*M*	RSD	*M*	RSD
FBZ drug ($n = 5$)						
k_a, h^{-1}	0.031	6.7	0.038	1.7	0.034	3.2
$t_{1/2ka}$, h	25.62	6.7	18.36	1.6	19.08	3.2
CL, l/h	0.92	6.6	1.56	3.2	1.36	3.3
C_{max}, ng/ml	19.86	1.6	16.68	1.6	18.14	1.7
T_{max}, h	40.64	6.2	27.62	2.4	27.84	3.0
$T_{1/2}$, h	28.84	6.5	20.12	3.0	21.60	3.1
AUC_{0-t}, ng/(ml · h)	1156.26	7.0	930.10	2.2	1012.16	3.5
MRT, h	61.62	8.2	55.26	2.5	56.24	3.2
SDF with Na_2GA ($n = 5$)						
k_a, h^{-1}	0.058	5.6	0.032	2.2	0.024	4.2
$t_{1/2ka}$, h	13.90	8.4	21.54	1.8	26.34	4.4
CL, l/h	0.20	7.2	0.48	5.4	0.38	6.5
C_{max}, ng/ml	50.80	4.2	41.76	4.2	42.12	4.8
T_{max}, h	42.84	9.4	31.70	2.4	40.16	4.2
$T_{1/2}$, h	102.26	12.3	24.62	3.5	28.63	3.6
AUC_{0-t}, ng/(ml · h)	3042.82	3.6	2484.70	4.0	2468.26	4.7
MRT, h	364.26	9.5	69.10	2.5	80.44	4.2

Note. k_a — absorption rate constant, $t_{1/2ka}$ — absorption half-life (time to absorb a half of the administered dose), CL — clearance (the volume of plasma from which a substance is completely removed per unit time), C_{max} — maximum drug concentration in blood, T_{max} — time of maximum concentration, $T_{1/2}$ — elimination half-life, AUC_{0-t} — area under the plasma drug concentration-time curve, MRT — mean residence time; *M* — means, RSD — relative standard deviation, %.

3. Concentration (ng/ml) **of fenbendazole** (FBZ) **and its metabolites in blood of Stavropol Merino sheep after administration of FBZ and its solid dispersion** (SDF) **with disodium salt of glycyrrhizic acid** (Na_2GA) (a.i. 2.0 mg/kg, model test)

Time after administration, h	Fenbendazole		Fenbendazole sulfoxide		Fenbendazole sulfone	
	M	RSD, %	*M*	RSD, %	*M*	RSD, %
FBZ drug ($n = 5$)						
0	< LOQ		< LOQ		< LOQ	
1	< LOQ		< LOQ		< LOQ	
2	< LOQ		< LOQ		< LOQ	
4	6.4	3,1	< LOQ		< LOQ	
6	6.6	8,2	6,2	4,6	6,0	4,6
8	6.7	6,0	8,4	6,2	8,5	6,0
12	8.2	11,6	12,5	6,8	13,0	6,5
24	15.8	6,2	19,4	9,6	20,8	3,6
33	22.1	7,0	16,6	8,2	18,6	4,0
48	23.3	6,4	12,4	6,0	15,4	4,2
72	12.4	9,6	8,8	4,6	8,2	8,4
SDF with Na_2GA ($n = 5$)						
0	< LOQ		< LOQ		< LOQ	
1	< LOQ		< LOQ		< LOQ	
2	9.8	8,0	6,4	6,0	6,0	6,4
4	12.4	7,2	16,2	8,4	8,0	7,2
6	20.0	10,4	16,4	7,8	13,0	8,4
8	26.6	11,2	17,6	9,2	20,4	9,3
12	34.2	13,6	28,2	10,0	22,2	10,6
24	41.2	12,6	30,6	11,6	30,8	11,4
33	58.4	12,2	64,0	12,8	54,0	12,2
48	49.6	11,0	37,8	10,6	44,2	11,4
72	44.2	10,3	24,6	9,8	32,0	9,6

Note. LOQ — Limit of Quantification, RSD — relative standard deviation, %.

4. Efficacy of solid dispersion of fenbendazole with disodium salt of glycyrrhizic acid upon experimental trichinellosis and hymenolepiosis of white mice BALB/c ($n = 10$, $M \pm SEM$)

Group	Average number of helminths	Efficacy, %	p
Trichinellosis (*Trichinella spiralis*)			
I	0	100	< 0.0001
II	2.1±0.2	98.05	< 0.001
III	7.8±0.8	92.74	< 0.001
IV	76.5±7.0	28.71	< 0.01
Control	107.3±5.6		
Hymenolepiosis (*Hymenolepis nana*)			
I	0	100	< 0.0001
II	0.05±0.002	98.67	< 0.001
III	0.41±0.06	89.04	< 0.001
IV	2.66±0.3	28.88	< 0.01
Control	3.74±0.4		

Note. For description of the groups, see *Materials and methods.*

The data for laboratory models showed an increase in the efficacy of SDF with Na_2GA in comparison with basic FBZ. For this reason, we tested SDF with Na_2GA in the field trials on sheep naturally infected by *Nematodirus* spp., gastrointestinal strongylates and *Moniezia* spp. (Table 5). Coproovoscopic examination revealed a significant increase in the efficacy of SDF with Na_2GA compared to FBZ. SDF with Na_2GA had 2.8-, 2.4- and 5.2-fold efficacy against nematodiruses, other gastrointestinal strongylates and moniesia compared to FBZ at 2.0 mg/kg. The highest anthelmintic activity of SDF with Na_2GA was registered at a dose of 3.0 mg/kg, while FBZ showed low efficacy.

An increase in the efficacy of SDF with Na_2GA in our experiments was due to higher solubility, higher absorption, and, as a consequence, a higher bioavailability of fenbendazole in the solid dispersion with Na_2GA. A similar manifestation of the effect was noted for solid compositions of drugs and Na_2GA [18, 20, 30-32]. Since glycyrrhizic acid contains hydrophilic and hydrophobic components, it is capable of forming complexes with organic molecules [33] and self-

5. Efficacy of solid dispersion of fenbendazole with disodium salt of glycyrrhizic acid against natural helminthiasis of Stavropol Merino sheep (*M*±SEM, control test, field trials, Agroresurs LLC farm, Samara Province, Pestravsky District, 2016-2017)

Group	Dose a.i. mg/kg	Infected prior to trial, heads			Average number of helminths' eggs per 1 g feces						Efficacy, %		
					prior to test			after treatment					
		1	2	3	1	2	3	1	2	3	1	2	3
I	3.0	10	9	7	266.0±9.8	328.3±8.9	356.2±9.3	0	0	13.6±1.4	100*	100*	96.44*
II	2.0	10	12	8	260.8±9.3	334.0±9.5	364.0±9.4	12.1±1.1	15.3±1.4	32.0±2.3	95.37*	95.42*	91.61*
III	1.0	11	10	9	257.2±9.6	341.4±9.8	357.8±9.2	20.4±1.9	31.6±2.1	72.0±4.7	92.07*	90.75*	81.12
IV	2.0	10	11	8	270.4±8.8	325.3±8.9	370.3±9.1	180.3±5.1	198.0±5.3	314.3±8.8	33.33**	39.14**	17.55**
Control		9	10	7	265.4±9.0	330.6±9.2	364.0±8.9	271.0±8.8	342.3±9.6	381.2±9.4			

N o t e. For description of the groups, see *Materials and methods*; 1 — *Nematodirus* spp., 2 — *Strongylata*, 3 — *Moniezia* spp.

*, ** Differences between the treatment and the control (animals not administered the drug) are statistically significant at $p < 0.001$ and $p < 0.01$, respectively.

associates in aqueous-alcoholic and aqueous solutions [34, 35]. Glycyrrhizic acid derivatives increase lability of lipids in biological membranes [17, 34], which facilitates the penetration of drug molecules into the cell. By PAMPA assay method with the use of artificial membrane, it was shown that the diffusion rate of the anthelmintic praziquantel molecules from its composition with Na_2GA significantly increases compared to pure praziquantel. The incorporation of praziquantel molecules into Na_2GA micelles provides an increased concentration of praziquantel molecules in the premembrane layer, that is, the drug delivery occurs faster, and the rate of absorption into the bloodstream is higher [19]. GA disodium salt acts as a solubilizing agent and a carrier for drug molecules, since in its native form it does not penetrate the walls of the gastrointestinal tract, but undergoes enzymatic hydrolysis in the intestine [20]. In addition, the interaction of HA with the lipid bilayer of cell membranes can also improve bioavailability [16], including through interaction with the intestinal epithelium. This can increase its permeability to drug molecules and contribute to an increase in the concentration gradient of the anthelmintic directly on the cell wall, which, in turn, enchases absorption. This increase in bioavailability contributes to a significant reduction in the drug dose [19, 36].

Thus, the technology proposed here to produce a solid dispersion of fenbendazole (SDF) is simple one-stage active mechanochemical process, i.e. mixing the powdery substance of the fenbendazole drug and disodium salt of glycyrrhizic acid (Na_2GA) in a grinder of abrasive type to the formation of particles 0.1-10 microns in size. The resulting powder is a solid dispersion of components that form supramolecular complexes when dissolved in water, and has a higher water solubility, absorption and anthelmintic efficacy. As compared to the fenbendazole substance applied separately, the SDF with Na_2GA at doses 2.5-3.5 times less than the therapeutic dose had greater anthelmintic efficacy in mice experimentally infected with *Trichinella spiralis*, *Hymenolepis nana* and in sheep naturally infected with gastrointestinal nematodes and moniesia. This is due to the fact that under mechanochemical treatment the molecules of the anthelmintic substance are distributed in the pores and on the surface of the carrier macromolecules. This improves the absorption of the active substance in the digestive tract at oral administration due to its rapid release and delivery through biological membranes. An increase in the efficacy of SDF with Na_2GA is due to a higher rate of intake and a 2.5-2.9-fold increase in the maximum blood concentration of fenbendazole and its metabolites, a decrease in the rate of drug excretion from the body and an increase in the time of drug residence in bloodstream.

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