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Research paper

The efficacy of the supramolecular complexes of niclosamide obtained by mechanochemical technology and targeted delivery against cestode infection of animals

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ABSTRACT

Niclosamide is an anthelmintic that is widely used to treat cestode infection of animals. The efficacy of the supramolecular complexes of niclosamide obtained by mechanochemical technology and targeted delivery was studied in hymenolepiosis of mice and monieziosis of sheep. The efficacy of new substances of niclosamide with polyvinylpyrrolidone polymer in different ratios (1:10; 1:5; 1:2) was determined by the results of helminthological necropsy of the small intestine of sheep and mice. Pre-treatment eggs per gram (EPG) were not significantly different ($P > 0.1$) among groups. The controlled test was used to evaluate the efficacy. A high efficacy (> 95% efficacy) of the supramolecular complexes of niclosamide with PVP (SCoNwPVP) was shown in different ratios (1:10; 1:5 and 1:2) at a dose of 20 mg/kg of body weight at oral administration against *Hymenolepis nana* in mice and *Moniezia expansa* in sheep. Whereas the basic drug − substance of niclosamide was effective at a dose of 100 mg/kg of b/w. No adverse effects of the drugs on animal health were detected during the study.

1. Introduction

Cestodoses of animals are widespread and prevalence of infection of animals ranges from 60 to 100% in some regions of the Russian Federation (Akbayev, 1983; Magomedov, 2000; Belova, 2011). Anoplocephalatosis and especially monieziosis of animals cause significant losses of production because of high prevalence and mortality of young infected animals (Tsoloev, 1999; Chetvertnov et al., 2009). Lambs are more susceptible and massive infections can cause diarrhea, reduced weight gain and intestinal obstruction. It was determined that the body weight gain of the infected lamb is 1.62 times less than the body weight of a healthy animal when calculating the economic damage in monieziosis of sheep. Infection with *Moniezia expansa* was associated with reduced weight gain estimated at 4.16 kg, with reduced wool yield estimated at 0.42 kg and mortality was 7.1% per animal (Safiullin, 1997).

Niclosamide (syn.: 5-Chloro-N-(2-chloro-4-nitrophenyl)-2 hydroxybenzamide, monsonil, fenasal, yomezan) is a chlorinated salicylanilide anthelmintic that is used to treat tapeworm infection in a wide range of animals (Reynolds, 1989). Niclosamide is also used against aquatic vertebrates and crustaceans (Goldsmith, 1984). It is a white to yellowish odorless crystalline powder; melting point 224 °C; practically insoluble in water; sparingly soluble in ether, ethanol, chloroform, and soluble in acetone; it is routinely administered orally to pets and livestock. The commercially available ethanolamine salt dissolves in distilled water (The Merck Index, 2001).

The mode of pharmacological action of niclosamide is associated with uncoupling of oxidative phosphorylation or stimulation of ATPase activity (Weinbach and Garbus, 1969). Niclosamide is used to treat cestodosis of sheep, goats and cattle (monieziosis, avitellinosis, thysanieziosis) at a dose of 100 mg/kg. It is also used at the same dose against anoplocephalatosis of horses, dipylidiosis and other taeniosis of carnivores. Side effects are infrequent, mild and transitory. Adverse events include nausea, vomiting, diarrhea, and abdominal pain (Campbell and Rew, 1986).

In addition niclosamide is an anticancer and protonophric agent. It

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is STAT3 inhibitor (IC50 = 0.25 μM) (Ren et al., 2010; Li et al., 2013a,b). It is shown to inhibit a number of downstream signaling pathways including mTORC1, Wnt/β-catinin, NF-κB, Notch and ROS, most of which are closely involved with cancer stem cells (Balgi et al., 2009; Wang et al., 2009; Jin et al., 2010; Osada et al., 2011).

The disadvantages of this drug are low solubility, poor absorption by the intestinal mucosa and as a result poor bioavailability and the requirement for high doses in treatment of cestodosis (Garin et al., 1964; Reynolds, 1989). In this regard, researchers have tried to reduce the doses and create new dosage forms of the drug with increased efficacy used micronization (microsal), different polymers (fenapeg, likvofen) (Arkhipov, 2009) and nanoparticles for intravenous administration (Yanghuan et al., 2014). However, sometimes these technologies require large material costs, prolonged preparation and formation of a large amount of waste products.

Therefore, we have prepared the supramolecular complexes of niclosamide by mechanochemical technology with targeted delivery to enhance the solubility and bioavailability of the drug. The aim of our study was to examine the efficacy of new supramolecular complexes of niclosamide with polyvinylpyrrolidone (SCoNwPVP) in different ratios against hymenolepiosis of white mice and monieziosis of sheep. The information obtained from the present study should provide a foundation for future investigations.

2. Materials and methods

The study was randomized, blinded, placebo-controlled and was conducted according to the Guidance for the experimental (preclinical) study of new pharmacological substances (Habriev, 2005), the rules of good clinical practice of the Russian Federation (2003) and the rules adopted by European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123), Strasburg, 1986. Inbred white mice experimentally infected with *H. nana* and sheep naturally infected with *M. expansa* were used in this study.

2.1. Preparation of supramolecular complexes of niclosamide

The supramolecular complexes of niclosamide were prepared by mechanochemical technology. Solid phase was carried out in one step by mechanochemical processing of the drug (niclosamide) and water-soluble polymer (polyvinylpyrrolidone) using a Ball Drum Mill LE-101 (Russia) to form particles of aggregates with sizes ranging from 0.1 to 10 micrometers. The drum was charged with niclosamide and PVP in a weight ratio of 1:10, 1:5 and 1:2 with 700 g of metal balls with diameter of 12 mm. The drum was set on rolls and the mixture was processed for two hours at a speed of 90 revolutions per minute. Loose powder of beige color was discharged from the drum for further examination of its physicochemical and anthelmintic properties.

2.1.1. Characterization

The thermal analysis of the investigated samples was carried out by differential scanning calorimetry on the DSC-550 (Instrument Scientific Specialists Inc., USA) in argon atmosphere with heating of samples from 20 to 250 °C at a rate of 10 °C/min. The morphology of these samples was observed via scanning electron microscopy (Hitachi TM-1000, Japan). X-ray analysis was carried out on a DRON-4 diffractometer (Russia) using CuK_{α}-radiation at a counter rotation speed of 2 deg/min (l = 1000). The granulometric composition of the precipitates of the initial substances and their supramolecular complexes was tested using a laser particle size analyzer Microsizer-201 (VA Insult, Russia). Infrared spectral studies of the initial drug substances and their supramolecular complexes with polymers were performed on a Shimadzu-2600 spectrophotometer (Japan). The solubility of complexes was determined by dissolving samples of 0.33; 0.6 and 0.8 g in 10 ml of distilled water for three hours in a shaker-incubator at 25 °C and 180 rpm. The concentration of drug substances in solutions was determined by high-performance liquid chromatography on an Agilent 1200 chromatograph with a reversed-phase chromatographic column LUNA C18 (250 × 4.6 mm) with a sorbent diameter of 5 μm; eluent: acetonitrile/dist. water (pH 3.0-3.5); the flow rate of the eluent was 1 cm^3/min; the volume of the injected sample was 50 μl; UV spectrum: 340 nm.

More detailed description of the methods has been presented previously (Dushkin et al., 2013; Arkhipov et al., 2016).

2.2. Study animals

2.2.1. Hymenolepiosis of mice

Fifty inbred white mice of both sexes weighing 16–18 g were used at studying the efficacy of the supramolecular complexes of niclosamide against hymenolepiosis. Mice were kept in the quarantine and adaptation room for 7 days prior to the start of the experiment. Then animals were divided into polycarbonate cages of 6 animals each. Mice were maintained on standard rat feed (from LLC Laboratorkorm) according to the daily feed rate of the Russian Federation (1983). Water was provided *ad libitum* throughout the course of the study. The animals were placed in the vivarium with natural and artificial light in a controlled environment at temperature of 20–22 °C and humidity of 60–70%. Animals were randomly selected in groups by the method of random numbers and with the same body weight for the experiment. The weight was used as ranking criterion during group allocation. The weight range prior to initial experimental infection was 16.75-18.93 g (arithmetic mean 17.33 g). The groups were homogenous at time of inclusion with no significant ($P > 0.05$) difference in weight among groups.

2.2.2. Experimental mice infection and feces examination

The eggs of *H. nana* were collected by the sedimentation method of Astafyev et al. (1989). Each mouse was inoculated orally with 200 infective eggs of *H. nana* using a disposable 1 ml syringe connected to a special long needle of 25 mm. After infection, the feces were examined daily by the sedimentation method on the 5th day after inoculation in order to find eggs of *H. nana*. The prepatent period and intermittent shedding pattern of eggs were determined.

2.2.3. Monieziosis of sheep

Fifty six 6–8-month-old sheep of Stavropol breed weighing 28–36 kg, spontaneously infected with *Moniezia* spp. were used in the experiment in the farm Krasnyi pyut of Samarskaya region. Sheep were not grazed on pasture during the experiment, but were kept indoors and fed according to the norms and rations of feeding livestock (Kalashnikov et al., 2003). Water was provided *ad libitum* throughout the course of the study.

Fecal examinations were made by the McMaster technique (MAFF, 1986) for 2–3 days before the study for random distribution of sheep into the experimental groups with the same number of eggs per gram of feces and for the determination of the geometric mean of eggs of cestodes per gram of feces (Wood et al., 1995).

2.3. Materials

Niclosamide (2′,5-Dichloro-4′-nitrosalicylanilide), 99.9% and polyvinylpyrrolidone (PVP) powder, average $M_w \sim 55.000$ ((C6H9NO)x) were purchased from Sigma-Aldrich (UK). All other chemicals and solvents were of analytical reagent grade and were used as received.

2.4. Experimental groups

The mice were divided into 4 experimental groups and one control group of 10 animals each on the 13th day after infection. The SCoNwPVP were administered into the stomach of mice of the 1–3 experimental groups in a ratio of 1:10; 1:5 and 1:2 respectively in a