WASHOUT OF RADIOACTIVE CESIUM AND STRONTIUM BY ENTEROSGEL IN LABORATORY ANIMALS

Lysenko N.P., Volkov M.Yu.

Ministry of Agriculture of the Russian Federation,

Federal State-funded Educational Institution of higher professional education "Moscow State Academy of Veterinary Medicine and Biotechnology named after K.I. Skryabin", Moscow, Russia, Akademika Skryabina str., 23, 109472 Moscow, Russia, www.mgavm.ru

Nikolay Lysenko - +79036734176 (Doctor of biological sciences, Professor, Head of Department of Radiobiology and Biophysics named after A.D. Belov, FSI "MSAVMB named after K.I. Skryabin")

Mikhail Volkov - +79161457101 (Doctor of biological sciences, Professor, Department of Biotechnology, FSI "MSAVMB named after K.I. Skryabin")

ABSTRACT

Enterosgel® medical device, paste for oral administration (polymethylsiloxane polyhydrate), 225 g (manufacturer LLC "TNK Silma", Russia, series 730313, production date 03.2013, shelf life 03.2016) was selected an object of study.

The purpose of the study is to define the ability of Enterosgel® medical device, paste for oral administration for removing cesium and strontium radionuclides from the body.

Investigations were carried out in the Department of Radiobiology and Biophysics named after Academician of the RAAS, Professor A.D. Belov, FSI "MSAVMB named after K.I. Skryabin", Moscow, from June to September 2013.

Males of white nonlinear laboratory mice 21 g of weight, two months of age were selected as target objects.

Studies have established that Enterosgel® medical device, paste for oral administration, effectively outputs radiostrontium -90 from the body of laboratory animals in case of a single dosing, that is why it stands out in comparison with other known adsorbents. During the experimental period 65% of radiostrontium-90 was excreted from the organism of laboratory animals of the experimental group vs. 33 % in the control group.

Enterosgel® medical device, paste for oral administration, accelerates removal of radioactive cesium -137 from the body of laboratory animals. Effective period of biological elimination of radiocaesium -137 was 1.4 days, which is 5.4 times less than in the control group, where the effective period of biological removal was 7.5 days.

***Keywords**: Enterosgel, adsorbent, radiospectrometry, radiocaesium, radiostrontium, polymethylsiloxane polyhydrate.*

INTRODUCTION

Over the past 70 years, radiation background of the Earth has increased by 50 times. Nuclear testing, in-plant accidents, work in disadvantaged areas, high level of radiation and just radiological examinations in hospitals - all this leads to the fact that the radionuclides (strontium, cesium and other harmful elements) are accumulated in the human organism.

Excretion of radionuclides from the body and removal of radioactive contamination is one of the greatest challenges of the present days.

Radioactive substances concentrated in the soft tissues and internal organs (cesium, molybdenum, ruthenium, iodine and tellurium) are rapidly cleared from the body, unlike those firmly fixed in the bones (strontium, plutonium, barium, yttrium, zirconium, niobium, lanthanides). Of a large number of radionuclides, the most important source of irradiation is strontium-90 and cesium-137.

Despite the fact that after the Chernobyl disaster more than 25 years have passed, ^{137}Cs and ^{90}Sr are continuously identified in animal products due to the global fallout as well as radionuclide migration of groundwater from deep to the upper soil layers. Cesium-137 (half-life 30 years) is by far the main dose forming radionuclide.

One of the most urgent problems of modern medicine is to find effective methods of prevention and pathogenic treatment of radiation injuries. Acuteness of the problem is determined by an expanding contingent of persons exposed to ionizing radiation as well as virtual absence of effective anti-radiation drugs.

Medical device Enterosgel®, paste for oral administration (polymethylsiloxane polyhydrate), can be one of these drugs.

In this regard, the **purpose** of the research was to study Medical device Enterosgel®, paste for oral administration (polymethylsiloxane polyhydrate) for removing cesium and strontium radionuclides from the body.

Research objectives:

To determine effectiveness of using Enterosgel® medical device, paste for oral administration as an adsorbent for removing radioactive cesium-137 and radiostrontium-90 from the bodies of laboratory mice;

To determine excretion percentage of radiocesium-137 and radiostrontium-90 using Enterosgel® medical device, paste for oral administration, and without the drug from the body of laboratory mice.

MATERIALS AND METHODS

Studies on evaluation of the effectiveness of Enterosgel® as a radiosorbent, made at the Department of Radiobiology and Biophysics named after Academician of the RAAS, Professor A.D. Belov, FSI “MSAVMB named after K.I. Skryabin”.

Object of research

Enterosgel® medical device, paste for oral administration (polymethylsiloxane polyhydrate), 225 g (LLC "TNK Silma”), series 730,313, manufacturing date 03.2013, shelf life till 03.2016.

Materials and equipment

Radiospectrometry complex "Progress- 320 " (Russia) consisting of the following devices: gamma-spectrometer with solid scintillation detector based on NaI, 63 x 63mm; registered energy range 0.03 - 3.0 MeV; fundamental measurement error - no more than 30% ; standard exposure time of countable sample 1800 s. Beta-spectrometer with solid scintillation detector 70x8 mm; mass of the sample in a standard ditch - 1-15 g; standard exposure time of countable sample 1800 s.

137CsCl working solution with an activity of 200 Bq.

90SrCl2 working solution with an activity of 730 Bq.

Animals:

Animal species - white nonlinear laboratory mice;

Gender - males;

Age - 2 months;

Weight - 21 g;

Vivarium of FSI “MSAVMB named after K.I. Skryabin”.

Adaptation and selection of the animals for research

Duration of the adaptation period for all animals was 19 days. During the adaptation period, clinical condition of the animals was controlled by visual inspection every day.

Animals with deviations detected during the inspection were not included in the experimental group.

Before the study began, the animals meeting criteria for inclusion in the experiment were divided into groups.

Grouping

Animals without characteristic deviations of appearance were selected for the experimental group, so that the individual mass value would not deviate from the average by more than 10%.

Animals were divided into 4 groups (see table 1).

Animal identification

Cage marking encoded protocol number of bioethical committee, animal gender, breed, date of the beginning and the end of the experiment, experimental group number, name of study director and laboratory assistant. Each animal in the group was labeled by applying a mark on the body. A unique number was assigned to each animal selected for the study.

Animal management

The animals were kept under standard conditions in the vivarium of the department of radiobiology of FSI “MSAVMB named after K.I. Skryabin” in accordance with *Guidelines for the Care and Use of Laboratory Animals*, National Academy press. -Washington, D.C. 1996 All Union State Standard R 53434-2009, with the rules approved by the USSR Ministry of Health 07/06/73, on the device, equipment and maintenance of experimental and biological clinics (vivaria) .

Cages

The animals were housed in polycarbonate cages of *Techiplast* company (Italy) with steel grating cover with feed hollow. Five animals were put into each cage, cage floor area per animal was 300 cm^2.

Bedding

As a bedding wooden pellets were used (LLC " ZooSPb ", St. Petersburg, Russia) .

Diet

Feeding of the animals was carried out on the basis of "Sanitary rules for maintenance of experimental biological clinics (vivaria) " approved on 06/07/73 , by USSR Ministry of Health and the order #755 of USSR Ministry of Health from 08/12/77.

" Diet for lab animals ", QF-120- 1 , prepared according to GOST 50258-92 , in accordance with the rules approved by the order of USSR Ministry of Health # 755 from 08/12/77, was provided *ad libitum* in the feed hollow of the cage steel grating. Veterinary certificate #247 0187041 from 3.10.12 , certificate # ROSRU.PR98.N00093/0051289 valid from 17.05.11 to 05.16.14 .

Water

Animals were given purified water according to SOP – OG H 3, with normalized organoleptic properties in terms of pH, solids, reducing substances, carbon dioxide, nitrates and nitrites, ammonia, chloride, sulfate, calcium, heavy metals according to SOP AB -38, on the basis of GOST 51232-98 "Drinking Water . General requirements for organization and quality control methods."

Water was given in standard drinking bowls with steel spout-type caps, *ad libitum*.

Environmental conditions

Animals were kept under controlled environmental conditions (18-22 ° C and relative humidity 50-70%). The light regime was 12 hours of light and 12 hours dark. The set ventilation

regime provided about 15 airspaces per hour, CO_2 concentration was not more than 0.1 % of volume, ammonia - not more than 0.001 mg/m^3.

Temperature and humidity were recorded daily. No significant deviation of these parameters happened in the acclimatization period and during the experiment.

Contents and the research met the requirements of the European Convention for Protection of Vertebrate Animals used for Experimental and other Scientific Purposes from March 18, 1986 (The text was modified in accordance with the provisions of the Protocol (ETS № 170), effective date December 2, 2005) . Manipulations with experimental animals were performed in accordance with provisions of the Helsinki Declaration on humane treatment of animals, guidelines on their removal from the experiment and euthanasia (Guiding principles for research, 2002).

Method of administration

Method of drug administration in mice was oral administration with the diet corresponding to the planned oral use in the clinical practice.

Study design

The experimental schedule is shown in Table 1.

Administration procedure

Oral administration of the prepared objects was performed daily, strictly maintaining the volume administered in accordance with the administration volume checklists. Doses of Enterosgel®, paste for oral administration were prepared by weighing, selecting the weighed quality, focusing on previous experiments with similar drugs.

Clinical observations

During the adjustment period, daily inspection of animals in the cages was carried out to identify morbidity and mortality.

In the experimental period inspection of animal cages was conducted to detect deviations of animal behavior and mortality.

Preparing radiolabeled diet

Crackers 1 g of weight were prepared.

Each cracker was provided with particular label added activity in amount of 1 ml $^{137}CsCl$ with activity 200 Bq and $^{90}SrCl_2$ with activity 730 Bq per 1 g of food.

Ready crackers were dried in the power condition, and each group of mice was fed once.

Cooking the diet with the drug

Crackers 1 g of weight were prepared and then sprayed with water and rolled in 250 mg of of Enterosgel®, paste for oral administration. Ready crackers with study drug were dried, and the animals were fed to in accordance with the objectives of the study at doses one cracker for each mouse once a day.

The research procedure

Feeding with the study drug was carried out under the scheme (see Table 1).

Upon completion of the experiment all the animal groups were euthanized in a specific time interval . Mice were dissected, and bones, muscles and internal organs were separated from the carcass. The parts of the carcasses were ashed.

Dry ashing mineralization is based on the complete decomposition of organic materials by heat treatment of the sample at a controlled temperature regime and consists of three successive stages - drying, charring and ashing. At each step the degree of concentration of the radionuclides increases.

The meat samples were separated from the fat, tendons and bones and then dried to constant weight in the oven at a temperature of 800-1000°C.

Bones and soft tissues were separated and dried in the oven at a temperature of 1000-1500° C for 2-3 hours.

After the establishment of permanent sample mass of the dry residue it was charred by roasting on a hotplate. Charring process is considered complete at the termination of the sample

swelling and disappearance of smoke. Dry charred remains were ashed in the muffle furnace at a temperature of 4000°C.

The main advantages of thermal concentration of sample activity by dry mineralization is their versatility and relative simplicity of the procedures. Typical drawbacks include their long duration, energy consumption and foul odors that accompany charring and ashing.

The resulting ash was placed in the special aluminum pans, mixed with ethanol and measured by radiometric spectrometer with software "Progress-320 ."

For beta and gamma - radiation from counting samples gamma and beta - spectrometric complex paths "Progress -320 " with scintillation detector blocks were used. For protection from external radiation the blocks are arranged in a special lead screen.

Calculation of the percentage of radiocesium and radiostrontium excretion from the body of the mice was carried out using a formula:

$$X = 100 - \frac{A}{A_0} \cdot 100 ,$$

where:

A - activity of one mouse (Bq) on the last day of the experiment;

A_0 - activity of one mouse (Bq) immediately after fusing

Calculation of the biological half-life:

$$T_{eff} = \frac{T_{phys} \cdot T_{bio}}{T_{phys} + T_{bio}}$$

where:

T_{eff} - effective half-life;

T_{phys} - physical half-life of radiocesium (30 years);

T_{bio} - biological half-life of radiocesium in the body.

Statistical analysis of the results

Statistical analysis was carried out work on the computer using EXCEL program.

RESULTS AND DISCUSSION

Dynamics of accumulation and elimination of radiocaesium and radiostrontium from the animal organism when using Enterosgel®, paste for oral administration.

The results are presented in Tables 2-12.

The studies have shown that on the first day after a single oral administration of radioactive cesium-137 with food specific activity of 200 Bq / g the highest concentration of radioactive cesium-137 was in the gastrointestinal tract (hereafter GIT), with specific activity of 5.45 Bq / g, which is 2 , 7% of the administered specific activity. Specific activity of radioactive cesium-137 in the muscles was 20 Bq / g on the first day, which was 10 % of the administered specific activity. Given that muscle mass is significantly more than GIT weight, a larger quantity of radioactive cesium-137 was concentrated in this tissue.

In the kidneys of control animals specific activity of radioactive cesium-137 was 1.3 Bq / g on the first day, which is 0.65 % of the administered specific activity; toward the end of the experiment specific activity of radioactive cesium -137 in the kidneys was equal to 9.3 Bq / g, which was 4.6 % of the administered activity.

In the kidneys of the experimental group on the first day specific activity of radioactive cesium -137 was 1.3 Bq / g, which was 0.6 % of the administered specific activity, at 14 days of the specific activity of cesium -137 in the kidneys was 15 Bq / g, which amounted to 7.5 % of the administered activity.

Studies have shown that in the experimental group # 3 there was an inversely proportional relationship between the level of radioactive cesium -137 in the muscles and kidneys, through

which radiocesium -137 was mainly excreted from the body. The less was radiocesium concentration in the muscles, the more it became in the kidneys during the study period (Table 2, 5). The effect of reducing the concentration of radioactive cesium-137 was also observed in the gastrointestinal tract of animals in the experimental group during the study period (Table 3) .

The declining trend in the concentration of radioactive cesium-137 was also observed for the other investigated organs (liver, heart) when using Enterosgel®, paste for oral administration (Table 4, 6).

Dynamics of accumulation and excretion of radiostrontium from the body of the animals when using Enterosgel®, paste for oral administration.

The results are presented in Tables 7-12.

It was found that the levels of radiostrontium absorption from the gastrointestinal tract ranges from 5 to 100 %. Radiostrontium soluble compounds are well absorbed from the gastrointestinal tract. The magnitude of radionuclide absorption from the gastrointestinal tract decreases with increasing age, with an increase in calcium and phosphorus content in the diet, with high doses of thyroxine.

According to the studies, on the first day after a single oral administration of radiostrontium-90 with food ,specific activity 730 Bq / g, the highest concentration of radiostrontium-90 was observed in the gastrointestinal tract, specific activity 60 Bq / g, which is 30% of the administered specific activity. Specific activity Radiostrontium – 90 in the bones was 30 Bq / g on the first day, corresponding to 15% of the administered specific activity. Given that the bone mass is much smaller than the mass of gastrointestinal tract, the total amount of radiostrontium-90 was in the gastrointestinal tract in the time of the isotope administration in the body. For a long time , the basic amount will be concentrated in the bones due to the slow exchange of radiostrontium in the bone tissue.

In the group # 4 radiostrontium-90 absorption blockade from the gastrointestinal tract was observed, in contrast to the group number 2 (control). 1 day after administration of Enterosgel®,

paste for oral administration, specific activity decreased to 6 Bq / g, and 0.8% of the administered specific activity (Table 8). In the kidneys of mice of the experimental group there was an increase in the concentration of radiostrontium -90 by the end of the experiment, the specific activity to 14 days was 55 Bq / g , which corresponded to 25 % of the administered activity, and proved that the removal of radiostrontium-90 from the animal organism was more intensive compared with the control group (Table 10). In this context, the total concentration of radiostrontium was reduced, which provided an overall positive effect by reducing the radiation load to the body when using Enterosgel®, paste for oral administration (Table 7 , 9, 11). .

Unlike radioactive cesium-137, radiostrontium -90 is worse excreted both in a natural way and using radiosorbentos. The main place of deposit of radiostrontium-90 is bone tissue. While using Enterosgel®, paste for oral administration as an enterosorbent of radiostrontium-90 in the body, the value of the specific activity of bone in the experiment group # 4 was 2 times lower by the end of the experiment than the value of specific activity of bone tissue in the animals of the control group # 2 (Table 12).

Study of ability of Enterosgel®, paste for oral administration, to descrete long-lived radionuclides from the mice.

The main parameters of the drug inflence on the rate of excretion of radioactive cesium from the body of mice are biological half-life and effective half-life of a radionuclide from the body of the test animals. The faster the product reduces twice radioisotope levels in the body (i.e. the shorter is biological half -life) and the less is the effective exposure period, the higher is the efficacy.

Biological half-life (T1) is the time during which the radionuclide activity in the body is reduced by 2 times by its biological excretion. It was determined as follows: separately for each group of animals, the time at which specific activity of ^{137}Cs decreased, was determined compared with the initial two times and calculates the number of days that have passed to obtain this value.

Second half-life (T2) is the time during which the radiocesium output is twice larger, i.e. 4 times compared with the initial activity. Then the effective half-life was calculated taking into account physical half-life of the radioisotope - the time during which the activity of the radionuclide is reduced by half due to the natural decay. T_{phys} of ^{137}Cs is 30 years.

Effective half-life period of radioactive cesium-137 in the body when using Enterosgel®, paste for oral administration, was 5.4 times less than the period of effective half-life of radioactive cesium-137 without it and was equal to 1.4 days. The experiment was carried out to 70% removal of radionuclides from the body of mice in the experimental and the control group (Table 13).

It was established that the percentage of radiostrontium -90 excretion from the body of patients receiving Enterosgel®, paste for oral administration, was higher compared with the control group and was 65% , which is 1.9 times greater than excretion percent in the control group without administration of Enterosgel®. Effective half-life of radiostrontium-90 in the bones is very long and was not included in the definition of experiment tasks.

CONCLUSIONS

Enterosgel®, paste for oral administration, effectively outputs radiostrontium-90 in laboratory animals (mice) after a single dose that distinguishes it among many well-known sorbents. During the period of the experiment, excretion in the experimental group of animals was 65 %, while in the control group it was 33%.

Enterosgel®, paste for oral administration accelerates removal of radioactive cesium-137 from the body of laboratory animals. Effective period of biological elimination of radiocaesium - 137 was 1.4 days, which is 5.4 times less than in the control group, where the effective period of the biological removal was 7.5 days.

These experiments allow widespread use Enterosgel®, paste for oral administration as a radioprotector and means for effective removal of long-lived radionuclides from humans and animals .

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