BioGecko

Vol 12 Issue 03 2023

ISSN NO: 2230-5807

DETERMINATION OF THE LOCAL IRRITIVE EFFECT OF ANTIBACTERIAL POLYMER BIOCARRIERON LABORATORY ANIMALS

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Abstract: The purpose of the study: to evaluate the toxicity and local irritant action of an antibacterial biocarrier with silver nanoparticles based on an acrylic polymer.
Methods:The antibacterial biocarrier is a volatile liquid that leaves an elastic transparent film on the skin. The study included an experimental assessment of acute toxicity according to the Noakes and Sanderson method, an assessment of skin resorptive action, a single local irritant effect, multiple local irritant effects on the skin and cumulative properties, as well as a local irritant effect.
The results of experiments on white rats showed that no signs of intoxication were detected during the 3-week observation period. Animals retained activity, appetite and adequate response to external stimuli. Thus, it has been proven that the studied agent does not have a toxic and skin-resorptive effect. There were no statistically significant differences in the state of the "mink reflex" between experimental and control animals. The data obtained indicate the absence of a local irritating effect in the studied medical device.
The antibacterial biocarrier based on acrylic polymer is a low-toxic substance that does not have a skin-resorptive, locally irritating effect on the skin, does not have a cumulative effect during repeated chronic exposure to the skin, and does not irritate the skin.

Keywords: acrylic polymer, biocarrier, toxicity, skin irritation.

Introduction. Products with a therapeutic and prophylactic effect are steadily gaining ground in one of the leading positions in the pharmaceutical market. In recent years, there has been an increasing demand for products containing components of natural origin, such as emollients, film formers, thickeners, preservatives, and dyes [1,2]. At the same time, products are required to have such properties as an instant effect in the form of softening, moisturizing, imparting a certain color, tone and masking skin imperfections, but they must also have an attractive appearance and contain substances that have other properties. such as antioxidant activity, wrinkle reduction and stimulation of collagen synthesis [3,4,5].
Polymeric materials are used in the composition of many therapeutic and prophylactic agents to improve their quality characteristics. Polymers function as gelling agents, stabilizers, emulsifiers, film formers and wetting agents. They are also often used as adjuvants to accelerate the precipitation and fixation of other active substances and ingredients and/or to facilitate these processes. In this case, polymers must have a number of properties, one of the most important of which is the ability to form a film during application. [6, 7, 8].
In this case, it is necessary to develop the specifics of the interaction of polymers with human skin and a possible side reaction. Thus, there is currently a need to develop optimal polymers and/or optimal combinations with the most complete set of necessary properties, which, among other things, can be used to create innovative cosmetics [9, 10].
MATERIALS AND METHODS Experimental studies were carried out at the Scientific Center for Standardization of Medicines of the Republic of Uzbekistan and at the National University of Uzbekistan named after MirzoUlugbek.
10 white mice of both sexes weighing 19-22 g were chosen as the object of the study; 40 rats of both sexes weighing from 140 to 200 g; 10 guinea pigs of both sexes weighing from 250 to 350 g; 10 rabbits of both sexes weighing from 2.0 to 2.5 kg according to the requirements of the "Guidelines for the experimental (preclinical) study of new pharmacological substances" (Khabriev R.U.). The formation of groups of animals

receiving different doses of the test drug of comparison, carried out by random sampling (sample unit - animal).
The LD50 value is calculated based on the results of observations after 30 minutes, 24 hours and 7 days and is expressed in units of mass per 1 kg of animal weight. The body weight of the animals selected for the study differed from the average body weight by no more than 20% (±10%).
The number of animals was sufficient for complete statistically significant registration of the studied effects and was minimally rational from the point of view of ethical principles.
The formation of experimental and control groups was carried out by the method of uniform distribution of animals with equivalent clinical manifestations into groups.
Chronic toxicity was investigated at two to three doses. When choosing the maximum dose based on the results of acute toxicity studies, the minimum dose should be close to the intended therapeutic dose recommended for the clinical study.
Acute toxicity of the drug was determined by the method of Noakes and Sanderson on 6 white rats weighing 180-200 g [1].
The day before the experimental studies, wool was cut off on the skin of the back in an area measuring 7.5x4 cm. The test drug was applied to the sheared area of the skin of rats with the concentration of the active substance 0.2 g/kg, 0.4 g/kg, 0.6 g/kg, 0.8 g/kg and 1.0 g/kg of animal body weight. During the first day of the experiment, the animals were observed every hour. Then, daily for 2 weeks, the animals of both groups were observed for the general condition and activity, taking into account behavioral reactions.
Acute toxicity was assessed by changes in body weight and neurosomatic parameters (general condition of the animal, behavioral characteristics, intensity and nature of motor activity, the presence and nature of seizures, coordination of movements, response to tactile, pain, sound and light reactions). irritants, frequency and depth of respiratory movements), condition of hair and skin.
The assessment of acute toxicity also included the study of skin-resorptive and local irritant effects with single and repeated exposure, as well as determining the degree of cumulative properties of the drug.
The general irritant effect was studied when applying a preparation containing the active substance at a concentration of 0.2 g/kg, 0.4 g/kg, 0.6 mg/kg, 0.8 g/kg and 1.0 g/kg of animal body weight, on intact skin of animals. experimental animals (albino rabbits weighing 2.5-2.7 kg), previously quarantined. A day before experimental studies, hair was cut out on the skin of the thoracic back in a 10x12 cm area on both sides of the spine. The drug was sprayed onto a clipped skin area on the lateral surface of a rabbit. The exposure time was 4 hours. After acute (single) exposure to the test product, skin condition was assessed every 2 hours on the first day and every 6 hours on the second day according to the skin reaction classification system [2].
Determination of a single local irritant action was carried out on 140-160 g weighing 6 white rats. The drug was applied to a clipped skin area 2x2 cm in size. Animals were fixed for 4 hours. Skin reaction was recorded at the end of exposure, 1 and 16 hours after application.
The determination of multiple local irritating effects on the skin and the study of cumulative properties were carried out on 140-160 g weighing 8 female white rats 1 time per day for 1 month. The remaining 8 white rats served as controls. The criteria for toxicity were: animal behavior, survival, time of death, the appearance of symptoms of intoxication, local changes on the skin, the dynamics of changes in body weight, "mink reflex".
The study of skin-resorptive action was carried out on 6 white rats weighing 140-160 g according to the standard method. The tails of the animals were immersed in test tubes with the test solution of the biocarrier for 2/3 of the length of the tail. The test tubes were placed in a water bath at a temperature of 28–30°C. The exposure time was 4 hours. After the end of the experiment, the skin of the tails was washed with warm water and soap to remove the substance. The animals were observed for 3 weeks.
Determination of anaphylactogenic activity was carried out on 230-250 g weighing 10 guinea pigs (5 experimental and 5 control).
Experimental studies were carried out in accordance with generally accepted principles of humanity based on existing international rules and regulations for working with laboratory animals.

Used equipment: electronic universal desktop scales MK-3.2-A20; single-channel dispenser 100-1000 µl, Thermo Scientific; single-channel dispenser 20-200 µl, Thermo Scientific; Laminar bacteriological; CO2 - incubator.

RESULTS AND DISCUSSION The results of studies on the determination of acute toxicity in white rats showed that during the observation period (3 weeks) signs of intoxication in the studied animals and their death were not detected. Animals retained activity, appetite and adequate response to external stimuli. ThesestudiesarepresentedinFigure 1.

	iocarrier volume, ml	oncentration of active ingredients, grams
		26
		53
		798
		06
	)	33
	2	59
	5	99
	)	66

Fig. 1 The concentration of the drug in determining acute toxicity.

Table 1 shows that after a single application to the skin of a liquid biocarrier at concentrations of 2 ml -20 ml, we did not observe visible changes in the behavior and functional state of the animals of the experimental group. All rats were active,there were no signs of intoxication. The dynamic increase in the concentration of the biocarrier did not have a negative effect and did not cause side effects. The rats responded adequately to tactile, sound, and light stimuli. Macroscopic changes in the skin and pathological changes in the hairline of animals are not observed. There were no deaths of rats within 2 weeks. An increase in the concentration of active substances from 0.5 to 2 Gy showed that the use of a biocarrier with a maximum concentration of 2 g adversely affected the skin, causing changes in the skin of the animal. At the next stage of research, we studied the body weight of the studied animals.

The data obtained are presented in table 2.

When assessing the irritating effect and studying the cumulative properties, the general condition of the experimental animals was not disturbed, signs of intoxication were not detected, and there was no death of the animals. No local changes were found on the skin, alopecia areata and ulcers were not noted. The animals were neat, the coat was smooth and shiny, they willingly ate food, were active, and adequately responded to external stimuli. The dynamics of body weight during repeated exposure of the biocarrier to the skin of white rats is presented in Table. 2.

Table 2 .Body weight (in g) in white rats after repeated exposure biocarrier to the skin (M±m)

xamination time, weeks	ain group, gr	ontrol group, gr
	30±2,1	31±1,9
	36±1,5	39±1,7

Note: * - differences compared to the control group are statistically significant (P>0.05);

The results of the studies showed that the weight gain of the experimental animals did not differ from the control ones.

Animal observations at the stage of studying the irritating effect of the drug showed that there were no visible changes on the skin of the albino rabbit of the experimental group 1, 24 and 72 hours after the drug was used. The state of the "mink reflex" in experimental and control animals is presented in Table. 3.

Table 3
The state of the "mink reflex" (the number of animals in the object per unit time) in white rats after repeated skin exposure
(within 4 min.), (M±m)

xamination time, weeks	ain group, gr	ontrol group, gr
	±0,43	±0,28
	±0,72	±0,86
	±1,01	±028
	±0,28	±1,16

Note: * - differences compared to the control group are statistically significant (P>0.05);
The frequency of preference was assessed visually, i.e., the NOR indicator was the number of animals in the object per unit time. Time was recorded using a stopwatch. There were no statistically significant differences in the state of the "mink reflex" between experimental and control animals.
According to the classification of skin reactions according to GOST ISO 10993.10-2011, the maximum score in animals of the experimental group was 0, i.e. the data obtained indicate the absence of a local irritating effect in the studied biocarrier.
According to the results of studies to determine the anaphylactogenic activity of the biocarrier, no reaction to the drug was observed in any animal. During the experiment, it was found that the biocarrier at a concentration of 1.33 g/kg in 10 ml does not have anaphylactogenic activity.
CONCLUSION. Thus, based on the toxicological and physiological studies of the biocarrier, it can be concluded that the biocarrier with active ingredients based on an acrylic polymer with silver nanoparticles is a low-toxic substance that does not have a skin-resorptive, locally irritating effect on the skin, with repeated chronic exposure to the skin it does not has a cumulative effect, does not irritate the skin and does not cause an anaphylactogenic reaction.

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