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STUDY OF THE PHARMACOLOGY OF SAFETY AND TOXICITY OF THE GEL "XELIOGEL" IN THE EXPERIMENT

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Abstract

Given the progressive urgency of the problem of burns, the search for new drugs is obvious. In Ukraine, in this regard, a method of treating burns with the use of xenografts has been proposed, the introduction of which into clinical practice in Ukraine as a skin substitute has reduced the mortality of patients with severe burns by 30%. An integral part of drug development is a toxicity study that reflects the relationship between dose and effect. The experiment was performed on white Wistar rats weighing 250-260 g. The acute toxicity of the developed gel was studied in accordance with the recommendations of the State Expert Center of the Ministry of Health of Ukraine. Therefore, to establish the non-toxicity of the developed gel "Xeliogel" it was evenly applied at a dose of 2810 mg/kg. The gel was applied to a pre-shaved area of the skin back, which was at least 10% of the total surface area of the animal. The dose of the drug was applied in 3 doses with an interval of 2 hours. Observations of animals were performed twice a day (morning and evening) for 14 days. A study of the pharmacology of the safety of "Xeliogel" gel showed that the functional indicators of potential toxicity are absent in the animals of the study group and are identical to intact animals. After completion of the observation, no massometric or visual changes were detected. The test gel is classified as class V toxicity of compounds (Practically Non-Toxic).

Keywords: *acute toxicity, gel, xenoderma, burn, wounds*

Introduction

Burns is one of the most common types of injuries. According to the WHO, the world annually registers 180 thousand deaths from this type of injury. The proportion of burns ranges from 5.6 % to 10 %, ranking third places among all types of injuries in peacetime [1-3]. The use of semisolid water-soluble medicines for the treatment of burns and wounds in all phases of the wound process is relevant and quite popular in recent years [4-6].

In Ukraine, a method of treating burns with the use of xenotransplantant, the introduction of which into clinical practice in Ukraine as a substitute for the skin, has reduced the mortality of patients with severe burns by 30 % (patent of Ukraine 65673 A, 2004). Cryolyophilized xenoderm is a material of natural origin, which contains a large number of amino acids that improve regeneration processes [7-9]. Xenoderma is developed by a special lyophilization technology (patent of Ukraine 55636 A, 2003).

On the basis of cryolyophilized xeroderma`s, which is intended for local closure of burn wounds, a gel was developed under the conditional name "Xeliogel" [10], (as an active pharmaceutical ingredient gel contains 16 amino acids, of which 9 are essential [11], and lidocaine hydrochloride) (patent of Ukraine 138600, 2019).

Before starting the study of specific pharmacological action according to the guidelines of the State Export Center of the Ministry of Health of Ukraine "Preclinical safety evaluation of biotechnology-derived pharmaceuticals" it is recommended to study the pharmacology of safety [12]. Namely, the developed gel based on a water extract from lyophilized xenoderma powder and one of the active pharmaceutical ingredient is lidocaine hydrochloride [13]. Therefore, it is important at this stage to establish the possibility of unexpected toxic effects, and if necessary, to carry out detailed monitoring of such effects during toxicological studies [14, 15]. The study of safety pharmacology involves the determination of functional indicators of potential toxicity, which contribute to the establishment of mechanisms of specific organ toxicity of the test gel and should be taken into account with respect to human use or indications for use [16, 17].

Determining the safety of the developed gel "Xeliogel" is an important stage of preclinical studies, which included the study of general toxic properties and macroscopic studies [17].

With this in mind, it was decided to initiate a single-dose toxicity study, which provides information on the relationship between dose and systemic and/or local toxicity.

The aim of this work was to confirm the safety pharmacology and the absence of toxic effects when using the developed gel "Xeliogel".

Methods

The experiments was performed on white Wistar rats weighing 250-260 g. Animals were kept on a standard I.Horbachevsky Ternopil National Medical University vivarium diet, kept under standard conditions at room temperature in isolated cages with a 12-hour day/night, with access to water and food ad libitum. The studies were carried out in accordance with national and international recommendations for the protection of animals used for experimental and other scientific purposes (Strasbourg, 1986; Law of Ukraine № 3447-IV, 2006). In accordance with the requirements of the Bioethics Commission of I.Horbachevsky Ternopil National Medical University (Protocol, No. 56 of January 8, 2020).

Acute toxicity

The acute toxicity of the developed gel was studied in accordance with the recommendations of the State Expert Center of the Ministry of Health of Ukraine. The experiment was performed on mature white rats of Wistar females, which are more sensitive to toxic effects than males, 6 individuals per group. The animals were divided randomly, equally. The Pharmacological Committee of the Ministry of Health of Ukraine and according to the classification of Hodge NS and Sterner LH recommends using the maximum dose of toxicity class IV according to the route of administration (external use) for the study of acute toxicity [19, 20]. Dermal DL50 of drugs in rats regarding the toxicity class IV is reported to be 350-2810 mg/kg in rats. Therefore, to establish the non-toxicity of the developed gel "Xeliogel" it was evenly applied at a dose of 2810 mg/kg [15]. So, if it is found that the

studied gel is non-toxic at a dose of 2810 mg/kg by cutaneous application, further studies can be considered inappropriate. The application of the developed gel was chosen in accordance with the intended use of the gel in clinical practice. The gel was applied to a pre-shaved area of the skin back, which was at least 10% of the total surface area of the animal. The dose of gel was applied in three doses with an interval of two hours. Animals of the negative control group were appropriately applied a gel base in an equivalent volume.

Observations of animals were performed twice a day (morning and evening) for 14 days. Clinical observations included control of motor activity, skin condition, changes in respiration, food and water intake, changes in weight, and any changes in animal behavior. To control body weight was performed individual weighing for 0, 3, 7 and 14 days.

At the end of the observation period, namely 14 days, the animals were euthanized. Subsequently, biochemical studies of the blood of rats were performed, as well as macroscopic examination of their internal organs.

Statistical analysis

The obtained results were subjected to statistical analysis by methods of variation statistics. For all studies, the arithmetic means, as well as the standard error, were calculated. For statistical analysis of the obtained results, a one-way analysis of variance ANOVA was performed using the Student's t-test [20]. Statistical analyzes were performed using GraphPad Prism, version 5.0 (GraphPad Software, Inc.). To assess the probability of the obtained results, the significance level $p \leq 0.05$ was taken.

Results and Discussion

Before the start of all biochemical and histological studies, the acute toxicity of the studied gel "Xeliogel" was determined to ensure its safety.

In the study of acute toxicity of drugs, an integral indicator is the survival/mortality of animals, which allows you to calculate the average lethal dose (LD50) of the drug.

No signs of clinical intoxication or death were observed during the observation of the animals' behavior, appearance, coat condition, activity, and appetite. Reflex excitability, urination, defecation are within the physiological norm. All animals showed weight gain without significant differences between groups (Table 1). No visible pathological changes in the appearance and behavior of experimental animals on the 1st, 7th and 14th days after the start of application of the developed tools were registered.

At the end of the observation period, namely 14 days, the animals were euthanized. During the experiment, there was also no difference in the values of the relative mass of animal organs, the results of the studies are shown in table 2.

All animals had a neat thick coat of fur, no external changes were recorded, and the external mucous organs were unchanged. All internal organs are anatomically correct. The surface of the organs is smooth, shape, color and size are characteristic and unchanged. The liver is represented by lobes of normal size. The spleen is full-blooded, elastic, normal size. The kidneys of all animals are symmetrical with a characteristic structure. The lungs are airy, the leaves of the pleura are unchanged. Thymus without changes. The ovaries are normal. The data of macroscopic observations are confirmed by histological studies, which indicate the preservation of the microstructure of the organs without signs of pathological changes.

According to the results of acute toxicity studies of the developed gel "Xeliogel" at a dose of 2810 mg/kg in the internal organs, no signs of pathological changes or inflammatory reactions were detected. Therefore, according to the parameters of acute toxicity, the developed gel belongs to the IV class of toxicity (Slightly Toxic) by the recommendations of the State Export Center of the Ministry of Health of Ukraine. Thus, the LD50 of studied gel "Xeliogel" is beyond the limits of the IV toxicity class ($LD50 \geq 2810$ mg/kg), which allows them to be classified as Practically Non-Toxic (V toxicity class).

Conclusions

According to the results of the experiment to determine the safety and acute toxicity of the animals during the observation period behaved identically intact. After completion of the observation, no massometric or visual changes were

detected. According to the parameters of acute toxicity, the developed gel "Xeliogel" can be attributed to the V class of Practically Non-Toxic (LD50≥ 2810 mg/kg).

Conflict of interest

The authors declare that there are no conflicts of interest.

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Table 1. Dynamics of body weight of animals in the study of acute toxicity of the gel "Xeliogel" (M±m)

Group of animals	White rats	Weight of animals (M ± m), g			
		beginning of the experiment	3 rd day	7 th day	14 th day
Control group	Female	247.17±0.70	249.00±0.68	254.17±0.65	260.0±0.37
"Xeliogel" (2810 mg/kg)	Female	249.17±1.35	251.83±1.35	256.0±1.39	262.17±1.25 $p_1 \geq 0.05$

Table 2. Mass coefficients of the internal organs of female rats in the study of acute toxicity of the gel "Xeliogel" (M±m)

Internal organs		Group of animals (M ± m), g	
		Control group	"Xeliogel"
White rats-(female)			
Liver		2.99±0.03	3.02±0.04 $p_1 \geq 0.05$
Kidneys	Right	0.34±0.01	0.33±0.01 $p_1 \geq 0.05$
	Left	0.33±0.01	0.32±0.01 $p_1 \geq 0.05$
Heart		0.41±0.01	0.42±0.01 $p_1 \geq 0.05$
Lungs		0.77±0.02	0.78±0.01 $p_1 \geq 0.05$
Spleen		0.46±0.02	0.47±0.01 $p_1 \geq 0.05$
Adrenal glands		0.041±0.001	0.039±0.001 $p_1 \geq 0.05$
Thymus		0.15±0.009	0.17±0.01 $p_1 \geq 0.05$