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Short Communication

Acute toxicity study of a new complex drug with anti-inflammatory activity

I. V. Nizhenkovska[1] and L. V. Zinchenko[2,*]

[1]Professor, Head at Department of Pharmaceutical, Biological and Toxicological Chemistry, Bogomolets National Medical University; [2]Assistant, Department of Pharmaceutical, Biological and Toxicological Chemistry, Bogomolets National Medical University, Ukraine.

ABSTRACT

Acute toxicity of a new complex anti-inflammatory agent, which is administered in the form of a suppository for the treatment of vaginosis of different etiologies, was studied. The absence of toxic properties in the studied drug with both intravenous and intravaginal administration was established.

KEYWORDS: suppositories, vaginosis, anti-inflammatory drugs.

INTRODUCTION

Female genital infectious inflammatory diseases caused by various sexually transmitted pathogens or by non-specific microflora are a serious medical and social problem in obstetrics and gynecology [1, 2].

Successful treatment of bacterial vaginosis, as any other disease, depends on the correct and timely diagnosis and pathogenetically appropriate therapy. The purpose of treatment of bacterial vaginosis is to restore the normal microflora of vagina, to stop inflammation and to delay the growth of microorganisms not peculiar to this microcenosis [1].

Metronidazole and Clotrimazole are widely used for the treatment of infectious diseases of genitals [3].

A new alternative and effective drug for intravaginal use was created in an early study based on our previous research conducted at the Department of Pharmaceutical Technology and Biopharmacy Department, Shupyk National Medical Academy of Postgraduate Education, Ukraine under the direction of Professor L. L. Davtyan for the treatment of female genital diseases, the effectiveness of which is the result of three components: Ibuprofen, Metronidazole and Clotrimazole [4].

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) from the group of propionic acid derivatives that has painkilling, antipyretic and anti-inflammatory effects.

Metronidazole is active against protozoa such as *Entamoeba histolytica*, *Giardia lamblia*, and *Trichomonas vaginalis*, as well as against Gram-negative anaerobic bacteria such as *Bacteroides fragilis*, *Fusobacterium* spp, *Prevotella* (*Prevotella Bivia*, *Prevotella buccae*, *Prevotella disiens*), and *Gardnerella vaginalis*, and some Gram-positive pathogen such as *Clostridium Perffringens*, *Clostridium difficile*, *Peptococcus* spp, and *Peptostreptococcus* spp.

Clotrimazole has a fungicide and bactericidal effect. It slows down the biosynthesis of ergosterol, which regulates the permeability of the cellular wall of microorganisms. Small concentrations are fungistatic and large concentrations result in a fungicide effect. At fungicide concentrations, it interacts with peroxidases and mitochondrial enzymes, resulting in an increase in hydrogen peroxide concentration to the toxic level, which also leads to the death of the fungi. It is highly active against fungi such as *Blastomyces dermatitidis*

*Corresponding author: lucy.on04@gmail.com

Candida spp, *Coccidioides immitis*, *Cryptococcus neoformans*, *Dermatophytes* (*Trichophyton mentagrophytes*, *Microsporum canis*, *Epidermophyton foccosum*), *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, and *Sporothrix schenckii*; protozoa such as *Trichomonas vaginalis*; Gram-positive bacteria such as *Streptococcus* spp, *Staphylococcus* spp, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* [5, 6].

To investigate the safety of this new integrated drug that is used to treat vaginoses of different etiology, a study was carried out on its acute toxicity through epicutaneous and intravaginal introductions.

MATERIALS AND METHODS

The acute toxicity of the drug was studied according to the requirements of GOST (set of technical standards maintained by the Euro-Asian Council for Standardization, Metrology and Certification) 12.1.007-76: "System of labor safety standards. Harmful substances. Classification and general safety requirements." The experiments were carried out using the Balb\c female mice. As the drug is a medication for external use, possible toxic effects as a result of different ways of entry into the organism were investigated. The medication was administered orally in doses by increasing the amount of active ingredients by 10 times (with consideration of animal mass). After the measurement of LD_{50} (oral introduction of the medication), acute and chronic toxicity was investigated. Medications were administered once at a dose corresponding to LD 50 for the study of acute toxicity and for 14 days at a dose of 20% of LD 50 for the study of chronic toxicity (49,152 mg/mouse). The drug administered both orally and intravaginally was studied.

The irritating effects of the drug on the skin were studied through a one-time and multiple (14 days) drug application onto the skin and eye mucous membrane. At the end of the irritant study, the following was evaluated:

1. Changes in blood indicators (blood formula);

2. Changes in weights (body mass, liver, spleen, lungs, heart, brain, gonads, adrenal glands, kidneys, and thymus).

RESULTS AND DISCUSSION

For the entire duration of the study, no clinical symptoms of intoxication were registered in the animals receiving the suppositories. The appearance, behavior, and consumption of food and water by animals were not different from those of the control group.

Application of the suppositories in the native form once and repeatedly (within 14 days) on the skin of the research mice (4-hour exposure) did not lead to the changes in behavior of the test animals; the drug did not produce irritating effects on the skin and did not cause any intoxication. The skin appeared normal, without hyperemia, edema and sores. The appearance, behavior and orientation in space of the research animal were not different from those of the control group. Changes in the mass of the internal organs and the morphological state of the blood, with one-time and multiple applications of the drug on the skin of the female mice are presented in Tables 1 and 2.

Table 1. The average mass of internal organs of the female mice when the drug was applied to the skin.

Name of organs	Intact animals	Observation (single application)	Observation (repeated application)
Heart	0.41 ± 0.022	0.42 ± 0.024	0.44 ± 0.023
Lungs	0.85 ± 0.048	0.87 ± 0.053	0.88 ± 0.056
Liver	5.670 ± 0.36	5.73 ± 0.43	5.77 ± 0.48
Spleen	0.74 ± 0.09	0.76 ± 0.11	0.77 ± 0.11
Kidneys	0.67 ± 0.017	0.7 ± 0.019	0.7 ± 0.019
Thymus	0.18 ± 0.013	0.2 ± 0.016	0.2 ± 0.015

Table 2. Parameters of the morphological state of the blood of female mice when the drug was applied to the skin.

Name of metrics	Intact animals	Observation (single application)	Observation (repeated application)
Hemoglobin, g / l	96 ± 8.34	98 ± 9.0	104 ± 11.3
Erythrocytes, x 10^6/ml	6.9 ± 0.5	7.0 ± 0.62	7.2 ± 0.68
Leukocytes, x 10^3/ml	16.3 ± 0.4	18.2 ± 0.4	19.0 ± 0.52
Platelets, x 10^6/ml	6.0 ± 0.2	7.1 ± 0.3	0.9 ± 0.36
Neutrophils, %	40.0 ± 6.2	40.0 ± 8.2	40 ± 7.8
Monocytes, %	3.9 ± 0.4	3.9 ± 0.6	3.9 ± 0.56
Lymphocytes, %	55.3 ± 7.4	56.4 ± 8.1	56.8 ± 8.7

Table 3. Average mass of internal organs of the female mice after intravaginal administration of suppositories.

Name of organs	Intact animals	Observation (single application)	Observation (repeated application)
Heart	0.41 ± 0.022	0.4 ± 0.028	0.42 ± 0.018
Lungs	0.85 ± 0.048	0.86 ± 0.056	0.85 ± 0.048
Liver	5.670 ± 0.36	5.81 ± 0.55	5.96 ± 0.53
Spleen	0.74 ± 0.09	0.79 ± 0.12	0.81 ± 0.13
Kidneys	0.67 ± 0.017	0.69 ± 0.017	0.68 ± 0.018
Thymus	0.18 ± 0.013	0.17 ± 0.015	0.16 ± 0.017

Table 4. Parameters of the morphological state of the blood of female mice with intravaginal administration of suppositories.

Name of metrics	Intact animals	Observation (single application)	Observation (repeated application)
Hemoglobin, g / l	96 ± 8.34	100.0 ± 12.0	109 ± 14.2
Erythrocytes, x 10^6/ml	6.9 ± 0.5	7.2 ± 0.67	7.9 ± 0.81
Leukocytes, x 10^3/ml	16.3 ± 0.4	18.8 ± 0.6	20.0 ± 0.59
Platelets, x 10^6/ml	6.0 ± 0.2	7.9 ± 0.4	0.9 ± 0.44
Neutrophils, %	40.0 ± 6.2	40.0 ± 7.8	40 ± 8.8
Monocytes, %	3.9 ± 0.4	3.8 ± 0.7	3.8 ± 0.6
Lymphocytes, %	55.3 ± 7.4	56.6 ± 7.8	56.3 ± 6.7

Through the intravaginal introduction of suppositories (once and repeatedly), changes were investigated in the mass of internal organs and in the morphological state of the blood. The obtained data are presented in tables 3 and 4.

CONCLUSION

From the data received, it was established that the one-time and multiple intravaginal and epicutaneous introductions of the new complex drug did not result in significant changes in the mass of the internal organs of intact animals, nor did it significantly affect the morphological conditions of the peripheral blood of the mice. The data also indicate that under such introduction and in the appropriate doses, vaginal suppositories do not show toxic, locally irritating effects and may be further effective in treating vaginoses of different etiology.

CONFLICT OF INTEREST STATEMENT

We assert that there are no conflicts of interest.

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