Investigation of Acute Toxicity of Tilorone Ointment for Topical Treatment of Herpes Virus Infection

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Abstract:
Tilorone (amixin) is a low molecular interferon inducer that is effective against a wide range of viral infections including herpes viruses. The mechanism of tilorone antiviral action is associated with inhibition of virus-specific peptides translation in infected cells resulting in the suppression of virus replication. An appropriate therapy of the viral infections with external rash besides the systemic preparations must include topical drugs. Considering this, we developed a new topical antiviral 2% tilorone preparation in the form of ointment. The aim of investigation was to study the parameters of acute toxicity of the 2% tilorone ointment. The acute toxicity of the ointment was studied by intragastric administration and cutaneous application. Acute intragastric toxicity was carried out in white outbred mice and white Vistar rats that were given intragastrically an aqueous solution of ointment at a dose of 0,5g/kg. Acute dermal toxicity was studied in white outbred mice. The animals were monitored daily for clinical signs in the course of 14 days. It was determined that an effective dose of the 2% test ointment which does not cause toxic effects on human body is DL₀≤0,4 g/kg. The results obtained enable us to attribute the developed drug product to the practically non-toxic.

Keywords: tilorone, ointment, acute toxicity.
Introduction:
We developed new semi-solid antiviral preparation for topical treatment of herpes virus infection.

Active pharmaceutical ingredients:tilorone and menthol.

Pharmacological effect of tilorone:

- Infected cell
  - Viral assembly
  - Viral RNA
  - Release of new virions
  - Attachment of interferon to specific receptors
  - Inhibition of viral proteins translation

- Contiguous cell
  - Destruction of viral RNA
  - Synthesis of antiviral proteins
  - Signal for defense gene activation
  - Prevention of viral release
From previous results based on physical-chemical and biopharmaceutical tests, polyethylene oxide base was selected for the tilorone ointment.

For adults and children from 7 years

It inhibits virus-specific peptides translation in infected cells

Tilorone ointment

Local effect

It reduces complications from exogenous interferon

It induces endogenous interferon synthesis in affected cells that is a non-specific factor of antiviral resistance
Ointments for topical treatment of herpes simplex virus infections are applied directly on the affected skin or mucous membranes. Therefore those preparations are to be highly safe to minimize risk of complications.

The aim of investigation to study the parameters of acute toxicity of the 2% tilorone ointment

Investigations were performed in compliance with standards of humanitarian treatment with animals according to the European Convention for the Protection of Vertebrate Animals.

Selection of laboratory animals for studies was based on the requirements of International Council on Laboratory Animal Science, World Society for the Protection of Animals and Ukrainian instructive documents.
Results and discussion:

Methods for measuring acute toxicity of 2% tilorone ointment

In 27 white outbred mice (Mus musculus L.) (20-25 g)

In 27 white Vistar rats (Rattus Norvegicus) (200-220 g)

Cutaneous application of the test drug product

Intragastric administration of the test drug product
All animals were cared for humanely, in accordance with the European convention for the protection of vertebrate animals used for experimental and other scientific purposes [Council of Europe, Strasbourg, 2006].

**Investigation of intragastric acute toxicity**

**White outbred mice**
- Control intact animals (7) were given physiological solution per os;
- Control animals (10) were given ointment base per os;
- Experimental animals (10) were given tilorone ointment per os.

**White Vistar rats**
- Control intact animals (7) were given physiological solution per os;
- Control animals (10) were given ointment base per os;
- Experimental animals (10) were given tilorone ointment per os.

\[ v(\text{tilorone ointment}) = v(\text{ointment base}) = v(0.9\% \text{ NaCl solution}) = 5 \text{ mL (dose of tilorone)} = 0.5 \text{ g/kg} \]

Dose of tilorone for both species of rodents was the same.
Results of investigation of the acute intragastric toxicity of 2% tilorone ointment

Single dosing of 2% tilorone ointment and ointment base did not cause the death of the experimental animals.

Transient reactions of cardiovascular system, decrease of muscle tone and physical activity in the first 10-20 min after administration of preparations were related to stress response. Complications were not observed in the further days of the experiment.

Differences in the weight dynamics between the control intact animals and other groups were not reported.

Peroral administration of 2% tilorone ointment at a dose of 0.5 g/kg (equivalent to tilorone) did not permit to determine the DL₅₀ in the experiment.

Dose of ointment – 0.5 g/kg (equivalent to tilorone) – has been accepted as an effective dose of the substance – DL₅₀.
Investigation of acute dermal toxicity of 2% tilorone ointment

Control intact animals (7) were cutaneously applied with physiological solution
Control animals (10) were cutaneously applied with ointment base
Experimental animals (10) were cutaneously applied with 2% tilorone ointment

Application area was 5.5-6.0 cm² (10% of the body area)

Volumes of the ointment, the ointment base and the physiological solution were equal 0.35-0.4 mL (equivalent to 7-8 mg of tilorone for an animal or 350 mg/kg)
Results of investigation of the acute dermal toxicity of 2% tilorone ointment

Cutaneous application of 2% tilorone ointment did not cause any critical changes in the skin, animal behavior, gastrointestinal symptoms, and diuresis.

Weight dynamics in the all groups of animals was the same.

An effective dose of 2% tilorone ointment is $DL_0 \leq 0.4$ (g/kg).

Extrapolation of obtained results on humans.

Application of higher doses was inappropriate.
Conclusions:

1. The results indicated that the developed 2% tilorone ointment was practically non-toxic.

2. It is reasonable to perform further investigations with the developed 2% tilorone ointment due to its safety.
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