The Investigation of Pharmacological Activity of Antihistaminic Gels with Dimebon

Mayorova Alena Valentinovna1, Sokolova-Merkuryeva Anna Vladimirovna2, Koryanova Ksenya Nikolayevna3, Tretyakova Ekaterina Vasilievna4

1Candidate of Pharmaceutical Sciences, Associate Professor, Head of the Chair of Aesthetic Medicine of the Peoples Friendship University of Russia.

2Candidate of Medical Sciences, associate Professor of the Chair of Aesthetic Medicine of the Peoples Friendship University of Russia.

3Candidate of Pharmaceutical Sciences, Lecturer of the Chair of Drugs Technology at Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University of the Ministry of Health of the Russian Federation.

4Associate Professor at the Department of Esthetic Medicine of the Faculty of Continuing Medical Education at the Medical Institute of Peoples’ Friendship University of Russia (Moscow), Associate Professor of Pharmaceutical Sciences, Russian Federation.

Abstract

The article covers the review of results of investigation for application safety of traditional gel with dimebon and gel with microcapsules dimebon of prolonged anti-allergic action. We have estimated the irritant action is situ and in vivo, studied acute toxicity by the survivability of mice considering calculated LD50. We have determined a signified antihistaminic (anti-oedemic) effect of gels with external application by the reduction of time and intensity of modelled limb swelling of rats. We have established regenerative action of gels by the reduction of burned skin area, augmentation of tissues epithelization and eschar rejection.

Keywords: Dimebon, Gel, Anti-allergic action, Antihistaminic, Anti-inflammatory action.

Introduction

Treatment and prevention of clinical manifestations of allergy is one of the urgent issues of modern medicine which have a social value. According to WHO, allergic diseases now occupy one of leading places in morbidity, with the number of patients increased.

As a rule, allergic responses are treated using combined therapy with peroral and external medicinal anti-allergic drugs. They include H1-antihistaminic, anti-serotonin, and anti-bradykinin compounds, anticholinergic drugs, however they frequently do not have therapeutic effects with their full use, and it is connected with insufficient complex form of the treatment and absence of necessary external dosage forms [1-5]. Application of glucocorticoid hormones is not always justified, since the final result is accompanied with possibility of side effects [6].

The range of external anti-allergic drug dosages is not wide. Therefore the working out of new Russian produced dermatological drug dosages is an urgent task. One of the anti-allergic drugs is dimebon, which now is only represented on the pharmaceutical market by pills. Production of external dimebon drug dosages would allow the possibility of their choice and widen a pharmacological spectrum of this Russian drug, which is no doubt important for the treatment and prevention of allergic responses.
Previously we worked out a gel with dimebon and gel with microcapsuled dimebon, carried out technological, biopharmaceutical researches of gels, developed the methods of qualitative and quantitative determination of an active substance in the external dosage forms under study [7,8,9,10].

Materials and Methods

Dimebon was chosen as an active substance.

**DIMEBON**

3,6-Dimethyl-9-[2,2'·methylpyridyl-5·ethyl]-1,2,3,4-tetrahydro-γ-carboline dihydrochloride

*Registration number:* LSR-004911/08 – 250608, FS 42-2794-91.

Evaluation of Mice Survivability

The study for general toxic action, which includes determination of acute toxicity and local irritant action is an obligatory test of external drugs use safety.

While investigating for the acute toxicity of dimebol gel samples, gels were administered topically on skin. The determination of acute toxicity was carried out following Karber’s method, described in official guidelines for experimental (preclinic) study of new pharmacological substances under direction of V.P. Fisenko [11].

Animals were dehained in the back area 100 m² square. Mice had their fur cut and then shaved with razor to hair length 1mm maximum. After that fur residues were eliminated with a wet wipe. Gel samples and gel base were administered in a thin layer with maximum dose at 5 g/kg of animal weight, which is 10 time bigger the dose of external dosage forms (ointments, gels) which are implemented in medicines [12, 13].

The mice condition was evaluated after the gels administration on skin at different dosages. Pattern of intoxication and survivability of the animals during 48 hours were the evaluation criteria of acute toxicity. Further observation was conducted during two weeks, and beginning with a first day, animals were under constant observation. To determine the action of gel base on the mice’s organism we carried out parallel researches using gel base in equal volume [14].

We have conducted 9 series of experiments (3 series on mice-traditional gel at different doses, 3 series on mice–gel with microcapsules at different doses, 3 series- gel base at different doses). We calculated acute toxicity following the recommendations of state pharmacological committee for study of general toxic action of biologically active substances [15-17].

**Calculation of Media Lethal Dose**

Medical lethal dose (LD₅₀) was calculated by means of evaluation of animals mortality during the following 48 hours, first 6 hours after gel samples implementations it was calculated non-stop.

Gel samples under study were administered on the epilated back area 5000 mg/kg, 2500 mg/kg, and 1250 mg/kg, which corresponds to 0.1 g, 0.05 g, and 0.025 g per one mouse, weighed 20 g. To determine the action of gel base on the mice organism, we conducted parallel researches using gel base in equal volume [14].

**Local Irritant or Skin Irritant Action**

Local irritant or skin irritant action of worked out gels was determined in situ, and in vivo.

In situ work was implemented using 18 Legron white chicken embryos 9-10 days old within 7 days in thermostat at 37.8°C, at stable air humidity 62.5%.

The estimation of irritant action of gels was done using test at chorioallantoic membrane of chicken embryo, HET-CAM test [1, 18]. The substance was tested in 18 replications (12 – experiments, 6 – control). The work was implemented using 18 Legron white chickens 9-10 days old, which were kept in thermostat at 37.8°C for 7 days, at stable average air humidity 62.5%. Before the start, egg was fixed at a
stand, pointing broad part up; shell was opened in the center of broad part, releasing entire air chamber from shells, after that, the surface of the open air chamber was moistened with isotonic solution of sodium chloride (0.89%) at 37°C gel at dose 0.3 g and observed the substance’s action during 240 seconds.

Experiments in vivo were carried out using 29 guinea pigs, weighed 350-400 g, kept in standard vivarium conditions: with ambient temperature 22±2°C, 12 hour synchronized change of light period, combined feed and water was given to animals ad libitum.

Animal experiments were carried out following a scheme used nowadays in Germany. The worked out gels were given a trial on the mucous membrane of guinea pigs eye, administering them on the anterior segment, making a final conclusion after that [11]. Index of an irritant action was estimated integrally: the swelling degree and hyperemia were summed up.

Evaluation of Antioedemic Effect

Acute inflammation response (swelling) was reproduced by intraplantar administration of histamine hydrochloride at 0.1 ml of 0.1% solution dorsoventrally into right back limb of a rat. The studied samples of gels with dimebon were administered locally in the paw at 250 mg. Second measuring of rat paw volume was done after every 10 minutes, observing the development of swelling peak, fixed swelling peak, and the final measurement of a paw volume was done every three hours, at the stage of acute exudative inflammation cancellation of rat’s paw. Paw’s volume increase was the degree of exudation stage intensity.

This method is simple, easy to reproduce, gives possibility to obtain qualitative characteristics of the results for further statistic processing, the device gives possibility to register paw volume alterations in dynamics with no necessity for anesthesia for animals under study. The intensity of exudative inflammation was evaluated using oncometry at maximum volume for this model (in 30 minutes) and after 3 hours of inflammation induction by histamine.

The paw’s volume was evaluated in dynamics by mechanic oncometer following A.S. Zakharevsky [11].

Anti-inflammatory efficiency of the samples under study was calculated using the following formula [19]:

\[ P = \frac{v_k - v_0}{v_0} \cdot 100\% \]  \( \text{(4)} \)

where: \( P \) - percent of inflammation suppression,

\( v_k \) – average increase of paw’s volume in control group,

\( v_0 \) – average increase of swelled paw volume in treated animals.

Modelling of Thermal Burn of Guinea Pigs Skin

Day before the trials, the animals were thoroughly epilated in the upper part of their body. Before the burn, animals were anesthetized by abdominal injection of chloral hydrate at dose 350 mg/kg, and then fixed. To limit the burn area we put the foil sheet with equal size holes (1×1 cm) on skin, the initial burn area amounted to 100 mm². After that, a thermal burn was inflicted with a contact, temperature at 100°C, and 10 seconds operation time. Thus, the burns were equal on their size and severity level.

Pathology process development criteria and reparative properties manifestation of the gel samples under study were the following: wound square measurement, regeneration dynamics, eschar rejection time, general condition of the animals, visual evaluation of the defect condition and nearby tissues. We carried out computer microphotography of skin defects using Intel® Play(tm) QX3(tm) Computer Microscope program.

Statistic processing of the results obtained was carried out using Student’s t-test for independent rows. The experiments results were compared with initial indexes, with non-treated animals [20, 21]. The calculations were done using Microsoft Excel 2000 program [22]. The changes of the indices under study were statistically relevant at p<0.05 [22, 23, 24].
Results and Discussion

Study for the Safety of External Drugs Application

Study for General Toxic Action of the Worked Out Dosage Forms

For this study we used worked out gels with dimebon: traditional and with microcapsules. Experiments were carried out following Karber's method on mice, weighed 20±1.0 g [11,20,25]. Acute toxicity was determined following the evaluation of mice survivability and by the calculated medial lethal dose.

While determining acute toxicity, we observed the alteration of general condition of mice during 14 days (visual appearance, behavioral response, their activity, respiratory rhythm, food intake) and death of the animals [26]. First 6 hours, animals were observed non-stop. There were no changes in animals’ behavior, which showed their normal general state.

First 6 hours and following 48 hours we were determining a medial lethal dose (LD50), calculated by the animals mortality evaluation. None of animals were dead in this period.

During this time we observed animals by the indexes of locomotion activity, spasms presence, movement’s coordination, response for irritants, skeleton muscles tone and respiration, condition of skin, fur, and coloration of visible mucous membranes, as well as feed and water intake, body weight alterations. There were no visible declinations in comparison with the control group of animals. The results are shown in the table 1.

### Table 1: Determination of acute toxicity of dimebon gel, gel with dimebon microcapsules, and gel base on male mice (skin application)

<table>
<thead>
<tr>
<th>Results</th>
<th>Gel with dimebon</th>
<th>Dimebon gel with microcapsules</th>
<th>Gel base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection volume of 0.1 g per animal weighed 250 g</td>
<td>0.025 g</td>
<td>0.05 g</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Number of Animals Died</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Survived</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Z</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0.025 g</td>
<td>0.05 g</td>
<td>0.025 g</td>
</tr>
<tr>
<td>DZ</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\[
\text{n}=6 \sum (dZ) = 0 LD_{20} = LD_{100} - \frac{\sum (dZ)}{n} > 5000 \text{mg/kg}
\]

Note: Z - index of the difference between the number of dead animals after using of two adjacent doses; D - показатель разницы между количеством index of difference between the quantity

Group of animals, which were applied with gel with dimebon and gel base did not have dead animals.

Discussing the results of the experiment data, the absence toxicity in gel samples at skin application should be noted. Lethal dose, which would kill all experimental animals was not revealed in this method.

The studies showed, that dimebon gel samples can be related to class IV – safe agent, following Hodge and Sterner classification of K.K Sidorov [15, 27].

Determination of Irritant Action of Gels with Dimebon

The research for irritant action of gel compositions with dimebon was carried out in 2 stages: in situ and in vivo.

Evaluation of Irritant Action in Situ

Оценку раздражающего действия гелей in situ при использовании методики ХЕТ-КАМ тест chorioallantoic оболочке куриного эмбриона, Результаты приведены в таблице 2. The evaluation of irritant action of gels in situ with the use of HET-CAM method at the chorioallantoic membrane of chicken embryo. The results are shown in the table 2.
Table 2: Degree of irritant action of gel samples and gel base on chorioallantoic membrane

<table>
<thead>
<tr>
<th>No of experiment</th>
<th>Class of compounds by the irritation degree</th>
<th>Gel with dimebon</th>
<th>Dimebon gel with microcapsules</th>
<th>Gel base</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0 weak</td>
<td>2.0 weak</td>
<td>2.0 weak</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.0 weak</td>
<td>1.0 absence of irritation</td>
<td>1.0 absence of irritation</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.0 absence of irritation</td>
<td>2.0 weak</td>
<td>1.0 absence of irritation</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.0 absence of irritation</td>
<td>1.0 absence of irritation</td>
<td>1.0 absence of irritation</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.0 weak</td>
<td>1.0 absence of irritation</td>
<td>2.0 weak</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.0 absence of irritation</td>
<td>1.0 absence of irritation</td>
<td>2.0 weak</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Stand.deviation</td>
<td>0.547723</td>
<td>0.547723</td>
<td>0.547723</td>
<td></td>
</tr>
</tbody>
</table>

As the table 2 shows, after the application of gel with dimebon at chorioallantoic membrane of chicken embryo there were no changes in the chorioallantoic membrane in 3 out of 6 cases, 3 cases showed vessel constriction with temporary blood circulation stop in certain capillaries.

This corresponds to 2 class by the irritation degree, with irritant action coefficient \(1.5 \pm 0.548\). While investigation of gel with dimebon microcapsules and gel base we obtained similar results, which corresponds to the 2 class of irritation degree, with irritant action coefficient \(1.5 \pm 0.548\).

From the experiment result it follows that the presence of weak irritant action is conditioned by the properties of gel base.

Preliminary results give evidence about the fact that substance under study in corresponding concentrations may not have a signified toxic and irritant action on the mucous membranes of mammals and can be experimented in further trials on warm-blooded animals following the scheme of Spielmann and co-authors (1996).

**Evaluation of irritant action in Vivo**

The index of irritant action was evaluated integrally: the swelling and hyperemia degree was summed up. The results of mammals conjunctiva damage was evaluated by 5 points scale, recommended by P. Mikhaylov (1985) [28].

Eight was the highest point of primary irritation [28].

The index of primary irritation was calculated by the relations between the area with erythema and swelling on the experimental mucous membrane of conjunctiva, and the square of control group.

Evaluation criteria are shown in the table 3.

Table 3: Irritant action criteria in Vivo

<table>
<thead>
<tr>
<th>Points</th>
<th>Hyperemia</th>
<th>Swelling formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>erythema is absent</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>very weak erythema</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>well-marked erythema</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>moderate or strong erythema</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>strong erythema or low-grade formation of burn eschar</td>
<td>4</td>
</tr>
</tbody>
</table>

Drug substances with irritant action index 1-2 are weak irritants of skin and mucous membrane, drugs with moderate irritant action are characterized by 3-5 index.

Drugs with 6-8 index have strong irritant action [28]. The estimation of primary irritation was done in 30 seconds, 2 minutes, 6 hours, and 24 hours. The second eye of an animal was taken as the control.

Results of the studies are shown in the table 4.
Table 4: Estimation of irritant action of samples of gel with dimebon and gel base on the anterior segment of guinea pigs eye

<table>
<thead>
<tr>
<th>Action No of experiment</th>
<th>Swelling 30 seconds</th>
<th>Hyperemia 30 seconds</th>
<th>Swelling 2 minutes</th>
<th>Hyperemia 2 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel with dimebon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Average value</td>
<td>0.33</td>
<td>1.0</td>
<td>0.33</td>
<td>1.0</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.577</td>
<td>0.0</td>
<td>0.577</td>
<td>0.0</td>
</tr>
<tr>
<td>Dimebon gel with microcapsules</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>5.</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>6.</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Average value</td>
<td>1.0</td>
<td>2.0</td>
<td>1.0</td>
<td>2.33</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.577</td>
</tr>
<tr>
<td>Gel base</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9.</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Average value</td>
<td>0.33</td>
<td>1.0</td>
<td>0.33</td>
<td>1.0</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.577</td>
<td>0.0</td>
<td>0.577</td>
<td>0.0</td>
</tr>
</tbody>
</table>

The value of irritant action of traditional gel with dimebon was obtained during the experiments 1, 2, and 3: the swelling after 30 seconds (at first minute) amounted to 0.33±0.577 points, as well as hyperemia with 0.33±0.577 points, 0.66 on 30 second in sum, which corresponds to the characteristics of a weak irritant action. In 2 minutes, irritant action was marked by the swelling 1.0±0.0 and hyperemia 1.0±0.0, which in sum equaled to 2 points, which is a weak irritant action.

The value of irritant action of the gel with dimebon microcapsules was obtained during the experiments 4, 5, and 6: the swelling after 30 seconds (at first minute) amounted to 1.0±0.0 points, as well as hyperemia with 1.0±0.0, which was equal to 2.0 points at 30 second, which corresponded to the characteristics of a weak irritant action.

The increase of irritant activity of the gel with dimebon microcapsules, apparently is explained by the mechanic irritation of an eye conjunctiva by its particles.

Thus, experimental data allowed us to make a conclusion about the fact that traditional gel with dimebon, and gel base have weak irritant action at contact with mucous membrane. The gel with dimebon microcapsules had moderate irritant action.

After 2 minutes the irritant action was expressed by the swelling 1.0±0.0 and hyperemia 1.0±0.0, which summed up to 2 points showing the moderate irritant action.

After 2 minutes the irritant action was expressed by the swelling 2.0±0.0 and hyperemia 2.33±0.577, which summed up to 4.33 points showing the moderate irritant action.

The value of irritant action of the gel base was obtained during the experiments 7, 8, and 9: the swelling after 30 seconds (at first minute) amounted to 0.33±0.577 points, as well as hyperemia with 0.33±0.577, which was equal to 0.66 points at 30 second, which corresponded to the characteristics of a weak irritant action.

After 2 minutes the irritant action was expressed by the swelling 1.0±0.0 and hyperemia 1.0±0.0, which summed up to 2 points showing the moderate irritant action.

Thus, experimental data allowed us to make a conclusion about the fact that traditional gel with dimebon, and gel base have weak irritant action at contact with mucous membrane. The gel with dimebon microcapsules had moderate irritant action.

The increase of irritant activity of the gel with dimebon microcapsules, apparently is explained by the mechanic irritation of an eye conjunctiva by its particles.

Study for Specific Activity of the Worked Out Gels

Evaluation of Anti-Oedemic Action of Samples of Dimebon Gels

Anti-oedemic action of the gels was evaluated by the dynamics of modelled swelling of rats’ limbs. Fenistil antihistamine gel was used as a comparative drug. For comparison, we studied the influence on inflammation process of gel base.

During experiments, we discovered that traditional gel with dimebon had a signified anti-exudative activity, with no relevant difference from the anti-allergic drug of Fenistil (table 5, table 6).
Table 5: Dynamics of inflammation swelling, obtained by the volume of water in absolute units (ml)

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Paw's volume initial</th>
<th>Swelling peak time</th>
<th>Paw's volume at peak</th>
<th>Paw's volume in 3 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated animals, n=6</td>
<td>0.93±0.24</td>
<td>40 min</td>
<td>2.06±0.084</td>
<td>1.12±0.097</td>
</tr>
<tr>
<td>Animals treated with gel with dimebon, n=6</td>
<td>1.15±0.04</td>
<td>30 min*</td>
<td>1.54±0.045</td>
<td>1.18±0.058</td>
</tr>
<tr>
<td>Animals treated with gel with dimebon microcapsules, n=6</td>
<td>1.03±0.053</td>
<td>40 min</td>
<td>1.41±0.063</td>
<td>1.12±0.054</td>
</tr>
<tr>
<td>Animals treated with gel base, n=6</td>
<td>0.96±0.044</td>
<td>40 min</td>
<td>2.04±0.21</td>
<td>1.18±0.076</td>
</tr>
<tr>
<td>Animals treated with Fenistil gel, n=6</td>
<td>0.98±0.238</td>
<td>30 min*</td>
<td>1.29±0.053</td>
<td>1.03±0.074</td>
</tr>
</tbody>
</table>

* – index of differences relevance to the paws' volume at the initial level to the group of non-treated animals.

The initial paws' volume of non-treated animals amounted to 0.93±0.24 ml, the swelling peak took place in 40 minutes at 2.06±0.084 ml, the final paw's volume amounted to 1.12±0.097, in 3 hours the volume gain amounted to 0.19±0.02 ml.

Paw volume of animals with modelled swelling, which were treated with gel base amounted to 0.96±0.44 ml at the beginning of the experiment, the swelling peak took place in 40 minutes and amounted to 2.04±0.21 ml, the final paw volume after 3 hours amounted to 1.18±0.076 ml.

Paw volume gain at the swelling peak amounted to 1.08 ml ±0.018, after 3 hours the paw volume amounted to 0.22±0.02 ml from the initial volume.

After application of gel with microcapsules, swelling peak took place in 40 minutes. Paw volume of animals with modelled swelling amounted to 1.03±0.053 ml at the beginning of the experiment, the paw volume at the peak amounted to 1.12±0.054 ml. Paw volume gain amounted to 0.38 ml ±0.015 at the swelling peak, after 3 hours the paw volume gain amounted to 0.11±0.008 ml.

Table 6: Dynamics of the inflammation swelling, obtained by the volume of displaced water in relative units (%)

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Initial paw volume</th>
<th>Paw volume gain at the peak</th>
<th>% and R to control</th>
<th>Paw volume gain after 3 hours</th>
<th>% and R to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated animals, n=6</td>
<td>100</td>
<td>+121.5%</td>
<td>+20.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals treated with gel with dimebon, n=6</td>
<td>100</td>
<td>+33.9%</td>
<td>27.9 p&lt;0.05</td>
<td>+2.6%</td>
<td>2.14 p&lt;0.05</td>
</tr>
<tr>
<td>Animals treated with dimebon microcapsules gel, n=6</td>
<td>100</td>
<td>+36.9%</td>
<td>30.4 p&lt;0.05</td>
<td>+8.7%</td>
<td>7.16 p&gt;0.05</td>
</tr>
<tr>
<td>Animals treated with the gel base, n=6</td>
<td>100</td>
<td>+112.5%</td>
<td>92.6 p&lt;0.001</td>
<td>+22.9%</td>
<td>18.8 p&lt;0.001</td>
</tr>
<tr>
<td>Animals treated with Fenistil gel, n=6</td>
<td>100</td>
<td>+31.6%</td>
<td>26.0 p&lt;0.01</td>
<td>+5.1%</td>
<td>4.2 p&lt;0.05</td>
</tr>
</tbody>
</table>

Notes:
R is an index of differences relevance to the paws' volume at the initial level to the group of non-treated animals (control); % - to the indexes of non-treated animals.
R of gel [(121.5-27.9)/121.5] * 100% = 77.04% 
R of gel with microcapsules [(121.5-36.9)/121.5] * 100% = 69.63% 
R of gel base [(121.5-92.6)/121.5] * 100% = 23.79% 
R of Fenistil gel [(121.5-26.0)/121.5] * 100% = 76.6%

The initial paw volume with a swelling of non-treated animals amounted to 0.93±0.24 ml, the peak of the swelling took place in 40 minutes with 2.06±0.084 ml, the final paw
volume amounted to 1.12±0.097. The non-treated animals paw volume gain amounted to 1.13 ml ±0.03 at the peak, after 3 hours paw volume gain amounted to 0.19±0.02 ml from the initial volume.

The paw volume of animals with modelled swelling treated with gel base amounted to 0.96±0.044 ml at the experiment start, the peak took place in 40 minutes and amounted to 2.04±0.21 ml, the final paw volume after 3 hours amounted to 1.18±0.076 ml. The paw volume gain amounted to 1.08 ml ±0.018 at the swelling peak, after 3 hours the paw volume gain amounted to 0.22±0.02 ml from the initial volume.

Animals treated with gel with microcapsules had the swelling peak in 40 minutes. The paw volume gain amounted to 0.22±0.02 ml from the swelling peak, after 3 hours the paw volume gain amounted to 0.38 ml ±0.015 at the peak, the volume gain after 3 hours amounted to 0.11±0.008 ml from the initial volume.

Antioedemic action of traditional gel with dimebon is the most signified: the paw volume gain in this group amounted to 2.14%, which differed relevantly from the indexes of the experimental groups of animals, which received a comparison drug of Fenistil 4.2% (table 24).

Anti-inflammator local efficiency of the gel with dimebon amounted to 77.04%, which is as good as indexes of Fenistil gel. Anti-inflammator efficiency of the gel with dimebon microcapsules at local application amounted to 69.63%. This can be explained by the prolonged release of active substance from microcapsules.

Anti-inflammator activity of the gel is provided by the active substance in its composition, which is explained by the fact that gel base at equal dose had 3.2 less activity.

**Study for Regenerative Activity of Gels with Dimebon**

Every day after burn wounding of guinea pigs, we measured the burned area. We also treated animals until the complete healing.

Animals were divided into 4 groups:
1 group – traditional gel of dimebon,
2 group – gel with dimebon microcapsules,
3 group – gel base,
4 group – Fenistil comparison gel.

Control group consisted of non-treated animals.

The time of burn eschar discharge showed the process of capillary network formation.

The regeneration of epithelial tissue was calculated following the percent of healing, which determined the degree of normal skin formation instead of damaged skin. We calculated the difference between the initial and final are after 7 and 14 days (table 7, figure 1).

**Table 7: Evaluation of the regenerative activity of gels**

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Eschar discharge time, days</th>
<th>Burn square mm²</th>
<th>% заживления и Р к контролю</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7th day</td>
<td>14th day</td>
<td>7th day</td>
</tr>
<tr>
<td>Non-treated animals, n=4</td>
<td>10th day</td>
<td>84.25±4.35</td>
<td>69.5±3.79</td>
</tr>
<tr>
<td>Animals treated with gel with dimebon, n=4</td>
<td>4th day</td>
<td>48.5±4.43</td>
<td>13.25±1.5</td>
</tr>
<tr>
<td>Animals treated with gel with dimebon microcapsules, n=4</td>
<td>8th day</td>
<td>64.25±2.63</td>
<td>35.25±2.51</td>
</tr>
<tr>
<td>Animals treated with gel base, n=4</td>
<td>8th day</td>
<td>68.5±4.12</td>
<td>51.0±1.15</td>
</tr>
<tr>
<td>Animals treated with Fenistil gel, n=4</td>
<td>6 сутки</td>
<td>65.25±3.40</td>
<td>40.75±1.5</td>
</tr>
</tbody>
</table>

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Capillaries formation in the group of animals treated with traditional gel of dimebon took place on 4th day. This index exceeded the index of officinal anti-allergic drug of Fenistil by 2 days. The group treated with gel with dimebon microcapsules had the eschar discharged on the 8th day.

Capillary strengthening effect is conditioned by the active component in the gel composition. The group of animals which was treated with the gal base had the eschar discharged on the 8th day. Eschar discharge in the group of non-treated animals took place on the 10th day.

**Figure 1: Influence of gels with dimebon in comparison with Fenistil gel on the change of burned area**

**Conclusion**

- Following the results of irritant action on the chorioallantoic membrane and eye anterior segment of guinea pigs, we have established that samples of dimebon gels may be included with the drugs with a weal irritant action.

Gels with dimebon are not dangerous for mammals: LD$_{50}$ (topically)>5000 mg/kg.

- We have determined a signified antioedemic effect of external use of the gels able to shorten the time of modelled rat limbs swelling development. The index of anti-inflammatory activity of traditional gel amounted to 77.04%.

- We have established the regenerative action of gels with dimebon able to decrease the burned area, augment tissues epithelization and eschar discharge.
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