Biochemical and planimetric investigations of hydrophilic creams containing ceramides or dexpanthenol on the model of chemical burns

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ABSTRACT

Introduction and aim. Chemical burns of the skin are common type of injuries both in private life and in industries. Local treatment of chemical burns using wound healing creams and ointments is predominant. Hydrophobic wound healing medicinal products dominate the Ukrainian pharmaceutical market but their hydrophobic base disturbs the healing process of skin. The aim of this work was biochemical and planimetric investigation of treatment efficacy of chemical burns with hydrophilic creams containing ceramides and dexpanthenol.

Material and methods. The experiments were performed on 30 rats weighing 190–220 g. In a rat skin burn model, animals were exposed to 9% acetic acid solution. Treatment was initiated after wound appearance and included application of creams containing ceramides and dexpanthenol. The effectiveness of treatment was estimated using planimetric parameters, such as: surface area of necrotic tissue (S, mm²) and cumulative reparative effect. Levels of the biochemical markers such as total protein, creatinine, C-reactive protein (CRP) and content of SH-groups were measured in the rats' blood serum.

Results. It was established that cream developed with ceramides and cream with dexpanthenol exhibits reparative properties at the level of 29 % and 4.5 %, respectively. Biochemical investigations demonstrated the treatment efficacy of creams containing ceramides and dexpanthenol. In terms of CRP level and content of SH-groups, the therapeutic action of cream with dexpanthenol was highly significant by a factor of 1.45 and 1.35, respectively in contrast to the cream with ceramides.

Conclusion. Using the chemical burn model and results of planimetric and biochemical research it was found that cream with ceramides and cream with dexpanthenol exhibit wound-healing properties. In-depth study on the wound-healing mechanism of investigated creams with the aim of creating effective hydrophilic creams for use in burn treatment is prospective.

Keywords. ceramides, chemical burns, dexpanthenol
Chemical burns of skin were induced by 0.5 ml subcutaneous injection of 9% acetic acid solution. On the third day of acetic acid injection coagulation necrosis appeared, leading to ulceration and skin inflammation. When the wound occurred, cream-based treatment was started. The investigated creams were applied on the wound once a day to complete healing.

A wound area was measured as follows: transparent linear graph paper was applied on the wound outlines and area of wounds (cm²) was measured at different time of observation (initial wound area, 5, 7, 9, 11, 13, 15, 17 day of treatment).

The effectiveness of treatment was estimated using planimetric parameters, such as: surface area of necrotic tissue (S, mm²) and cumulative reparative effect.

Surface area of necrotic tissue (S, mm²) was calculated according the following formula:

\[ S = S_{\text{initial}} - S_{(0)} \]

Cumulative reparative effect was calculated using the statistical package «MedCalc, v. 9.3.7.0» and used as a value of integrated index of area under curve «surface area of healing – time».

The level of total protein (TP) and creatinine was determined in blood plasma by photometry using the kits made by "Filisist-Diagnosis", Ukraine. Level of C-reactive protein (CRP) was quantified by ELISA test («UkrMedService», Ukraine) using analyzer «Libline-90» (Austria); content of SH-groups was determined using the specific thiol reagent – 5,5ʹ-Dithiobis (2-nitrobenzoic acid) (also called DTNB or Ellman’s reagent).

All laboratory animal experiments were performed according to the rules of humane treatment of laboratory animals and as per the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes" and the Decree of the First National Congress on Bioethics.

Laboratory animals got a nutritionally balanced diet (combined feed of PF "Vita" Kharkiv, Ukraine). During the experiments laboratory animals were kept in the plastic cages in an experimental animal room at temperatures between 18-24°C, relative humidity not more than 55% and a normal day/night cycle.

All interventions and euthanasia of animals was performed in accordance with animal bioethical standards. Results of analysis were processed using the program "Statistica 8" with p<0.5 (StatSoft, Tulsa, USA).
**Results**

Penetration of acetic acid in the skin resulted in the formation of coagulation necrosis (ulceration) with an eschar area 259-290 mm$^2$. Analysis of planimetric parameters showed that in the group of untreated rats (CP) healing of wounds during five days was slow as evidenced by the small healing area of wounds (13.50 mm$^2$) (table 1). On the ninth day, the healing process in animals was more intensive as evidenced by the increase of healing area up to 94.83 mm$^2$. In the course of the treatment of animals with burns intensive healing takes place already on day 5 – healing area in the group of animals treated by the cream with ceramides was 33.17 mm$^2$, but in the group of animals treated by the cream with dexpanthenol the healing area was 35.33 mm$^2$, which is significantly higher by a factor of 2.45 and 2.62 in comparison with CP, respectively.

On day 13 of treatment by a cream with dexpanthenol healing area (260 mm$^2$) was significantly higher than at the case of treatment by the cream with ceramides (233.33 mm$^2$).

Total reparative effect, calculated as area under curve «area of healing – time», for group of animals which were treated by the cream with ceramides was 2092.83 (difference is 29%), by the cream with dexpanthenol – 2295.82 (difference is 41.5 %) in comparison with group of control pathology – 1623.02 (Fig. 1).

Skin damage due to the burn can be caused by the direct loss of protein because of hemorrhaging and necrosis. This state is accompanied by an increased protein and carbohydrate supply resulting in a metabolism change. Major disturbances of protein metabolism results in arrested development of granulation tissue, and epithelialization. Also, it disturbs the healing process. That's why it makes sense to determine level of total protein (TP) and C-reactive protein (CRP) in the blood to estimate the treatment efficacy.

A statistically significant decrease of the total protein level (1.4×) took place after the simulation of burns caused by acetic acid in comparison with control group (Table 2). Increase of total protein level (1.1×) had been noticed after the application of cream with ceramides, but the level of TP did not get the intact values. The level of TP increased significantly (1.24×) in the group treated by the cream with dexpanthenol and it was close to the intact values as evidenced by the suppression of irritation in necrotic areas of skin.

| Table 1. Dynamic pattern of healing area (mm$^2$) under the influence of investigated medicinal products on the chemical burn model (n=6) *
<table>
<thead>
<tr>
<th>Days of treatment</th>
<th>Control pathology</th>
<th>Cream with ceramides</th>
<th>Cream with dexpanthenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial wound area</td>
<td>Healing area</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>263.83±14.94</td>
<td>33.17±2.02 *</td>
<td>35.33±2.65 *</td>
</tr>
<tr>
<td>7</td>
<td>258.50±9.41</td>
<td>36.00±6.47</td>
<td>36.67±6.4 *</td>
</tr>
<tr>
<td>9</td>
<td>269.83±5.97</td>
<td>35.33±4.95 *</td>
<td>129.33±5.75 *</td>
</tr>
<tr>
<td>11</td>
<td>174.83±10.98</td>
<td>96.67±6.42 *</td>
<td>192.83±8.35 *</td>
</tr>
<tr>
<td>13</td>
<td>197.83±10.98</td>
<td>233.33±8.99 *</td>
<td>260.00±5.66 * **</td>
</tr>
<tr>
<td>15</td>
<td>217.17±14.91</td>
<td>251.33±9.25 *</td>
<td>280.00±7.33 * **</td>
</tr>
<tr>
<td>17</td>
<td>233.83±13.03</td>
<td>258.50±9.41</td>
<td>287.17±8.43 *</td>
</tr>
</tbody>
</table>

* – deviation is statistically significant in relation to the group of control pathology, p<0.05; ** – deviation is statistically significant in relation to the cream with ceramides, p<0.05 (Mann-Whitney test); n – number of animals in the group

Statistically significant increase of the CRP level was 23.4× in comparison with the intact group which provides evidence of the development of necrotic inflammatory process at the peak of pathology. After completion of the experiment, the level of CRP in all groups decreased significantly but in different manner – 4.2× in the group of control pathology; after the treatment by the cream with ceramides – 10.6×; after the treatment by the cream with dexpanthenol – 15.4×. Such changes are evidence of a reduction of adverse reactions.

A significant increase of creatinine level (2×) in rats’ blood serum shows the intensity of destructive changes in the development of chemical burn (peak of pathology). This parameter in the group of CP changed little as evidenced by the stability of necrotic processes. After
the treatment of animals, a lowering of the creatinine level occurred as evidenced by the completion of necrotic phase of wound process. Creatinine level decreased significantly in the group of animals treated by the cream with dexpanthenol – 1.3× compared to the group of maximum pathology and this was significantly less than in the group of intact animals. Normalization of creatinine level was observed after the treatment by the ceramide-based cream but the values were unreliable.

**Table 2.** Biochemical parameters after the treatment by investigated medicinal products using the model of chemical burn (n=6) *  

<table>
<thead>
<tr>
<th>Test group</th>
<th>Total protein (C, g/L)</th>
<th>CRP (C, mg/L)</th>
<th>Creatinine (C, µmol/L)</th>
<th>SH-(C, mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Intact group (healthy animals – basic data)</td>
<td>73.70±3.5</td>
<td>0.48±0.04</td>
<td>55.11±3.86</td>
<td>12.74±0.74</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum pathology (necrosis occurs – third day after subcutaneous injection of 9% acetic acid solution)</td>
<td>51.1±1.76*</td>
<td>11.24±0.31*</td>
<td>111.34±9.01*</td>
<td>22.46±1.22*</td>
</tr>
<tr>
<td>Control pathology (17th day of observation)</td>
<td>58.24±3.83*</td>
<td>2.66±*</td>
<td>109.66±7.83*</td>
<td>18.28±1.12*</td>
</tr>
<tr>
<td>Group 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream with ceramides (17th day of observation)</td>
<td>58.98±3.76*</td>
<td>1.06±*</td>
<td>96.16±6.33*</td>
<td>15.70±0.89*</td>
</tr>
<tr>
<td>Cream with dexpanthenol (17th day of observation)</td>
<td>63.52±</td>
<td>0.73±*</td>
<td>87.73±</td>
<td>11.67±</td>
</tr>
</tbody>
</table>

**Discussion**

The variation in the severity of certain pharmacological effects of hydrophilic creams containing dexpanthenol or ceramides can be explained by the difference in their pharmacological action.

When tissues are damaged, the necessity for structural (proteins, carbohydrates, lipids, water), energy (vitamins, particularly pantothenic acid) and other biomaterials increases sharply. This is due to the fact that these materials take part in different biochemical processes, accelerating them and, thereby, restoring damaged tissues. It is known that when applied topically, dexpanthenol is readily absorbed and rapidly converted enzymatically to pantothenic acid, a constituent of coenzyme A, which plays an important role in cellular metabolism (synthesis of ATP, acetylglucosamines and mucopolysaccharides, disposing of products of amino acid deamination, optimization of fatty acids and phospholipid metabolism).

Apart from reparative properties, pantothenic acid exhibits anti-inflammatory (it takes part in the synthesis of anti-inflammatory hormones) and immunomodulatory (it stimulates antibody production) effects. The main components of ceramides are glyceroceramides, cholesterol, and phospholipids. Glycoceramides decrease metabolic cost and loss of structural material for synthesis, renewing the content of endogenous glyceroceramides, as well as work within lipid lamellar systems of the intercellular space facilitating restoration of epidermis and reducing dehydration. Phospholipids and cholesterol play an important role in the regeneration of the lipid bilayer. Pantothenic acid and cholesterol are necessary for synthesis of steroid hormones. It should be noted that hydration of skin is considered to be one of the most promising approaches for optimization of regeneration processes as it prevents extremely dry skin and enhancement of necrosis, preventing the development of cicatricial deformities.

**Conclusion**

In planimetric studies on the model of chemical burn it was shown that application of cream with dexpanthenol and cream with ceramides facilitate healing necrotic ulcers.
cers on the skin. Cumulative reparative effect of cream with ceramides was 29%, but the reparative effect of cream with dexpanthenol was 41.5%. We consider the advantages of cream containing dexpanthenol is associated with its penetration in all skin layers and its ability to accelerate cell fission. This active substance effects the strength of collagen and restores the skin structure while ceramide-cream acts in the surface layers of epidermis and promotes cell repair.

Biochemical research proved an efficacy of treatment with investigated creams. In terms of CRP level and content of SH-groups therapeutic action of cream with dexpanthenol was highly significant by a factor of 1.45 and 1.35, respectively, compared to the cream with ceramides.

Application of drugs containing dexpanthenol and ceramide, that are wound-healing agents with different reparative action, is a promising and reasonable approach for the treatment of burn wounds.

Declarations
Funding
Authors have no commercial interest and financial interest. The costs of the research were covered by the researchers.

Author contributions
Conceptualization, Y.B. and T.T.; Methodology, Y.B.; Software, T.T.; Validation, Y.B.; Formal Analysis, YB; Investigation, Y.B.; Resources, T.T.; Data Curation, Y.B.; Writing – Original Draft Preparation, Y.B. and T.T.; Writing – Y.B. and T.T.; Visualization, Y.B. and T.T.; Supervision, Y.B.; Project Administration, Y.B.; Funding Acquisition, Y.B.

Conflicts of interest
The authors have no conflict of interest.

Data availability
The datasets used and/or analysed during the current study are open from the corresponding author on reasonable request.

Ethics approval
The ethical approval was obtained from Ethics Committee of Clinical and Diagnostics Center of National University of Pharmacy (NUPh), Kharkiv (protocol No. 2 dated February 19, 2019).

References