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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, mm^2) 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

Introduction

Currently, chemical burns are a very common trauma.[1-3] Many substances that are freely available in the community, either occupational or domestic items, have the potential to cause chemical burns. Every year up to 10000 cases of chemical burns are registered in Ukraine and 50-70% patients have no need for operative intervention.[4-6]

It should be noted that chemical burns commonly occur as a result of chemical ingestion that causes tissue-necrosis (for example, concentrated acids – acetic, sulfuric, hydrochloric and others). Depending on the concentration and exposure time to substance, deep lesions on skin can form as coagulation necrosis that caused by the coagulation of proteins due to quick elimination of water from the epidermis.[1-3] Significant intoxication is characteristic for deep burns due to the formation of toxic protein cleavage products and its absorption.[1,4] On the other hand, diluted acids result in edema and blisters on the skin because of migration of cellular water under epidermis. Such kinds of burns are interfacial and have a large area.

Local treatment of burn lesions using wound-healing agents is a beneficial. For now, dexpanthenol is a drug of choice because it is absorbed quickly by skin, converting to pantothenic acid that is a component of coenzyme acetyl-CoA that plays a significant role in cellular metabolism (synthesis of ATP, acetylglucosamines, mucopolysaccharides, elimination of waste products of deamination of amino acids, optimization of fatty acid and phospholipids metabolism).[6] It is known that pantothenic acid exhibits reparative properties that facilitate division of cells, make collagen fibers stronger, rejuvenates skin and also has anti-inflammatory action.[7] Nowadays, there are a lot of hydrophobic creams and ointments with dexpanthenol. This hydrophobic base promotes formation of a lipid layer on the skin and inhibits qualitative wound healing.[4] This is why development of hydrophilic topical medications is currently important.

Humidification of skin is considered to be one of the most prospective approaches to the optimization of regenerative processes of skin because it prevents excessive dryness of skin, deepening of necrosis and development of cicatricial malformations. One of the methods of skin correction is an application of ceramide-based cosmetic products because ceramides facilitate restoration of the epidermis and a decrease in skin dehydration.[8-10]

Aim

The aim of this research was a comparison of treatment efficacy of hydrophilic creams containing ceramide or dexpanthenol on the model of chemical burn.

Material and methods

Experiments were performed in 30 rats, weighing 190–220 g, which were divided in 5 groups of animals (n=6): 1 group – control group (healthy animals); 2 – peak of pathology (formation of chemical burn); 3 – control pathology (CP) – chemical burn was produced in animals and they were not treated in the experiment; 4 – animals with chemical burn treated with cream containing ceramides; 5 – animals with chemical burn treated with cream containing dexpanthenol. The technique for manufacturing creams with ceramides (composition: 0.5% of ceramides, hydrophilic base up to 100 g) and dexpanthenol (composition: 5 % of dexpanthenol, hydrophilic base up to 100 g) was developed in the State Scientific and Research Center of Medicinal products under supervision of Prof. M.O. Lyapunov.

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.[4] 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 (cm^2) 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, mm^2) and cumulative reparative effect.

Surface area of necrotic tissue (S, mm^2) was calculated according the following formula:

$$S = S_{initial} - S_{(t)}, \text{ where}$$

$S_{initial}$ – initial wound area, mm^2;

$S_{(t)}$ – wound area at the day of measurement, mm^2.

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

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 – 5,5′-Dithiobis (2-nitrobenzoic acid) (also called DTNB or Ellman′s reagent).[4]

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.[11]

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.[11] 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).

Table 1. Dynamic pattern of healing area (mm^2) under the influence of investigated medicinal products on the chemical burn model (n=6) [a]

Days of treatment	**Study groups**		
	Control pathology	**Cream with ceramides**	**Cream with dexpanthenol**
Initial wound area	263.83±14.94	258.50±9.41	287.17±8.43
		Healing area	
5	13.50±6.34	33.17±2.02 *	35.33±2.65 *
7	36.00±6.47	96.67±6.4 *	100.67±7.27 *
9	94.83±5.97	123.33±4.95 *	129.33±5.73 *
11	135.17±10.98	174.67±6.52 *	192.83±8.56 *
13	197.83±10.98	233.33±8.99	260.00±6.56 */**
15	217.17±14.91	251.33±9.25	280.00±7.33 */**
17	233.83±13.03	258.50±9.41	287.17±8.43 *

[a] * – 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

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).

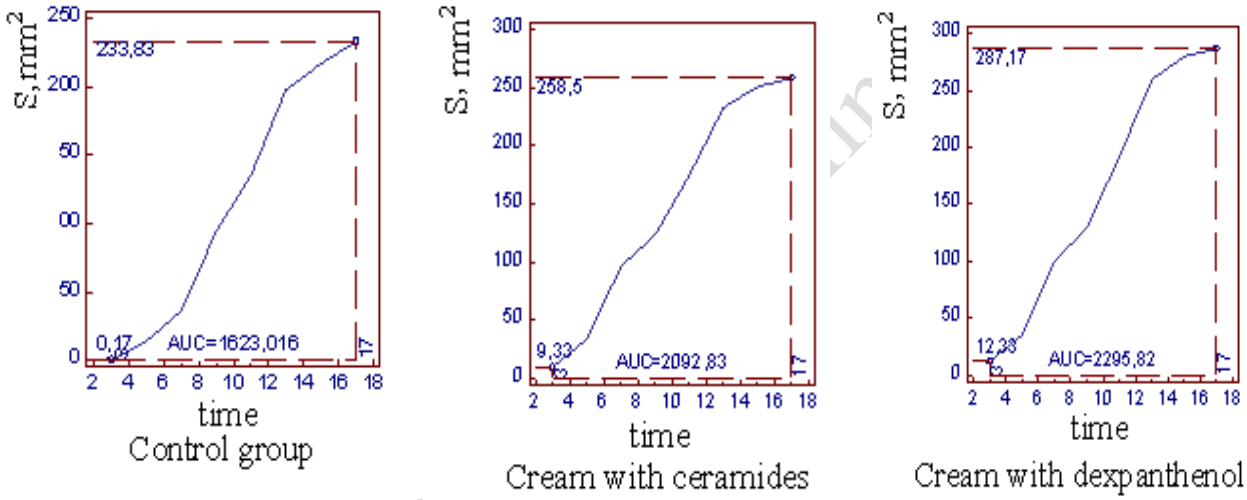

Fig. 1. Total reparative effect for the cream with dexpanthenol and cream with ceramides on the model of chemical burn

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

Test group	Total protein (C, g/L)	CRP (C, mg/L)	Creatinine (C, μmol/L)	SH- (C, mmol/L)
Group 1 Intact group (healthy animals – basic data)	73.70±3.5	0.48±0.04	55.11±3.86	12.74±0.74
Group 2 Maximum pathology (necrosis occurs – third day after subcutaneous injection of 9% acetic acid solution)	51.11±1.76 *	11.24±0.31 *	111.34±9.01 *	22.46±1.22 *
Group 3 Control pathology (17th day of observation)	58.24±3.83 *	2.66± 0.08*/**	109.66±7.83 *	18.28±1.12 */**
Group 4 Cream with ceramides (17th day of observation)	58.98±3.76 *	1.06± 0.03 */**/***	96.16±6.33 *	15.70±0.89 */**
Group 5 Cream with dexpanthenol (17th day of observation)	63.52± 1.19 */**	0.73± 0.04 */**/***	87.73± 3.26 */***	11.67± 0.82 **/***

[a] * – significant difference in relation to intact animals, $p<0.05$; ** – in relation to maximum pathology, $p < 0.05$; *** – in relation to control pathology, $p<0.05$ (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.

Changes of the state of antioxidant system (AOS) evidence the intensity of the inflammatory necrotic processes in the course of the experiment. After the formation of burn (peak of pathology) statistically significant increase of the content of SH-group (1.76×) was observed in comparison with intact animals (Table 2). These changes provide evidence of the activation of lipid peroxidation and elevation of antioxidant defense during the development of inflammatory necrotic process.

After the completion of the experiment state of AOS was normalized as evidenced by the lowering of the content of SH-group in all test groups (Table 2). Thus, this parameter decreased 1.2× in the group of control pathology; 1.4× in the group treated by the cream with ceramides; 1.9× in the group treated by the cream with dexpanthenol.

Summing up what has been said according the results of the planimetric and biochemical investigations, it was found that developed hydrophilic medicinal products (cream with ceramides and cream with dexpanthenol) have evident reparative properties as evidenced by promoted healing compared to control group. The therapeutic action of cream with ceramides matches the action of cream with dexpanthenol for first 11 days of treatment course. On day 13 and 15 the reparative action of cream with dexpanthenol was significantly better than the action of cream with ceramides. Biochemical research demonstrated an efficacy of treatment with investigated creams. In accordance with the results of CRP level determination and SH-group content, the efficacy of cream with dexpanthenol vs. cream with ceramides was significantly better.

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.[4] 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.[5] The main components of ceramides are glycoceramides, cholesterol, and phospholipids. Glycoceramides decrease metabolic cost and loss of structural material for synthesis, renewing the content of endogenous glycoceramides, 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.[9]

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 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).

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