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THE STUDY OF THE SPECIFIC PHARMACOLOGICAL ACTIVITY OF DERMABIN ON THE MODEL OF THE EXPERIMENTAL CONTACT DERMATITIS IN RATS

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Key words: psoriasis; contact dermatitis; DERMABIN; betamethasone dipropionate; salicylic acid

In spite of a great number of the existing drugs and methods, as well as introduction of the new ones, the problem of improving the treatment of patients with psoriasis and psoriatic arthritis is still one of the most burning and topical in modern dermatology. In case of such disorders, glucocorticoids are the most effective if combined with keratolytics – the effective agents facilitating the drug penetration directly to the affected area. The aim of the work was to study the specific pharmacological activity of Dermabin medicine on the model of contact dermatitis in rats compared to Diprosalik. In order to reproduce this pathology rats were applied 2,4-dinitrochlorobenzene in the form of 5% ethanol–acetone solution twice a day for 4 days. In 4 days after the first application the area affected by contact dermatitis (CD) was covered with Dermabin or the reference medicine Diprosalic for 14 days. The severity of psoriasis developed was assessed daily based on the change of the skin covering indices: the skin fold thickness and the severity of skin manifestation. It was registered that in animals with the test and reference medicines applied the hemorrhagic crusting began exfoliating on the 4th day of the treatment, which was twice faster than in rats of the control group, and the skin fold thickness significantly decreased. On the 7th and 10th days of the treatment the progressive improvement of the CD affected area was observed in these animals. On the 14th day all rats receiving the active treatment showed significant restoration of their appearance, and the ESR index decreased almost to the level observed in intact animals. At the same time the severity of leucocytosis also reduced. Therefore, the results obtained indicate the marked anti-inflammatory activity of Dermabin medicine in rats with the experimental model of contact dermatitis.

Psoriasis is a chronic recurrent genetically determined disease of multifactorial nature, which is characterized by staging, immune-dependent inflammation, benign hyperproliferation of epidermal cells with their abnormal differentiation accompanied with papulosquamous rash, as well as the possible involvement of the locomotor apparatus and visceral organs to the pathological process. The main peculiarities of this dermatosis are the etiology of the disease that has not been entirely understood, complexity of pathogenic mechanisms of development, frequent recurrence with short disease-free survivals, impairment of the patient's life, decrease of their professional activity and social integration, the frequent development of complicated forms, the increase in the number of cases of the patients' long-term incapacity for employment and disability [10, 13].

The main cause of this autoimmune disease is considered to be disorders of the immune system, which attacks the cells of the own organism by mistake leading to skin inflammation and excessive cell proliferation [17]. The results of many studies prove the leading role of T-lymphocytes and macrophages in development of autoimmune aggression upon skin cells [11, 18]. In recent years much data on the important role of chemokines in the processes of leukocyte migration and distribution in psoriasis have appeared. It has been shown that populations of effector T-lymphocytes release CLA and CCR4 chemokines [8, 21]. CCL20, CCL27 and monokine induced by γ-interferon (MIG) participate in involvement of T-cells to the psoriatic rash zone [14, 15, 21]. Moreover, in psoriasis the release of angiogenic factors and pro-inflammatory cytokines intensifies, and the level of anti-inflammatory cytokines decreases [9, 22].

In spite of a great number of the existing drugs and methods, as well as introduction of the new ones, the problem of improving the treatment of patients with psoriasis and psoriatic arthritis is still one of the most burning and topical in modern dermatology. The treatment success criteria are the time of the clinical effect onset, the skin cleansing from rash, remission duration, improvement of quality of the patient's life. Not least important is the high level of safety, tolerability and comfort of application [4, 19]. The most active drugs of the anti-inflammatory therapy are glucocorticoids possessing the vasoconstrictive and membrane-stabilizing action; they have the suppressor effect on immune factors, inhibit production of pro-inflammatory cytokines and reduce cell proliferation in the affected area [20].

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In case of the marked keratinisation disorders the efficiency of the therapy with topical steroids is significantly increased if combined with keratolytics – the effective agents facilitating the drug penetration directly to the affected area. Combined medicines of corticosteroids with keratolytics, in particular with salicylic acid, provide more significant therapeutic action in case of psoriasis than each drug individually. Salicylic acid has the keratolytic effect at the stratum corneum level of the epidermis, and a corticosteroid penetrates to deeper skin layers and inhibits the synthesis of pro-inflammatory cytokines and reduces the vessel wall permeability in the uppermost layer of the dermis [16]. Dermabin ointment (produced by "Pharmaceutical Plant "Biofarma" LLC) contains salicylic acid and betamethasone dipropionate. Therefore, the aim of the work was to study the specific pharmacological activity of Dermabin medicine on the model of contact dermatitis in rats compared to Diprosalik.

Materials and Methods

The specific pharmacological activity of Dermabin ("Pharmaceutical Plant "Biofarma" LLC, Bila Tserkva, Ukraine, ointment in tubes, 15 g; its 1 g contains betamethasone dipropionate, being equivalent to 0.5 mg of betamethasone, and 30 mg of salicylic acid) was assessed on the model of contact dermatitis (CD). In order to reproduce this pathology rats were applied 2,4-dinitrochlorobenzene in the form of 5% ethanol–acetone solution on the skin areas (9 cm^2) previously clipped twice a day for 4 days [2]. In 4 days after the first application the affected CD area was covered with Dermabin or the reference medicine Diprosalic (Schering-Plough Labo N.B., Belgium, ointment in tubes, 15 g; its 1 g contains betamethasone dipropionate, being equivalent to 0.5 mg of betamethasone, and 30 mg of salicylic acid). The medicines were applied daily once a day for 14 days. The severity of psoriasis developed was assessed daily based on change of the skin covering indices: the skin fold thickness and the severity of skin manifestation. The skin manifestation severity was assessed in points from 0 to 5 by the skin testing scale [6]: 0 – no reaction; 0.5 – appearance of local foci of hyperemia; 1 – marked hyperemia; 2 – hyperemia and edema; 3 – abrupt rubefaction and edema; 4 – formation of erosions; 5 – hemorrhagic crusting formation.

The experiments were carried out on 40 male white non-pedigree rats weighing 190–230 g. All experimental animals were kept in the vivarium in compliance with sanitary standards on the required diet [7]. All studies were performed in accordance with regulations of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" [12]. After 10-days isolation period the experimental rats were divided into the following groups by the randomization method: group 1 – the intact control (n=10); group 2 – animals with the experimental CD where Vaseline (ointment in tubes, 30 g, produced by "Phytopharm" PJSC, Ukraine) was applied on the affected areas (n=10); group 3 – animals with the experimental CD + Dermabin (n=10); group 4 – animals with the experimental CD + Diprosalic (n=10).

The assessment of the manifestation severity in the CD area and measurement of the skin fold thickness were performed on the 1^{st}, 4^{th}, 7^{th}, 10^{th} and 14^{th} day of the experiment. The anti-inflammatory activity of the test and reference medicines was determined on the 14^{th} day of the experiment [1].

The peripheral blood indices were determined before application of the medicines and on the 14^{th} day of the experiment. The erythrocyte sedimentation rate (ESR), white blood count and the percentage ratio of certain leukocyte subpopulations in the peripheral blood was determined using common methods [3].

Statistical processing of the results was performed by STATISTICA 6.0 application program package (StatSoft, USA) using Student's t-test, Mann-Whitney U-test and Wilcoxon signed-ranks test [5]. At $p<0.05$ the differences between control and experiment were considered to be significant.

Results and Discussion

The results showed that after 4 days of 2,4-dinitrochlorobenzene application the CD area was formed on the rats' skin, it was characterized by vesicular skin rash, excoriation and formation of a thick, heavy and deep hemorrhagic crusting. In the affected area and dependent areas the edema and inflammatory cells developed, the skin fold increased, its thickness reached 5–6 mm, while in the intact animals it was on the average 2.2 mm ($p<0.05$, Table 1). The skin manifestation severity in three experimental groups was 4.8±0.13 points ($p<0.05$ compared to the group of intact animals where it was 0). This day was considered to be the first day of the therapy, when application of Vaseline, Dermabin and the reference medicine Diprosalic was started directly on the CD area.

In the animals of group 2 (CD + Vaseline) improvements occurred on the 10^{th} day of observation and were evident by the decrease of the skin damage severity on the average up to 4.1 points (Table 1). The skin fold thickness decreased by 25% compared to the corresponding index on the 1^{st} day. In that period in 30% of rats of this group almost compete exfoliation of hemorrhagic crusting formed during dinitrochlorobenzene application was observed, and in the rest of animals only some fragments remained. Complete exfoliation in all animals was over by the 14^{th} day of observation, however, in that period hyperemia and a slight increase of skin

fold thickness was observed compared to intact animals.

In the animals of groups 3 and 4 with application of the test and reference medicine on the CD area the hemorrhagic crusting began exfoliating on the 4th day of the treatment, which was twice faster than in rats of CD + Vaseline group. In that period the skin fold thickness decreased by 10.9% each in CD + Dermabin group and CD + Diprosalic group ($p<0.05$; Table 1). On the 7th and 10th days of the treatment the progressive improvement of the CD affected area was observed. In the groups of the test and reference medicine the skin fold thickness on the 7th day was 17.8 and 15.6% ($p<0.05$), and on the 10th day – 20.9 and 18.6% ($p<0.05$) less compared to the corresponding indices of animals of group 2 (Table 1). On the 14th day all rats receiving active treatment showed a significant restoration of their appearance. In CD + Dermabin group the skin fold thickness decreased by 27.8% ($p<0.05$), and in CD + Diprosalic group by 22.2% ($p<0.05$; Table 1). Therefore, both medicines have the same effect on the abovementioned parameters of the experimental CD in rats. Comparative values of the anti-inflammatory activity of both medicines confirm this: Dermabin – 55.2% and Diprosalic – 58.6%.

Table 1

The effect of Dermabin and Diprosalic on the severity of contact dermatitis (CD) in rats (M±m), n=40

Day of the study	The group of animals	The severity of skin manifestations, score	The skin fold thickness, mm
	Intact	0	2.2 ± 0.13
Day 1	CD + Vaseline	4.8 ± 0.13#	5.7 ± 0.15#
	CD + Dermabin	4.8 ± 0.13#	5.6 ± 0.16#
	CD + Diprosalic	4.8 ± 0.13#	5.5 ± 0.17#
Day 4	CD + Vaseline	4.7 ± 0.15	5.5 ± 0.17
	CD + Dermabin	4.4 ± 0.16	4.9 ± 0.10*
	CD + Diprosalic	4.3 ± 0.15	4.9 ± 0.18*
Day 7	CD + Vaseline	4.5 ± 0.17	4.5 ± 0.22
	CD + Dermabin	3.9 ± 0.18*	3.7 ± 0.15*
	CD + Diprosalic	3.8 ± 0.20*	3.8 ± 0.13*
Day 10	CD + Vaseline	4.1 ± 0.10	4.3 ± 0.21
	CD + Dermabin	3.0 ± 0.15*	3.4 ± 0.16*
	CD + Diprosalic	3.0 ± 0.21*	3.5 ± 0.17*
Day 14	CD + Vaseline	2.9 ± 0.18	3.6 ± 0.16
	CD + Dermabin	1.3 ± 0.15*	2.6 ± 0.16*
	CD + Diprosalic	1.2 ± 0.13*	2.8 ± 0.20*

Note. # – $p<0.05$ in relation to the intact control group; * – $p<0.05$ in relation to CD + Vaseline group.

During the experiment the changes of the peripheral blood indi-

Table 2

Indices of the peripheral blood of rats with the experimental contact dermatitis (CD) after dermal application of Dermabin and Diprosalic for 14 days (M±m), n=40

Index	The group of animals			
	Intact	CD + Vaseline	CD + Dermabin	CD + Diprosalic
Day 1				
ESR, mm/h	2.3 ± 0.30	5.3 ± 0.50#	5.3 ± 0.30	5.4 ± 0.43
Leucocytes, $\times 10^9$/l	7.29 ± 0.49	11.29 ± 0.81#	11.5 ± 0.66	11.32 ± 0.96
Segmented neutrophils, %	21.2 ± 0.89	32.3 ± 1.26#	32.6 ± 1.51	33.2 ± 1.43
Banded neutrophils, %	1.5 ± 0.17	3.7 ± 0.30#	3.3 ± 0.37	3.5 ± 0.34
Eosinophils, %	2.0 ± 0.33	3.1 ± 0.23#	2.9 ± 0.35	3.0 ± 0.26
Lymphocytes, %	72.2 ± 0.93	57.4 ± 1.31#	57.8 ± 1.49	56.7 ± 1.46
Monocytes, %	3.1 ± 0.31	3.5 ± 0.22	3.4 ± 0.16	3.6 ± 0.16
Day 14				
ESR, mm/h	2.1 ± 0.23	4.1 ± 0.28#	3.0 ± 0.26*	3.1 ± 0.31*
Leucocytes, $\times 10^9$/l	7.29 ± 0.43	9.50 ± 0.49#	7.99 ± 0.45*	7.88 ± 0.49*
Segmented neutrophils, %	22.3 ± 1.16	30.3 ± 0.88#	27.0 ± 0.97*	27.5 ± 0.95*
Banded neutrophils, %	1.7 ± 0.15	2.4 ± 0.16#	2.0 ± 0.21	1.9 ± 0.23
Eosinophils, %	2.1 ± 0.23	2.5 ± 0.27	2.3 ± 0.21	2.4 ± 0.16
Lymphocytes, %	70.7 ± 1.13	61.4 ± 0.99#	65.1 ± 1.13*	64.9 ± 1.11*
Monocytes, %	3.2 ± 0.20	3.6 ± 0.31	3.3 ± 0.30	3.4 ± 0.43

Note. # – $p<0.05$ in relation to the intact control group; * – $p<0.05$ in relation to CD+Vaseline group.

ces in all animal groups were also studied (Table 2). Before the treatment all animals with the simulated CD showed a typical reaction on inflammation, namely the double increase of ESR, leucocytosis, the increase of the neutrophil percentage with a slight, but significant shift to the left.

The statistically probable increase in the eosinophil count was also registered. The lymphocyte count in animals of the experimental groups was significantly less than in intact animals.

The study of ESR and the peripheral blood cell composition conducted on the 14th day of the treatment showed a significant improvement in the group of rats with application of Dermabin or Diprosalic medicines on the CD affected area (Table 2). The ESR index in rats of this group decreased almost to the level observed in intact animals. At the same time the severity of leucocytosis also reduced. The percentage of the main leucocyte subpopulations reduced almost to the level of intact rats. Both medicines (Dermabin and Diprosalic) had almost the same effect on the peripheral blood indices of rats with the experimental CD. Concerning the indices in CD + Vaseline group there were significant differences from the indices of intact animals: the high ESR and leucocytosis were observed. The percentage of segmented and banded neutrophils was much higher, and that of lymphocytes was lower than in intact animals (Table 2).

CONCLUSIONS

Therefore, the results obtained indicate the marked anti-inflammatory activity of Dermabin medicine ("Pharmaceutical Plant "Biofarma" LLC) in rats with the experimental model of contact dermatitis. Under conditions of the local inflammatory response induced by 2,4-dinitrochlorobenzene application this medicine significantly reduces the severity of dermatitis developed and decreases the system indices of the inflammatory process. By its properties and composition Dermabin medicine is highly competitive with the reference medicine Diprosalic.

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ДОСЛІДЖЕННЯ СПЕЦИФІЧНОЇ ФАРМАКОЛОГІЧНОЇ АКТИВНОСТІ ПРЕПАРАТУ «ДЕРМАБІН» НА МОДЕЛІ ЕКСПЕРИМЕНТАЛЬНОГО КОНТАКТНОГО ДЕРМАТИТУ У ЩУРІВ

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Ключові слова: псоріаз; контактний дерматит; дермабін; бетаметазону дипропіонат; саліцилова кислота

Незважаючи на значну кількість вже існуючих та появу нових лікарських засобів та методів терапії, проблема вдосконалення лікування хворих на псоріаз та псоріатичний артрит залишається однією з найгостріших та найактуальніших у сучасній дерматології. Найбільш активними засобами протизапальної терапії при цих захворюваннях є глюкокортикостероїдні гормони в комбінації з кератолітичними засобами – ефективними агентами, які полегшують проникність ліків безпосередньо у вогнище ураження. Метою роботи було дослідження специфічної фармакологічної активності препарату «Дермабін» у порівнянні з препаратом «Дипросалік» на моделі контактного дерматиту у щурів. Для відтворення цієї патології щурам протягом 4-х днів двічі на добу робили аплікації 2,4-динітрохлоробензолу у вигляді 5% етанол-ацетонового розчину. Через 4 доби після першої аплікації на вогнища уражень наносили препарат «Дермабін» або референтний препарат «Дипросалік» протягом 14 днів. Тяжкість розвиненого дерматиту оцінювали щодня за змінами показників шкірного покриву: величиною шкірної складки і тяжкістю шкірних проявів. Показано, що у тварин, яким на вогнище ураження наносили дані препарати, вже на 4-ту добу лікування почала відокремлюватись геморагічна кірка вдвічі швидше, ніж у щурів контрольної групи, а також спостерігалося вірогідне зменшення товщини шкірної складки. На 7-му та 10-ту добу терапії у цих тварин відзначено поступове поліпшення стану вогнища контактного дерматиту. На 14-ту добу у всіх щурів, які отримували активне лікування, спостерігалося значне відновлення зовнішнього вигляду, а значення ШОЕ знизилося майже до рівня, який спостерігався в інтактних тварин. Одночасно зменшилася і вираженість лейкоцитозу. Таким чином, отримані нами результати свідчать про виражену протизапальну активність препарату «Дермабін» у щурів з експериментальною моделлю контактного дерматиту.

ИССЛЕДОВАНИЕ СПЕЦИФИЧЕСКОЙ ФАРМАКОЛОГИЧЕСКОЙ АКТИВНОСТИ ПРЕПАРАТА «ДЕРМАБИН» НА МОДЕЛИ ЭКСПЕРИМЕНТАЛЬНОГО КОНТАКТНОГО ДЕРМАТИТА У КРЫС

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Несмотря на значительное количество уже существующих и появление новых средств и методов терапии, проблема совершенствования лечения больных псориазом и псориатическим артритом остается одной из самых острых и актуальных в современной дерматологии. Наиболее активными средствами противовоспалительной терапии при этих заболеваниях являются глюкокортикостероидные гормоны в сочетании с кератолитическими средствами – эффективными агентами, которые облегчают проницаемость лекарств непосредственно в очаг поражения. Целью нашей работы было исследование специфической фармакологической активности препарата «Дермабин» в сравнении с препаратом «Дипросалик» на модели контактного дерматита у крыс. Для воспроизведения этой патологии крысам в течение 4-х дней дважды в сутки делали аппликации 2,4 динитрохлорбензола в виде 5% этанол-ацетонового раствора. Через 4 дня после первой аппликации на очаги поражений наносили препарат «Дермабин» или референтный препарат «Дипросалик» в течение 14 дней. Тяжесть развившегося дерматита оценивали ежедневно по изменениям показателей кожного покрова: величине кожной складки и тяжести кожных проявлений. Показано, что у животных, которым на очаг поражения наносили исследуемый и референтный препараты, уже на 4-е сутки лечения начала отторгаться геморрагическая корка вдвое быстрее, чем у крыс контрольной группы, а также наблюдалось достоверное уменьшение толщины кожной складки. На 7-е и 10-е сутки терапии у этих животных отмечено постепенное улучшение состояния очага контактного дерматита. На 14-е сутки у всех крыс, получавших активное лечение, наблюдалось значительное восстановление внешнего вида, а значение СОЭ снизилось почти до уровня, который наблюдался в интактных животных. Одновременно уменьшилась и выраженность лейкоцитоза. Таким образом, полученные нами результаты свидетельствуют о выраженной противовоспалительной активности препарата «Дермабин» у крыс с экспериментальной моделью контактного дерматита.

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