THE EFFECT OF DICLOFENAC SODIUM ON BONE MARROW CELLULARITY, THE LSK CELL POOL, AND CYTOKINE PROFILES IN MICE WITH EXPERIMENTAL DERMATITIS

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Nonsteroidal anti-inflammatory drugs (NSAIDs), one of which is diclofenac sodium, are widely used in clinical practice. This study aimed to assess the effect of diclofenac sodium on bone marrow cellularity, the population of hematopoietic stem and progenitor cells (Lin-Sca-1+ c-Kit+, LSK), as well as the levels of tumor necrosis factor — (TNFa) and interleukin-1b (IL1b) in laboratory mice with experimental dermatitis. The study was a randomized controlled trial; the experimental phase lasted 96 hours. Male BALB/c mice (n = 6, age 46 weeks, 18–25 g) were randomized into five groups: intact, untreated dermatitis, dermatitis + diclofenac 1.5 mg/kg, dermatitis + diclofenac 3 mg/kg, diclofenac 3 mg/kg without dermatitis. The drug was administered intramuscularly 2 times a day for 96 hours. Dermatitis was modeled using sodium dodecyl sulfate and *Dermatophagoides farinae*. Bone marrow was collected after 96 hours. The total number of cells was determined by an automatic counter, the LSK cell population by flow cytometry, and cytokine concentrations by ELISA. Statistical analysis was performed using ANOVA ($p < 0.05$). We observed a decrease in bone marrow cellularity in dermatitis groups (0.95 ± 0.12 versus 1.20 ± 0.15 × 10^6/ml; $p < 0.05$). Diclofenac 1.5 mg/kg decreased cellularity moderately (0.80 ± 0.10 × 10^6/ml) and an increased LSK cells (3.8 ± 0.5% vs. 2.1 ± 0.3%; $p < 0.05$). Diclofenac 3 mg/kg markedly brought down cellularity (0.60 ± 0.08 × 10^6/ml) and LSK cells (1.4 ± 0.3%; $p < 0.01$), and brought up IL1b (15.5 ± 1.2 pg/ml) and TNFa (82.1 ± 4.5 pg/ml). Our findings indicate a dose-dependent effect of diclofenac sodium on hematopoiesis and inflammatory response.

Keywords: bone marrow, diclofenac sodium, cellularity, undifferentiated cells, tumor necrosis factor α, interleukin-1, inflammation

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Compliance with ethical standards: the study was approved by the Ethics Committee of St. Luka LSMU (Minutes No. 1 of January 27, 2026), conducted in accordance with the Recommendations of the Board of the Eurasian Economic Commission of November 14, 2023 No. 33 "Guidelines for working with laboratory (experimental) animals during preclinical (non-clinical) studies," in compliance with the principles of the European Convention for the Protection of Vertebrates used for Experimental and Other Scientific Purposes, as well as in accordance with the Directive of the European Parliament and the Council of the European Union on the protection of animals used for scientific purposes.

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ДИКЛОФЕНАК НАТРИЯ ВЛИЯЕТ НА КЛЕТОЧНОСТЬ КОСТНОГО МОЗГА, ПУЛ LSK-КЛЕТОК И ЦИТОКИНОВЫЙ ПРОФИЛЬ У МЫШЕЙ С ЭКСПЕРИМЕНТАЛЬНЫМ ДЕРМАТИТОМ

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В клинической практике широкое применение имеют нестероидные противовоспалительные препараты (НПВП), одним из которых является диклофенак натрия. Целью исследования было оценить влияние диклофенака натрия на клеточность костного мозга, популяцию гемопоэтических стволовых и прогениторных клеток (Lin– Sca-1+ c-Kit+, LSK), а также уровни фактора некроза опухоли (TNFα) и интерлейкина-1β (IL1β) у лабораторных мышей с экспериментальным дерматитом в ходе рандомизированного контролируемого исследования на протяжении 96 ч. Самцов мышей линии BALB/c (n = 6, 46 недель, 18–25 г) рандомизировали на пять групп: интактная, дерматит без лечения, дерматит + диклофенак 1,5 мг/кг, дерматит + диклофенак 3 мг/кг, диклофенак 3 мг/кг без дерматита. Препарат вводили внутримышечно 2 раза в сутки в течение 96 ч. Дерматит моделировали с использованием додецилсульфата натрия и аллергена *Dermatophagoides farinae*. Через 96 ч проводили забор костного мозга. Общее количество клеток определяли автоматическим счетчиком, популяцию LSK-клеток — методом проточной цитометрии, концентрации цитокинов — методом ELISA. Статистический анализ выполняли с использованием ANOVA ($p < 0,05$). Установлено снижение клеточности костного мозга при дерматите (0,95 ± 0,12 против 1,20 ± 0,15 × 10^6/мл; $p < 0,05$). Применение диклофенака 1,5 мг/кг сопровождалось умеренным снижением клеточности (0,80 ± 0,10 × 10^6/мл) и увеличением доли LSK-клеток (3,8 ± 0,5% против 2,1 ± 0,3%; $p < 0,05$). Доза 3 мг/кг вызывала выраженное снижение клеточности (0,60 ± 0,08 × 10^6/мл) и LSK-клеток (1,4 ± 0,3%; $p < 0,01$), а также повышение IL1β (15,5 ± 1,2 пг/мл) и TNFα (82,1 ± 4,5 пг/мл). Полученные данные свидетельствуют о дозозависимом влиянии диклофенака натрия на гемопоэз и воспалительный ответ.

Ключевые слова: костный мозг, диклофенак натрия, клеточность, недифференцированные клетки, фактор некроза опухолей α, интерлейкин-1, воспаление

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The continued search for NSAIDs that cause minimal toxicity to rapidly dividing cells — especially bone-marrow cells — is an important objective [1]. Long-term use of NSAIDs can stimulate immune system and change blood concentration of cytokines, many of which are factors or catalysts for bone marrow cell differentiation [2].

NSAIDs may be prescribed for indications besides inflammation. Due to their capability to relieve pain post-surgery, NSAIDs are widely used as analgesics, which subsequently modifies the frequency and the dose of opioid painkillers [1, 3]. Prolonged, uncontrolled use of NSAIDs can damage the gastrointestinal tract, liver, and kidneys, and may also adversely affect the bone marrow, whose cells are in constant mitosis. One of the most popular NSAIDs is diclofenac sodium, which has a pronounced anti-inflammatory effect. In clinical practice, it is most often prescribed for inflammatory conditions like rheumatoid arthritis, arthrosis, ankylosing spondylitis, gynecological, neurological pathology, and also as a post-injury analgesic [2, 4, 5].

The condition of the bone marrow after prolonged use of diclofenac sodium deteriorates: the number of hematopoietic cells decreases, which results in pancytopenia, anemia, and an increased risk of infections [6, 7]. Biotransformation of diclofenac sodium occurs mainly in hepatocytes; 60–65% of the drug is eliminated with urine, 30–35% — with bile through the gastrointestinal tract [8, 9]. Diclofenac sodium can cause bone marrow suppression, resulting in neutropenia or agranulocytosis and reduced erythrocyte, leukocyte, and platelet counts, as well as altered cytokine levels [10]. The latter are small proteins whose primary function is to transmit signals between cells via specific receptors. The signaling mechanism employed by cytokines is similar to that of some protein hormones. The difference is that cytokines, unlike hormones, are produced by many different cells of the body and can act either at a distance (endocrine) or locally (paracrine or autocrine) [11, 12]. Cytokines are typically classified as pro-inflammatory (e.g., interleukin-1β [IL1β] and tumor necrosis factor α [TNFα]) or anti-inflammatory. IL1β are synthesized by myeloid cells as a response to the detected patterns indicating pathogens or damage. These signaling proteins induce synthesis of other cytokines associated with inflammation, in particular TNFα and IL6 (enable bone marrow cell differentiation), and stimulate megakaryocyte progenitor cells, pre-B lymphocytes [13].

TNFα was named for its capability to lead some tumor cells to hemorrhagic necrosis, which gave hope for a cure from cancer. Further studies have established that TNFα promotes the progression of neoplasms and their metastasis [14]. This proinflammatory protein, produced primarily by monocytes and macrophages, stimulates proliferation and differentiation of neutrophils and macrophages and promotes their rapid release into the bloodstream. In case of inflammation or stress, the cytokine inhibits production of erythrocytes and lymphocytes, and promotes generation of myeloid cells [15]. TNFα directly increases the concentration of IL1 and other cytokines, and when an infection enters the body, it makes leukocytes active [16]. Depending on the condition of the body, TNFα acts as both a pro-inflammatory and anti-inflammatory agent, and plays a leading role in the COVID-19-associated cytokine storm [17–21]. Cytokines play an important role in the pathogenesis of inflammatory diseases such as rheumatoid arthritis and ankylosing spondylitis, for which NSAIDs are often prescribed for long-term use [22, 23].

Although atopic dermatitis primarily appears as local skin lesions, current concepts view it as a systemic disease involving primary immune organs. Excessive production of peripheral inflammatory mediators can remotely modulate the functional state of the bone marrow. In particular, a chronic inflammatory signal leads to hyperstimulation of the pool of the earliest hematopoietic stem cells and precursors, LSK cells (lineage (Lin), Sca-1+ c-Kit+ markers), shifting their differentiation towards myelopoiesis and exacerbating systemic cytokine imbalance (including hyperproduction of IL1β and TNFα).

In the context, investigating the effects of diclofenac sodium is a particularly interesting task. Being a non-selective cyclooxygenase inhibitor (COX-1 and COX-2), diclofenac blocks the synthesis of prostaglandins, which are recognized as important regulators of both the skin's barrier properties and the bone marrow's microenvironment. In this study, we used diclofenac sodium as a pathogenetic tool that allows determining the contribution of COX-dependent mechanisms to the realization of the systemic response from bone marrow (its cellularity and pool of LSK cells) in a skin pathology.

This study aimed to evaluate the effect of diclofenac sodium at doses of 1.5 and 3 mg/kg on bone marrow cellularity, the percentage of undifferentiated blasts, and the concentration of pro-inflammatory cytokines (IL1β and TNFα) in mice with experimental dermatitis.

METHODS

The experimental part of the work was performed at St. Luka LGMU in accordance with the requirements of the Recommendations of the Board of the Eurasian Economic Commission of November 14, 2023 No. 33 "Guidelines for Working with Laboratory (Experimental) Animals During Preclinical (Non-clinical) Studies," and in compliance with the principles of the European Convention for the Protection of Vertebrates used for Experimental and Other Scientific Purposes [24, 25].

The experimental model

For the study, we selected 64 male BALB/c mice aged 6 weeks and weighing 18–25 g. The animals were kept in standard vivarium conditions at a temperature of 22 ± 2 °C, relative humidity of 50–60% and a 12–hour light cycle, with unlimited access to food and water.

The mice randomized into groups using a random number generator. The researcher assessing the morphological and biochemical indicators was not aware which group the respective animal belonged to (blind assessment). The groups were as follows: group 1 (n = 12) — control, no dermatitis modeling and no active substance injection; group 2 (n = 10) — experimental dermatitis modeling, no injection of the active substance; group 3 (n = 16) — experimental dermatitis modeling, injection of diclofenac sodium at a dose of 1.5 mg/kg; group 4 (n = 16) — experimental dermatitis modeling, injection of diclofenac sodium at a dose of 3 mg/kg; group 5 (n = 10) — no dermatitis modeling, injection of diclofenac sodium at a dose of 3 mg/kg in order to assess the direct toxic effect of the active substance on the bone marrow.

The rationale behind the selected doses: compared to the dose of 3 mg/kg, the dose of 1.5 mg/kg of diclofenac sodium (Solopharm, Russia) has a less pronounced myelosuppressive effect and triggers a compensatory reaction of blast cells [1, 2, 4, 5]. The active substance was injected intramuscularly into the quadriceps femoral muscle twice a day every 12 hours (8.00 and 20.00) for 96 hours. We also selected dosages to enable comparison, to elicit moderate and pronounced pharmacological effects, and to assess dose–response relationships. The control group received an equivalent volume of saline solution.

Table 1. Bone marrow cellularity and population of undifferentiated cells (LSK) in experimental animals of various groups (M ± SD)

Group	*n*	Total number of cells in 1 ml of bone marrow homogenate	Undifferentiated cells (LSK), %
Group 1 (intact, control)	12	$1.1 \pm 0.1 \times 10^6$	2.0 ± 0.2
Group 2 (modeled dermatitis)	10	$0.95 \pm 0.12 \times 10^{6*}$	2.3 ± 0.4
Group 3 (modeled dermatitis + 1.5 mg/kg diclofenac sodium)	16	$0.4 \pm 0.1 \times 10^{6*\#}$	5.1 ± 0.2*#
Group 4 (modeled dermatitis + 3.0 mg/kg diclofenac sodium)	16	$1.0 \pm 0.3 \times 10^{5*\#\dagger}$	0.8 ± 0.2*#†
Group 5 (3.0 mg/kg diclofenac sodium)	10	$0.7 \pm 0.09 \times 10^{6*\#}$	1.6 ± 0.4*#

Note: * — $p < 0.05$ compared with the control group; # — $p < 0.05$ compared with group 2; + — $p < 0.05$ compared with group 3. In the groups treated with diclofenac sodium at a dose of 3 mg/kg, the cellular index was significantly lower, and therefore the results are presented as $\times 10^5$ cells/ml.

Contact dermatitis was chosen as the experimental inflammatory model because it is a rapidly inducible, validated model of a local immune response accompanied by changes in the cytokine profile. The disease was modeled on the dorsal surface of the skin after hair removal. The primary damage to the skin barrier was done by applying a 10% solution of sodium dodecyl sulfate (Ecotech, Russia), which destroyed the lipid layer of the epidermis and increased skin permeability. The initial inflammatory response was assessed in the first 24–48 hours after application. To maintain the inflammatory process and control its development time-wise, we used Dermatophagoides farinae Biostir-AD (Biostir Inc., Japan), an allergen, in the form of a cosmetic ointment based on house dust mite. It was applied at a dose of 100 mg to the damaged skin 2–3 times a week for 7–14 days. The development of dermatitis was assessed by the signs of the inflammatory process: hyperemia, edema, local fever, peeling and itching. They were measured on days 7–10 of the experiment.

At the end of the experimental period, the animals were euthanized under general anesthesia using inhaled isoflurane mixed with oxygen. This anesthetic was chosen because of its minimal effect on cytokine profile compared to injectable drugs. Once the depth of anesthesia was adequate, the animals were decapitated and their femurs were extracted in aseptic conditions. Bone marrow was isolated by mechanical disruption of the bone and the preparation of a cell suspension. In the suspension, we counted the total number of cells per 1 ml, the number of undifferentiated cells, and the concentration of proinflammatory cytokines (IL1β and TNFα).

The drug was administered for 96 hours because the animals tended to die in a longer term. This was factored in as a limitation of the model, and ensured compliance with the ethical requirements for minimizing the suffering of experimental animals.

Flow cytometry was used to quantify the population of undifferentiated bone marrow cells. The cells were stained with a panel of monoclonal antibodies: lineage (Lin) markers and Sca-1+ c-Kit+ markers (LSK), enabling identification of hematopoietic stem and progenitor cells. The analysis was performed on a BD FACSCanto II flow cytofluorimeter (BD Biosciences, USA). The results were expressed as a percentage of the total number of nucleated cells [26–27].

Immunomagnetic cell separation

For additional enrichment of the progenitor cell population, we used immunomagnetic separation with commercially available kits (Miltenyi Biotec, Germany). The resulting fraction increased the accuracy of the analysis of cellular characteristics. Immunomagnetic separation was only an auxiliary method; it was not used to quantify the population of undifferentiated cells [28–30].

Determination of cytokine concentration

The concentrations of IL1β and TNFα in the bone marrow homogenate were determined by ELISA using commercially available kits: species-specific test systems of the Quantikine ELISA Kits series (R&D Systems, USA), Mouse IL1 beta/IL1F2 kit (Cat. No. MLB00C) and TNFalpha Mouse Kit (Cat. No. MTA00B). The sensitivity of the method was < 5 pg/ml. The measurements were carried out in duplicates with the generation of calibration curves. The results were expressed in pg/ml.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.

The sample distribution was tested for normality using the Shapiro–Wilk test. One-way ANOVA with a Tukey post-hoc test enabled comparison of more than two groups. For non-normal distributions, we used the Kruskal–Wallis test with Dunn's post hoc test. The data is presented as M ± SD. The differences were considered statistically significant at $p < 0.05$. Additionally, 95% confidence intervals and effect size (n^2) were calculated. Statistical processing of the experiment results and the generated graphs and diagrams was performed in Statistica 10 (StatSoft Inc., USA).

RESULTS

The study revealed the dose–dependent effect of diclofenac sodium on bone marrow cellularity, the population of undifferentiated cells (LSK), and concentrations of pro-inflammatory cytokines.

In group 2 (modeled dermatitis, no treatment), we observed a significant decrease in the total number of bone marrow cells compared with the control group ($p < 0.05$), which reflects the inhibition of hematopoiesis with a systemic inflammatory process in the background.

The administration of diclofenac sodium was accompanied by a further decrease in cellularity. For the dose of 1.5 mg/kg, this indicator dropped moderately ($p < 0.05$ compared with the control group), whereas in group 4, the decrease was more pronounced compared with both the control group and group 2 ($p < 0.01$), which indicates a dose-dependent myelosuppressive effect of the drug. Table 1 presents the cellular composition of the bone marrow.

The number of undifferentiated cells in group 2 increased by 1.15 times ($p < 0.05$) in comparison with the control group. In group 3, the growth was 2.55-fold ($p < 0.05$) compared with the control group and 2.21-fold ($p < 0.05$) compared with group 2. Group 4 showed a 60% lower mean count of undifferentiated cells compared with the control group ($p < 0.05$) and a 65.3% lower mean count versus group 2 ($p < 0.05$). In group 5, the

Table 2. Cytokine concentrations in the bone marrow of experimental animals (pg/ml, M ± SD)

Group	*n*	IL1β (pg/ml)	TNFα (pg/ml)
Group 1 (intact, control)	12	12.8 ± 0.3	64.6 ± 1.5
Group 2 (modeled dermatitis)	10	13.8 ± 1.0*	68.5 ± 4.2*
Group 3 (modeled dermatitis + 1.5 mg/kg diclofenac sodium)	16	13.3 ± 0.1*#	79.6 ± 0.5*#
Group 4 (modeled dermatitis + 3.0 mg/kg diclofenac sodium)	16	14.9 ± 0.1*#†	72.8 ± 0.4*#†
Group 5 (3.0 mg/kg diclofenac sodium)	10	12.8 ± 1.1*#	70.2 ± 3.8*#

Note: * — $p < 0.05$ compared with the control group; # — $p < 0.05$ compared with group 2; + — $p < 0.05$ compared with group 3.

cellular index decreased by 20% ($p < 0.05$) in comparison with the control group, by 30.5% ($p < 0.05$) in comparison with group 2, by 68.7% ($p < 0.05$) in comparison with group 3, and increased 2-fold ($p < 0.05$) versus group 4.

In group 2, we observed increased concentration of IL1β и TNFα. In group 3, the studied cytokine levels also showed a clear upward trend compared with the control group. In group 4, the concentration of IL1β reached its maximum, and the level of TNFα remained relatively high. In group 5, the mean counts of IL1β and TNFα were close to those recorded in the control group. Table 2 shows the described changes in the concentrations of cytokines.

The analysis of IL1β concentration showed that in group 2, it was 1.08-fold higher ($p < 0.05$) than in the control group. Group 3 exhibited a growth of 3.7% ($p < 0.05$) compared with the control group, and a drop of 3.7% ($p < 0.05$) versus group 2. In group 4, the concentration of the cytokine increased to the maximum value in the study, becoming 1.16-fold higher ($p < 0.05$) than that in the control group, and 1.07 times higher ($p < 0.05$) compared with group 2. In group 5, the concentration of IL1β remained similar to the control group, decreased by 7.3% ($p < 0.05$) in comparison with group 2, by 3.8% ($p < 0.05$) versus group 3, and by 14.1% ($p < 0.05$) compared with group 4.

The concentration of TNFα in group 2 increased by 1.06 times ($p < 0.05$) compared with the control group. In group 3, the increase was 1.23-fold ($p < 0.05$) versus the control group, and 1.16-fold ($p < 0.05$) in comparison with group 2. Group 4 showed a 1.13-fold growth of TNFα level ($p < 0.05$) versus the control group, and 1.06-fold growth ($p < 0.05$) in comparison with group 2. In group 5, the concentration of the cytokine increased by 1.09 times ($p < 0.05$) relative to the control group and 1.02 times ($p < 0.05$) compared with group 2; it decreased by 11.9% ($p < 0.05$) versus group 3 and 3.6% ($p < 0.05$) versus group 4.

Figures 1–2 visually represent the dynamics of changes in the studied bone marrow parameters in all the studied groups.

DISCUSSION

Diclofenac sodium has a pronounced dose-dependent effect on hematopoiesis and the cytokine profile of the bone marrow against the background of inflammation [1, 11]. This study has shown a decrease in total bone marrow cellularity in animals dermatitis models, which is consistent with data on the suppressive effect of systemic inflammation on hematopoiesis [2]. The analysis of the LSK cell population showed that in the group with dermatitis and no treatment, their proportion did not differ significantly from the control values (group of intact animals), but was still higher. This allows an assumption that hematopoiesis activates when there is an ongoing inflammatory process, and the basic pool of stem cells is preserved in the early stages of the response to that process.

In the group where animals with dermatitis received diclofenac at a dose of 1.5 mg/k the total number of cells went down, which suggests some toxicity of the active substance to individual groups of cells [4, 5]. At the same time, there was a significant increase in the proportion of LSK cells ($p < 0.05$), which may indicate

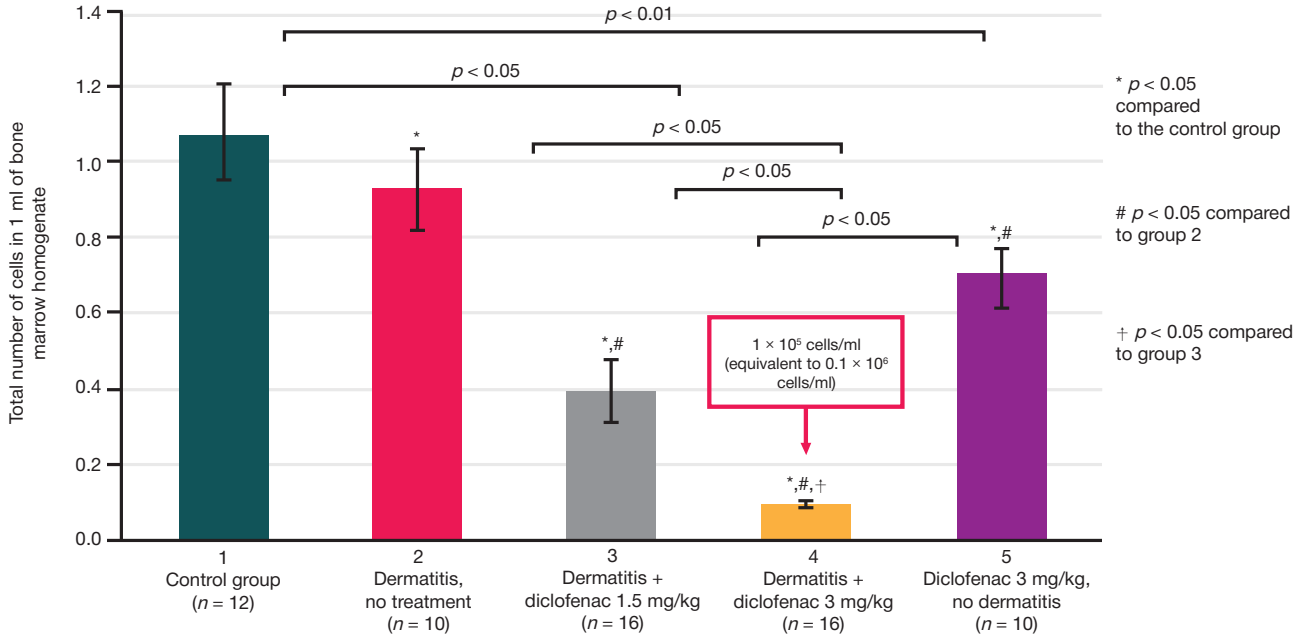

Fig. 1. Dynamics of changes in the total number of bone marrow cells (×10^6/ml) in experimental animals of various groups on the 3rd day of the study. In group 5, the absolute value of cellularity corresponded to 1 × 10^5 cells/ml, which is equivalent to 0.1 × 10^6 cells/ml on the graph. The data is presented as M±SD. The statistical analysis was performed using one-way ANOVA with Tukey post-hoc test. The differences were considered significant at $p < 0.05$.

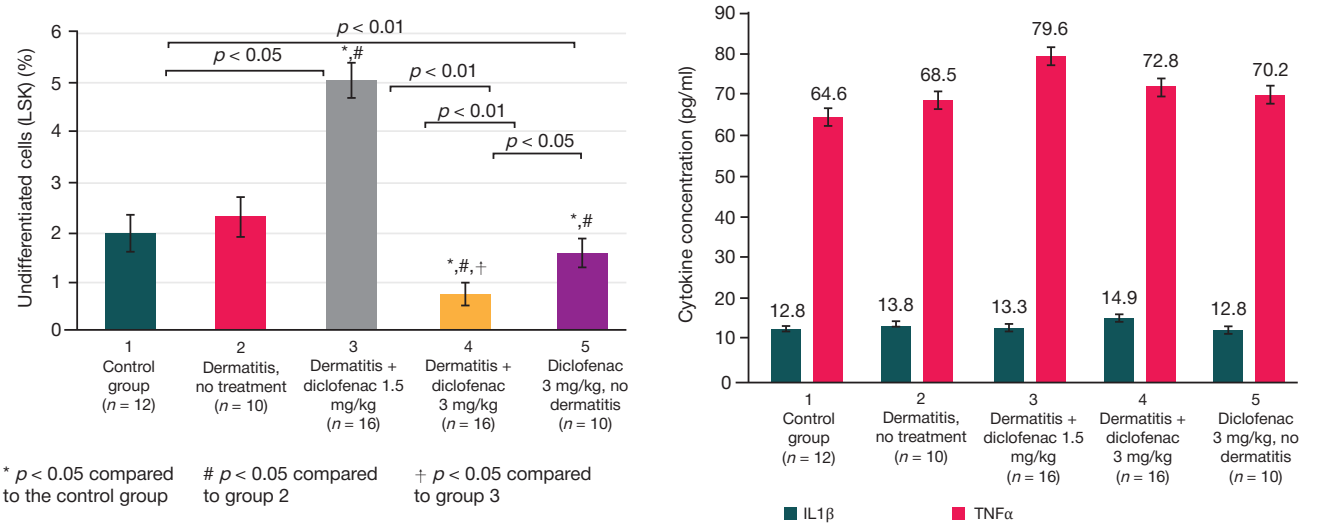

Fig. 2. Dynamics of changes in the population of LSK cells (%) in the bone marrow of experimental animals of various groups on the 3rd day of the study. The data is presented as M±SD. One-way ANOVA with a Tukey post-hoc test enabled the assessment ($p < 0.05$)

compensatory activation of hematopoietic stem and progenitor cells as a response to diclofenac sodium administration.

At the same time, the injection of the dose of 3.0 mg/kg for 96 hours yielded a significant decrease in the number of cells in 1 ml of bone marrow suspension and the percentage of LSK cells compared with the group that received 1.5 mg/kg and with the control group ($p < 0.01$), which indicates an inhibition of the proliferative potential of the bone marrow. A similar trend was observed in the group of animals treated with diclofenac without modeling dermatitis, which indicates a direct toxic effect of the drug [7, 8].

In the group that received 3 mg/kg of diclofenac without modeled dermatitis, the bone marrow cellularity decreased, but in compared to the dermatitis and no treatment group, the indicator was at a higher level since the administered dose was the same, which suggests the toxic effect of the active substance that, against the background of inflammation, only gets stronger with the growing dosage. Similar changes were observed for the proportion of LSK cells, which also indicates the presence of a direct myelotoxic effect of diclofenac sodium, and not exclusively associated with the inflammatory process.

The use of diclofenac sodium also affects the profile of IL1β and TNFα. In the group with modeled dermatitis and without treatment, the level of TNFα increases against the background of isolated inflammation, same as IL1β. In the group that received 1.5 mg/kg of diclofenac sodium, the concentration of IL1β decreased due to the anti-inflammatory properties of diclofenac sodium. The concentration of TNFα grows; under these conditions, it realizes its anti-inflammatory properties, and such changes may indicate compensatory activation of immunoregulatory mechanisms with moderate exposure to the drug [29].

In the dermatitis group that received 3 mg/kg of diclofenac, the concentration of IL1β reached its maximum value and exceeded that in all other groups, leading only to the progression of the inflammatory process in the body. The concentration of TNFα decreased relative to the group where the dose was 1.5 mg/kg, but remained consistently high relative to the control group and group with dermatitis and no treatment. Such changes may stem from pro-inflammatory properties of TNFα, an increased pro-inflammatory response, and the development of a dose-dependent toxic effect of diclofenac sodium [5].

In the group with dermatitis that received 3 mg/kg of diclofenac, the IL1β concentration did not change in comparison with the control group, which may point to the direct effect of the drug on the bone marrow's cytokine profile. The concentration of TNFα remained high compared with the control group and the group with isolated dermatitis, but lower than in the groups with modeled dermatitis and diclofenac treatment.

Study limitations

It should be noted that the interpretation of changes in TNFα levels is a complicated task, since this cytokine has pleiotropic effects and is able to participate in both the activation and regulation of the inflammatory process. In the present study, an increase in its concentration combined with the growing an increase in IL1β levels and a marked decrease in bone marrow cellularity probably reflects aggravation of the inflammatory and cytotoxic effects of the drug [14, 15, 18].

CONCLUSIONS

In this study, we have shown that diclofenac sodium had a dose-dependent effect on the cellular composition of bone marrow, the population of hematopoietic stem and progenitor cells (LSK), and the cytokine profile in experimental dermatitis and in healthy animal models. The use of diclofenac sodium at a dose of 1.5 mg/kg led to toxic changes, which were manifested by a decrease in total bone marrow cellularity while increasing the proportion of LSK cells, which may reflect compensatory activation of hematopoietic stem and progenitor cells. The administration of diclofenac sodium at a dose of 3 mg / kg had the most pronounced effects, including inhibition of hematopoiesis: the number of bone marrow and LSK cells decreased while the concentration of proinflammatory cytokines increased, indicating the development of myelotoxic and proinflammatory effects of the high dose of the drug. Our data confirm that a high dose of diclofenac sodium has a pronounced negative effect on the bone marrow and enhances the inflammatory response, while a low dose is accompanied by less pronounced changes and signs of compensatory activation of stem cells. Further research may investigate long-term exposure to diclofenac sodium, consider wider ranges of cytokines, include molecular markers of hematopoiesis regulation, such as microRNAs, in order to more accurately assess the mechanisms of the drug's effect and potential clinical significance.

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