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The effect of CO_2 therapy and its combinations on inflammatory activity and immune status: an experimental study

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Osteoarthritis (OA) is a chronic degenerative disease characterized by cartilage destruction, inflammation, and autoimmune processes. The limited effectiveness of current therapies has generated interest in alternative approaches, such as carboxytherapy, which possesses anti-inflammatory and regenerative properties. Objective. To evaluate the effects of carboxytherapy (CO_2) in mono- and combination therapy on a carrageenan-induced model of inflammation in rats by assessing leukocyte differential counts and integral indices. Methods. The study was conducted on 56 white rats divided into 7 groups ($n = 8$): control (intact and carrageenan-induced), mono- and combination therapy with CO_2 in conjunction with diclofenac or chondroitin. Changes in the leukocyte differential counts and integral indices (NMR, NLR, LSI, LI, IIR) were evaluated. Results. CO_2 monotherapy (Group V) reduced the NMR (Neutrophil-to-Monocyte Ratio) by 25.4 % (15.60 ± 2.95 vs. 20.92 ± 8.89 in Group II, $p < 0.001$). The Leukocyte Index (LI) increased by 82.2 % (1.471 ± 0.155 vs. 1.791 ± 0.191, $p = 0.001$). The combination of CO_2 with diclofenac (Group VI) reduced NMR by 68.3 % (6.61 ± 2.72, $p < 0.001$) and increased LI by 103.1 % (3.637 ± 0.770, $p < 0.001$). CO_2 combined with chondroitin (Group VII) reduced the NLR (Neutrophil-to-Lymphocyte Ratio) by 48.6 % (0.290 ± 0.0938, $p < 0.001$) and increased LI by 121.7 % (3.847 ± 1.421, $p < 0.001$). In Groups VI and VII, there was a significant decrease in band neutrophils (by 79.2 % and 75.0 %, respectively) and an increase in lymphocytes (by 72.2 % and 70.9 %, $p < 0.001$). Conclusions. Carboxytherapy modulates the inflammatory response and enhances the immune response. The best results were observed with combined CO_2 therapy with diclofenac or chondroitin, confirming their potential in reducing inflammation and stimulating regeneration.

Остеоартрит (ОА) — хронічне дегенеративне захворювання суглобів, патогенез якого пов'язаний із запаленням і дисфункцією імунної системи. У свою чергу, карбокситерапія (CO_2) є перспективним методом лікування остеоартриту завдяки своїм протизапальним і регенеративним властивостям. Мета. Оцінити вплив карбокситерапії як монотерапії та в комбінації з диклофенаком натрію та хондроїтину сульфатом на показники лейкоцитарної формули, інтегральні індекси запалення й імунної відповіді у щурів із карагеніновою моделлю запалення. Методи. Дослідження проведено на 56 білих щурів, поділених на 7 груп ($n = 8$). Для моделювання запалення використовували карагенін. Групи отримували CO_2 (0,5 мл), диклофенак натрію (4–8 мг/кг), хондроїтину сульфат (3 мг/кг) або їх комбінації. Аналіз лейкоцитарної формули проводили за стандартною методикою; розраховували інтегральні індекси лейкограми (ІСНМ, ІСЛМ, ІСНЛ, ІЗЛ, ЛІ). Дані обробляли за допомогою дисперсійного аналізу ($p < 0,05$). Результати. Монотерапія CO_2 знизила ІСНМ на 25,4 % ($p < 0,001$) та ІСНЛ на 46,9 % ($p < 0,001$), тоді як комбіноване застосування CO_2 із диклофенаком натрію зменшило ІСНМ на 68,3 % ($p < 0,001$) і підвищило ЛІ на 103,1 % ($p < 0,001$). CO_2 із хондроїтину сульфатом знизив ІСНМ на 45,5 % ($p = 0,026$), а ЛІ зріс на 121,7 % ($p < 0,001$). Частка лімфоцитів у групі CO_2 + диклофенак натрію збільшилася на 42,9 % ($p < 0,001$), що вказує на активацію адаптивного імунітету. Висновки. Карбокситерапія ефективно модулює запальні процеси, знижуючи нейтрофільну активність і посилюючи адаптивну імунну відповідь. Комбінація CO_2 із диклофенаком натрію або хондроїтину сульфатом демонструє синергічний ефект, що дозволяє зменшити дози традиційних препаратів і мінімізувати побічні ефекти. Отримані результати підтверджують перспективність використання CO_2-терапії у лікуванні остеоартриту. Ключові слова. Карбокситерапія, вуглекислий газ, запалення, нейтрофіли, лімфоцити, диклофенак натрію, хондроїтину сульфат, карагенінова модель

Key words. Carboxytherapy, carbon dioxide, inflammation, neutrophils, lymphocytes, diclofenac, chondroitin sulfate, carrageenan model

Introduction

Osteoarthritis (OA) is a long-term degenerative joint disease, with its pathogenesis remaining incompletely understood. It is characterized by cartilage destruction, osteophyte formation, and inflammatory processes [1]. OA was previously considered a disease caused mainly by mechanical wear of the joints. However, modern studies emphasize the significant role of the immune system in its development. Infiltration of immune cells into the joint, production of inflammatory mediators, and autoimmune processes indicate that OA has an immunological nature [2].

The release of pro-inflammatory mediators (cytokines, adipokines, growth factors) has been proven to determine inflammatory reactions in joint tissues, which are accompanied by loss of their structure and function [3].

Studies have shown that changes in hematological parameters, in particular leukocyte (neutrophil, lymphocyte, monocyte) and platelet levels, as well as erythrocyte distribution width and acute phase protein content, can reflect the degree of systemic inflammatory response in OA [4, 5]. On this basis, it has been proposed to use different ratios of blood cellular elements, neutrophil to lymphocyte (NLR) or monocyte to lymphocyte (MLR), as markers that correlate with the course of OA [6]. The study of hematological parameters is a convenient, minimally invasive and economically available method for detecting systemic inflammation in osteoarthritis [4].

Modern treatment of OA mainly includes chondroprotectors, nonsteroidal anti-inflammatory drugs (NSAIDs), and in case of progression, arthroplasty [7]. Recently, more and more attention has been paid to the use of natural substances that block molecules involved in the progression of inflammation and cartilage destruction [8]. Modern therapy is increasingly focused on a comprehensive approach, supplemented by alternative treatment methods [7].

One of the promising areas in the treatment of OA is carboxytherapy, a method based on the introduction of carbon dioxide (CO_2). This approach attracts attention due to the anti-inflammatory and regenerative properties of CO2 [9]. The mechanism of action includes improving microcirculation, stimulating tissue metabolism and activating regenerative processes [10]. The introduction of CO_2 promotes vasodilation, improves oxygen transport to tissues and the removal of metabolic products [11, 12].

It is assumed that CO_2 may have an anti-inflammatory effect, modulating the immune response and promoting the recovery of damaged tissues [13, 14]. However, the mechanisms of action of CO_2 during the treatment of OA remain poorly understood.

Purpose: to evaluate the effect of carboxytherapy both as monotherapy and in combination with traditional anti-inflammatory drugs (diclofenac sodium and chondroitin sulfate) on leukocyte formula and integral intoxication indices in case of carrageenan inflammation in rats.

The study aims to determine whether carboxytherapy is able to modulate inflammatory processes and promote tissue regeneration, as well as to investigate its ability to enhance the anti-inflammatory effect of traditional drugs. We assume that combination therapy will contribute to a more effective immune response, reduce inflammatory activity and trigger recovery processes, which may become the basis for the development of new therapeutic approaches in the treatment of osteoarthritis.

Material and methods

The study, conducted at the vivarium of Poltava State Medical University, was approved by the ethical committees of Poltava State University (protocol No. 225 dated 21.03.2024) and Uzhhorod National University (protocol No. 9/2 dated 07.06.2023). All procedures complied with the main provisions of the Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, as well as Directive 2010/63/EU of the European Parliament and of the Council of the EU.

This fragment is part of the initiative research topic of the Department of Pharmacology, Clinical Pharmacology and Pharmacy of Poltava State Medical University "Pharmacological study of biologically active substances and drugs for the development and optimization of indications for their use in medical practice" (state registration number 0120U103921), as well as the research topic of the Department of General Surgery of the State Higher Educational Institution "Uzhhorod National University" "Monitoring of traumatic disease against the background of chronic stress" (state registration number 0124U002167).

The study used 56 white rats (n = 56), weighing 285–315 g, of which 34 (60.7 %) were females. The animals were kept in standard vivarium conditions at a temperature of (22 ± 2) °C, relative humidity (55 ± 5) % and a 12-hour light regime. They had free access to water and standard food. The animals were randomized and divided into 7 groups of 8 rats each (n = 8): group I — intact control (saline); group II — control pathology (carrageenan 1 %, 0.1 ml); group III — control pathology + diclofenac sodium (8 mg/kg, intraperitoneally); group IV — control pathology + chondroitin sulfate (3 mg/kg, intraperitoneally); group V — control pathology + carbon dioxide (CO_2, 0.5 ml, p/s); group VI — control pathology + diclofenac sodium (4 mg/kg) + CO_2 (0.5 ml); group VII — control pathology + chondroitin sulfate (3 mg/kg) + CO_2 (0.5 ml).

To simulate acute inflammation, a carrageenan model was used in rats. Animals were injected subplantarly with a 1 % solution of carrageenan in a volume of 0.1 ml. Diclofenac sodium in doses of 8 and 4 mg/kg, and chondroitin sulfate in a dose of 3 mg/ kg, were administered intraperitoneally one hour before the carrageenan test. Subcutaneous administration of CO_2 was performed using an INDAP Insuf apparatus (Czech Republic) using a BD Mikrolance 3.30 G ½ 0.3 × 13 mm needle in a dose of 0.5 ml per animal one hour before the administration of carrageenan. The intact control group was administered saline in an equivalent volume.

Six hours after the administration of carrageenan, the rats were euthanized under thiopental anesthesia (50 mg/kg), and blood was collected from the heart until it stopped. The blood was fixed in test tubes with a layer of 2-substituted salt of ethylenediaminetetraacetic acid. For cytological examination, blood smears were prepared, fixed and stained according to the standard Romanovsky-Giemza method. Their analysis was performed using a light microscope with an immersion system (lens ×100, eyepiece ×10). The number of leukocytes of different types in the field of view per 100 cells (cells/μl) was counted.

To identify the intensity of the inflammatory process and the general state of the immune system, the following integral leukocyte indices were calculated: INMR (neutrophil-monocyte ratio = neutrophils/monocytes); ILMR (lymphocyte-monocyte ratio = lymphocytes/monocytes); INLR (neutrophil-lymphocyte ratio = (rod-nucleated neutrophils + segmented neutrophils)/lymphocytes)); ILS (leukocyte shift index = (eosinophils + basophils + segmented neutrophils + rod-nucleated neutrophils) / (lymphocytes + monocytes)); LI (leukocyte index = lymphocytes/neutrophils); IIR (immunoresistency index) = (lymphocytes + eosinophils)/monocytes [15, 16].

Digital data were processed using Jamovi 2.3.21 version. Results are presented as mean values ± standard deviation. The Shapiro-Wilk test was used to check normality. Group comparisons were performed using Welch's t-test or Tukey's test for normal distribution, and for non-normal distributions, the Kruskal-Wallis test with Bonferroni correction. Results were considered statistically significant at $p < 0.05$.

Results

Analysis of the results (Tables 1 and 2) demonstrates the modulating effect of CO_2 on the hemogram, which is manifested in changes in both individual leukocyte indices and integral indices.

The tables show relative indicators. The values are presented in percentages per 100 cells, and "< 0.01" indicates a level that does not exceed the detection limit of the analysis method. Statistical analysis was performed using one-way analysis of variance followed by Tukey's a posteriori test. Statistically significant difference $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ compared to the control pathology group (carrageenan).

In order to find new effective methods for the treatment of inflammatory diseases, a study was conducted to study the anti-inflammatory activity of combined therapy of traditional drugs using CO_2 and to analyze changes in the leukocyte formula and immune indices, which allowed to identify the mechanisms of the positive effect of CO_2 during its use as part of both mono- and complex therapy with traditional drugs. The results obtained may contribute to the development of new approaches to the treatment of osteoarthritis and other diseases associated with inflammatory processes. In group II (carrageenan), an increase in the level of neutrophils was observed: rod-shaped by 12.0 %, segmented by 24.4 %, and the proportion of lymphocytes decreased by 30.1 % compared to intact animals ($p < 0.05$). The INMR increased almost threefold (by 190.9 %, $p < 0.001$), the INLR by 97.2 % ($p < 0.001$), and the ILS by 118.5 % ($p < 0.001$), which indicates the activation of the acute phase of inflammation.

In group III (diclofenac), a decrease in neutrophil activity was observed: rod-shaped neutrophils decreased by 62.8 %, segmented neutrophils by 24.6 %, while the proportion of lymphocytes increased by 31.9 % ($p < 0.001$). The INMR decreased by 28.6 % ($p < 0.001$), the INLR by 47.3 % ($p < 0.001$), and the ILS by 43.9 % ($p < 0.001$), which indicates a decrease in the intensity of inflammation and the activation of adaptive immunity.

Group IV (chondroitin sulfate) had the highest level of segmented neutrophils, which increased by 52.9 % ($p < 0.001$), while lymphocytes decreased by 23.3% ($p < 0.001$) compared to group II. INMR and INLR remained at high levels, indicating an increased neutrophilic response characteristic of acute inflammation.

In group V (CO_2 monotherapy), there was a decrease in the INMR by 25.4 % ($p < 0.001$), the INLR by 46.9 % ($p < 0.001$), and the ILS by 43.5 % ($p = 0.015$), which indicates a decrease in neutrophil activity.

The combination of CO_2 with diclofenac sodium (group VI) provided a decrease in the INMR by 68.3 % ($p < 0.001$), the INLR by 49.3 % ($p < 0.001$), and the ILS by 51.9 % ($p < 0.001$). LI increased by 103.1 % ($p < 0.001$), and the proportion of lymphocytes by 42.9 % ($p < 0.001$), which confirms the activation of adaptive immunity.

In group VII (CO_2 + chondroitin sulfate), the INMR decreased by 45.5 % ($p = 0.026$), the INLR by 48.6 % ($p = 0.014$), and the ILS by 49.1 % ($p < 0.001$). The LI increased by 121.7 % ($p < 0.001$),

Table 1

Effect of carboxytherapy on leukocyte formula in carrageenan inflammation in rats (M ± SD, n = 8)

Animal group	Changed, %	Rods, %	Segmented, %	Eosinophils, %	Monocytes, %	Basophils, %	Lymphocytes, %
Intact	not detected (< 0.01) ± 0	not detected (< 0.01) ± 0	21.60 ± 3.543	2.13 ± 0.641	3.13 ± 0.991	not detected (< 0.01) ± 0	73.00 ± 3.550
II	12.00 ± 0.586	4.38 ± 0.916	24.40 ± 3.623	6.00 ± 1.069	1.75 ± 1.035	0.125 ± 0.354	51.00 ± 2.730
III	5.25 ± 1.035	1.63 ± 0.518***	18.40 ± 1.408**	5.38 ± 1.923	1.50 ± 0.535	0.125 ± 0.354	67.30 ± 1.040***
IV	3.75 ± 1.282	3.50 ± 0.756	37.30 ± 3.732***	14.00 ± 2.507***	3 ± 0.756	not detected (< 0.01) ± 0	39.10 ± 2.170***
V	3.00 ± 1.195	2.75 ± 1.165**	33.00 ± 0.926***	6.50 ± 1.309	2.38 ± 0.518	not detected (< 0.01) ± 0	52.40 ± 3.160
VI	not detected (< 0.01) ± 0	2.50 ± 1.309***	18.30 ± 2.765**	3.38 ± 0.518*	3 ± 1.069	not detected (< 0.01) ± 0	72.90 ± 2.590***
VII	0.50 ± 0.535	3.00 ± 0.756*	17.60 ± 5.097**	4.25 ± 1.488	2.50 ± 1.604	not detected (< 0.01) ± 0	72.40 ± 4.720***
Probable intergroup differences	$\chi^2 = 51.20$ $p < 0.001$	$\chi^2 = 37.90$ $p < 0.001$	$F = 118.00$ $p < 0.001$	$F = 38.40$ $p < 0.001$	$F = 5.23$ $P = 0.002$	$\chi^2 = 5.09$ $p = 0.532$	$F = 224.00$ $p < 0.001$
Normality Test (Shapiro-Wilk)	$W = 0.836$ $p < 0.001$	$W = 0.955$ $p = 0.036$	$W = 0.988$ $p = 0.862$	$W = 0.968$ $p = 0.149$	$W = 0.982$ $p = 0.589$	$W = 0.382$ $p < 0.001$	$W = 0.991$ $p = 0.953$

Notes: * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$.

Table 2

Effect of carboxytherapy on integral immune response indices in carrageenan inflammation in rats (M ± SD,

Animal group	INMR	ILMR	INLR	ILS	LI	IIR
Intact	7.19 ± 2.31	25.4 ± 7.75	0.286 ± 0.0603	0.303 ± 0.0651	3.623 ± 0.738	26.1 ± 7.97
II	20.92 ± 8.89	38.3 ± 17.90	0.564 ± 0.0660	0.662 ± 0.0486	1.791 ± 0.191	43.0 ± 20.24
III	14.94 ± 5.27	50.5 ± 18.20	0.297 ± 0.0220***	0.371 ± 0.0279***	3.376 ± 0.246***	54.9 ± 20.75
IV	14.53 ± 4.78	13.8 ± 3.79	1.048 ± 0.1502***	1.306 ± 0.1472	0.970 ± 0.124	18.7 ± 4.81
V	15.60 ± 2.95	23.0 ± 5.08	0.686 ± 0.0738*	0.776 ± 0.0835	1.471 ± 0,155	25.9 ± 5.67
VI	6.61 ± 2.72***	22.7 ± 5.86	0.286 ± 0.0641***	0.318 ± 0.0587***	3.637 ± 0.770***	23.7 ± 6.04
VII	11.47 ± 8.69*	37.1 ± 22.89	0.290 ± 0.0938***	0.337 ± 0.0843***	3.847 ± 1.421***	39.0 ± 23.44
Probable intergroup differences	$F = 11.40$ $p < 0.001$	$F = 9.32$ $\chi^2 = 25.7$ $p < 0.001$	$\chi^2 = 44.1$ $p < 0.001$	$F = 101.00$ $p < 0.001$	$\chi^2 = 44.1$ $p < 0.001$	$F = 5.63$ $p = 0.001$
Normality Test (Shapiro-Wilk)	$W = 0.983$ $p = 0.594$	$W = 0.963$ $p = 0.086$	$W = 0.942$ $p = 0.010$	$W = 0.985$ $p = 0.687$	$W = 0.917$ $p < 0.001$	$W = 0.973$ $p = 0.244$

Notes: * — $p < 0.05$; *** — $p < 0.001$.

and the proportion of lymphocytes increased by 42.0 % ($p < 0.001$), which indicates a decrease in inflammation and activation of the immune response.

Comparison of groups III (diclofenac sodium) and VI (diclofenac sodium + CO_2) showed that the combined therapy significantly enhances the anti-inflammatory effect. The INMR in group VI decreased by 55.8 % compared to group III ($p < 0.001$), the INLR by 3.7 % ($p < 0.001$), and the ILS by 14.3 % ($p < 0.001$). The LI increased by 7.7 % ($p < 0.05$), and the proportion of lymphocytes increased by 8.3 % ($p < 0.001$). These results indicate a synergistic effect of CO_2 with diclofenac, which reduces neutrophil activity and enhances the adaptive immune response.

Comparison of groups IV (chondroitin sulfate) and VII (chondroitin sulfate + CO_2) demonstrates a similar trend. The INMR in group VII decreased by 21.1 % compared to group IV ($p < 0.05$), the INLR by 72.3 % ($p < 0.001$), and the ILS by 25.8 % ($p < 0.001$). The LI increased by 194.2 % ($p < 0.001$), and the proportion of lymphocytes increased by 85.2 % ($p < 0.001$). These changes confirm that the addition of CO_2 to chondroitin enhances the anti-inflammatory effect and significantly activates the adaptive immune response.

The results obtained emphasize that CO_2 is important both in monotherapy and in combination with anti-inflammatory drugs, enhancing their effect and contributing to the reduction of inflammatory activity.

Discussion

Our studies confirm that carboxytherapy (CO_2) is a promising method in modulating inflammatory and regenerative processes. The observation also showed that the combined use of CO_2 with diclofenac sodium or chondroitin sulfate is more effective in reducing inflammatory activity and enhancing the adaptive immune response than the use of each component separately. The greatest effect was demonstrated under the conditions of combined therapy of CO_2 with diclofenac, where a decrease in the INMR index by 68.3 % ($p < 0.001$) and an increase in LI by 103.1 % ($p < 0.001$) was observed compared to the pathology group. In the "CO_2 + chondroitin sulfate" group, the INMR decreased by 45.5 % ($p = 0.026$), and the LI increased by 121.7 % ($p < 0.001$). It is important that CO_2 enhances the anti-inflammatory effect of traditional drugs, allowing to reduce their dosage by 2 times and, thus, to reduce the risk of side effects. A similar synergistic effect of CO_2 was also observed in clinical studies involving patients with post-traumatic and postoperative lesions [9]. The mechanisms of action of CO2 include a decrease in the activity of NF-kB and MAPK, which suppresses the expression of pro-inflammatory cytokines [17, 18]. In addition, hypercapnic acidosis inhibits ERK1/2, contributing to a decrease in the inflammatory response, which confirms the role of CO_2 in the modulation of cellular metabolism [19].

CO_2 is actively studied and used in many areas of medicine, as it helps to accelerate fracture healing and increase bone strength by stimulating microcirculation, tissue oxygenation, as well as reducing the levels of pro-inflammatory cytokines (HIF-1α, IL-1β, IL-6) and increasing the expression of factors that produce regeneration (VEGF, TGF-β) [20]. A study by P. Chou et al. demonstrated that the use of a decellularized matrix treated with supercritical CO_2 in combination with stem cells significantly improves tissue regeneration and reduces inflammation. This confirms the potential of CO_2 in stimulating reparative processes [21]. In the treatment of muscle injuries, the use of CO_2 paste helps to increase the expression of MyoD and myogenin, reduce the levels of IL-1β, IL-6 and TGF-β, and accelerate muscle regeneration through the induction of the Bohr effect [22]. In addition, the use of CO_2 stimulates angiogenesis by regulating the expression of VEGF and TGF-β, which in turn improves the repair and regeneration of damaged tissues [23, 24]. Thus, the available data demonstrate the broad therapeutic potential of the use of carbon dioxide due to its ability to modulate key molecular and cellular processes that underlie reparative mechanisms in the body. The use of carboxytherapy in orthopedics is becoming increasingly relevant due to its ability to modulate inflammatory and regenerative processes. Osteoarthritis and other degenerative joint diseases are often accompanied by chronic inflammation, impaired blood circulation and progressive damage to cartilage tissue. Since neutrophils play a central role in the pathophysiology of osteoarthritis, the modulating effect of CO_2 on their activity through the effect on pH and inhibition of NF-κB opens up new perspectives for therapeutic intervention [25]. In addition, CO_2 enhances the anti-inflammatory effect of traditional drugs, allowing to reduce their dosage and reduce the risk of side effects. Thus, the use of carboxytherapy in orthopedics is of great importance for the individualized and effective treatment of osteoarthritis and other degenerative joint diseases.

Conclusions

Carboxytherapy demonstrated a significant anti-inflammatory effect in the carrageenan model of inflammation. The use of CO_2 as monotherapy reduced the neutrophil-monocyte ratio index by 25.4 % ($p < 0.001$), the neutrophil-lymphocyte index by 46.9 % ($p < 0.001$), and the leukocyte shift index by 43.5 % ($p = 0.015$), indicating a decrease in neutrophil activity.

The combination of CO_2 with diclofenac reduced the neutrophil-monocyte ratio index by 68.3 % ($p < 0.001$) and the neutrophil-lymphocyte ratio index by 49.3 % ($p < 0.001$), while increasing the leukocyte index by 103.1 % ($p < 0.001$). The combination of CO_2 with chondroitin also demonstrated a positive effect: a decrease in the neutrophil-monocyte ratio index by 45.5 % ($p = 0.026$), the neutrophil-lymphocyte index by 48.6 % ($p = 0.014$) and an increase in the leukocyte index by 121.7 % ($p < 0.001$). These results indicate an increase in the anti-inflammatory effect of traditional drugs due to the addition of CO_2.

The use of CO_2 contributed to an increase in the proportion of lymphocytes by 42.9 % ($p < 0.001$) in the CO_2 + diclofenac sodium group and by 42.0 % ($p < 0.001$) in the CO_2 + chondroitin sulfate group, which indicates the activation of the adaptive immune response.

The results obtained in the carrageenan model of inflammation in animals confirm the promising potential of combined use of carboxytherapy with diclofenac or chondroitin for modulating inflammatory processes. Given the effectiveness of reducing neutrophil activity, improving integral indices and stimulating the adaptive immune response, further clinical studies are appropriate.

The prospective use of such combined therapy can provide a more targeted correction of the inflammatory process in patients with degenerative joint diseases, in particular osteoarthritis of the knee and hip joints. This will allow reducing the dosage of traditional anti-inflammatory drugs, minimizing side effects and enhancing regenerative processes in the affected tissues.

Conflict of interest. The author declares the absence of a conflict of interest.

Prospects for further research. A preclinical study is planned on a model of monoiodoacetate-induced osteoarthritis (MIA-OA) to assess the effectiveness of carboxytherapy both as monotherapy and in combination with diclofenac or chondroitin. The study will assess the impact of combined CO_2 use on the main mechanisms of inflammation in the joints, in particular: analysis of the expression of key pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) to determine the anti-inflammatory effect of combined carboxytherapy and its potential ability to reduce the level of chronic inflammation in joint tissues and assessment of the level of transforming growth factor β (TGF-β), in order to determine the impact of CO_2 and its combinations on the stimulation of cartilage tissue regeneration.

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THE EFFECT OF CO_2 THERAPY AND ITS COMBINATIONS ON INFLAMMATORY ACTIVITY AND IMMUNE STATUS: AN EXPERIMENTAL STUDY

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