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The effect of carboxytherapy and its combination with diclofenac sodium and chondroitin sulfate on TNF-A and TGF-B1 expression in monoiodoacetate-induced osteoarthritis in rats

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Osteoarthritis remains one of the leading causes of disability worldwide, associated with chronic inflammation and progressive destruction of articular cartilage. Current therapeutic approaches show limited efficacy in restoring damaged tissues, which drives interest in novel adjuvant methods, particularly physiotherapeutic techniques such as carboxytherapy. Objective. To investigate the effects of carbon dioxide monotherapy and its combinations with diclofenac sodium or chondroitin sulfate on the expression of the pro-inflammatory cytokine TNF-α and the regenerative factor TGF-β1 in rats with experimentally induced osteoarthritis. Methods. An osteoarthritis model was induced by intra-articular injection of monoiodoacetic acid. Treatment included CO_2 monotherapy or its combination with chondroitin sulfate or diclofenac sodium. On days 14 and 28, serum levels of TNF-α and TGF-β1 were measured using the ELISA method. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test; 95 % confidence intervals and effect sizes (Cohen's d) were calculated. Results. All interventions involving CO_2 led to a statistically significant reduction in TNF-α levels on days 14 and 28 (ANOVA: $F = 2551$ and $F = 4057$, respectively; $p < 0.001$), with the most pronounced effect observed in the «CO_2 + diclofenac» group (a decrease of –54.7 %). Concurrently, an increase in TGF-β1 levels was noted across all treatment groups (ANOVA: $F = 1492$ and $F = 3492$; $p < 0.001$), particularly in the «CO_2 + chondroitin» group (+99.4 % compared to the pathology group). Large effect sizes were recorded for key comparisons (Cohen's $d > 0.8$), indicating the clinical relevance of the observed changes. Conclusions. The combined application of carboxytherapy with diclofenac sodium or chondroitin sulfate promotes a reduction in inflammatory response and activation of regenerative mechanisms in an osteoarthritis model, supporting the rationale for further preclinical and clinical investigations.

Остеоартрит залишається однією з провідних причин інвалідизації у світі через хронічні запалення і поступову деструкцію суглобового хряща. Сучасні терапевтичні підходи мають обмежену ефективність у відновленні уражених тканин, що зумовлює інтерес до нових ад'ювантних методів, зокрема фізіотерапевтичних, таких як карбокситерапія. Мета. Дослідити вплив монотерапії вуглекислим газом і його комбінацій з диклофенаком натрію або хондроїтином сульфатом на експресію прозапального цитокіну TNF-α і регенераторного фактора TGF-β1 у щурів із експериментальним остеоартритом. Методи. Модель остеоартриту відтворювали шляхом внутрішньосуглобового введення монойодоцтової кислоти. Лікування включало монотерапію CO_2 або його поєднання з хондроїтином сульфатом чи диклофенаком натрію. На 14-й і 28-й день визначали рівні TNF-α і TGF-β1 у сироватці крові методом ELISA. Для статистичного аналізу застосовували однофакторний дисперсійний аналіз (ANOVA) з пост-хок тестом Тьюкі; обчислювали 95 % довірчі інтервали й ефекти розміру (Cohen's d). Результати. Усі втручання, що включали CO_2, спричиняли статистично значуще зниження рівня TNF-α на 14-й і 28-й день (ANOVA: $F = 2551$ та $F = 4057$ відповідно; $p < 0,001$), із найбільш вираженим ефектом у групі «CO_2 + диклофенак» (зниження до –54,7 %). Паралельно в усіх терапевтичних групах відзначено підвищення рівня TGF-β1 (ANOVA: $F = 1492$ і $F = 3492$; $p < 0,001$), особливо в групі «CO_2 + хондроїтин» (+99,4 % до патології). Для основних порівнянь зафіксовано великі розміри ефекту (Cohen's $d > 0,8$), що вказує на клінічну релевантність змін. Висновки. Комбіноване застосування карбокситерапії з диклофенаком натрію або хондроїтином сульфатом спричинює зниження запального компонента й активацію регенераторних механізмів у моделі остеоартриту, що обґрунтовує доцільність подальших доклінічних і клінічних досліджень. Ключові слова. Остеоартрит; карбокситерапія; CO_2; диклофенак натрію; хондроїтину сульфат; TNF-α; TGF-β1; експериментальна модель; монойодоцтова кислота; комбіноване лікування.

Keywords. Osteoarthritis; carboxytherapy; CO_2; diclofenac sodium; chondroitin sulfate; TNF-α; TGF-β1; experimental model; monoiodoacetic acid; combined treatment

Introduction

Osteoarthritis (OA) is one of the most common musculoskeletal conditions, affecting more than 300 million people worldwide, experiencing a significant increase due to the aging of the population [1]. Characterized as a chronic degenerative-inflammatory disease of the joints, OA leads to significant limitation of mobility, chronic pain and a decrease in the quality of life of patients [2]. Its pathogenesis is extremely complex and involves the interaction of local (mechanical) and systemic (metabolic and immunological) factors that cause progressive degeneration of articular cartilage, remodeling of subchondral bone, synovitis and neoangiogenesis [3, 4].

One of the key components of the pathophysiology of OA is inflammation mediated by pro-inflammatory cytokines, among which tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) play a leading role [5]. They activate the NF-κB and p38MAPK signaling pathways, which induce the expression of matrix metalloproteinases (MMPs), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and lead to chondrocyte apoptosis and extracellular matrix degradation [4, 6]. In addition, TNF-α is able to disrupt TGF-β-dependent signal transduction in chondrocytes by inhibiting Smad signaling, which in turn prevents cartilage regeneration and extracellular matrix synthesis [7, 8]. Despite significant progress in understanding the pathogenesis of OA, effective disease-modifying agents are still lacking [9]. The mainstay of treatment remains nonsteroidal anti-inflammatory drugs (NSAIDs), particularly diclofenac sodium, which, although effective in relieving pain, has serious side effects (gastrointestinal, renal, cardiovascular) with prolonged use [10, 11]. This has prompted the scientific community to search for safer alternative or adjuvant therapies [12]. One promising approach is carboxytherapy, the therapeutic use of carbon dioxide (CO_2), which has historically been used in vascular diseases and is now considered a potential anti-inflammatory agent. CO_2 therapy is increasingly gaining attention as a tool for modulating the tissue microenvironment by improving oxygenation, blood flow, stimulating angiogenesis, and inhibiting pro-inflammatory cytokines such as TNF-α and IL-6 [15]. The mechanisms of action of CO2 therapy include activation of VEGF and TGF-β, induction of nitric oxide synthase, and reduction of HIF-1α levels, suggesting its potential efficacy in the treatment of hypoxia-induced and chronic inflammatory damage.

Of particular scientific interest is the potential for combining carboxytherapy (CO_2) with traditional treatments for osteoarthritis. One promising avenue is the combination of CO_2 with nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac sodium. This combination may enhance the anti-inflammatory effects of NSAIDs, allowing for a reduction in the required dosage and, consequently, minimizing the risk of side effects — an essential consideration given the chronic use of NSAIDs in osteoarthritis management.

Additionally, the use of CO_2 in combination with chondroitin sulfate, a well-known chondroprotector with regenerative and partially anti-inflammatory properties, could potentially promote chondrogenesis and help stabilize the tissue microenvironment. Some research suggests that CO_2 might simultaneously reduce inflammation and stimulate tissue repair mechanisms, potentially through the regulation of cytokines. This dual action, both anti-inflammatory and regenerative, opens up new possibilities for improving the clinical management of osteoarthritis, particularly in cases where conventional treatments fall short.

At the same time, the effect of combining CO_2 with diclofenac sodium or chondroitin sulfate on the expression of key pro-inflammatory and anti-inflammatory markers in an animal model of osteoarthritis (OA) remains insufficiently studied and requires further investigation and verification. The model of osteoarthritis induced by intra-articular injection of monoiodoacetic acid (MIOA) is widely used to study the effectiveness of new therapeutic approaches. This model reliably reproduces the key pathomorphological signs of OA, including synovitis, cartilage erosion, synovial hyperplasia, and increased expression of TNF-α [13, 14]. Utilizing this model enables an objective assessment of inflammatory biomarkers and allows for the monitoring of morphological changes in joints under the influence of experimental interventions.

In previous experimental studies, we demonstrated the effectiveness of carboxytherapy (CO_2) both in monotherapy and in combination with traditional anti-inflammatory agents (diclofenac sodium, chondroitin sulfate) in formalin and carrageenan models of inflammation in rats. In particular, analgesic [16], anti-inflammatory [17], and antioxidant properties of CO_2 were revealed [18, 19]. The combined use of CO_2 with NSAIDs or chondroprotectors demonstrated a synergistic effect, which was manifested in a decrease in the intensity of pain, edema, temperature, a decrease in pro-inflammatory markers (integrated indices of inflammation and immune response in rats with a carrageenan model of inflammation) and an

increase in antioxidant protection [20, 21]. The results obtained became the basis for further study of the effect of CO_2 in the OA model in rats.

Objective: to experimentally study the effect of carbon dioxide monotherapy, as well as its combined use with diclofenac sodium or chondroitin sulfate on the expression level of tumor necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β1) in a model of osteoarthritis induced by monoiodoacetic acid in rats.

Material and methods

The study used 80 sexually mature white outbred male rats (body weight 180–220 g), which were kept in standard vivarium conditions: air temperature — 21–23 °C, relative humidity — 50–60 %, light regime — 12 h light / 12 h dark. The animals had free access to water and standard laboratory food. Before the start of the experiment, all rats underwent 7-day acclimatization. All animal manipulations were carried out in accordance with the International Directive on the ethical use of laboratory animals (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes).

The osteoarthritis model was reproduced according to the modified protocols of M. Udo et al. [22] and R. Riewruja et al. [23]. Under ether anesthesia, rats were injected once with 0.05 ml of 3 % solution of MIOA into the cavity of the right knee joint of the hind limb. The solution was prepared ex tempore in 0.9 % NaCl. The control (intact) group was injected with an equivalent volume of saline.

Twenty-four hours after the induction of osteoarthritis, the animals were randomized into eight groups (five animals per subgroup) to assess the effects of therapy on the 14th and 28th days:

– intact animals (I) — without interventions;

– intact + NaCl (II) — intraperitoneal administration of 0.9 % NaCl;

– MIOA (III) — pathology group (osteoarthrosis without treatment);

– MIOA + diclofenac 8 mg/kg (IV) — intraperitoneally;

– MIOA + chondroitin sulfate 3 mg/kg (V) — intraperitoneally;

– MIOA + CO_2 0.5 ml (VI) — subcutaneously, periarticularly;

– MIOA + diclofenac sodium 4 mg/kg + CO_2 0.5 ml (VII) — combination therapy;

– MIOA + chondroitin sulfate 3 mg/kg + CO_2 0.5 ml (VIII) — combination therapy.

Diclofenac sodium (4 or 8 mg/kg) and chondroitin sulfate (3 mg/kg) were administered intraperitoneally every 3 days according to the protocol, based on the effectiveness of such doses in animal models of osteoarthritis [11, 32]. Carbon dioxide was applied locally — subcutaneously above the affected knee (0.5 ml every 3 days) for 14 or 28 days.

On the 14th or 28th day of the experiment, the animals were euthanized in accordance with the recommendations of the AVMA Guidelines for the Euthanasia of Animals (2020). Thiopental sodium was used to induce anesthesia at a dose of 50 mg/kg intraperitoneally. The state of deep anesthesia was determined by the absence of reflexes (corneal and painful) and a decrease in respiratory rate. After confirmation of deep anesthesia, cardiopuncture was performed with subsequent blood sampling until cardiac arrest.

Serum was obtained by centrifugation at 3000 rpm for 10 min at +4 °C and stored at −20 °C until analysis.

The concentrations of cytokines TNF-α and TGF-β1 in serum were determined by sandwich ELISA in triplicate using commercial kits FineTest® (China):

– TNF-α — Rat TNF-α ELISA Kit, cat. no. ER1 393; measurement range: (3.906–250) pg/ml; sensitivity: 2.344 pg/ml.

– TGF-β1 — Rat TGF-β1 ELISA Kit, cat. no. ER1 378; measurement range: (31.25–2000) pg/ml; sensitivity: 18.75 pg/ml.

Before the analysis, all reagents were incubated at room temperature for 20 min. The washing buffer was prepared by diluting with distilled water in a ratio of 1 : 25 according to the manufacturer's instructions.

Optical density was measured at a wavelength of 450 nm using a LabLine-026 microplate photometer.

Cytokine concentrations were calculated based on calibration curves using CurveExpert 1.4 software, which provides automatic selection of a mathematical model for constructing a standard curve.

The changes in the levels of the pro-inflammatory cytokine TNF-α and the anti-inflammatory mediator TGF-β1 in the serum of rats with experimental osteoarthritis was studied on the 14th and 28th day of treatment with different therapeutic regimens using carboxytherapy both in the case of monotherapy and combined use with traditional drugs (diclofenac sodium, chondroitin sulfate).

Statistical data processing was performed using Jamovi software, version 2.3.21. The results are presented as mean ± standard deviation (M ± SD). Normality of distribution was checked using the Shapiro–Wilk test.

For intergroup comparisons with normal distribution, Welch's t-test or ANOVA with Tukey's post hoc test was used. In case of deviation from normality, the Kruskal–Wallis test with Bonferroni correction was used. Results were considered statistically significant at $p < 0.05$. For the main comparisons, 95 % confidence intervals (CI) and effect sizes (Cohen's d) were calculated, which allows assessing both the statistical and clinical significance of the results.

Results and their discussion

The study showed that under the conditions of a single injection of 0.05 ml of 3 % MIOA solution into the knee joint cavity of experimental animals, a long-term significant increase in both TNF-α and TGF-β1 levels was observed, which indicates the presence of an inflammatory process (Table 1).

Thus, on day 14, a significant increase in TNF-α levels was observed in group III — (29.97 ± 0.50) pg/ml, which was 4.36 times higher than the value of the intact group ((6.87 ± 0.44) pg/ml, $p < 0.001$). All interventions that included the use of CO_2 — both as monotherapy and in combination with diclofenac or chondroitin — significantly reduced TNF-α levels compared to group III (ANOVA: $F = 2,551$, $df = 7$; 13.5; $p < 0.001$). The greatest decrease in TNF-α levels was found in group VII — (18.81 ± 0.28) pg/ml (37.3 % reduction relative to pathology; Mean difference = –11.16, 95 % CI [–11.66; –10.66], Cohen's $d = –27.5$; $p < 0.001$). The combination of chondroitin with CO_2 was also effective: (22.15 ± 0.35) pg/ml (–26.1 %, $p < 0.001$, Cohen's $d = –18.1$).

The difference in TNF-α levels between group III and all treated experimental groups (IV; V; VI; VII; VIII) was statistically significant (ANOVA: $F = 2,551$, $df = 7$; 13.5; $p < 0.001$).

On day 28, TNF-α levels remained elevated in group III ((29.59 ± 0.10) pg/ml). The lowest values were observed in group VII — (13.41 ± 0.52) pg/ml (–54.7 %, $p < 0.001$; Cohen's $d = –43.2$), while in group VIII the TNF-α level was (20.17 ± 0.45) pg/ml (–31.8 %; $p < 0.001$; Cohen's $d = –24.3$).

The difference in TNF-α levels between group III and all experimental groups remained statistically significant on the 28th day of observation (ANOVA: $F = 4,057$; $df = 7$; 12.8; $p < 0.001$).

Thus, the use of MIOA led to a significant increase in TGF-β1 levels — from (567.12 ± 19.4) pg/ml (intact) to (840.56 ± 7.87) pg/ml in group III (+48.2 %; $p < 0.001$).

On day 14, the highest level of TGF-β1 was recorded in group VIII — (1570.12 ± 18.50) pg/ml (an increase of 86.9 % compared to MIOA, $p < 0.001$; Mean difference = +730, 95 % CI [704.1; 755.9], Cohen's $d = 22.0$). The combination of CO_2 with diclofenac also showed efficacy: (1202.30 ± 11.56) pg/ml (+43.1 %; $p < 0.001$; Cohen's $d = 11.6$).

The difference in TGF-β1 levels between group III and all experimental groups receiving treatment (IV; V; VI; VII; VIII) was statistically significant (ANOVA: $F = 1,492$; $df = 7$; 13.5; $p < 0.001$).

On day 28, the highest value of TGF-β1 levels was again found in group VIII — (1658.91 ± 14.66) pg/ml, which is 99.4 % higher than pathology ((831.89 ± 6.19) pg/ml; $p < 0.001$, Mean difference = +827, CI [805.7; 848.3], Cohen's $d = 26.7$). The combined regimen with diclofenac + CO_2 maintained high values ((1226.35 ± 5.91) pg/ml, +394.5 pg/ml, Cohen's $d = 12.7$, $p < 0.001$).

The difference in the level of TGF-β1 between group III and all experimental groups remained statistically significant on the 28th day of observation (ANOVA: $F = 3,492$; $df = 7$; 12.6; $p < 0.001$).

In the groups that received treatment (IV; V; VI; VII; VIII), the level of TNF-α significantly decreased between the 14th and 28th days ($p < 0.001$). The largest decrease was recorded in group IV — by 9.28 % (from 25.43 to 23.07 pg/ml).

In parallel, a statistically significant increase in TGF-β1 levels was observed in all the indicated therapeutic groups, most significantly in group V by 206.2 pg/ml (+19.5 %, $p < 0.001$).

In this case, the results obtained indicate the prospects for the use of carboxytherapy (CO_2) as a monotherapeutic approach, as well as in combination with NSAIDs (diclofenac sodium) and chondroprotectors (chondroitin sulfate) in the conditions of an experimental model of OA induced by monoiodoacetic acid. In the groups receiving CO_2, a significant decrease in TNF-α levels was observed on the 14th and 28th day of the experiment, which indicates the anti-inflammatory potential of carboxytherapy. The most pronounced decrease (–54.7 %) relative to the pathology group was recorded in animals that were administered CO_2 in combination with diclofenac, which suggests the presence of a synergistic effect between these compounds.

One possible mechanism for this synergy is the effect of CO_2 on MAPK-dependent regulation of proinflammatory cytokines, including TNF-α and IL-6 [24]. In addition, CO_2 is known to promote local vasodilation, improve microcirculation and activate endothelial NO synthase, which leads to improved tissue oxygenation and create conditions for the repair

Table

Effect of CO_2 in monotherapy and combinations on the levels of pro-inflammatory cytokine TNF-α and anti-inflammatory factor TGF-β1 in the serum of rats with experimental osteoarthritis (14^{th} and 28^{th} day of observation)

Animal group	Day 14 TNF-α, pg/ml	Day 28 TNF-α, pg/ml	Day 14 TGF-β1, pg/ml	Day 28 TGF-β1, pg/ml
I	6.87 ± 0.44	6.87 ± 0.44	567.12 ± 19.40	567.12 ± 19.40
II	6.85 ± 0.33	6.85 ± 0.32	572.15 ± 21.25	572.15 ± 21.25
III	29.97 ± 0.50	29.59 ± 0.10	840.56 ± 7.87	831.89 ± 6.19
IV	25.43 ± 0.71***	23.07 ± 0.56***	917.93 ± 7.73***	907.25 ± 1.81***
V	27.90 ± 0.20***	26.55 ± 0.57***	1055.69 ± 26.13***	1261.88 ± 20.63***
VI	28.09 ± 0.66***	26.42 ± 0.35***	1133.62 ± 13.59***	1192.39 ± 20.42***
VII	18.81 ± 0.28***	13.41 ± 0.52***	1202.30 ± 11.56***	1226.35 ± 5.91***
VIII	22.15 ± 0.35***	20.17 ± 0.45***	1570.12 ± 18.50***	1658.91 ± 14.66***
Statistical significance of between-group difference	F (7; 13.5) = 2 551, p < 0.001	F (7; 12.8) = 4 057, p < 0.001	F (7; 13.5) = 1 492, p < 0.001	F (7; 12.6) = 3 492, p < 0.001
Shapiro-Wilk test	W = 0.985; P = 0.877	W = 0.986; P = 0.892	W = 0.985; P = 0.849	W = 0.969; P = 0.331

Notes: Data are presented as mean ± standard deviation (n = 5 in each group). No statistically significant deviations from normal distribution were found for all variables (Shapiro–Wilk test, p > 0.05), which allowed the use of one-way analysis of variance (ANOVA) with Tukey's post hoc test. *** p < 0.001 — significant difference compared to the pathology group.

of damaged structures [25, 26]. Although most of these effects have been studied in the skin, similar microvascular responses may also be relevant for joint tissues, especially the subchondral plate. In our study, an increase in the level of TGF-β1, a cytokine that plays an important role in chondrogenesis and cartilage matrix repair, was also recorded. The highest levels of TGF-β1 (1658.91 ± 14.66) pg/ml were found in the group receiving chondroitin sulfate in combination with CO_2. It is known that TGF-β1 stimulates the production of aggrecan, proteoglycans and type II collagen in chondrocytes, which ensures the renewal of the extracellular matrix [27].

The obtained data emphasize the potential of carboxytherapy as an adjuvant approach in the treatment of OA. High expression of TGF-β1 in group VIII correlates with the activation of reparative processes in articular cartilage, which is consistent with the literature data on the role of TGF-β1 as a key mediator of chondrogenesis, maintenance of tissue homeostasis and cartilage remodeling [28]. At the same time, it should be noted that with prolonged stimulation, TGF-β1 may also play a fibrogenic role, which necessitates further observation and morphological analysis of its long-term effects.

Indirect confirmation of the reparative potential of CO_2 is provided by the data of R. Amano-Iga et al. [29], where it was proven in a skin injury model that percutaneous CO_2 administration stimulates the expression of VEGF and TGF-β, and also suppresses HIF-1α, IL-6 and IL-1β, which is accompanied by accelerated tissue healing. Despite the different type of model, the general mechanisms — anti-inflammatory effect, activation of growth factors — may be relevant for cartilage tissue. Similarly, in the study of K. Takeshita et al. [30] it was shown that hypercapnic state inhibits cytokine-induced activation of NF-κB, and the work of C. Brandi et al. [31] demonstrated the ability of CO_2 to improve microcirculation and stimulate tissue regeneration. These effects create a favorable microenvironment for repair, in particular through stimulation of TGF-β1.

In addition to statistical significance, the effects obtained are potentially clinically relevant: the effect size values (Cohen's d > 0.8) for the reduction in TNF-α and the increase in TGF-β1 indicate pronounced biological changes. This may provide the basis for the development of combined therapeutic strategies with the possibility of reducing the dosage of NSAIDs, which in turn will reduce the risk of side effects and potentially enhance the chondroprotective effect of the treatment.

At the same time, the interpretation of the obtained results should take into account certain limitations. First, the study was conducted on an animal model, which only partially reflects the pathophysiology of human osteoarthritis. Second, the number of animals in each group was limited (n = 5), which reduces statistical power. Third, the study did not include morphological analysis of tissues (histology, immunohistochemistry), which narrows the completeness of the morphofunctional interpretation. In addition,

only two cytokines (TNF-α, TGF-β1) were studied, while other important mediators of inflammation and matrix degradation, such as IL-1β, IL-6, IL-17, MMP-13, remained outside the scope of the analysis.

Given this, further studies should be aimed at expanding the molecular profile, morphological confirmation of changes in tissues, increasing the sample, and studying the effectiveness of combined CO_2 therapy in clinical models.

Conclusions

Carboxytherapy (CO_2) demonstrated a pronounced anti-inflammatory effect, as evidenced by a significant decrease in the level of the pro-inflammatory cytokine TNF-α in rats with experimental osteoarthritis on both the 14th and 28th day of observation.

The use of CO_2 in combination with diclofenac sodium or chondroitin sulfate enhanced the anti-inflammatory effect, providing greater efficacy compared to monotherapy. The most pronounced decrease in TNF-α was recorded in group VII.

Simultaneously with the anti-inflammatory effect, combined treatment with CO_2 promoted the stimulation of reparative processes, which was confirmed by a significant increase in the level of TGF-β1, especially in group VIII, where the highest values of this indicator were recorded.

The effect sizes (Cohen's $d > 0.8$) in key comparisons confirm not only the statistical but also the potential clinical significance of the use of CO_2 as an adjuvant therapeutic factor in osteoarthritis.

The obtained data confirm the feasibility of further preclinical studies aimed at elucidating the molecular mechanisms of action of CO_2, with the prospect of transitioning to clinical trials for the development of combined strategies for the treatment of OA using carboxytherapy.

Conflict of interest. The authors declare the absence of a conflict of interest.

Prospects for further research. In the future, further studies are needed, in particular, morphological verification of the effects of CO_2 both in monotherapy and in combination with diclofenac sodium or chondroitin sulfate, assessment of long-term effects on cartilage tissue, as well as expansion of the panel of studied mediators, including cytokines (IL-1β, IL-6, VEGF) and cartilage matrix degradation enzymes, in particular MMP-1, MMP-3, MMP-13, as well as aggrecanases ADAMTS-4 and ADAMTS-5. Of particular importance is the clinical validation of the results obtained, by conducting studies on the effectiveness of carboxytherapy in combination with diclofenac or chondroitin in real clinical practice of treating osteoarthritis.

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THE EFFECT OF CARBOXYTHERAPY AND ITS COMBINATION WITH DICLOFENAC SODIUM AND CHONDROITIN SULFATE ON TNF-A AND TGF-B1 EXPRESSION IN MONOIODOACETATE-INDUCED OSTEOARTHRITIS IN RATS

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