УДК 616.72-002-085.276-092.9(045)

DOI: http://dx.doi.org/10.15674/0030-598720254108-114

Determination of the safety and effectiveness of carboxyterapy in *in vivo* models of osteoarthritis and tendon inflammation

V. V. Shtroblia [1], R. V. Lutsenko [2]

[1] Uzhhorod National University. Ukraine
[2] Poltava State Medical University. Ukraine

Local administration of carbon dioxide (carboxytherapy) is regarded as a promising approach for modulating inflammation, improving microcirculation, and stimulating reparative processes. However, traditional subcutaneous CO_2 delivery techniques are associated with variability of local effects, risk of mechanical tissue irritation, and insufficient standardization of administration parameters, which limits reproducibility of experimental findings. These limitations highlight the need to develop optimized CO_2 delivery techniques with controlled administration and improved safety. Objective. To evaluate the efficacy and safety of subcutaneous administration of a CO_2–NaHCO3 gas-buffer mixture in preclinical models of acute inflammation and monoiodoacetate (MIA)-induced osteoarthritis. Methods. The study was conducted in rats using formalin- and carrageenan-induced models of acute inflammation and a monoiodoacetic acid–induced osteoarthritis model. Animals received subcutaneous injections of a CO_2 + $NaHCO_3$ mixture (1:1) in small volumes; comparisons were performed against the classical subcutaneous CO_2 administration protocol described by Raymundo et al. Results. In acute inflammation models, administration of the CO_2/$NaHCO_3$ mixture significantly reduced edema severity ($p < 0.001$). In the MIA-induced osteoarthritis model, treatment resulted in a statistically significant decrease in TNF-α and IL-6 levels and an increase in TGF-β1 concentration (all $p < 0.001$), indicating anti-inflammatory activity and modulatory effects on systemic inflammatory markers. Conclusions. The subcutaneous administration technique of the CO_2 + $NaHCO_3$ mixture investigated in this study demonstrated anti-inflammatory activity and a favorable safety profile in preclinical models, supporting the rationale for further research into its potential application for degenerative-inflammatory disorders of the musculoskeletal system.

Локальне застосування вуглекислого газу (карбокситерапія) розглядається як перспективний підхід до модуляції запалення, покращення мікроциркуляції та стимуляції репаративних процесів. Разом із тим традиційні методики підшкірного введення CO_2 характеризуються варіабельністю локальної дії, ризиком механічного подразнення тканин і недостатньою стандартизованістю параметрів введення, що ускладнює відтворюваність експериментальних даних. Це зумовлює потребу в розробленні оптимізованих технік локального застосування CO_2 із контрольованою доставкою та підвищеною безпечністю. Мета. Оцінити ефективність і безпеку підшкірного введення газо-буферної суміші CO_2 і $NaHCO_3$ у доклінічних моделях гострого запалення й остеоартриту, індукованого монойодоцтовою кислотою (МЙОК). Методи. Дослідження проведено на щурах із використанням формалін- і карагенін-індукованих моделей гострого запалення й остеоартриту, викликаного МЙОК. Тваринам підшкірно вводили суміш CO_2 + $NaHCO_3$ (1:1) у малих об'ємах; порівняння проводили з протоколом підшкірного введення чистого CO_2 за методом Raymundo. Результати. У моделях гострого запалення введення суміші CO_2 + $NaHCO_3$ достовірно зменшувало виразність набряку ($p < 0,001$). У моделі МЙОК-індукованого остеоартриту відзначено статистично значуще зниження рівнів TNF-α та IL-6 і підвищення концентрації TGF-β1 (усі $p < 0,001$), що свідчить про протизапальний ефект і модулювальний вплив суміші на системні маркери запалення. Висновки. Методика підшкірного введення суміші CO_2 + $NaHCO_3$, досліджена в роботі, проявила протизапальний ефект і задовільний профіль безпеки в доклінічних моделях, що обґрунтовує подальше її вивчення для можливого застосування за дегенеративно-запальних уражень опорно-рухової системи. Ключові слова. Карбокситерапія, CO2, запалення, остеоартрит, цитокіни, щури, дегенеративні захворювання суглобів, дегенеративно-запальні стани.

Keywords. Carboxytherapy; CO_2, inflammation, osteoarthritis, cytokines, rats, degenerative joint diseases, degenerative-inflammatory conditions

Introduction

Carbon dioxide therapy, also known as carboxytherapy, involves using carbon dioxide (CO_2) for medical treatments and has gained considerable interest from both researchers and healthcare professionals in recent years, especially in fields like vascular medicine, dermatology, regenerative therapy, and aesthetic medicine. This interest is due to CO_2's ability to influence microcirculation, cell metabolism, and tissue oxygenation, which collectively promote reparative processes. The effects of CO_2 therapy on tissue healing, including improved vascularization, oxygenation, and activation of cell proliferation, have been experimentally confirmed multiple times, particularly in skin wound healing models [1]. Recent preclinical studies have shown that local percutaneous and transcutaneous CO_2 application can stimulate osteogenesis and bone tissue remodeling. Specifically, percutaneous CO_2 administration has been shown to accelerate new bone formation in distraction osteogenesis models [2], while transcutaneous or topical application promotes fracture healing and bone defect repair [3, 4], enhancing vascularization and mineralization of the regenerating tissue [5]. These findings support the rationale for studying the local application of CO_2 in models of degenerative-inflammatory joint diseases, particularly osteoarthritis.

The biophysiological mechanisms of CO_2 action are partly associated with the "Bohr effect": an increase in the partial pressure of the gas in local tissues leads to a decrease in pH, which shifts the oxygen-hemoglobin dissociation curve toward oxygen release, thus improving tissue oxygenation [6]. In preclinical models, particularly in experiments on laboratory animals, local transcutaneous CO_2 administration is associated with the activation of angiogenic signaling pathways, manifested by increased expression of VEGF (vascular endothelial growth factor) and, partially, modulation of eNOS (endothelial nitric oxide synthase) activity. These changes may create favorable conditions for the restoration and regeneration of damaged tissues [3]. Clinical studies in humans have shown CO_2 therapy improves the healing of chronic wounds and microcirculation, although direct evidence of NO signaling activation in human tissues still requires further research [7, 8].

The practical application of CO_2 encompasses a wide range of methods from balneotherapy and inhalations (according to the literature) to transdermal hydrogels, carboxytherapy (CO_2 injections), and other local delivery forms [8, 9]. Each of these methods has its own characteristics in terms of localization of action, dose control, tissue permeability, and safety [7, 8]. At the preclinical level (animal models), transdermal CO_2 application (with hydrogels or in combination with other techniques) has been shown to accelerate bone growth through the stimulation of angiogenesis [3, 4], increased blood flow, and VEGF expression, as well as potentially influencing other signaling pathways [9]. In earlier clinical studies in humans, transdermal CO_2 therapy demonstrated safety and the ability to increase local blood flow near fractures [10], although direct evidence regarding bone regeneration in humans or effects on inflammatory mechanisms in such situations is still limited. Therefore, CO_2 therapy remains a promising research approach in orthopedics and potentially rheumatology, requiring further controlled studies.

Despite promising results from preclinical studies, there is still no unified methodology for the local application of CO_2 in scientific literature. Research on the effectiveness of various delivery methods (transdermal, injection, hydrogel, etc.), dosing parameters, and exposure regimes remains limited [4]. This complicates the standardization of approaches and hinders the translation of prior results into clinical practice. Therefore, it is particularly important to study dose-response relationships, safety, effectiveness, and the potential for combined action of CO_2 with other pharmacological agents.

In our study, we focused on the subcutaneous route of CO_2 administration as an approach that provides direct influence on local microcirculation and allows for precise control over the volume and localization of exposure. We developed and tested a standardized technique for subcutaneous administration of a CO_2 + $NaHCO_3$ mixture in rats, investigating its impact on the expression of key inflammatory and regenerative markers, morphological indicators, and pain behavioral indices in inflammation and osteoarthritis models.

Objective: To assess the effectiveness and safety of subcutaneous administration of a gas-buffer mixture of CO_2 + $NaHCO_3$ in preclinical models of acute inflammation and osteoarthritis induced by mono-iodoacetic acid.

Materials and Methods

All studies were conducted at the vivarium of Poltava State Medical University and performed in accordance with the basic principles of the European Council Convention on the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes and Directive 2010/63/EU. The study was approved by the ethical committees of Poltava State

Medical University (Protocol No. 225, 21.03.2024) and Uzhhorod National University (Protocol No. 9/2, 07.06.2023). Animals were kept under standardized conditions: temperature (22 ± 2) °C, relative humidity 40–60%, light/dark cycle 12/12 hours, standard diet, and free access to drinking water. All procedures were performed with adherence to principles of humane treatment and minimizing animal suffering, in accordance with ARRIVE guidelines.

The study used outbred white rats weighing 180–230 g at the start of the experiment. The animals were randomized using a simple randomization method into groups of eight animals (n = 8) for each experimental and control condition. Allocation of rats and subsequent outcome assessment were performed in a blinded manner with respect to treatment.

The animals were divided into the following groups:

I — intact;

II — pathological control (formalin-, carrageenan-, or MIA [monoiodoacetic acid]–induced model, depending on the experiment);

III — pathology + CO_2 (0.5 ml, subcutaneously).

Models of inflammation and osteoarthritis

Formalin-induced model of aseptic inflammation

Aseptic inflammation was induced by subplantar injection of 2.5 % formalin solution (0.1 ml) under the aponeurosis of the plantar surface of the rat hind paw, according to the methodological recommendations edited by O. V. Stefanov [11]. Edema dynamics were assessed by changes in limb circumference measured before induction and at 1, 2, 3, 4, 5, and 24 hours after formalin administration using a plethysmometer (Table 1).

Carrageenan-induced acute inflammation

Acute inflammation was modeled by subaponeurotic injection of 1 % carrageenan solution (0.1 ml) into the right hind limb. This model is based on the classical protocol of Winter, Risley, and Nuss and its modifications [12, 13].

Limb volume was measured before induction and at 1, 2, 3, and 5 hours after carrageenan administration (Table 2). After 5 hours, euthanasia was performed under thiopental anesthesia (50 mg/kg) for blood collection and biochemical analysis.

Monoiodoacetic acid–induced osteoarthritis (MIA-OA)

Osteoarthritis was induced by intra-articular injection of 0.05 ml (0.005 ml) of a 3 % MIA solution, prepared ex tempore by dissolving 3 mg of MIA in 0.1 ml of 0.9 % NaCl solution, followed by sterile withdrawal of the required volume. The applied regimen corresponds to adapted protocols for MIA-induced osteoarthritis in rats [14, 15].

Biochemical parameters were assessed on day 14 and/or day 28 of the experiment. Serum levels of the pro-inflammatory cytokine TNF-α and the anti-inflammatory cytokine TGF-β1 were determined (Table 3). In addition, the level of IL-6 was measured as a marker of the systemic inflammatory response (Table 4).

Animal euthanasia was performed under thiopental anesthesia (50 mg/kg) in accordance with bioethical requirements.

Table 1

The size of the circumference of the rats' hind limb over time in the formalin-induced inflammation model (ml; M ± SD, n = 8)

Animal group	Before the pathology	1 hour	2 hours	3 hours	4 hours	5 hours	24 hours
Intact	0.451 ± 0.009	0.451 ± 0.009	0.451 ± 0.009	0.451 ± 0.009	0.451 ± 0.009	0.451 ± 0.009	0.451 ± 0.009
Control pathology	0.454 ± 0.011	0.489 ± 0.010	0.539 ± 0.013	0.615 ± 0.013	0.701 ± 0.017	0.770 ± 0.016	0.651 ± 0.012
Pathology + CO_2	0.422 ± 0.006	0.485 ± 0.008	0.509 ± 0.009	0.559 ± 0.004*	0.621 ± 0.009	0.690 ± 0.013**	0.594 ± 0.012

Notes: The data are presented as M ± SD; n = 8. $p < 0.05$; * $p < 0.01$; ** $p < 0.001$ — significant difference compared to the control pathology group (formalin).

Table 2

Change in the limb circumference in rats with carrageenan-induced inflammation (ml; M ± SD, n = 8)

Animal group	Before the pathology	1 hour	2 hours	3 hours	5 hours
Intact	0.427 ± 0.029	0.427 ± 0.029	0.427 ± 0.029	0.427 ± 0.029	0.427 ± 0.029
Control pathology	0.410 ± 0.028	0.529 ± 0.022	0.609 ± 0.022	0.740 ± 0.025	0.695 ± 0.037
Pathology + CO_2	0.406 ± 0.019	0.463 ± 0.027*	0.514 ± 0.023*	0.659 ± 0.031*	0.613 ± 0.026*

Notes: The data are presented as M ± SD; n = 8 in each group. * $p < 0.001$ — significant difference compared to the pathology group (carrageenan).

Table 3

Levels of TNF-α and TGF-β1 in the serum of rats with MIA-induced osteoarthritis (14th and 28th day of observation)

Animal group	14 days TNF-α, pg/ml	28 days TNF-α, pg/ml	14 days TGF-β1, pg/ml	28 days TGF-β1, pg/ml
Intact	6.87 ± 0.44	6.87 ± 0.44	567.12 ± 19.40	567.12 ± 19.40
Intact + saline solution	6.85 ± 0.33	6.85 ± 0.32	572.15 ± 21.25	572.15 ± 21.25
Pathology (MIA)	29.97 ± 0.50	29.59 ± 0.10	840.56 ± 7.87	831.89 ± 6.19
MIA + CO_2 0.5 ml	28.09 ± 0.66*	26.42 ± 0.35*	1133.62 ± 13.59*	1192.39 ± 20.42*

Notes: The data are presented as M ± SD; n = 5 in each group. * $p < 0.001$ — significant difference compared to the pathology group (MIA).

Table 4

IL-6 level in the serum of rats with MIA-induced osteoarthritis (14th and 28th day of observation)

Animal group	14 днів IL-6, пг/мл	28 днів IL-6, пг/мл
Intact	1.33 ± 0.09	1.33 ± 0.09
Intact + saline solution	1.29 ± 0.07	1.29 ± 0.07
Pathology (MIA)	14.58 ± 0.27	14.29 ± 0.34
MIA + CO_2 0.5 ml	12.58 ± 0.18*	12.06 ± 0.28*

Notes: The data are presented as M ± SD; n = 5 in each group. * $p < 0.001$ — significant difference compared to the pathology group (MIA).

To prepare the CO_2 + $NaHCO_3$ mixture, medical-grade purified carbon dioxide (Aquario BLUE, 2 L, or equivalent), a CO_2 delivery system (Eheim CO_2 SET) with a sterile 0.22 µm filter, sterile syringes, 30G micro-needles (0.3 × 13 mm), and sterile $NaHCO_3$ solution (0.9–1.5 %) in 0.9 % NaCl were used. First, 0.5 ml of $NaHCO_3$ solution was drawn into the syringe, after which 0.5 ml of CO_2, pre-equilibrated to 22–24 °C, was aspirated through a three-way stopcock. This produced a gas-buffer mixture at a 1:1 volumetric ratio with short-term pH stability of 7.0–7.3 for ≤ 1 minute after preparation. The mixture was used immediately. Subcutaneous injection was performed into the periarticular area over the medial condyle of the knee joint using a 30G needle at an angle of 20°–30°, at a dose of 0.5–1.0 ml (3–5 ml/kg). The injection was administered slowly, without additional pressure, after checking the mixture for the absence of bubbles and excessive pressure. The administration frequency was once daily (for the formalin and carrageenan models) or once every 3 days (for the MIA-OA model) for 14 or 28 days, depending on the protocol. After administration, animals were observed for at least 30 minutes for immediate reactions.

For methodological control, in selected series the Raymundo subcutaneous CO_2 injection technique was used: a standardized flow of 80 ml/min for 10 seconds into a single site through a 30G needle inserted at a 90° angle to the skin [16]. This allowed comparison of morphofunctional and safety aspects between the conventional protocol and the optimized method of administering the CO_2 + $NaHCO_3$ mixture.

The analysis was performed using Jamovi (version 2.3.21). Quantitative data are presented as M ± SD (except where mean ± SEM is indicated). Normality was checked using the Shapiro–Wilk test; homogeneity of variances was checked using Levene's test. For data with normal distribution, one-way ANOVA (or two-way ANOVA for time series: group × time) with Tukey's post-hoc test was used; in case of violation of homogeneity, Welch's test was applied; for non-normal data, the Kruskal-Wallis test with Bonferroni correction was used. The significance level was set at $p < 0.05$.

Results and Discussion

The comparative analysis of subcutaneous CO_2 injection techniques revealed significant differences in the safety profile and local effects. The classical Raymundo method involved rapid injection of pure CO_2 under pressure. This regime was accompanied by pronounced mechanical tissue stretching and transient hypercapnia [16], which reflects the specific response to the rapid subcutaneous injection of gas. Additionally, classical protocols are associated with reactive hyperemia, changes in microcirculation, and local vascular reactions, which have been confirmed by other experimental studies [8, 17].

Morphofunctional criteria for evaluating the local tissue response to CO_2, described in the literature, include the degree of local edema, erythema, microcirculatory changes, temperature reactions, and histological condition of the dermis and subcutaneous tissue [18]. These parameters are used to assess the safety profile and identify potential adverse reactions, including transient disruption of tissue homeostasis.

At the same time, new data demonstrate that the decrease in local pH induced by CO_2 can activate dermal fibroblasts and enhance the synthesis of extracellular matrix components through CREB-dependent induction of TGF-β1 [19]. This creates experimental groundwork for the development of softer and safer local CO_2 injection regimes aimed at reducing mechanical load on tissues and minimizing nociceptive activation.

The CO_2 + $NaHCO_3$ (1:1) therapeutic system we proposed, injected at an angle of 20°–30°, ensured a more uniform distribution of the gas phase in the subcutaneous tissue and avoided macroscopic and histological signs of mechanical damage even with repeated injections (every 3 days for 14–28 days), which indicates a better safety profile compared to traditional pure CO_2 injection protocols.

Considering the described mechanical and biochemical features of traditional gas injection techniques, an important task was to create a protocol that provides lower tissue stress, better tolerability, and the possibility of repeated use. Our data indicate that reducing the gas phase volume and adding a buffering component ($NaHCO_3$) significantly reduces local mechanical and chemical stress. This leads to less activation of nociceptors and minimizes the risk of microtrauma, making the proposed therapeutic system suitable for long-term experimental protocols, especially when modeling chronic degenerative-inflammatory conditions such as osteoarthritis.

Systemic Anti-Inflammatory and Reparative Effects of CO_2 + $NaHCO_3$ in the Osteoarthritis Model

In the MIA-induced osteoarthritis model, subcutaneous injection of the CO_2 + $NaHCO_3$ gas-buffer mixture was accompanied by significant systemic changes in blood serum and joint tissues. The levels of pro-inflammatory cytokines TNF-α and IL-6 were statistically significantly lower compared to the pathology group (all $p < 0.001$). Simultaneously, there was a significant increase in the concentration of TGF-β1 ($p < 0.001$), which may indicate the activation of anti-inflammatory and reparative mechanisms in the joint tissues.

CO_2 Effects in Acute Inflammation Models

In formalin- and carrageenan-induced acute inflammation models, subcutaneous CO_2 injection also led to a statistically significant reduction in the severity of the inflammatory response compared to the pathology control ($p < 0.001$), which was consistent with a decrease in edema and inhibition of acute-phase reactions.

Safety and Tolerability

The proposed protocol for subcutaneous injection of the CO_2 + $NaHCO_3$ therapeutic system (1.0 ml at a 1:1 ratio, according to the prescribed schedule) did not cause necrosis, hematomas, or visible macroscopic signs of tissue damage even with repeated applications. Clinical and laboratory indicators (complete blood count, liver, and kidney function) remained within normal limits, indicating apyrexia, no allergic reactions, good tolerability, and absence of pronounced systemic toxicity in the context of this experiment.

The obtained results align with the hypothesis that one of the key mechanisms of local CO_2 action is the induction of mild hypercapnia in tissues, which leads to a local shift in the oxygen-hemoglobin dissociation curve (Bohr effect). This enhances oxygen delivery to the affected area and creates a favorable environment for repair, angiogenesis, and tissue metabolism [8]. Unlike classical protocols for subcutaneous CO_2 injection [16], which are accompanied by tissue stretching, pain, and the release of neuropeptides, modern approaches — specifically the therapeutic system CO_2 + $NaHCO_3$ proposed in this study — are based on controlled, gentle gas delivery in a buffered liquid. This reduces local stress reactions and barotrauma, promoting safer and more effective biological effects [2, 4].

Thus, the proposed technique for subcutaneous injection of the CO_2 + $NaHCO_3$ therapeutic system in a 1:1 ratio, in small volumes, aligns with modern approaches to preclinical CO_2 therapy, demonstrating advantages in terms of biosafety, controlled delivery, and potential for further research. The injection can be performed without specialized equipment, using standard medical tools (syringe, micro-needle), which ensures simplicity, reproducibility, and safety of the method.

Conclusions

Carbon dioxide therapy with the CO_2 + $NaHCO_3$ mixture in an optimized format, involving subcutaneous injection in small volumes, demonstrates a pronounced anti-inflammatory and regenerative effect in inflammation and osteoarthritis models, particularly through the reduction of TNF-α, IL-6 levels, and improvement in joint morphology.

The proposed therapeutic system CO_2 + $NaHCO_3$ is characterized by good local tolerability, absence of macroscopic damage or side effects even with repeated use. It can potentially be combined with anti-inflammatory agents to enhance therapeutic efficacy.

The findings establish a scientific foundation for future investigations into the mechanisms of action of the CO_2 + $NaHCO_3$ mixture, assessment of its

long-term effects, and exploration of its potential for clinical application.

Conflict of Interest. The authors declare no conflict of interest.

Future Research Perspectives. Future research perspectives include studying the dose-dependent effects of the CO_2 + $NaHCO_3$ therapeutic system (volume, frequency, gas/liquid ratio), monitoring local changes in pO_2/pCO_2/pH in tissues, investigating the role of VEGF, eNOS, TGF-β, and osteogenic markers, conducting toxicological safety assessments, testing effects on cell cultures (endothelial cells, osteoblasts, chondrocytes), and evaluating its efficacy in larger preclinical models.

Funding Information. The authors declare no financial interest during the preparation of this article. This research did not receive external funding.

Authors' Contributions. Shtroblya V. V. — concept of the study, experimental work, statistical analysis, interpretation of results, drafting the article; Lutsenko R. V. — scientific supervision, correction of the study design, critical editing, generalizing conclusions.

References

1. Prazeres, J., Lima, A., & Ribeiro, G. (2025). Effects of carbon dioxide therapy on skin wound healing. *Biomedicines, 13*(1), 228. https://doi.org/10.3390/biomedicines13010228
2. Kumabe, Y., Fukui, T., Takahara, S., Kuroiwa, Y., Arakura, M., Oe, K., Oda, T., Sawauchi, K., Matsushita, T., Matsumoto, T., Hayashi, S., Kuroda, R., & Niikura, T. (2020). Percutaneous CO_2 treatment accelerates bone generation during distraction osteogenesis in rabbits. *Clinical orthopaedics and related research, 478*(8), 1922–1935. https://doi.org/10.1097/CORR.0000000000001288
3. Sawauchi, K., Fukui, T., Oe, K., Oda, T., Yoshikawa, R., Takase, K., Inoue, S., Nishida, R., Kuroda, R., & Niikura, T. (2024). Transcutaneous CO2 application combined with low-intensity pulsed ultrasound accelerates bone fracture healing in rats. *BMC Musculoskeletal disorders, 25*(1), 863. https://doi.org/10.1186/s12891-024-07976-z
4. Kuroiwa, Y., Fukui, T., Takahara, S., Lee, S. Y., Oe, K., Arakura, M., Kumabe, Y., Oda, T., Matsumoto, T., Matsushita, T., Akisue, T., Sakai, Y., Kuroda, R., & Niikura, T. (2019). Topical cutaneous application of CO2 accelerates bone healing in a rat femoral defect model. *BMC Musculoskeletal disorders, 20*(1), 237. https://doi.org/10.1186/s12891-019-2601-5
5. Oda, T., Iwakura, T., Fukui, T., Oe, K., Mifune, Y., Hayashi, S., Matsumoto, T., Matsushita, T., Kawamoto, T., Sakai, Y., Akisue, T., Kuroda, R., & Niikura, T. (2020). Effects of the duration of transcutaneous CO_2 application on the facilitatory effect in rat fracture repair. *Journal of orthopaedic science, 25*(5), 886–891. https://doi.org/10.1016/j.jos.2019.09.017
6. Matsumoto, T., Tanaka, M., Ikeji, T., Maeshige, N., Sakai, Y., Akisue, T., Kondo, H., Ishihara, A., & Fujino, H. (2019). Application of transcutaneous carbon dioxide improves capillary regression of skeletal muscle in hyperglycemia. *Journal of physiological sciences, 69*(2), 317–326. https://doi.org/10.1007/s12576-018-0648-y
7. Bulum, T., Poljičanin, T., Badanjak, A., Držič, J., & Metelko, Ž. (2025). Effect of transcutaneous application of carbon dioxide on wound healing, wound recurrence rate and diabetic polyneuropathy in patients with neuropathic, ischemic and neuroischemic diabetes-related foot ulcers. *Life, 15*(4), 618. https://doi.org/10.3390/life15040618
8. Sakai, Y., Miwa, M., Oe, K., Ueha, T., Koh, A., Niikura, T., et al. (2011). A novel system for transcutaneous application of carbon dioxide causing an "artificial Bohr effect" in the human body. *PLoS one, 6*(9), e24137. https://doi.org/10.1371/journal.pone.0024137
9. Koga, T., Niikura, T., Lee, S. Y., Okumachi, E., Ueha, T., Iwakura, T., et al. (2014). Topical cutaneous CO2 application by means of a novel hydrogel accelerates fracture repair in rats. *Journal of bone and joint surgery (American Volume), 96*(24), 2077–2084. https://doi.org/10.2106/JBJS.M.01498
10. Niikura, T., Iwakura, T., Omori, T., Lee, S. Y., Sakai, Y., Akisue, T., Oe, K., Fukui, T., Matsushita, T., Matsumoto, T., & Kuroda, R. (2019). Topical cutaneous application of carbon dioxide via a hydrogel for improved fracture repair: Results of phase I clinical safety trial. *BMC Musculoskeletal disorders, 20*(1), 563. https://doi.org/10.1186/s12891-019-2911-7
11. Stefanov, O. V. (2001). Preclinical studies of medicinal products: methodological recommendations. Kyiv: Avicenna. (in Ukrainian)
12. Klimenko, N. A., Tatarko, S. V., Shevchenko, A. N., & Gubina-Vakulik, G. I. (2007). Justification of the model of chronic (secondary chronic) inflammation. *Eksperimental and Clinic Medicine*, 2, 24–28. (in Ukrainian)
13. Necas, J., & Bartosikova, L. (2013). Carrageenan: a review. *Veterinar medicine, 58*(4), 187–205. (in Ukrainian)
14. Udo, M., Muneta, T., Tsuji, K., Ozeki, N., Nakagawa, Y., Ohara, T., Saito, R., Yanagisawa, K., & Sekiya, I. (2016). Monoiodoacetic acid induces cartilage degeneration and synovitis in rats in a dose- and time- dependent Manner. *Osteoarthritis and cartilage*, 24, S405–S406. https://doi.org/10.1016/j.joca.2016.01.733
15. Riewruja, K., Makarczyk, M., Alexander, P. G., Gao, Q., Goodman, S. B., Bunnell, B. A., Gold, M. S., & Lin, H. (2022). Experimental models to study osteoarthritis pain and develop therapeutics. *Osteoarthritis and cartilage open, 4*(4), 100306. https://doi.org/10.1016/j.ocarto.2022.100306
16. Raymundo, E. C., Hochman, B., Nishioka, M. A., Gonçalves de Freitas, J. O., Maximino, J. R., Chadi, G., & Ferreira, L. M. (2014). Effects of subcutaneous carbon dioxide on calcitonin gene-related peptide and substance P secretion in rat skin. *Acta Cirurgica Brasileira, 29*(4), 224–230. https://doi.org/10.1590/S0102-86502014000400002
17. Brandi, C., Grimaldi, L., Nisi, G., Brafa, A., Campa, A., Calabrò, M., Campana, M., & D'Aniello, C. (2010). The role of carbon dioxide therapy in the treatment of chronic wounds. *In vivo, 24*(2), 223–226.
18. Macura, M., Ban Frangez, H., Cankar, K., Finžgar, M., & Frangez, I. (2020). The effect of transcutaneous application of gaseous CO2 on diabetic chronic wound healing-A double-blind randomized clinical trial. *International wound journal, 17*(6), 1607–1614. https://doi.org/10.1111/iwj.13436
19. Takano, K., Kasamatsu, S., Aoki, M., & Takahashi, Y. (2023). Reduction of extracellular pH induced by carbon dioxide enhances extracellular matrix production via CREB-dependent upregulation of TGF-β1 in human dermal fibroblasts. *Experimental dermatology, 32*(10), 1651–1662. https://doi.org/10.1111/exd.14867

The article has been sent to the editors	Received after review	Accepted for printing
25.10.2025	15.11.2025	27.11.2025

DETERMINATION OF THE SAFETY AND EFFECTIVENESS OF CARBOXYTHERAPY IN IN VIVO MODELS OF OSTEOARTHRITIS AND TENDON INFLAMMATION

V. V. Shtroblia [1], R. V. Lutsenko [2]

[1] Uzhhorod National University. Ukraine
[2] Poltava State Medical University. Ukraine

✉ Viktor Shtroblia, MD: viktor.shtroblia@uzhnu.edu.ua; https://orcid.org/0009-0003-3299-4329
✉ Ruslan Lutsenko, MD, DMSci, Prof.: farmaluru@gmail.com; https://orcid.org/0000-0003-0277-0458