

REVIEW ARTICLE

Ozone therapy and blood oxygen transport: A comprehensive narrative review of physiological mechanisms and clinical aspects

Viktor V. Zinchuk^{id}, **Elena S. Biletskaya***^{id}, and **Pavel E. Voloshko**

Department of Normal Physiology, Faculty of Medicine, Grodno State Medical University, Grodno, Belarus

Abstract

Ozone therapy is increasingly used across clinical fields, yet the biological mechanisms underlying its effects on tissue oxygenation remain incompletely understood. This work aimed to synthesise current evidence on how ozone affects blood oxygen transport, focusing on haemoglobin oxygen affinity, gasotransmitters (nitric oxide [NO], hydrogen sulfide [H₂S]), erythrocyte rheology, and oxidative–antioxidant balance. We searched PubMed, Scopus, and Web of Science (1995–2026; search date: August 2026) using terms related to ozone therapy, oxygen transport, haemoglobin affinity, p50, 2,3-diphosphoglycerate [2,3-DPG], NO, H₂S, and erythrocyte deformability. We included peer-reviewed *in vitro*, animal, and human studies, as well as relevant narrative and systematic reviews. We applied no language restriction *a priori*; however, inclusion was practically limited to languages accessible to the authors. Two authors performed study selection and data extraction; they resolved discrepancies through discussion. At therapeutic concentrations, ozone induces mild, transient oxidative stress that may trigger adaptive responses, including a rightward shift of the oxyhaemoglobin dissociation curve (decreased oxygen affinity), increased erythrocyte deformability, and upregulation of antioxidant enzymes. These effects appear dose-dependent and are most consistently observed in *in vitro* and animal models, with more limited and heterogeneous human mechanistic data. Proposed mechanisms involve NO, H₂S, 2,3-DPG, and adenosine triphosphate, but their relative contributions in humans remain uncertain. Available experimental and early clinical data suggest that NO- and H₂S-mediated modulation of haemoglobin affinity, improved erythrocyte rheology, and enhanced antioxidant capacity may contribute to ozone's effects on oxygen transport. Robust, standardised human studies are needed to clarify mechanisms and clinical relevance.

*Corresponding author:

Elena S. Biletskaya
(biletskaya.e@inbox.ru)

Citation: Zinchuk, V. V., Biletskaya, E. S., & Voloshko, P. E. Ozone therapy and blood oxygen transport: A comprehensive narrative review of physiological mechanisms and clinical aspects. *Hypoxia & Medicine*.
<https://doi.org/10.36922/HM026300006>

Received: July 21, 2026

Revised: September 10, 2026

Accepted: September 15, 2026

Published online: October 8, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Keywords: Ozone therapy; Haemoglobin oxygen affinity; Gasotransmitters; Nitric oxide; Hydrogen sulfide; Oxidative stress; Erythrocytes; Oxygen transport

1. Introduction

Ozone (O₃)—a triatomic form of oxygen—has a long history in medicine, dating back to the nineteenth century when it was used to disinfect wounds and treat infections. It is used today in various countries, particularly in parts of Europe, Latin America, and

Asia, although acceptance within conventional medicine remains heterogeneous and guideline-driven adoption is limited. Its potent oxidising capacity naturally raises safety concerns; however, when administered carefully as a mixture of medical oxygen and O₃ (typically 1–5% O₃ in 95–99% oxygen [O₂]), it has shown therapeutic benefits across diverse clinical contexts in some settings. The most common delivery method is major autohaemotherapy: a portion of the patient's blood (usually 50–200 mL) is exposed to the oxygen–O₃ mixture outside the body and then reinfused (Smith *et al.*, 2017; Wen *et al.*, 2022; Zinchuk & Biletskaya, 2023a).

Today, O₃ therapy is routinely used in orthopaedics and traumatology—for osteoarthritis, fracture healing, chronic low back pain, and other musculoskeletal issues (Jeyaraman *et al.*, 2024). During the COVID-19 pandemic, it also drew attention as an adjunctive treatment, with some studies reporting improvements in laboratory parameters and peripheral oxygen saturation (Franzini *et al.*, 2020). Still, many aspects of its biological activity—particularly its effects on blood oxygen transport—remain poorly characterised. This gap limits our ability to design rational treatment protocols and hinders broader acceptance in conventional medicine. (Cattel *et al.*, 2021; Cenci *et al.*, 2022; de Sire *et al.*, 2022; Fernández-Cuadros *et al.*, 2020; Harapan & Harapan, 2023; Magalhaes *et al.*, 2012).

The therapeutic logic of O₃ therapy hinges on hormesis: a low-grade, transient stressor triggers a beneficial adaptive response. A growing body of evidence supports this view. One key point deserves emphasis: O₃'s biological effects are non-linear and dose-dependent. Low and intermediate concentrations promote beneficial adaptations; excessive doses deplete antioxidant systems and cause damage (Inguscio *et al.*, 2026). Moreover, the primary products of O₃ interaction with biological fluids—ozonides and hydrogen peroxide—act as signalling molecules that engage cellular sensors and set off stress-activated cascades, ultimately shaping the therapeutic outcome (Khalid *et al.*, 2026).

When O₃ contacts biological fluids, it reacts with unsaturated fatty acids in plasma and cell membranes, generating ozonides and hydrogen peroxide (H₂O₂). These primary products act as signalling molecules, activating redox-sensitive transcription factors—especially nuclear factor erythroid 2-related factor 2 (Nrf2)—which in turn upregulate a range of cytoprotective enzymes (Alimohammadi *et al.*, 2025). The net effect is an enhanced antioxidant reserve and improved cellular stress tolerance (Alimohammadi *et al.*, 2025). The shift towards “low-dose” regimens has significantly reduced clinically used O₃ concentrations, underscoring the need for precise

dosing to maximise benefit while minimising risk (Smith *et al.*, 2017). Recent work has explored dose–response relationships across cell types and animal models, revealing a narrow hormetic window and individual variability that can influence the optimal dose.

Against this background, the present review aims to consolidate current knowledge of O₃ effects on blood oxygen transport. It focuses on gasotransmitters, the prooxidant–antioxidant balance, and erythrocyte properties. By integrating *in vitro* and *in vivo* findings, we aim to provide a mechanistic framework to guide clinical practice and future research. We also consider the translational relevance of these mechanisms for hypoxia-related diseases—cardiovascular conditions, chronic wounds, and ischaemic disorders.

2. Methods

This narrative review focuses on physiological mechanisms linking O₃ therapy to blood oxygen transport. To improve transparency and reproducibility, we summarise our literature search and study selection approach.

2.1. Search strategy

We searched PubMed, Scopus, and Web of Science for peer-reviewed articles and reviews published between 1995 and 2026. We conducted the final search in August 2026. Search terms included combinations of “ozone therapy” OR “oxygen–ozone” OR “ozonated autohemotherapy” AND “oxygen transport” OR “haemoglobin oxygen affinity” OR “oxyhaemoglobin dissociation curve” OR “p50” OR “2,3-DPG” OR “nitric oxide” OR “hydrogen sulfide” OR “erythrocyte deformability” OR “erythrocyte rheology” OR “oxidative stress” OR “antioxidant enzymes.” No language restrictions were applied *a priori*; however, practical inclusion was limited to languages accessible to the authors (primarily English, with selective inclusion of Russian/Belarusian/German sources where full texts were available).

2.2. Inclusion and exclusion criteria

We included peer-reviewed original studies (*in vitro*, animal, and human) and narrative or systematic reviews that examined O₃'s influence on: (i) the oxyhaemoglobin dissociation curve or p50; (ii) gasotransmitter pathways (nitric oxide [NO], hydrogen sulfide [H₂S]); (iii) 2,3-diphosphoglycerate [2,3-DPG], adenosine triphosphate [ATP], or related allosteric modulators; (iv) erythrocyte deformability, aggregation, or blood viscosity; and (v) oxidative stress biomarkers and antioxidant enzyme activities. We excluded conference abstracts without full texts, case reports, and non-peer-reviewed sources.

2.3. Study selection and data extraction

Two authors screened titles and abstracts, retrieved potentially relevant full texts, and assessed them for eligibility. Discrepancies were resolved by discussion. Data extracted included study design, model (*in vitro*/animal/human), O₃ concentration/dose and route, key outcomes (p50, 2,3-DPG, NO, H₂S, deformability, oxidative/antioxidant markers), and main findings. Given the heterogeneity of methods, populations, and outcomes, we did not perform a formal meta-analysis; instead, we synthesised findings narratively, grouping evidence by experimental level (*in vitro*, animal, human mechanistic, clinical outcomes).

2.4. Scope and limitations

This review prioritises mechanistic data directly relevant to blood oxygen transport. Clinical applications are discussed briefly to illustrate potential translational relevance, with an emphasis on conditions where oxygen delivery and microcirculatory perfusion are central to pathophysiology.

3. The hormetic nature of ozone's action

The hormesis principle is central to O₃ therapy (Smith *et al.*, 2017). Experimental data consistently show an inverted U-shaped dose-response curve—a hallmark of hormetic agents (Inguscio *et al.*, 2026). When O₃ encounters organic substrates, it generates reactive oxygen species (ROS) and lipid peroxidation products. Far from being mere toxic by-products, these molecules act as effector signals that shape the therapeutic outcome (Khalid *et al.*, 2026). They engage a variety of cellular sensors, setting off stress-activated signalling cascades that ultimately confer a protected phenotype (Khalid *et al.*, 2026).

At the molecular level, the initial reaction involves O₃ attacking the double bonds of polyunsaturated fatty acids in plasma lipoproteins and membrane phospholipids. This yields relatively stable ozonides and, crucially, H₂O₂. At sub-toxic concentrations, H₂O₂ serves as a second messenger, promoting Nrf2 translocation to the nucleus, where it drives the transcription of antioxidant response element-bearing genes—including those encoding haem oxygenase-1, glutathione peroxidase, and superoxide dismutase (Alimohammadi *et al.*, 2025). This strengthens the antioxidant defence system and increases capacity to handle subsequent oxidative challenges (Alimohammadi *et al.*, 2025; Travaglini & Iorio, 2023).

Other transcription factors—nuclear factor kappa B and activator protein 1, for instance—can also be influenced by O₃-generated ROS, affecting inflammatory and immune responses. Whether the net effect is protective or harmful depends on how these pathways interact. Mild oxidative

stress tends to dampen excessive inflammation, whereas severe oxidative stress can worsen it. This duality makes dosing critical.

In practice, the response to O₃ hinges on the concentration used. Excessive exposure leads to uncontrolled oxidation and cellular injury, which is why current clinical guidelines recommend 10–40 µg/mL for autohaemotherapy. The lowdose approach has gained broad acceptance and considerably improved the safety profile. Recent meta-analyses confirm that these concentrations yield meaningful improvements in oxidative stress markers without causing haematological toxicity in the studied cohorts (Alimohammadi *et al.*, 2025; Viebahn-Haensler & León Fernández, 2021).

4. Ozone and the oxygen transport function of blood

4.1. Modulation of haemoglobin–oxygen affinity

A substantial body of evidence indicates that O₃ reduces haemoglobin's affinity for oxygen, shifting the oxyhaemoglobin dissociation curve to the right. *In vitro* studies show that ozonation decreases O₂ affinity in blood from healthy individuals and from patients with type 2 diabetes and peripheral arterial disease (Coppola *et al.*, 1995). The effect appears all-or-nothing, and the magnitude of the shift correlates with the O₃ concentration in these experimental systems (Coppola *et al.*, 1995). These findings are derived primarily from *in vitro* models and may not fully reflect *in vivo* conditions. In animal experiments, a single intravenous dose of O₃ (10 µg/kg) increased arterial pO₂, oxygen saturation (SO₂), and the half-saturation pressure (p50_{real}) compared to controls (Zinchuk & Biletskaya, 2023b). These changes were accompanied by elevated circulating NO and H₂S levels, which in turn stimulate 2,3-DPG and ATP production—both well-known allosteric modulators of haemoglobin affinity (Zinchuk & Biletskaya, 2023b). Notably, sub-threshold and supra-optimal concentrations had no effect, reinforcing the importance of precise dosing. These data are derived from animal models and should be interpreted as hypothesis-generating for human physiology.

In patients with peripheral occlusive arterial disease, ozonated autohaemotransfusion significantly raised p50 values, signalling improved O₂ delivery (Giunta *et al.*, 2001). Interestingly, some researchers observed this improvement without a concurrent increase in 2,3-DPG, hinting that alternative pathways—particularly gasotransmitter-mediated—might be involved (Zinchuk & Biletskaya, 2023a). This prompted further exploration of NO and H₂S.

The rightward shift is multifactorial. O_3 -generated ROS can oxidise haemoglobin to methaemoglobin, but at therapeutic doses this is minimal. The primary mechanism involves allosteric modulation. 2,3-DPG binds to deoxyhaemoglobin, stabilising the T (tense) conformation and lowering oxygen affinity. Experimental observations suggest O_3 boosts 2,3-DPG levels by stimulating glycolysis, possibly via phosphofructokinase activation. ATP, also elevated, competes with 2,3-DPG for binding sites and further promotes oxygen unloading.

Another gasotransmitter—carbon monoxide (CO)—has also been implicated in haemoglobin affinity regulation, though its role in O_3 therapy remains unexplored. The interplay between NO, H_2S , and CO could yield synergistic or antagonistic effects worth investigating. Additionally, post-translational modifications of haemoglobin, such as S-nitrosylation and S-sulphydration, directly alter its conformation and oxygen-binding kinetics, as suggested by *in vitro* and animal data (Smith *et al.*, 2017).

4.2. Involvement of gasotransmitters: Nitric oxide and hydrogen sulfide

Nitric oxide and H_2S are now established as ubiquitous signalling molecules that regulate vascular tone, inflammation, and tissue oxygenation. They may also influence haemoglobin's oxygen-binding properties through both direct and indirect routes (Figure 1).

4.2.1. Nitric oxide

Ozone exposure stimulates NO production through several mechanisms. It may upregulate endothelial and inducible

NO synthases (eNOS and iNOS), boosting endogenous NO synthesis. NO can directly interact with haemoglobin, altering its conformation and allosteric regulation; its reaction products (e.g., peroxynitrite) can also induce post-translational modifications of haemoglobin. Consistent with this, co-administration of a NO donor (nitroglycerin) with O_3 potentiated the enhancement of O_2 transport, reflected in further increases in pO_2 , SO_2 , and $p50$ real (Akbudak *et al.*, 2019). Conversely, blocking NO synthase with L-NG-nitroarginine methyl ester attenuated the O_3 -induced responses, confirming the involvement of NO-mediated signalling (Akbudak *et al.*, 2019). Moreover, NO activates soluble guanylate cyclase, raising cyclic guanosine monophosphate levels and promoting vasodilation, which may improve microcirculatory flow and complement the effects on oxygen unloading.

4.2.2. Hydrogen sulfide

Emerging evidence suggests H_2S is equally important in mediating O_3 's effects. O_3 can upregulate endogenous H_2S production, particularly under hypoxic conditions. H_2S may act through S-sulphydration of haemoglobin, altering its oxygen affinity, and by stimulating glycolysis to increase 2,3-DPG formation. In experimental models, pharmacological inhibition of H_2S synthesis with DL-propargylglycine diminished the rightward shift normally induced by O_3 ; conversely, combined administration of an H_2S donor (NaHS) and an NO donor produced a synergistic effect (Zinchuk & Biletskaya, 2023a). This cross-talk between NO and H_2S pathways appears essential for the full expression of O_3 's action on oxygen transport.

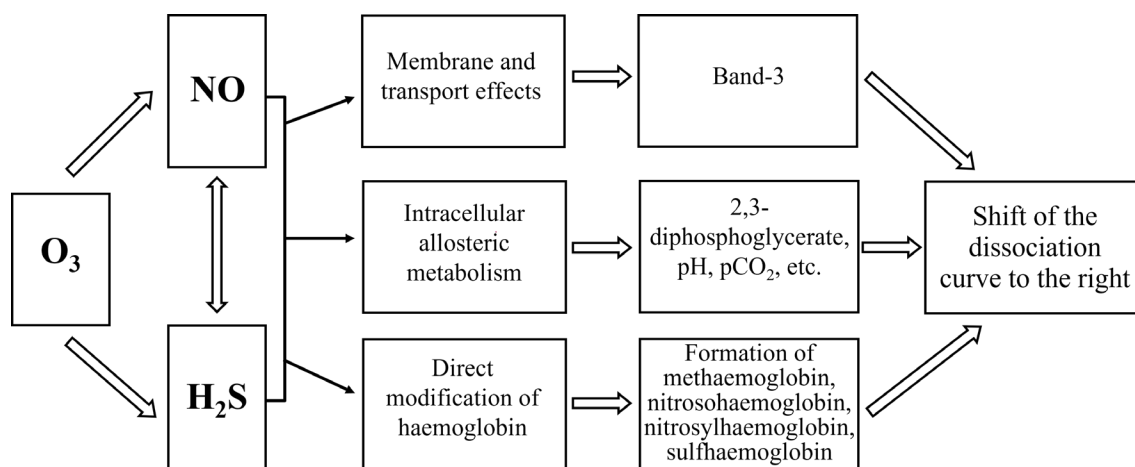


Figure 1. Ozoneinduced mechanisms of the rightward shift in the haemoglobin dissociation curve. The figure summarises proposed mechanisms based primarily on *in vitro* and animal data; their relative contributions in humans remain to be fully established.

Abbreviations: H_2S : Hydrogen sulfide; NO: Nitric oxide; O_3 : Ozone; pCO_2 : Partial pressure of carbon dioxide.

The relationship between NO and H₂S is intricate. Each can modulate the other's production and bioavailability. For instance, H₂S can enhance eNOS activity and NO release, while NO can influence H₂S-generating enzymes. This reciprocal regulation enables fine-tuning of vascular and erythrocyte function. Both gasotransmitters also inhibit platelet aggregation and adhesion, potentially reducing thrombotic complications in ischaemic disease.

4.3. Erythrocyte rheology and microcirculatory perfusion

Beyond altering haemoglobin affinity, O₃ improves red blood cell flow properties. One study reported that ozonation at 50 µg/mL increased erythrocyte deformability, facilitating passage through narrow capillaries and enhancing tissue perfusion. The tested concentrations did not change red cell aggregation (Akbulak *et al.*, 2019). The improved flexibility likely stems from O₃-induced modifications in membrane lipid organisation and surface charge, which reduce cell–cell adhesion and create a more favourable microcirculatory environment. The net result—enhanced O₂ unloading coupled with improved rheology—translates into better oxygen delivery to metabolically active tissues.

Mechanistically, O₃ may affect the erythrocyte membrane by altering lipid bilayer fluidity and the activity of membrane transporters such as Na⁺/K⁺-ATPase. Increased deformability reduces the critical capillary radius needed for passage, thereby improving perfusion of hypoxic regions. O₃ also lowers blood viscosity through mild oxidative depolymerisation of fibrinogen and other plasma proteins, further enhancing flow dynamics.

Rheological improvements have been documented in various clinical settings. In patients with diabetes and peripheral artery disease, O₃ therapy not only increased p50 but also reduced whole-blood viscosity and erythrocyte aggregation, improving tissue oxygenation (Giunta *et al.*, 2001). These combined effects position O₃ as a promising adjunct in managing chronic limb-threatening ischaemia.

5. Prooxidant–antioxidant homeostasis

A distinctive feature of O₃ therapy is its dual effect on redox balance: it generates a controlled oxidative challenge, yet paradoxically, this very challenge strengthens endogenous antioxidant defences.

5.1. Generation of oxidative stress and lipid peroxidation

Exposing blood to O₃ produces ROS and secondary products such as malondialdehyde (MDA) and conjugated dienes in a dose-dependent fashion (Inguscio *et al.*, 2023). Elevated MDA and free haemoglobin reflect oxidative

damage to membrane lipids and red cell integrity. However, at low O₃ concentrations, lipid peroxidation remains moderate and reversible, triggering adaptive responses rather than causing irreversible harm. Higher concentrations overwhelm cellular safeguards, resulting in sustained oxidative injury (Inguscio *et al.*, 2023). This non-linearity again underscores the critical importance of dose selection.

The temporal dynamics of oxidative stress markers are also noteworthy. Immediately after ozonation, ROS and MDA show a transient peak, followed by a decline as endogenous antioxidant systems are activated. This hormetic pattern resembles ischaemic preconditioning, where a brief stressor confers tolerance to a subsequent lethal insult. In clinical practice, repeated O₃ sessions may progressively enhance baseline antioxidant capacity, yielding cumulative benefits.

5.2. Upregulation of the antioxidant defence system

Ozone exposure leads to measurable increases in catalase, superoxide dismutase, and glutathione peroxidase activities, as summarised in a recent meta-analysis (Alimohammadi *et al.*, 2025). These enzymes are essential for scavenging ROS and maintaining a reduced intracellular environment. Nrf2 largely drives this transcriptional upregulation; upon activation, it binds to antioxidant response elements in the promoter regions of target genes (Smith *et al.*, 2017).

Metabolomic profiling shows that low O₃ concentrations affect multiple metabolic networks—including those of amino acids, carbohydrates, lipids, and nucleotides—with most altered metabolites linked to redox homeostasis and energy production (Khalid *et al.*, 2026). Therefore, O₃ appears to act as a broad-spectrum metabolic modulator, not merely a simple antioxidant.

Non-enzymatic antioxidants—glutathione, uric acid, and bilirubin—also increase. Glutathione, in particular, plays a central role in detoxifying H₂O₂ and lipid peroxides. The reduced-to-oxidised glutathione ratio is a sensitive indicator of redox status, and O₃ therapy has been shown to improve this ratio across several patient cohorts (Alimohammadi *et al.*, 2025). These adaptations protect not only erythrocytes but also other blood cells and vascular endothelium, contributing to systemic resilience.

6. Clinical applications and therapeutic outlook

6.1. Musculoskeletal disorders

Ozone has shown encouraging results in musculoskeletal conditions—osteoarthritis, fractures, and chronic pain (Jeyaraman *et al.*, 2024). Its anti-inflammatory and analgesic

effects, attributed to suppression of pro-inflammatory cytokines (interleukin-1 beta [IL-1 β], tumour necrosis factor alpha) and elevation of anti-inflammatory mediators (interleukin-10), are complemented by improved local oxygen supply. Intra-articular O₃ injections have been reported to reduce pain and improve function for up to six months in knee osteoarthritis. However, response rates vary, and clinicians must carefully manage oxidative burden, warranting further protocol refinement.

Recent randomised trials have compared O₃ injections with hyaluronic acid or corticosteroids, showing comparable or superior efficacy in some endpoints with fewer side effects. A meta-analysis of 12 studies concluded that O₃ therapy significantly improves Western Ontario and McMaster Universities Osteoarthritis Index scores and visual analogue scale pain ratings in knee osteoarthritis, with effects lasting at least three months (Jeyaraman *et al.*, 2024). However, the optimal injection frequency and concentration remain debated.

6.2. COVID-19 and infectious diseases

During the pandemic, researchers explored O₃ therapy as an adjunct to standard care. Several systematic reviews suggest that it aids SARS-CoV-2 RNA clearance, as indicated by negative PCR results on follow-up, though robust evidence for shorter hospital stays or reduced mortality is lacking (Harapan & Harapan, 2023). Immunomodulatory and antioxidant actions could plausibly mitigate the cytokine storm and improve microcirculatory oxygenation, but larger, well-controlled trials are needed before firm recommendations.

One proposed mechanism involves direct oxidation of viral envelope lipids, along with modulation of host immune responses. O₃ may also improve oxygen saturation in COVID-19 patients through the same mechanisms—rightward shift and improved rheology. Preliminary case series data suggest faster SpO₂ recovery and reduced need for supplemental oxygen, but these findings require confirmation in larger cohorts.

6.3. Cardiovascular and peripheral vascular diseases

In patients with lower-extremity ischaemia, O₃ autohaemotherapy has yielded meaningful gains: increased pain-free walking distance, alleviation of rest pain, and accelerated ulcer healing (Giunta *et al.*, 2001). These benefits arise from enhanced O₂ delivery (higher p50), reduced oxidative stress, and NO-mediated vasodilation. Rheological improvements also contribute to better tissue perfusion (Akbulduk *et al.*, 2019).

A pilot study in patients with intermittent claudication

reported that 10 sessions significantly increased ankle-brachial index and walking distance, with effects sustained for up to six months (Giunta *et al.*, 2001). In diabetic foot ulcers, topical O₃ (ozonated oil) or systemic therapy has been reported to promote granulation and epithelialisation, possibly through enhanced oxygen supply and antimicrobial activity (Martinez-Sanchez, 2005).

Recent studies have also explored O₃'s potential in oncology. The rationale rests on its ability to increase tumour oxygenation, potentially sensitising hypoxic tumours to radiotherapy and chemotherapy. Preliminary *in vitro* studies suggest that O₃ can induce apoptosis in some cancer cell lines, but *in vivo* evidence remains sparse (Rahimzadeh *et al.*, 2022; Re *et al.*, 2008).

Furthermore, O₃ has shown beneficial effects on lipid profiles, haemostatic parameters, and inflammatory markers in patients with coronary artery disease, suggesting a potential role in secondary prevention (Martinez-Sanchez *et al.*, 2012). These observations, combined with improved oxygen delivery, make O₃ a promising candidate for integrated cardiovascular rehabilitation.

6.4. Other emerging applications

Ozone therapy is being investigated in wound care (pressure ulcers, venous stasis ulcers), post-stroke rehabilitation, and as an adjuvant in various conditions. In neurological conditions, O₃ may improve cerebral blood flow and mitochondrial function, offering potential benefits in neurodegenerative diseases. However, these applications are speculative and require thorough investigation.

Alternative routes of administration, such as rectal insufflation, have been studied in experimental models of inflammatory bowel disease and show promising antioxidant and anti-inflammatory effects (Osikov *et al.*, 2022). This non-invasive approach could broaden O₃'s therapeutic reach to gastrointestinal and systemic conditions.

7. Safety, contraindications and future research

Despite its promise, O₃ therapy carries risks. Strict adherence to published protocols is essential to avoid adverse events—oxidative damage to blood components, venous irritation, and, very rarely, gas embolism (Smith *et al.*, 2017). Current guidelines recommend O₃ concentrations of 10–40 μ g/mL for autohaemotherapy, with gradual escalation based on patient tolerance. Contraindications include glucose-6-phosphate dehydrogenase deficiency (which predisposes to haemolysis), acute myocardial infarction, hyperthyroidism, and pregnancy.

Common side effects—transient fatigue, headache, and local pain at the injection site—are usually mild and self-limiting. Severe adverse events are extremely rare when proper procedures are followed. Nonetheless, the lack of internationally harmonised protocols remains a major barrier to wider acceptance. Different countries use varying concentrations, volumes, and routes of administration, complicating cross-study comparisons.

International consensus on concentrations, administration routes, and treatment schedules would greatly benefit the field. High-quality, long-term studies are also needed to define optimal indications and assess the benefit–risk ratio across diverse patient populations.

Emerging applications include wound care, diabetic foot ulcers, post-stroke rehabilitation, and oncology. Combining O₃ with hyperbaric oxygen or photobiomodulation could open new therapeutic avenues. Novel delivery systems—ozonated oils, ozonated saline, rectal insufflation—are making the therapy more accessible and acceptable. These alternatives may reduce invasiveness and expand the patient population that can benefit (Martinez-Sanchez *et al.*, 2005).

Future research priorities should include:

- Large, multicentre randomised controlled trials with sham or placebo controls.
- Biomarker-guided dose individualisation based on baseline antioxidant status.
- Mechanistic studies using advanced omics technologies to map the full spectrum of O₃-induced

molecular changes.

- Exploration of synergistic combinations with other physical therapies or pharmacological agents.
- Long-term safety surveillance to detect rare adverse events.

8. Conclusion

Ozone therapy improves blood oxygen transport through three interconnected mechanisms: right-shifting the haemoglobin–oxygen dissociation curve via NO/H₂S pathways, enhancing erythrocyte deformability, and upregulating antioxidant defences. The rightward shift boosts O₂ delivery to ischaemic tissues, which likely accounts for many of the reported clinical benefits (Table 1).

The mild oxidative stress induced by O₃ acts as a hormetic trigger, activating cytoprotective enzymes and reprogramming cellular metabolism. Because these responses are dose-dependent, precise control of O₃ concentration is essential for both safety and efficacy.

A mechanistic understanding of how O₃ interacts with gasotransmitters and redox systems should facilitate the design of optimised therapeutic regimens and may extend its use to a broader range of hypoxia-associated conditions. As ongoing research continues to unravel these complex molecular networks, the full therapeutic potential of O₃ will become increasingly attainable, provided that future studies clearly distinguish between experimental hypotheses and clinically validated mechanisms.

Table 1. Summary of key studies on ozone and blood oxygen transport parameters

Study/model	Ozone dose/route	p50/2,3-DPG/NO/H ₂ S	Rheological parameters	Oxygenation endpoint	Main findings	Study design/model
Coppola <i>et al.</i> , 1995	<i>In vitro</i> ozonation of whole blood (various µg/mL)	Decreased O ₂ affinity (rightward shift); 2,3-DPG unchanged	Not reported	<i>In vitro</i> O ₂ dissociation curve	Ozone reduced haemoglobin–O ₂ affinity in healthy and diabetic blood samples	<i>In vitro</i>
Zinchuk & Biletskaya, 2023b	Rats, intravenous ozone 10 µg/kg	Increased p50real; ↑ NO, ↑ H ₂ S; ↑ 2,3-DPG, ↑ ATP	Not reported	↑ arterial pO ₂ , SO ₂	Ozone improved oxygen-affinity parameters and gasotransmitters	Animal
Giunta <i>et al.</i> , 2001	Patients with peripheral occlusive arterial disease; autohaemotransfusion (10–40 µg/mL, multiple sessions)	↑ p50; 2,3-DPG unchanged in some subgroups	↓ whole-blood viscosity; ↓ erythrocyte aggregation	Improved tissue oxygenation indices (clinical)	Ozonated autohaemotransfusion improved haemorheology and oxygen delivery markers	Human clinical (small cohort)
Zinchuk & Biletskaya, 2023a	Red blood cell suspension, <i>in vitro</i> ozone	Proposed ↑ NO/H ₂ S signalling; ↑ 2,3-DPG	Not reported	<i>In vitro</i> O ₂ affinity	H ₂ S inhibition attenuated ozone-induced rightward shift	<i>In vitro</i>

(Cont'd...)

Table 1. (Continued)

Study/model	Ozone dose/route	p50/2,3-DPG/NO/ H ₂ S	Rheological parameters	Oxygenation endpoint	Main findings	Study design/ model
Akbudak <i>et al.</i> , 2019	Humans; major autohaemotherapy (10–40 µg/mL)	Not directly measured	↑ erythrocyte deformability; no change in aggregation	Not directly measured	Ozone improved red blood cell deformability without affecting aggregation	Human mechanistic (small)
Inguscio <i>et al.</i> , 2023	Humans; low-dose ozone	Oxidative stress markers transiently ↑; antioxidant enzymes ↑	Not reported	Not directly measured	Low-dose ozone induced hormetic redox response	Human clinical (biomarkers)

Notes: p50: Partial pressure of oxygen at which haemoglobin is 50% saturated with oxygen. P50real: Measured/actual partial pressure of oxygen at which haemoglobin is 50% saturated.

Abbreviations: 2,3-DPG: 2,3-diphosphoglycerate; ATP: Adenosine triphosphate; H₂S: Hydrogen sulfide; O₂: Oxygen; NO: Nitric oxide; pO₂: Partial pressure of oxygen; SO₂: Oxygen saturation.

Acknowledgments

The authors thank their colleagues at the Department of Normal Physiology, Grodno State Medical University, for stimulating discussions during the preparation of this manuscript.

Funding

This work was supported by institutional grants from Grodno State Medical University (grant no.: 0170643)

Conflict of Interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: Viktor V. Zinchuk, Elena S. Biletskaya

Funding acquisition: Viktor V. Zinchuk

Project administration: Elena S. Biletskaya

Supervision: Viktor V. Zinchuk

Visualization: Elena S. Biletskaya

Writing—original draft: Elena S. Biletskaya

Writing—review & editing: All authors

All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All data are derived from the cited publications. No new datasets were generated.

References

- Akbudak, I. H., Kucukatay, V., Kilic-Erkek, O., & Gokalp, G. (2019). Investigation of the effects of major ozone autohemotherapy application on erythrocyte deformability and aggregation. *Clinical Hemorheology and Microcirculation*, 71(3), 365–372. <https://doi.org/10.3233/CH-180417>
- Alimohammadi, M., Khoshnazar, S. M., Khajepour, H., Einollahi, B., & Hushmandi, K. (2025). Effect of ozone therapy on oxidative stress indices in chronic inflammatory diseases: A systematic review and meta-analysis of randomized clinical trials. *Advances in Redox Research*, 17, 100143. <https://doi.org/10.1016/j.arres.2025.100143>
- Cattel, F., Giordano, S., Bertiond, C., Lupia, T., Corcione, S., Scalfaferri, M., Angelone, L., & De Rosa, F. G. (2021). Ozone therapy in COVID-19: A narrative review. *Virus Research*, 291, 198207. <https://doi.org/10.1016/j.virusres.2020.198207>
- Cenci, A., Macchia, I., La Sorsa, V., Sbarigia, C., Di Donna, V., & Pietraforte, D. (2022). Mechanisms of action of ozone therapy in emerging viral diseases: Immunomodulatory effects and therapeutic advantages with reference to SARS-CoV-2. *Frontiers in Microbiology*, 13, 871645. <https://doi.org/10.3389/fmicb.2022.871645>
- Coppola, L., Giunta, R., Verrazzo, G., Luongo, C., Sammartino, A., Vicario, C., & Giugliano, D. (1995). Influence of ozone on haemoglobin oxygen affinity in type-2 diabetic patients with peripheral vascular disease: In vitro studies. *Diabetes & Metabolism*, 21(4), 252–255.
- de Sire, A., Marotta, N., Ferrillo, M., Agostini, F., Sconza, C., Lippi, L., Respizzi, S., Giudice, A., Invernizzi, M., & Ammendolia, A. (2022). Oxygen-Ozone Therapy for Reducing Pro-Inflammatory Cytokines Serum Levels in Musculoskeletal and Temporomandibular Disorders: A Comprehensive Review. *International Journal of Molecular*

Sciences, 23(5), 2528.

<https://doi.org/10.3390/ijms23052528>

Fernández-Cuadros, M.E., Albaladejo-Florín, M.J., Peña-Lora, D., Álava-Rabasa, S., & Pérez-Moro, O.S. (2020). Ozone (O₃) and SARS-CoV-2: Physiological bases and their therapeutic possibilities according to COVID-19 evolutionary stage. *SN Comprehensive Clinical Medicine*, 2(8), 1094-1102.

<https://doi.org/10.1007/s42399-020-00328-7>

Franzini, M., Valdenassi, L., Ricevuti, G., Chirumbolo, S., Depfenhart, M., Bertossi, D., & Tirelli, U. (2020). Oxygen-ozone immunocutaneous therapy for patients with COVID-19: Preliminary evidence reported. *International Immunopharmacology*, 88, 106879.

<https://doi.org/10.1016/j.intimp.2020.106879>

Giunta, R., Coppola, A., Luongo, C., Sammartino, A., Guastafierro, S., Grassia, A., Giunta, L., Mascolo, L., Tirelli, A., & Coppola, L. (2001). Ozonized autohemotransfusion improves hemorheological parameters and oxygen delivery to tissues in patients with peripheral occlusive arterial disease. *Annals of Hematology*, 80(12), 745-748.

<https://doi.org/10.1007/s002770100377>

Harapan, B. N., & Harapan, T. (2023). The role of ozone therapy in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and coronavirus disease 2019 (COVID-19): A review. *Medical Gas Research*, 13(4), 165-171.

<https://doi.org/10.4103/2045-9912.369237>

Inguscio, C.R., Cisterna, B., Carton, F., Barberis, E., Manfredi, M., & Malatesta, M. (2023). Modifications of blood molecular components after treatment with low ozone concentrations. *International Journal of Molecular Sciences*, 24(24), 17175.

<https://doi.org/10.3390/ijms242417175>

Inguscio, C. R., Pozza, E. D., Dando, I., Tabaracci, G., Angelini, O., Picotti, P. M., Malatesta, M., & Cisterna, B. (2026). Dose-related structural and functional modifications of mitochondria are induced in vitro by low ozone concentrations. *International Journal of Molecular Sciences*, 27(5), 2267.

<https://doi.org/10.3390/ijms27052267>

Jeyaraman, M., Jeyaraman, N., Ramasubramanian, S., Balaji, S., Nallakumarasamy, A., Patro, B. P., & Migliorini, F. (2024). Ozone therapy in musculoskeletal medicine: A comprehensive review. *European Journal of Medical Research*, 29(1), 398.

<https://doi.org/10.1186/s40001-024-01976-4>

Khalid, A., Sala, A., Forlino, A., & Besio, R. (2026). Impact of oxygen-ozone mixture on cellular functions: A biochemical perspective across cell types. *Molecular Medicine*, 32, 82.

<https://doi.org/10.1186/s10020-026-01475-8>

Magalhaes, F. N. O., Dotta, L., Sasse, A., Teixeira, M. J., & Fonoff,

E. T. (2012). Ozone therapy as a treatment for low back pain secondary to herniated disc: A systematic review and meta-analysis of randomized controlled trials. *Pain Physician*, 15(2), E115-E129.

<https://doi.org/10.36076/ppj.2012/15/E115>

Martinez-Sanchez, G., Al-Dalain, S. M., Menendez, S., Re, L., Giuliani, A., & Leon, O. S. (2005). Therapeutic efficacy of ozone in patients with diabetic foot. *European Journal of Pharmacology*, 523(1-3), 151-161.

<https://doi.org/10.1016/j.ejphar.2005.08.020>

Martinez-Sanchez, G., Delgado-Roche, L., Diaz-Batista, A., Perez-Davison, G., & Re, L. (2012). Effects of ozone therapy on haemostatic and oxidative stress index in coronary artery disease. *European Journal of Pharmacology*, 691(1-3), 156-162.

<https://doi.org/10.1016/j.ejphar.2012.07.010>

Osikov, M.V., Davydova, E.V., Kaigorodtseva, N.V., Fedosov, A.A., Il'inykh, M.A., Savchuk, K.S., & Vorgova, L.V. (2022). Influence of rectal ozone application on the intensity of free radical destruction of lipids and intestinal proteins in the dynamics of experimental colitis. *Bulletin of Experimental Biology and Medicine*, 173(1), 24-27.

<https://doi.org/10.1007/s10517-022-05485-4>

Rahimzadeh, P., Imani, F., Azad Ehyaei, D., & Faiz, S. H. R. (2022). Efficacy of oxygen-ozone therapy and platelet-rich plasma for the treatment of knee osteoarthritis: A meta-analysis and systematic review. *Anesthesia and Pain Medicine*, 12(4), e127121.

<https://doi.org/10.5812/aapm-127121>

Re, L., Mawsouf, M. N., Menendez, S., Leon, O. S., Sanchez, G. M., & Hernandez, F. (2008). Ozone therapy: Clinical and basic evidence of its therapeutic potential. *Archives of Medical Research*, 39(1), 17-26.

<https://doi.org/10.1016/j.arcmed.2007.07.005>

Smith, N. L., Wilson, A. L., Gandhi, J., Vatsia, S., & Khan, S. A. (2017). Ozone therapy: An overview of pharmacodynamics, current research, and clinical utility. *Medical Gas Research*, 7(3), 212-219.

<https://doi.org/10.4103/2045-9912.215752>

Travagli, V., & Iorio, E. L. (2023). The biological and molecular action of ozone and its derivatives: State-of-the-art, enhanced scenarios, and quality insights. *International Journal of Molecular Sciences*, 24(10), 8582.

<https://doi.org/10.3390/ijms24108465>

Viebahn-Haensler, R., & León Fernández, O. S. (2021). Ozone in Medicine. The Low-Dose Ozone Concept and Its Basic Biochemical Mechanisms of Action in Chronic Inflammatory Diseases. *International Journal of Molecular Sciences*, 22(15), 7890.

<https://doi.org/10.3390/ijms22157890>

Wen, Q., Liu, D., Wang, X., Zhang, Y., Fang, S., Qiu, X., & Chen, Q. (2022). A systematic review of ozone therapy for treating chronically refractory wounds and ulcers. *International Wound Journal*, 19(4), 853-870.

<https://doi.org/10.1111/iwj.13687>

Zinchuk, V. V., & Biletskaya, E. S. (2023a). Impact of ozone on the oxygen affinity blood properties and prooxidant-

antioxidant balance under effect of H₂S-generating system. *Journal of Evolutionary Biochemistry and Physiology*, 59, 960-968.

<https://doi.org/10.1134/S0022093023030274>

Zinchuk, V., & Biletskaya, E. (2023b). Effect of ozone on oxygen transport and pro-oxidant-antioxidant balance of red blood cell suspension. *Acta Chimica Slovenica*, 70(2), 226-230.

<https://doi.org/10.17344/acsi.2023.8032>