RESEARCH ARTICLE

Umit Karadeli [1]
Meliha M. Cicekliyurt [2]

Ozone therapy in organ preservation and ischemia–reperfusion injury: mechanistic and translational perspectives

Authors' addresses:
[1] Department of Bioinformatics and Systems Biology, Canakkale Onsekiz Mart University, Canakkale, Turkey
[2] Department of Medical Biology, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey

Correspondence:
Umit Karadeli
Department of Bioinformatics and Systems Biology, Canakkale Onsekiz Mart University, Canakkale, Turkey;
E-mail :umitkaradeli@comu.edu.tr
Tel: ++902862635950/1846

Article info:
Received: 29 April 2026
Accepted: 08 June 2026

ABSTRACT
Kidney transplantation remains the best method for kidney failure. However, ischemia-reperfusion injury (I/R) is one of the main problems in graft survival. The University of Wisconsin (UW) solution, which made history with great hope, has shown its protective effect, but coping with oxidative stress is not easy. Ozone, which is frequently used in alternative medicine, has been studied as a potential adjunctive strategy in solid organ transplantation, particularly to improve organ preservation and reduce ischemia-induced damage. This review summarizes the current experimental and translational evidence regarding the potential role of ozone therapy in organ protection, organ transplantation, and the regulation of oxidative stress. Examples of transplantation and clinical applications are provided, highlighting the effects of ozone.

Key words: Kidney transplantation, Oxidative stress, Ozone therapy, UW

Introduction

Ozone (O_3) is a three-atom oxygen molecule first identified by Christian Friedrich Schönbein in 1840; due to its powerful oxidative properties, it has been used for many years in various industrial and medical applications. Its initial applications included water purification, sterilization, and disinfection, and it was also used during World War I for wound cleansing and infection control. In subsequent years, its effects on chronic wounds, circulatory disorders, infectious diseases, and inflammatory conditions were investigated (Sagai & Bocci, 2011; Bocci et al., 2020). Experimental and clinical studies conducted over the past twenty years have shown that controlled low-dose ozone therapy can function not only as an oxidative agent but also as a biological regulator that activates cellular adaptation mechanisms. This effect, particularly defined as hormesis or oxidative stress, has been associated with the activation of endogenous antioxidant defense systems, the regulation of inflammatory signaling pathways, and the maintenance of cellular redox balance (Calabrese & Mattson, 2017; Bocci et al., 2020; Malatesta et al., 2024). These scientific findings have led to ozone being regarded not merely as a simple oxidizing molecule, but as a redox regulator capable of modulating biological responses. Consequently, in recent years, ozone's potential protective effects have been increasingly investigated in fields where oxidative stress plays a central role, such as ischemia-reperfusion injury, organ protection, and transplantation medicine (García et al., 2023; Re et al., 2023; Viebahn-Haensler & León Fernández, 2025).

When it comes to tissue and organ transplantation, kidney transplantation is the first thing that comes to mind. This is because it is still one of the most frequently performed and effective treatment methods. I/R damage is still unavoidable during organ removal, during transfer, and even during the transplantation procedure (Chen et al., 2008). The ischemia that occurs during this process disrupts the mitochondrial system, leading to succinate accumulation in cells. This condition, caused by cells being deprived of oxygen, disrupts cellular balance(Fukushima et al., 2024; Sorby-Adams et al., 2024).

In fact, the severity of damage increases proportionally with reperfusion. This is because mitochondrial activity increases in the first few minutes (Şengül & Şengül, 2021). Experimental studies have shown that succinate formation may contribute to the formation of superoxide radicals by disrupting the electron transport system(Fukushima et al., 2024; Sorby-Adams et al., 2024). Therefore, the current evidence suggests that succinate accumulation plays a central role in the development of ischemia-reperfusion injury.

In the reperfusion event occurring in the first few minutes, the cause of mitochondrial ROS production is succinate formation (Fukushima et al., 2024). The rapid

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20261035

increase in these oxidants leads to the release of key cytokines such as TNF-α and IL-1β. This process then progresses step by step to necrosis (H. Meng et al., 2017; W. Meng & et al., 2017). Preventing succinate formation could block all these negative mechanisms.

The use of UW cannot prevent the early activation of oxidative stress mechanisms. In addition, it has begun to be used in solutions such as PERLA®. However, this also cannot completely prevent I/R damage (Belzer & Southard, 1988). For this reason, there is growing interest in researching complementary strategies, such as ozone therapy, that may help reduce oxidative stress and support the body's own defense mechanisms (Bejaoui et al., 2024; García et al., 2023).

For these reasons, the potential applicability of ozone therapy in kidney transplantation warrants further evaluation. This review includes extensive research on the effect of ozone on pre-transplant and post-transplant ischaemia/reperfusion (I/R) injury. Accordingly, this review synthesises mechanical, preclinical, and translational evidence to evaluate the applicability and therapeutic potential of ozone therapy in kidney transplantation. This review focuses particularly on early succinate-derived ROS biology, the time-dependent risks of prolonged CIT, and the limitations of current protection strategies.

Literature Search Strategy

This narrative review is based on literature obtained from Google Scholar, Web of Science, and PubMed. Publications related to ozone therapy, ozone preconditioning, ischemia-reperfusion injury, organ protection, kidney transplantation, oxidative stress, ferroptois, and redox signaling were evaluated. The literature search primarily focused on recent studies published between 2000 and June 2025. However, several key publications from before 2000 that provide essential background information on the principles of organ preservation, the development of the University of Wisconsin (UW) solution, and the pathophysiology of ischemia-reperfusion injury were also included. Priority was given to studies relevant from experimental, translational, and clinical perspectives.

Mechanistic Basis of Ozone Therapy in Organ Protection

During ischemia, the electron transport chain ceases due to oxygen deprivation, leading to the accumulation of succinate in the mitochondria. Upon the onset of reperfusion, the accumulated succinate is rapidly oxidized, resulting in reverse electron transfer (RET) via Complex I. This process leads to the production of large amounts of superoxide and other reactive oxygen species (ROS) in the first minutes of reperfusion. The resulting ROS trigger lipid peroxidation, protein oxidation, and DNA damage, leading to impaired cellular function. Simultaneously, it initiates the inflammatory response through the activation of NF-κB, MAPK, and inflammatory cytokine networks. Therefore, the succinate-induced ROS burst that occurs in the early phase is considered one of the primary biological triggers of ischemia-reperfusion injury.

The controlled oxidative stress induced by low-dose ozone therapy leads to the formation of reactive oxygen species (ROS) and lipid oxidation products (LOPs). Rather than causing direct cellular damage, these molecules act as secondary messengers and activate cellular adaptation mechanisms. In particular, the formation of ROS and LOPs triggers the activation of the Nrf2/Keap1 signaling pathway, thereby increasing the expression of antioxidant defense systems such as glutathione peroxidase 4 (GPx4), superoxide dismutase (SOD), catalase, and glutathione (GSH). As a result, cellular redox homeostasis is maintained, mitochondrial function is supported, and lipid peroxidation is limited. Additionally, it had been indicated that low-dose ozone therapy suppresses NF-κB and MAPK-mediated inflammatory signaling pathways and reduces the production of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8. The combination of antioxidant and anti-inflammatory effects contributes to the inhibition of ferroptosis and apoptosis, thereby helping to preserve cellular integrity and maintain organ function.

To protect against damage caused by oxidative stress, ozone, which has an antioxidant effect even at low doses (10-40 µg/mL), directly activates antioxidant mechanisms. However, doses above 80 µg/mL may be toxic (Ye et al., 2025). Ozone therapy may contribute to a rapid increase in defence mechanism elements such as GSH (glutathione) and SOD (superoxide dismutase), resulting in a significant decrease in stress parameters such as MDA (malondialdehyde)(Viebahn-Haensler & León Fernández, 2025).

Due to these effects, ozone produces signals that activate antioxidant mechanisms through preconditioning (Calabrese & Mattson, 2017; Bocci et al., 2020). This adaptive mechanism, termed oxidative eustress, stimulates the Nrf2/Keap1 pathway and up-regulates antioxidant enzymes such as SOD, catalase, and glutathione peroxidase (GPx) (Zhu et al., 2024).

Activation of the Nrf2/SLC7A11/GPx4 signalling mechanisms suppresses ferroptosis, the pathway leading to necrosis (Wang & et al., 2024; Petrosillo & et al., 2022). In these sequential processes, NF-κB- and MAPK-mediated inflammatory signaling is suppressed, leading to a decrease in the production of proinflammatory cytokines such as TNF-α, IL-6, and IL-8 (Zhu et al., 2024). In mitochondrial studies

using ozone, it had been indicated that membrane potentials are preserved and apoptosis is prevented (H. Meng et al., 2017; Tasset & et al., 2021). Numerous in-depth studies had indicated that ozone has a more potent effect beyond its antioxidant mechanism. This reveals that the energy mechanism involves an almost complete reshaping of the mitochondrial structure (Petrosillo & et al., 2022). In short, the biological effect of ozone is a controlled preconditioning that stimulates endogenous defence mechanisms and can exhibit toxic effects at high doses (Ye et al., 2025; Calabrese & Mattson, 2017; Bocci et al., 2020). In a stressful environment, significant reductions in peroxidation markers are observed with ozone therapy (Zhu et al., 2024). The way to reverse this is by enhancing the Nrf2–SLC7A11–GPx4 mechanism. Subsequently, lipid peroxidation is halted and ferroptosis resulting from I/R injury is prevented (Wang & et al., 2024; Zhang et al., 2022; Petrosillo & et al., 2022). Inflammatory signalling shifts in parallel: ozone blunts NF-κB and MAPK activation with corresponding decreases in TNF-α, IL-6, and IL-8 (Zhu et al., 2024). Mitochondrial readouts show a more stable ΔΨm, fewer mPTP openings, and reduced apoptosis under preconditioning; later, indices of biogenesis and metabolic recovery increase (W. Meng & et al., 2017; Tasset & et al., 2021; Viebahn-Haensler & León Fernández, 2025). Together, these changes are compatible with attenuation of the succinate/RET-dependent ROS surge during early reperfusion and improved energetic recovery in the graft.

Figure 1 provides a schematic overview of these pathways, demonstrating that controlled ozone exposure activates Nrf2/Keap1/GPx4, inhibits NF-κB and MAPK, and ultimately may help prevent ferroptosis and apoptosis.

Rodent work has been central to mapping the protective window of ozone exposure (Chen et al., 2008; Kal & et al., 2016). In Wistar and Sprague–Dawley rats, both in vivo preconditioning and ex vivo use of ozonated preservation solutions lowered oxidative-stress readouts—malondialdehyde (MDA) fell and histopathology improved (Qiu et al., 2017). Similar protection has been reported in hepatic and renal I/R models, with a clear dose–response consistent with hormesis(Re et al., 2023). Enriching PERLA® with ozone increased total antioxidant capacity and preserved tissue architecture without losing compatibility with UW-based media (H. Meng et al., 2017). Organ-specific signals point in the same direction: in the liver, reduced Kupffer-cell activation and faster regeneration (Fernández & et al., 2008; García et al., 2023; Gültekin & et al., 2013); in the heart, steadier mitochondrial function and less apoptosis and in neurological I/R models, activation of the NRF2/SLC7A11/GPX4 axis with restraint of ferroptosis (Tasset & et al., 2021; Zhu et al., 2024). From these findings, a practical therapeutic window becomes apparent, as summarized in Table 1. Low-dose ozone (~15–25 µg/mL) induces beneficial eustress, whereas higher concentrations may approach oxidative toxicity (Table 1; Fukushima et al., 2024; Martínez-Sánchez et al., 2021).

Strategies for Ozone Therapy in Organ Preservation

Two complementary routes have been tested so far. In vivo preconditioning—a brief, low-dose exposure before retrieval—primes systemic antioxidant defences and improves tolerance to ischemia (Qiu et al., 2017). Ex vivo exposure—short ozonation of the organ or the preservation medium—reinforces local redox control before cold storage; 5–10 minutes at ~20 µg/mL is the most reported setting (Bejaoui et al., 2024). Using both tends to add up: less tubular necrosis, better glomerular architecture, lower MDA, and higher total antioxidant capacity have been reported when preconditioning and ex-vivo ozonation are combined (Kal & et al., 2016). Hypothermia alone slows metabolism but leaves redox balance unsettled; ozone appears to bridge this gap by carrying Nrf2/SLC7A11/GPx4 activity into early reperfusion (Petrosillo & et al., 2022; Re et al., 2023).

The hepatology literature offers a concrete read-out. Adding ozone to UW solution during liver perfusion lowered the release of hepatocellular enzymes. For example, ALT/AST levels were 35/31 U/L at 0 h, 415/295 U/L at 6 h, and 546/372 U/L at 12 h, compared to 77/82, 680/461, and 1027/682 U/L with standard UW(Aydın et al., 2022). Less leakage is in line with the histology signal and shows that UW-based media can be used with it.

It is evident that centres are not exclusively reliant on ozone to exert its influence on redox-bioenergetic processes. The utilisation of active oxygenation during preservation has garnered significant attention across a comprehensive array of 17 human and 48 animal studies, encompassing hypothermic oxygenated machine perfusion (HOPE), oxygen carriers, the two-layer method, venous per sufflation, and hyperbaric oxygenation(Mesnard et al., 2022). Oxygenation reduces the build-up of succinate, shrinking the fuel for the ROS burst on reperfusion and restoring cellular energy. In clinical planning, normothermic and hypothermic perfusion should be seen as complementary tools for assessment, protection, and reconstitution of high-risk grafts (Foguenne et al., 2023).

Outside the kidney–liver space, preclinical work points to cross-organ effects: in the liver, less Kupffer-cell activation and faster regeneration (García et al., 2023; Gültekin & et al., 2013); in the heart, steadier mitochondrial indices and less apoptosis (W. Meng & et al., 2017; Tasset & et al., 2021) in neural I/R, reinforcement of the Nrf2–SLC7A11–GPx4 axis with restraint of ferroptosis (Zhu et al., 2024).

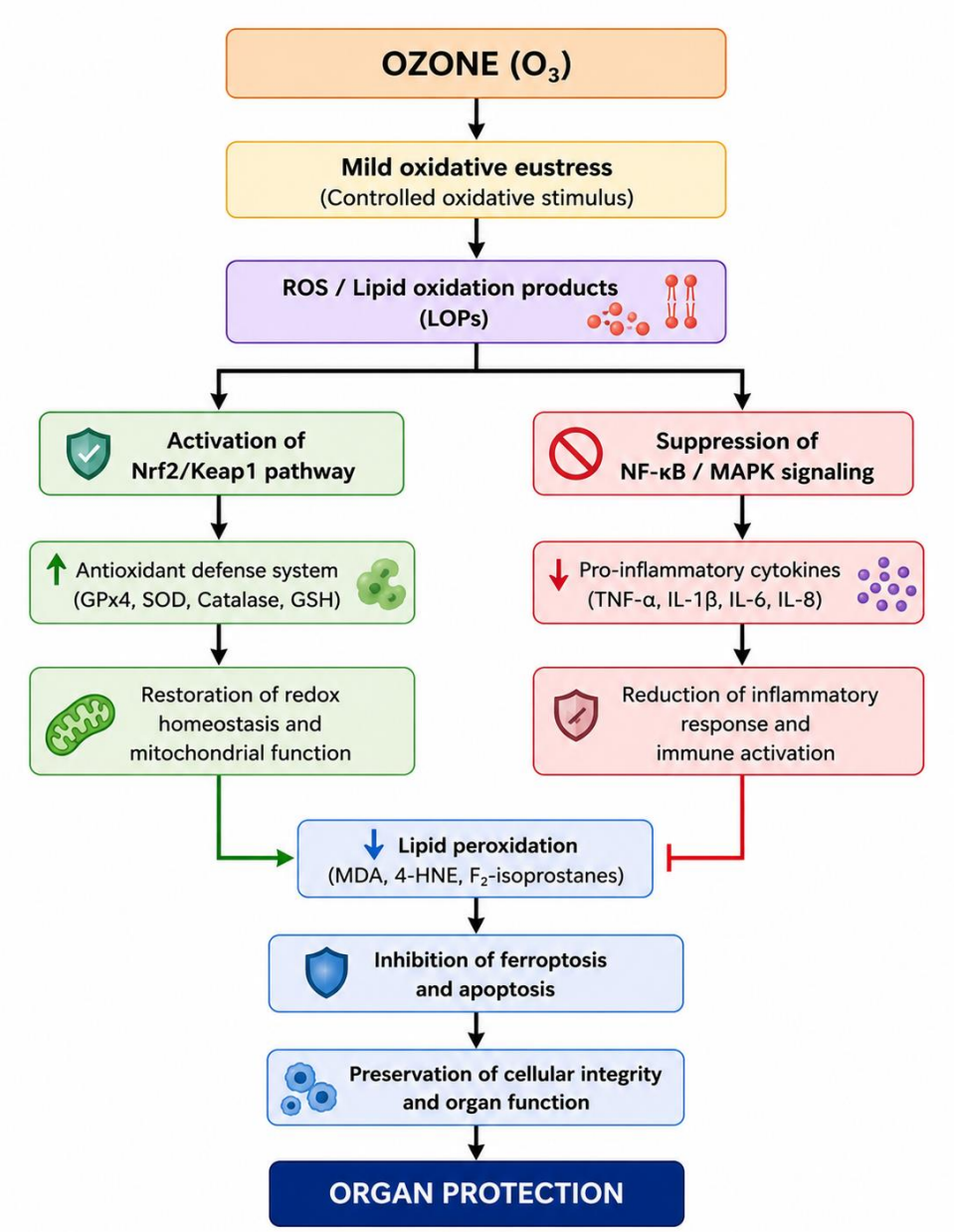

Figure 1. *Schematic representation of the molecular mechanisms mediating the antioxidant and anti-inflammatory effects of ozone in the organ protection process. Low-dose ozone therapy induces the formation of reactive oxygen species (ROS) and lipid oxidation products (LOPs) by creating a controlled oxidative stimulus (oxidative eustress). These molecules activate the Nrf2/Keap1 signaling pathway, thereby increasing the expression of antioxidant defense elements such as glutathione peroxidase 4 (GPx4), superoxide dismutase (SOD), catalase, and glutathione (GSH). As a result, cellular redox homeostasis and mitochondrial function are preserved. At the same time, ozone therapy suppresses NF-κB and MAPK-mediated inflammatory signaling pathways, thereby reducing the production of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8. The combination of antioxidant and anti-inflammatory effects contributes to reduced lipid peroxidation and the suppression of ferroptosis and apoptosis. As a result, cellular integrity is preserved, organ functions are supported, and organ-protective effects against ischemia-reperfusion injury emerge.*

Abbreviations: *ROS, reactive oxygen species; LOPs, lipid oxidation products; Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; GPx4, glutathione peroxidase 4; SOD, superoxide dismutase; GSH, glutathione; NF-κB, nuclear factor kappa B; MAPK, mitogen-activated protein kinase; TNF-α, tumor necrosis factor alpha; IL, interleukin; MDA, malondialdehyde; 4-HNE, 4-hydroxy-nonenal.*

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20261035

Table 1. *Comparison of Experimental In Vivo and Ex Vivo Ozone Preconditioning Studies in Ischemia–Reperfusion and Transplantation Models.*

Study	Organ / Tissue	Study Type	Experimental Model	Ozone Dose / Concentration	Route of Administration	Preconditioning Protocol	Exposure Duration	Main Findings
Chen et al., 2008	Kidney	In vivo	Renal ischemia–reperfusion injury	1 mg/kg	Ozone oxidative preconditioning	Single preconditioning before ischemia	Not reported	Improved renal function and attenuated reperfusion injury
Kal et al., 2016	Kidney	In vivo	Renal ischemia–reperfusion injury	1 mg/kg (60 µg/mL)	Intraperitoneal administration	Once daily before I/R	7 consecutive days	Reduced oxidative stress and histopathological injury
Gültekin et al., 2013	Liver	In vivo	Hepatic ischemia–reperfusion injury and partial hepatectomy	1.2 mg/kg	Intraperitoneal administration	Once daily before surgery	5 consecutive days	Reduced liver injury and improved regeneration
Wang et al., 2018	Kidney graft (donor rats)	In vivo	Kidney transplantation model	1 mg/kg; 50 µg/mL	Rectal insufflation	Donor preconditioning before organ retrieval	15 consecutive days	Reduced NF-κB, HMGB1, IL-6 and IL-18 expression; improved graft preservation
Zhu et al., 2024	Brain tissue / neurons	In vivo	Cerebral ischemia–reperfusion injury	40 µg/mL; 2.5 mL/kg/day	Intraperitoneal administration	Once daily before ischemia	5 consecutive days	Reduced ferroptosis through NRF2/SLC7A11/GPX4 activation
Aydın et al., 2022	Liver	Ex vivo	Organ preservation model	Ozonated UW solution	Direct addition to preservation solution	No preconditioning; ozone added during preservation	6–12 h cold preservation	Reduced preservation-related injury and enzyme leakage

Summary of experimental in vivo and ex vivo studies investigating the protective effects of ozone therapy against ischemia–reperfusion injury and transplantation-associated tissue damage. The table summarizes the organ or tissue studied, experimental model, ozone dose or concentration, route of administration, preconditioning strategy, duration of ozone exposure, and principal outcomes reported in each study. Most in vivo studies employed repeated ozone preconditioning protocols prior to ischemia induction or organ retrieval, whereas ex vivo studies incorporated ozone directly into preservation solutions during organ storage. Overall, the findings indicate that ozone therapy may attenuate oxidative stress, inflammation, apoptosis, and tissue injury while enhancing antioxidant defense mechanisms in various ischemia–reperfusion settings.

Abbreviations: I/R, ischemia–reperfusion; UW, University of Wisconsin solution; NF-κB, nuclear factor kappa B; HMGB1, high mobility group box 1; IL, interleukin; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; NRF2, nuclear factor erythroid 2-related factor 2; GPX4, glutathione peroxidase 4**.**

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20261035

Studies conducted to date have shown that while an ozone dosage of ~15–25 µg/mL is beneficial for the antioxidant mechanism, concentrations of ≥ 80 µg/mL and higher may lead to the development of toxicity (Martínez-Sánchez et al., 2021; Re et al., 2023; Ye et al., 2025). In short, more research is still needed to determine the optimal dosage and select the best option.

Rationale for Ozone Use in Kidney Transplantation and Organ Transport

Cold ischaemia developing before and after transplantation remains a key indicator affecting organ survival (Belzer & Southard, 1988; Peralta & Roselló-Catafau, 2022). Therefore, minimizing this injury remains an important objective in transplantation medicine. Preventive measures taken before transplantation can provide significant protection against ischemic damage. Even hypothermia, by slowing down metabolism, significantly may help prevent the accumulation of ROS. However, its inability to prevent it entirely leaves it lacking in such a sensitive matter as transplantation. The distinguishing feature of ozone compared to other solutions is its rapid change. Moreover, during this change, the osmolarity remains constant. Therefore, it can be safely added to solutions such as PERLA®, especially UW (Bejaoui et al., 2024).

Adding ozone to preservation solutions during prolonged pre-transplant periods may help protect the graft by supporting cellular homeostasis. This had been indicated in recent studies. This is because a significant reduction in fibrosis has been observed (Delgadillo-Valero & et al., 2023; García et al., 2023).

Mechanistically, low-dose exposure (approximately 10–40 µg/mL) functions as a redox stimulant by promoting Nrf2 translocation with subsequent SLC7A11/GPx4 induction, constraining lipid peroxidation and ferroptosis, while inducing the TLR4–IKK–NF-κB axis and modulating cytokine release (Ye et al., 2025; Zhu et al., 2024). Functional read-outs align with this mechanism: when ozone is added to UW during liver perfusion, hepatocellular enzyme leakage is consistently lower (ALT/AST 35/31 U/L at 0 h, 415/295 U/L at 6 h, and 546/372 U/L at 12 h versus 77/82, 680/461, and 1027/682 U/L with standard UW) mirroring histology and indicating that UW-based media can safely carry the (Aydın et al., 2022). The overall pattern fits a hormetic model in which moderate ROS act as signals that up-regulate cytoprotective programs, whereas excessive dosing (≥80 µg/mL) trends toward oxidative injury; in practice this translates to short ex vivo ozonation (~15–25 µg/mL for 5–10 min) layered onto hypothermia to bridge the redox gap and carry Nrf2/SLC7A11/GPx4 activity into early reperfusion, with concurrent microvascular protection and improved mitochondrial efficiency (Martínez-Sánchez et al., 2021; Petrosillo & et al., 2022; Re et al., 2023; Ye et al., 2025).

Limitations

Despite these available findings, routine use should be controlled. This is because it should not be forgotten that while low doses have a positive effect, high doses lead to lipid peroxidation (Bocci et al., 2020; Sagai & Bocci, 2011). The standardisation of dosing remains to be achieved; experimental exposures currently range from 10–50 µg/mL and vary according to route, duration, and target compartment. Human evidence is still limited and varied, with only a few controlled trials available (Malatesta et al., 2024; Re et al., 2023). The safety of gas-phase ozonation in human perfusion circuits remains unverified, and there is no agreement on delivery pathways or operational parameters. Longer-term consequences for graft immunogenicity, fibrotic remodelling, and endothelial phenotype also remain uncertain. Addressing these gaps will require mechanistic readouts—redox-directed proteomics and metabolomics, paired with single-cell transcriptomics—to define dose–response behavior and adaptive thresholds (Petrosillo & et al., 2022; Re et al., 2023; Zhang et al., 2022). The necessity for ethical and regulatory guardrails to agree with multicentre collaboration and harmonised trial designs prior to wider adoption is equally apparent.

Discussion

Cold storage remains the weakest point in organ transplantation: hypothermia slows metabolism but does not completely suppress succinate accumulation, ROS production, or the inflammatory surge that occurs during reperfusion (Belzer & Southard, 1988; Foguenne et al., 2023). In this context, it is best to view ozone not as a wholesale replacement for existing applications, but as an adjuvant that "bridges" the redox gap during the protection-reperfusion transition. At low concentrations, short-term exposures trigger a coordinated preconditioning response, leading to the nuclear translocation of Nrf2, induction of SLC7A11 and GPx4, improved glutathione management, and tighter control of lipid peroxidation (Martínez-Sánchez et al., 2021; Sagai & Bocci, 2011; Ye et al., 2025; Zhu et al., 2024), while simultaneously suppressing NF-κB/MAPK signaling. These molecular signals have functional counterparts. In perfused livers, the addition of ozone to UW reduces hepatocellular enzyme leakage throughout the entire process, consistent with histology (Aydın et al., 2022). Kidney and liver models also show improved microcirculation and reduced interstitial fibrosis, along with faster functional recovery after storage (Delgadillo-Valero & et al., 2023; García et al., 2023; Petrosillo & et al., 2022; Re et al., 2023).

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20261035

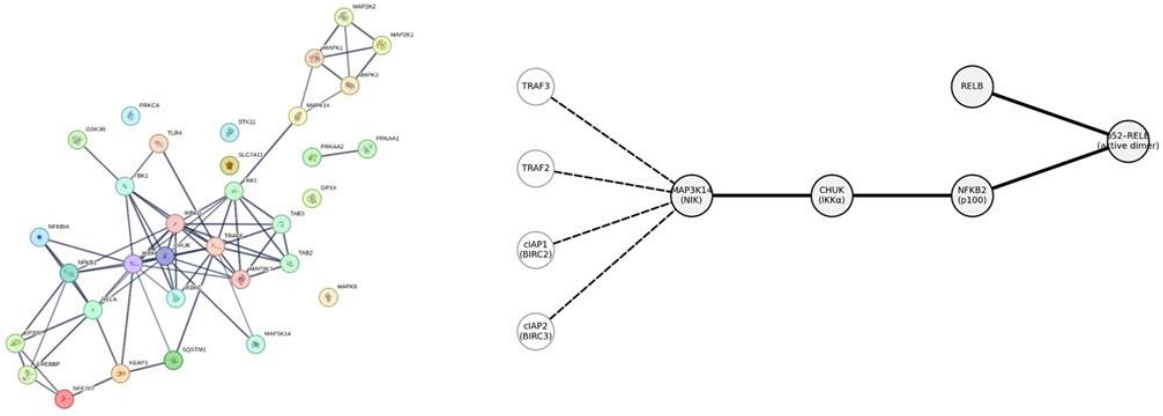

Figure 2. *Protein interaction network and non-canonical NF-κB signaling pathway associated with ozone-mediated redox regulation.*
(Left panel) Protein–protein interaction (PPI) network generated using the STRING database. Nodes represent proteins involved in oxidative stress, inflammatory signaling, antioxidant defense, and NF-κB-related pathways, while edges indicate experimentally validated or predicted functional interactions.
(Right panel) Simplified schematic representation of the non-canonical NF-κB signaling pathway. Activation of NF-κB-inducing kinase (NIK/MAP3K14) promotes IKKα-mediated processing of p100 (NFKB2) to p52, leading to the formation of the p52–RELB transcriptionally active complex. This pathway is associated with inflammatory regulation, cellular adaptation, and redox-responsive signaling mechanisms relevant to ischemia–reperfusion injury.

Situated within contemporary preservation strategies, active oxygenation (HOPE, NMP, oxygen carriers, two-layer methods) already reduces the succinate reservoir that fuels the early ROS burst and supports mitochondrial ATP reconstitution (Foguenne et al., 2023; Mesnard et al., 2022). Ozone seems more complementary than unnecessary: by adding a layer of controllable hormetic signaling to oxygenation and hypothermia, it shifts Nrf2/SLC7A11/GPx4 activity and endothelial protection to early reperfusion. Practically, most reports converge on short-term ex vivo ozonation of the organ or preservation medium (≈15-25 µg/mL for 5-10 minutes) and a low-dose in vivo preconditioning step before collection in some protocols (Bejaoui et al., 2024; García et al., 2023; Kal & et al., 2016; Qiu et al., 2017). However, the therapeutic window is narrow: the risk of oxidative cytotoxicity increases above approximately 80 µg/mL, so the dose, route of administration, and timing cannot be left to conventional methods (Martínez-Sánchez et al., 2021; Re et al., 2023; Sagai & Bocci, 2011).

Three gaps limit immediate clinical adoption. First, standardisation is incomplete. Studies report gas-phase concentrations and exposure times but seldom the dissolved ozone at the point of tissue contact, nor do they routinely capture inline telemetry (pO_2, ORP, temperature, flow). Closed-loop ozonation of UW/HTK/PERLA® with harmonised reporting would make results portable across centres (Bejaoui et al., 2024). Second, evidence quality skews preclinical. Controlled human data are scarce, and safety for gas-phase ozonation within clinical perfusion circuits remains to be established (Malatesta et al., 2024; Re et al., 2023). Third, long-term biology is under-characterised: we lack a clear view of how repeated redox nudging shapes allo-immunity, endothelial remodelling, and fibrosis months after implantation. Mechanistic read-outs—time-resolved redox proteomics/metabolomics, single-cell transcriptomics focused on endothelial and tubular compartments—should be embedded alongside clinical endpoints to define dose–response behaviour and adaptive thresholds (Petrosillo & et al., 2022; Re et al., 2023; Zhang et al., 2022).

Looking forward, a cautious, stepwise path seems the most defensible. Organ-on-chip systems can de-risk parameters under controlled shear and oxygenation before escalation to animal or human circuits, with read-outs such as TEER, NO bioavailability, glycocalyx shedding, and mitochondrial respiration. In the perfusion lab, ozone layered onto HOPE/NMP should be tested head-to-head against oxygenation alone using paired-graft designs, with prespecified markers of lipid peroxidation (MDA/4-HNE), ferroptosis restraint, succinate handling, and microvascular function. Combinatorial strategies—e.g., ozone with N-acetylcysteine or glutathione donors, and context-specific ferroptosis modulators—warrant small factorial trials to determine additivity versus redundancy (Tasset & et al., 2021; Wang & et al., 2024; Zhang et al., 2022). Under ethical conditions, in order to achieve optimisation, multi-centre studies must be undertaken, or large databases must be created for this study, and extensive data analysis must be performed.

Conclusion

However, when administered at appropriate doses, ozone therapy can serve as a beneficial preconditioning strategy capable of regulating redox reactions. The control of mitochondrial processes and the careful monitoring of pathways must be supported by numerous experiments, and these supports must be consistent with each other (Aydın et al., 2022; Petrosillo & et al., 2022; Re et al., 2023). Because the disadvantages are obvious. These disadvantages are the short range of usage doses, limited number of study data, and the use of different doses. Considering the above, it can be said that the priority is to evaluate consistent and overlapping results. To this end, controlled ex vivo applications and monitoring of graft function are important. If those studies confirm safety and reproducible benefit, ozone could take a defined place alongside oxygenation and perfusion technologies as part of an integrated preservation toolbox. Until then, careful dosing, transparent reporting, and mechanism-anchored endpoints should guide its use.

Conflict of interest

The authors declare no conflict of interest. The funding institution had no involvement in the design of the study, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

All aspects of the study were conducted jointly by MMC and UK, and both authors have read and approved the final version of the manuscript.

Funding

This study was financially supported by the Scientific Research Coordination Unit of Çanakkale Onsekiz Mart University (Project No: TSA-2025-5114).

References

Aydın, H. O., Ayvazoğlu Soy, E. H., Tezcaner, T., Avcı, T., Erkent, M., Yıldırım, S., & Haberal, M. (2022). Effect of ozone added to University of Wisconsin solution on preservation damage in perfused liver. *Experimental and Clinical Transplantation*.

Bejaoui, M., Dammacco, F., Doria, C., Spada, M., & Bejaoui, M. (2024). PERLA preservation solution in kidney transplantation: A systematic review and meta-analysis of randomized controlled trials. *Transplant International*, *37*, 12154.

Belzer, F. O., & Southard, J. H. (1988). Principles of solid-organ preservation by cold storage. *Transplantation*, *45*(4), 673–676.

Bocci, V., Zanardi, I., & Travagli, V. (2020). Ozone acting on human blood yields a hormetic dose-response. *Journal of Translational Medicine*, *18*, 322.

Calabrese, E. J., & Mattson, M. P. (2017). How does hormesis impact biology, toxicology and medicine? *NPJ Aging and Mechanisms of Disease*, *3*, 13.

Chen, H., Sun, H., You, G., & et al. (2008). Ozone oxidative preconditioning protects the rat kidney. *Journal of Surgical Research*, *149*, e199–e207.

Delgadillo-Valero, L. F. & et al. (2023). The protective role of ozone therapy in kidney disease. *Journal of Translational Medicine*, *21*, 102.

Fernández, O. S. L. & et al. (2008). Ozone oxidative preconditioning mediated by A1 adenosine receptors. *European Journal of Pharmacology*, *581*, 306–314.

Foguenne, M., MacMillan, S., Kron, P., Nath, J., Devresse, A., De Meyer, M., Michel, M., Hosgood, S., & Darius, T. (2023). Current evidence and future perspectives to implement continuous and end-ischemic use of normothermic and oxygenated hypothermic machine perfusion in clinical practice. *Journal of Clinical Medicine*, *12*(9), 3207.

Fukushima, C. T., Dancil, I. S., Clary, H., Shah, N., Nadtochiy, S. M., & Brookes, P. S. (2024). Reactive oxygen species generation by reverse electron transfer at mitochondrial complex I under simulated early reperfusion conditions. *Redox Biology*, *70*, 103047.

García, S. J., Borroto, B. A., Guerra, B. C., Menéndez, S. S., & Morales, R. N. (2023). Ozone therapy in organ preservation and transplantation: A systematic review. *International Immunopharmacology*, *123*, 110620.

Gültekin, F. A. & et al. (2013). Effects of ozone oxidative preconditioning on liver regeneration. *Turkish Journal of Gastroenterology*, *24*, 104–111.

Kal, O. & et al. (2016). Prophylactic ozone administration reduces renal ischemia/reperfusion injury. *International Journal of Clinical and Experimental Medicine*, *9*, 14338–14347.

Malatesta, M., Tabaracci, G., Pellicciari, C., & et al. (2024). Low-dose ozone as a eustress inducer: Experimental evidence of molecular mechanisms. *International Journal of Molecular Sciences*, *25*(23), 12657.

Martínez-Sánchez, G., Al-Dalain, S. M., & Menéndez-Cepero, S. (2021). Ozone therapy in human disease: A narrative review of clinical trials. *Archives of Medical Research*, *52*(1), 7–18.

Meng, H., Ding, J., Zhu, F., He, Z., & Li, J. (2017). Ischemia–reperfusion injury in renal transplantation: From pathogenesis to therapeutic strategies. *Frontiers in Physiology*, *8*, 717.

Meng, W. & et al. (2017). Ozone protects rat heart against ischemia-reperfusion injury. *Archives of Cardiovascular Diseases*, *110*, 146–154.

Mesnard, B., Thuillier, R., & Hauet, T. (2022). New insights into oxygenated machine perfusion in organ transplantation: From energy metabolism to mitochondrial protection. *International Journal of Molecular Sciences*, *23*(21), 12894.

Peralta, C., & Roselló-Catafau, J. (2022). The future of organ preservation solutions. *Transplantation Reviews*, *36*, 100654.

Petrosillo, G. & et al. (2022). Cross-talk between Nrf2 and mitochondrial biogenesis in ozone-induced preconditioning. *Redox Biology*, *55*, 102442.

Qiu, Y., Li, F., Yu, P., Ding, H., Tang, B., & Zhang, Y. (2017). The role of reactive oxygen species in ischemia-reperfusion injury in kidney transplantation. *Oxidative Medicine and Cellular Longevity*, *2017*, 1–10.

Re, L., Martínez-Sánchez, G., & Bordicchia, M. (2023). Clinical and preclinical perspectives on ozone therapy as a redox modulator. *Free Radical Biology and Medicine*, *210*, 116–128.

Sagai, M., & Bocci, V. (2011). Mechanisms of ozone action on the organism. *British Journal of Biomedical Science*, *68*(1), 1–13.

Şengül, D., & Şengül, İ. (2021). Connection of reactive oxygen species as an essential actor for the mechanism of phenomena; ischemic preconditioning and postconditioning. *North Clinical Istanbul*, *8*(6), 644–649.

Sorby-Adams, A., Prime, T. A., Miljkovic, J. L., Prag, H. A., Krieg, T., & Murphy, M. P. (2024). A model of mitochondrial superoxide production during ischaemia-reperfusion injury for therapeutic development and mechanistic understanding. *Redox Biology*, *72*, 103161.

Tasset, I. & et al. (2021). Mitochondrial effects of ozone therapy in ischemia-reperfusion injury. *Biomedicine & Pharmacotherapy*, *142*, 112023.

Viebahn-Haensler, R., & León Fernández, O. S. (2025). Protection of mitochondria, cells and organs from ischemia-reperfusion damage through preventive redox bioregulation by ozone. *International Journal of Molecular Sciences*, *26*(12), 5557.

Wang, L. & et al. (2024). Research progress on ferroptosis in pathogenesis and therapy. *Frontiers in Cellular Neuroscience*, *18*, 1359453.

Ye, Y., Yu, Z. H., Nie, L., Wang, F. X., & Mu, G. (2025). Beyond paradox: Ozone therapy as a hormetic modulator in ischemia-reperfusion injury – An update. *Cell Biochemistry and Biophysics*.

Zhang, M. Y., Dugbartey, G. J., Juriasingani, S., Akbari, M., Liu, W., Haig, A., & Sener, A. (2022). Sodium thiosulfate-supplemented UW solution protects renal grafts against prolonged cold ischemia-reperfusion injury in a murine model of syngeneic kidney transplantation. *Biomedicine & Pharmacotherapy*, *145*, 112435.

Zhu, F., Ding, S., Liu, Y., & et al. (2024). Ozone-mediated cerebral protection via NRF2/SLC7A11/GPX4. *Journal of Chemical Neuroanatomy*, *136*, 102387.