Ozone therapy addresses neuropathic pain in ulcerous wounds

Giuseppe Masiello, Marianno Franzini, Tommaso Richelmi, Umberto Tirelli, Luigi Valdenassi, Salvatore Chirumbolo*

Neuropathic pain (NPP) in the context of ulcerous wounds arises from nerve damage or dysfunction, often occurring alongside chronic wounds such as diabetic foot ulcers, pressure sores, or venous leg ulcers. Unlike nociceptive pain, which results from tissue damage and inflammation, NPP originates from the nervous system, characterized by abnormal signaling due to injury to peripheral or central nerves.[1] Chronic wounds, particularly diabetic ulcers, can cause direct nerve damage due to ischemia, infection, or compression. Ulcers are associated with chronic inflammation, leading to the release of pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-alpha, interleukin 1-beta (IL-1β), which sensitize nerve endings and contribute to pain. Moreover, bacterial colonization or infection in ulcerous wounds may exacerbate nerve irritation and lead to NPP.

NPP can impair wound healing by increasing stress and catecholamine levels, which disrupt the inflammatory and proliferative phases of repair. Poor pain management may lead to reduced mobility and compliance with wound care protocols, further delaying healing.

Ozone therapy in ulcerous wounds appears as a really promising approach.[1,2]

But how ozone addresses NPP?

The effects of oxygen-ozone therapy on the patient's immune system are becoming increasingly clear in the scientific field to date, as various pieces of evidence suggest that ozone, at hormetic doses, is capable of inhibiting the formation of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome.[3] This occurs by blocking its interaction with NIMA-related kinase 3 through the most important electrophilic of ozone-induced lipid peroxidation, 4-hydroxynonenal.[3,4] Inhibiting the inflammasome reduces the maturation of IL-1β, prevents pyroptosis, and consequently inhibits the activation of sodium voltage-potential channels, which underlies ozone's anti-nociceptive effect used in anti-inflammatory and pain-relief therapies. Additionally, the hormetic doses used in oxygen-ozone therapy, such as those established by the clinical protocols of the Italian Scientific Society of Oxygen-Ozone Therapy,[5,6] promote, through the production of reactive oxygen species as biochemical signaling molecules, the activation of the nuclear factor erythroid 2–related factor 2/Kelch-like ECH-associated protein 1/antioxidant response element (Nrf2/Keap1/ARE) pathway. This induces the synthesis of heme oxygenase 1 and the production of carbon mono-oxide, which in turn inhibits the activation of nuclear factor kappa-light-chain-enhancer of activated B cells, blocking the gene expression of pro-inflammatory cytokines. It also enhances the anti-inflammatory action mediated by M2 macrophages and upregulates the surface marker Macrophage Receptor with Collagenous Structure.[7]

A recent study investigates the therapeutic potential of ozone in alleviating NPP, a chronic condition often unresponsive to conventional treatments.[8] NPP is driven by mechanisms such as the accumulation of apoptotic cells and neuroinflammation, which impair macrophage-mediated efferocytosis, a process essential for clearing dying cells and reducing inflammation. Using a chronic constriction injury mouse model, some authors administered ozone at varying concentrations and assessed its impact on pain symptoms, apoptotic cell clearance, and neuroinflammatory markers.[8]

Ozone treatment at an optimal dose of 30 μg/mL effectively reduced mechanical hypersensitivity and thermal hyperalgesia, hallmark symptoms of NPP. This analgesic effect was linked to the ability of ozone to promote the phagocytic activity of macrophages by enhancing the AMP-activated protein kinase/growth arrest-specific protein 6/Mer tyrosine kinase (AMPK/Gas6-MerTK) signaling pathway.[8]

Although many studies were conducted on laboratory animals, specifically, ozone activated AMPK and increased levels of Gas6, which facilitated MerTK expression in macrophages.[8] This enhanced efferocytosis led to the efficient clearance of apoptotic cells and a subsequent decrease in pro-inflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor-alpha in the sciatic nerve and spinal cord. Importantly, Ruan et al.[8] also demonstrated that the therapeutic effects of ozone were abolished by inhibitors of AMPK or MerTK, confirming the centrality of this pathway in mediating ozone's benefits.

In addition to improving macrophage efferocytosis, ozone upregulated suppressor of cytokine signaling 3 (SOCS3), an anti-inflammatory protein that suppresses cytokine signaling, further contributing to its neuroprotective effects. However, higher concentrations of ozone, i.e., > 45 μg/mL, induced oxidative stress, negating its therapeutic benefits, highlighting the importance of precise dosing. These findings suggest that ozone therapy, by targeting the AMPK/Gas6-MerTK/SOCS3 axis, could serve as an effective intervention for NPP, offering a novel approach to modulating immune responses and reducing neuroinflammation. Ruan et al.[8] provided a strong foundation for further clinical exploration of ozone therapy as a treatment for NPP.

The activity induced on macrophage efferocytosis by ozone in the oxygen-ozone therapy is particularly crucial in an injury site, represented, for example, by highly inflamed anatomical districts, such as wounds or musculoskeletal damages, i.e., regions the efficient clearance of cells undergoing apoptosis is a crucial prerequisite for the timely resolution of the inflammatory response via macrophage-induced efferocytosis, even involving other signaling regulators such as microRNAs.[9] What is particularly interesting is that microRNA-21 is a mediator of cellular signaling and regulation strongly promoted by biological contact with ozone, making it one of the key factors connecting ozone to the anti-inflammatory and modulatory response mediated by macrophage efferocytosis.[9,10] The ability of ozone to move multiple pieces on the vast chessboard of immune-mediated response regulation makes this molecule a key component in the antinociceptive properties that characterize oxygen-ozone therapy. In the case of ulcerative wounds, ozone not only exerts an antiseptic and anti-inflammatory action but also an antinociceptive effect thanks to its influence on macrophage efferocytosis, as mentioned a few lines earlier.

Figure 1 summarizes this issue.

At least two major mechanisms lead to the anti-inflammatory and anti-nociceptive role of ozone, of which one related to the Nrf2/Keap1/ARE pathway and the other to the AMPK/Gas-6/MerTK/SOCS3 pathway, besides to the role of 4-hydroxynonenal on NLRP3.

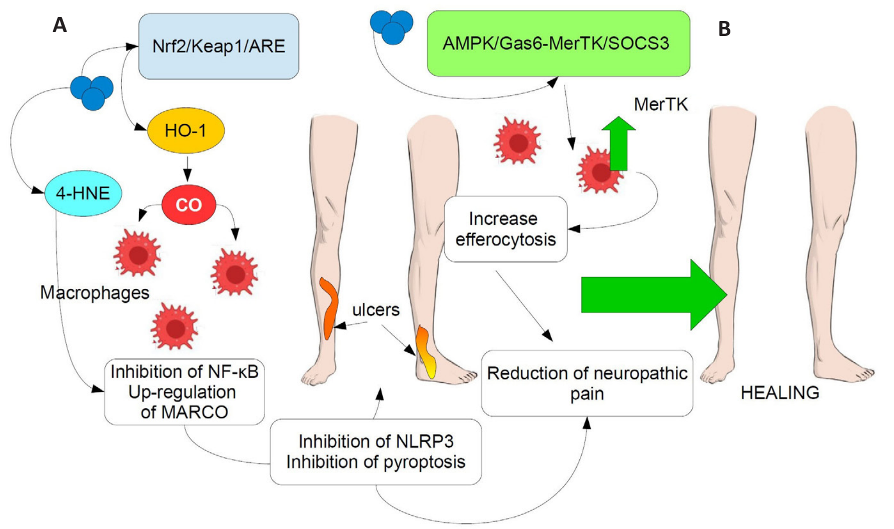

Figure 1 | Carton shows two possible ways used by oxygen-ozone therapy in addressing ulcerous wounds.
(A) Ozone in hormetic doses activates the Nrf2/Keap1/ARE pathway, inducing the synthesis of HO-1, which in turn activates CO in inactivating NF-κB (promoting an anti-inflammatory action) and skewing M1 pro-inflammatory macrophages to MARCO-expressing M2 macrophages. (B) Ozone increases macrophage-mediated efferocytosis via the activation of the AMPK/Gas-6/MerTK/SOCS3 pathway. Carton drawn with the assistance of T.R. via the software CorelDRAW Graphics Suite: The latest version is 2024.1 (version 25.1), Apache OpenOffice, v. 4.1.15, Paintbrush, 2.6.0 (revised). 4-HNE: 4-Hydroxynonenal; AMPK: AMP-activated protein kinase; ARE: antioxidant response element; CO: carbon monoxide; Gas6: growth arrest-specific protein 6; HO-1: heme oxygenase-1; Keap1: Kelch-like ECH-associated protein 1; MARCO: macrophage receptor with collagenous structure; MerTK: Mer tyrosine kinase; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; Nrf2: nuclear factor erythroid 2–related factor 2; SOCS3: suppressor of cytokine signaling 3.

Ozone therapy represents a promising and innovative approach to managing ulcerous wounds, addressing both the physical and physiological challenges of chronic wound care. Its dual action as a potent antimicrobial agent, a modulator of tissue oxygenation, and an anti-inflammatory agent, sets it apart from conventional therapies. By enhancing local oxygen availability and promoting the generation of reactive oxygen species at controlled levels, ozone therapy stimulates cellular mechanisms essential for wound repair, including angiogenesis, collagen synthesis, and the resolution of chronic inflammation. Additionally, its capacity to reduce microbial load and biofilm formation tackles one of the primary impediments to wound healing.

Moreover, in cases of NPP associated with ulcerous wounds, ozone's potential to modulate inflammatory pathways and improve microvascular circulation offers a novel avenue for pain relief and nerve recovery. Moreover, its ability to synergize with other therapeutic modalities, such as advanced dressings and physical therapies, positions ozone as an integral component of a comprehensive wound care strategy.

Anyway, while the evidence supporting its efficacy is encouraging, further large-scale clinical studies are needed to standardize ozone application protocols, optimize dosing, and confirm its long-term safety and efficacy. With its multi-faceted benefits and relatively low-risk profile when used correctly, ozone therapy holds the potential to transform the management of ulcerous wounds, ultimately improving patient outcomes and quality of life.

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