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REVIEW ARTICLE

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Ozone therapy for skin diseases: Cellular and molecular mechanisms

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Abstract

Ozone is a highly reactive oxidant molecule consisting of triatomic oxygen atoms. Ozone therapy can be achieved using ozonated hydrotherapy, ozonated oil, ozone autohemotherapy, and other innovative dosage forms of ozone products. Ozone is frequently used as a complementary therapy for various cutaneous diseases, including infectious skin diseases, wound healing, eczema, dermatitis, psoriasis, axillary osmidrosis, diabetic foot, and pressure ulcers. In addition, several studies have reported the superior potential of ozone therapy for improving skin and gut microbiomes, as well as antitumour and antiaging treatment. Ozone therapy is an emerging treatment strategy that acts via complex mechanisms, including antioxidant effects, immunomodulatory capacity, and modulation of local microcirculation. Studies assessing the mechanism of ozone have gradually expanded in recent years. This review article aims to summarise and explore the possible molecular biological mechanisms of ozone in cutaneous diseases and provide compelling theoretical evidence for the application of ozone in cutaneous diseases.

KEYWORDS

anti-infective agents, antioxidants, immunoregulation, ozone, skin diseases

Key Messages

- ozone is frequently used as a complementary therapy for various cutaneous diseases
- this review article aims to summarise and explore the possible molecular biological mechanisms of ozone in cutaneous diseases

1 | INTRODUCTION

The skin, which is the largest organ in the human body, constitutes the first natural line of defence against external stimulation and pathogens and prevents the loss of nutrition and water.[1] Frequently, skin diseases exhibit complex etiologies that cannot be effectively addressed using currently available therapeutic agents. Furthermore, unavoidable and significant side effects may be experienced, resulting in multiple physical and psychological adverse events during a prolonged treatment course. Accordingly, there is a need to explore and develop novel therapeutic strategies.

Ozone (O_3) therapy is a non-invasive and low-cost treatment that elicits limited side effects. Ozone was considered a toxic gas until the 16th century when Wolff first proposed the therapeutic effect of ozone at low concentrations.[2] Since then, ozone has been explored as a potential therapeutic agent. Currently, several ozone-related medical agents have been examined, developed, and employed to circumvent contraindications, including hyperthyroidism, hemorrhagic or coagulation dysfunction, glucose-6-phosphatase-α (G6Pase-α or G6PC) deficiency, or ozone allergy.[3] Ozone therapy has been widely used in more than 50 pathological processes, including skin diseases, intervertebral disc herniation,[4] diabetic complications,[5] oral mucosal diseases, cardiovascular and cerebrovascular diseases,[6] and cancer. In terms of skin diseases, topical ozone therapy can be employed to treat infectious skin diseases (e.g., tinea capitis and herpes zoster),[7,8] inflammatory skin diseases (e.g., eczema, psoriasis, atopic dermatitis, and contact dermatitis),[9-11] autoimmune-related skin diseases (e.g., pemphigus, bullous pemphigoid, and dermatomyositis)[12,13] and chronic ulcers (e.g., diabetic foot and pressure ulcers).[14,15] In addition, ozone therapy can be used to clean burns and scald wounds,[16] accelerate wound healing,[17] and promote skin repair after laser cosmetology. Furthermore, systemic ozone therapy has shown great potential as an analgesic, antiaging,[18] and antitumour agent,[19] along with the capacity to alleviate chemoradiotherapy resistance. Moreover, some studies have explored the potential of ozone therapy as a treatment strategy for coronavirus disease 2019 (COVID-19).[20]

O_3 is a bluish gas with a fishy smell and is composed of three oxygen atoms that are easily broken into oxygen and oxygen atoms. Reportedly, O_3 can affect gene expression of hypoxia-inducible factors (HIFs) and activate vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) to improve the hypoxic tissue conditions.[21,22] Notably, O_3 can be distinguished by its reactive and robust oxidation capacity, second only to fluorine, which forms the basis for the pharmacological effects mediated by ozone therapy. When ozone dissolves into plasma or serum, a rapid reaction occurs within 1–2 min, producing reactive oxygen species (ROS), such as hydrogen peroxide (H2O2) and lipid oxidation products (LOPs) containing 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA).[23] These molecules can regulate the nuclear factor erythroid-derived 2–like 2 (Nrf2) signalling and nuclear factor kappa B (NF-κB) pathways,[9,24] which play crucial roles in modulating the intracellular redox reaction and inflammatory balance. The interactions between these pathways depend on a complex set of molecular interactions influenced by multiple cell and tissue types. Accumulated data has revealed that the pharmacological effects of ozone are mediated via these two pathways. Notably, the eye and respiratory system are susceptible to ozone; therefore, ozone should be used under strict regulation and supervision.

In this review, we aim to clarify the pharmacological effects and potential molecular mechanisms of ozone treatment in cutaneous diseases. In addition, this study provides theoretical evidence for the clinical applications of ozone.

2 | THE APPLICATION OF OZONE IN DERMATOLOGY

2.1 | Ozonated hydrotherapy

Ozonated hydrotherapy involves physically dissolving ozone in water (rather than chemically). Ozonated hydrotherapy can aid in treating dermatological diseases, such as infectious skin diseases,[25,26] atopic dermatitis,[11] psoriasis,[9] pemphigus,[12] and diabetic foot.[15] In addition, ozone water can be used to clean burns and scald wounds, accelerate wound healing,[14] and promote skin repair after laser cosmetology. Based on the location, scope, and other related conditions of specific skin lesions, as well as for diagnosis and treatment, the operator can select different methods, including debridement, soaking, and wet compression. In summary, the use of ozonated hydrotherapy is simple and flexible. Moreover, ozonated hydrotherapy has no age limitation.[27] However, restrictions owing to the instability of O_3 should be considered. At 20°C, the half-life of O_3 in water is only 27 min,[28] so ozonated water cannot be shipped or kept in storage for a long time. Ozonated water is produced on the spot; thus, hospitals or clinics need the appropriate equipment and a suitable area. To achieve effectiveness, the O_3 concentration, water temperature, and treatment time must all be precisely controlled and monitored. This implies that ozonated hydrotherapy must be administered by a physician or nurse under the direction of a qualified engineer. In addition, given the astringent effect of ozone water, patients can employ a combination of moisturisers when necessary.

2.2 | Ozonated oil

Ozonated oil is prepared by the direct reaction of O_3 with vegetable oil rich in unsaturated fatty acids, which are critical substances that maintain the activity of ozone. Ozone oxidises unsaturated fatty acids to form 1,2,4-trioxolane, gradually releasing O_3 on the surface of skin lesions.[29] When stored at 4°C, ozonated oil can maintain stable properties and pharmacological activities

for 2 years.[25,30] Therefore, ozonated oil is considered an excellent supplement to unstable ozonated water. Moreover, ozonated oil is convenient to carry and use. In addition, ozone oil exhibits a good affinity for the skin and can afford an emollient effect. However, it is imperative to control the degree of peroxidation, as it can affect the efficacy of ozonated oil.[29] In addition, repeated topical application of ozonated oil may result in excessive ozone concentrations in the skin lesion, occasionally inducing irritant pain. Ozonated oil can promote wound healing[17] and topically treat atopic dermatitis,[11] psoriasis,[9] superficial bacterial infections,[31] and fungal infections.[32]

2.3 | Ozonated autohemotherapy (OAHT)

Ozonated autohemotherapy (OAHT) refers to a process in which a limited amount of blood is exposed to a precisely controlled ozone dose in a sterile environment and then infused back into the body. The antioxidant system can quickly neutralise the transient oxidative stress occurring during this process. In terms of safety and effectiveness, precise exposure time and dosage remain critical. For successful OAHT, the antioxidant system must reach a certain threshold to initiate the cytokine cascade; simultaneously, the antioxidant capacity should not be exceeded. Di Paolo et al. proposed a safety standard for ozonated autohemotherapy.[33] The therapeutic concentration of ozone for OAHT should range between 10 and 80 μg/mL.[30] Studies have reported that the optimal ozone concentration to stimulate distinct cytokines tends to vary. Therefore, some researchers feel that 20–40 μg/mL ozone can effectively trigger the immune system.[27] Moreover, years of clinical experience with OAHT indicate a lack of adverse reactions.[34] However, some patients reportedly experience tiredness following the first OAHT session, especially when a high ozone dose is employed. Therefore, OAHT should be initiated at a low dose, and the patient's response should be closely monitored. Currently, OAHT is considered a supplementary therapy for diabetic foot, certain tumours, herpes zoster, and some autoimmune-related diseases, such as lupus erythematosus. In addition, OAHT can be employed as an antiaging strategy.

2.4 | Other novel dosage forms

2.4.1 | Ozonated oil-based emulsions

Ozonated oil-based emulsion is a colloidal dispersion prepared from oil and water and an emulsifier or surfactant, with a typical particle size of 1–5 μm; this product is further employed to formulate ointment creams, gels, and lotions. Optimization of dosage forms can afford patients a better user experience and expand the scope and scenarios of application.[35]

2.4.2 | Microemulsions and nanoemulsions

Ozonated microemulsions and nanoemulsions emulsify or load ozone oil with microemulsions. This product results in moderate skin and mucosal surface irritation and demonstrates good antibacterial properties.[36]

2.4.3 | Macro-, micro-, and nanoencapsulation of oils

Microcapsules are obtained by coating or embedding ozone oil in homogeneous or heterogeneous substrates.[37,38] Microcapsules help regulate drug release, improve physical stability, and prevent chemical reactions between the drug content and environment.[35] Microcapsules of ozonated oil are used in medical textiles, resulting in high antibacterial activity. This method extends the range of ozone applications.[39]

2.5 | Wearable and flexible ozone generating system

Large ozone generating equipment limits its application scope. Small, portable equipment can be used in low-dose or long-term therapy for wound infections, reducing side effects and irritants. The portable generator consists of a flexible and disposable ozone delivery patch, an ozone generating unit, and a flexible tube. The study confirmed the effective bactericidal properties of Pseudomonas aeruginosa and Staphylococcus epidermidis in wound infections.[40]

3 | MOLECULAR BIOLOGICAL MECHANISM OF OZONE THERAPY FOR CUTANEOUS DISEASES

3.1 | Antioxidant capacity

Oxidative stress refers to the excessive production of ROS and reactive nitrogen species (RNS), overwhelming the intracellular antioxidant defence system, and resulting in an imbalance between the two systems. In addition, the structure and function of cells may be influenced by

oxidative damage, which decreases mitochondrial activity.[19] Nrf2 is a critical signalling pathway responsible for intracellular redox reactions and inflammatory balance. Under primary conditions, Nrf2 binds to its repressor, Kelch-like ECH-associated protein 1 (Keap1), the linker between Nrf2 and Cullin 3 proteins, and its ubiquitination results in proteasome-mediated Nrf2 degradation.

Following ozonated systemic therapy (mainly through ozonated autohemotherapy), ozone dissolves in serum or plasma and rapidly reacts with polyunsaturated fatty acids, inducing downstream second messengers such as H_2O_2 and 4-HNE, which, in turn, stimulate the overregulation of the antioxidant system. It should be noted that ozone disappears after only a few seconds. Thus, ozone acts as a regulator without following the standard principles of pharmacology, that is, absorption, distribution, metabolism, and excretion. As a primitive and transitory messenger, H_2O_2 has a plasma lifespan of approximately 2 min. Notably, 4-HNE is more stable than H_2O_2[41] and is a later and persistent messenger. Accordingly, 4-HNE can send transient signals to different tissues to stimulate oxidative stress. These effects depend on the O_3 concentration and tissue type. Interestingly, the O_3 concentration and outcomes do not exhibit a linear relationship: low concentrations may demonstrate no effect, and extremely high concentrations could induce effects opposite to those of middle and lower concentrations.[42]

H_2O_2 and 4-HNE can stimulate the Nrf2 pathway via an indirect pathway.[43,44] Studies have shown that H_2O_2 activation is associated with the dose-dependent activation of extracellular signal-regulated kinase (ERK1/2) and P38 MAP kinase (P38).[45] In addition, O_3 induces the modification of Keap1 cysteine residues,[46,47] which inhibits the ubiquitination of the Keap1 complex and results in the nuclear accumulation of Nrf2. In the nucleus, Nrf2 dimerizes and induces transcription of antioxidant response elements (ARE).[48] The transcription of ARE regulates the constitutive and inducible expression of antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT), heme oxygenase-1 (HO-1), NAD(P)H-dependent quinone oxidoreductase 1 (NQO-1), and heat shock proteins (HSPs).[49,50]

HO-1 is a genetically encoded enzyme that catalyses the degradation of carbon monoxide (CO) and heme in free iron, and biliverdin is converted to bilirubin. CO, another essential inhibitor of the NF-κB pathway, contributes to the downregulated expression of pro-inflammatory cytokines. In addition, bilirubin functions as a lipophilic antioxidant. In summary, HO-1 balances inflammation by inhibiting pro-inflammatory cytokines and activating anti-inflammatory cytokines.[51]

In addition, casein kinase 2 (CK2) is a negative regulator of NADPH oxidase. Recent studies have revealed that ozone can afford a therapeutic effect in multiple sclerosis by influencing CK2 levels through the Nrf2 pathway.

It is well-established that mitochondria produce energy for most cellular processes. Mitochondrial dysfunction can induce excessive ROS production, resulting in increased oxidative protein and lipid damage, decreased ATP production, and accumulation of DNA damage.[52] Ozone can decrease mitochondrial damage caused by ischemia–reperfusion injury.[52] Furthermore, ozone can repair mitochondrial damage by activating the Nrf2 signalling pathway and increasing the expression levels of downstream antioxidant proteins, including ischemia–reperfusion (GCL) and SODs.[53] Moreover, ozone contributes to the remodelling of cellular structures and increases the length of mitochondrial cristae,[54] thus increasing mitochondrial function and decreasing mitochondrial autophagy. Microtubule-associated protein 1 light chain 3 (LC3) is essential for autophagy. Ozone treatment was found to reduce the proportion of autophagy-related proteins LC3B-II and LC3B-I. The conversion of LC3B-I to LC3B-II indicates the formation of autophagosomes, typically deemed a marker for autophagy.[55,56] Meanwhile, ozone therapy reportedly decreases PTEN-induced putative kinase 1 (PINK1) levels and the mitochondrial accumulation of Parkin, which indicates a decrease in mitochondrial autophagy. PINK1 is degraded by proteasomes within normal mitochondria, retaining low PINK1 levels to monitor abnormal mitochondria. However, following mitochondrial damage, PINK1 degradation is suppressed, leading to PINK1 accumulation in damaged mitochondria.[57,58] PINK1 recruits and activates the ubiquitin-proteasome system, eliciting the autophagosome-mediated phagocytosis of damaged mitochondria.

3.2 | Immunoregulatory functions

O_3 plays a role in regulating the immune system during disease treatment. On the one hand, ozone increases the number of leukocytes and enhances the phagocytic ability of granulocytes.[59] Macrophages exhibit higher levels of mitochondrial ROS following ozone treatment. O_3 promotes the formation of monocytes and activates T cells. Simultaneously, it stimulates the release of cytokines, such as interferon and interleukins (IL), to trigger antibody-dependent cell cytotoxicity (ADCC).[60,61] Conversely, O_3 inhibits the NF-κB pathway,[62] which regulates the inflammatory response. Typically, resting-state NF-κB exists in an inactive form in the cytoplasm, a trimeric complex, composed of p65, p50, and an inhibitor of NF-κB-α (IκB-α). Various factors can induce the activation of the trimeric complex, including lipopolysaccharide (LPS), cytokines (IL-6, IL-17,

and tumour necrosis factor [TNF]-α), and physical and chemical factors (X-rays, oxidants, and chemotherapy drugs). Under the stimulation by listed activators, IκB kinase β (IκKβ) is phosphorylated within the cell, which then results in the phosphorylation of the amino-terminal serine residue of IκB. Phosphorylated IκB is ubiquitinated, followed by conformational changes and degradation. Then, the heterodimer p50–65 translocates to the nucleus and initiates the transcription and expression of the target gene. Activation of the NF-κB pathway contributes to the release of various pro-inflammatory cytokines and the expression of pro-inflammatory genes, such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS),[63] resulting in an inflammatory response and tissue injury. Therapeutic O_3 doses suppress the NF-κB pathway. In contrast, high doses of O_3 promote inflammation by activating the NF-κB pathway.[64] For example, during the onset of psoriasis, an abnormal increase in pathogen-associated molecular patterns (PAMPs, such as LPS and peptidoglycan) and structurally activated multiple inflammatory cytokines via the NF-κB pathway can be detected in psoriatic lesions.[65] The results in excessive keratinocyte proliferation and robust inflammatory response in local lesions.[66] We previously reported that topical ozone therapy significantly reduced expression levels of TLR2, P50, and P65 in the imiquimod (IMQ)-induced psoriasis-like mouse model. Accordingly, local ozone therapy can significantly inhibit TLR2/NF-κB signal transduction in psoriatic lesions.[9] Interestingly, topical ozone therapy also significantly suppressed IMQ-induced activation of TLRTNF and IL-17 signalling pathways. In addition, our study revealed that ozone therapy inhibited the expression of various chemokines, including chemokine (C-X-C motif) ligand 1(CXCL1), CXCL2, and CXCL3, as well as inflammatory cytokines associated with psoriasis, such as IL-17A, IL-17C, IL-17F, IL-1β, IL-8, IL-22, TNF-α, VEGF, defensin B14, S100A7, S100A8 and S100.[9]

3.3 | Anti-infection

3.3.1 | Antibacterial activity

Bacterial infection-related dermatosis has a high incidence rate. Accordingly, the discovery and application of antibiotics have afforded radical benefits. Most infections caused by planktonic bacteria have been brought under rapid control. However, improper use of antibiotics, the spread of resistant bacteria, and infections induced by invasive medical devices remain major concerns. These infections are often associated with bacterial biofilm formation. Bacteria adhere to the contact surface, secrete polysaccharide matrix, fibrin, and lipid–protein, and wrap themselves around it to form bacterial aggregation membranes.[67] Bacterial biofilm can resist the host immunity; the sensitivity to the antibiotic is lower than the same kind of planktonic bacteria. However, many antimicrobial agents were initially developed for planktonic bacteria. Ozone has a broad spectrum, efficient, and safe bacteriostatic effect. Reports have confirmed that ozone can efficaciously kill *S. aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA), Streptococcus, *P. aeruginosa*, *Escherichia coli*, and mycobacteria.[31,68-72] Reportedly, bacterial biofilms were shown to be destroyed following exposure to ozonated water and oil.[73] Notably, the bactericidal effect of ozone is related to its high oxidative activity. The specific mechanism of ozone sterilisation may be as follows: ozone destroys the bacterial cell wall by acting on the outer membrane lipoprotein and the inner LPS. In this case, ozone invades the bacteria, oxidising glycoproteins and glycolipids, affecting enzyme functions and damaging DNA and RNA, and disrupting the metabolic and reproductive processes of microorganisms.[74] In addition, ozone enhanced immune defence is elicited via the activation of a large number of anti-inflammatory factors (IL-4, IL-10, IL-13) to neutralise the inflammatory factors (IL-1, IL-12, IL-15).[75] Furthermore, ozone therapy improved tissue hypoxia and increased the content of fibroblast growth factor and epidermal growth factor in infected skin to promote wound healing.

3.3.2 | Antiviral activity

We postulated the potential role of ozone in viral suppression. Ozone could interfere with viral replication and exert an antiviral effect. Ozone damages four polypeptide chains on viral capsid proteins, damages RNA, disrupts the reproductive cycle, and inhibits viral replication.[76] However, ozone has been shown to oxidise intracellular cysteine residues.[77] The NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome is a cytoplasmic complex responsible for the production of IL-1β and IL-18 and plays a vital role in initiating inflammatory processes, including viral infections. Reportedly, ozone exhibits anti-inflammatory activity by modulating the NLRP3 inflammasome.[32] Ozone can inactivate herpes simplex virus type 1 without affecting cell survival. Previously, we reported that ozone therapy, used as adjuvant therapy for herpes zoster treatment, could afford pain relief during the acute phase of herpes zoster and rapidly reduce viral inclusion bodies, thereby shortening the course of herpes zoster disease.[8] In addition, ozone can influence the activity of angiotensin-converting enzyme 2 (ACE2) receptors by controlling the Nrf2 signalling pathway. In addition, ozone may block the endogenous replication of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by preventing the interaction between SARS-CoV-2 and the

ACE2 receptor. However, it is worth noting that ozone enhances antiviral drugs and cannot comprehensively replace antiviral agents.[78]

3.3.3 | Antifungal activity

Fungi are conditional pathogens, and their spores are strongly resistant to the external environment. Thus, fungal infections have a long cycle and high recurrence rate. Unfortunately, long-term use of traditional antifungal drugs has well-reported side effects. Ozone can enter the fungal cytoplasm, disrupt critical cellular functions, increase nutrient leakage, and ultimately inhibit the production of fungal urease, amylase, alkaline phosphatase, lipase, and keratin.[79] Khatri et al. confirmed that ozone could reduce the number of Candida colonies.[80] Furthermore, ozone can kill *Trichophyton mentagrophytes* and *Trichophyton rubrum*, the pathogens responsible for onychomycosis and tinea pedis, respectively. Previously, we reported that ozone therapy could treat tinea pedis safely and effectively.[7] In addition, ozone can be conjugated with the double bond of the furan ring at the end of aflatoxin B1 (AFB1) and can attack the double bond in trichothecene mycotoxins, eliminating the toxicity-related groups.[81,82] These mycotoxins are widely found in nature and can cause renal and liver injury or even death if ingested by humans via contaminated grains. In conclusion, ozone has shown potential as an antifungal agent, and its mechanism needs to be further elucidated.

3.4 | Improving wound healing

Numerous studies have confirmed that ozone can facilitate wound healing by improving skin microcirculation, especially in pressure ulcers, diabetic foot, burn wounds, and other ulcers associated with peripheral vascular disease.[83-85] Typically, wound healing comprises four stages: rapid haemostasis, appropriate inflammation, proliferation, and maturation/reconstruction.[86,87] These phases overlap and influence each other. Haemostasis occurs initially within seconds to minutes after injury. Platelets are indispensable during this stage and the entire healing process. Notably, ozone promotes platelet aggregation.[88] The inflammatory phase is characterised by sequential infiltration of neutrophils, macrophages, and lymphocytes.[89] Ozone can induce and regulate leukocytes and macrophages, thus positively impacting the wound healing process. The proliferative phase overlaps the inflammation phase, and its main feature is re-epithelialization, which refers to epithelial cells proliferating and migrating to the temporary wound matrix.[90] The repair phase also includes capillary sprouting and extracellular matrix production.[86] Studies have shown that moderate oxidative stress generated by ozone therapy could induce the upregulation of HIF-1α, promote the release of excessive transforming growth factor-β (TGF-β) and PDGF, and facilitate keratinocytes to release VEGF,[91] thus implying that ozone could accelerate vascular repair and acute wound healing. Moreover, an increased number of new blood vessels were detected in the ozone-treated group, which may be attributed to the direct induction of VEGF expression by H_2O_2 and/or indirect induction of VEGF expression by HO-1.[92] Additional, ozone therapy promotes wound healing via facilitating fibroblast migration. It has demonstrated ozone oil promotes fibroblast migration and EMT process via PI3K/Akt/mTOR signalling pathway.[93] The transdifferentiation of epithelial cells into motile mesenchymal cells, a process known as epithelial-mesenchymal transition (EMT), is integral in wound healing.[94] Transforming growth factor-β (TGF-β) regulates EMT by various signal pathways, such as Smads, RhoA, MAP and PI3K.[95,96] Ozone treatment accelerates the wound healing process through increasing the proliferation and migration abilities as well as α-SMA and collagen I protein levels in fibroblasts, resulting from miR-21-5p overexpression on fibroblasts.[97] MiR-21-5p plays a role by knockdown the level of RASA1, known as a Ras GTPase-activating protein (RasGAP) that regulates fibroblast migration via negatively regulating Ras activity.[98,99]

O_2 is essential for almost all wound healing processes. Some chronic ischemic and hypoxic wounds are prone to prolonged healing and enter a pathological inflammatory state. O_2 can facilitate angiogenesis, upregulate keratinocyte differentiation and migration, and enhance fibroblast proliferation and collagen synthesis. In addition, the level of superoxide production (a critical factor in oxidation-mediated pathogen killing) by polymorphonuclear leukocytes is highly dependent on available O_2 levels. Notably, ozone increases erythrocytic ATP and 2,3-diphosphoglycerate (2,3-DPG) and thus expands the oxygen-carrying capacity of erythrocytes, shifts the oxygen dissociation curve of haemoglobin to the right, and reduces the affinity of haemoglobin to O_2, which is conducive for O_2 release and expanding the O_2 supply to peripheral tissues.[75] Accordingly, ozone therapy is beneficial in reducing wound hypoxia, especially in pressure ulcers, diabetic foot, and other ulcers associated with peripheral vascular disease.

3.5 | Regulation of skin and gut microecology

The "gut-skin axis" may affect skin health through the immune system.[100] The skin hosts various microbes, including bacteria, fungi, mites, and viruses, which occupy different environmental niches and appendages

on the skin.[101,102] Bacteria are prominent members comprising Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes.[103] The gut microbiome is a highly complex ecosystem, including bacteria, eukaryotic viruses, fungi, and some archaea, with bacteria playing a dominant role.[104] The gut microbiota composition is dynamic, depending on various factors, such as diet, age, and environmental conditions. Quantitative studies have confirmed that the dynamic balance of the skin and gut microbiomes is closely associated with the incidence and severity of skin diseases, such as acne, rosacea, atopic dermatitis, and psoriasis.[105-110] For example, the microbial diversity decreased while the colonisation density of *S. aureus* increased in atopic dermatitis lesions. *S. aureus* may invade Pseudomonas acnes, an important member of the skin microbiota, by secreting virulence factors[111]; this, in turn, results in skin barrier destruction. We previously reported that topical ozone treatment reduced the burden of *S. aureus* and increased the proportion of Acinetobacter species in atopic dermatitis.[112] In atopic dermatitis lesions, ozone therapy not only exhibits a bactericidal effect but also participates in restoring the skin microbial diversity.[11] In addition, we assessed the potential of ozone therapy in axillary hyperhidrosis and observed that ozonated oil could effectively decrease the skin pH value and inhibit the growth of Corynebacterium, the pathogenic bacteria of axillary hyperhidrosis. A few possible mechanisms can be postulated: (1) ozone plays an antibacterial role (as mentioned in the anti-infection section), directly affecting microbial composition and distribution. (2) Ozone impacts microecology through immunoregulatory effects. IL-1B, IL-8, and IL-6 can induce the growth of normal flora, such as *Lactobacillus*, while simultaneously inhibiting opportunistic pathogenic bacteria, such as *S. aureus* and *E. coli*. If the bacterial community is disordered, the abundance of these cytokines increases, thus inhibiting the growth of normal flora and stimulating the growth of opportunistic pathogens; ozone can moderate the production of these cytokines. (3) Ozone therapy affects microecology by affecting the microenvironment. The colonisation of microorganisms is affected by the local microenvironment. Topical ozone therapy can interfere with skin moisture, oil secretion, and pH, subsequently affecting microecology. In conclusion, ozone therapy can potentially improve skin and gut microecology, and the specific mechanisms need to be elucidated.

4 | CONCLUSIONS

Ozone can activate the antioxidant and immune systems of the body. Accordingly, ozone therapy has shown considerable promise in clinical applications, presenting anti-infective properties, improving microcirculation, and relieving pain. Meanwhile, ozone therapy has great potential for enhancing skin microecology, antiaging, and treating COVID-19. In addition, ozone therapy has advantages, such as low cost, high efficiency, and few side effects. Therefore, ozone is an excellent supplementary treatment for cutaneous diseases. In future studies, we plan to explore the value of ozone application in treating other skin diseases. Moreover, clarifying the underlying molecular mechanism is crucial for promoting the application of ozone therapy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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