Review

Mechanisms of action involved in ozone-therapy in skin diseases

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ABSTRACT

Ozone-therapy initially applied in medicine by an empirical approach, has now reached a new stage where most of the biological mechanisms of ozone action have been clarified, that refers to antimicrobial effects, immunoregulation, antioxidant defenses and epigenetic modification. Current ozone medical preparation in dermatology mainly classified as ozone hydrotherapy, ozonated oil externally used and ozone autohemotherapy (OAHT). Admittedly, ozone is widely used in various fields against gram-negative and gram-positive bacteria, viruses, and fungi. More recently, great progress has been obtained in wound healing which is a multiphase process that consists of three overlapping but distinct stages: inflammation, tissue proliferation and remodeling. While the exact mechanisms of ozone-therapy still remain unclear. Therefore, more evidence is required before ozone can be presented as a promising method for the management and prevention of various skin diseases. In this review, we review the application status of ozone in dermatology and summarize possible mechanisms of ozone-therapy on skin diseases, aims to shed a light on providing a series of theoretical basis for its applications.

1. Introduction

Ozone was originally applied in medicine in an empirical and rather imprecise manner for the last about 200 years since the first report for sterilization in 1826, fortunately, during the last decade, great progress has been made owing to new medical ozone generators allowing the determination of precise ozone concentrations in real time and the clarification of mechanisms of ozone action on diseases treatment [1,2]. Ozone is an unstable molecule consisting of three oxygen atoms which can quickly break down into oxygen and single oxygen atom acting as a strong oxidant to kill microorganisms. Therefore, in proper concentrations, it serves as an ideal drug [3–5]. Notably, due to its easy quenching, ozone can be used safely in medicine even though it is released into the blood where it possesses potent antioxidant capacity composed of a number of lipophilic, hydrophilic compounds and a variety of antioxidant enzymes [6]. While reports showed monthly exposure to even low troposheric ozone concentrations was toxic for the pulmonary system [7], which implies we should supervise and manage its application more effectively in medicine. This controversial molecule has been widely used as a treatment agent of more than 50 pathological processes [8–10] as well as skin diseases [11]. Currently, there are correspondingly simple application forms and biological mechanisms (Table 1) to be known in medicine. (See Table 2.)

Ozone medical preparations were mainly classified as ozone hydrotherapy, ozonated oil externally used and ozone autohemotherapy (OAHT) in dermatology. Recently, it has been used to treat four types of skin diseases: (1) infectious skin diseases containing virus, bacteria and fungi such as herpes zoster, abscess and athlete's foot [12–15]; (2) allergic diseases such as atopic dermatitis, eczema, urticarial (ozone autohemotherapy) and prurigo [16,17]; (3) erythema scaly diseases such as psoriasis and palmoplantar pustulosis [18,19]; (4) wound healing and ulcer recovery [3]. The mechanisms of ozone's action are omnifarious involved in direct antimicrobial effect, immunoregulation, antioxidant defenses, epigenetic modification, even more other potent properties such as biosynthetic, analgesic and vasodilative effects [11]. There are several statements for its antimicrobial effect, firstly, ozone directly disrupts nucleic acid or liposome shell of microorganisms. After the membrane is damaged, permeability increases and ozone molecules can easily enter into the cells [11]. Moreover, it generates molecular-level reactions in the medium where it releases oxygen-free radicals and then indirectly destroys the living micro-environment [20,21]. The immunoregulation of ozone in the treatment of diseases is generally accepted that ozone on the one hand, increases the quantity of leukocytes, enhances the phagocytic capacity of granulocytes, facilitates the formation of monocytes and activates T cells. Simultaneously, it boosts the release of cytokines such as interferon and interleukin triggering antibody dependent cellular cytotoxicity (ADCC) [22,23]. On the other hand, ozone augments the production of hydrogen peroxide (H_2O_2) derived from immune cells of body [24] to kill pathogens. While the strength of the oxidative stress determines the effectiveness and toxicity of ozone. Severe oxidative stress activates nuclear transcriptional factor kappa B (NF-κB), leading to inflammatory responses and tissue injury

Table 1
Ozone therapy in the management and prevention of skin diseases.

Medical preparations types	Effects	Skin diseases included	Ref.
Ozonated hydrotherapy	Antimicrobial, relieving itching,	Infectious, allergic, erythema scaly diseases, wound healing and ulcer recovery	[3] [9,11]
Ozonated oil	Antimicrobial, moisturizer, reducing exudate		[13,36]
Ozonated autohemo-therapy	Antioxidant defenses, immunoregulatory effect, epigenetic modification	Chronic systemic conditions, autoimmune diseases, postherpetic neuralgia, beauty care items	[37] [19,44,45,48]

by the production of COX2, PGE2, and cytokines. Conversely, moderate oxidative stress activates nuclear factor-erythroid 2-related factor 2 (Nrf2) and represses NF-κB and inflammatory responses. Additionally, moderate oxidative stress induced the production of hypoxia inducible factor-1a (HIF-1a) which has been elucidated in vascular and degenerative diseases as well as skin lesions [25]. In recent years, the mystery of epigenetic modification induced by ozone therapy is gradually unveiling.

Unfortunately, there is not enough solid theoretical foundation and clinical evidence to support the ozone-therapy in dermatology at present, and most of the current applications just depend on clinical experience, which presents a great challenge in this field. Thus, a more exact mechanism of action how ozone works and a more reliable evidence-based medical data are in great need. In this review, we summarize the application status of ozone-therapy in dermatology, discuss the possible mechanisms of action and strive for providing more evidence for ozone applications.

2. The application status of ozone-therapy in dermatology

Ozone was first performed for treating German soldiers suffering gaseous gangrene during World War I owing to its strong bactericidal effect on *Clostridium* anaerobic [26], while this approach is very empirical and unprecise. Until 2001, Werkmeister [27] mastered the use of ozone in several skin ulcers affected by atherosclerosis and diabetes, however, he just used a polythene-bag (the so-called bagging system) or using an ozone-resistant plastic cup to store ozone but difficult to control its concentration. Later on, Werkmeister [27] could release a continuous gas flow with a moderate pressure that improved the vasodilation of the ulcer's site and enhanced blood circulation. Via such strategy he treated plenty of extensive and otherwise incurable lesions within 50–200 days. Remarkable, ozone functions well only in a water vapour-saturated bag because it must dissolve into superficial water or exudate to react precisely. And during the treatment process normal skin did not suffer from any damage. Now owing to the drawback of cumber and air contamination in use these procedures have been abandoned. Thus, various medical preparations have been manufactured to more accurately and conveniently serve for patients.

2.1. Ozonated hydrotherapy

With the good knowledge of medical equipment, we have developed an ozone generator which allowed us to control and measure the precise ozone concentrations in real time during treatment procedure and maintain optimum therapeutic dose instead of respiratory injury [28]. This instrument was designed for patients to take a bath or soak the skin lesions in clinic through producing ozone water. Generally, the

Table 2
The exact studies of ozone therapy applied in medicine included.

Diseases	Patients number	Patients' criteria	Assessment indicators	Medical preparations types	Frequency of treatments	Outcomes	Ref.
Diabetic foot	101	Type 2 diabetes and diabetic feet	Glycemic index, the area and perimeter of the lesions and biochemical markers of oxidative stress and endothelial damage	Ozonated Hydrotherapy	Twice weekly	The healing of the lesions improved	[102]
Wound healing	18	Free gingival graft surgery	Wound sizes and shape factor	Ozonated oil	Daily for 1 week.	A significant improvement in wound size and epithelial healing	[103]
Peripheral arterial occlusive disease	152	Ankle-brachial index below 0.40	Walking distance	OAHT	Once or thrice weekly for 12 weeks	A significant improvement in blood flow	[104]

treatment time continues about 15 min every time, and one course contains 3–5 times for acute dermatitis and infections, also, it can be adjusted according to age, entity and serious degree of pathogenetic condition. Better yet, there is no age limit on ozone-therapy which supplies a very good option for refractory skin diseases, especially for children, old man and those who cannot tolerate the adverse effect of drugs. Now ozone hydrotherapy is wildly used for treatment of infectious skin diseases including bacterial, virus and fungi and itch dermatoses such as eczema, atopic dermatitis, prurigo nodularis et al. Studies showed ozone hydrotherapy can ameliorate effusion of tissue fluid, reduce inflammatory response, promote wound healing, ease the pain and pruritus [11]. However, what's the drawback of ozone hydrotherapy is its inconveniency to carry on because of its rapid degradation feature. Therefore, ozonated oil agent meets a great need for distant patients.

2.2. Ozonated oil externally used

Ozonated oil consists of ozone and unsaturated fatty acids, among the later, it can be classified into three types: ω-9 series of unsaturated fatty acids represented by oleic acid in tea oil, ω-6 series of unsaturated fatty acids represented by sub-acid in vegetable oils and ω-3 series of unsaturated fatty acids represented by eicosapentaenoic acid (EPA)and docosahexaenoic acids (DHA) in fish oil. Notably, the direct ozonation of vegetable oils with unsaturated fatty acids leads to the formation of the 1,2,4-trioxolane moiety [29,30], which represents the active form of ozone in these substrates. The trioxolane ring within the vegetable ozonated matrices quickly produces some compounds accountable for the healing process when cured either a humid wound or an ulcer [11,31–33]. Moreover, it is responsible for antimicrobial and antimycotic treatments [34–36]. In addition, the oil itself acts as moisturizer and protectant particularly for those patients with impaired skin barrier function. More importantly, it is storable and portable in daily life to meet great need of more patients.

2.3. Ozonated autohemotherapy (OAHT)

For treatment of systemic conditions, the ozonated autohemotherapy (OAHT) is the form of good choice [37,38]. Now in clinic ozone major autohemotherapy (100–150 ml blood venoclysis) is more common than minor autohemotherapy (3–5 ml blood intramuscular) whose applications are justified in broad pathological conditions, covering a potential antimicrobial effect, the activation of the immune system and the induction of wound healing as well as the improvement of erythrocyte metabolism and the regulation of body's antioxidant capacity [25,39]. Common diseases involve in chronic inflammatory conditions [19], diabetic foot ulcers [40], herpes zoster and postherpetic neuralgia [41,42], autoimmune diseases (AID) [43–45] as well as psoriasis and atopic dermatitis [19]. It is worth noting that the concentration of ozone-therapy cannot exceed 80 μg/ml in serum, suggesting that the key point during treatment process is precise control of ozone concentration because the induction of distinct cytokines needs different ozone concentration such as 11.5 μg/ml for IFN-γ, 25 μg/ml for IL-6 and TNF-α [46,47]. In short, range from 20 to 40 μg/ml is the optimum concentration to activate immune system [25,48]. Additionally, a regular and adequate duration of the treatment is essential to ensure the efficacy. Generally, 10–15 times are recommended in a course and 1–2 courses are demanded every year [48,49]. Fortunately, OAHT can be regarded as a strategy management of anti-aging and health care benefiting many people owing to its antioxidant defenses such as inducing and activating the antioxidant enzyme system of body to produce SOD, a free-radical scavenger, clearing excessive free-radicals formed in chronic joint and vascular inflammation. In contrast with conventional medical therapeutic modalities, ozone therapy is quite economical leading to an obvious decrease of both medical costs and also prevents the aggravation and recurrence.

3. The possible mechanisms of ozone-therapy in skin diseases

3.1. Antimicrobial effect

Although ozone therapy is extensively applied in antimicrobial effect and wound repair, little is known about cellular mechanisms regarding this process. There are mainly existed two hypothesis to clarify the antimicrobial mechanism of ozone action. One is the oxygen free-radicals released by ozone acting as a strong oxidant to directly kill microorganisms such as *Candida albicans* [50] and *Staphylococcus aureus* [51]. The other is an increase of O_2 tension within skin lesion also conforming to the emerging theory that hyperbaric oxygen therapy on chronic inflammatory conditions [52]. As we all known, *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) which are the most dominant bacterial community found in skin may easily develop infections. While ozone can forcefully inactivate bacteria, viruses and spores within a few minutes [53] so it is proved clinically effective in the treatment of infected wounds [54]. In repeated animal experimental models, ozone-therapy has been confirmed to have beneficial effects when used as an adjunct to standard antibiotic treatment [55]. A recent study identified the antimicrobial effect of ozonated oils by testing in vitro four bacterial species and one yeast within or without different amounts of human serum, which suggested that ozonated oil owned a moderate and continuous elimination of microorganisms and exudate is an essential condition for the potent bactericidal effect of the agent [13]. This study indicated a great promise in a variety of skin and mucosal infections. In addition, ozonated oils are far less expensive and lower resistance than antibiotic preparations. Unfortunately, patients must clean the damaged skin surface to remove necrotic tissue, pus, loose fibrin deposition, and excess of fluid exudates before the oil application because of the poor diffusion of ozonated oil throughout the medium [13]. Recently, several studies have emphasized the importance of thiol disulphide homeostasis in infection [56,57]. Indeed, a study by Dodd et al. also reported ozone to cause stoichiometric elimination of antibacterial activity of many antibacterial molecules including vancomycin [58]. This may be due to that the ozone molecules may attach to vancomycin molecules similarly to the thiols, since vancomycin molecule also has electron emitting potential as thiols.

3.2. Antioxidant defenses

As mentioned above, moderate oxidative stress is good for health whereas excessive one is not [25]. Moderate oxidative stress activates Nrf2, a nuclear transcriptional factor. During the last decade, overwhelming evidence demonstrated that the activation of Nrf2 induced the transcription of antioxidant response elements (ARE) [59]. Transcription of ARE leads to the production of numerous antioxidant enzymes, such as SOD, GPx, glutathione-*S*-transferase (GSTr), catalase (CAT), heme-oxygenase-1 (HO-1), NADPH-quinoneoxidoreductase (NQO-1), phase II enzymes of drug metabolism and heat shock proteins (HSP) [60–64]. This vital cell response occurs in cardiovascular, degenerative and chronic infective diseases aggravated by a chronic oxidative stress [65]. In a previous report conducted by Alessandra et al. [65] shown that ozonated plasma is able to up-regulate HO-1 expression in endothelial cells and the activation of Nrf2 reacted with a dose-dependent on concentration of ozone in serum. Moreover, the treatment with ozonated serum was associated with a dose dependent activation of extracellular-signal-regulated kinases (ERK1/2) and p38 MAP kinases (p38), not directly involved in Nrf2 activation [66,67]. Not only that, ozone therapy may also suppress the activity of NF-κB and inflammatory responses as well as activate another nuclear transcriptional factor, hypoxia inducible factor-1α (HIF-1α), which plays a critical role in treating vascular and degenerative diseases [68–70]. Increasing evidence confirmed thiol-disulphide homeostasis was a requisite condition for antioxidant protection and immune response in our body. The conversion of thiols and disulphides is affected by

oxidation which depends on the oxidant-antioxidant balance of the organism. Once severe oxidative stress take place, the reversible disulphide bonds are difficult to break into thiol [71,72]. Recent studies indicated that both native and total thiol even the ratio of native thiol/total thiol reduced, while disulphide/native thiol and disulphide/total thiol ratio increased in acute appendicitis. Proteins also have functional −SH groups such as albumin which has a critical role in serum potent antioxidant capacity [12]. Generally, the level of albumin rapidly degrades in inflammation and oxidative stress [72].

However, ozone cannot oxidize pathogens inside organisms either free in exudates or intracellular because they are well protected by the serum and cellular antioxidants, that is the potent antioxidant capacity of serum existing hydrophilic (ascorbic acid, uric acid, free Cysteine, GSH and albumin) and lipophilic (vitamin E, thioredoxin, α-lipoic acid and bilirubin) compounds. Consequently, most of ozone is neutralized, whereas the bulk rapidly reacts with n-3 and n-6 polyunsaturated fatty acids (PUFA) engendering its vital messengers: hydrogen peroxide (H_2O_2) and active aldehydes, principally 4-hydroxy-2,3-*trans*-nonenal (4-HNE) [73]. H_2O_2 is a reactive oxygen species (ROS) but it has a half-life of about 20 s in the blood and activates several relevant biochemical pathways [74]. While 4-HNE forms adduct with the Cys34 of albumin or with glutathione. The sudden infusion of ozonated blood into patients prepares for the entrance of 4HNE into cells. When this electrophile binds to Cys151 of Keap1, it suppresses the constitutive inhibition of Nrf2, which then translocates into the nucleus as mentioned above [61,75,76].

3.3. Immunoregulatory effect

It is well known that T-cells, serving as very crucial soldiers, defend our body against foreign pathogens: a tyrosine-phosphorylation response takes place immediately in the ZAP-70 molecule when the T-cell antigen receptor (TCR) recognizes any invaders, and then activates phospholipase C γ1 (PLCγ1) [77]. Membrane lipid phosphatidylinositol-4,5-bisphosphate (PIP2) can be hydrolyzed by the activation of PLCγ1, therefore, producing two critical second messengers including inositol triphosphate (IP3) and diacylglycerol (DG). Then, IP3 binds to its receptor (IP3R) located in the endoplasmic reticulum (ER) membrane, leading to Ca^{+2} transformed from ER into the cytosol. The elevated levels of Ca^{+2} in cytosolic will activate calcineurin (CN), a phosphatase dependent on Ca^{+2}/calmodulin, which dephosphorylates nuclear factor activated T-cells (NFAT) and transports it into the nucleus. NFAT then induces the transcription of cytokines, such as IL-2, TNFα, IL-6 and IFNγ, participating in the immune response of our body [78]. As mentioned above, these nuclear factors produced in mild oxidative stress are induced by ozone therapy and then activate immune functions. Several studies [79–81] have been conducted via using human normal blood treated with proper ozone concentrations, called "therapeutic window". And according to these parameters ozone works well without any toxicity [82]. In above studies they found several cytokines including IFNγ, TNFα, IL-2, IL-6 and IL-8 were synthesized and released by immune cells, showing a dose-dependent on ozone concentration. Torossian et al. [83] showed that cytokine TNFα level was significantly enhanced with ozone pretreatment in septic rats. Tandara et al. [84] and Barcin et al. [12] reported that the H_2O_2 formed by ozone treatment to increase production of growth factors and IL-4, mainly VEGF, can be an indicator of protective effects of ozone in inflammatory processes. Interestingly enough, the amount of lymphocytes and monocytes presenting in the blood exposed to ozone *ex vivo* was only activated about 4% during each ozone therapy session [85], which suggested that the small portion of immunocytes activated by H_2O_2 *ex vivo*. It is assumed NF-κB may play a vital role of transmitting the activation effect in vivo after infusing ozonated blood into the donor patient and then activates other cells [85]. Indeed, in consistent with antimicrobial effects of ozone therapy in both acute and chronic bacterial and viral infections ozone can serve as an ideal therapeutic

method without any toxicity in an appropriate dosages.

On the other hand, a recent study carried on by Frank A. et al. showed levels of IgE and HLA-DR obviously decreased in asthma patients treated with systemic ozone therapy. Lung function and symptoms test were markedly improved. This study demonstrates the effectiveness of ozone therapy in reducing IgE and inflammatory mediators along with the induction of antioxidant elements by means of its immunomodulatory and oxidative stress regulation properties [86]. Study showed ozone-therapy enhanced the expression levels of innate immune surface proteins CD14, CD11b and TLR4, antigen presentation markers CD80, CD86, and HLA-DR, and immunoglobulin receptors CD23, CD16 and FcϵR I [87]. Simultaneously, it also increased oxidative burst and phagocytic functions, which suggested that ozone exposure might increase the inflammatory milieu of body and improve the response to biologic agents. Thus, it is postulated that the diseases, present in pathological IgE increase in serum or skin lesions, can be treated with ozone-therapy such as atopic dermatitis and eosinophilia. There are also some conflicting reports on ozone's immunological effects. Burleson et al. showed that pulmonary ozone exposure caused a suppression of naturel killer cells activity [88]. Collectively, the immunoregulatory mechanism of ozone in the treatment of diseases is complex and omnifarious (Fig. 1) and needs more effort from us to explore it.

As we all known, ozone is clinically effective in the treatment of infected wounds [3,12]. Wound healing, as a synthetic disease, is a multiphase process that consists of three overlapping but distinct stages: inflammation, tissue proliferation and remodeling which illustrates the comprehensive functions better contributed by ozone in its treatment process. Wound forms at the moment of skin barrier broken, and the blood clot is responsible for hemostasis and cell recruitment. Firstly, neutrophils prevent bacterial invasion and activate keratinocytes, fibroblasts as well as immune cells. In this stage, ozone provides an aid to kill microorganisms and activates immune system. The increase in O_2 tension within the wound site also justifies the use of ozone, considering that it increases the formation of granulation tissue, accelerating the wound closure. Secondly, the proliferation stage begins 2–10 days after injury, when macrophages adopt an anti-inflammatory,
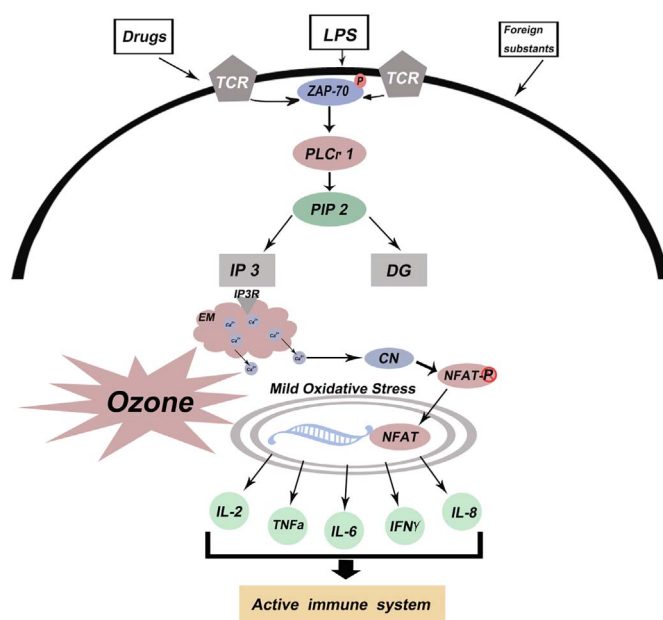

Fig. 1. Mild oxidative stress induced by ozone therapy to activate immune functions. In our adaptive immunity, T cells serve as very crucial soldiers to defend our body against foreign pathogens. And ozone therapy activates the production of some nuclear factors as mentioned in part of antioxidant defenses to induce cytokines transcription, such as IL-2, TNFα, IL-6, IFNγ and IL-8, participating in the immune response of our body.

profibrotic phenotype, producing growth factors such as transforming growth factor-β (TGF-β) and recruiting fibroblasts from the wound borders [89]. Ozone therapy enhances a higher expression of growth factors TGF-β and vascular endothelial growth factor (VEGF), which play important roles in the wound repair process, after 2–3 weeks of injury, remodeling of the extracellular matrix (ECM) begins and collagen fibers proliferated and reorganized into a stronger network [90,91]. In these situations, ozone promotes the release of NO, endothelium-independent vasodilator, which increases blood circulation for tissue remodeling.

3.4. Epigenetic modification

Cumulative evidence identified that epigenetic modifications, such as DNA methylation, noncoding RNAs, and histone modifications, play a critical role in the molecular mechanism of oxidative stress induced by ozone exposure, mostly focusing on the effect of surfactant protein A (SP-A) expression in pulmonary diseases, an important lung host defence molecule, as well as its functions [92–94]. Epigenetic remodeling of chromatin packaging existed in various biological processes such as microbial environment, ozone exposure and other forms of oxidant stress. Ozone, as a strong oxidant, posttranslationally modifies the histone deacetylase (HDACs) and creates histone acetyl transferase (HAT)/HDAC stoichiometry imbalance, contributing to the enhancement of IL-1β-stimulated inflammatory cytokines production including IL-8, IL-6, CXCL1, CXCL2, and CXCL3 in allergic diseases [95,96]. Additionally, ROS generated by oxidative stress can affect DNA methylation via the hydroxylation of pyrimidines and 5-methylcytosine (5mC) [97] and influence DNA demethylation by DNA oxidation and TET-mediated hydroxymethylation [98]. Remarkably, ROS can also indirectly regulate the activity of the epigenetic machinery since histone-modifying enzymes depend on intracellular levels of essential metabolites, such as Acetyl-CoA, Fe, ketoglutarate, NAD +, and S-adenosylmethionine, indicating that epigenetic changes are tightly linked to global cellular metabolism and energy levels of the cell [99]. In addition, functional assays revealed that assessment of potential IL-6-targeting microRNAs, miR-149, miR-202, and miR-410 showed differential expression in primary cultures based on animal ozone exposure experiments. Clay CC et al. [100] suggested differentially expressed microRNAs such as miR-149 was capable of binding to the IL-6 3′ UTR and decreased IL-6 protein synthesis in airway epithelial cell lines, which played a critical role in the persistent modulation of the epithelial innate immune response towards microbes in the mature lung. As we all known, ozone is an abiotic elicitor of reactive oxygen species in plants. Another research conducted by Niranjani J. Iyer, et al. [101] identified 22 miRNAs that were differentially expressed early in response to ozone in a plant miRNA array which played an important role in regulating oxidative stress responses of plants to adapt to the changeful environment. Therefore, mild oxidative stress caused by ozone-therapy in a proper concentration can globally influence the cell on multiple levels, serving as a brilliant method applied in medicine. While it is still a great challenge for us to completely understand this molecular process and we should evoke more programme and suitable clinical trials to reveal the full efficacy of ozone therapy by evidence-based medicine.

4. Conclusion

Ozone serves for a practical drug in dermatology working destructively against bacteria, fungi, and viruses, activating cellular and humoral immune system as well as acting as antioxidant to defend various pathologic conditions. However, not much research has been done to uncover other physico-chemical properties of ozone such as biosynthetic, analgesic, vasodilators secretion such as NO effects. Additionally, as epigenetics era is coming, it is opening new horizons in the comprehension of the molecular mechanism of ozone-therapy on

skin diseases. Remarkably, the therapeutic effects of ozone depend on its concentration, thus, an accurate ozone generator and ideal agents seem to be very essential. So far, ozone medical preparations are classified into three types including ozonized water, ozonized oil and ozonized gas. Fortunately, the ozone therapy is quite inexpensive, predictable and conservative with little side effects. And the elucidation of molecular mechanisms of ozone further benefits practical application in dermatology. Furthermore, the clinical trials in this area should be more collaborative in a multi-centric fashion to ensure its reliability and practicability.

Conflict of interests

The authors declare no conflict of interests.

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