OZONE AS U-SHAPED DOSE RESPONSES MOLECULES (HORMETINS)

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□ Redox environment involves a broad network of pro-oxidant and antioxidant components. Health benefit or damage can be induced as a consequence of an adaptive cellular stress response. A consequence of hormetic amplification is an increase in the homeodynamic space of a living system in terms of an increased defense capacity and a reduced load of damaged macromolecules. Ozone, when used at appropriate doses, promotes the formation of reactive oxygen species and lipid peroxides allows them to become late and long-lasting messengers. Healthy aging may be achieved by hormesis through mild and periodic, but not severe or chronic, physical and mental challenges, and by the use of nutritional hormesis incorporating mild stress-inducing molecules called hormetins. The paradoxical concept that ozone eventually induces an antioxidant response capable of reversing a chronic oxidative stress is common in the animal and vegetal kingdom; it is already supported by findings of an increased level of antioxidant enzymes during ozone therapy. Those facts can include ozone as a hormetin. The established scientific foundations of hormesis are ready to pave the way for new and effective approaches in redox-related disease research and intervention; ozone therapy can be a good candidate.

Keywords: ozone, hormesis, adaptive responses, age, oxidative stress, skin

INTRODUCTION

The acceptance of the concept of hormesis, a specific type of non-monotonic dose response, has accelerated in recent years (Cook and Calabrese 2006). Although given many names, hormesis has been observed in the fields of medicine (Celik et al. 2005, Calabrese and Blain 2009) molecular biology (Randic and Estrada 2005), pharmacology (Chiueh et al. 2005, Zhang et al. 2009), nutrition (Lindsay 2005), aging and geriatrics (Rattan et al. 2009), agriculture (Calabrese and Blain 2009), microbiology (Brugmann and Firmani 2005) immunology (Dietert 2005), toxicology (Shanker 2008) exercise physiology (Radak et al. 2008 ), and carcinogenesis (Cox 2009) literally, across the biological
It has also been observed in relation to disparate outcomes from the isolated single cellular process to the more holistic (e.g., growth, longevity, disease, death) that likely result from a complex interplay of multiple factors and mechanisms (Calabrese 2005).

Prolonged inhalation of ozone can be very deleterious firstly for the lungs and successively for the whole organism (Kajekar 2007). On the other hand, a small ozone dose well-calibrated against the potent antioxidant capacity of blood can trigger several useful biochemical mechanisms and reactivate the antioxidant system. In detail, firstly ex vivo and secondly during the infusion of ozonated blood into the donor, the ozone therapy approach involves blood cells and the endothelium which, by transferring the ozone messengers to billions of cells will generate a therapeutic effect (Bocci et al. 2009). Thus, in spite of a common prejudice, single ozone doses can be therapeutically used in selected human diseases without any toxicity or side effects. Moreover, the versatility and amplitude of beneficial effect of ozone applications have become evident in orthopedics (Re et al. 2008b), pain control (Re et al. 2008a), diabetes (Martinez-Sanchez et al. 2005) cutaneous and mucosal infections (Bocci et al. 2009) as well as in dentistry (Loncar et al. 2009).

The present article is focus on the issue of if, and to what extent, hormesis mechanisms mediate health benefits of ozone. Examples of human, animal and cell culture data supporting hormetric features of biological actions of ozone, together with findings that elucidate specific adaptive stress response signaling pathways that mediate ozone-induced hormesis.

**THE PARADOX OF OZONE**

That ozone, one of the most potent oxidizer, may induce an antioxidant response capable of reversing a chronic oxidative stress at first sight seems a paradoxical concept. However, this concept has become common in the animal and vegetal kingdoms (Kangasjarvi et al. 1994, Desikan et al. 2000, Ranieri et al. 2000). Any change of the external or internal environment disturbs cell homeostasis, but if the stress is tolerable, or carefully calibrated in intensity, the cell or the organism can adapt to it and survive. If it is excessive or the cell is already damaged, the cell programmes its own death. Stresses include hyperthermia, hyperoxia, ischemia, hypoglycemia, pH modifications, radiation, very likely mental and hormonal derangement and chronic infections which imply an excessive Reactive Oxygen Species (ROS) and Lipid Hydroperoxides (LH) production. Obviously, ozone has to be included and the phenomenon of ozone tolerance is now well-known. The concept of ischemic preconditioning for the heart, which after undergoing a brief, non-lethal period of ischemia can become resistant to infarction from a subsequent ischemic insult was pioneered by (Murry et al. 1986). The concept of oxidative preconditioning was
enunciated first in 1998 (Leon et al. 1998) and has been successively also well-demonstrated (Candelario-Jalil et al. 2001, Ajamieh et al. 2004, Ajamieh et al. 2005). Therefore, it is of interest that small amounts of ROS and LH can elicit the up-regulation of antioxidant enzymes on the basis of the phenomenon described under the term of *hormesis* (Calabrese 2008). On the basis of this phenomenon that says: the exposure of an organism to a low level of an agent, harmful at high levels, induces an adaptive and beneficial response (Stark 2008), it has been postulated that LH, by acting as long-distance messengers, can transmit to all organs the information of an acute oxidative stress (Bocci 2002).

Typical sigmoid shape observed in pharmacology respond to a defined mechanism of interaction drug/receptor. However, when the action mechanisms is more complex, the dose effect behavior became more complex (Fig. 1). The ozone treatment is now envisaged as a transitory and miniaturized oxidative stress resulting in a sort of therapeutic “shock” for the ailing organism. Ozone acting as a pro-drug, realizes this shock because generates a number of messengers able to reach all cells in the organism. Sub-micromolar levels of LH and other bio-molecules oxidation byproducts act as key mediators and in still responsive cells may activate a sequence of biochemical mechanisms able to reactivate gene expression leading to a renewed synthesis of Heat Shock Protein (HSP) and antioxidant enzymes (Fig. 2). If the disease has gone too far, cells become anergic and are unable to respond to the treatment. Indeed, it was observed that after intensive chemotherapy, pre-terminal cancer patients do not improve with ozone therapy. That is also the reason why always it should be start using low ozone concentrations just above the threshold level to better achieve the ozone tolerance and in-line with the old concept “start low, go slow”.

The variability of the clinical responsiveness of the patients introduces further difficulties in the set up of standardized studies. Therapeutic dose of ozone can up-regulate the synthesis of antioxidant enzymes and hemoxygenase 1 (Ross and Hu 2003, Bocci 2006). In all case the synthesis of enzymes need the presence of micronutrients. Indeed, recent advances on the ozone effects, suggest the key role of trace elements. Studies are in progress to evaluate the role of Se, Mn, Cu, Zn and other essential elements for the enzymatic activity of Superoxide Dismutase (SOD) and other enzymes induced by ozone. A dietary insufficiency or impairment either in food supply or metabolic pathways may play a negative role in the efficacy of the ozone treatment. Another origin of the variability of the clinical responsiveness to O$_2$/O$_3$ therapy comes from the lack of an appropriate clinical diagnostic of the redox status. Patients with severe disruption in the redox systems may not respond to the oxidant stimulus of ozone (Re et al. 2008a).
DOSE-RESPONSE IN THE TOXICITY OF OZONE

The toxicological effects of $O_3$ take place in subject inhaling ozonated air because the respiratory tract lining fluids of the mucosa have a very
weak buffering and antioxidant capacity. It must be emphasized that the toxicity of ozone for the respiratory tract cannot be extrapolated to blood owing to quite different anatomical, biochemical and metabolic conditions (Bocci 2006). After prolonged breathing of air contaminated with ozone, the seriousness of symptoms and pathological changes are in relation to the ozone concentration and the exposure time (Table 1). It is important to note that under clinical practice ozone is not given by inhalation and is safe within the therapeutic windows.

For a single toxicant like O₃, it is possible to explore the nature of exposure-response for short-term exposures and acute responses in humans under controlled laboratory conditions and in field studies (Lippmann and Schlesinger 2000). Furthermore, O₃ is a very strong oxidant and has a limited range of reactions with lung cells and lung surface fluids. There are many variations in human responses to O₃ exposure that remain poorly understood. Well documented responses to short-term exposures include: deficits in pulmonary function; increased respiratory symptoms; increased hospital admissions for respiratory diseases such as

FIGURE 2. Main biological effects elicited during exposure to ozone. This representation correspond with the systemic exposition of the drug (non for inhaled O₃). Box A correspond with immediate effect of O₃ treatment and box B with surrogate effects.
Ozone as hormetin

Studies involving 6.6 h of O₃ exposure in chambers at 0.16, 0.20 and 0.24 µg/L with moderate exercise in young adult volunteers, the average deficit in forced expiratory volume in one second (FEV1) grew steeper with each hour of exposure in a concentration related fashion (Fig. 3). However, when examining the variability of responses among those exposed in the same protocol it can be seen that some individuals exposed at 0.20 µg/L and 0.24 µg/L had very little, if any, response, while others had FEV1 reduced by half. It has also been shown that individuals have reproducible responses (Horstman et al. 1990). Susceptibility factors that could account for such extreme variability have been sought, but not yet found, other than that the FEV1 response varies with age. It is also of interest that other health effects endpoints were measured in some of the volunteers who were exposed to O₃ in the series of 6.6 h studies. It has been reported that a significant inflammatory response, as indicated by increased levels of neutrophils, was also observed in bronchohalveolar lavage fluid from subjects exposed to either 0.16 µg/L or 0.24 µg/L O₃ for 6.6 h. Exposition 6.6 h at 0.20 µg/L O₃ produced a 3.8-fold increase in neutrophils at 18 h after the exposure, whereas the 6.6 h at 0.16 µg/L produced a 2.1-fold increase. The amounts of O₃ inhaled in the 0.16 µg/L and 0.20 µg/L protocols were about 2.0 µg and 2.5 µg, and about 3.6 µg in the 0.8 µg/L protocol. Thus the effect of concentration was apparently somewhat greater than that of exposure duration. The signif-

### TABLE 1. Toxic effects of gaseous ozone in humans, when inhaling.

| O₃ concentrations in air, µg/L | Toxic effects |
|-------------------------------|---------------|
| 0.2                           | Lachrymation and irritation of upper respiratory airways |
| 2.0-4.0                       | Rhinitis, cough, headache, occasionally nausea and retching |
| 4.0-10.0 (10-20 min)          | Progressively increasing dyspnoea, bronchial spasm, retrosternal pain |
| 10.0 (60 min)                 | Acute pulmonary oedema and occasionally respiratory paralysis |
| 20.0                          | Death within 4 h |
| 100.0                         | Death within minutes |

Note: Our odour perception threshold for ozone is about 0.02 µg/L.
significant increase in neutrophils at a concentration as low as 0.16 µg/L suggests that lung inflammation from inhaled O₃ has no threshold down to ambient background O₃ levels (Devlin et al. 1991).

Other studies indicate that the inflammatory process caused by O₃ exposure is promptly initiated (Seltzer et al. 1986) and persists for at least 18 h (Koren et al. 1989). The time course of this inflammatory response and the O₃ exposures necessary to initiate it, however, have not yet been fully elucidated. Furthermore, these studies demonstrate that cells and enzymes capable of causing damage to pulmonary tissues were increased, and the proteins that play a role in the fibrotic and fibrinolytic processes were elevated as a result of O₃ exposure. Of further interest is the fact that different individuals exhibit different kinds of responses to these controlled O₃ exposures. The levels of O₃-induced symptoms and respiratory tract injury and inflammation have not been closely correlated with the levels of decrements seen for FEV1 or other functional parameters.

In terms of epidemiological studies focused on associations between community air concentrations to O₃ and health effects there is little evidence for non-linearity. For instance it has been demonstrated a linear relationship between daily O₃ 1 h peak levels and hospital admissions for respiratory diseases (Burnett et al. 1994). In addition, it has been shown that effect size for respiratory hospital admissions varies with age, as noted earlier for controlled chamber exposure studies, with some differences for the elderly (age > 65 years) according to socioeconomic status (Yang et al. 2003).

In summary, for a well-defined pollutant such as O₃, which tends to be relatively uniform in outdoor concentration over broad geographic

**FIGURE 3.** Mean forced expiratory volume in one second (FEV1) after each 50 min of exercise during exposures to O₃ at 0 (open circles), 0.16 µg/L (squares), 0.2 µg/L (triangles), and 0.24 µg/L (solid circles). Asterisks indicate significant reduction in FEV1 from corresponding values at 0 µg/L. From (Horstman et al. 1990).
areas, it is difficult to determine if non-linear exposure-response relationships for general populations. Thus, the assumption of a linear, non-threshold relationship when establishing ambient air quality standards or issuing public health advisories appears to be reasonable and prudent (Lippmann 2005).

**DOSE-RESPONSE IN THE PHARMACOLOGICAL ACTION OF OZONE**

The hormetic response, oxidative preconditioning or the adaptation to the chronic oxidative stress, during ozone therapy regimen, has been now demonstrated experimentally (Bocci 1996, Al-Dalain et al. 2001, Martinez-Sanchez et al. 2005). The increased synthesis of enzymes such as SOD, GPx, GR and CAT has been repeatedly determined in experimental animals and in patients (Bocci 2008). It has been demonstrated that 4-hydroxynonenal (4-HNE), by inducing the expression of γ-glutamate cysteine ligase, causes an intracellular increase of reduced glutathione (GSH), which plays a key role in antioxidant defense (Iles and Liu 2005). Furthermore LH induce oxidative stress proteins, one of which is hemeoxygenase I (HO-1 or HSP-32) which, after breaking down the heme molecule, delivers very useful compounds such as CO and bilirubin (Abraham and Kappas 2008). Bilirubin is a significant lipophilic antioxidant and a trace of CO cooperates with NO in regulating vasodilation by activating cyclic GMP. Fe$^{2+}$ is promptly chelated by the up-regulated synthesis of ferritin (Balla et al. 1992). The bone marrow is particularly relevant because it can up-regulate antioxidant enzymes during erythropoiesis and may allow the release of staminal cells for possibly regenerating infarcted organs (Bocci et al. 2009).

The induction of HO-1 after an oxidative stress has been described in thousands of papers as one of the most important antioxidant defense and protective enzyme. Both mild ozone inhalation and ozonated plasma induce HSP-70 (Bocci et al. 2007). When ozone is judiciously used in small doses, can become a useful drug able to correct an otherwise irreversible state of oxidative stress. There are serious pathologies such as chronic infections, neurodegenerative and autoimmune diseases in which a vicious imbalance between overproduced oxidants and depleted antioxidant defenses become established and lead to death (Bocci et al. 2009).

Ozone can be administered with great flexibility but it should not be injected intravenously as a gas because of the risk of provoking oxygen embolism, given the fact that the gas mixture contains always no less than 95% oxygen. So far the most advanced and reliable approach has been the major ozonated Autohaemotherapy (AHT) because, on the basis of the patient’s body weight, a predetermined volume of blood (50-270 mL) can be exposed to an equal volume of gas ($O_2/O_3$) in a stoichiometric fashion, with the ozone concentration precisely determined. Moreover,
the therapeutic modalities, until now restricted to major AHT and to rectal insufflation of gas, have been extended: they include the quasi-total body exposure to $O_2/O_3$ and the extracorporeal blood circulation against $O_2/O_3$. Clearly, today we can select the most suitable method for different pathologies, their stage and the patient’s condition. A discussion on its own is needed for the minor AHT, which basically consists of withdrawing 3-10 mL of blood to be immediately and vigorously mixed for 1 min with an equal volume of $O_2/O_3$ at an ozone concentration ranging between 10 µg/mL and 60 µg/mL of gas per mL. The strongly oxidized blood, including the foam, is promptly injected into the gluteus muscle without the need of any anesthetic.

On the basis of experimental data obtained during the last decade (Bocci 2007) and on the average antioxidant capacity of human blood, it has determined the so-called therapeutic window, that is the range of ozone concentrations (expressed as µg/mL of gas per mL of blood) within which ozone can exert therapeutic effects without toxicity with regard to major AHT. The range is surprisingly wide: 10-15 µg/mL as a minimum and 80 µg/mL as a maximum. Above 90 µg/mL, an incipient hemolysis (4–5%) warns about toxicity. The threshold level varies between 15 µg/mL and 20 µg/mL, depending upon the individual antioxidant capacity.

It is clear that the ozone oxidative activity is efficiently counteracted by the wealth of plasmatic and intracellular antioxidants so that an ozone concentration of 5-10 µg/mL per mL of blood is practically neutralized: only a trace of ROS and LH become detectable and therefore, at this very low level of ozonation, AHT may only have a placebo effect. Ozone doses are always apply by the physician using the strategy start low go slow and, depending on the stage of the disease and the patient’s condition, usually scale up the concentrations from 15, then 20, 30 and 40 µg/mL, and more when necessary, during the 1st, 2nd, 3rd and 4th weeks, respectively. By using this strategy, after many thousands of autotransfusions, therapist have never recorded any acute or chronic toxicity.

Ozone doses, within the therapeutic range (10-80 µg/mL of gas per mL of blood), are partly neutralized by well-known sacrificial reactions e.g.: ascorbic, uric acids, thiol compounds with -SH groups such as cysteine, GSH and albumin. Albumin-SH groups undergo oxidation and in fact albumin is considered the main sacrificial molecule and surely prevents lipoprotein damage. The small amount of oxidized albumin is rapidly removed from the circulation and does not affect the plasma level. There are evidence that $O_2/O_3$ behaves similarly when this gas mixture comes in contact with a moist human skin and the rabbit colon–rectal mucosa (Bocci et al. 2000): ozone dissolves immediately in the water overlaying the epithelium and reacts with sebum, mucoproteins, feces and any other biomolecules present in the liquid film generating hydrogen peroxide ($H_2O_2$), possibly other ROS and lipid ozonation products
(LOP). Only LOP are absorbed via lymphatics and venous capillaries and reach first the liver and then enter into the general circulation where these have been measured.

After the instantaneous reactions of the dissolved ozone with biomolecules (antioxidants and polyunsaturated fatty acid), the newly formed \( \text{H}_2\text{O}_2 \) and a heterogeneous number of LOP represent the chemical mediators of the totally extinct ozone (Bocci et al. 1999). The behavior and pharmacodynamic of \( \text{H}_2\text{O}_2 \) have been ascertained: the initial formation of a gradient between plasma and intracellular water allows its entrance into the erythrocytes and lymphocytes but its concentration remains around a few micromoles because it is quickly reduced to \( \text{H}_2\text{O} \) by free GSH, catalase and GPx (Bocci and Aldinucci 2005). Its half-life is of about 1 s and yet its intracellular concentration is critical because, in order to activate some biochemical pathways (formation of GSSG with consequent activation of the pentose cycle in the red cell and activation of a tyrosine kinase in lymphocytes), it must reach a critical threshold that, nonetheless, is not cytotoxic. The concept of threshold is physiologically important and means that an ozone dose below 10 \( \mu \text{g/mL} \) of gas per \( \text{mL} \) of blood, in most cases, is biologically ineffective because the ozone dose is totally neutralized by the plasma antioxidants (Bocci 2006). In other words, the concept of a threshold helps to understand that a too low ozone dose may be ineffective (placebo effect) while a dose higher than the therapeutic one can be toxic. The transitory presence of \( \text{H}_2\text{O}_2 \) in the cytoplasm means that it acts as one of the ozone chemical messengers and that its level is critical: it must be above a certain threshold to be effective but not too high to become noxious.

The ozone dose is within a well-defined, experimentally determined range (10-80 \( \mu \text{g/mL} \) or 0.21-1.68 mM per \( \text{mL} \) of blood), there is only a transitory decrease (no more than 25\%) of the potent antioxidant capacity of plasma, fully reconstituted within 20 min owing to the efficiency of the redox system. There is neither damage to erythrocytes: hemolysis is negligible (from 0.4 up to 1.2\%) and methemoglobin remains normal (Travagli et al. 2007) nor to other blood cells. It must be added that ozonated erythrocytes show an improved glycolysis with an increase of ATP and 2,3-DPG levels, which are able to shift the dissociation curve of \( \text{HbO}_2 \) to the right, confirming the observation of an improved delivery of oxygen in peripheral obstructive arterial disease. Extensive data have been reported and shown clearly that a pharmacological ozone dose (most frequently ranges between 10 and 40 \( \mu \text{g/mL} \) or 0.21 and 0.84 mM per \( \text{mL} \) of blood) triggers an acute and precisely calculated oxidative stress able to activate several biological processes.

It may be said that the adaptive response induced by ROS, LH products, and physical stimuli is a general phenomenon endowed inherently to aerobic organisms during evolution to cope with oxidative stress. The
adaptive response to low levels of radiation has been known for many years as hormetic effects (Renn 2008). The effects of concentrations of \( \text{H}_2\text{O}_2 \) on cellular response may be also important in this context. A human being is prepared to respond to the oxidative stress and capable of coping with it by inducing an adaptive response to maintain homeostasis or even to stimulate ourselves to promote a healthy state, both physically and emotionally (Niki 2009).

**Induction of adaptive response by lipid hidroperoxides products**

It has been shown that LH products exert various biological effects either directly by reacting with proteins, enzymes, and nucleic acids or indirectly through receptor-mediated pathways (Noguchi 2008). LH alters chemical characteristics and physical organization of cellular membranes to induce functional loss and modifies lipoproteins to proatherogenic and proinflammatory forms. LH products are assumed to be pathogenic and contribute to the etiology of various diseases, including neurodegenerative diseases (Sultana et al. 2006). Some of the LH products are chemically reactive and readily react with proteins, sugars, and DNA. In particular, the \( \alpha, \beta \)-unsaturated carbonyl compounds such as HNE, acrolein, and 15d-PGJ2 are highly reactive and react directly with thiol groups of cysteine, histidine, and lysine residues in proteins and peptides to form stable adducts. The reactivity of thiols is governed by pKa and also by steric factors, allowing for biological specificity to be conferred to individual proteins. These adducts have been detected in various tissues and peripheral blood and considered to be pathogenic. In fact, the levels of these adducts quantified by using immunoassays have been associated with the level of oxidative damage and diseases (Niki 2009).

Many studies have shown that aldehydes react with nucleic acid bases to give exocyclic adducts with a five-member ring (etheno adducts) and a six-member ring (propane adducts) (Blair 2008). These products are accepted to contribute to the mutagenic and carcinogenic effects associated with oxidative stress. DNA repair enzymes, such as those involved in base excision repair, are able to excise the DNA adducts so that they can be excreted in urine. Thus, the analysis of urinary levels of DNA adducts may be applied for monitoring the oxidative DNA damage in vivo and used as biomarkers for cancer risk assessment of carcinogenic chemicals and environmental agents and also for the assessment of beneficial effects of antioxidants. Oxidative modification of nucleic acids has been also implicated in neuro-degeneration (Pratico 2008).

It is known that LH products, both chemically reactive and stable compounds, exert cytotoxic effects. Reactive unsaturated carbonyl compounds, lipid hydroperoxides, hydroxyl-fatty acids, hydroxycholesterols, ketocholesterol, epoxycholesterol, and lysoPC all induce cell death (Piga et al. 2007). At the same time, it has been shown over recent years that
LH products at low concentrations activate the cellular cytoprotective signaling pathways and increase antioxidant capacity (Gutierrez et al. 2006). It is becoming increasingly clear that the pretreatment of cells with these LH products at sublethal level enhances the cell tolerance against forthcoming oxidative stress induced by ROS and LH products. Ozone at therapeutic doses generate LH that induce antioxidant mechanism, however toxic doses of ozone generate toxic concentration of LH.

The adaptive response to low levels of LH products requires the transcriptional regulations of antioxidant genes, including enzymes related to glutathione synthesis, GST, NADPH dehydrogenase quinone 1 (NQO1), thioredoxin reductase 1 (TR1), and HO-1. It was shown that low levels of LDL oxidized by copper, SIN-1, and metmyoglobin was cytoprotective through mechanisms by which intracellular glutathione was increased. The increased protein levels of the subunit of glutamate-cysteine ligase (GCL), a rate-limiting enzyme in glutathione synthesis, and increased activity of GCL were observed. It was found also that mildly oxidized LDL induced HO-1 (Ishikawa et al. 1997). This mechanism will be explain the effect of $O_2/O_3$ as inductor of HO-1.

The important issue is the physiological significance of the action and role of LH and its products as cellular signaling messengers in vivo. The experimental evidence supporting the potential role of LH products as a regulator and modulator of cellular signaling and gene expression has been accumulated in this decade, mainly in the studies using cultured cells. Many reports suggest the involvement of LH products in enzyme modulation, cell signaling, and gene expression. The concentrations of LH products are one of the important factors that determine their effects. The physiological concentrations of LH products in human blood are below 1 μM. On the other hand, most of the LH products exerted cytotoxicity above 10 μM in the cell culture systems and they induced adaptive responses above 1 μM (Niki 2009). Acute application of $O_2/O_3$ increase the concentration of LH, rapidly buffering by the antioxidant system, but when the acute stimulus is repeated induce an antioxidant response (e.g. increment in SOD enzyme) Fig. 4 (Bocci 2005).

**Adaptive response of ozone on cutaneous tissues**

Recent literature points out that although a long cutaneous exposure to $O_3$ is certainly deleterious, transitory exposure at low and precisely controlled $O_3$ concentrations can have useful effects (Valacchi et al. 2005). In addition, the well known activity of $O_3$ as a potent disinfectant and oxygen donor has been also studied for therapeutic use. Two approaches have been described. The first consists of a quasi-total body exposure in a thermostatically controlled cabin or local bag application. This treatment has proved to be useful in patients with chronic limb ischaemia. The second approach is based on the topical application of
ozonated oil in several kinds of skin infection (from soreness to diabetic ulcers, burns, traumatic and surgical wounds, abscesses and skin reactions after radiotherapy). There are observed a striking cleansing effect with improved oxygenation and enhanced healing of these conditions. It is now clear that, on the skin, O₃, like other drugs, poisons and radiation, can display either a damaging effect from a long exposure or a beneficial effect after a brief exposure to O₂ and O₃ or to the application of ozonated oil to chronic wounds (Valacchi et al. 2005).

Within the skin, the stratum corneum (SC) has been identified as the main target of oxidative damage. The effects of O₃ at toxic levels of exposition on cutaneous tissues have recently been evaluated using a murine model. While no effect of O₃ on endogenous antioxidants was observed in full thickness skin (dermis, epidermis and SC), it could be demonstrated that a single high dose of O₃ (10 µg/g 2 h) significantly depleted topically applied vitamin E (Thiele et al. 1997b). When the skin was separated into upper epidermis, lower epidermis and papillary dermis, and dermis, O₃ induced a significant depletion of tocopherols and ascorbate followed by an increase in the lipid peroxidation measured as malondialdehyde (MDA) content. O₃ is known to react readily with biomolecules and does not penetrate through the cells; therefore, it was hypothesized that O₃ mainly reacts within the SC (Thiele et al. 2001). This hypothesis was supported by further experiments, where hairless mice were exposed to varying levels of O₃ for 2 h. Depletion of SC lipophilic (tocopherols) as well as hydrophilic (ascorbate, urate, GSH) antioxidants was detected upon O₃ exposure and it was accompanied by a rise in lipid peroxidation.
as an indicator of increased oxidative stress (Thiele et al. 1997a). Furthermore, a recent study has shown the increase of 4-HNE content in murine SC using both Western blot and immunohistochemical analysis (Valacchi et al. 2002).

To evaluate the effect on cutaneous tissues of O₃ exposure, hairless mice were exposed for 6 days to 0.8 µg/g for 6 h/day) and the homogenized whole skin was analyzed. Under these experimental conditions an increase of proinflammatory marker cyclooxygenase-2 (COX-2) expression was detected confirming the role that O₃ can play in skin inflammation. This induction was accompanied by an increase in the protein level of HO-1, confirming that HSP are sensitive markers of O₃-induced stress in cutaneous tissues (Valacchi et al. 2003).

As HSP are involved in cell proliferation, apoptosis and inflammatory response, O₃-mediated HSP induction can affect normal skin physiology. Thus, HSP might provide an adaptive cellular response to O₃; enhancing the expression of HSP might turn out to be a new way to deal with the immediate and long-term consequences of O₃ exposure. A prerequisite for the utilization of this concept is the development of nontoxic HSP inducers and their evaluation for clinical efficacy and safety. Furthermore, increased levels of metalloproteinase-9 (MMP-9; mRNA and activity) was observed after O₃ exposure (0.8 µg/g for 6 h/day) (Valacchi et al. 2004). MMP have been associated with the degradation of the basal membrane and play important roles in wound healing and in tumor development. In addition, MMP may contribute to the enhancement of skin ageing and formation of wrinkles (Sato et al. 2001).

It is not surprising that exposure of the skin to toxic doses of O₃ can trigger several biochemical pathways leading to inflammation and affecting skin biology. On the other hand basic and clinical work developed during the last 20 years has shown that transient treatment and small O₃ doses can reanimate useful body functions and might display therapeutic activity (Bocci 2005).

The dual behavior of O₃ fits well the concept of *hormesis* that says the exposure of a living organism to a very low level of an agent harmful at high or chronic levels induces an adaptive and beneficial response (Calabrese 2008). The paradoxical concept that ozone eventually induces an antioxidant response capable of reversing a chronic oxidative stress is already supported by findings of an increased level of antioxidant enzymes during ozone therapy. Those facts can include ozone as a hormetin.

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