Ozone as an oxidant and its influence on free radical activity and antioxidant levels in the human environment in disease and health

Thomas Marshall-Manifold
Wimbledon Clinic of Natural Medicine, London, UK.

Abstract

A number of free radical species fulfil physiologically important roles within the body, for example, superoxide and nitric oxide function as second messengers. However, free radical levels in the body must be carefully controlled as they are highly reactive and can cause tissue destruction.
Antioxidants help regulate and control the levels of free radicals at the required physiological concentrations. When the production of free radicals and their removal by the antioxidant system becomes unbalanced, tissue damage and disease can occur.
The use of ozone as a therapeutic modality to counteract the disease process in the body would seem to be paradoxical at the least, ozone being an oxidant, and by definition a procurer of free radical activity.
This paper reports the effect of ozone on both free radical and antioxidant levels in the blood of subjects both in disease and health before, during, and after ozone therapy

Introduction

Extensive research in the field of free radicals and ROS has linked them with a wide range of chronic and acute diseases. Some of the major diseases have been discussed in this brochure, however the list is growing daily and includes other diseases such as HIV, genetic mutations, motor neurone disease, hypocuprosis, adult respiratory distress syndrome (ARDS).
A number of free radical species fulfil physiologically-important roles within the body, for example, superoxide and nitric oxide function as second messengers. However, free radical levels in the body must be carefully controlled as they are highly reactive and can cause tissue destruction. Antioxidants help regulate and control the levels of free radicals at the required physiological concentrations. When the production of free radicals and their removal by the antioxidant system becomes unbalanced, tissue damage and disease can occur.

1: Primary healthcare screening – to detect and identify individuals with lowered antioxidants defences who may be at greater risk of developing ROS-induced disease. People identified as being “at risk” could be targeted for intervention before presentation of overt disease, thus reducing healthcare costs. Recent studies have demonstrated that individuals with a family history of heart disease, but without obvious disease themselves, have lowered antioxidant defences compared to individuals with no family history of heart disease.
2: Diagnostic monitoring – of diseased patients for the assessment of antioxidant levels. This assists the clinician in determining optimal treatment and possible outcome. Reduction in antioxidant defences has been correlated with poor outcome in acute myocardial infarction.

Free Radicals

The human body is constantly under attack from free radicals. Free radicals are highly reactive molecules generated by the biochemical redox reactions that occur as part of normal cell metabolism, and by exposure to environmental factors such as UV light, cigarette smoke, environmental pollutants and gamma radiation. Some toxic compounds can result in the production of free radicals which include anti-cancer drugs, anaesthetics, analgesics, etc.

The main free radical species which occur in the human body include:

Superoxide radical (°O2') Hydroxyl radical (OH°)
Nitric oxide radical (NO°) Peroxyl radical (ROO°)

Once formed, free radicals attack cell structures within the body. As a result, free radicals have been implicated in numerous diseases, examples of which are listed below:

- Atherosclerosis • AIDS • Cancer • Inflammatory bowel disease • Diabetes
- Central nervous system disorders•Respiratory disease•Parkinson's disease
- Liver damage • Motor neurone disease • Rheumatoid arthritis • Cataracts
- Conditions associated with premature birth

The antioxidant system

In healthy individuals, the antioxidant system defends tissues against free radical attack. Three classes of antioxidants have been identified:

- **Primary antioxidants** (e.g. Superoxide Dismutase, Glutathione Peroxidase, Caeruloplasmin, Transferrin, Ferritin) prevent the formation of new free radical species.
- **Secondary antioxidants** (e.g. Vitamin E, Vitamin C, B-carotene, Uric Acid, Bilirubin, Albumin) remove newly formed free radicals before they can initiate chain reactions. These chain reactions can lead to cell damage and further free radical formation.
- **Tertiary antioxidants** (e.g. DNA repair enzymes, Methionine Sulphoxide Reductase) repair cell structures damaged by free radical attack.

Deficiencies in the antioxidant system can develop for a number of reasons.

- Low intake of dietary antioxidants. • Renal dialysis. • Total parenteral nutrition
- Diseases that reduce the absorption of antioxidant nutrients from food (e.g. Crohn's Disease).

In these situations the antioxidant system struggles to protect the body from free radical attack and, as a result, the risk of free radical-mediated disease increases.

FRAS – Free radical Analytical System – d – Roms Test (1) (2)

This test is based on spectophotometer studies on increases in red colour intensity after the addition of a small amount of human blood to a solution of N, N-diethyl-para-phenilendiamine (chromogen) and buffered at PH 4.8. Such colouring is attributed to the formation of the cation radical of the amine which forms due to alcoxylic and peroxylic radicals. These derive from the reaction of the Fe2 and Fe3 ions released by protein in acidic conditions.
The test allows for the determination of free radicals using a single drop of capillary blood. It permits a precise evaluation of plasma oxidation capacity. This is primarily determined through the process of oxygen releasing free radicals. The production of these radicals is a normal event during metabolic processes in living beings. However,
In conditions of oxide stress it has been demonstrated that free radicals can cause even serious damages by modifying the working order and structures of living tissue. The d-ROMs test is considered a simple rapid and accurate method to monitor the free radical status and the effects of therapeutic attempts to alter that status.
The test may be carried out using either whole blood or capillary blood. The results are expressed in conventional arbitrary units U.CARR, where a reference interval of 250 to 300 U.CARR. was obtained from 4000 tests on healthy persons and values ranging from 300 to 320 U.CARR. were interpreted as borderline. 2000 patients with diverse pathologies were tested with results in the range 320 to 340 U.CARR.

The following are reference ranges for test evaluation:

250 – 300 U.CARR.	Normal
320 – 340 U.CARR.	Slight Oxide Stress
340 – 400 U.CARR.	Oxide Stress
400 – 500 U.CARR	High Oxide Stress
Above 500 U.CARR.	Very High Oxide Stress

Ozone as a Mediator in Conditions of Oxide Stress

The mechanism by which ozone, as an oxidant, could positively influence levels of oxide stress in the body is one of the arguments made against its therapeutic use. Consideration of the process of ozone within the body is important to understand how this seeming paradox could work.
Ozone increases the glycolysis rate in red blood cells, this in turn leads to the activation of 2,3-diphosphoglycerate, which in turn causes increased oxygen release into tissue. Ozone also stimulates the production of the antioxidants glutathione peroxidase, catalase, and superoxide dismutase and activates the Kreb cycle by enhancing oxidative carboxylation of pyruvate, stimulating production of ATP. Ozone Causes reduction in NADH and induces oxidation of cyochrome C. Prostacyline. Ozone reacts with unsaturated fatty acids in cellular membranes to form hydro peroxides. The result of which has a synergistic effect with cellular formed H2O2.
The beneficial products of such lipid peroxidation produces alkoxyl and peroxyl radicals, single oxygen, ozonides, carbonides, carbonyls, alkanes and alkenes.

Ozone oxidises the outer lipid layer of malignant cells and destroys them through cell lysis. Phagocytes produce H2O2 and hydroxyl to kill bacteria and virus. The development of hydroxyl by killer cells is critical to there cytotoxic capability.
Ozone stimulates conversion of L-Arginine to citrulline, nitrite and nitrate by the process of phagocytosis
Studies on the biological effects of ozone have shown that the induction of tumor necrosis factor (TNF-alpha) on human leucocytes is however dependent on the concentration of the applied ozone, where high ozone concentrations were required to be effective in order to release factors with antiviral and immunomodulatory activity by leucocytes. (3)
It has also been found that the as erythrocytes constitute the bulk of blood cells and represent the main target of ozone they have been taken as a useful marker of its oxidative activity. It appears that transient exposure (30 sec) of blood of up to 78 micrograms ozone per ml. Of blood does not depress the production of cytokines even though there is a slight increase of haemolysis and a small decrease of intracellular reduced glutatathione. In contrast a constant (up to 30 sec) exposure to an ozone flux or a high ozone concentration (108 micrograms/ml) markedly decreases reduced glutathione levels and depresses cytokine production. (4)
A Japanese study investigated the susceptibilities of blood plasma antioxidants and erythrocyte constituents to low levels of ozone after rapid mixing of human whole blood with ozone at 20, 40, 60, and 100 microg/ml blood. Ascorbic acid, uric acid, and alpha-tocopherol in plasma decreased as ozone increased, but bilirubin was unaffected. The content of thiobarbituric acid-reactive substances in plasma was increased by ozone. However, the content of thiobarbituric acid-reactive substances and alpha-tocopherol in the erythrocyte membrane was not significantly affected.
No significient changes occurred in the content of methemoglobin, cytoskeleton proteins or erythrocyte enzymes such as Na /K –ATPase, acetycholinesterase, catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase at all the ozone levels tested. A decrease in reduced glutathione in erythrocytes was the only significant change caused by the ozone level used for autohaemotherapy. It is suggested that this may be one of the chemical events responsible for the beneficial effects of ozonated autohaemotherapy. (5)
A further study on the influence of ozone on human red cells, compared other mechanisms of oxidative stress with that of ozone. This study concluded that the hydroperoxide and photosensitisers plus light, are quite dissimilar. The conclusion of a review by Bocci V. entitled “ Biological and clinical effects of ozone. Has ozone therapy a future in medicine?” (6) concludes that although ozone therapy has been used as an alternative medical approach for four decades, it has encountered scepticism, if not outright objection, by orthodox medicine. This prejudice is not unjustified because ozone therapy often has been used without rational basis or appropriate controls. With the advent of precise medical ozone generators, it is now possible to evaluate mechanisms of action and possible toxicity. In contrast with the respiratory tract, human blood exposed to appropriate ozone concentrations is able to tame its strong oxidant properties and neither acute nor chronic side effects have ensued in millions of patients treated with ozonated autohaemotherapy. In an earlier paper the same author concludes that “ An exciting new aspect is that ozone, being a strong oxidiser, can stimulate the increase of cellular anti-oxidant enzymes, eventually inhibiting the oxidative stress” (7)

The protocol followed for the administration of ozone by rectal insufflation to the subjects studied for the purpose of this report

Four subjects were studied who presented with symptom pictures consistent with ME (myalgic encephalitis), systemic yeast overgrowth, IBS (irritable bowel syndrome), or exhibiting high ESR counts with disordered white cell count and differential.
All subjects had undergone one or more of the following clinical assessment:

CDSA (Comprehensive Digestive Stool Analysis) Great Smokies Laboratory, USA.
Health Risk Profile - BioLab – Weymouth St. London. W1N 3FF
Candida Profile – Antibody Assay Laboratories – CA92705 –USA
Analysis of Blood, Saliva, Urine – Bio-Vincent analyser- MedTronik GmbH – Germany

24 hours prior to the administration of ozone the subjects underwent total bowel evacuation following the procedure used for large intestine clinical investigation using Magnesium Citrate 2 sachets (this prerequisite allows for a considerable increase in the amount ozone administered with better absorption on the intestinal wall).

The ozone was generated by the HÄNSLER OZOSAN PM 80 ozone generator (8) using a bar pressure of between 1 to 2 bars, with concentration setting at step 3, for a period of 60 seconds. This setting produces an ozone concentration of 133,500µg O_3 for a bar pressure of 1.5. Time and pressure would vary slightly to patient tolerance. Ozone was “bubbled” through a water filled view glass during the process of administration.

Free radical assessment was made using the d-ROMS Test as previously discussed

(1) 1 hour before ozone administration.
(2) 1 hour after ozone administration
(3) 24 hours following ozone administration.
(4) 1 week following ozone administration.

Test results on 4 patients receiving ozone rectal insufflation over a three week period their free radical activity being monitored with the FRAS d-ROMs test.

Week 1	1 hour before ozone	1 hour after ozone	24 hours after ozone
Patient No.1	286 U.Carr	216 U.Carr	328 U.Carr
Patient No.2	402 U.Carr	271 U.Carr	378 U.Carr
Patient No.3	396 U.Carr	321 U.Carr	362 U.Carr
Patient No.4	430 U.Carr	311 U.Carr	386 U.Carr

Week 2 7 days later	1 hour before ozone	1 hour after ozone	24 hours after ozone
Patient No.1	301 U.Carr	257 U.Carr	289 U.Carr
Patient No.2	379 U.Carr	285 U.Carr	363 U.Carr
Patient No.3	373 U.Carr	302 U.Carr	351 U.Carr
Patient No.4	410 U.Carr	290 U.Carr	311 U.Carr

Week 3 7 days later	1 hour before ozone	1 hour after ozone	24 hours after ozone
Patient No.1	291 U.Carr	263 U.Carr	277 U.Carr
Patient No.2	324 U.Carr	300 U.Carr	328 U.Carr
Patient No.3	359 U.Carr	307 U.Carr	363 U.Carr
Patient No.4	367 U.Carr	322 U.Carr	339 U.Carr

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Conclusion

There is to be expected a normal fluctuation in free radical activity from day to day, or following the stress and strains of daily living. On previous studies, it has been shown that smokers and women on the contraceptive pill exhibited higher levels of free radical activity than the general population. High levels of free radical activity were also encountered following physical “work out” in the gymnasium or following dental treatment. These fluctuations however are generally transient and return to the normal range within 12 to 24 hours.

The striking results of the four patients partaking in the experiment was the virtual immediate reduction in free radical levels following ozone application, even though ozone was not applied directly to the blood as in autohaematherapy (the fact that the patients had total bowel evacuation prior to the ozone has to be taken into account).

An unexpected result was a substantial rise in levels 24 hours following ozone, (especially after the first ozone application) with a subsequent fall in levels 7 days following ozone application (measurements taken 1 hour before the next ozone application).

Although the observed fall and subsequent rise in free radical levels was consistent, the variations became less marked following the second and third ozone application. It was also observed that the overall free radical levels were consistently falling ozone application.

Patients No.2 and No.4 were considered to be the most immune compromised as determined by Health Risk Profile (red cell antioxidant levels) and Bio-Vincent (redox potential) results. It was these patients that showed more striking results following ozone.

Although the trends observed in this limited study suggest the beneficial effect of ozone on the immune system further research on a wider scale especially on the observation of the transient rise in free radical activity 24 hours following ozone application would be welcome. As there is the possibility, that in patients suffering from autoimmune conditions, free radical activity may not always fall following this observed 24 hour transient rise.

One precaution to eliminate this risk would be to prescribe high antioxidant medication for 24 to 48 hours following ozone treatment.

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