

Review

The involvement of free radicals in burn injury: a review

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1. Introduction

Burn traumas, are more commonly induced in tissues by the sudden application of excessive thermal energy or by caustic chemicals. The effects of the injury and its clinical sequelae are widespread and long lasting. It is customary to classify burn injuries etiologically as thermal, electrical or chemical in origin [1]. Thermal burns may be further subdivided into flame burns, flash burns, scald burns and contact burns.

2. Thermal burns

The local thermal wound is the result of heat necrosis of cells. The content of cellular destruction depends upon several factors: the intensity of heat tissue involved. The conductance of the tissue involved determines the rate of dissipation or absorptions of heat and depends upon several factors. These include the peripheral circulation, water content of the tissue, thickness of the skin and its pigmentation, of the presence or absence of external insulting substances such as hair and skin oil. Of these factors perhaps the most important in determining the degree of injury is the peripheral circulation [1]. The rate of blood flow through the heat exposed tissues can be altered rapidly. This mechanism is of major importance in determining the amount of cellular destruction associated with the transfer of heat to the tissue.

3. Electrical burns

Electrical injuries result from the heat produced by the flow of electrical current through the resistance of body tissues. Factors of primary importance in determining the effect of the passage of an electric current through the human body include the type of circuit, voltage, amperage, resistance of the tissues involved, the path of the current through the body and duration of contact with the current. The chief reason for considering these wounds as a category distinct from the more common thermal burn is the volume of tissue that is often involved in high voltage electrical injuries.

4. Chemical burns

A wide variety of agents may be responsible for chemical burns. The majority of chemical agents produce skin destruction through chemical reactions rather than hyperthermic injury [2,3]. Included among these reactions are coagulation of protein by reduction, corrosion, oxidation, formation of salts, poisoning of protoplasm and desiccation. Acids promote collagen denaturation and subsequent degradations [4]. Heat production is often a by-product of the chemical reactions with tissues and may worsen the injury.

5. Burn and wound healing

Burn wound healing is a normal reaction to injury and the formation of scar tissue occurs through a series of cellular and biochemical processes. During cutaneous thermal injury several factors contribute to further tissue damage and important of these are the oxygen free radicals. Oxidative stress contribute to secondary tissue damage and impaired immune functions

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in patients after burn injury [5]. At this point the early intervention of antioxidant therapy will significantly help to restore cell mediated immunity, decrease free radical mediated damage and minimize tissue destruction during extensive burn injury.

The burn wound is thought as the pivotal factor mediating many local and systemic disturbances that characterize burn injury. These include fluid and protein loss, local and systemic sepsis, gross metabolic, endocrine, haematological and immune disturbances [6] and is the major cause of the morbidity and mortality associated with burn injury. The initial local effect of a burn is that of tissue damage and destruction. It is suggested [7] that the local effect could be divided into several zones of differential damage and blood flow as the zone of coagulation, the zone of stasis and the zone of hyperaemia and if the burn wound is large enough the entire body becomes a zone of oedema [8]. The behaviour and progression of lesion in turn determines the amount of subsequent tissue necrosis. Oedema is an abnormal accumulation of fluid in the tissue spaces and serous cavities. This accumulation may be local or general, a distinction which holds great importance.

6. Inflammatory oedema

The reaction of vascularized tissue to local injury is defined as inflammation. The characteristic feature of the inflammatory process in higher life forms is the reaction of blood vessels, leading to the accumulation of fluid and blood cells. Inflammation serves to destroy, dilute or wall off the injurious agent and sets into motion a series of events that heal and reconstitute the damaged tissue [9].

The swelling which is one of the cardinal signs of inflammation is largely due to the oedema. Owing to the action of the irritant the permeability of the capillaries is increased and fluid pours out into the intercellular spaces. This fluid is rich in protein. Perhaps due to the formation of a network of fibrin the fluid is entrapped and unable to move within the tissues [9].

This rapid oedema formation is due to increased microvascular permeability [10,11] vasodilation, increased extravascular osmotic activity [12] and accumulation of leukocytes [13]. These effects are probably the result of a complex interplay between the direct effects of heat on the microcirculation and the action of chemical mediators such as histamine, serotonin, prostoglandins, kinins and complement system byproducts [14]. The pathophysiological mechanisms underlying the development of oedema are mainly due to the inflammatory response [15] which activates cytokines with subsequent stimulation of phagocytic cells that results in the formation of oxygen free radicals leading to lipid peroxidation. The generated oxygen derived

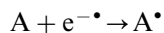
free radicals subsequently cause peroxidation of membrane phospholipids. Polymorphonuclear cell degeneration has been considered as a primary source of oxygen free radical after burn injury. The oxidation of polyunsaturated fatty acids from membrane phospholipids may produce additional free radicals which in turn stimulate further lipid peroxidation and tissue injury [16]. A close relationship between the intensity of lipid peroxidation and complications after burns has been demonstrated [17] and it documents the role of oxygen free radicals leading to lipid peroxidation as a causative agent in the mechanism of local wound response, development of burn shock and distant organ injury [18]. These oxygen free radicals have also been implicated in a variety of ischaemic and inflammatory diseases [19–21] and it is now apparent that oxygen metabolites may in many situations be deleterious to wound healing [22,23].

7. Oxygen derived free radicals

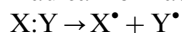
In response to a particular stimuli such as bacteria or bacterial fragments and to non-particulate agents and molecular species such as aggregated IgG, components of IL-1 polymorphs initiate the complex sequence of events termed as phagocytosis [24]. Phagocytosis is an energy dependent process. As the cell moves and enzymes are activated, oxygen is quickly consumed. This response is exceedingly rapid and constitutes a 'respiratory burst'. During oxygen consumption free radicals are produced [25,26]. A free radical can be defined as a molecule with an unpaired electron in the outer orbit and is capable of an independent transient existence [20]. This odd electron is frequently represented by a dot (*) in chemical formulas (e.g. O_2^* , OH^*) and imparts a potent oxidizing and or/reducing potential to the molecular species (i.e. it receives or donates electrons respectively). The major pathway of oxygen metabolism that occurs in man involves the tetravalent reduction of molecular oxygen by the cytochrome oxidase system in the mitochondria, with the resultant production of ATP and water [27]. Only 1–2% of oxygen substrate may 'leak' from the system to become metabolized by univalent reduction [28] producing various oxygen radicals. As such free radicals can be formed in three ways: (1) By the homolytic cleavage of a covalent bond of a normal molecule, with each fragment retaining one of the paired electrons. (2) By the loss of a single electron to a normal molecule. (3) By the addition of a single electron to a normal molecule. The latter, electron transfer, is a far more common process in biological systems than is homolytic fission, which generally requires high energy input from either high temperatures, UV light or ionizing radiation. Free

radicals can be positively charged, negatively charged or electrically neutral. The process by which free radicals and ions are formed are illustrated as follows [29]:

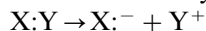
Radical formation by electron transfer:



Radical formation by homolytic fission:

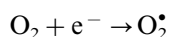


Ion formation by heterolytic fission:

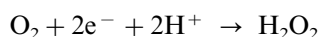


8. Reactive oxygen species

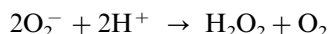
The most important free radicals in biological systems are radical derivatives of oxygen. The addition of one electron to the oxygen molecule results in the superoxide radical:



The addition of two electrons yields hydrogen peroxide:



In the biological systems hydrogen peroxide is generated via the production of superoxide: two superoxide anions can react together to form hydrogen peroxide and oxygen:



Hydrogen peroxide is not a free radical but is also referred to as a reactive oxygen species (ROS) that includes not only oxygen free radicals but also non-radical oxygen derivatives that are involved in oxygen radical production.

Hydrogen peroxide is an important compound in free radical biochemistry because it is easily broken down, particularly in the presence of transition metal ions, to produce the most reactive and damaging of the oxygen free radicals, the hydroxyl radical (OH):



9. Free radicals production in cells

Free radicals are generated deliberately by cells in certain special circumstances because they can be useful entities if constrained and targeted. Some enzymes, e.g. ribonucleotide reductase [30,31], utilize a free radical at their active site in the process of catalysis. Activated phagocytes during phagocytosis generate superoxide as part of their bactericidal role [32]. Though the free radicals are produced only at the interface of phagocyte plasma membrane and bacterium, some leakage of superoxide, hydrogen peroxide and other reactive oxygen species is inevitable. Under normal circumstances, the major source of free radicals in cells is electron

'leakage' from electron transport chains such as those in mitochondria and in endoplasmic reticulum, to molecular oxygen generating superoxide. Another source of superoxide in animal cells is the autooxidation of certain compounds including ascorbic acid (vitamin C), thiols (e.g. glutathione, cysteine), adrenaline and flavin co-enzymes. These autooxidation reactions are greatly enhanced by the involvement of transition metal ions. This accidental production of free radicals is kept to a minimum by the high efficiency of enzyme-mediated electron transfer and by keeping metal ions highly sequestered [29].

Increased intracellular generation of free radicals has been implicated in:

1. Hyperoxygenation syndromes such as hyperbaric oxygen toxicity from respirator dependency.
2. Ischemia reperfusion syndrome.
3. Ageing.
4. Drug-induced haemolytic anaemias.
5. Vitamin E and vitamin A deficiency.
6. Chemical-induced tissue injury (carbon tetrachloride, paraquat, chemotherapeutic agents, or carcinogens).

10. Cellular damage by free radicals

Almost all the major classes of biomolecules may be attacked by free radicals but lipids are probably the most susceptible. Cell membranes are rich sources of polyunsaturated fatty acids (PUFAs), which are readily attacked by oxidising radicals. The oxidative destruction of PUFAs known as lipid peroxidation, is particularly damaging because it proceeds as a self-perpetuating chain reaction [33]. The superoxide radical may be toxic to cells by direct attack at the molecular level or indirectly by generating secondary radicals such as hydroxyl [27]. In addition, the superoxide radical may be secondarily cytotoxic by evolving tissue responses such as initiating the inflammatory response [34]. Hydroxyl radicals are extremely reactive metabolites capable of reacting with almost all biological substrates [21]. These reactive oxygen metabolites have been implicated in the following responses.

1. Endothelial cell damage with resultant increased vascular permeability for example, activation of C5a in vivo causes neutrophilic aggregation, endothelial cell damage, and increased permeability of lung capillaries [35].
2. Inactivation of antiproteases, such as α_1 -antitrypsin may lead to unopposed protease activity with increased destruction of structural components of tissue such as elastin. Inactivation appears to result from oxidation of methionyl residues on the antiprotease molecule to the sulfoxide with loss of biologic activity [21,36].

- Injury to other cell types (parenchymal cells, red cells, tumour cells) are ascribed to a variety of toxic metabolites.

The oxygen derived radicals cause cellular injury by:

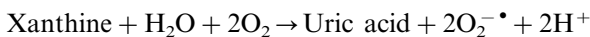
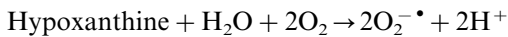
- Degrading hyaluronic acid and collagen.
- Destroying cell membranes through the peroxidation of fatty acids within the phospholipid membrane.
- Disrupting organelle membranes such as those surrounding lysosomes and mitochondria [37].
- Interfering with important protein enzyme systems (e.g. Na^+/K^+ ATPase, $\text{Ca}^{++}/\text{ATP}$ ase, glutamine synthetase, and α -1-proteinase inhibitor) [21].

11. Other sources of reactive oxygen species

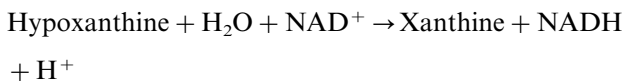
There are other potential sources of oxygen metabolites in vivo, including radiation and chemical injury, and the other two enzymatic systems which seem to be most important clinically at present are xanthine oxidase and NADPH oxidase.

11.1. Xanthine oxidase

It is a well known documented biologic source of oxygen free radicals [38]. Xanthine oxidase in the presence of oxygen, oxidises either hypoxanthine or xanthine to the respective products xanthine or uric acid:



In the above reactions, both the superoxide radical and hydrogen peroxide can be generated. In normal tissues, xanthine oxidase exists as xanthine dehydrogenase which utilizes NAD^+ instead of oxygen as the electron acceptor:



In the reaction no free radicals or oxygen metabolites are generated. In normal tissues, highly reactive oxygen metabolites are not routinely produced. During traumatic conditions, xanthine dehydrogenase is converted to xanthine oxidase and therefore, is capable of generating oxygen free radicals [39]. Additional oxidants can be generated by the xanthine oxidase system that also react in biologic systems, causing a cascade effect [21].

11.2. NADPH dehydrogenase

Neutrophils and other inflammatory cells (macrophages, monocytes, eosinophils) during immune response may activate a plasma membrane-associated NADPH oxidase system capable of oxidizing NADPH

to NAD^+ and in the process generating superoxide radicals [32]:



Unlike the xanthine oxidase system, the NADPH oxidase complex of the neutrophil predominately generates superoxide as compared to hydrogen peroxide [21]. Spontaneous dismutation of the superoxide radical yields hydrogen peroxide and molecular oxygen at physiologic pH.

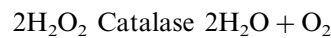
In addition to the generation of oxygen radicals, human neutrophils are able to generate additional oxygen metabolites such as the hypohalous acids [40]. Catalysed by lysosomal myeloperoxidase, hydrogen peroxide oxidises circulating halides to their corresponding hypohalous acids, which are powerful oxidants capable of injuring a variety of biologically sensitive mechanism [21].

12. Defensive mechanism against free radical damage

To prevent the destructive potential of oxygen radicals cells are able to defend themselves by preventing or limiting oxidative injury. These cytoprotective mechanisms known as antioxidant defences include several enzyme systems designed to scavenge oxygen radicals and detoxify them [41]. They exist in both the aqueous and membrane compartments of cells and can be either enzymatic or non-enzymatic antioxidants. One such enzyme, superoxide dismutase (SOD) catalyses the reaction:

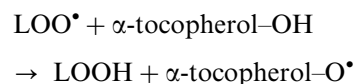


The superoxide radical is reduced to the less reactive hydrogen peroxide and oxygen. Hydrogen peroxide similarly can be reduced to oxygen and water by either catalase or glutathione peroxidase [42]:



By generating O_2 and H_2O , however the generation of hydroxyl radicals can be regulated.

In cell membranes, the best characterised the non-enzymatic antioxidant is α -tocopherol, the major member of vitamin E family [43,44]. This molecule is known as a 'chain-breaking antioxidant' because it functions to intercept lipid peroxyl radicals ($\text{LOO}\cdot$) and so terminate lipid peroxidation chain reactions:



The resultant tocopheroxyl radical is relatively stable and in normal circumstances it is insufficiently reactive to initiate lipid peroxidation. Other lipid soluble chain

breaking antioxidants, such as ubiquinol [45] is under severe experimental study. Ascorbic acid (vitamin C) is an important antioxidant both within cells and in the plasma [46] Uric acid in plasma and glutathione [41] in cell cytosol also possess strong radical scavenging properties. Other antioxidants include β -carotene, dimethyl sulphoxide (DMSO) and ceruloplasmin [20].

13. Assessment of antioxidant status during burns

Thermal injury initiates systemic inflammatory reactions producing burn toxins, oxygen radicals and finally leads to peroxidation. The relationship between the amount of products of oxidative metabolism and natural scavengers of free radicals determines the outcome of local and distant tissue damage and further organ failure in burn injury [47]. During normal body conditions there exists a balance between free radicals and the natural scavengers of the body but during traumatic state the balance is lost and reactive oxygen metabolites outnumber. At this stage therapeutic application of enzymatic and non-enzymatic antioxidants become essential. Increased generation of oxygen free radicals in the extracellular space is seen in inflammatory state in which the relatively low concentrations of SOD and catalase increase the susceptibility of extracellular components to oxygen radical injury and may stimulate chemotaxis for other inflammatory cells [37,48]. The generation of free radicals in the absence of scavenging defences might be the major cause of some acute and chronic diseases [49]. At this stage assessment of enzymatic antioxidants namely superoxide dismutase is carried out by the method of Misra and Fridovich [50]. Catalase can be assessed by the method of Caliborne [51] and the levels of glutathione peroxidase (Gpx) and glutathione-S-transferase (GST) can be monitored by the method of Rotruck et al. [52] and Habig et al. [53], respectively. Along with this the non-enzymatic antioxidants ceruloplasmin, ascorbic acid and tocopherol can be assessed by the methods of Ravin [54], Omaye et al. [55] and Quaipe and Dju [56], respectively. The study on lipid peroxidation product, the malondialdehyde levels by the method of Yagi [57] will give us the extent of free radical damage in the cells. All the above studies will prove to be effective as it forms an index for the therapeutic supplementations of enzymatic and non-enzymatic antioxidants depending on the free radical damage.

14. Therapeutic interventions to improve antioxidant status during burn injury

Recent studies demonstrate that there is a close relationship between a lipid peroxide reaction and sec-

ondary pathological changes following burns [17,58]. Undoubtedly, great attention should be paid to antioxidant therapy during the treatment of burns. Initially attempts were made to prevent the development of oedema with various drugs like antihistamines [59], benzopyrones [60] and antiprostaglandins [61] which were only partially successful. When treated with hydrocortisone the very early post-burn oedema formation was reduced which might partly be due to its membrane-stabilizing influence and partly to a direct effect on the microvasculature, causing a reduction of the vasodilatation [62]. Latha et al. [63] have demonstrated that the enzyme preparation (trypsin:chymotrypsin) when administered orally to the burn patients reduced the oedema formation by maintaining a balanced antioxidant status. It has been shown that conjugated dienes rise in the plasma after thermal injury. These dienes serve as a marker of oxygen-radical mediated tissue injury. Burn patients when received the antioxidant enzyme polyethylene glycol conjugated superoxide dismutase (PEG-SOD) the conjugated dienes formation was prevented and it appeared that 1000 units/kg of SOD was more effective than 500 U/kg in preventing conjugated diene formation [64]. Several studies have shown superoxide dismutase to be effective in various inflammatory models [65,66] possibly by inhibiting the formation of an $O_2^{\cdot-}$ dependent chemotactic factor [37,67]. Hilton [68] reported a decrease in plasma loss after thermal injury as a result of pretreatment with superoxide dismutase and catalase. In skin ulcers or severely inflamed and erosive lesions due to burn wounds lipid peroxides are markedly increased and during this stage topical application of free manganese-SOD or copper zinc-SOD extracted from bovine source was dramatically effective in skin lesions [69]. Two agents that have been extensively used to modify oxidant release after tissue trauma or ischemia are ibuprofen and allopurinol. Ibuprofen, besides being a cyclooxygenase inhibitor, is also known to inhibit the release of oxidants from neutrophils by an undefined mechanism [70]. It was also shown that ibuprofen decreases lipid peroxidation in the lungs after burns [71]. Allopurinol is a xanthine oxidase inhibitor that decreases oxidant release by the ischemia reperfusion pathway [72].

Reduced glutathione protects the cells against free radicals and reactive oxygen species and is synthesized from amino acid precursors, a limiting precursor being the availability of cysteine [73]. *N*-acetyl cysteine has been reported to stimulate the production of glutathione [74–76]. It is reported that water soluble antioxidants glutathione, *N*-acetyl cysteine and vitamin C given orally after burn injury prevent the altered cell energetics, strongly suggesting a cause-and-effect relationship between increased oxidant release with inflammation, decreased antioxidant activity and altered cell

energetics [77]. Cetinkale et al. [78] have investigated the effect of antioxidant therapy on postburn immunosuppression following burn injury in a rat model with well known antioxidants such as allopurinol (50 mg/kg per day), desferrioxamine (15 mg/kg per day), PEG-catalase (1200 U/kg per day), *N*-acetylcysteine (1 mg/kg per day) and vitamin C (0.5 mg/kg per day) administered over 7 days following thermal injury. This study demonstrated that a large burn was profoundly immunosuppressive and early intervention of antioxidant therapy was able to significantly restore cell-mediated immunity.

Burn injuries cause an increase in capillary permeability with subsequent plasma leakage into the interstitial space, resulting in hypovolaemia. Massive fluid resuscitation is required during the first 24 h for extensive burns in order to prevent hypovolaemic shock [79]. Experimental data [80] documents that a continuous intravenous infusion of 340 mg/kg per 24 h of vitamin C therapy beginning at 0.5 h postburn reduces the resuscitation fluid volume requirements by 75% in extensive full thickness burns and maintained better hematocrit and cardiac output values. Another report suggests that high dose vitamin C infusion maintains haemodynamic stability in the presence of reduced resuscitation fluid volume provided vitamin C was administered for a minimum of 8 h post burn [81]. It is also shown that vitamin C therapy diminished early post burn lipid peroxidation [82,83] due to its capacity to scavenge superoxide ($O_2^{\cdot-}$) [84], hydroxyl radicals (OH^{\cdot}) [85] and singlet oxygen ($^1O_2^{\cdot}$) [86].

The protective effect of vitamin E supplementation to severely burned patients showed that it acted as an efficient free radical scavenger and protected the neutrophil function by elevating SOD values, lowering the malonaldehyde values and restored the impairment of the neutrophil function [87]. A further report [88] showed that supplementation of vitamin E (100 mg) given to the burn patients increased blood concentration of vitamin E and decreased the lipid peroxide levels to a certain extent. During burn injury α -tocopherol possesses a protective stabilizing effect on the red blood cell membrane. This stabilizing action is observed when α -tocopherol was injected both before the skin burn and immediately after it [89]. Vitamin E terminates free radical reactions by donating its reactive hydrogen atom at the carbon-6 position to the oxygen radical to form a tocopheroxy radical. The α -tocopherol radical may then react with another oxygen radical to form a stable adduct, with another tocopherol radical to form a dimer, or with ascorbic acid or glutathione to regenerate reduced α -tocopherol. Thus α -tocopherol appears to be a crucial cellular defence agent against loss of normal membrane integrity from oxidative attack as well as from phospholipase activity [90,91]. Since α -tocopherol prevents lipid peroxidation

and has been proven beneficial in inhibiting various cell and tissue toxicity in vitro [92] it has been used successfully as an antioxidant. Willimore and Rubin [93] demonstrated that α -tocopherol reduced both iron-catalyzed lipid peroxidation and subsequent seizures. Experimental evidence also showed that the combined application of α -tocopherol and FC-43 perfluorocarbon emulsion immediately after thermal skin injury in rats increased plasma antioxidant capacity, decreased free radical mediated damage of erythrocytes and suppressed their aggregation on the third hour after the injury [94].

Apart from these natural biological antioxidants several chemical based antioxidants are in use which have the potential to act as an efficient free radical scavenger. It is reported that a zinc-carnosine chelate compound Z-103, attenuated gastric mucosal injuries and inhibited the increase of lipid peroxide in the gastric mucosa induced by burn shock or ischemia-reperfusion. The antioxidative property of Z-103 is attributed to its inhibition of superoxide generation from polymorphonuclear leukocytes stimulated by opsonized zymosan and also to inhibition of the generation of hydroxyl radicals by fenton reaction [95]. Another study revealed the importance of coumarin (a benzopyrone) for its antioxidative effect in thermal oedema [96]. In addition, application of topical glycolic acid has also been reported to be an effective antioxidant during inflammation [97].

15. Trace elements

It is well known that burn injury necessitates increased nutritional requirements associated with the resulting hypermetabolic state. Among the nutrients required for supplementation trace elements need greater attention because stress alters the level of these trace elements due to altered intestinal absorption, altered body losses, altered distribution among body proteins and altered protein concentration [98]. Different trace elements have a specific role to play during healing and performs vital functions in the body. Trace elements are directly involved in free radical scavenging and they are potentially important in burns. The trace element status is altered after major burns, especially during the first week post injury with severely decreased serum level [99–101]. Iron is essential for erythropoiesis, which is markedly disturbed after burn injury, since patients become anaemic with no evidence of haemorrhagic losses or deficiency of iron supply in the diet [102]. Iron is important in energy metabolism because of its structural role in oxygen-carrying proteins. It is also associated with various enzymes, including catalase, Gpx and various dehydrogenases [103]. Zinc is an essential co-factor for collagen biosyn-

thesis such that preservation of an adequate zinc status might be considered an essential part of promoting wound healing and tissue repair [104]. Copper is an essential nutrient and it has several functions as an integral component of numerous enzymes, one such enzyme is ceruloplasmin which has ferroxidase activity [105]. Selenium functions primarily as an integral component of Gpx and functions as part of the cellular antioxidant defence system [106] and also needed for oxygenation and for protection against lipid peroxidation [107].

Burn patients are characterized by an intense hyper-metabolic response, maximal between 7 and 12 post-burn days with enormously increased nutritional requirements, which endogenous trace element stores and normal dietary intakes are insufficient to satisfy and that necessitates the supplementation of trace elements [108].

The free radicals are involved in major physiological mechanisms such as phagocytosis, the inflammatory reaction and the reperfusion ischaemia phenomenon observed during organ storage. The therapeutic use of enzymatic and non-enzymatic antioxidants and several chemical based antioxidants plays a vital role and is yet to be further evaluated. Antioxidants given post-burn restore antioxidant defences attenuated by the altered cell energetics and prevents mortality. Though each of these scavengers have been suggested to play a role in preventing peroxidation, the competition and balance among these scavengers has to be defined according to their consumption and deficiency and this can be established by studying the total antioxidant status. The current state of our knowledge on free radicals indicate the extreme complexity of these systems and calls for caution in the therapeutic use of antioxidant substances.

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