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Mini Review

Oxidative stress in severe acute illness

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ABSTRACT

The overall redox potential of a cell is primarily determined by oxidizable/reducible chemical pairs, including glutathione–glutathione disulfide, reduced thioredoxin–oxidized thioredoxin, and NAD^+–NADH (and NADP–NADPH). Current methods for evaluating oxidative stress rely on detecting levels of individual byproducts of oxidative damage or by determining the total levels or activity of individual antioxidant enzymes. Oxidation–reduction potential (ORP), on the other hand, is an integrated, comprehensive measure of the balance between total (known and unknown) pro-oxidant and antioxidant components in a biological system. Much emphasis has been placed on the role of oxidative stress in chronic diseases, such as Alzheimer's disease and atherosclerosis. The role of oxidative stress in acute diseases often seen in the emergency room and intensive care unit is considerable. New tools for the rapid, inexpensive measurement of both redox potential and total redox capacity should aid in introducing a new body of literature on the role of oxidative stress in acute illness and how to screen and monitor for potentially beneficial pharmacologic agents.

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Introduction

The change in the Gibbs free energy (ΔG) of a chemical reaction can be described in terms of the equilibrium constant of the reaction and the electromotive force of each half reaction under standard conditions. This electromotive force is simply the tendency of each half reaction to lose or gain electrons. Another name for the ΔG of a reaction is the redox potential. Such measurements have been documented for many isolated biochemical reactions and constitute part of our understanding of biological chemistry, analogous to the way the measurement of pH has led to an understanding of H^+ ions in biology. Because human cells derive energy using electron transfer from donor species to oxygen, and

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because the structural integrity of the cell membrane and the enzymatic activity of many proteins also depend on the redox potential of the cellular environment, an understanding how this milieu changes in certain disease states is of great interest.

The overall redox potential of a cell is primarily determined by oxidizable/reducible chemical pairs, including glutathione–glutathione disulfide, reduced thioredoxin–oxidized thioredoxin, and NAD^+–NADH (and NADP–NADPH). There are, nonetheless, some areas of heterogeneity with respect to redox potential inside the cell. In particular, whereas the overall ratio of reduced to oxidized glutathione in a cell is less than 30:1 (and usually at least 100:1), this does not seem to be true for the endoplasmic reticulum, where this ratio has been reported to range from 1:1 to 3:1 [1]. This difference is remarkable as the endoplasmic reticulum is precisely the cellular compartment in which correct disulfide linkages (oxidation) must be formed in proteins as they are synthesized in order for them to be active. James Watson has argued that imposing too many anti-oxidants on an organism could lead to poor protein folding because the redox potential might be too low for correct disulfide bond formation [2].

In addition, the major anti-oxidant mechanisms, as well as the overall manner of generating reactive oxygen species, vary a great deal between the intra-cellular and extra-cellular milieu [3]. For example, reduced glutathione is the major intra-cellular thiol compound (between 3 and 10 mM) whereas this compound is about 1000-fold less concentrated in extra-cellular fluid (about 2.8 μM). The extra-cellular fluid possesses cysteine as its major thiol compound [4]. There are three superoxide dismutases (SOD), one mitochondrial, one intra-cellular (but not in the mitochondria), and one extracellular. The three have different properties but all catalyze the same reactions, eliminating the superoxide anion and producing molecular oxygen and hydrogen peroxide (which is then removed by catalase). Despite the differences in extra-cellular and intra-cellular mechanisms of free radical generation and removal, the two systems are in a general equilibrium, with most of the free radicals being generated intra-cellularly, especially in mitochondria, while most of the measurements cited here of oxidative stress in severe acute disease are of extra-cellular fluids, especially serum/plasma.

The utility of knowing the redox potentials of biological fluids exists on two levels. First, the overall measure of the redox potential in a biological fluid, such as blood, urine, or cerebrospinal fluid (CSF), is a result of the myriads of reactions (glutathione synthesis, glutathione oxidation, NAD^+ and NADP reduction, thioredoxin regeneration and synthesis, nutrition, pathological processes, etc.). The combination of these reactions provides a single redox potential, which is measured in millivolts. Multiple studies have shown that this measurement can be useful in evaluating acute diseases, such as traumatic brain injury, severe sepsis, stroke, and myocardial infarction, as well as chronic diseases, such as Alzheimer's disease and atherosclerosis. Redox potential was predominantly used only as a research tool in the past, perhaps because redox potential measurements were recorded on cumbersome devices that used large electrodes, required large volumes, produced slow readouts, and were not adapted to the easy use and fast turnaround times required in an emergency room (ER) or intensive care unit (ICU) setting. Current methods for evaluating oxidative stress rely on detecting levels of individual byproducts of oxidative damage or by determining the total levels or activity of individual antioxidant enzymes. Oxidation–reduction potential (ORP), on the other hand, is an integrated, comprehensive measure of the balance between total (known and unknown) pro-oxidant and antioxidant components in a biological system. An ORP measurement that is significantly higher than that of a reference sample indicates the presence of oxidative stress. In the clinical setting, assessment of ORP can provide a global measure of the redox status of a biological sample. Furthermore, assessing the ORP of a biological sample does not rely on any individual marker.

Recent clinical studies in patients with traumatic brain injury (TBI) and multiple trauma have shown the utility of ORP as an indicator of redox status. A clinical study in patients with isolated TBI demonstrated that ORP values from plasma were significantly elevated at the time of admission to the trauma center in patients with mild-to-moderate isolated TBI compared with healthy individuals [5]. Consistent with ORP accurately reflecting redox status, this study detected significantly higher levels of oxidized human serum albumin in patients with TBI compared with healthy individuals. Additionally, a study of redox status in trauma patients found that plasma ORP values were significantly elevated in patients with severe trauma compared with healthy individuals [6]. Total levels and activity of the antioxidant paraoxonase–arylesterase were also reduced in these patients with severe trauma compared with healthy individuals, indicating the presence of oxidative stress. Furthermore, ORP values in the patients with severe trauma approached normal levels by the time of discharge following recovery. Importantly, a separate study reported that plasma ORP levels in patients with moderate-to-severe trauma correlated positively with the severity of injury [7]. Taken together, these results demonstrate the potential for using ORP to assess oxidative stress, severity of injury, and overall health status in patients with TBI and severe trauma.

All published research on ORP monitoring in critical illness was performed using a bench top ORP microelectrode suitable for a research laboratory only. Therefore, after demonstrating that ORP monitoring provides the clinician with valuable information on the redox status in a patient, it became imperative that a point-of-care device suitable for a clinical setting be developed.

Once the influence of redox potential has been established for a disease process or therapeutic intervention, one can hone in on the exact damage done to the tissue by various redox imbalances and study their specific deleterious effects. Before we examine this idea for specific clinical entities below, we first review the types of molecular insults generated by free radicals during oxidative stress.

Nucleic acids

In biological systems, the ferric ion is reduced to the ferrous ion by the superoxide free radical, generating oxygen. The ferrous ion can then react with hydrogen peroxide, which itself is a product of cell respiration, to produce the very reactive hydroxyl radical (the Fenton reaction). The hydroxyl radical can further generate other free radical species but also oxidizes the nucleotides in DNA and RNA as well as the bases and sugars in mono-nucleosides and mono-nucleotides. Although multiple products have been isolated from damaged nucleic acids, the major products and most widely-studied are 8-hydroxydeoxyguanosine in DNA and 8-hydroxyguanosine in RNA. In DNA, this base can be mutagenic and, thus, carcinogenic in principle. Although less studied, the RNA base can lead to accelerated degradation and a reduced capacity for protein translation. A variety of DNA repair enzymes exist to remove oxidized deoxyguanidine from DNA and thus eliminate its potential mutagenicity. Perhaps more intriguing from the point of view of acute illnesses is the fate of oxidized guanidine in RNA. RNA miscoding or mRNA degradation are rapid events, rendering them more significant to acute disease development than the effects of DNA mutation. Suggestions from the literature indicate RNA oxidization leads to the binding of proteins that may either act as or recruit nucleases to that site [8,9]. Further research into this possibility for acute diseases may be accomplished with an interesting technology that was previously described for the global analysis of RNA oxidation in *Saccharomyces cerevisiae* [10]. Here, the authors

isolated all of the mRNA species with augmented levels of 8-hydroxyguanosine residues and sequenced them, looking particularly at the large number of mRNAs whose oxidized guanosine residues were increased after a brief treatment of the yeast cells with hydrogen peroxide. Such techniques could become available for the cell types involved in various acute human diseases.

Lipids

Reactive oxygen species react with multiple, long-chain, unsaturated fatty acids in cell membranes to generate lipid peroxidation products, particularly malondialdehyde (MDA). These products can then react with other functional groups, especially amines, to generate another reactive aldehyde, 4-hydroxynonenal (HNE), which has been well studied with respect to its downstream reactions [11]. The chemistry of both the damage done by these free radical-generated compounds and their reduction by anti-oxidants has been the subject of many reviews [12,13]. The biological consequences of lipid peroxidation are primarily concerned with membrane function [14]. For example, HNE is toxic to neurons and impairs glutamate uptake as well as mitochondrial function at synapses, leading to the excitotoxic death of the neurons [15]. A factor that must be taken into account is the metabolism of these oxidative compounds in various tissues. Recently, for example, the lipid peroxidation product HNE was shown to be rapidly degraded in the liver but almost not at all in the lung and brain [16]. Thus, free radical generation would be expected to have much more severe consequences in the lung and brain than in the liver.

Proteins

Amino acids, both as free monomers and as the building blocks of polypeptide chains, can be oxidized by free radicals to produce a variety of compounds, including peroxides, keto-acids, and a large number of derivatives. Protein oxidation can occur at the amino group, the carboxyl group, or the various side chains that impart each amino acid with its unique properties. In terms of causing disease, such changes can induce enzyme inactivation, protein degradation, altered binding properties, the faulty transport of small molecules across membranes (a property shared with oxidized lipids), and the oxidation of cysteine residues to cysteine dimers through disulfide bonds for example. It has been suggested that protein oxidation can facilitate ubiquitination, which then targets these proteins for degradation by the proteasome [17]. In addition, accumulated oxidized protein may play a role in aging, although there is no direct evidence for this. Clearly, undegraded, misfolded, and oxidized proteins can be causative agents in chronic diseases, such as Alzheimer's disease and amyotrophic lateral sclerosis (ALS), but it is not yet known if the protein abnormalities induced by reactive oxygen species are etiologic factors in acute diseases.

Role of reactive oxygen species in acute disease

It is well established that any oxygen-consuming organism will generate free radicals during respiration and such free radicals interact with compounds in tissues to generate a variety of oxidized species with a concomitant reduction of the oxidizing compound. Many of these oxidized species are deleterious to tissues and organs in humans, but not all have been thoroughly studied. Of note, but which we have not discussed in this article, is that there are anti-oxidant compounds, both generated by metabolism and ingested as food and food supplements, which can sometimes mitigate the deleterious effects of free radical interactions. Therefore, attempting to understand the role of free radical damage in human disease is complicated by the flux of reactive species, which is influenced by the flux of anti-oxidant compound production and ingestion. Perhaps the best approach for studying the role of oxidative stress in disease, then, is to first measure the overall redox capacity of the tissue and blood before evaluating the effect of specific compounds.

Expending the known reduced species ($NADH^+$, reduced glutathione, etc.) to "neutralize" the deleterious compounds weakens the organism's capacity to combat further oxidative stress. Thus, the easy measurement of the redox capacity, as well as the redox potential, should not only help us to understand the current state of the organism but should also be predictive and prognostic for evolving diseases.

The role of continuing oxidative stress has been studied in a variety of chronic diseases, including diabetes mellitus, Alzheimer's disease, ALS, and atherosclerosis, but less attention has been paid to the role of oxidative stress in the progression and prognosis of acute disease. Next, we shall review four acute conditions that are likely to be seen in the ER and ICU, and evaluate the contribution of measuring redox potential in their management.

Traumatic brain injury (TBI)

The brain is vulnerable to injury by oxidative stress as its rate of oxygen consumption is high, nerve cells are not easily replenished, and the high levels of unsaturated fatty acids in the brain membranes are targets of the lipid peroxidation reactions initiated by reactive oxygen species. A seminal study in a pediatric setting (trauma is the leading cause of death in children) compared CSF from children aged two months to 16 years with severe TBI (Glasgow Coma Scale ≤ 8) with the CSF from children undergoing lumbar puncture to rule out a diagnosis of meningitis. The presence of a TBI led to a sustained reduction of the total anti-oxidant reserve and a decrease in the levels of the anti-oxidants ascorbate and reduced glutathione. In addition, there were increased levels of lipid peroxidation products and protein oxidation [18]. Moreover, in a larger pediatric population, the amount of nerve damage, as measured via the activity of neuron-specific enolase, was correlated with the level of the lipid peroxidation product F2-isoprostane after day 1 of TBI [19]. Reactive oxygen species also promote the formation of peroxynitrite, which reacts with tyrosine to yield 3-nitrotyrosine. In an adult population, the oxidative product nitrotyrosine was not detected in normal CSF. In severe TBI, on the other hand, nitrotyrosine was present in most of the CSF samples. Thus, the consistent presence of nitrotyrosine in CSF samples from TBI patients has been suggested to be a prognostic indicator of poor outcome [20]. Most recently, peroxiredoxin VI has come to the forefront in the list of potential prognostic markers in TBI. Peroxiredoxin VI is a major anti-oxidant enzyme normally found in astrocytes. While this enzyme is present in the CSF of both normal and TBI patients, its enzymatic activity is high in the CSF of normal patients but is greatly diminished in the CSF of TBI patients, signifying that the enzyme is oxidized after the traumatic event. Significantly, recovery of the enzymatic activity at 24 h after admission was shown to be a prognostic sign of recovery, whereas continued low enzymatic activity after 24 h was shown to be a prognostic sign of poor outcome [21]. Thus, specific oxidation products as well as total redox capacity are worth investigating in the search for biomarkers of successful treatments of TBI in the near future.

Sepsis

Sepsis and the multiple organ failure often seen in response to sepsis are the most common causes of death in the ICU. Oxidative

stress that is induced by the inflammatory cycle as a consequence of sepsis has been well documented, and oxidized species of lipids and proteins are prominent in this disease state. Increased lipid oxidation was observed in patients who presented with three or more dysfunctional secondary organs compared to patients with two or fewer dysfunctional secondary organs [22]. In septic patients, both plasma superoxide dismutase (SOD) and plasma catalase are elevated compared to healthy normals. This difference is expected because of the increased production of the superoxide ion and hydrogen peroxide in septic patients. Plasma SOD is high in non-survivors of sepsis but tends to decrease to about a third of these high levels in survivors [23]. When total redox capacity is measured in sepsis instead of individual free radicals or oxidized cell components, a clear decrease is seen in septic patients compared to healthy controls. This low redox capacity stays low in patients who succumb to sepsis, whereas it tends to return to normal in patients who survive the disease. Thus, one could imagine that the total redox potential could measure the efficacy of treatment in severe sepsis [24]. Increased levels of xanthine oxidase, SOD, and glutathione peroxidase were also seen in a pediatric septic population, although no prognostic correlation with outcome was observed [25]. More recently, emphasis has been placed on the oxidative stress that occurs at the point of production of most reactive oxygen radicals, namely the mitochondrion. It has been suggested that mitochondrion-targeted anti-oxidant therapy may be more effective for patients with sepsis or septic shock than therapy with global anti-oxidant compounds [26].

Stroke

Before discussing the role of reactive oxygen species in stroke, it is important to clarify an apparent paradox of free radical production in tissues. This is especially pertinent to the discussion of stroke but is also relevant for discussion of a number of diseases in which tissues become hypoxic but free radical damage is invoked. The superoxide anion and, subsequently, hydrogen peroxide are produced in the mitochondria during respiration. In general, the higher the oxygen partial pressure, the higher the production of free radicals [27]. Paradoxically, when cells become hypoxic (for example, in cultured cells when the ambient oxygen is reduced from 21% to 1–3% oxygen), the production of reactive oxygen species increases. This seems to be related to the stabilization of hypoxia-inducible factor 1 (HIF 1) at low oxygen pressures. HIF 1 is a transcription factor that induces the transcription of erythropoietin, vascular endothelial growth factor (VEGF), and a variety of other mRNAs, which re-adjusts the metabolism of the cell. Through a mechanism that has not yet been elucidated, these metabolic changes interact with the mitochondrial respiratory chain to increase the release of the superoxide anion and hydrogen peroxide from this chain [27–29].

Stroke accounts for 9% of deaths worldwide. The majority (80–85%) of strokes are ischemic, in which a blood clot cuts off the blood supply to a region of the brain. The other 15–20% are hemorrhagic, in which a blood vessel bleeds into a region of the brain, severely disturbing brain function. By measuring oxidative stress via the serum MDA concentration and total redox capacity via the level of free thiol groups in serum, a recent study showed that reactive oxygen species were increased and total redox capacity decreased on days 1 and 7 after ischemic stroke [30]. In this study, the highest MDA concentration and the lowest total redox capacity were associated with poor outcome. Also, the MDA level on day 7 after the stroke was reported to be an independent predictive factor for three-month outcomes. The lipid peroxidation product 8-isoprostane was elevated in the sera of patients who had suffered an ischemic stroke. In this study, it was reported that a statin, simvastatin, showed an anti-oxidative effect in inhibiting the rise of 8-isoprostane [31]. In another study, 8-isoprostane was elevated and the natural anti-oxidant vitamin C was lowered in the blood of patients who had suffered an ischemic stroke; interestingly, the decrease in vitamin C was proportional to the increase in 8-isoprostane [32]. The demonstration that reactive oxygen species play a role in the brain damage observed after an ischemic stroke has led to the introduction of many anti-oxidants as potential medicines to ameliorate outcomes.

Combination vitamin C and vitamin E therapies have been evaluated for ischemic stroke patients in a number of settings, and some suggestive positive results have been found [33,34]. A study using B group vitamins has shown that inflammatory markers are decreased after stroke, but no effect on clinical outcomes was reported [35]. Oxidative stress is equally implicated in the other group of stroke patients, those suffering from hemorrhagic strokes. For instance, total superoxide dismutase levels were found to be higher in brain samples from patients after sub-arachnoid hemorrhages than in those with unruptured aneurysms [36].

Myocardial infarction

Myocardial infarction is caused by the lack of blood supply to regions of heart muscle. The lack of blood supply is usually preceded by chronic atherosclerosis, diminishing blood supply, and, thus, oxygen to the heart muscle. As mentioned above, this hypoxia leads to a paradoxical increase in reactive oxygen and nitrogen species. Moreover, once an infarct is treated, the reperfusion to the damaged myocardium also leads to increased oxidative stress [37,38]. The extent of myocardial damage and subsequent clinical outcome seems to be related to the severity of the original oxidative stress, and the anti-oxidative milieu brought to bear on these reactive species. This is best illustrated in patients with diabetes mellitus, who tend to have more severe atherosclerosis, more frequent myocardial infarcts and worse outcomes from these MI's, than non-diabetic patients. This has been postulated to be caused by the more severe oxidative stress in tissues of diabetics than in others [39]. More recent evidence supports this idea. Diabetic patients suffer twice the mortality after MI than do non-diabetic patients, and this is correlated with increased levels of Ca^{++}–calmodulin dependent protein kinase II delta, which is known to be involved in redox control [40]. In an experimental rodent model, the increased mortality associated with diabetes can be eliminated by using a "knock-in" technique rendering this enzyme insensitive to oxidation (it is methionine oxidation which causes the damage) [41]. Also in a rat model of diabetes, the consequences of MI could be drastically reduced by using a cloned thioredoxin-1 gene to express this enzyme to reduce oxidative stress in heart muscle. This gene therapy reduced mortality by increasing expression of thioredoxin-1 and heme oxygenase-1 as well as of VEGF; this led to increased angiogenesis in the heart muscle and decreased ventricular remodeling [42]. Such promising results have led to a clinical trial in human MI to test whether the anti-oxidants Vitamins C and E might reduce the infarct size in MI patients undergoing percutaneous coronary angioplasty. Vitamin C will be given directly to heart muscle and Vitamin E will be given orally. This trial should be fully enrolled in 2016 [43].

Quite recently, a study has been published which probably exemplifies all of the points we have tried to make here. In heart muscle (as well as in other organs), Chouchani et al. showed that ischemic changes included a cytosolic change in xanthine oxidoreductase, which does not influence mitochondrial reactive oxygen species, and a huge increase in succinate, which is an intermediate in the mitochondrial citric acid cycle [44]. They went on to show that this hypoxic change was induced by formation of succinate from fumarate by a reversal of the succinate dehydrogenase enzyme. When reperfusion occurred the succinate

oxidation led to a great over-production of superoxide radical, and thus ischemia–reperfusion injury in the heart muscle. They showed that dimethylmalonate, a precursor to malonate, which inhibits succinate dehydrogenase, leads to a reduction in ischemia–reperfusion injury. This seems to be a clear demonstration that mitochondrial production of reactive oxygen species leads to at least some of the extra-cellular injury associated with MI.

Multiple trauma

Reactive oxygen species and oxidative stress are also associated with many other acute diseases likely to be seen in the ER or ICU. These include multiple trauma cases and burn patients. For example, increased levels of lipid oxidation products have been observed in patients undergoing cardio-pulmonary bypass while their circulation and blood oxygenation are extra-corporeal [45]. In patients with extensive burns, the plasma levels of the antioxidants vitamin E and retinol are decreased at admission but are increased over time during recovery [46]. Many of these findings suggest that common pathways of reactive oxygen species production and common biochemical targets of these species may be present in multiple organs. For example, endothelial stem cells and polymorphonucleated leukocytes (PMNLs) accumulate at trauma sites of all varieties. Trauma-activated PMNLs produce reactive oxygen species, which kill the stem cells, and this may be a common mechanism of organ failure in trauma and burn patients [47].

Conclusion

Research on reactive oxygen species and their role in disease causation has taken parallel paths. On the one hand, the enumeration of all oxidized products, including those resulting from nitrogen-free radical compounds, has been more or less divorced from the identification of specific pathways for specific diseases. Those studying diseases in which reactive oxygen species play at least a partial etiologic role tend not to characterize all of the responsible species nor focus on specific pathways. In many ways, this is understandable because both undertakings are enormous, as is the pharmacologic search for reducing agents that may help to fight some of these diseases. Much emphasis has been placed on the role of oxidative stress in chronic diseases, such as Alzheimer's disease and atherosclerosis, but as we have shown here, the role of oxidative stress in severe acute diseases often seen in the ER and ICU is considerable. Moreover, the pharmacologic effort to identify compounds that can relieve oxidative stress in acute illness is likely to be somewhat different than the therapeutic compounds that are successful in fighting chronic disease. New tools for the rapid, inexpensive measurement of both redox potential and total redox capacity should aid in introducing a new body of literature on the role of oxidative stress in acute illness and how to screen for potentially beneficial pharmacologic agents.

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