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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