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Generation of free radical by interaction of iron with thiols in human plasma and its possible significance

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Abstract It has been reported that iron can generate reactive oxygen species (ROS) with thiols. In this study, we examined the interaction of iron with thiols in plasma and the generation of ROS. In human plasma, unlike with Fe^{3+}, treatment with Fe^{2+} increased lucigenin-enhanced chemiluminescence in a concentration-dependent manner, and this was inhibited by superoxide dismutase. Boiling of plasma did not affect chemiluminescence generation induced by Fe^{2+}. Thiol depletion in plasma by pretreatment with *N*-ethylmaleimide (NEM) decreased chemiluminescence significantly. Consistent with these findings, albumin, the major thiol contributor in plasma, also generated ROS with Fe^{2+}. Treatment with Fe^{2+} resulted in significant reduction of oxygen radical absorbance capacity (ORAC value) in plasma followed by an increase in low-density lipoprotein (LDL) oxidation. These results suggest that generation of ROS by nonenzymatic reaction of Fe^{2+} with plasma thiols could lead to reduction of total antioxidant capacity in plasma, thereby enhancing susceptibility of plasma LDL to oxidation under iron overload conditions.
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Introduction

Oxidative stress, defined as the imbalance between oxidants and antioxidants in favor of oxidants that potentially lead to tissue damage [1], has been

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associated with a number of diseases in humans. These diseases include cancer, coronary heart disease, arthritis, diabetes, cataract, and the degenerative processes associated with aging [2,3]. Over lifetimes, humans are continuously exposed to oxidants of both exogenous and endogenous origin. Examples of external sources of oxidants are natural dietary constituents, UV radiation, and environmental pollutants. Endogenous sources of oxidants include mitochondrial respiration, enzymes, such as lipoxygenase and xanthine oxidase, and the NADPH oxidase/myeloperoxidase system of phagocytes and metal ions [4].

One of these sources of oxidants is iron, an essential mineral nutrient. In solution, iron exists in two oxidation states. Ferrous (Fe^{2+}) donates and ferric (Fe^{3+}) accepts an electron and these activities generate oxidative stress [5]. Iron becomes nontoxic when it binds to the transport protein, transferrin, in plasma. Another protein, ceruloplasmin, has ferroxidase activity and catalyzes the oxidation of ferrous irons and certain ferrous complexes to the less reactive ferric state [6]. Humans have a very limited capacity to excrete iron. When iron enters the body in excess of metabolic requirements, it accumulates in tissues and body fluids and can exceed the body's capacity to sequester it in a relatively harmless form [7]. Individuals with certain diseases or medical conditions, such as homozygous hemochromatosis, sickle cell disease, rheumatoid arthritis, adult respiratory distress syndrome, and cardiopulmonary bypass, show significant amounts of non-transferrin-bound iron (NTBI) in their blood [7–9]. NTBI is a potentially redox-active form of iron. NTBI-mediated oxidative stress is reported to reduce the antioxidant capacity found in plasma [10]. Furthermore, several epidemiological and animal studies suggested the key role of NTBI in many diseases caused by oxidative stress [11]. However, the mechanism for iron generating oxidative stress in plasma has not been conclusively explained.

Some oxidative stress-related diseases, such as blood cardioplegia, atherosclerosis, thrombosis, and rheumatoid arthritis [12,13], are reported to be associated with thiols. Thiols, abundant in human plasma and other tissues, are endogenous molecules that assist aerobic cells in maintaining a reducing state, despite an oxidizing environment [14]. There is also some evidence that thiols in vitro can act as a nonenzymatic pro-oxidant by redox cycling [12]. For example, it was reported that thiols stimulated lipid peroxidation in vitro and in vivo in the presence of iron [7,15]. Moreover, many enzymes have been shown to lose catalytic activity by thiol-dependent oxidation reaction with iron [16]. This system is also capable of damaging DNA, incorporating carbonyl groups into protein and fragmenting peptide chains.

The purpose of this study was to investigate the mechanisms of oxidative stress induced by iron in plasma and the physiological significance of iron overload. We examined the role of plasma thiols in iron-induced generation of free radicals and determined the change of total antioxidant capacity and Cu(II)-catalyzed plasma LDL oxidation following iron overload to plasma.

Materials and methods

Materials

Ferrous sulfate heptahydrate, ferric citrate, anhydrous dimethyl sulfoxide (DMSO), luminol, lucigenin, superoxide dismutase (SOD), albumin (human, fraction V), 2,2′-azobis(2-methyl propionamidine) dihydrochloride (AAPH), R-phycoerythrin (R-PE) or B-phycoerythrin (B-PE), trolox, *N*-ethylmaleimide (NEM) were purchased from Sigma (St. Louise, USA). All other reagents used were of the highest purity available.

Preparation of plasma and thiol solution

Following approval by the Ethics Committee of Health Service Center at Seoul National University, human blood was obtained from healthy male donors (18–25 years old) using a heparinized vacutainer and a 21 gauge needle (Becton Dickinson, USA). The plasma was freshly prepared by centrifugation of blood at 1500×*g* for 20 min. In order to deplete thiols in plasma, plasma was treated with 10 mM NEM and incubated for 1 h at 37°C, as described previously [17]. To inactivate protein in plasma, the plasma was heated for 30 s at 100°C using a multiblock heater (Lab-line, USA). To prepare various thiol solutions under physiological conditions, plasma was added into Centriprep 50 (Amicon, USA) and proteins over 50,000 molecular weight were removed by centrifugation at 1500×*g*. The resulting plasma was then incubated at 80°C for 30 min followed by centrifugation at 2100×*g* for 30 min. For experimental use, the pH was adjusted to 7.4 with 1 N HCl. L-Cysteine, GSH, and albumin were dissolved to the final concentrations of 10 μM, 5 μM, and 4.5%, respectively.

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