Opinion Article

Can selenite be an ultimate inhibitor of Ebola and other viral infections?

ABSTRACT

It is known that the virulence of Ebola and other RNA enveloped viruses involves in the first step their attachment to host cell membranes. Following this initial step the virus enters the target cell cytoplasm by forming hydrophobic spikes that make holes in the membrane lipid bilayer. Formation of such spikes is catalyzed by the reduced form of viral protein disulfide isomerase (PDI_{red})thus initiating chain of disulfide exchange reactions. Consequently, hydrophobic protein epitopes become exposed, which in the absence of proper chaperones form hydrophobic 'spikes' capable of penetrating the host cell membranes. In this communication evidence is discussed showing that the chain of disulfide exchange events can be inhibited by a small redox molecule – sodium selenite. It is suggested that this inexpensive and readily available food supplement can be an ultimate inhibitor of Ebola and other enveloped viral infections.

Key words: Ebola virus, hydrophobicity, protein disulfide exchange, sodium selenite

Introduction

Selenium (Se) is a ubiquitous element that participates in various biochemical reactions in human body. This metalloid, akin to sulfur, is present in numerous proteins forming so-called mixed disulfides [1] that participate in thiol-disulfide exchange reactions [2]. Although physiological function of selenium compounds is not well known, it has been recognized that Se deficiency is associated with certain degenerative diseases[3, 4]. However, not all Se derivatives are biologically active, and amongst various inorganic forms of Se, the best studied is its four-valention (selenite) that readily interacts with protein sulfhydryls (*P*-SH). Selenite can also interfere with and/or modify thiol/disulfide exchange reactions, particularly those occurring during the attachment of viral glycoproteins to the host cell membranes catalyzed by protein disulfide isomerase (PDI). Therefore, this specific form of inorganic selenium, which is

inexpensive and readily available as a food supplement, can be used as an effective inhibitor of Ebola and other enveloped virus infections.

Disulfide exchange reactions in human proteins

Properties and functions of native proteins are maintained by intra-molecular disulfide bonds that are positioned in such a way as to hide hydrophobic regions inside their tertiary structure[5]. However, when one or more of the disulfides is reductively cleaved, the hydrophobic groups of polypeptide chain(s) are unmasked and allow them to react readily one with another [6]. Consequently, in the absence of proper chaperones the unfolded polypeptide chains become incorrectly refolded with the formation large hydrophobically bonded aggregates (Fig.1).

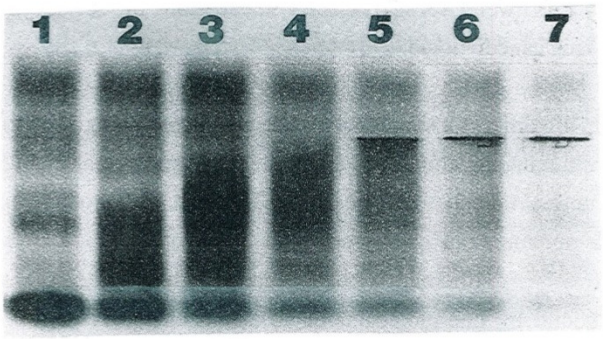

Figure 1 shows that the limited reduction of plasma proteins results in the formation of large aggregates that are not dissociable during gel electrophoresis and that are remarkably resistant to

dissolution by chaotropic solvents and proteolytic enzymes. We have previously shown that similar insoluble aggregates are formed from fibrinogen under the reductive influence of iron-generated hydroxyl radicals [7] that was suggested to form a protective coat (parafibrin) around the tumor cells and prevent their elimination by NK cells [8]. It is important for this paper to note that the formation of parafibrin was demonstrated by us to be preventable by sodium selenite and other oxidizing agents [9]. The unfolded polypeptides can also form complexes with the hydrophobic tails of lipid molecules forming holes in the cell membrane bilayer [10]. It is generally believed that this mechanism is responsible for the virus entry into the host cytoplasm thus allowing its survival and multiplication [11].

It is well established that RNA-virus multiplication starts with a critical event of its attachment to the host cell membrane followed by virus entry into the cell [12, 13, 14]. To achieve this goal Ebola and other double-stranded RNA viru are equipped with a relatively simple albeit not obvious mechanism involving modulation of *gp*120 glycoprotein moiety [15]. After the initial viral contact with the host cellular membrane a number of enzymes are activated that lead to the reduction of at least one disulfide bond in the *gp*120 molecule [16, 17, 18]. Importance of this reaction for the human HIV infection was demonstrated in experiments with the use of agents that interfered with the thiol/disulfide interchange [19]. So far, two enzymes were shown to be of importance in this exchange, namely protein disulfide isomerase (PDI) [20,21], and thioredoxin reductase (Trx-R) [22, 23].

Selenium and its therapeutic potential

Selenium (Se) is a ubiquitous albeit not uniformly distributed element in the soil of various regions of Earth [24]. It is generally known that Se deficiency, both in the agricultural food products and in the human organism, is associated with various degenerative diseases, notably in viral infections [25,26,27]. In view of the fact that supplementation with this element proved to be beneficial for human healthe.g. Keshan disease in China [28], numerous studies have been undertaken to document beneficial effects of Se in human pathology. However, so far no unequivocal results have been reported, most likely because there is very little understanding of the relationship between chemical forms of Se and its biological activity.

There are two classes of selenium compounds – inorganic and organic. In the former, the element occurs as a four-valent (Se^{4+}) and six-valent (Se^{6+}) cations. In the organic compounds selenium exists as selenide mostly in the form of selenocysteine. In human body there are several selenoproteins, the function of which is not clearly understood. It should be, however, strongly emphasized that the biological activity of inorganic forms of Se depends on their electronic structure. Thus, only Se^{4+} (selenite) abut not Se^{6+} (selenate) is a redox active entity. This basic chemical fact is a source of misunderstanding when researches lump all forms of Se and just label them 'selenium'. It should be born in mind that only *four-valent* Se in the form of sodium selenite can interact with free sulhydryl (-SH) groups of proteins to oxidize them to disulfides according to the following formula:

$(P\text{-SH})_2 + Se^{4+} \rightarrow P\text{-S-S-}P + Se^{2+}$, where

P stands for protein polypeptide chain, and Se^{2+} is the reduced form of selenite. This chemical equation is applicable to the mechanism by which selenite inhibits thiol/disulfide exchange initiated by the viral PDI (Fig.2).

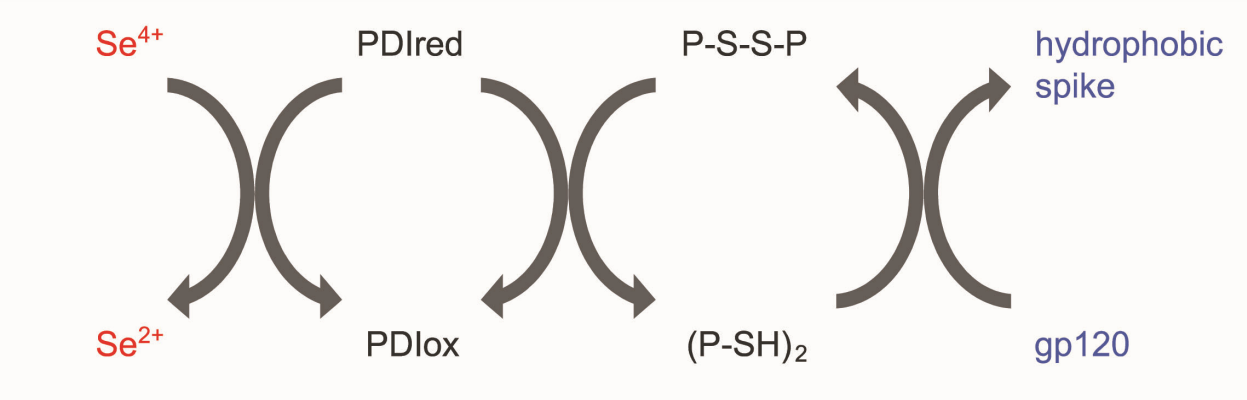

Fig.2

The chain of events depicted in Fig.2 starts with the PDI-catalyzed reduction of protein disulfides in the virus glycoprotein that opens up its hydrophobic epitopes. In the absence of specific chaperones the hydrophobic "spike" of *gp*120 makes a hole in the host bilayer membrane through which the virus can enter into the host cytoplasm with all its pathological consequences.

The most effective way to prevent this event is by inhibition of protein disulfide reduction that can readily be achieved with a timely administration of *sodium selenite*. This concept provides an explanation, however tentative, why Se deficiencies are associated with enhanced infectivity of Ebola and other viruses [29]. Additionally, it is of interest to note that selenite inhibits TrxR activity in a dose dependent manner [30], and at the same time increases NK cells potency [31]. The latter may explain the fact that those ca. 30 percent of people who are resistant to Ebola infections may have adequate blood concentrations of selenite and/or other electrophilic substances that can inhibit disulfide exchange and/or activate NK cells. An important argument in favor of the importance of PDI activation in virus infections is the recently reported finding that the inhibition of protein disulfide exchange prevents thrombo-hemorrhagic events so characteristic for the Ebola-induced disease [32,33].

Of note, another small molecule, melatonin, was recently suggested as a potential therapeutic agent in Ebola virus infections [34]. In addition to this hormone's main functions in sleep and circadian rhythm regulations, melatonin is known to scavenge hydroxyl radicals by virtue of aromatic hydroxylation [35,36]. Hence it is possible that PDI-catalyzed disulfide exchange reaction involves intermediate free radicals vulnerable to the scavenging action of melatonin [37].

In view of the fact that sodium selenite is readily available as an inexpensive food supplement, the concept presented in this article is particularly important for the protection of large populations against threat of Ebola epidemics [38]. However, unjustified fear of selenite "toxicity" has to be overcome, with the understanding that not all forms of this chemical exhibit similar beneficial health effects. Although small doses of 100 μg/day of sodium selenite were reported to increase the rate of poliovirus clearance, they may not be sufficient to prevent other viral infections [39, 40]. Other researchers have also reported that larger than recommended doses of sodium selenite including continuous *i.v.* infusions were well tolerated by humans [41, 42] and by experimental animals [43]. However, it has to be noted that sodium selenite should not be administered together with ascorbic acid, because this vitamin reduces selenite to an inactive divalent selenium ion.

Finally, it is not surprising albeit not immediately obvious that sodium selenite has been used to in the prevention and/or treatment of various forms of cancer [44, 45]. Similarly to viral PDI,

selenite inhibits disulfide exchange reactions in plasma proteins (specifically fibrinogen) and in this way prevents the formation of a hydrophobic protective coat around tumor cells [8].

Conclusions

In spite of enormous complexity of biology and pathology of Ebola and other enveloped RNA viruses,there is one reaction that deserves our closer attention. This is the attachment of viral *gp*120 glycoprotein, which initiates the fusion and entry of virus to the host cell. This process is controlled by a redox modulation of protein disulfide exchange that can be a target for antiviral therapies. In this article evidence is presented that a specific chemical form of selenium, sodium selenite, may offer an effective weapon against Ebola and other viral infections by the inhibition of thiol/disulfide exchange reactions thus preventing virus entry and proliferation. Although this article reveals only tip of the iceberg, it is hoped that it will stimulate research efforts aimed at preventing the emerging threat of Ebola virus epidemics by the use sodium selenite and other redox-active molecules.

Legend to figures:

Fig.1.

Agarose-gel electrophoretic pattern of human plasma incubated at 37^{o}C with a reducing agent *dithiothreitol* at various times from 0 (line 1) to 30 min. (line 7). This figure shows that the reduction of disulfide bonds in plasma proteins results in a time-dependent formation of huge hydrophobic aggregates that are not dissociable in the electric field. Other experiments (data not shown) have revealed that such aggregates are remarkably resistant the proteolytic degradation.

Fig.2.

Chain of events initiated by viral protein disulfide isomerase (PDI_{red}). It should be noted that a singular event of the withdrawal of two electrons from the sulfhydryl groups of PDI_{red} and their transfer to the selenite cation (Se^{4+}) results in the inhibition of protein disulfide reduction in the *gp*120 molecules and subsequently prevents the formation of a hydrophobic spike.

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