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Iron and Nitric Oxide Balance in African Trypanosomosis: Is there Really a Link?

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ABSTRACT

Iron is essential for living cells, including pathogenic microorganisms. So altered iron availability is often a key component in the host-pathogens interplay. Secondary to infection with pathogens, there is the activation of macrophages. Activated macrophages by action of inducible Nitric Oxide Synthase (iNOS) produce Nitric Oxide (NO) as part of host defense mechanism. However, production of nitric oxide as obtained in *Trypanosoma brucei* infection strongly correlates with increased cellular iron. The expanded free iron pool may become available to invading parasites and thus promotes proliferation. Establishing the link between these two important molecules may offer new treatment strategies for African trypanosomosis in the face of increasing toxicity and parasite resistance to drugs currently available for treatment of African trypanosomosis.

Key words: Cellular iron, nitric oxide, African trypanosomosis, iNOS

INTRODUCTION

Trypanosoma brucei is a unicellular parasite transmitted by the bite of tsetse fly and is the causative agent of sleeping sickness in humans and related diseases in animals (Adeyemi *et al.*, 2009; Sulaiman and Adeyemi, 2010). Over 60 million people living in 36 Sub-Saharan countries are threatened with sleeping sickness (Ekanem *et al.*, 2002) and 48,000 deaths were reported in 2002 (Johnson and Ekanem, 2003). Chemotherapy of African trypanosomosis still relies on drugs developed decades ago and some of these drugs display serious toxic side effects (Adeyemi *et al.*, 2009). In addition, drug resistance in African trypanosomes is increasing (Ekanem, 2001) thus underscoring the need for development of new strategies to treat African trypanosomosis. Studies directed at host-parasite interaction in African trypanosomosis may play a key role in this search for newer and safer chemotherapy for the disease.

Upon invasion of a mammalian system, trypanosomes proliferate rapidly through binary cell division to establish its population in the infected host (Anosa, 1980; Ekanem *et al.*, 1996). A major requirement of rapidly dividing cells including trypanosomes is the intracellular availability of free iron required by ribonucleotide reductase (RNR) (Lieu *et al.*, 2001; Johnson and Ekanem, 2003), in the synthesis of deoxyribonucleotides (dNTPs) from ribonucleotides (NTPs). The RNR is

responsible for the production of all the four dNTPs required for replication; a necessary step in cell division prior to proliferation (Ekanem, 2001; Richardson, 2002; Harhaji *et al.*, 2004; Nishimura *et al.*, 2008). Subsequent to parasite proliferation, the severity of disease observed in cases of African trypanosomosis is usually associated with increased levels of inflammatory mediators, which include Tumor Necrosis Factors (TNF- α) and nitric oxide derivatives. Blood monocytes and tissue macrophages play a key role in the control of parasite population and an increased number of activated hematopoietic cells are observed during trypanosomosis (Vincendeau *et al.*, 1992). TNF- α and NO fulfil important functions in host-parasite interactions as they control infections by various pathogens, including *T. brucei* (Vincendeau *et al.*, 1992). More so, chronic secretion of macrophage-derived mediators is in part responsible for the pathogenic aspects of African trypanosomosis (Lejon *et al.*, 2002). Nitric Oxide (NO) is generated to eliminate parasites but chronic exposure to NO may also contribute to the pathophysiology of African trypanosomosis (Clark and Rockett, 1996). The increased level of NO oxidation products have been shown to contribute to the pathophysiology of African trypanosomosis (Clark and Rockett, 1996; Ekanem *et al.*, 2009). Besides the generation of free radicals secondary to over production of NO, it should also be noted that studies have established that Nitric Oxide (NO) affects some proteins involved in iron metabolism which could cause iron release mobilization (Kim and Ponka, 2003; Richardson and Lok, 2008). A recent report by Ekanem *et al.* (2009), showed a close correlation between nitric oxide production and iron status in experimental infection with *T. brucei*. Although, one may want to propose that production of nitric oxide, secondary to infection may contribute to free iron release which essentially promotes proliferation and aggravates infection, how does the NO induce free iron pool mobilization in trypanosome infection? Understanding the link between nitric oxide production and iron status balance as a result of infection due to pathogens may reveal more about the delicate host-parasite interaction and would be helpful in developing new strategies for treatment of infection related diseases.

Host-iron availability plays a crucial role in the host-pathogen relationship. As a general anti-microbial defence mechanism, mammals possess an elaborate iron-withholding system that effectively reduces the amount of iron accessible to invading pathogenic microorganisms. Iron is predominantly intracellular and the limited amount of extracellular iron is tightly bound to proteins such as transferrin and lactoferrin (Dunn *et al.*, 2007). Therefore, invading pathogens require specialized and effective mechanisms to acquire iron and iron availability is often found to have an important role in virulence (Weinberg, 2000). Based on available reports this review aims to appraise the relationship between nitric oxide production and iron balance and its relevance to infection by *Trypanosoma brucei*.

Iron (Fe) and Nitric Oxide (NO) interactions in cellular systems: Iron is the most abundant transition metal present in the body in the protein-bound form such as heme and non-heme proteins. It is an essential nutrient for almost all living organisms playing a major role in electron transfer and oxygen utilization. The significance of iron stems from the facts that it possess a flexible redox potentials; interact with coordinating ligands; undergo electron transfer and acid-base reactions (Kim and Ponka, 2002; Sutak *et al.*, 2008). Iron in living cells may function as a metal cofactor for many heme or non heme iron containing proteins (Richardson and Milnes, 1997). Hemoproteins have crucial biological functions, such as oxygen binding (haemoglobin), oxygen metabolism (oxidase, peroxidase, catalase, etc.) and electron transfer (cytochromes). Many non heme iron-containing proteins catalyze key reactions involved in energy metabolism (e.g.,

mitochondrial aconitase and iron-sulfur (Fe-S) proteins of the electron transport chain) and DNA synthesis (ribonucleotide reductase). In aqueous solution, iron may exist in two oxidation states, Fe^{2+} and Fe^{3+} , which can donate and accept electrons, respectively. At physiological pH and oxygen tension, Fe^{2+} is readily oxidized to Fe^{3+} , which rapidly forms essentially insoluble $\text{Fe}(\text{OH})_3$ precipitate. Moreover, unless appropriately chelated or removed, iron, due to its catalytic action in one electron redox reactions, plays a key role in the formation of harmful oxygen radicals that may ultimately cause oxidative damage to vital cell structures (Halliwell and Gutteridge, 1990). To ensure iron availability and to eliminate the toxicity of free iron in addition to its accessibility for invading pathogens, mammals have evolved a strictly regulated system for iron homeostasis (Dunn *et al.*, 2007; Sutak *et al.*, 2008). Cellular systems are equipped with exclusive mechanisms that maintain adequate amounts of iron for synthesis of physiologically functional iron-containing molecules and yet keep “free iron” at its lowest possible concentration. Physiologically, the cellular system acquires iron from plasma glycoprotein, transferrin (Tf). In plasma, circulating iron is bound to transferrin, whereas, in external secretions such as colostrum, iron is bound to lactoferrin (Sutak *et al.*, 2008). The uptake of iron bound transferrin is mediated by transferrin receptor-1 (TfR1), which facilitates the internalization of the transferrin-TfR1 complex by endocytosis (Sutak *et al.*, 2008). Release of iron from Tf follows a decrease in endosomal pH and the iron thus released passes through the endosomal membrane via Divalent Metal Transporter 1 (DMT1) (Fleming *et al.*, 1997), into the poorly characterized intracellular labile iron pool. Generally, sensitive regulatory mechanisms exist that monitor iron levels in the intracellular labile pool and prevent its undue expansion, while still making the metal available for synthesis of iron-dependent proteins. It should be noted that excess intracellular iron that is above the requirement for the synthesis of functional heme and non heme iron-containing proteins is stored within ferritin (Richardson and Ponka, 1997), a protein polymer of 24 subunits that can accommodate up to 4500 iron atoms (Sutak *et al.*, 2008).

Iron metabolism is meticulously regulated (Aisen *et al.*, 1999; Sutak *et al.*, 2008) and major players in this regulation are cytosolic iron regulatory proteins (IRP1 and IRP2) which are responsive to iron levels in the labile pool. A previous study has been able to show that IRP1 and IRP2 perform their function by binding to specific nucleotide sequences, termed Iron-Responsive Elements (IREs), which are present in the mRNAs for numerous proteins involved in iron metabolism (Fast *et al.*, 1999; Kim and Ponka, 2002). These proteins bind to Iron-Responsive Elements (IREs) of mRNAs that encode proteins involved in iron uptake (TfR1 and DMT1), utilization (erythroid d-aminolevulinic acid synthase), storage (ferritin) and export (ferroportin-1). Another key player in the regulation of systemic iron homeostasis is the circulating peptide hormone, hepcidin (Nemeth *et al.*, 2004; Sutak *et al.*, 2008). Hepcidin regulates cellular iron efflux by binding to ferroportin-1 and inducing its internalization (Sutak *et al.*, 2008). IRP1 is a 98 kDa bifunctional protein with mutually exclusive functions of RNA binding and aconitase activity (Haile *et al.*, 1992). It shares 30% identity with mitochondrial aconitase (Kennedy *et al.*, 1992), an enzyme of the Krebs cycle. Importantly, some active site residues of mitochondrial aconitase which are conserved in IRP1, (Philpott *et al.*, 1993) are also involved in the binding of Fe in the $[\text{4Fe-4S}]$ cluster (Hirling *et al.*, 1994). The $[\text{Fe-S}]$ cluster has been known to play a crucial role in regulating the enzymatic activity of mitochondrial aconitase as well as IRP1 (cytosolic aconitase). The holo-form of IRP1 contains a $[\text{4Fe-4S}]$ cluster and has aconitase activity but cannot bind to IREs. On the other hand, when the $[\text{Fe-S}]$ cluster is disassembled, IRP1 loses aconitase activity and acquires IRE binding activity (Sutak *et al.*, 2008). Thus, the aconitase and RNA binding activities of IRP1

are mutually exclusive (Kim and Ponka, 2000). IRP2, a second IRE-binding protein, which was initially identified in rat hepatocytes, had been cloned from a variety of mammalian tissues and cells subsequently (Guo *et al.*, 1994). Kim and Ponka (2000) showed that IRP2 shares 62% amino acid sequence identity with IRP1 but differs in a unique way; possessing a 73-amino acid insertion in its N-terminal region and lacks the [Fe-S] cluster. This additional region contains a cysteine-rich sequence that is known to be responsible for targeting the protein for degradation via the ubiquitin-proteasome pathway when cellular Fe levels are high (Guo *et al.*, 1995; Iwai *et al.*, 1995, 1998). IRP2 does not have aconitase activity, probably due to the absence of the [Fe-S] cluster (Kim and Ponka, 2000). Observations from the study by Richardson and Milnes (1997) showed that cellular iron is an important factor in the interactions of IRPs with IREs which subsequently affect the regulation of iron metabolism. When cellular iron becomes depleted, IRP2 remains in the cytosol and IRP1 is converted into the high affinity binding state. The binding of IRPs to the IRE in the 5'-UTR of ferritin mRNA blocks the translation of ferritin, whereas the association of IRPs with IREs in the 3'-UTR of TfR mRNA stabilizes this transcript. On the other hand, when intracellular iron is abundant, IRP1 is unable to bind to IREs and IRP2 is degraded, resulting in efficient translation of ferritin mRNA and rapid degradation of TfR mRNA (Kim and Ponka, 2000). It has also been reported that iron may not be the only player that modulates IRP1 binding activity, IRP2 levels and, consequently, transferrin receptor and ferritin expression. A recent study by Richardson and Lok (2008) revealed that Nitric Oxide (NO) also plays a key role in RNA binding activities of both IRP1 and IRP2.

The neuronal nitric oxide synthase (nNOS) isoform was the first to be purified (Bredt *et al.*, 1991). It is also known as type I NOS (nNOS or NOS-1). Shortly thereafter inducible NOS, also known as type II NOS (iNOS or NOS-2; (Nathan, 1995) and then by endothelial NOS or type III NOS (eNOS or NOS-3) (Bian and Murad, 2003) were identified and purified. NOS converts L-arginine to L-hydroxyarginine and subsequently to nitric oxide and citrulline. The NOS enzyme catalyzes the 5 electron oxidation of one N-atom of the guanidino of L-arginine to NO and L-citrulline through the cofactors including: NADPH; Flavin Adenine Dinucleotide (FAD); flavin mononucleotide (FMN) and tetrahydrobiopterin (H₄B). The endothelial and neuronal isoforms of NOS are expressed constitutively (cNOS) and the production of NO by cNOS is regulated on a moment-to-moment basis by calmodulin binding, triggered by transient elevations in intracellular-free calcium levels (Bredt, 1999; Ignarro *et al.*, 1999). In contrast, NOS-2 is unique because it requires de novo synthesis in most cells and upon exposure to stimuli such as endotoxin (LPS) and proinflammatory cytokines, it is rapidly expressed. This results in the production of much larger quantities of NO relative to the two other isoforms. It is widely distributed in various cell types (Gottesfeld *et al.*, 1998; Weisbrodt *et al.*, 1999). NOS-2 or iNOS does not appear to be present under normal conditions in most cells. The activation of NOS-2 or iNOS and the subsequent production of large amounts of free radical gas NO has been viewed as an important anti-infectious and anti-tumor mechanism of innate immunity (Stamler *et al.*, 1992; Marletta, 2001; Bian and Murad, 2003). Also, overproduction of NO has been implicated in several pathological conditions including tissue injury and various inflammatory disorders (Abramson *et al.*, 2001; Calatayud *et al.*, 2001); neuronal disease (Heneka and Feinstein, 2001); auto-immune diseases (Amin *et al.*, 1999; Liu *et al.*, 2001); cancer or tumor cell proliferation (Aaltoma *et al.*, 2001; Kagoura *et al.*, 2001; Kong *et al.*, 2001; Kondo *et al.*, 2002); angiogenesis and related pathological changes (Wilson, 2002; Franchi *et al.*, 2002) and diabetes mellitus (Johannesen *et al.*, 2001; Morris *et al.*, 2002) amongst others. Thus, selective inhibition of NOS-2 or iNOS may have therapeutic potential for treatment

of conditions mediated by the overproduction of NO (Bian and Murad, 2003). Earlier studies have revealed that NO exists in a variety of redox states which may be responsible for its diverse effects on a variety of molecular targets (Pantopoulos *et al.*, 1996; Kim and Ponka, 2002; Richardson and Lok, 2008) in the biological systems. The reduced form of Nitric Oxide (NO) also known as NO radical (NO^\bullet) can form complexes with iron. The binding of NO^\bullet radical, can modulate iron-containing proteins by direct coordination to iron centers and in the presence of superoxide anion ($\text{O}_2^{\bullet-}$), it produces peroxynitrite (ONOO^-) which has cytotoxic effect. The second important species of NO, the nitrosonium ion (NO^+), can nitrosylate thiol groups of proteins, a modification that may have important regulatory functions (Vanin *et al.*, 1967; Vanin, 1991; Mohr *et al.*, 1994; Pantopoulos *et al.*, 1996). The NO has two principal roles, which include regulation of important physiological functions in cells and cytotoxicity. Under physiological conditions, production of NO in small amounts mediates vasorelaxation and controls the adhesion and aggregation of platelets and neutrophils. Also this molecule is involved in neurotransmission (Vanin, 1998). Most of these actions are mediated via the binding of NO to iron in the heme prosthetic group of soluble guanylate cyclase, which catalyzes the conversion of GTP to cyclic GMP (Vanin *et al.*, 1998). The importance of iron in mediating the functions of NO is also apparent when examining the cytotoxic effects of the molecule. When produced in larger quantities by activated macrophages, hepatocytes, Kupffer cells and other cells following exposure to cytokines or microbial products, NO may become cytotoxic (Lepoivre *et al.*, 1991). NO produced via such high-output systems inhibits proliferation of intracellular pathogens and tumor cells (Lee *et al.*, 1994; Griscavage *et al.*, 1994). These effects can be explained by the reactivity of NO radical (NO^\bullet) with iron in the [Fe-S] centers of several important macromolecules, including aconitase and complex I and complex II of the electron transport chain (Ignarro, 1991). The high affinity of NO^\bullet radical for iron probably results in both the removal of iron from [Fe-S] centers and the formation of nitrosyl-iron species within [Fe-S] proteins. It is important to note that, the interaction of NO with intracellular iron-containing proteins is vital for its many diverse effects (Richardson, 2005; Richardson and Lok, 2008). Vanin *et al.* (1967, 1998) and Richardson and Lok (2008) showed that the NO^\bullet radical forms intracellular complexes within cells and this plays important roles in biological processes. Apart from the formation of heme-NO complexes in guanylate cyclase, previous reports revealed that effect of NO is also mediated, at least in part, due to its interaction with iron containing proteins which, include:

- The rate-limiting enzyme in DNA synthesis, ribonucleotide reductase (Lepoivre *et al.*, 1991)
- The iron storage protein, ferritin (Lee *et al.*, 1994)
- Heme-containing proteins (Ignarro, 1991; Khatsenko *et al.*, 1993; Griscavage *et al.*, 1994)
- The [Fe-S] cluster enzyme ferrochelatase that catalyses the final step in the heme synthesis pathway
- Other [Fe-S] cluster proteins involved in energy metabolism such as mitochondrial aconitase and those in complex I and II of the electron transport chain (Drapier and Hibbs, 1986, 1988)
- The [Fe-S] cluster containing molecule, iron-regulatory protein-1 (IRP1), that plays a role in regulating iron homeostasis (Drapier *et al.*, 1993; Weiss *et al.*, 1993; Richardson *et al.*, 1995a; Wardrop *et al.*, 2000; Cairo *et al.*, 2002; Soum *et al.*, 2003; Richardson and Lok, 2008)

The production of NO also have been reported to induce over-expression of heme catabolism enzyme heme oxygenase which hitherto may contributes toward expanding labile iron pool concentration (Chenais *et al.*, 2002).

According to reports by Richardson and Ponka (1996), the high affinity of NO for iron depicts that it will affect the activity of many iron-containing proteins and a significant example of NO interaction with iron-containing molecules is its effect on IRP1 that is known to play a role in the regulation of iron metabolism (Cairo *et al.*, 2002). Various studies have been able to show that, NO can in deed activate the RNA-binding activity of IRP1 which plays a role in the homeostatic regulation of cellular iron metabolism (Drapier *et al.*, 1993; Weiss *et al.*, 1993; Richardson *et al.*, 1995b; Richardson and Lok, 2008). It also has been demonstrated that NO increases the size of the Labile Iron Pool (LIP) (Kim and Ponka, 2002; Ramachandran *et al.*, 2004; Lipinski *et al.*, 2005). The NO-mediated iron release is physiologic, as observed in the interaction of activated macrophages with tumour cells and other target cells (Nestel *et al.*, 2000). NO can enter cells and act to some degree like a chelator by binding iron and inducing its release (Watts and Richardson, 2001, 2004). Recent studies by Richardson and Lok (2008) demonstrated that NO mechanism for cellular iron release may be glutathione (GSH) - and energy-dependent and involves the uptake and metabolism of D-glucose (D-Glc) (Watts and Richardson, 2001, 2002). These authors in their studies reported that only sugars that can be taken up and metabolised by cells were effective at increasing NO-mediated Fe release (Watts and Richardson, 2001). Glucose enters the cell by the well characterised family of glucose transporters (Joost and Thorens, 2001) and is subsequently phosphorylated to glucose-6-phosphate (G-6-P) (Watts *et al.*, 2003). Glucose-6-phosphate is metabolised by two major pathways, either through glycolysis and/or the Tricarboxylic Acid Cycle (TCA) to generate ATP, or through the pentose phosphate pathway (PPP; that is also known as the hexose monophosphate shunt) to form reduced NADPH (e.g., for GSH synthesis) and pentose sugars (Watts and Richardson, 2004). These studies further demonstrated that D-Glc uptake and metabolism by the PPP was essential for NO-mediated iron release (Watts and Richardson, 2001). However, significant depletion of GSH using the specific GSH synthesis inhibitor, buthionine sulfoximine (BSO) (Griffith and Meister, 1979), prevented NO mediated iron release from cells (Watts and Richardson, 2001, 2002, 2004). Furthermore, reported by Kim and Ponka (2002) revealed that production of nitric oxide promotes the expression of heme oxygenase mRNA which also contribute toward free iron mobilization from heamoglobin degradation. It is possible that overproduction of NO leads to generation of free radicals making the erythrocytes more liable to lysis which thus flood the heamoglobin degradation pathway. Under normal physiological condition, relationship between iron and nitric oxide operates like an auto loop (Kim and Ponka, 2002; Watts *et al.*, 2006); when the concentration of iron is high, the expression of nitric oxide synthase is repressed and vice versa. This corroborates an earlier observation by James (1995), which revealed that iron chelators up-regulates the expression of nitric oxide synthase. While there appear to be no dispute in the relationship between iron and nitric oxide balance under normal physiological condition as had been studied and reviewed by several authors (James, 1995; Kim and Ponka, 2000, 2002, 2003; Watts *et al.*, 2003; Watts and Richardson, 2001, 2002, 2004; Lipinski *et al.*, 2005; Richardson and Lok, 2008). It is not clear if the same relationship holds for pathogen-infected status. Studies evaluating the effects of nitric oxide production on iron homeostasis in the presence of an infection by parasites may be required. Such clarification may be necessary to understanding the host-parasite interaction as it affects iron homeostasis. It is possible that chronic production of nitric oxide by iNOS secondary to infection by pathogens contributes to the expansion of labile iron pool with the consequence of promoting parasite proliferation which further elicit host defense response and thereby loosen the control of iron level on nitric oxide synthase expression (Kim and Ponka, 2002; Richardson and Lok, 2008).

Iron, nitric oxide and *Trypanosoma brucei*: Iron is essential for living cells, including pathogenic microorganisms. So, altered iron availability is often a key component in the host-microorganisms interplay. Consequent upon which iron chelation therapy has been proposed in the control of infectious diseases. Several studies have demonstrated that pathogens require iron to establish their infection in the host. Ekanem *et al.* (2005) suggested that withholding unbound iron via the use of exogenous iron chelating agents could have deleterious effects on the growth of these pathogens. Like all living organisms, trypanosomes require iron for growth (Merschjohann and Steverding, 2006). Iron plays a pivotal role in the synthesis of DNA in trypanosomal system through the enzyme ribonucleotide reductase (Ekanem *et al.*, 2005). The delivery of iron into bloodstream forms of *Trypanosoma brucei* is mediated by host transferrin that is taken up via a unique receptor. The transferrin receptor of *T. brucei* is a heterodimeric complex which shows no homology to the homodimeric mammalian transferrin receptor (Chaudhri *et al.*, 1994; Salmon *et al.*, 1994; Fast *et al.*, 1999). If a cellular system is starved of iron, the enzyme ribonucleotide reductase becomes inactivated (Barrett *et al.*, 2007). Earlier studies have revealed that growth of cells even those of trypanosomes can be successfully inhibited by depriving the cells of iron (Ekanem *et al.*, 2005; Merschjohann and Steverding, 2006). And indeed iron chelation has been reported to inhibit ribonucleotide reductase (Lonsdale-Eccles and Grab, 1987; Ekanem *et al.*, 1996; Barrett *et al.*, 2007) and the enzyme has been suggested as a prime target for controlling African trypanosomiasis (Clayton *et al.*, 1980; Ekanem *et al.*, 2005). Macrophages play a central role in the anti-parasitic defense, mainly due to the production of Nitric Oxide (NO) (Silva *et al.*, 2003). The NO is a diffusible messenger which has been implicated in different physiological functions, including vasodilation, inhibition of platelet aggregation, neurotransmission and immune regulation (Mariotto *et al.*, 2004), among others. In infectious or inflammatory conditions, large amounts of NO can be produced after the induction of inducible NOS (iNOS) in different cell types, notably macrophages. During infection involving protozoa parasites, NO production, measured by its end products nitrite (NO_2^-) plus nitrate (NO_3^-) in biological fluids and culture supernatants, increased early after the infection and remains high throughout infection (Petray *et al.*, 1994; Vespa *et al.*, 1994; Naviliat *et al.*, 2005; Ekanem *et al.*, 2009). Although, we have seen evidences from various authors on the mechanisms by which nitric oxide effect iron release but these studies mostly are carried in non-infective states or condition. And while one may want to extrapolate these data to support the observations by Ekanem *et al.* (2005, 2009) and also from our laboratory (not published) that production of nitric oxide in *T. brucei*-infected animals correlated with increased iron status, further clarifications may still be necessary to work out the mechanisms involved. Available data from various authors support a close link between Nitric Oxide (NO) production and cellular iron homeostasis. Nitric oxide generation by inducible nitric oxide synthase (iNOS) enzyme may contributes to increased iron status in experimental infection with *T. brucei* [as observed by Ekanem *et al.* (2005, 2009)] by any of the afore mentioned mechanisms. This process of iron release essentially may promote proliferation of invading parasites and thus necessitating therapy that include iron chelation and selective inhibition of iNOS. Further studies to demonstrate the relevance of NO generation to iron metabolism in infective states or conditions are urgently needed to bridge the gap.

IMPLICATION

There is a chain of events in the susceptibility of host tissues to infection that largely or entirely depends upon the availability of free iron accessible to the pathogens. Natural resistance to

infection operates in an environment where the amount of freely available iron is extremely low. Recent studies from our laboratory (not published) have demonstrated increased iron status in *Trypanosoma brucei* infected animals when compared to their uninfected counterparts. This observation corroborates previous reported by Ekanem *et al.* (2005) and (2009). Recently, Ekanem *et al.* (2009) reported that increased iron status correlated with increased Nitric Oxide (NO) production in experimental infection with *T. brucei*. From the foregoing evidence available from previous reports, one may speculate that increases in iron status observed in *T. brucei* infected animals might have been precipitated by production of Nitric Oxide (NO) secondary to infection by the pathogen. But what is the mechanism by which the generated NO lead to increased free iron pool? Data available from previous reports only revealed links between nitric oxide and iron homeostasis in a non-infective condition. Studies revealing such links between the duo in the presence of an infection by pathogens are needed. Nevertheless looking at available data; it is plausible to hypothesize that production of Nitric Oxide (NO) by inducible nitric oxide secondary to *T. brucei* infection may increase iron status thereby promoting parasite proliferation and subsequently aggravates infection. It is therefore imperative to suggest that employing selective inhibition of inducible nitric oxide synthase (iNOS) and/or use of iron chelators to remove free iron from the biological system may be relevant in parasite chemotherapy. While some school of thought advanced that, the host possess a natural iron withholding system that keeps free iron away from invading pathogens, however as we have been able to summarise here from previous studies; pathogen invasion induces nitric oxide production which is a normal process secondary to infection. On the hand, the NO so produced among its effector functions may mobilize for cellular iron release which may expand the labile iron pool and thus aggravate infection-related conditions by promoting parasite proliferation beside the generation of free radicals which also may cause cellular damage. Another implication of a sustained or prolonged generation of Nitric Oxide (NO) is that it could cause release of free iron and hence expands the labile iron pool. The expanded pool of free iron may thus become available to invading parasites thereby promoting proliferation of parasite in a process suspected to involve ribonucleotide reductase (Ekanem *et al.*, 2009). More so correlation has been reported between reduced parasitaemia, iron levels and nitric oxide levels in experimental infection by *T. brucei* following treatment with iron chelators. One may be justified to extrapolate data from these foregoing studies to support the assertion that; NO production subsequent to infection may lead to increases in free cellular iron status. Nonetheless studies are required to examine whether effects of nitric oxide on iron metabolism in the presence and absence of an infection are the same. Further studies to (1) establish the relationship between iron homeostasis and nitric oxide production in the presence of an infection by African trypanosomes and (2) examine the mechanism of iron release in the presence of an infection by pathogens, are suggested as this might lead to the development of novel chemotherapeutic strategies.

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