Hypothesis

Does myoglobin protect *Trypanosoma cruzi* from the antiparasitic effects of nitric oxide?[1]

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Abstract The hemoflagellate protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas disease, a progressive fatal cardiomyopathy widespread in South and Central America. Here, we postulate that the preferential colonization of cardiomyocytes by *T. cruzi* may reflect the role of myoglobin (Mb) as a nitric oxide (NO) scavenger, protecting the parasite from the trypanocidal effects of NO. The proposal of this novel function of Mb is based on knowledge that ferrous oxygenated Mb reacts rapidly and irreversibly with NO yielding nitrate and ferric oxidized Mb, which is reduced back to the physiologically active form by an intracellular reductase. The postulated protective role of Mb on the viability of *T. cruzi* is reminiscent of that postulated for hemoglobin in protecting intraerythrocytic *Plasmodia* from the parasiticidal effect of NO.

Key words: Myoglobin; Nitric oxide; *Trypanosoma cruzi*

The hemoflagellate protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease, afflicts more than 20 million people in Central and South America. A much larger population is considered at risk worldwide. Acute Chagas disease is a generalized infection with lesions located anywhere in the mammalian host, consisting of parasite-related focal lesions. However, acute Chagas disease is only rarely fatal in naturally infected humans (5% or less). The majority of infected people in endemic areas present the intermediate elusive form of Chagas disease; a fraction of these (approximately 30%) will evolve to the progressive fatal chronic cardiac form. Remarkably, Chagas disease, for which no therapy is currently available, represents the main cause of heart attack among infected people [1].

T. cruzi has a complex life cycle, involving a triatomine bug as hematophagous vector and a mammalian host. It is usually transmitted to humans by contamination of abraded skin, with the infective, penetrative trypomastigote forms that occur in the feces of infected bugs. Having gained access to the mammalian host, trypomastigotes penetrate non-phagocytic or phagocytic cell lines, transform to the amastigote stage which multiplies, and eventually emerge from ruptured cells as trypomastigotes. Parasites penetrate again in cells to renew the cycle of intracellular division. A fraction of trypomastigotes may circulate in the blood to be picked up by triatomine bugs taking a blood meal. Transmission by transfusion of infected blood and by renal or heart transplantation is taken as epidemiologically significant [1].

Nitric oxide (NO) has been reported to display antiparasitic activity [2]. Macrophages from *Trypanosoma*-infected mice produce high levels of NO to kill developing trypomastigotes [3–5]. Levels of NO and NO metabolites increase during *T. cruzi* invasion of cardiomyocytes [6] which express the main isoforms of NO synthase [7,8]. Consistently, an inverse relationship has been observed between trypanosomiasis and NO levels, in vivo and in vitro [9–11]. Moreover, susceptibility to *T. cruzi* in mice deficient in inducible NO synthase [12] increases significantly. Treatment of infected mice with inhibitors of inducible NO synthase has adverse effects, while NO donors kill *T. cruzi* trypomastigotes [9,10]. Furthermore, NO has been reported to play a role in apoptosis induction during the acute phase of *T. cruzi* infection in mice [13], in the reduction of the host immune response [13] and in parasite cell influx, contributing to the pathogenesis of chagasic cardiomyopathy [14,15].

NO may affect *T. cruzi* by chemical modification of cysteine-containing proteins and/or by binding to metalloproteins. Recently, the inhibitory effect of NO or NO donors on the catalytic activity of cruzipain, the major papain-like cysteine proteinase from *T. cruzi*, has been reported. This dose-dependent effect was attributed to *S*-nitrosylation of Cys^{25}, a catalytic residue present in the active site of cruzipain [16]. Ribonucleotide reductase inhibition has been suggested to account for the cytostatic effect of NO on *T. brucei gambiense* and *T. brucei brucei*. In addition the antiparasitic effect of NO has been also attributed [2] to inhibition of aconitase, aldolase, cytochrome *c* oxidase and cytochrome P450.

Tissue tropism of *T. cruzi* varies and multiplication may

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[1] This paper is dedicated to the memory of Matti Saraste.

Abbreviations: Mb, myoglobin; deoxyMb, ferrous deoxygenated Mb; MbO_2, ferrous oxygenated Mb; MbNO, ferrous nitrosylated Mb; metMb, ferric oxidized Mb; metMbNO, nitrosylated metMb

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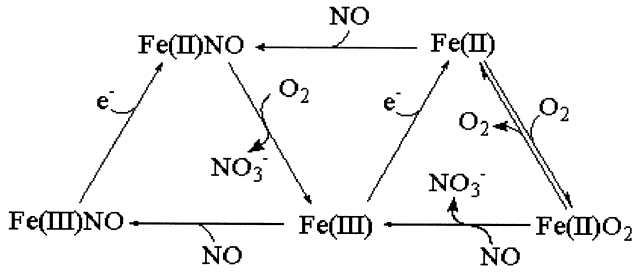

Fig. 1. The reactions of Mb with O_2 and NO. The reversible binding of O_2 to deoxyMb (Fe(II)) yields MbO_2 (Fe(II)O_2) involved in O_2 transport and storage [22]. MbO_2 (Fe(II)O_2) reacts rapidly with NO to yield nitrate (NO_3^-) and metMb (Fe(III)) [20]. Moreover, deoxyMb (Fe(II)) binds NO yielding MbNO (Fe(II)NO) which in the presence of O_2 may be converted to metMb (Fe(III)) and NO_3^- [23]. These reactions quench free NO, which may inactivate cysteine-containing and metallo-enzymes [2,16,24]. NO detoxification facilitated by Mb may thus protect *T. cruzi* colonizing the cardiomyocytes from the adverse effects of NO. NO may bind also to metMb (Fe(III)) yielding metMbNO (Fe(III)NO), which in the presence of metMb reductase and O_2 may be converted to metMb (Fe(III)) and NO_3^-. However, this process does not appear of physiological relevance, being thermodynamically and kinetically unfavorable [19,23–25].

occur in various non-phagocytic and phagocytic cell types. However, in mammals there seems to be a general preference for muscle, especially heart but also skeletal and smooth muscle [1]. Although factors controlling tissue tropism are poorly understood, it has been suggested that cardiomyocyte mannose receptors localized at the sarcolemma mediate recognition and can be down-modulated by parasite infection [17,18]. Interestingly, the preference of *T. cruzi* for cardiomyocyte infection is positively correlated with the severity of the lesions in the heart, generally more diffused than in other organs [1].

Here, we postulate that the preferential colonization of the heart and skeletal muscle by *T. cruzi* depends on the role of myoglobin (Mb) as a NO scavenger, protecting cellular respiration [19]. The proposal of this novel function of Mb is based on knowledge that ferrous oxygenated myoglobin (MbO_2) reacts rapidly and irreversibly with NO yielding nitrate and ferric oxidized Mb (metMb) [20], which is reduced back to the physiologically active form by an intracellular reductase [21]. Accordingly, the cycle may repeat over and over again and the scavenging effect may be considered 'pseudo-enzymatic' (Fig. 1). Given the high concentration of Mb in the heart and skeletal muscle (0.2–0.4 mM), this reaction should efficiently intercept NO and thereby reduce or abolish NO-related pathophysiological effects. Since NO was shown [26,27] to be a potent (albeit reversible) inhibitor of cytochrome *c* oxidase, the terminal enzyme of the mitochondrial respiratory chain, the scavenging function of Mb may lead to effective protection of cellular respiration and thus maintain energy production in red muscle [19,28]. Along the same line of thought, we propose that Mb may protect *T. cruzi* from the adverse effects of NO, including eventually inhibition of the respiratory chain of the parasite, and thereby explain its preferential localization in the cardiomyocytes.

This hypothesis has some predictive value. First of all, it is expected that the severity of the chagasic heart disease would significantly increase if tested with Mb knock-out mice which display a benign phenotype [29,30]. Second, it may stimulate an investigation to find out if *T. brucei* (the parasite responsible for sleeping disease) is preferentially localized in the brain areas which express the newly discovered neuroglobin [31]. This Mb-like hemoprotein, expressed predominantly in the frontal lobe, the subthalamic nucleus and the thalamus, is likely to be irrelevant in O_2 transport because of its low concentration (submicromolar), but may be involved in NO metabolism. Moreover, it may demand some explanation to account for the reported localization of *T. cruzi* in smooth muscle fibers [1], which are known to express Mb to a very small extent [32]. In these cells, a role of Mb in NO metabolism is possible but this hypothesis needs additional investigation. In this respect, it is of interest that some prokaryotes and unicellular eukaryotes encode NO-inducible flavo- and truncated hemoglobins that act as effective NO scavengers to escape host defenses and to survive in infected macrophages [33,34].

An additional consideration may be in order. *Plasmodium falciparum* is known to be killed by NO [35], and the mosquito *Anopheles stephensi*, a natural vector of human malaria, limits parasite development with inducible synthesis of NO [36]. Therefore, it seems likely that NO exerts an antiparasitic activity in both the vector and the mammalian hosts. The postulated protective effect of MbO_2 on *T. cruzi* colonizing cardiomyocytes may therefore be analogous to that assigned to oxygenated hemoglobin in protecting intraerythrocytic *Plasmodia* from the parasiticidal effect of NO [37].

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