Reactivity of the Labile Iron with Peroxynitrite

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INTRODUCTION.Varying concentrations of the labile iron pool (LIP) is present in all cell types and it isknown to reduce hydrogen peroxide to hydroxyl radical, causing oxidative stress. However, the reaction of LIP with other peroxides and their consequences have never been investigated. We set a model using murine macrophage cells and oxidizable fluorescent probes to investigate the possible reaction of LIP and peroxynitrite(ONOOH/$ONOO^-$), an important reactive species formed by the diffusion controlled recombination of nitric oxide ($NO^\bullet$) and superoxide ($O_2^{\bullet-}$). OBJETIVES.The aim of the proposal is to study the possible reaction between peroxynitrite and LIP. MATERIAL AND METHODsRaw 264.7 macrophage cells (ATCC) were treated with 30 µM H_2DCF -DA for 30 min in PBS and exposed to peroxynitrite generated by the "peroxynitrite donor" SIN-1(250 µM) or the combination of a $NO^\bullet$ donors [sper/NO (20 µM) or Deta/NO (1000µM)]and the redox cycling compound/ $O_2^{\bullet-}$ generator paraquat(20 µM). The reaction of LIP towards peroxynitrite was evaluated by comparisons of the intracellular H_2DCF oxidation rate in control cells with cells where LIP was removed by the known, strong iron/LIP chelator SIH. The oxidation of intracellular H_2DCF was followed in real time by fluorescence spectroscopy using conventional ormultiwell plate reader instruments. RESULTS AND DISCUSSION. The peroxynitrite dependent oxidation of the intracellular H_2DCF probe increases considerably in presence of SIH (≅ 100 %), suggesting that LIP reacts with peroxynitrite rapidly and produces non-oxidant species under the two protocols tested. CONCLUSIONS.LIP may reduce ONOOH/$ONOO^-$ and/or its derived oxidant species ($NO_2^\bullet$, $CO_3^{\bullet-}$ and $OH^\bullet$) to non-oxidant species;LIP may protect macrophage cells from peroxynitrite and its derived oxidants;The finding may change the generalized concept that LIP is just a pro-oxidative cellular iron source.

KEYWORDS. Labile Iron pool, chelatable iron pool, superoxide, nitric oxide, peroxynitrite, oxidative stress, oxidation.
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