INTERCELLULAR DIFFUSION OF MACROPHAGE-DERIVED NITRIC OXIDE AND PEROXYNITRITE.

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Macrophage-derived nitric oxide (·NO) has cytotoxic effects mediated either directly by ·NO or its derived species such as peroxynitrite, arising from the reaction of ·NO with superoxide radical ($O_2^{\cdot-}$). To propose a predominant ·NO-dependent cytotoxicity mechanism, the different diffusion properties of ·NO and peroxynitrite should be considered as they will affect their radii of action. Herein, we evaluated how the diffusion of ·NO derived from activated RAW 264.7 macrophages is modulated by the simultaneous formation of $O_2^{\cdot-}$. Our experimental model consists in the co-incubation of differentially activated macrophages for the production of ·NO, $O_2^{\cdot-}$ or both (and hence peroxynitrite) with red blood cells (RBC) as targets. Diffusion of ·NO/peroxynitrite to the RBC was evaluated as intracellular oxyhemoglobin oxidation and nitrosylhemoglobin formation. Our results showed that: i) oxyhemoglobin oxidation yields obtained with ·NO + $O_2^{\cdot-}$ are smaller than with only ·NO and are dependent on diffusion distances; ii) nitrosylhemoglobin was partially decreased when macrophages also produced $O_2^{\cdot-}$**,** indicating that diffusion of ·NO to RBC only partially outcompetes peroxynitrite formation and iii) macrophage-derived peroxynitrite can diffuse and reach target cells. Computer assisted-simulations were performed and are on line with the experimentally-obtained results. Our data supports an *in vivo* scenario where the cytotoxic effects of ·NO will be strongly influenced by the intercellular diffusion distances and by the concomitant formation of $O_2^{\cdot-}$.