TEMPOL MODULATES OXIDATIVE BURST AND MICROBICIDAL ACTIVITY OF HUMAN NEUTROPHILS AGAINST Mycobacterium tuberculosis

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ABSTRACT

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), remains as serious public health problem in least-developed countries. The innate immune response following Mtb infection plays a crucial role in preventing the onset of active TB. Phagocytes-derived reactive oxygen species (ROS) are essential for an effective response against Mtb. However, the indiscriminate use of antioxidant supplements or their use as adjuvant therapy during TB treatment can decrease ROS levels, thus, potentially increasing the host's susceptibility to Mtb/TB. This study investigated the ex vivo effect of the cyclic nitroxide tempol (4-hydroxy-2,2,6,6-tetra-methyl-1-piperidinyloxy), an antioxidant with superoxide dismutase mimetic properties, on neutrophils activity against Mtb (type-strain H37Ra/ATCC 35177). Human neutrophils were isolated from venous blood of healthy volunteers by Ficoll density gradient centrifugation (Ethical approval CAAE17064713.0.0000.5142). To evaluate the neutrophil oxidative burst triggered by heat-inactivated Mtb (multiplicity of infection=10), total and extracellular ROS were determined using the luminol- and Isoluminol-amplified chemiluminescence method. The microbialic activity of neutrophils was evaluated through the microbial killing assay ("two-step" protocol) and rate constants for phagocytosis (k_p) and intracellular Killing (k_k) were calculated. Treatment of neutrophils with 450 µM tempol significantly decreased Mtb-induced ROS generation (total and extracellular ROS, p < 0.05). Furthermore, this concentration was also able to reduce neutrophils microbicidal activity, since colony-forming units of Mtb in the treated group were significantly higher than those for the untreated group. Interestingly, tempol decreased the k_k of neutrophils, but had no effect on their k_p. To modulate ROS generation and microbicidal activity in Mtb-stimulated neutrophils, 450 µM tempol was equivalent to 32 µM diphenyleneiodonium. Notably, viability of neutrophils exposed to 450 µM tempol was equivalent to non-exposed. This study provides insights of the influence of antioxidants on the immune response to Mtb, so that clinical implications for the prevention and treatment of TB should take into account these findings.

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Key Words: Tempol; Mycobacterium tuberculosis; Neutrophils.