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The Effects of Molecular Hydrogen and Suberoylanilide Hydroxamic Acid on Paraquat-Induced Production of Reactive Oxygen Species and TNF-α in Macrophages

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Abstract

The aim of this study is to investigate the effects of molecular hydrogen (H_2) and suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, on paraquat (PQ)-stimulated production of reactive oxygen species (ROS) and tumor necrosis factor alpha (TNF-α) in macrophages. First, the PQ optimal concentration was determined in RAW264.7 macrophage by treating serum-starved cells with PQ at 0, 0.001, 0.01, 0.1, 1, and 10 mM. We evaluated at 1, 2 and 8 h (1) cell viability (by means of trypan blue exclusion method), (2) intracellular ROS levels (with a fluorescent DCFH-DA probe), and (3) TNF-α level in the culture media (determined by enzyme-linked immunosorbent assay, ELISA). Subsequently, mouse RAW267.4 macrophages were treated with PQ in combination with SAHA and/or H_2 for 8 h. PQ exerted a significant stimulatory but nontoxic effect on RAW267.4 macrophages at 0.1 mM. This PQ concentration was used in the subsequent experiments. H_2 and H_2 combined with SAHA evoked a greater reduction in PQ-induced ROS production than SAHA alone, especially at 2 and 8 h. At 1 and 2 h, treatments involving H_2 caused a greater decrease in PQ-induced production of TNF-α than the corresponding treatments without H_2. However, at 8 h, treatment with SAHA evoked more pronounced effects on TNF-α than treatment without SAHA. H_2 decreases PQ-induced ROS production and attenuates early PQ-induced TNF-α production whereas SAHA reduces the late phase of the PQ-induced TNF-α production in macrophages. The effects are enhanced by the combination of H_2 and SAHA.

Keywords: SAHA; TNF-α; hydrogen; macrophage; paraquat; reactive oxygen species.

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