



Short Communication

Thiol-reactivity of the fungicide maneb

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ABSTRACT

Maneb (MB) is a manganese-containing ethylene bis-dithiocarbamate fungicide that is implicated as an environmental risk factor for Parkinson's disease, especially in combination with paraquat (PQ). Dithiocarbamates inhibit aldehyde dehydrogenases, but the relationship of this to the combined toxicity of MB + PQ is unclear because PQ is an oxidant and MB activates Nrf2 and increases cellular GSH without apparent oxidative stress. The present research investigated the direct reactivity of MB with protein thiols using recombinant thioredoxin-1 (Trx1) as a model protein. The results show that MB causes stoichiometric loss of protein thiols, reversibly dimerizes the protein and inhibits its enzymatic activity. MB reacted at similar rates with low-molecular weight, thiol-containing chemicals. Together, the data suggest that MB can potentiate neurotoxicity of multiple agents by disrupting protein thiol functions in a manner analogous to that caused by oxidative stress, but without GSH depletion.

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Introduction

Maneb (MB), a manganese-containing dithiocarbamate fungicide, is used in agriculture to treat a variety of crop pathologies. Epidemiological studies associate repeated human exposure to agricultural chemicals with increased risk for Parkinson's disease (PD) [1,2] and recent reports indicate that occupational and/or residential exposure to MB significantly increases the risk of developing PD [3–5]. Rodent models of pesticide-mediated PD show that combined effects of paraquat (PQ) and MB exposure leads to deficits in motor function, loss of tyrosine hydroxylase staining in the substantia nigra, and altered striatal dopamine metabolism [6,7]. Mechanistic studies showed that the increased toxicity of PQ + MB could be due, at least in part, to altered toxicokinetics of PQ in mice exposed to MB [8].

In a recent *in vitro* study, we obtained evidence that MB and PQ operate through divergent mechanisms of toxicity [9]. Results showed that PQ acted via a mechanism involving reactive oxygen species (ROS) while MB did not. MB did not increase ROS production or oxidize thiol-containing antioxidants (thioredoxin and peroxiredoxin), but did activate Nrf2. Hong et al. have reported a strong correlation between alkylation of critical Cys residues on Keap-1 and the potency of Nrf2 activation [10]. This report and our data lead to the speculation that MB could be a direct thiol-modifying agent causing Nrf2-Keap-1 dissociation, nuclear translocation and gene transcription. Currently, there is no explicit evidence demonstrating that MB modifies Cys

residues, e.g. Keap-1. Therefore, the purpose of this study was to characterize the thiol binding activity of MB utilizing N-acetylcysteine (NAC) and thioredoxin-1 (Trx1) as model thiol containing agents.

Materials and methods

Materials

Recombinant human thioredoxin-1 protein was obtained from Lab Frontier (Korea). All other reagents were obtained from Sigma.

Reactivity of MB with free thiol

To investigate thiol reactivity of MB, we incubated MB (0–100 μ M) with 50 μ M NAC for 15 min at 37 °C and measured free thiols using Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) as described [11].

Reactivity of MB with protein-bound thiols

To assess the ability of MB to bind thiols in proteins, we utilized Trx1 as a model protein. Briefly, Trx1, in its fully reduced state [12], was incubated with increasing concentrations of MB for 1 h at 37 °C. Following MB incubation, samples were desalted using a spin column (Pierce) and the free thiols were labeled using mPEC₂-biotin (Pierce), a biotinylated N-ethylmaleimide compound. The samples were then separated via SDS-PAGE, and biotinylated protein was visualized by Western blotting with fluorescently labeled streptavidin. Reactivity of MB to primary amines was also assessed using sulfo-NHS-biotin (Pierce) and application of streptavidin blotting.

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