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SCIENCE DIRECT

Toxicology and Applied Pharmacology 212 (2006) 89 – 98

Toxicology and Applied Pharmacology

www.elsevier.com/locate/ytaap

Molecular mechanisms underlying mancozeb-induced inhibition of TNF-alpha production

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Received 1 April 2005; revised 5 July 2005; accepted 6 July 2005
Available online 19 August 2005

Abstract

Mancozeb, a polymeric complex of manganese ethylenebisdithiocarbamate with zinc salt, is widely used in agriculture as fungicide. Literature data indicate that ethylenebisdithiocarbamates (EBDTCs) may have immunomodulatory effects in humans. We have recently found in agricultural workers occupationally exposed to the fungicide mancozeb a statistically significant decrease in lipopolysaccharide (LPS)-induced tumor necrosis factor-alpha (TNF) production in leukocytes. TNF is an essential proinflammatory cytokine whose production is normally stimulated during an infection. The purpose of this work was to establish an in vitro model reflecting in vivo data and to characterize the molecular mechanism of action of mancozeb.

The human promyelocytic cell line THP-1 was used as in vitro model to study the effects of mancozeb and its main metabolite ethylenthiourea (ETU) on LPS-induced TNF release. Mancozeb, but not ETU, at non-cytotoxic concentrations (1 – 100 μg/ml), induced a dose- and time-dependent inhibition of LPS-induced TNF release, reflecting in vivo data. The modulatory effect observed was not limited to mancozeb but also other EBDTCs, namely zineb and ziram, showed similar inhibitory effects. Mancozeb must be added before or simultaneously to LPS in order to observe the effect, indicating that it acts on early events triggered by LPS. It is known that nuclear factor-κB (NF-κB) tightly regulates TNF transcription. We could demonstrate that mancozeb, modulating LPS-induced reactive oxygen species generation, prevented IκB degradation and NF-κB nuclear translocation, which in turn resulted in decreased TNF production. To further understand the mechanism of the effect of mancozeb on TNF transcription, THP-1 cells were transfected with NF-κB promoter – luciferase construct, and the effect of mancozeb on luciferase activity was measured. Cells transfected with promoter constructs containing κB site showed decreased LPS-induced luciferase activity relative to control after mancozeb treatment, confirming NF-κB binding as an intracellular target of mancozeb.

Overall, this study contributes to our understanding of the mechanism underlying mancozeb-induced immunotoxicity.

Keywords: Immunotoxicity; Ethylenebisdithiocarbamates; Fungicides; Cytokine; NF-κB; ROS; Pesticides; Cytokine; In vitro

Introduction

Pesticides are designed to interfere with certain living species, and are inevitably characterized by variable levels of toxicity. A number of data are at present available suggesting that the immune system may be a target of the toxic effect of some pesticides (Barnett and Rodgers, 1994; Repetto and Baliga, 1996).

With the exemption of ziram and thiram introduced during the 1930s, the development of dithiocarbamate derivatives with pesticidal properties occurred during and

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0041-008X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2005.07.002

after the Second World War. Dithiocarbamates are mainly used in agriculture as insecticides, herbicides, and fungicides. Additional use are as biocides for industrial or other commercial applications, as vulcanization accelerators, and antioxidants in rubber industry, and because of their chelating properties, they are also used as scavengers in waste-water treatment (World Health Organization, 1988). Ethylenebisdithiocarbamates (EBDTCs) are widely used worldwide due to the low acute toxicity and their short environmental persistence. EBDTCs are generally unstable and are rapidly degraded in the presence of oxygen, moisture, or biological systems, and decompose rapidly in water to form a number of compounds, some of which (i.e. ethylenthiourea and propylenethiourea) are toxicologically important (World Health Organization, 1988). Ethylenthiourea (ETU) is fairly stable, highly water soluble, and it is of particular importance because of its specific reversible thyroid toxicity.

In the last decades, evidence supporting an immunomodulatory effect of dithiocarbamates and EBDTCs has been collected (Pruett et al., 1992; Padget et al., 1992; Lombardi et al., 1991; Renoux et al., 1988). Sodium diethyldithiocarbamate has been demonstrated to be a potent in vivo immunomodulator, influencing maturation and activation of T cells, NK cells, IgG secretion, and prolonging immunological memory (Renoux and Renoux, 1980; Renoux et al., 1988). An increase in serum IgG, IgE and β_2-macroglobulin was observed in workers occupationally exposed to mancozeb (Vergova et al., 1988) and to maneb (ethylenebisdithiocarbamate associated with manganese). We also demonstrated in a group of manufacturers exposed to mancozeb an increase in T cell proliferative responses, suggesting an immunostimulatory effect in conditions of low level, prolonged occupational exposure (Colosio et al., 1996). In contrast to mancozeb, zinc diethyldithiocarbamate (zineb) was devoid of immunoenhancing influence on the response to T cell mitogens, and exerted a cytotoxic effect on spleen lymphocytes (Renoux et al., 1988). Sodium methyldithiocarbamate has also been reported to cause a significant immunosuppression in mice following in vivo exposure (Pruett et al., 1992; Padget et al., 1992). More recently, in agricultural workers, we found that occupational exposure to mancozeb resulted in a dose-related increase in lymphocyte counts and proliferative responses to mitogens, and in a reduction in LPS-induced TNF release and decrease in $CD25^+$ cells (Corsini et al., 2005).

The purpose of this work was to establish an in vitro model reflecting in vivo data (i.e. cytokine production), to characterize the molecular mechanism of action of mancozeb and to determine if the observed effects were due to mancozeb or to its principal metabolite ETU. In this paper, we focused our attention on TNF, one of the major proinflammatory cytokine produced during an infection. A decrease in the ability of cells to produce this cytokine is likely to contribute to increased susceptibility to infections. LPS, a component of $Gram^-$ bacteria wall, is a potent activator of the immune system and in particular of monocytes/macrophages. It has been shown that LPS stimulates the production of reactive oxygen species (ROS) such as hydrogen peroxide, superoxides, and NO as well as a battery of signal transduction pathways leading to gene expression (Caroff et al., 2002; Beutler et al., 2003; Miyake, 2004). There are several indications that ROS may act as a cellular second messenger and it has been demonstrated that H_2O_2 can activate NF-κB (Schreck et al., 1991), which in turn regulates the expression of many immune and inflammatory molecules, including TNF (Sanlioglu et al., 2001).

The human promyelocytic cell line THP-1 resulted to be a useful in vitro model toward understanding the mechanism of the effects of mancozeb on cytokine production. We found that mancozeb, but not ETU, caused a dose- and time-related inhibition of LPS-induced TNF release reflecting in vivo data. We hypothesized that the reduction in TNF production caused by mancozeb resulted from alterations in NF-κB activation. We could demonstrate that mancozeb interferes with NF-κB activation, consistent with observed reductions in TNF production. Results from an NF-κB-dependent reporter system also demonstrated that mancozeb alters the ability of NF-κB to direct transcription. Given the role of NF-κB in transcription of numerous genes, the effects of mancozeb on NF-κB is likely to be an important mechanism of the immunotoxicity of the chemical.

Materials and methods

Chemicals

Mancozeb, zineb, and ziram were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany), lipopolysaccharide from *Escherichia coli* serotype 0127:B8, and pyrrolidine dithiocarbamate (PDTC) were from Sigma (St Louis, MO, USA); antibodies against IκB and β-actin as all cell culture reagents from Sigma. Electrophoresis reagents were from Bio-Rad (Richmond, CA, USA). All reagents were purchased at the highest purity available.

Cells

For all experiments, THP-1 cells (Istituto Zooprofilattico di Brescia, Brescia, Italy) were diluted to 10^6 cells/ml in RPMI 1640 containing 2 mM L-glutamine, 0.1 mg/ml streptomycin, 100 IU/ml penicillin, β-mercaptoethanol 50 μM, supplemented with 10% heated-inactivated fetal calf serum (media), and cultured in 37 °C in 5% CO_2 incubator. For TNF release, 0.5×10^6 cells were seeded in 1.5 ml sterile polypropylene microcentrifuge tubes, while for Western blot analysis, mRNA expression, and transcription factor activation, 4×10^6 cells were plated in 60-mm Petri dishes. Cells were incubated for different times with or without different concentrations of lipopolysaccharide