SUPPRESSION OF BLEOMYCIN-INDUCED INCREASED PRODUCTION OF NITRIC OXIDE AND NF-κB ACTIVATION BY TREATMENT WITH TAURINE AND NIACIN

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INTRODUCTION

Bleomycin (BL)-induced pulmonary fibrosis is initially characterized by alveolar inflammation, influx of inflammatory cells followed by progressive proliferation of septal cells, increased production of septal matrix and loss of lung architecture. The process of cellular injury in lung fibrosis is thought to be mediated by oxygen radicals produced by infiltrating inflammatory cells. Peroxynitrite is a potent oxidant produced by the rapid reaction of nitric oxide (NO) and superoxide. In addition, BL itself is capable of producing superoxide and hydroxy radicals by binding to DNA:Fe^{2+} and forming a DNA: Fe^{2+}:BL complex which undergoes redox cycling and generates these reactive oxygen species (ROS). Recent evidence suggests that macrophage and neutrophil-derived ROS may have important roles in lung inflammation and fibrosis by stimulating the production of proinflammatory and fibrogenic cytokines that mediate enhanced fibroproliferative response. The roles of these cytokines in the BL-induced lung fibrosis have been well characterized and it is commonly...
understood that not a single cytokine but a network of cytokines controls the inflammatory and fibrotic responses.

A role for NO in the physiologic processes of the lung is suggested by the findings of nitric oxide synthase (NOS) activity in lung tissues and by demonstration of vasodilatory effects of inhaled NO in pulmonary vessels and airways and by observations that lung epithelial cells produce factor(s) capable of causing smooth muscle relaxation. In lung tissues, NOS has been localized and identified in two different forms: constitutive form present in endothelial cells and brain and inducible form found in macrophages. Several studies have shown that inducible nitric oxide synthase (iNOS) mRNA expression, protein and nitric oxide production can be induced in macrophages and neutrophils by specific stimuli. It has been demonstrated by Huot and Hacker that macrophages activated in vivo by BL secrete nitrite spontaneously and this secretion can be blocked by a substrate-specific analogue of the L-arginine-dependent effector mechanism, N\textsuperscript{6}-monomethylarginine.

The production of proinflammatory and profibrogenic cytokines in macrophages is controlled in part at the level of gene transcription by a number of DNA binding proteins which interact with specific sequence motifs in the promoter region of the gene. A widely distributed DNA binding nuclear factor kB (NF-kB) which is normally sequestered in the cytoplasm as an inactive multiunit complex bound to inhibitory protein, I\textsuperscript{kB-\alpha}, is known to regulate the production of many cytokines. A number of stimuli including ROS can activate this complex by causing phosphorylation and degradation of I\textsuperscript{kB-\alpha} and thus allowing the translocation of the active dimer into the nucleus, where it binds to the promoter region of genes such as IL-1\alpha, IL-6 and TNF-\alpha containing the NF-kB motif and stimulates the expression of their genes.

Since a large part of bleomycin (BL)-induced lung damage is due to an excess production of ROS either by macrophages and neutrophils or by the DNA:Fe\textsuperscript{2+}:BL complex, we hypothesized that intratracheal (IT) instillation of BL increases the production of nitric oxide and cytokines secondary to upregulation of inducible nitric oxide synthase (iNOS) and activation of NF-kB in the lungs, respectively; and the antifibrotic effects of the combined treatment with taurine and niacin as demonstrated in our laboratories may reside in their ability to suppress the BL-induced upregulation of iNOS and activation of NF-kB. In order to test this hypothesis, we investigated the effects of saline or BL instillation on the expression of iNOS mRNA, protein, NO production, NF-kB activation, I\textsuperscript{kB-\alpha} levels and changes in the levels of some cytokines during the course of development of lung fibrosis with and without taurine and niacin treatment.