Inhibition Of Acetylation Of The Pmn Chemoattractant Peptide Pgp By Carbocysteine And N-Acetyl Cysteine

M. D. Brown[1], M. T. Hardison[2], J. E. Blalock[1], P. L. Jackson[1]

[1]University of Alabama at Birmingham, Birmingham, [2]University of Alabama Birmingham (UAB), Birmingham

Rationale

Cigarette smoke contains over 4000 known compounds including free radicals, carcinogens, and other highly reactive compounds that create an inflammatory state in the lung. Airway epithelial damage from cigarette smoke stimulates neutrophil chemotaxis. Activated neutrophils subsequently invade the damaged airway and degranulate, releasing proteases which breakdown collagen.

Proline-Glycine-Proline (PGP) is a tripeptide degradation product of collagen proteolysis. In clinical samples from patients with chronic lung inflammation, increased levels of PGP correlate with disease severity. Our research has shown that PGP activates CXCR1/CXCR2 receptors on neutrophils. This activation stimulates neutrophil chemotaxis and enhances inflammation. PGP is degraded by the aminopeptidase activity of leukotriene A4 hydrolase, but an acetylated form of PGP (AcPGP) is not. Furthermore, AcPGP is more potently chemoattractive to neutrophils. We demonstrate that cigarette smoke components including acrolein, acetaldehyde, and methylglyoxal result in PGP acetylation.

N-acetyl cysteine (NAC) and carbocysteine are reducing agents currently used as mucolytics in chronic inflammatory lung diseases. These agents may diminish inflammation by inhibiting acetylation of PGP. We hypothesize that NAC or carbocysteine will diminish PGP acetylation by reactive cigarette smoke compounds.

Methods

Cigarette smoke extract (CSE) or condensate (CSC) was prepared by bubbling 1 cigarette/ml of PBS or DMSO respectively, then incubating with 100ng/ml unacetylated PGP with or without 10mM of NAC or carbocysteine. Samples were incubated at 37°C and analyzed at various time points. The concentration of AcPGP was measured with electrospray ionization liquid chromatography mass spectrometry/mass spectrometry (ESI-LC-MS/MS). This data was repeated in a vapor experiment, where PGP mixed with either reducing agent was placed in a separate well from the cigarette smoke in a 96-well plate and incubated at various time points.

Results

Our data demonstrate significant AcPGP generation by cigarette smoke extract, condensate, and vapor that is inhibited with either NAC or carbocysteine. At 24 hour incubation time, AcPGP from CSC and CSE resulted in 1.41ng/ml and 1.87ng/ml of AcPGP, respectively. This acetylation was ablated in the presence of carbocysteine, with CSC and CSE exposure values of 0.024ng/ml and 0.036ng/ml. This diminished acetylation was even more pronounced with exposure to NAC, as AcPGP values for CSC and CSE were 0ng/ml and 0.0072ng/ml.

Conclusions

Our data support a possible mechanism whereby reducing compounds such as NAC and carbocysteine may diminish lung inflammation in COPD and other chronic lung diseases by inhibiting PGP acetylation thereby facilitating PGP degradation.

This abstract is funded by: The National Heart, Lung, and Blood Institute (HL07783, HL090999, and HL087824)