Exposure and Biological Response Biomarkers of Cigarette Smoke

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Exposure to tobacco smoke (mainstream and environmental) is a leading cause of death in the US. Cigarette smoke is an extremely complex mixture, containing some 3800 constituents including numerous polycyclic aromatic hydrocarbons (PAHs). PAHs and other constituents are found both mainstream and sidestream (environmental) smoke fractions. Cigarette smokers provide an extreme model of PAH exposure that will permit both exposure and biological response biomarkers to be developed. There is substantial evidence that PAHs are causative agents in lung, skin, and bladder cancer. Furthermore, tobacco smoke is associated with oxidative stress, pancreatic cancer, cardiovascular disease, and chronic obstructive pulmonary disease (COPD); although the specific role of PAHs is not clear. Interestingly, the cardiovascular effects of sidestream smoke are almost as great as mainstream smoke.

The Penn UO-1 program stems from significant advances that have been made over the last six years in the quantification of protein, lipid, and DNA biomarkers using stable isotope methodology and our basic research into enzyme regulation during oxidative stress. Previous methods for the analysis of oxidative DNA damage have been fraught with numerous methodological problems and the current state-of-the-art involves the use of a COMET assay to measure 8-oxo-2'-deoxyguanosine (dGuo) lesions. We recently devised a more quantitative method based on immunoaffinity stable isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) that can be readily elaborated to studies of tobacco smokers. We also showed that oxidative stress could induce the formation of aldo-keto reductases (AKRs) of the 1C family. AKR1C3 is the enzyme, which we recently showed is responsible for the conversion of prostaglandin (PG) D_2 to the potent bronchoconstrictor 11β-PGF_2. This provides an additional potential link between oxidative stress and COPD, as well as the potential for a new therapeutic strategy involving AKR1C3 inhibition. Finally, preliminary studies have revealed that a DNA-adduct than can only arise from lipid peroxidation is present in the urine of cigarette smokers, but is completely absent in urine from non-smokers.

Penn investigators will build on these exciting new findings by developing panels of *in vivo* biomarkers of exposure and biological response, which they hypothesize, will make it possible to distinguish a cohort of non-smokers from a cohort of disease-free tobacco smokers. This hypothesis will be tested by conducting research under the following three specific aims:
Specific Aim 1: To discover whether B[*a*]P and B[*a*]P-7,8-dione induce AKR1C/2 in NHBE cells and increase oxidative stress to form 8-oxo-dGuo and HεdGuo in DNA, induce AKR1C3 in HASM cells and increase the biosynthesis of the potent bronchoconstrictor 11β-PGF_2, as potential urine and EBC biological response biomarkers of PAH exposure.
Specific Aim 2: To discover secreted proteins following treatment of NHBE and HASM cells with B[*a*]P and its oxidative metabolites as potential serum biological response biomarkers of PAH exposure.
Specific Aim 3: To conduct predictive and refinement analyses of *in vivo* exposure and response

biomarkers in urine together with biological response biomarkers in EBC and serum in order to distinguish non-smokers from disease-free tobacco smokers.

Program Implementation

The biomarker discovery and validation program will be conducted under the auspices of the Center of Cancer Pharmacology directed by Dr. Blair and Center of Excellence in Environmental Toxicology (CEET) directed by Dr. Trevor Penning at the University of Pennsylvania. Dr. Penning will also participate in the UO-1 program. He will provide expertise in the enzymology and etiology of activation of polycyclic aromatic hydrocarbon and nicotine-derived carcinogens. Dr. Blair is the Director of the Oxidative Stress and Molecular profiling Cores within the CEET. Resources from these Cores will be deployed in order to facilitate rapid implementation of the program. This will provide access to four triple quadrupole mass spectrometers, five linear ion trap mass spectrometers, and a high-resolution linear ion trap mass spectrometer. Drs. Blair and Penning already have a substantial collaboration on the activation of environmental chemicals through working closely together on individual RO-1 projects. They also collaborate on a program funded by the Commonwealth of Pennsylvania on development of a Center for the study of gene-environment interactions in lung cancer. We anticipate that the ability to identify biomarkers of exposure to cigarette smoke and elucidate the genetic susceptibility to lung cancer will provide a powerful way to identify individuals at increased risk.

Patient recruitment for the U0-1 program has been initiated by Co-Investigators Anil Vachani, MD, Assistant Professor of Medicine. It is anticipated that substantial new information will become available on the presence of various biomarkers so that Andrea Troxel, PhD, Associate Professor of Biostatistics, can assess their potential utility. In addition to determining conventional lipid and DNA biomarkers, a discovery program will be implemented in collaboration with Don Baldwin, PhD, Director of the Penn Microarray Core Facility, in order to identify potential protein biomarkers.

Significant progress has already been made on the implementation of exposure and biological response biomarkers. A validated stable isotope dilution LC-MS assay has been developed for urinary nicotine, cotinine, 3'-hydroxy-cotinine, and their glucuronide conjugates. This will make it possible to assess the dose response of other biomarkers to cigarette smoking. The major advance in biological response biomarkers has been in the preparation of a monoclonal antibody to 8-oxo-dGuo. The antibody has been used to develop a validated stable isotope dilution LC-MS assay for urinary 8-oxo-dGuo. Importantly, a linear regression line was obtained when a standard curve was prepared in urine, it was parallel to the standard curve obtained in buffer, and the intercept corresponded to the endogenous urinary 8-oxo-dGuo concentration.

Summary

Successful completion of the proposed research will provide a panel of biomarkers of exposure and biological response to tobacco smoke. This will have significant utility in future studies designed to elucidate the relationship between gene environment interactions and diseases such as cancer, cardiovascular disease, and COPD.