A Fusion of Field and Laboratory Studies in the Investigation of Environmental Chemicals Causing Oxidative Stress and Covalent Modification

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Arsenic is ubiquitously distributed in nature throughout Earth's crust and thus the major source of exposure to this metalloid for the general population is naturally polluted drinking water from wells. In East Asia, more than 30 million people are chronically exposed to arsenic. Interestingly, the manifestations of vascular diseases caused by prolonged exposure to arsenic are consistent with those induced by impaired production of endothelium-derived nitric oxide (NO) and/or oxidative stress. However, no information has been available on the relation among NO synthesis, oxidative stress and chronic arsenic poisoning in humans. A cross-sectional study in an endemic area of chronic arsenic poisoning in Inner Mongolia and experimental animal studies indicated that long-term exposure to arsenic by drinking water causes reduction of NO production in endothelial cells and oxidative stress through generation of reactive oxygen species (ROS). Subsequent examinations showed that decreased NO production and ROS generation during arsenic exposure is, at least in part, due to an "uncoupling" of endothelial NO synthase evoked by decreased levels of BH_4, leading to endothelial dysfunction. Furthermore, an intervention study in the area of chronic arsenic poisoning in Inner Mongolia suggested that decreased NO levels and peripheral vascular disease in arsenosis patients can be reversed by exposure cessation. In our cellular experiments, we found that arsenic exposure causes adaptive response against oxidative stress and arsenic cytotoxicity through Nrf2 activation. Blockage of GCLs, GSTs, or MRPs exacerbated arsenic toxicity in primary mouse hepatocytes, suggesting that these phase II xenobiotic-metabolizing enzymes and phase III transporters controlled by Nrf2, play a critical function in arsenic efflux into extracellular space to decrease its cytotoxicity. By contrast, pretreatment with sulforaphane, an Nrf2 activator, diminished cellular accumulation, thereby reducing its cellular toxicity in the cells, which supports the notion that Nrf2 is a critical transcription factor against arsenic toxicity. Thus, we suggest that daily intake with foods including such an Nrf2 activator may be an effective strategy for the treatment of chronic poisoning.

Diesel exhaust particles (DEP) containing numerous compounds are a major constituent of ambient particulate matter. Although several lines of evidence suggested that chemicals contaminated in DEP promote oxidative stress and impairment of NO-dependent vascular tone, no identification of component(s) involved in the deleterious effects has been reported. In 1997, we proposed for the first time that polycyclic aromatic hydrocarbon quinones (PAHQs) are potential constituent responsible for catalytic generation of ROS, resulting in oxidative stress. We also developed a method for quantitative determination of PAHQs as their diacetoxy derivatives in DEP and atmospheric $PM_{2.5}$ collected from Los Angeles Basin. Experiments with purified NO synthase, and aortic ring of rats, and *in vivo* studies revealed that 9,10-phenanthraquinone (PQ), a major PAHQ in atmospheric $PM_{2.5}$, interacts with the cytochrome P450 reductase domain on NO synthase, and thus inhibits NO formation, thereby occurring the suppression of NO-dependent vasorelaxation and elevation of blood pressure. We found that PQ undergoes two-electron reduction by AKR1C isozymes to form 9,10-dihydroxyphenanthrene (PQH_2) that initiates redox cycling to cause oxidative stress-mediated cytotoxicity; however, monoglucuronide of PQH_2 produced by UGTs was devoid of the redox activity and transports PQH_2 to extracellular space through MRPs. With primary hepatocytes from $Nrf2^{+/+}$ and $^{-/-}$ mice, it was also shown that Nrf2 is a critical transcription factor against PQ cytotoxicity as well. Our *ex vivo* studies indicated that 1,2-naphthoquinone (NQ) identified as a PAHQ of atmospheric $PM_{2.5}$ causes concentration-dependent contractions of guinea pig tracheal rings through increased phosphorylation of EGFR that is negatively regulated protein tyrosine phosphatases (PTPs). Consequently, we demonstrated that covalent binding of NQ to PTP1B through Cys121 is at least partially responsible for the reduction of PTPs activity, which leads to persistent transactivation of EGFR in A431 cells.