Progress toward the use of oxidative stress as a broadly observed key event in environmental toxicology

Presenters: Gary Hatch and Prasada Kodavanti

Contributing Organizations: ORD/NHEERL

LTG 1 Poster 09

Science Questions

- Is oxidative stress (OS) a common pathway for environmentally induced toxicity?
- Is disease-associated OS a common underlying condition for increased risk from environmental exposures?
- Are there any specific, reliable and noninvasive methods for measuring OS in both clinical and epidemiological studies?

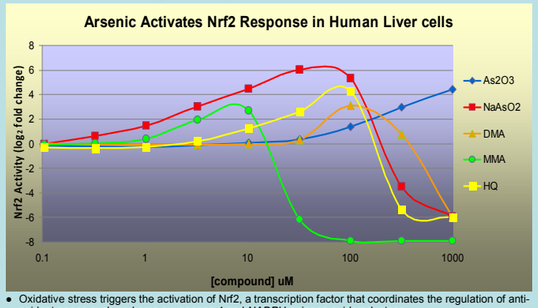
Research Goals

- Determine whether oxidative stress is an indicator of the biological effects of a variety of toxicants.
- Evaluate 'background' oxidative stress levels under various disease conditions.
- Improve non-invasive measurements of oxidative stress and apply these as exposure biomarkers in epidemiological studies.

Findings and Conclusions

Oxidative stress has been shown to be a common pathway for environmentally induced toxicity

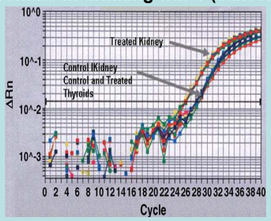
Arsenic in drinking water (cancer)



- Oxidative stress triggers the activation of Nrf2, a transcription factor that coordinates the regulation of anti-oxidant genes such as *heme oxygenase 1* and *NADPH quinone oxidoreductase*.
- Using human liver cells, we engineered a stable, cell-based reporter gene assay that measures Nrf2 activation in response to oxidative stressors.
- Human liver cells were treated for 24 hours with 10nM-1mM arsenic oxide (As_2O_3), sodium arsenite ($NaAsO_2$), dimethylarsinous acid (DMA), monomethylarsinous acid (MMA) or a known Nrf2 inducer, hydroquinone (HQ). Nrf2 activity was measured using luciferase assay normalized to stable green fluorescent protein expression. Nrf2 activity (y-axis) is plotted as $\log_2$ fold change compared to untreated controls, therefore positive values reflect increased Nrf2 activation, while negative values reflect loss of basal Nrf2 levels (cytotoxicity).

Simmons and Ramabhadran, submitted.

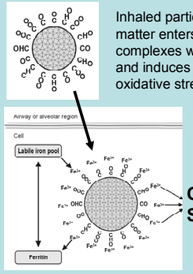
Bromate in drinking water (cancer)



Expression of 8-oxodeoxyguanosine glycolase (OGG1) an inducible DNA repair enzyme in response to oxidative stress, was up-regulated approximately 4-fold in rat kidney following bromate exposure.

Delker,D. et al. 2006. Molecular biomarkers of oxidative stress associated with bromate carcinogenicity. Toxicology 221: 158-165.

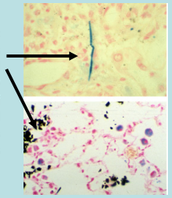
Iron dys-regulation resulting from PM and fiber exposure



Inhaled particulate matter enters the cell, complexes with iron and induces oxidative stress

Oxidative Stress

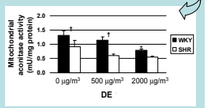
Microscopic images of lungs stained for iron show accumulation of the metal around retained articles and fibers



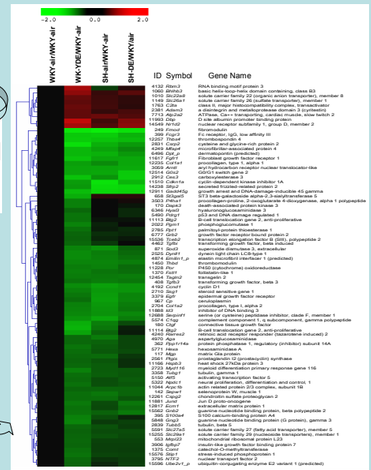
Concept: Turi JL, Yang F, Garrick MD, Piantadosi CA, Ghio AJ. The iron cycle and oxidative stress in the lung. Free Radic Biol Med 2004; 36 (7):850-857. (19 publications since 2005 expand this concept)

Particulate matter (PM) in air (non-cancer)

Diesel exhaust (DE) inhalation caused cardiac mitochondrial aconitase inhibition indicating oxidative stress in healthy Wistar Kyoto (WKY) and Spontaneously Hypertensive (SH) rats.

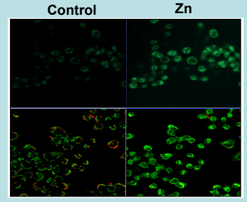


DE-induced cardiac oxidative stress is associated with development of a hypertensive gene expression pattern in healthy WKY rats making them appear like SH rats. Downregulation of genes involved in antioxidant compensatory response, mitochondrial function and cardiac cell structural integrity is noticed.



Gottipolu, et al Environ. Health Perspect., 2009, accepted.

Zinc induced injury to mitochondria



- Live cell imaging with the sensor, PG1, shows that Zn exposure elevates intra-cellular H_2O_2 levels.
- Zn exposure induces a loss of mitochondrial potential that precedes the increase in H_2O_2, as detected by the change of JC1 fluorescence from red to green.

Concepts: Kim, Y.M., Reed, W., Wu, W., Bromberg, P.A., Graves, L.M. and Samet, J. M. (2006). Am. J. Physiol.: Lung Mol. Cell. Biol.. 290: L1028-1035.

Ozone induced oxidative stress progress

Unique changes in expression of oxidative stress genes at different ozone exposure levels: Rat lung homogenates after 2 hour ozone exposures.

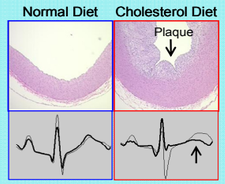
Table 5. List of induced (> 2-fold) genes that are unique to 2 or 5 ppm O_3.[a]

Accession no.[b]	Gene symbol[c]	Gene name[d]	Fold change[e]
2 ppm O_3			
J03933	*Thrb*	thyroid hormone receptor beta, c-erbA-β	5.32
U73174	*Gsr*	glutathione reductase	5.21
U08447	*Cd3z*	T-cell receptor CD3 zeta subunit	4.37
L46791	*Ces3*	liver carboxylase precursor 10 (carboxylesterase 3)	3.95
J02650	*Rpl19*	60S ribosomal protein L19	3.51
X96394	*Abcc1*	multidrug resistance protein	2.70
D29766	*Bcar1*	FAK substrate p130	2.53
U49062	*Cd24*	signal transducer CD24	2.39
D16554	*Ubb*	polyubiquitin	2.25
5 ppm O_3			
X17163	*Jun*	c-jun AP1	5.26
M84203	*Kcnc2*	potassium channel protein (Kshlll A)	5.20
D10862	*Id1*	inhibitor of DNA binding 1	4.33
M81855	*Abcb1*	multidrug resistance protein 1	2.74
D14051	*Nos2*	inducible nitric oxide synthase	2.61
U45965	*Cxcl2*	Mip-2 chemokine ligand 2	2.57
M86389	*Hspb1*	heat shock 27 kDa protein 1	2.55
L29232	*Igf1r*	IGF-1 receptor	2.50
D16237	*Cdc25b*	M-phase inducer phosphatase 2	2.48

Source: Nadadur, S.S et al. 2005. Environ. Health Perspect 113: 1717-22

Disease-associated oxidative stress has been shown to be a common underlying condition explaining increased risk from environmental exposures.

Rabbit cardiovascular disease model: effect of $ZnO/ZnSO_4$ instillation.



When compared to animals fed a normal diet, animals fed a cholesterol diet had:

1) plaques in the aorta

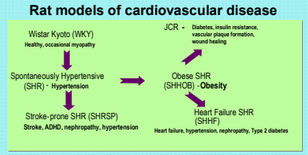
2) ECG waveforms that changed from baseline (gray line) minutes after the instillation of zinc (black line).

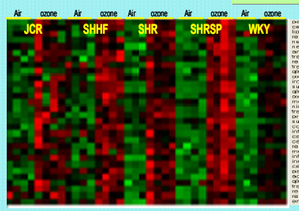
3) changes in the oxidative status of the lung days after zinc instillation (indicated by changes in lung lavage fluid oxidized glutathione).

LaGier, A.J et al, 2008 (accepted: Cardiovascular Toxicology).

Animal models of heart failure, stroke, diabetes and hyper-tension show increased oxidative stress gene expression in the lung immediately after ozone exposure (1.0 ppm, 4 hr).

Rat models of cardiovascular disease

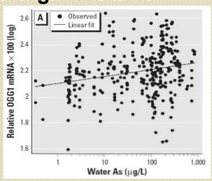




New markers useful for epidemiologic studies.

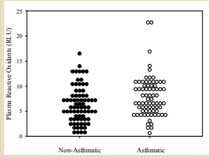
Measurement of induced repair gene (OGG1) expression for oxidized DNA detected toxicity of drinking water arsenic in Inner Mongolian residents in China (N=299)

Mo, J., Xia, Y., Wade, T.J., Schmitt, M., Le, X.C., Dang, R., Mumford, J.L. Environ. Health Perspect. 2006 Vol.114(6):835-41



- Measurement of reactive oxygen species in blood plasma in children showed that asthmatic children had higher levels than non-asthmatic.
- Measured by lucigenin chemiluminescence in blood plasma of 148 children residing in Detroit, Michigan.

Heidenfelder et al. Annals of Allergy, Asthma and Immunology accepted 2008



Impact and Outcomes

- NCEA will be able to use oxidative stress measures as part of the mechanistic underpinning in risk assessment for both cancer and non-cancer endpoints.
- Assessment of 'background oxidative stress' resulting from life style and pre-existing disease can explain and identify susceptible sub-groups.
- Future epidemiology studies will be able to incorporate newer non-invasive markers of oxidative stress as biomarkers of exposure.
- In vitro screening tests for oxidative stress can be extended to other 'stress pathways' (see LTG1 – 10)

LTG1-10 shows future directions building on the oxidative stress MOA which include:

- Extend and modify the reporter gene assays already developed to other cell lines and exposure scenarios
- Screen an array of pollutants relevant to Air and Water programs (see LTG 1-11).
- Develop a virtual lung model to determine the dose of reactive gases and particles to deep lung targets in order to link whole-body exposure data with toxicity pathway information obtained *in vitro*. (see LTG 1-113,14).

Researchers Involved

Steve Simmons, James Samet, Jane Gallagher, Ram Ramabhadran, Kirk Kitchin, Urmila Kodavanti, Joyce Royland, Andy Ghio, Judy Mumford, Adriana LaGier. NHEERL, NTD/ETD/ECD/HSD

Use of Mechanistic Data in Risk Assessment

This poster does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.