



*Tobacco Harm Reduction:
What does it Mean?*

**2007 Symposium
on
Tobacco Science and Health**

March 6-8, 2007

**Galt House Hotel
Louisville, KY**

Conference Agenda

8:00 Check-in (lobby outside of Carroll Ford)

8:30 Welcome (Carroll Ford)
Forum Chairperson

Presentation Session I – Health Issues Indicating Need for Tobacco Harm Reduction Strategies (Carroll Ford)

8:45 Dr. Irfan Rahman

University of Rochester Medical Center, Rochester, NY

"Redox Regulation of Chromatin Modeling in Response to Cigarette Smoke in COPD: Impact on Pro-inflammatory Gene Transcription"

9:30 Dr. Judy Zelikoff

New York University School of Medicine, Tuxedo, NY

"Prenatal Exposure to Cigarette Smoke and Chronic Airway Disease in the Juvenile and Adult Offspring"

10:15 Break (Carroll Ford)

10:45 Dr. Roger Jenkins

Consultant, Oak Ridge National Laboratories

"Harm Reduction for Environmental Tobacco Smoke: Holy Grail or Achievable Goal?"

11:30 Poster Session I (11:30 - 12:45, Laffoon)

12:45 Lunch (Wilkinson)

Presentation Session II – Current Research Linking Health Issues to Harm Reduction (Carroll Ford)

1:45 Dr. Dominique Balharry (2006 Dietrich Hoffmann Career Development Award Recipient)

Cardiff University, School of Biosciences, Cardiff, United Kingdom

"Using Toxicogenomics to Search for Biomarkers of Exposure and Harm in Respiratory Epithelia"

2:30 Dr. Franky Richter

Ludwig-Maximilians University

"Biomonitoring of Human Exposure to Tobacco-Specific Nitrosamines (TSNA): The Role of Adducts Releasing 4-Hydroxy-1-(3-Pyridyl)-1Butanone (HPB) from DNA and Hemoglobin"

3:15 Break (Carroll Ford)

3:45 **Dr. Ramesh Gupta**
University of Louisville, Louisville, KY
"Cigarette smoke-mediated DNA adducts and their Inhibition"

5:15 **Dinner Reception** (Fountain Room)

Day 2 (March 7, 2007)

Presentation Session III – Tobacco Harm Reduction: Strategies and Perceptions (Carroll Ford)

8:30 **Dr. Brad Rodu**
University of Louisville, Louisville, KY
"Tobacco Harm Reduction: Smokeless Tobacco Use Can Substitute for Smoking"

9:15 **Frank Sloan**
Duke University, Durham, NC
"Risk Perceptions and the Cost of Smoking"

10:00 **Break** (Carroll Ford)

10:30 **Poster Session II** (10:30 - 12:00, Laffoon)

12:00 **Lunch** (Wilkinson)

1:15 **Dr. David J. Doolittle**
Vice President of Product Evaluation, RJ Reynolds Tobacco Company, Winston-Salem, NC
"An Effective Product Evaluation Strategy for PREP Cigarettes"

2:00 **Dr. Adrian Payne**
British American Tobacco
TBD - *"Tobacco Harm Reduction Strategy"*

2:45 **Break** (Carroll Ford)

3:15 **Posters, Discussions, Free time**

Day 3 (March 8, 2007)

8:00 Introduction to Break-out Sessions (Carroll Ford)

Questions for discussion groups –

- 1) How should PREPs be assayed to determine if they actually reduce harm?
- 2) How does smoking behavior affect usage of PREPs and how does this behavior impact the harm reduction potential of these products?
- 3) What directions should we be looking toward to achieve harm reduction?

8:10 Break-out Session Discussions (Wilson, Segell, McCreary)

9:40 Break

10:00 Break-out Session - Group Reports (Carroll Ford)

11:55 Concluding Remarks (Carroll Ford)

12:00 Adjourn

Abstracts

Plenary Speakers

REDOX REGULATION OF CHROMATIN MODELING IN RESPONSE TO CIGARETTE SMOKE IN COPD: IMPACT ON PRO-INFLAMMATORY GENE TRANSCRIPTION

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(see additional abstract at Poster Board # 1)

Oxidative stress and inflammation are major hallmarks of various chronic inflammatory diseases and cancer. Reactive oxygen species (ROS) play a key role in enhancing the inflammation through the activation NF- κ B and alteration in nuclear histone acetylation and deacetylation (chromatin remodeling) leading to sustained gene expression of pro-inflammatory mediators in the lung. Histone acetylation is reversible and is regulated by a group of acetyltransferases (HATs) which promote acetylation, and deacetylases (HDACs) which promote deacetylation. Acetylation of specific lysine residues present in the N-terminal tails of the core histone (specifically histone, H4) results in uncoiling of the DNA leading to increased accessibility to transcription factor binding. Histone deacetylation represses genes by limiting access to transcription factors. Recent studies from our laboratory show that oxidative stress induced by cigarette smoke enhances lung inflammation through the activation of intrinsic HAT activity of co-activator molecules, and increased histone 3 phospho-acetylation leading to increased NF- κ B activation. Oxidative stress also inhibits the activity of HDACs, activates cells for NF- κ B transactivation and enhances inflammatory gene expression which leads to chronic inflammatory response both in monocytes and epithelial cells in vitro and in vivo in rodent lungs exposed to cigarette smoke. Inhibition of HDAC activity was associated with post-translational modifications of HDAC proteins by reactive aldehyde and nitric oxide products present in cigarette smoke. These events were associated with increased levels of pro-inflammatory mediators (MIP-1, MIP-2, IL-6, TNF- α and KC cytokines). We also show that oxidative stress interferes in glucocorticoid action to inhibit pro-inflammatory mediators in monocytes. These data provide new information on oxidant-mediated regulation of inflammatory response by chromatin remodeling at molecular level.

PRENATAL EXPOSURE TO CIGARETTE SMOKE AND CHRONIC AIRWAY DISEASE IN THE OFFSPRING

Judith Zelikoff

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(also presented at Poster Board # 1)

Epidemiologic and toxicologic data indicate that smoking during pregnancy increases the risk of offspring respiratory ailments (e.g., asthma) later in life. While these findings carry extensive clinical consequences, little data are available to provide insight into the possible mechanisms by which these effects might occur. We hypothesize that inhalation exposure to mainstream cigarette

smoke (MCS) by pregnant mice influences immune-mediated airway reactivity in the offspring. Pregnant CD1 mice were exposed daily for 4 hr/d (5 d/wk) from gestational day 4 to parturition to intact MCS at a concentration equivalent to smoking <1 pack of cigarettes per day. Changes in airway reactivity were evaluated in naive or ovalbumin (OVA)-sensitized, prenatally exposed offspring by a non-specific bronchoprovocation challenge with acetylcholine (Ach) at 5 or 8 and 15 or 18 wk of age, depending upon the protocol; immune parameters known to mediate airway reactivity were also evaluated. In the absence of effects on pregnancy incidence, gestational duration, litter size or offspring sex ratio, prenatal exposure to MCS enhanced airway reactivity and elevated plasma IgE concentrations (compared to the sex/age-matched, air exposed controls) in 5-wk-old naive female (but, not male) offspring. In contrast, airways of prenatally exposed 4-mo-old naive female offspring were significantly less responsive to Ach challenge than their matched controls. OVA-sensitized offspring exposed prenatally to MCS tended to have higher plasma IgE levels than their age-matched, air-exposed controls; sensitized 18-wk-old male offspring were more sensitive to Ach challenge than their age- and treatment-matched female counterparts. Results suggest that prenatal exposure to CS: affects airway health in a gender-dependent manner; increases offspring allergic antibody levels and airway reactivity; may play a role in the induction and/or exacerbation of allergy-related outcomes; and, has long-term effects on offspring respiratory health and may increase the risk of respiratory dysfunction later in life. (IFSH 28-B2600- 7893).

HARM REDUCTION FOR ENVIRONMENTAL TOBACCO SMOKE: HOLY GRAIL OR ACHIEVABLE GOAL?

Roger Jenkins

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Despite the prohibition of smoking in many workplaces and public areas, environmental tobacco smoke (ETS) remains a common indoor air pollutant in private residences and in those workplaces where smoking is permitted. Many public health organizations and the US Surgeon General have implicated ETS as a causative agent for lung and cardiac disease, as well as a number of cancer types. However, the actual estimated increases in relative risk are typically measured in only tens of percent, if they are statistically significant at all. From a risk measurement/attribution standpoint, the challenges to quantifying potential ETS harm reduction are substantial. Individual or even group exposures are difficult to quantify unless direct measurements are used. Estimated risks are low, which means quantification of risk reductions can be problematic. Results of many long term studies remain sensitive to never-smoker misclassification rates. For many populations, exposures to some of the toxins found in ETS may be much greater from normal backgrounds. And for some health outcomes, the disproportionate response to ETS, relative to the exposures associated with active smoking, make evidence interpretation difficult. Given that ETS is comprised of 70 – 90% aged and diluted sidestream smoke, it would seem that, if harm reduction from ETS is to be achieved and quantified, substantial changes in cigarette tobacco combustion during cigarette smoldering must occur. Presumed reduced exposure products (PREPs) which focus on this goal will be discussed.

USING TOXICOGENOMICS TO SEARCH FOR BIOMARKERS OF EXPOSURE AND HARM IN RESPIRATORY EPITHELIUM

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(also presented at Poster Board # 2)

A novel toxicological tool, consisting of a highly differentiated, 3-D, in vitro model of human respiratory epithelia (EpiAirway-100; MatTek Corporation), was utilized to examine the early gene responses following exposure to tobacco smoke components (TSC). The model is cultured to form a pseudo-stratified tissue-like appearance that closely resembles human respiratory tract epithelia, using cells that are geno- and pheno-typically more representative of normal epithelia than other commonly used models.

The EpiAirway tissue model was exposed at the air/liquid interface to representative particle (nicotine and cadmium) and vapour phase (formaldehyde and urethane) components of cigarette smoke. The dose and exposure time of the TSC was optimized to ensure biomarkers involved with toxicant stress were identified, rather than studying dead or dying cells.

This was achieved using toxicological analysis to assess epithelial integrity (trans-epithelial electrical resistance), cell viability (mitochondrial activity; MTT assay), secreted proteins (total protein and mucin) and morphology. Microarray technology (SuperArray) was then employed to compare patterns of mRNA expression of human genes associated with toxicology and drug resistance (n=5 per dose). Using gene array software and SAM analysis, candidate gene lists were generated for each TSC.

The resultant lists showed the majority of altered genes were encoding for proteins involved in drug metabolism. There were also a number of genes related to cell growth and proliferation, cell cycle and apoptosis. The transcriptional changes could also be classified according to carcinogenic category, where urethane, formaldehyde and cadmium (known carcinogens) demonstrated high gene homology, when compared to the non-carcinogen, nicotine. These findings could be important in understanding the mechanisms of toxicity and carcinogenicity, as well facilitating the discovery of intelligent biomarkers for exposure and harm.

BIOMONITORING OF HUMAN EXPOSURE TO TOBACCO-SPECIFIC NITROSAMINES (TSNA): THE ROLE OF ADDUCTS RELEASING 4-HYDROXY-1-(3-PYRIDYL)-1-BUTANONE (HPB) FROM DNA AND HEMOGLOBIN

Franky Richter

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(also presented at Poster Board # 3)

Tobacco-specific nitrosamines, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN) are classified as human carcinogens. From determination of urinary NNK metabolites it can be concluded that smokers take up at least 100-fold higher amounts of NNK than nonsmokers. No such data are available for NNN.

HPB-releasing DNA and hemoglobin adducts resulting from metabolic activation of both NNK and NNN are regarded as biomarkers of exposure and effect. In several studies hemoglobin adducts did not show the expected specificity for smoking. In lung cancer patients sixfold higher levels of HPB-releasing adducts were found in lung DNA from 22 smokers (Mean±SE: 385±56 fmol HPB/mg DNA)

compared to 12 nonsmokers (67 ± 18 fmol/mg; $p < 0.0001$). Hemoglobin adducts in a subset of 12 smokers and 7 nonsmokers (63 ± 53 versus 42 ± 13 fmol/g hemoglobin; n.s.) did not correlate with lung DNA adducts. In tumor-free sudden death victims adducts in 32 smokers and 51 nonsmokers were not significantly different in DNA from lung (90 ± 26 versus 65 ± 10 fmol/mg; n.s.) and from esophageal mucosa close to the gastric junction (138 ± 39 versus 133 ± 18 fmol/mg; n.s.). DNA adducts in the esophagus did not correlate with adducts in the lung.

Sources other than TSNA are postulated to explain the lack of correlation of HPB-releasing adducts with tobacco exposure. Myosmine could be an important tobacco-independent source of these adducts. Myosmine has been found in a large variety of edible plants and is present in human plasma and saliva in the lower ng/ml range indicating daily uptake of mg amounts of myosmine from the diet. Myosmine is not only nitrosated rapidly to NNN but gives rise to reactive precursors of HPB by nitrosation and peroxidation.

CIGARETTE SMOKE-MEDIATED DNA ADDUCTS AND THEIR INHIBITION

¹Ramesh C. Gupta and ²C. Gary Gairola

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Cigarette smoking has been strongly associated with lung cancer. Cigarette smoke is a complex mixture containing nearly 5,000 chemicals of which approximately 100 are known or suspected carcinogens, including polycyclic aromatic hydrocarbons (PAHs), tobacco-specific nitrosamines, aromatic amines, aldehydes and butadiene. To determine cigarette smoke exposure-associated DNA damage, a prerequisite for the initiation of cancer, we used nose-only and whole-body exposure models in which mice and rats were exposed to cigarette smoke 6 hours a day 5 days a week for several days to several months. To inhibit smoke-exposure-associated DNA damage, experiments were also performed in which groups of animals received diets supplemented with test chemopreventive agents. Respiratory (lung, nasal and trachea) and non-respiratory (liver, bladder and heart) tissues were analyzed for lipophilic DNA adducts by highly sensitive ³²P-postlabeling assay. Comparison of the groups revealed no qualitative difference in the adduct profile in the sham and smoke-exposed animals. However, adduct levels were substantially higher in the treated groups and the adduct levels increased with the duration of the exposure. The lung and the heart tissues showed the highest DNA adduction. Co-chromatography with reference adducts suggested that none of the smoke-associated adducts was related to typical PAHs or aromatic amines. Based on their lipophilicity, these adducts were presumably also not related to the tobacco-specific nitrosamines. Groups of animals intervened with known or potential chemopreventive agents (e.g. oltipraz and indole-3-carbinol) were found to significantly diminish the tissue DNA adduct load. When human lung tumor tissues from cigarette smokers, ex-smokers and non-smokers were analyzed for lipophilic DNA adducts, smokers showed significantly higher degree of adducts compared with ex-smokers or never smokers, with female smokers eliciting higher levels than the male counterparts. Again, these smoke-associated adducts were also found to be unrelated to typical PAHs and aromatic amines based on extensive co-chromatography. It is concluded that i) cigarette smoke exposure significantly enhances the tissue adduct burden; ii) these adducts do not seem to originate from metabolites of typical PAHs and aromatic amines; and iii) the adduct accumulation can be diminished by intervention with chemopreventive agents such as oltipraz and indole-3-carbinol. It is suggested that that the smoke-associated DNA adducts are related to either other smoke constituents such as butadiene and aldehydes or they result from some as yet unknown endogenous pathway.

TOBACCO HARM REDUCTION: SMOKELESS TOBACCO USE CAN SUBSTITUTE FOR SMOKING

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This presentation will discuss tobacco harm reduction, in which inveterate smokers are informed of alternative delivery systems for nicotine, a powerfully addictive drug that does not cause any of the diseases associated with smoking and may be used as safely as caffeine. A specific focus will be alternative nicotine delivery by smokeless tobacco (SLT) products, for which there is an abundant research literature regarding usage patterns and health consequences.

Oral cancer is the most frequently cited health risk from long-term SLT use, but there are many misconceptions regarding both the putative determinants and magnitude of this risk. Historical levels of tobacco-specific nitrosamines and other contaminants (such as cadmium, formaldehyde, benzo-*a*-pyrene and lead) in moist snuff, dry snuff and chewing tobacco will be compared with those in contemporary SLT products. SLT use in Eastern societies, associated with substantially elevated risk for head and neck cancers, will be compared with use in the US and Sweden. In addition, the evidence for risks for other smoking-related cancers, cardiovascular diseases and diabetes among SLT users will be reviewed.

There is strong and unambiguous evidence that tobacco harm reduction has been successful in Sweden, where for decades men have smoked at far lower rates than those in other European countries. Data on patterns of tobacco use among adults in northern Sweden from 1986 to 2004 will be presented, and a study on tobacco use among 15-16 year old Swedish schoolchildren will provide perspectives on the impact of prevalent SLT use as a gateway to, or from, smoking.

The presentation will conclude by briefly reviewing the growing discussion of tobacco harm reduction among public health and policy experts in the US and the EU.

RISK PERCEPTIONS AND THE COST OF SMOKING

Frank Sloan

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This talk describes some major results contained in two recently published books by the author. In *The Smoking Puzzle: Information, Risk Perception, and Choice*, Harvard University Press, 2003, coauthors and I study how people's beliefs about the probability that smoking will be harmful to their personal health and how these risk perceptions are modified by health events that they personally experience. Our findings suggest that more detailed and personalized risk messages focusing on the probabilities of dying from a smoking-related disease are likely to be ineffective. But information about the impact on quality of life of a smoking-related disease is news and does cause smokers to change their perceptions of the risk associated with smoking.

In *The Price of Smoking*, MIT Press, 2004, coauthors and I estimate the cost of smoking to the smoker, his or her family, and to society more generally. The bottom line is the cost to the smoker is \$32.78 per pack (2000 \$), to other family members is \$5.44 per pack, and for society at large, the per

pack cost is \$1.44 for a total cost per pack of \$39.66. A smoker who is age 24 can expect to spend \$141,181 on smoking over the life course on average. This cost includes many elements of cost of which the cost of cigarettes is only a minor component. Messages which display this cost information would seem to be a promising approach for discouraging tobacco use.

AN EFFECTIVE PRODUCT EVALUATION STRATEGY FOR PREP CIGARETTES

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Cigarette smoking has been shown to substantially increase the risk of a number of diseases; including lung cancer, pulmonary disease and heart disease. There is agreement among many stakeholders that the development of potentially reduced exposure cigarette products (PREPs) may offer the opportunity to reduce the risk of disease for smokers. The appropriate testing strategy for evaluating PREPs for potential to reduce the risk of smoking-related diseases is an ongoing subject of discussion within the scientific community. R.J. Reynolds Tobacco Company (RJRT) has developed an effective product evaluation strategy for PREPs that includes both stewardship and reduced exposure components. Product stewardship is a tiered approach focused on ensuring that the proposed product modifications do not increase the inherent biological activity of smoke from the modified cigarette. Stewardship studies may include smoke chemistry, in vitro toxicology and animal toxicology studies. Evaluation of reduced exposure may include data from multiple types of studies, including yield data under multiple machine regimens, yield-in-use data, and biomarker data from smokers. In conformance with the IOM guidelines it is critically important to demonstrate the biological plausibility of a PREP. Biological plausibility requires developing a sufficiently compelling argument based on scientific data to support the conclusion that the demonstrated reduction in exposure would be anticipated to result in a measurable reduction in morbidity and/or mortality in subsequent clinical or epidemiology studies. Approaches that demonstrate biological plausibility include biological and chemical evidence from preclinical toxicology studies, biomarkers of exposure and effect in smokers, and quantitative risk assessment. Finally, the scientific evidence developed in support of a potential PREP should be reviewed and the PREP conclusion verified by an external scientific body.

Poster Presentations

Poster Board # 1

PRENATAL EXPOSURE TO CIGARETTE SMOKE AND CHRONIC AIRWAY DISEASE IN THE OFFSPRING

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Epidemiologic and toxicologic data indicate that smoking during pregnancy increases the risk of offspring respiratory ailments (e.g., asthma) later in life. While these findings carry extensive clinical consequences, little data are available to provide insight into the possible mechanisms by which these effects might occur. We hypothesize that inhalation exposure to mainstream cigarette smoke (MCS) by pregnant mice influences immune-mediated airway reactivity in the offspring. Pregnant CD1 mice were exposed daily for 4 hr/d (5 d/wk) from gestational day 4 to parturition to intact MCS at a concentration equivalent to smoking <1 pack of cigarettes per day. Changes in airway reactivity were evaluated in naive or ovalbumin (OVA)-sensitized, prenatally exposed offspring by a non-specific bronchoprovocation challenge with acetylcholine (Ach) at 5 or 8 and 15 or 18 wk of age, depending upon the protocol; immune parameters known to mediate airway reactivity were also evaluated. In the absence of effects on pregnancy incidence, gestational duration, litter size or offspring sex ratio, prenatal exposure to MCS enhanced airway reactivity and elevated plasma IgE concentrations (compared to the sex/age-matched, air exposed controls) in 5-wk-old naive female (but, not male) offspring. In contrast, airways of prenatally exposed 4-mo-old naive female offspring were significantly less responsive to Ach challenge than their matched controls. OVA-sensitized offspring exposed prenatally to MCS tended to have higher plasma IgE levels than their age-matched, air-exposed controls; sensitized 18-wk-old male offspring were more sensitive to Ach challenge than their age- and treatment-matched female counterparts. Results suggest that prenatal exposure to CS: affects airway health in a gender-dependent manner; increases offspring allergic antibody levels and airway reactivity; may play a role in the induction and/or exacerbation of allergy-related outcomes; and, has long-term effects on offspring respiratory health and may increase the risk of respiratory dysfunction later in life. (IFSH 28-B2600- 7893).

Poster Board # 4

HUMAN BRONCHIAL EPITHELIAL CELL TRANSCRIPTOME: EFFECTS OF CIGARETTE SMOKE AND VAPOUR PHASE IN VITRO AND THE GENERATION OF THE SEEK DATABASE

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Associations between cigarette smoking and disease are well-documented and effects of cigarette smoke on the human lung epithelial transcriptome have been reported. However, little is known about the mechanistic basis of smoking-related disease at the cellular level. The gene expression changes observed in the lung epithelium of healthy smokers may be modulated by multiple parameters such as lifestyle, genetic variability and sub-clinical disease including mild inflammation. To begin to understand the cellular basis of smoke toxicity it is necessary to perform in vitro studies.

We have conducted transcriptomics studies using a 3-D air-liquid interface model of human tracheobronchial epithelium exposed to 2 sub-toxic doses of whole mainstream cigarette smoke and vapor phase. Whole genome data has been generated for 1, 6 and 24h post exposure time-points using Affymetrix HGU133-2 arrays.

For robust statistical analysis, 3 individual whole smoke experiments were performed with cells from each of 3 different donors. We have constructed an online relational database for storing, analyzing and downloading microarray data from these smoke exposure experiments. The 'SEEK' database (Smoke Exposure Experimental Knowledgebase), allows researchers without sophisticated bioinformatics and statistics training to execute queries and obtain biologically relevant results for further investigation. It also enables links for specific genes in the query results into internet based online resources such as NCBI's website. The features of the database means that investigators can download a selection or all data files from selected microarray experiments and perform their own analyses for biological interpretation. Part of the data from this database has already been uploaded onto EBI's ArrayExpress as supplementary data to a submitted manuscript. SEEK has a user friendly interface with the capability of data upload into the database as further experiments are performed by researchers in our laboratories, thus providing a 'one-stop' data management tool for real-time and future use.

Poster Board # 5

PARP-1 ACTIVITY AND EXPRESSION IN CULTIVATED HUMAN LUNG CELLS AFTER EXPOSURE TO STRESS FACTORS

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Poly(ADP-ribose)polymerase -1 (PARP-1), plays a primary role in the process of poly(ADP-ribose)ylation mainly via its activation in response to DNA damage reflecting the severity of genotoxic stress.

We have examined PARP activity in primary human lung cells under exposure to heavy metals, cigarette smoke condensate (CSC) and prolonged cultivation.

Normal human bronchial epithelial (NHBE) and peripheral lung cells (PLC) from lung cancer patients were grown as explant cultures and were seeded on glass cover-slides.

PARP-1 expression was proved by Western Blotting in (NHBE) and tumor lung cell A549.

To study PARP-1 activity in NHBE a functional assay has been used.

Induction of DNA damage by H₂O₂ (100 µM) in NHBE led to formation of ADP-ribose polymers which were detected by immunofluorescence.

Copper(II) (0.05 mM - 24h) did not trigger PARP-1 activity in NHBE but decreased PARP-1 activity induced by H₂O₂ (100 µM). Similarly mercury (II) (0.03 mM-24h) exerted no effect on PARP-1 activity and decreased the activity induced by H₂O₂ (0.1 mM). Treatment of NHBE with CSC (0.5 mg/L) for 24h induced PARP-1 activity by factor 1.3 compared to the control (basal cellular PARP activity) and increased H₂O₂ (100 µM) -induced PARP-1 activity from 1.4 fold to 1.8 fold.

We have analyzed PARP-1 activity in cultures of lung cells for 10 weeks. In higher passages and generations H₂O₂-induced PARP activity decreased, this reflects an adaptation of cells to cultivation. The expression of PARP-1 was almost the same in different generations of the same patient. This means that PARP-1 expression is not affected by prolonged cultivation and is stable.

This study is supported by IFSH and DFG Graduate College

A STUDY DESIGN TO INVESTIGATE THE INFLUENCE OF ISO TAR YIELD AND TAR BAND SWITCHING ON CIGARETTE SMOKE DOSE AS DETERMINED BY FILTER ANALYSIS AND BIOMARKERS OF EXPOSURE

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Two methodological approaches have previously been used to achieve estimates of human smoke exposure, namely filter analysis and biomarkers of exposure.

Filter analysis estimates the maximum amount of smoke that exits the cigarette filter and is taken into the mouth (mouth level exposure).

Conversely, biomarkers of exposure use the level of biomarkers of specific smoke components in biological media (urine, blood or saliva for example) to estimate smoke uptake into the body.

Past studies have shown both good and poor correlations between mouth level and biomarker exposure estimates, depending on the smoke component in question [Rickert et al. 1981, Russell et al. 1982]. Studies using filter analysis have shown that smokers of lower tar yield products have lower mouth level exposure of tar and nicotine than smokers of higher yield products [Shepperd & Mariner, 2001]. However, data from biomarker studies are less conclusive. Some have shown a dose response for nicotine and metabolites, but for other smoke components such as pyrene and NNK there appears to be no significant differences in the levels of biomarkers of exposure found in the body fluids in groups of cigarette smokers over a range of tar yields [Benowitz et al., 2005, Hecht 2005].

Since these studies have not taken into account smoke retention or metabolism differences between individual smokers, the correlation between mouth exposure and biomarkers may be partially dependent on these aspects.

Therefore, by incorporating smoke retention measures and tar band switching into a filter analysis/biomarkers of exposure study, a design has been developed to further investigate the level of correlation between these exposure measures.

The design includes comparison of the level of estimated human cigarette smoke exposure in smokers of a range of ISO/FTC tar bands and non-smokers as well as the influence of switching to a lower tar band.

CAN NATURAL ANTIOXIDANTS PROTECT HUMAN LUNG CELL CULTURES FROM DNA-DAMAGE BY CU(II) AND FE(II)?

Felix Glahn

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Although not being the major source of exposure to heavy metals, inhalation is a route of chronic uptake of low doses of heavy metals e.g. from ambient air or smoking. These metals are mainly bound to fine and ultra-fine particulate matter containing copper and iron among other metals. Transition metals like Cu and Fe generate reactive oxygen species via the Fenton-reaction and might thus lead to DNA-damage. Several naturally occurring compounds are known to be radical-scavengers.

We analyzed the effect of ascorbic acid, tocopherols and quercetin, a natural flavanoid in the human lung tumor cell line A549 treated with Cu(II) and Fe(II). We measured the effects on the DNA using the alkaline COMET-assay. We applied the MTT-assay to establish sub-toxic concentrations for treatment. In the first set of experiments we treated cells with the described antioxidants and either Cu or Fe for 72h. At a concentration of Fe(II) of 500 μ M there was a significant protective effect of the tested antioxidants. Ascorbic acid and tocopherols also exerted a significant protective effect at concentrations of 25 and 50 μ M of Cu(II). In a second set of experiments we pre-treated the cells with antioxidants for four days before applying either Cu(II) or Fe(II). Upon treatment with Fe (1000 μ M) ascorbic acid, quercetin and [δ]-tocopherol significantly reduced tail length, but not at 1500 μ M. At Cu(II) concentrations of 50 μ M cells pre-treated with quercetin or [α]-tocopherol showed increased DNA-damage, while at 75 μ M ascorbic acid and both tocopherols showed significant reduction of tail moment.

Supported by Philip Morris External Research 2002 Program

Poster Board # 8

U. S. SMOKELESS TOBACCO COMPANY SURVEY DATA FROM SIX COUNTRIES REGARDING ADULT CIGARETTE SMOKERS' PERCEPTIONS OF THE COMPARATIVE HEALTH RISKS OF TOBACCO PRODUCTS

James Dillard III

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A number of tobacco harm reduction proponents suggest that cigarette smokers who do not quit or use medicinal nicotine products should switch completely to smokeless tobacco, although others disagree. We sought to understand adult cigarette smokers' views of the comparative health risks of cigarettes and smokeless tobacco internationally, and assess opinion in the scientific and public health communities, as expressed in the published scientific literature, regarding how those views may affect implementing a tobacco harm reduction strategy that includes smokeless tobacco.

One significant impediment to effectively implementing such a tobacco harm reduction strategy, according to published opinion, is adult cigarette smokers' perceptions of the comparative health risks of tobacco products. To explore this issue, consumer research conducted in the USA, Canada, Taiwan, Japan, Australia, and New Zealand investigated adult cigarette smokers' opinions on the relative health risks of tobacco products. Adult smokers (size of groups ranged from 19 to 641) were asked to rate, on a ten point scale, how dangerous they thought certain tobacco products are to a person's health. The results varied among countries. They reflect that U.S. adult cigarette smokers sampled view cigarettes and smokeless tobacco as posing nearly equal health risks. While the adult cigarette smokers sampled in other countries believe that the difference in health risks is greater, they still do not believe that the difference is substantial. Data reflecting these results will be presented.

There is support in the scientific and public health communities for providing adult cigarette smokers with accurate and relevant information regarding the options available to reduce the potential risks to their health, although there are also those who disagree. Further, among proponents of tobacco harm reduction there is opinion that unless cigarette smokers are provided with such information the status quo with respect to their smoking will remain.

MEASUREMENT AND ASSESSMENT OF POLONIUM²¹⁰ AND LEAD²¹⁰ AS BIOMARKERS OF ACTUAL DOSE OF INHALED CIGARETTE SMOKE

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The assessment of disease risk from cigarette smoking requires valid estimates of the inhaled dose from both current and past smoking. The atmospheric radon decay product lead²¹⁰ (Pb²¹⁰) accumulates in tobacco leaves resulting in the in-growth of polonium²¹⁰ (Po²¹⁰), and both isotopes are transferred to cigarette smoke. We hypothesize that the urinary concentrations of Po²¹⁰ and Pb²¹⁰ correlate with cigarette smoking intensity. Pb²¹⁰ is potentially useful as a biomarker of long-term inhaled dose from smoking, while Po²¹⁰ may indicate short-term dose. To our knowledge, there are no biomarkers of long-term integrated dose currently available, and cotinine, a short-term exposure biomarker, derives from both the particle and gas phases.

We recruited 250 subjects (mean age 47.6 years, range 20-82 years) living in China and with different smoking intensities. Each subject provided a 24-hour urine sample, from which cotinine and creatinine levels were measured rapidly; Po²¹⁰ and Pb²¹⁰ are currently being assayed. Twelve brands of commonly smoked cigarettes were also examined for their Po²¹⁰ and Pb²¹⁰ contents.

The urine results indicate a weak regression ($R^2=26\%$) between the number of cigarettes smoked as predicted by the measured cotinine. In the cigarettes, the average (range) Po²¹⁰ activity is 24 (18-30) mBq/cigarette. Assuming a secular state of equilibrium between the Po²¹⁰ and Pb²¹⁰, it is estimated that a person smoking 20 cigarettes daily would inhale about 180 mBq of Po²¹⁰ and 120 mBq of Pb²¹⁰ per day. These inhalation estimates will be used with the urinary concentrations of Po²¹⁰ and Pb²¹⁰ to establish dose-response relationships that will allow for the development of a model predicting the inhaled doses of cigarette smoke from urinary excretion data.

VALIDATION OF BIOMARKERS IN EXHALED BREATH CONDENSATE (EBC) FOR EVALUATING THE SMOKING-RELATED AIRWAY INFLAMMATION

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Exhaled breath condensate (EBC) can non-invasively be collected and has high potential for investigating molecular changes in the respiratory tract.

Inflammation markers in EBC are determined for clinical purposes in patients with asthma, COPD, fibrosis and other lung diseases. The effect of tobacco smoking on EBC biomarkers has been also investigated, with up to now controversial results.

The purpose of our investigation was to establish, validate and apply methods for collecting EBC and measuring EBC biomarkers in order to determine the suitability of this technique for the evaluation of PREPs.

Analytical methods for the following EBC constituents were established and validated: Nitrite, nitrate, Na⁺, K⁺, Ca²⁺, Mg²⁺, urea, alpha-amylase, 3-nitrotyrosine, aldehydes, total proteins, interleukine-6, tumor necrosis factor alpha, adenosine, 8-isoprostane, leukotriene B4,

cadmium. Nitrogen oxide (NO_x) was also determined. These EBC markers were applied to a semi-controlled pilot study with 12 volunteers (6 nonsmokers, 6 smokers).

All EBC markers measured in the pilot study showed high (to extremely high) intra- and inter-individual variations. In addition, there was significant interference from background contamination. Nitrite and NO_x were inversely associated with smoking, whereas nitrate was not affected by smoking. 3-Nitrotyrosine tended to be elevated in smokers and showed a weak association with the smoking dose. Acrolein, crotonaldehyde and hexanal were significantly higher in EBC of smokers compared to nonsmokers. The effect of smoking was weaker and not significant for heptanal and malondialdehyde and almost absent for nonanal.

Aldehydes (in particular crotonaldehyde) and 3-nitrotyrosine show some potential as biomarkers of smoking-related effects in the lung. NO_x is a suitable medium- to long-term biomarker for smoking related changes in the respiratory tract. EBC technology requires further improvements before it can be applied for PREP testing.

Poster Board # 11

ANALYSIS OF MYOSMINE, COTININE AND NICOTINE IN HUMAN TOENAILS

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Myosmine, a minor tobacco alkaloid, was thought to be as specific for tobacco as nicotine. Recently, myosmine has been detected in dietary components including staple food such as potatoes, rice, corn and millet, and also in milk and cream. Under acid conditions and after nitrosation myosmine give rise to HPB-releasing adducts which are also formed after metabolic activation NNN and NNK. In human lymphocytes and nasal mucosa genotoxic effects of myosmine have been demonstrated in the comet assay.

Aim of this work was to measure myosmine beside cotinine and nicotine in human toenails for the first time. After digestion of the toenails in NaOH, the alkaloids were extracted with CH₂Cl₂ and analyzed by GC-MS. Thirty-two samples of toenails from self-reported nonsmokers (NS, n=10), passive smokers (PS, n=6) and smokers (S, n=16) have been analyzed so far.

Whereas myosmine was detectable in all samples and nicotine in all except one nonsmoker, cotinine could not be detected in all nonsmokers and one of six passive smokers. The concentrations (ng/mL) of nicotine (NS: 0.119 ± 0.023 ; PS 0.637 ± 0.237 ; S: 1.983 ± 0.208) and cotinine (NS: n.d.; PS 0.360 ± 0.138 ; S: 1.259 ± 0.202) were highly dependent on the smoking status. Myosmine concentrations (ng/mL) were not different between nonsmokers (0.023 ± 0.004) and passive smokers (0.031 ± 0.010) and less than threefold higher in smokers (0.062 ± 0.013). Myosmine correlated well with both nicotine ($r=0.647$, $p<0.0001$) and cotinine ($r=0.634$, $p<0.0001$) but less well than nicotine with cotinine ($r=0.831$, $p<0.0001$).

These results indicate that myosmine exposure in humans depends only partially on smoking and suggest an important contribution of other sources such as diet.

VALIDATION OF BIOMARKERS OF EXPOSURE AND HOST RESPONSE

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Cigarette smoking remains the most preventable cause of death in the U.S. and is the most important cause of COPD. Smoking elicits an inflammatory response believed to play a key role in pathogenesis of several lung diseases, thus measures of inflammation are plausible biomarkers related to disease risk.

The purpose of this study is to establish feasibility and validity of exhaled breath condensate (EBC) biomarkers for use in studies designed to evaluate harm reduction strategies of smoking. This will be accomplished by measuring selected markers, particularly H₂O₂ in EBC believed related to pathogenesis of lung disease before and after smoking cessation interventions using nicotine replacement therapy (NRT). Individuals who fail to quit will be allowed to continue on NRT and will have biomarkers assessed.

One hundred subjects have been enrolled based on the following criteria: ≥ 19 years of age, stable smoking habit > 3 months, ≥ 5 pack year history, and normal lung function. All subjects must have been willing to make a serious quit attempt and willing to use NRT.

Once enrolled, subjects established a quit date, were treated with NRTs based on individual preference, and received smoking cessation counseling. Scheduled visits included 12 visits over 25 weeks.

Evaluations include EBC, interval smoking history, exhaled carbon monoxide, blood drawn for chemistry and CBC, urine sample for isoprostane. Some visits include spirometry with and without bronchodilators, 24 hour urine collections for NNAL and NNAL-glc quantification, food frequency questionnaire, health status assessed using St. George's Respiratory Questionnaire, Functional Assessment of Chronic Illness Therapy-Fatigue, Leicester Cough Questionnaire, Clinical COPD Questionnaire and smoking related symptoms assessed with Breathlessness, Cough, and Sputum Scale.

Interim data are available, but no interim statistical analysis is planned. The study is expected to complete in summer of 2007 with final data analysis in fall of 2007.

THE EFFECT OF AIR POLLUTANTS ON RESPIRATORY AND CARDIOVASCULAR RESPONSES

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The control of gene function is an important level where environmental pollutants such as fine particles may be active. There is still a considerable lack of information on the mechanisms of toxicity by particulate matter. Various chemical components, such as metals and organic compounds, and other cooperating factors such as cigarette smoke may influence the inflammatory potential of ambient particles.

Data from both human and animal studies indicate a possible etiological relationship between exposure to air particles and the development of some respiratory and cardiovascular diseases. A low density lipoprotein receptor knock-out mouse model (C57 BL/6.LDLR^{-/-}) has been created and used to study the mechanisms of lesion formation in atherosclerosis, environmental effects on the extent of

lesions, and the role of candidate genes in the susceptibility. We use a diet that produces hypercholesterolemia and atherosclerosis in these mice.

We have designed an experimental system to study the possible interactive and synergetic effects of hypercholesterolemia and exposure to fine particles on the development of atherosclerosis. Two test groups of female LDLR^{-/-} mice are subjected to Arizona road dust and printex75 for 8 weeks in 1 m³ inhalation chambers after receiving the diet for 4 weeks. Atherosclerotic lesions will be measured as cross-sectional area at the aortic root and brachiocephalic artery.

In previous studies, we have proved that metal stress (copper, mercury) decreases the activity of the DNA damage indicator Poly(ADP-ribose)polymerase_1 (PARP-1) in primary human lung cells. Treatment of human bronchial cells with cigarette smoke condensate has significantly induced PARP-1 activity. Therefore, the effect of particles on cytokine-inducing potential (ELISA) and gene expression profiling with respect to DNA-repair (PARP1), detoxification mechanisms (glutathione) and lung CYP-450 will be analyzed by gene-array to verify the genetic background of the effects.

Poster Board # 14

USING THE EPIAIRWAY MODEL TO CHARACTERIZE TOBACCO CONSTITUENT TOXICITY

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Environmental tobacco smoke, along with many other inhaled materials is implicated in the aggravation of many respiratory and cardiac diseases. As such, the need for new and effective methods for evaluating pulmonary toxicity is ever increasing. This study investigates the potential of a differentiated, mixed population, 3-dimensional, in vitro model of human respiratory epithelia (EpiAirway-100 cells; MatTek Corporation) to act as a suitable model for pulmonary toxicology.

The EpiAirway tissue model (ETM) was dosed apically with four tobacco smoke components (TSC: nicotine, formaldehyde, cadmium, urethane). The TSC represent an overview of the compounds found in both the particulate and vapor phase of cigarette smoke that induce a range of toxic effects (e.g. cytotoxic, thrombogenic, carcinogenic). A range of TSC doses were used to elicit a classic dose response and to measure the capacity of the ETM to model different mechanisms of toxicity. The ETM was dosed with surrogate solutions of TSC. Conventional toxicological analysis was used, to measure changes in transepithelial electrical resistance (TEER), cell viability (MTT) and secreted surface proteins (Bradford assay).

All four TSC appeared to induce similar stress responses within the model. Distinct alterations in tissue integrity occurred in response to the toxins. At high doses MTT and TEER values decreased ($p < 0.05$) but at sub-toxic doses a peak in TEER measurements (20-60% increase from control) was observed ($p < 0.05$). This peak coincided with an increase in protein secretion, suggesting a role for secretory processes as an early protective response to TSC.

The EpiAirway system appeared to show a good capacity to model TSC toxicity. It provides the convenience of in vitro models, whilst generating more physiological responses than traditional single-cell culture systems. The ETM has good potential as an in vitro model for inhalation toxicology investigations.

DIFFERENTIAL EFFECTS OF CIGARETTE SMOKE ON OXIDATIVE STRESS AND PROINFLAMMATORY CYTOKINE RELEASE IN PRIMARY HUMAN AIRWAY EPITHELIAL CELLS AND IN A VARIETY OF TRANSFORMED ALVEOLAR EPITHELIAL CELLS

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Background: Cigarette smoke mediated oxidative stress and inflammatory events in the airway and alveolar epithelium are important processes in the pathogenesis of smoking related pulmonary diseases. Previously, individual cell lines were used to assess the oxidative and proinflammatory effects of cigarette smoke with confounding results. In this study, a panel of human and rodent transformed epithelial cell lines were used to determine the effects of cigarette smoke extract (CSE) on oxidative stress markers, cell toxicity and proinflammatory cytokine release and compared the effects with that of primary human small airway epithelial cells (SAEC).

Methods: Primary human SAEC, transformed human (A549, H1299, H441), and rodent (murine MLE-15, rat L2) alveolar epithelial cells were treated with different concentrations of CSE (0.2-10%) for 4-24 hr. Cytotoxicity was assessed by lactate dehydrogenase release assay, trypan blue exclusion method and double staining with acridine orange and ethidium bromide. Glutathione concentration was measured by enzymatic recycling assay and 4-hydroxy-2-nonenal levels by using lipid peroxidation assay kit. The levels of pro-inflammatory cytokines (e.g. IL-8 and IL-6) were measured by ELISA. Nuclear translocation of the transcription factor, NF- κ B was assessed by immunocytochemistry and immunoblotting.

Results: Cigarette smoke extract dose-dependently depleted glutathione concentration, increased 4-hydroxy-2-nonenal (4-HNE) levels, and caused necrosis in the transformed cell lines as well as in SAEC. None of the transformed cell lines showed any significant release of cytokines in response to CSE. CSE, however, induced IL-8 and IL-6 release in primary cell lines in a dose-dependent manner, which was associated with the nuclear translocation of NF- κ B in SAEC.

Conclusion: This study suggests that primary, but not transformed, lung epithelial cells are an appropriate model to study the inflammatory mechanisms in response to cigarette smoke.

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