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Award Number: DAMD17-00-1-0086

TITLE: The Effect of Cancer Chemopreventive Agents on DNA Adduct Formation by the Dietary Prostate Carcinogen PhIP

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REPORT DATE: April 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 2002	Annual (1 Apr 01 - 31 Mar 02)

4. TITLE AND SUBTITLE
The Effect of Cancer Chemopreventive Agents on DNA Adduct Formation by the Dietary Prostate Carcinogen PhIP

5. FUNDING NUMBERS
DAMD17-00-1-0086

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

This proposal aims to investigate chemopreventive strategies to reduce the genotoxic effects of the prostate carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). PhIP is considered to pose a significant prostate cancer risk to humans because it is found in cooked meat and epidemiology studies have linked meat consumption to prostate cancer. Importantly, PhIP causes prostate cancer in rats following high-dose exposures. Therefore, our purpose is to use the rat model to determine the risk posed by PhIP at levels found in the diet and to identify candidate chemopreventive agents that could be used to reduce prostate cancer risk as a result of exposure to PhIP. Over the last year, we have established several mechanisms by which the isothiocyanate PEITC reduces the genotoxic effects of PhIP and have investigated the dose-dependence of the chemopreventive effects . We have completed experiments using chlorophyllin and lycopene and have shown that chlorohyllin may also be useful in prostate cancer prevention. Importantly, albumin adduct levels in the blood were useful biomarkers of these chemopreventive effects. Consequently, this work may lead to the identification of effective chemopreventive strategies.

14. SUBJECT TERMS	15. NUMBER OF PAGES
prostate cancer, prevention, carcinogenesis, biomarker, accelerator mass spectrometry, PhIP, chemopreventive agents	12
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover	1
SF 298	2
Introduction	4
Body	4
Key Research Accomplishments	7
Reportable Outcomes	8
Conclusions	8
References	9
Appendices	10

Introduction:

This proposal aims to investigate chemopreventive strategies to reduce the genotoxic effects of the prostate carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). PhIP is considered to pose a significant prostate cancer risk to humans because it is found in relatively high concentrations in cooked meat (Zhang *et al.*, 1988; Felton and Knize, 1990) and some epidemiology studies have shown a correlation between meat consumption and prostate cancer incidence (Mills *et al.*, 1989; Talamini *et al.*, 1992; De Stefani *et al.*, 1995; Ewings and Bowie, 1996). Importantly, PhIP causes prostate cancer in rats following high-dose exposures (Shirai *et al.*, 1997). However, studies to establish carcinogenicity and determine the efficacy of chemopreventive agents generally employ chemical doses orders of magnitude higher than the average human daily intake, hence are of questionable relevance. Therefore, our purpose is to use the rat model and the highly sensitive technique of accelerator mass spectrometry (AMS) (Turteltaub *et al.*, 1990a) to determine the risk posed by PhIP at levels found in the diet. We are using DNA adduct formation by PhIP as a measure of risk, as it is a form of DNA damage which is considered an early event in the development of cancer. Subsequently, we aim to identify candidate chemopreventive agents and the effective doses that could be used to reduce DNA adduct formation, and consequently prostate cancer risk, as a result of dietary exposure to PhIP.

Body:

The progress made towards the specific aims of the research project completed in the period April 1, 2001 to March 31, 2002 is described as follows:

Specific aim #1: Determine the bioavailability of PhIP and the level of adduct formation in rat prostate following dietary levels of exposure.

These studies were completed in year 1 of this proposal.

Specific aim #2: Determine the metabolism of PhIP in rat prostate at dietary levels of exposure. In year 1 we established that circulating NOH-PhIP, an intermediate that results in DNA binding, may be a factor in the targeting of PhIP to the prostate. In year 2, we investigated if the prostate has the capacity to metabolize [^{14}C]PhIP to genotoxic metabolites, which will help determine which pathways of PhIP metabolism can be targeted in human prostate cancer chemoprevention.

Microsomes prepared from rat prostate did not generate oxidation products of PhIP. In contrast, liver microsomes generated multiple oxidation products from PhIP, including NOH-PhIP. We conclude that NOH-PhIP is not generated by cytochrome P450 at detectable levels in the prostate and is supplied by the circulation, probably from the liver.

Specific aim #3: Determine the effect of chemopreventives on PhIP bioavailability, metabolism and adduct formation in the prostate.

The goal of this aim is to identify several candidate chemopreventive agents and the effective doses that could be used to reduce human prostate cancer risk as a result of dietary exposure to PhIP.

A pilot study was conducted in year 1 in which we showed that the isothiocyanate PEITC reduced adduct formation in the prostate. Although preliminary, these results suggest that PEITC had a protective effect. This study was repeated in year 2 using larger groups of animals (5 per dose group), a lower dose of [^{14}C]PhIP (8 μg/Kg bw) and 2 doses of PEITC to look at the effect of a lower dose (816 mg/Kg and 82 mg/Kg in diet). We also examined the effect of genistein (found in soy; 500 mg/Kg diet), chlorophyllin (a stable, sodium salt of chlorophyll; 1% w/w in diet) and lycopene (as the dietary supplement Lycovit; 0.1% w/w lycopene in diet). DNA adduct levels were measured in the colon and prostate (the target organs for PhIP-induced cancer). Results are shown in figure 1. DNA adduct levels were reduced in the colon and prostate of animals pretreated with 816 mg/kg bw PEITC and chlorophyllin. The lower dose of PEITC (82 mg/kg bw) did not significantly reduce adduct levels. This data indicates that PEITC and chlorophyllin may be useful in prostate cancer prevention, although in the case of PEITC, there may be a dose-dependence for the protective effect.

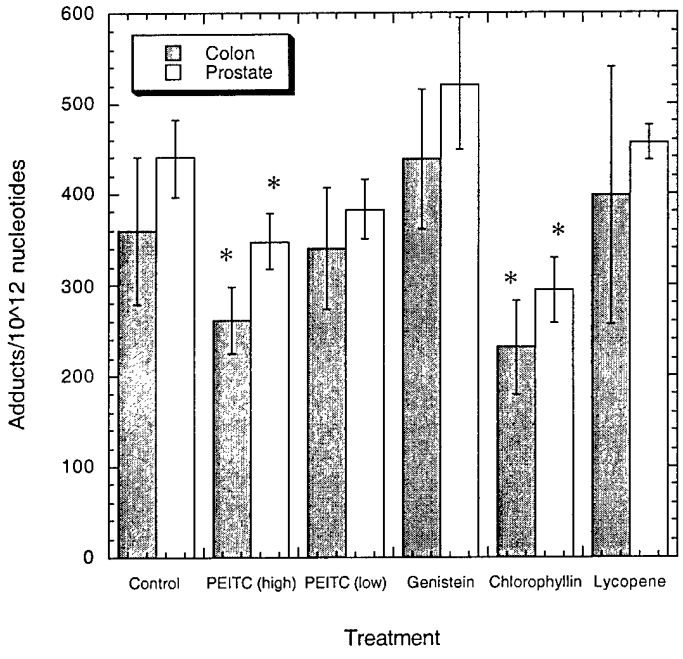

Figure 1. DNA adduct levels in the colon and prostate of rats pretreated with PEITC, Genistein, Chlorophyllin and lycopene and then dosed with [^{14}C]PhIP (*significantly different from controls, $P<0.05$)

To futher our understanding of the role of albumin adducts in the blood as a biomarker in chemoprevention studies, albumin was extracted from the blood and analyzed to

determine if DNA adduct levels were reflected by the albumin adduct levels. Data are shown in figure 2 and show that albumin adducts were also reduced in the high dose PEITC and chlorophyllin treated animals. Therefore, albumin adducts are good biomarkers for assessing the effect of chemopreventive agents in target organs.

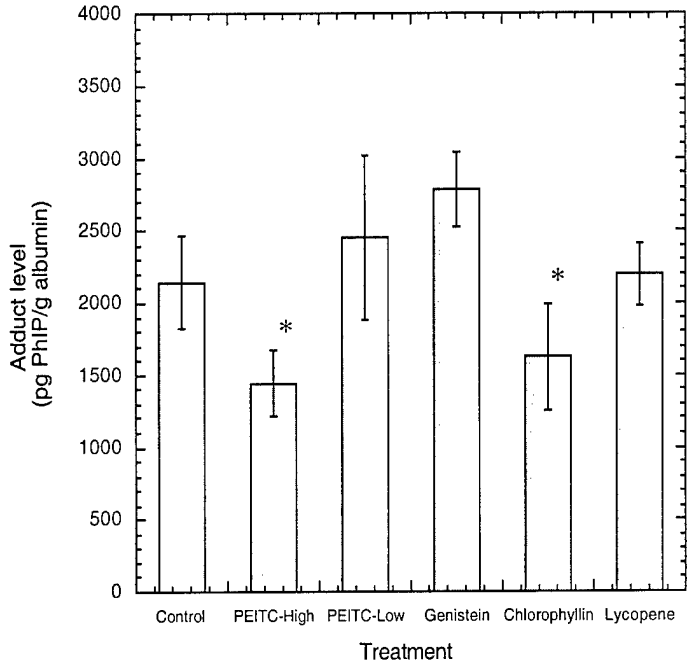

Figure 2. Albumin adduct levels in the blood of rats pretreated with PEITC, Genistein, Chlorophyllin and lycopene and then dosed with [^{14}C]PhIP (*significantly different from controls, $P<0.05$)

In year 2, the mechanism by which PEITC reduces the carcinogenic potential of PhIP was also investigated. To determine if PEITC interferes with PhIP bioactivation, microsomes from rat liver were prepared to measure the metabolism of PhIP in the presence of PEITC. PhIP metabolism in liver was inhibited by PEITC. With particular significance PEITC abolished the production of NOH-PhIP, while less reactive metabolites were still produced. We conclude that PEITC inhibits cytochrome P450 dependent metabolism of PhIP and that inhibition of the metabolic activation of PhIP to reactive intermediate such as NOH-PhIP may lead to the reduction of DNA adducts.

Inorder to investigate whether this chemoprevention mechanism may also apply to humans, the effect of PEITC on PhIP bioactivation was tested using recombinantly expressed human and rat cytochrome P450 1A1/1A2. The bioactivation of PhIP by expressed cytochrome P450 1A1 /1A2 produced two predominant metabolites, 4'OH-PhIP and NOH-PhIP. The ratio of 4'OH/NOH metabolites produced differed substantially by individual CYP450 isoforms and between rat and human. When PEITC was added to incubations containing expressed proteins, the bioactivation of PhIP was inhibited (figure 3). The inhibition appeared to be dose responsive, as greater

concentrations of PEITC inhibited PhIP bioactivation to a greater degree. We conclude from these studies that metabolism of PhIP differs greatly between human and rat P450 isoforms and PEITC inhibits bioactivation of PhIP.

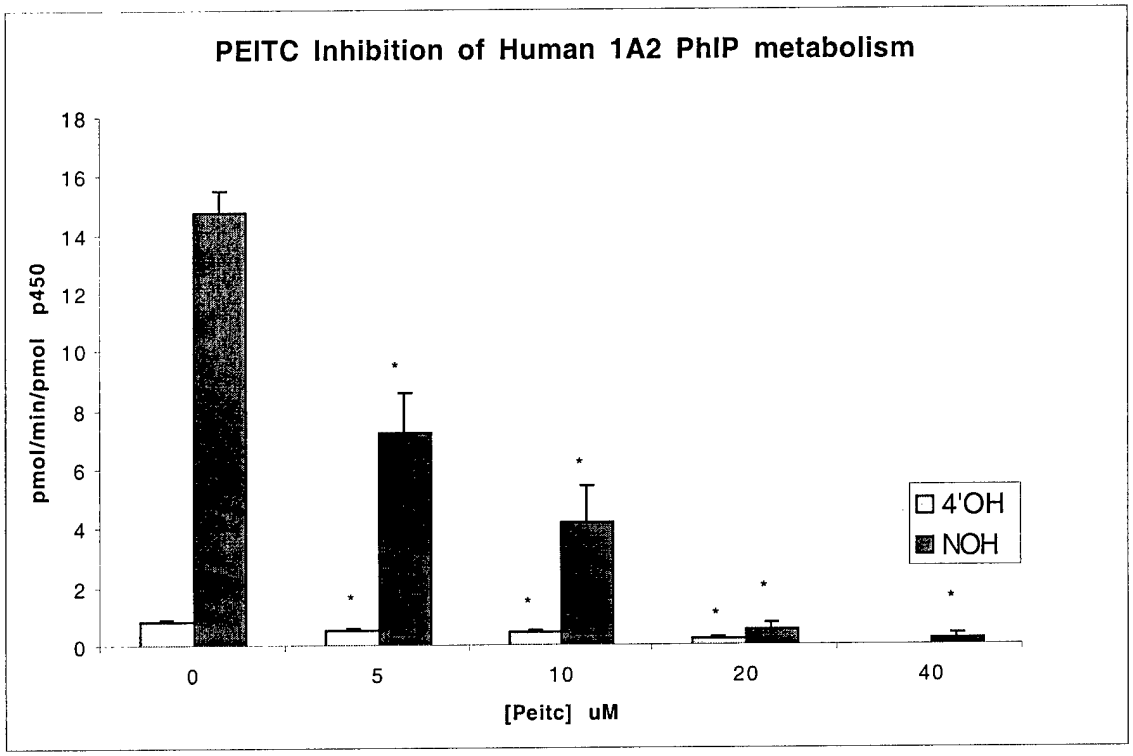

Figure 3. Inhibition of human cytochrome P450 metabolism by PEITC (*significantly different from controls, $P<0.05$)

Inorder to investigate the effect of PEITC on PhIP detoxification, rats were fed PEITC at 816 mg/Kg body-weight for nine days. Twenty-four hours later, microsomes from rat liver were prepared to determine if glucuronidation was induced. When rats were exposed to dietary concentration of PEITC over nine days, glucuronidation of NOH-PhIP was induced. Compared to control, rats fed PEITC appeared to induce detoxification of NOH-PhIP by glucuronidation approximately two-fold. Therefore, PEITC appears to induce the major route of detoxification for PhIP.

Key Research Accomplishments:

During the second year of this grant, we have shown that:

1. Rat prostate does not have the capacity to activate PhIP to N-OH PhIP by cytochrome P450. This metabolite most likely reaches the prostate through the circulation.
2. PEITC may inhibit DNA adduct formation through inhibition of bioactivation of PhIP by cytochrome P450, aswell as induction of detoxification enzymes.
3. The inhibition of adduct formation by PEITC is dependent on dose.
4. Genistein and lycopene do not reduce adduct formation by PhIP in the prostate.
5. Chlorophyllin reduces adduct formation in the prostate, as well as other organs.
6. Albumin adduct levels in blood reflect DNA adduct levels in the prostate.

Reportable Outcomes:

1. **Abstracts/poster presentations**

a) Abstract entitled 'Phenylethylisothiocyanate (PEITC) both inhibits cytochrome P450 1A1/1A2 bioactivation and induces glucuronide detoxification of the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)'. Presented by West, J.A., Turteltaub K.W., and Dingley K. at the Society of Toxicology Annual Meeting, Nashville, TN., March 17-21 2002.

b) Abstract entitled 'Phenylethylisothiocynate (PEITC) reduces 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) DNA adduct formation by inhibition of Cytochrome P450 dependent metabolism'. Presented by West, J.A., Turteltaub K.W., and Dingley K. at the Society of Toxicology Annual Meeting, Nashville, TN., March 17-21 2002.

2. **Oral presentations**

a) Presentation entitled 'Effect of dietary supplements with chemopreventive potential on adduct formation following a low dose of PhIP' was presented by K. Dingley at the 8th International Conference on Carcinogenic/Mutagenic N-Substituted Aryl Compounds, November 12-14, 2001. K. Dingley received a **Research Award for Outstanding Contribution of Original Research.**

3. **Manuscripts**

Four manuscripts containing work from this grant are currently in preparation. They are:

a) 'Metabolism And DNA Adduct Formation Of 2-Amino-1-Methyl-6-Phenylimidazo[4,5-*b*]Pyridine (PhIP) At Low Dose In The Male F344 Rat'

b) 'The Effect of Dietary Supplements with Chemopreventive Potential on Metabolism and DNA-Adduct Formation of the Heterocyclic Amine PhIP in Rats'.

c) 'Phenylethylisothiocyanate (PEITC) both inhibits cytochrome P450 1A1/1A2 bioactivation and induces glucuronide detoxification of the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)'

d) 'Phenylethylisothiocynate (PEITC) reduces 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) DNA adduct formation by inhibition of Cytochrome P450 dependent metabolism'.

4. **Employment/Research Opportunities**

Over the last year, we were able to hire a Summer student, Kristin Stoker from UC Berkeley, to work on this project.

Conclusions:

During the second year of this grant, we have made significant progress on our specific aims. We have shown that NOH-PhIP, a reactive intermediate involved in DNA adduct formation, most likely reaches the prostate through the circulation. PEITC inhibits adduct formation in the prostate, possibly through inhibition of cytochrome P450, aswell as induction of detoxification enzymes. However, this inhibition is dose-dependent.

Chlorophyllin may also be useful in prostate cancer prevention, as it reduces DNA adduct formation in the prostate. Furthermore, albumin adduct levels in blood reflect DNA adduct levels in the prostate and may be useful in future human studies for assessing the effectiveness of chemoprevention strategies.

"So What?"

As a result of the work completed over the last year, we have made the following contributions to conquering prostate cancer:

1. Identified chlorophyllin as a chemopreventive agent that could be used to reduce human prostate cancer risk as a result of dietary exposure to PhIP.
2. Identified mechanisms that may be involved in chemoprevention by PEITC. These mechanisms are likely to be relevant to humans, although dietary-relevant doses of PEITC may not be effective in chemoprevention *in vivo*.
3. Further validated albumin adduct formation in the blood as a biomarker that could be used for monitoring the effect of chemoprevention strategies.

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Appendices:

Copy of abstracts entitled 'Phenylethylisothiocyanate (PEITC) both inhibits cytochrome P450 1A1/1A2 bioactivation and induces glucuronide detoxification of the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)' and 'Phenylethylisothiocynate (PEITC) reduces 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) DNA adduct formation by inhibition of Cytochrome P450 dependent metabolism'. Presented by West, J.A., Turteltaub K.W., and Dingley K. at the Society of Toxicology Annual Meeting, Nashville, TN., March 17-21 2002.

quantities. These compounds have been found in sugar, salt, dairy products, meat, fish, shellfish, instant soup, cereal, and edible plant products. Although there is little carcinogenic and genotoxic information for DBP, other phthalate esters such as DEHP have been shown to be carcinogeneic and to some degree genotoxic. We propose that DBP may be genotoxic. To test this hypothesis two experiments were conducted with male F344 rats dosed with various concentrations of DBP (10 g/kg, 5 g/kg, & 1g/kg) by gavage. One group of rats were sacrificed twenty-four hours after dosing. Slides were prepared from testes, stained with 33258 Hoechest, and analyzed by fluorescence microscopy for micronuclei formation. The second group of rats were sacrificed four hours after dosing. DNA from the liver and testes were analyzed by P32 Post-labeling for DNA adducts. The results revealed that DBP produced micronuclei in a dose dependent fashion. One thousand cells from each group were counted. The high dose (10g/kg) produced micronuclei in 174 cells, the 5g/kg dose produced micronuclei in 133 cells, and the 1g/kg dose produced micronuclei in 56 cells. However, no DNA adducts were detected. The formation of micronuclei indicates that chromosome breakage has occurred. This is direct evidence that DBP is genotoxic *in vivo*. This genotoxicity can lead to cancer or other adverse effects.

LB22 INHIBITION OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION AND THE ACTIVATION OF MITOGEN ACTIVATED PROTEIN KINASE BY PCBS DEPENDS ON SUBSTITUTION OF CHLORINE IN THE ORTHO-POSITION.

B Upham, J Trosko. *Food Safety & Toxicology, Michigan State University, East Lansing, MI.*

The most tumorigenic polychlorinated biphenyls (PCBs) in rodent model systems are the non-planar, ortho-chlorinated congeners. These nonplanar PCBs are effective promoters and not initiators in two stage cancer models, which indicate that epigenetic mechanisms are involved. Three critical epigenetic events are needed at the promotional stages of cancer; one being the removal of an initiated cell from the suppression of growth by neighboring cells through the intercellular transfer of signal transductants *via* gap junctions, the second being the activation of a mitogenic pathway such as the extracellular receptor kinase (ERK) class of mitogen activated protein kinases (MAPK), and the third is the inhibition of apoptosis. We determined the effects of the coplanar PCB-126 (3,3',4,4',5-pentachlorobiphenyl), and the nonplanar PCB153 (2,2',4,4',5,5'-hexachlorobiphenyl) congeners on gap junctional intercellular communication (GJIC) and the phosphorylation of ERK in a rat liver epithelial cell line that has oval cell characteristics. The scrape load/dye transfer technique was used to measure GJIC, and a Western blot analysis with phospho-specific antibodies was used to measure activated ERK. The non-planar PCB reversibly inhibited GJIC at noncytotoxic doses with a threshold value of no effect at 35 μM and complete inhibition at 50 μM and 1 h. The coplanar PCB did not inhibit GJIC, even after 20 h, and up to cytotoxic levels. Similarly, the nonplanar and not the coplanar PCB activated ERK with similar kinetics as the GJIC study; however, experiments with the MEK inhibitor PD98059 indicated that inhibition of GJIC was independent of the MAPK pathway. These MAPK-results differ from endogenous growth factor induced-inhibition of GJIC but are similar to the effects of polycyclic aromatic hydrocarbons (PAHs) that contain bay-like regions. PAHs operate partially through a phospholipase C (PLC) mechanism. The selective inhibitor of diacylglycerol lipase, RHC-80267, partially reversed the effects of the nonplanar PCB on GJIC indicating that PLC was, in part, involved in the mechanism of GJIC-inhibition. Overall, our *in vitro* results are consistent with the tumor promotion properties of a compound in which the nonplanar carcinogenic PCB inhibits GJIC and activates a mitogenic signaling pathway. Funded by the National Institute of Environmental Health Science (NIEHS) Superfund grant USA #P42 ES04911-07 to JET.

LB23 PHENYLETHYLISOTHIOCYANATE (PEITC) BOTH INHIBITS CYTOCHROME P450 1A1/1A2 BIOACTIVATION AND INDUCES GLUCURONIDE DETOXIFICATION OF THE CARCINOGEN 2-AMINO-1-METHYL-6-PHENYLIMIDAZO[4,5-B]PYRIDINE (PHIP).

J West[1], K Turteltaub[2], K Dingley[2]. [1]*Sandia National Laboratory, Livermore, CA.* [2]*Lawrence Livermore National Laboratory, Livermore, CA.*

Exposure to heterocyclic amine mutagens, such as PhIP, result in DNA adducts that correlate with tumor formation. The chemopreventative agent PEITC appears to reduce the formation of DNA adducts. The mechanism by which this occurs is not completely understood. The current studies were designed to determine if PEITC interferes with metabolism routes relevant to PhIP bioactivation and detoxification. The effect of PEITC on PhIP bioactivation was tested using recombinantly expressed human and rat cytochrome P450 1A1/1A2. In a separate experiment, rats were fed PEITC for nine days. Twenty-four hours later, microsomes from rat liver were prepared to determine if glucuronidation was induced. Results: The bioactivation of PhIP by expressed cytochrome P450 1A1 /1A2 produced two predominant metabolites, 4'OH-PhIP and NOH-PhIP. The ratio of 4'OH/NOH metabolites produced differed substantially by individual CYP450 isoforms and between rat and human. When PEITC (5-40mM) was added to incubations containing expressed proteins, the bioactivation of PhIP was inhibited. The inhibition appeared to be dose responsive as greater concentrations of PEITC inhibited PhIP bioactivation to a greater degree; inhibition appeared maximal > 40mM. When rats were exposed to dietary concentration of PEITC over nine days, glucuronidation of NOH-PhIP (the toxic metabolite of PhIP) was induced. Compared to control, rats fed PEITC appeared to induce detoxification of NOH-PhIP by glucuronidation approximately two-fold. We conclude from these studies: 1) Metabolism of PhIP differs greatly between human and rat P450 isoforms, 2) PEITC inhibits bioactivation of PHIP and 3) Dietary PEITC appears to induce the major route of detoxification for the heterocylic amine carcinogen, PhIP.

LB24 DEFICIENCY OF DNA DOUBLE-STRAND REJOINING AND SPINDLE CHECK POINT IN MALIGNANT TRANSFORMED CELL LINES OF HUMAN BRONCHIAL EPITHELIAL CELLS GENERATED BY [ALPHA] PARTICLES EXPOSURE.

PK Zhou, J Sui, J Sun, Y Geng, Y Hu, and D Wu. *Department of Radiation Toxicology, Beijing Institute of Radiation Medicine, Beijing, PR China.* Sponsor: J Goodman.

Carcinogenesis is a multistage process prompted by the deficiency of mechanisms maintaining genomic integrity or chromosome stability. Malignant transformed cell lines were derived from the HPV 18-immortalized human bronchial epithelial cell line BEP2D generated by 1.5Gy of (alpha) particles imitted by a 238Pu source. The deletions of chromosome 2, 12, 13 and 17 were obsserved in the transformed cell lines. Transformed cell line BERP35T4 showed a significant higher proportion of polypoid cells (40.5%) compared with parental BEP2D cells and another transformed cell line BERP35T1 (5%). The capacity of χ-ray-induced DNA DSBs rejoining in malignant transformed cell lines BERP35T1 and BERP35T4 was significantly decreased compared with the parental BEP2D cells. The analysis of mRNA level revealed a 2.5- to 6.5-fold dow-regulated expression of the DNA repair gene XRCC-2, XRCC-3 and Ku80 in BERP35T1 and BERP35T4 cells. In contrast, the expression of DNA-PKcs was 2.4-fold up regulated in the transformed cell line BERP35T4. Point mutations of DNA-PKcs gene were also detected in the transformed cells. Consisting with the increased proportion of polyploides, BERP35T4 cell line also showed an abnormal spindle check-point mechansim. The methylation of CpG in the promoter region of some mitotic check-point genes were obsserved. This study has shown tht the karyotypic changes, and the deficiency of DNA double-strand break resjoining and spindle check-point could be involve in the malignant transformation process of BEP2D cells induced by (alpha) particles exposure.

hyaluronic acid concentrations at early time points after each LPS administration exhibited transient but significant increases that returned to normal values 24 hr after treatment. Conclusion: Our results indicate an increase in hepatic CYP2E1 activity with low-dose LPS in humans that is associated with enhanced but transient measures of systemic oxidative stress.

617 PHENYLETHYLISOTHIOCYNATE (PEITC) REDUCES 2-AMINO-1-METHYL-6-PHENYLIMIDAZO[4, 5-B]PYRIDINE (PHIP) DNA ADDUCT FORMATION BY INHIBITION OF CYTOCHROME P450 DEPENDENT METABOLISM.

J. A. West, K. H. Dingley and K. W. Turteltaub. *Biology and Biotechnology Program, Lawrence Livermore National Laboratory, Livermore, CA.*

Exposure to potent heterocyclic amine mutagens, such as PhIP, result in DNA adducts that correlate with tumor formation in target tissues of rats. Consumption of cruceferous vegetables, which contain PEITC, appear to reduce the carcinogenic potential of heterocyclic amines. The mechanism by which PEITC reduces the carcinogenic potential is unknown. To determine if chemopreventitives interfere with PhIP bioactivation, microsomes from rat liver, prostate and lung were prepared to measure the metabolism of PhIP in the presence of PEITC. In a separate experiment rats were administered PEITC prior to a PhiP exposure and DNA adducts were measured by accelerator mass spectrometry. Results: Microsomes prepared from neither rat prostate, nor lung tissue generated oxidation products of PhIP. In contrast, liver microsomes generated multiple oxidation products from PhIP, including NOH-PhIP, an intermediate that results in DNA binding. PhIP metabolism in liver was inhibited by PEITC. With particular significance PEITC abolished the production of NOH-PhIP, while less reactive metabolites were still produced. When rats were administered PEITC prior to PhIP exposure, DNA adducts were reduced. We conclude: 1) NOH-PhIP is not generated by cytochrome P450 at detectable leves in extra-hepatic tissue 2) PEITC inhibits cytochrome P450 dependent metabolism of PhIP, and 3) inhibition the metabolic activation of PhIP to reactive intermediate such as NOH-PhIP, leads to the reduction of DNA Adducts. This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48. Research supported by USDOD USAMRDC # 45559FLD

618 USE OF REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (QRT-PCR) METHODOLOGY TO STUDY THE INDUCTION OF CYTOCHROME P450 (CYP) mRNA LEVELS IN RAT LIVER.

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In this study QRT-PCR (TaqMan®) methodology has been employed to study the induction of CYP mRNA levels in rat liver and in cultured precision-cut liver slices. Homologenic (TaqMan®) probe/primer combinations specific for a range of CYP cDNAs were designed using Primer Express® software. The probe/primer sets were selected to span exon junctions in order to minimise the effect of any contamination from genomic DNA. Probes for CYP genes utilised a FAM reporter while the house-keeping (albumin) probe was labelled with VIC allowing duplex analysis for both CYP and albumin in the same reaction. To test the specificity of the TaqMan® probes and primers male Sprague-Dawley rats were treated with either a single 500 mg/kg ip dose of Aroclor 1254 (ARO) or four daily oral doses of 50 and 75 mg/kg/day dexamethasone (DEX) and methylclofenapate (MCP), respectively. Total RNA was extracted from liver samples from control rats and pooled samples from ARO, DEX and MCP treated rats and cDNA prepared. A series of duplex PCR amplifications was conducted using an ABI PRISM™ 7700 Sequence Detector. Compared to albumin (as a house-keeping gene) levels of CYP1A1, CYP1A2, CYP3A1, CYP3A2 and CYP4A1 mRNA were induced by 51, 44, 49, 13 and 62 fold, respectively. To assess CYP mRNA induction *in vitro*, rat liver slices (diameter 10 mm, thickness around 250 µm) were prepared using a Krumdieck tissue slicer. Liver slices were cultured in Williams medium E containing 0.1 µM insulin, 0.1 µM DEX, 2 mM L-glutamine, 50 µg/ml gentamicin and 2.5 µg/ml fungizone in a dynamic organ culture system at 37°C under an atmosphere of 95% O_2/5% CO_2. Treatment with ARO, but not MCP, for 24 hr markedly induced levels of CYP1A1 and CYP1A2 mRNA, whereas MCP induced levels of CYP4A1 mRNA. These results demonstrate the usefulness of TaqMan® QRT-PCR methodology for studying the induction of rat CYP mRNA levels *in vivo* and *in vitro*.

619 MEASUREMENT OF HEPATIC ENZYME INDUCTION AND β-OXIDATION IN RATS DOSED WITH CLOFIBRATE.

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The goals of this study were to evaluate the effects of various doses of clofibrate on inducible liver enzymes in the rodent and to assess possible increases in peroxisome proliferation at the higher doses. Groups of 5 male and 5 female [Crl:CD®(SD)BR (Sprague-Dawley)] rats received either 0, 300, 450 or 600 mg/kg/day clofibrate s.i.d. for 14 days. The animals were sacrificed, and the livers removed and frozen. Subsequently the livers were homogenized, and supernatants were prepared and later assayed for peroxisome proliferation based on cynanide-insensitive β-oxidation of palmitoyl CoA. In comparison with control animals, palmitoyl CoA oxidation increased significantly ($p < 0.01$) in both sexes at 300 mg/kg, but appeared to plateau at 450 and 600 mg/kg, indicating that a 300 mg/kg dose of clofibrate was adequate to generate an optimal response in the rat. Concurrently, liver microsomal samples were prepared from the same livers and later assayed for microsomal cytochrome P450, NADPH cytochrome c reductase, ethoxyresorufin O-dealkylase (EROD), methoxyresorufin O-dealkylase (MROD), pentoxyresorufin O-dealkylase (PROD), p-nitroanisole O-demethylase (P-NOD), p-nitrophenol hydroxylase (NP), and ethylmorphine N-demethylase (EMD) activities. For some of these endpoints, significant ($p < 0.05$) differences were found between the control means and those means obtained from animals dosed with clofibrate. In both sexes, significant elevations in PROD and P-NOD were seen at all three dose levels. Elevated cytochrome c reductase was noted in both sexes at the high dose. Both EROD and MROD were essentially unaffected in males and females. In males, elevations of total P450 were observed at 300 and 450 mg/kg. In females, EMD levels rose significantly at all three doses, and increases were also seen in NP at 300 and 450 mg/kg. Based upon results for several inducible rodent cytochromes, this study demonstrates that clofibrate is a hepatic microsomal enzyme inducer of CYP2B1 and/or CYP2B2 and CYP2E1 in both sexes and CYP3A1 in females.

620 IRON DEFICIENCY CAUSES MANGANESE ACCUMULATION IN DEVELOPING RAT BRAINS.

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Iron (Fe) deficiency (ID) is a prevalent nutritional disorder affecting nearly 2 billion people worldwide. It is well known that ID represents a risk factor for toxicity due to metal exposure (e.g., aluminum, cadmium, lead). Since Fe and manganese (Mn) share similar transport mechanism(s), studies were carried out to test the hypothesis that dietary ID leads to increased Mn accumulation in the brain, potentially predisposing the central nervous system (CNS) to toxicity. Previous studies have shown that ID increases brain Mn, but specific regional changes have, as of yet, not been reported. Thus, the goal of this study was to examine the effect of ID on [Mn] in Mn-rich brain regions. Twenty-one day old male Sprague-Dawley rats were fed for 6 weeks one of three semi-purified diets (AIN-93-G), (35 mg Fe/Kg, 10 mg Mn/Kg diet, CN n=7), (3 mg Fe/Kg, 10 mg Mn/Kg diet, ID n=7) and (3 mg Fe/Kg, 100 mg Mn/Kg diet, IDMn+ n=7). After 6 weeks of dietary treatment, the rats were euthanized, and the brains dissected into seven brain regions. Brain regions were analyzed for [Mn] with neutron activation analysis (NAA), and amino acid (glutamate, aspartate, glutamine, glycine, taurine, GABA) concentrations were measured with HPLC. Both Fe-deficient diets caused significant ($p<0.05$) increases in [Mn] in six of the brain regions compared to controls (ranging from cerebellum, 31%-globus pallidus 60% increase). The hippocampus was the only brain region in which the IDMn+ group accumulated significantly more Mn than both the CN and ID groups (CN<ID<IDMn+). While multiple alterations in brain regional amino acid concentrations were observed, two significantly correlated with CNS [Mn]. In the substantia nigra, [Mn] significantly correlated with increased taurine concentration ($r=0.65$; $p<0.05$), whereas in caudate putamen [Mn] negatively correlated with decreased g-amino butyric acid (GABA) ($r=-0.54$; $p<0.05$). These data show that ID increases brain Mn in a heterogeneous fashion, and that ID is a significant risk factor for CNS Mn accumulation. (Supported by NIEHS 10563).

621 PRENATAL LEAD (PB) EXPOSURE AND SCHIZOPHRENIA: METHODOLOGY FOR EXAMINING THE POTENTIAL RELATIONSHIP.

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Schizophrenia is a mental disorder affecting approximately 2.8 million Americans, characterized by hallucinations, delusions, emotional and social withdrawal, and disorganized thinking. The causes of the disease are thought to include hitherto unspecified genetic and environmental risk factors. Prior studies suggest that disruptions in prenatal development may be risk factors. The current study was designed