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

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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. Consequently, we have determined that PhIP is bioavailable to the prostate at dietary levels of exposure, where it damages DNA through the dose-dependent formation of DNA adducts. The mechanism leading to adduct formation may be bioactivation to N-OH PhIP, which reaches the prostate via the circulation. Treatment of animals with PEITC, an isothiocyanate, reduced the genotoxic effects of PhIP and may be useful in the prevention of PhIP-induced prostate cancer. Consequently, this work has provided further evidence linking PhIP to the development of human prostate cancer and may lead to the identification of effective chemopreventive strategies.

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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, 2000 to March 31, 2001 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.

The goal of this aim was to establish if [^{14}C]PhIP forms adducts with DNA, the effect of dose and the kinetics of formation and clearance of DNA adducts. Furthermore, in order to investigate the utility of blood protein adducts as a surrogate biomarker of DNA adduct formation in the prostate, adduct formation with albumin was measured. **These studies were completed in year 1 of this proposal.**

To determine if DNA adducts are formed in the prostate by PhIP and the effect of dose, a single oral dose of [^{14}C]PhIP was administered to male F344 rats in the dose range 5 ng-100 mg/kg, which includes the high doses shown to produce tumors in rats and dietary-levels of exposure. Tissues (prostate, colon and liver) and plasma were collected 6 hours following dosing. The levels of PhIP in tissues and plasma, as well as adduct formation with tissue DNA and plasma albumin were measured using AMS.

Dose-dependent levels of [^{14}C]PhIP and/or PhIP metabolites were present in plasma and tissues, including the prostate. (Figure 1). These results demonstrate that **PhIP is bioavailable to the prostate at dietary levels of exposure.**

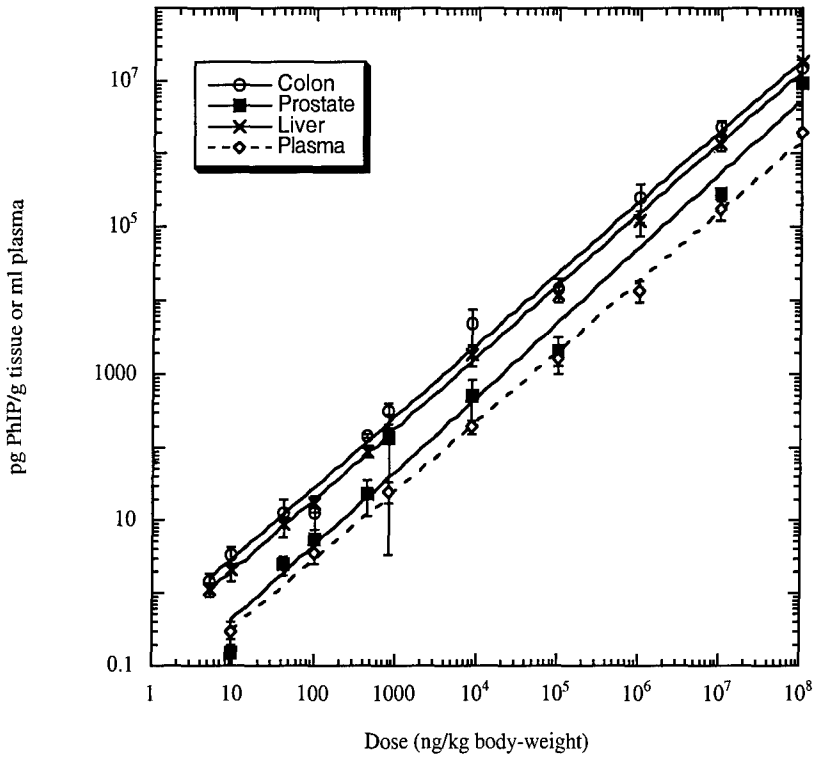

Figure 1. Levels of [^{14}C]PhIP and its metabolites in the plasma, prostate, liver and colon of F344 rats 6 hours following administration of a single oral dose of [^{14}C]PhIP.

Analysis of DNA from the tissues also demonstrated that the **PhIP damaged DNA in the prostate through the dose-dependent formation of DNA adducts** (Figure 2). Importantly, at low dose, the adduct levels were higher in the prostate than the other tissues analyzed. From these results, an optimal dose of approximately 100 μg/kg body-weight was chosen for chemoprevention experiments. This dose results in adduct levels that are high enough in the prostate to be able to quantify any adduct-reducing effects of the chemopreventives.

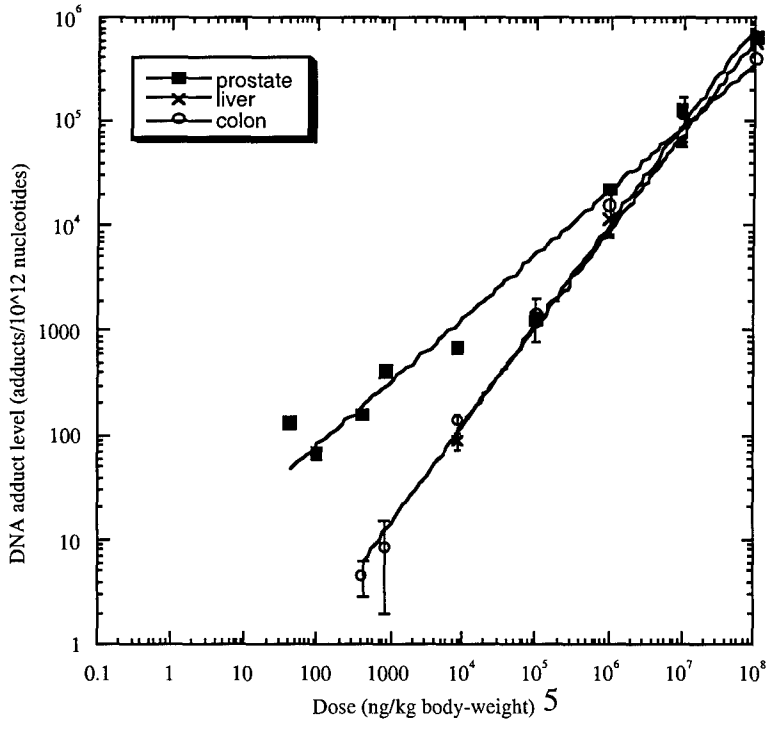

Figure 2. DNA adduct levels in the prostate, liver and colon of F344 rats 6 hours following administration of a single oral dose of [^{14}C]PhIP.

In order to determine if albumin adducts in the blood could be used as a biomarker of PhIP exposure and adduct formation in tissues, albumin from the dosed rats was analyzed for covalently bound [^{14}C]PhIP. A linear dose-response for albumin adduct formation was observed over the dose range 100 ng-100 mg PhIP/kg body-weight (Figure 3). These results indicate that albumin adduct formation by PhIP may be a good biomarker of exposure and adduct formation in tissues.

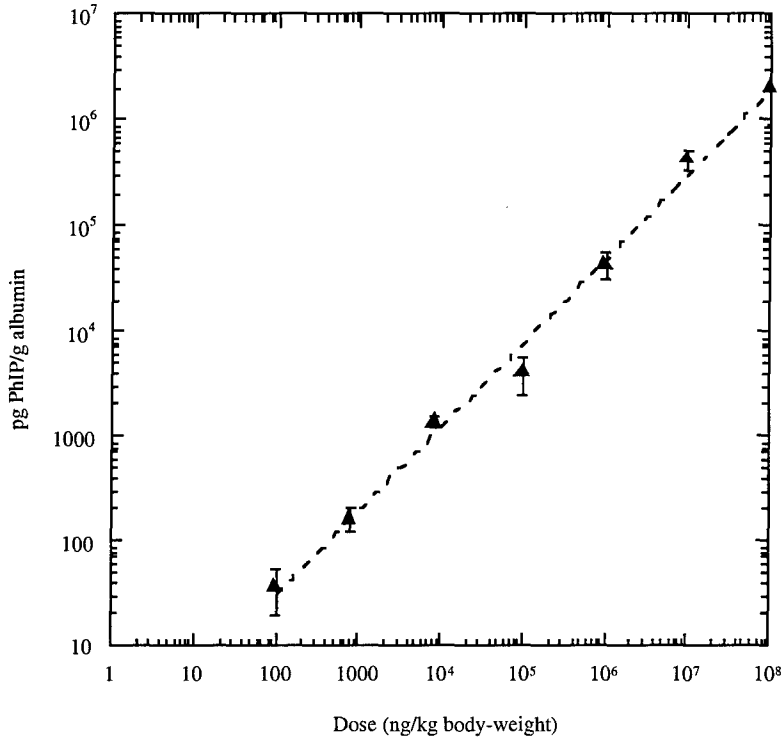

Figure 3. Albumin adduct levels in the blood of F344 rats 6 hours following administration of a single oral dose of [^{14}C]PhIP.

Adduct levels over time may provide a more accurate measure of risk compared to single time-point studies. Therefore, we also determined the clearance kinetics of PhIP in the plasma and prostate following administration of a single 15 μg/kg body-weight dose of [^{14}C]PhIP by gavage, and rats sacrificed at selected time points up to 72 hours post dosing. PhIP was rapidly absorbed with peak levels of PhIP detected in the plasma within 0.5 hours of exposure, followed by a rapid clearance (fig 4). The half-life of PhIP in the plasma was 18.9 ± 2.3 hours.

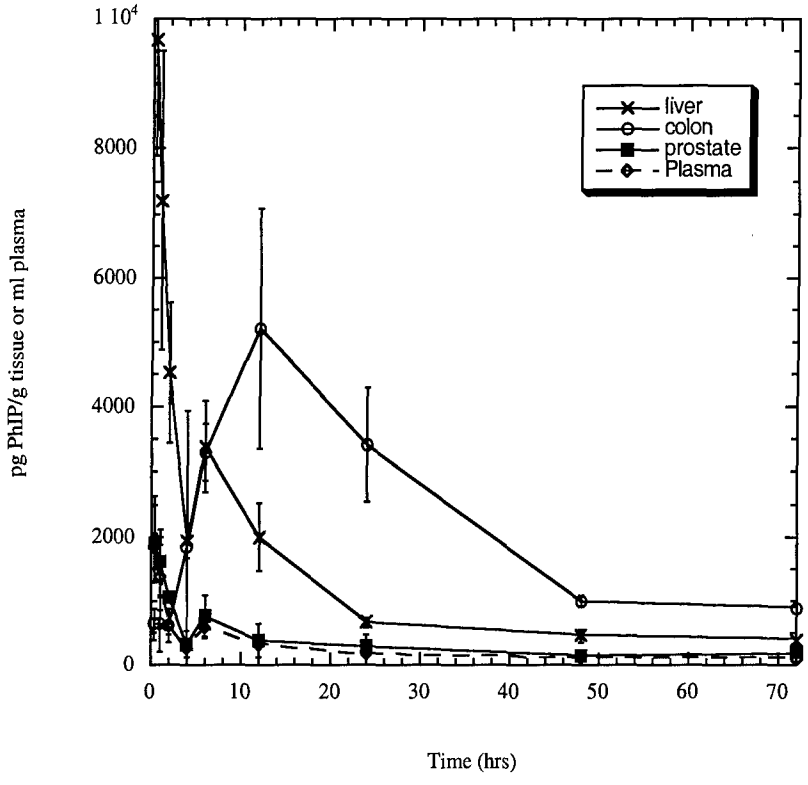

Figure 4. Levels of [^{14}C]PhIP and its metabolites in the plasma, prostate, liver and colon of F344 rats at time-points up to 72 hours following administration of a single oral dose of [^{14}C]PhIP.

DNA adduct levels, based on the measured ^{14}C content of the DNA at various time points following dosing are shown in figure 5. DNA adducts were detectable in the prostate within 0.5 hours and continued to increase until 6-10 hours. **At all time-points, adduct levels were highest in the prostate tissue.**

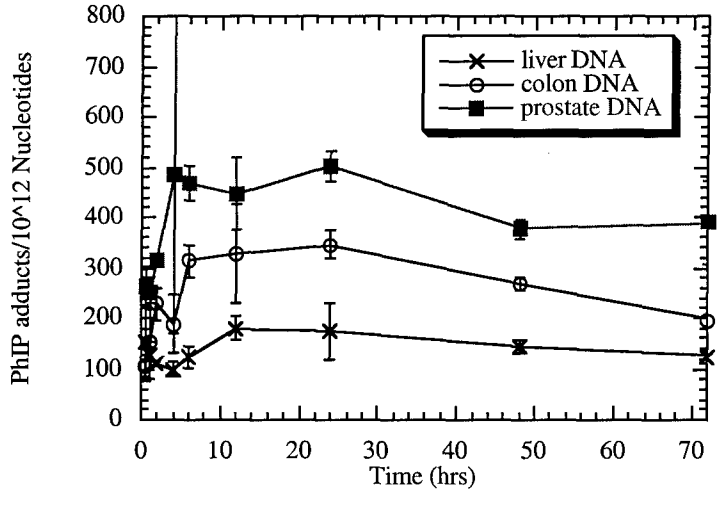

Figure 5. DNA adduct levels in the prostate, liver and colon of F344 rats at time-points up to 72 hours following administration of a single oral dose of [^{14}C]PhIP.

PhIP-albumin adducts in the blood followed a similar trend to the DNA adducts in tissues, reaching a peak at approximately 12 hours following exposure (fig 6).

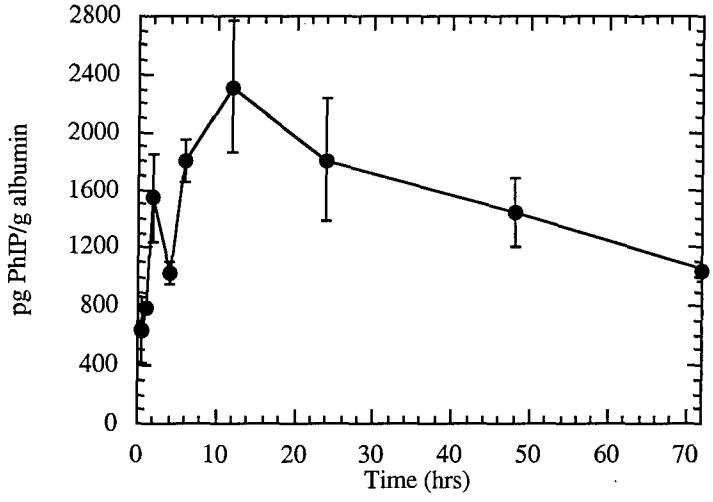

Figure 6. Albumin adduct levels in the blood of F344 rats at time-points up to 72 hours following administration of a single oral dose of [^{14}C]PhIP.

Specific aim #2: Determine the metabolism of PhIP in rat prostate at dietary levels of exposure. In order to understand which metabolic factors target chemicals to the prostate, we are establishing if the prostate has the capacity to metabolize [^{14}C]PhIP to genotoxic metabolites. In addition, we are investigating if metabolites produced in other organs may be factors in the targeting of PhIP to the prostate. This will help determine which pathways of PhIP metabolism can be targeted in human prostate cancer chemoprevention.

During year 1 of this proposal, we completed analysis of the metabolite profiles of PhIP in the plasma of rats. Plasma metabolites were analyzed by HPLC at defined time points between 0.5 and 24 hours following [^{14}C]PhIP exposure. At the 0.5, 1 and 2 hour time points, levels of ^{14}C in the HPLC fractions were measurable by Liquid Scintillation Counting. The 4, 6, 12, and 24 hour time points were analyzed for ^{14}C content by AMS. Representative results from analysis of HPLC fractions by AMS at 12 hours are shown in figure 7. Eight ^{14}C-containing peaks were detected (at least 10-times above background) at each of the time points measured. The identification of the plasma metabolites was made by matching the retention times of the radiocarbon peaks to those of authentic metabolite standards purified from rat urine following a 50 mg/kg dose of PhIP.

PhIP was the major component present in plasma at all time points followed by 4'-PhIP sulfate. The other metabolites present are given in table 1. While some variation in the relative peak height ratios was seen for the individual metabolites at the different time points, the relative proportions of each metabolite where the same at all time points. No relationship was found between individual metabolite kinetics and adduct kinetics.

Interestingly, a radiocarbon peak detected at 57 minutes corresponded to the retention time of N-OH-PhIP. To eliminate the possibility that this peak was due to other PhIP metabolites with similar retention characteristics, an isochratic HPLC method was developed which resolves N-OH-PhIP, NO_2-PhIP, and PhIP. Using this method, the

putative N-OH-PHIP peak seen in plasma eluted at the same time as the N-OH-PhIP authentic standard. While this is preliminary, **these results suggest that N-OH-PhIP circulates and does not need to be produced within the extra hepatic tissues. To our knowledge this is the first evidence that N-OH-PhIP circulates following administration of PhIP to a whole animal.** N-OH-PhIP is the result of PhIP oxidation by cytochrome P4501A2, an enzyme found in the liver (McManus *et al.*, 1989; Turesky *et al.*, 1991). **N-OH-PhIP is a potentially genotoxic metabolite which can be esterified via Phase II enzymes to form either N-acetoxy-PhIP N-sulfoxy-PhIP, metabolites thought to be responsible for the formation of PhIP-DNA adducts** (Lin *et al.*, 1992; Turteltaub *et al.*, 1990b; Frandsen *et al.*, 1992).

Fig. 7. PhIP plasma metabolite profile from the 12 hour time point.

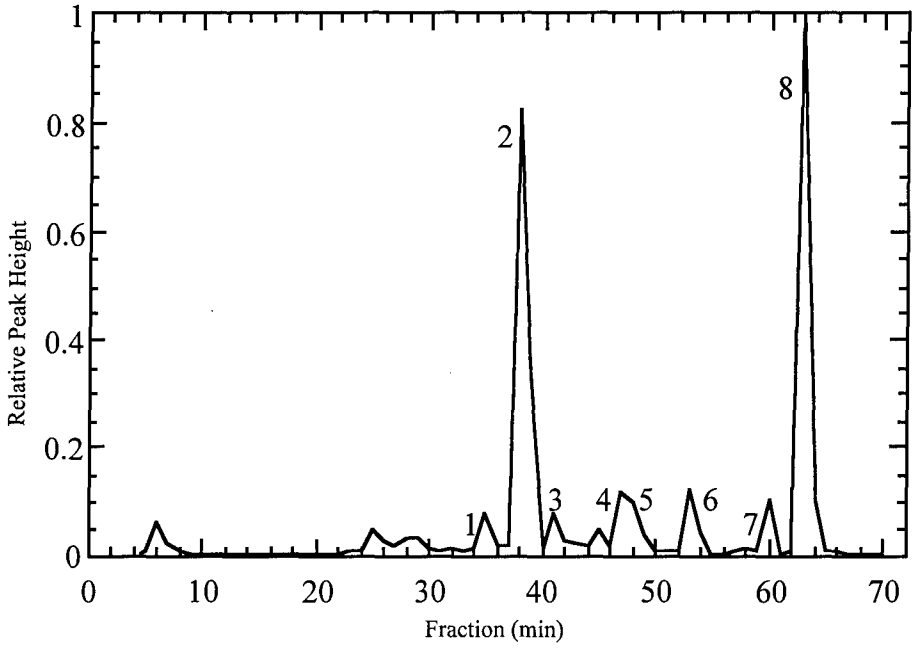

Table 1. Metabolite Identification from the plasma of rats dosed with [^{14}C]PhIP

Peak No.	Ret. Time (min)	$(M+H)^+$	Metabolite
1	31.5	401	4'-O-glucuronide
2	34.8	321	4'-PhIP-SO_4
3	37	---	unidentified
4	42.9	417	N-OH,N_2-glucuronide
5	44.4	241	4'-OH-PhIP
6	49.4	417	N-OH,N_3-glucuronide
7	57.6	N/A	N-OH-PhIP
8	60.1	225	PhIP

In addition to the above studies, we have started to conduct *in vitro* assays to determine the capacity of prostate to bioactivate PhIP to genotoxic species. As proposed in the statement of work, this aim will be complete in year 2.

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. A single dietary-relevant dose of [^{14}C]PhIP (90 μg/kg bw) was administered to F344 rats that have been fed chronically for 11 days diets containing several potential chempreventive agents (816mg phenylethylisothiocyanate/kg (PEITC), 140mg 3,3'4',5,7-pentahyroxyflavone/kg (quercetin), 906mg 1-isothiocyanato-(4R,S)-(methylsulfinyl) butane/kg (sulforaphane), or the de-alcoholized-dehydrated residue from one liter red wine/kg (wine solids)). The level of [^{14}C]PhIP in the plasma and tissues and bound to DNA were then measured (table 2).

Quercetin, and sulforaphane altered the concentrations of PhIP or its metabolites in some of the tissues, suggesting that these diet supplements significantly altered the bioavailability and/or the kinetic behavior of the [^{14}C]PhIP. However, only the **PEITC supplemented rats experienced significantly lower adduct formation in the prostate. Although preliminary, these results suggest that PEITC had a protective effect.**

Table 2. The effect of chemopreventive treatment on the concentration of PhIP in plasma and tissues and adduct levels. Values are means ± Standard Deviation.

Diet supplement	None (control)	PEITC	Quercetin	Sulforaphane	Wine solids
Rats/treatment	5	2	2	2	2
Plasma PhIP, (ng/L)	1038 ± 244	1065 ± 78	1825 ± 120	1535 ± 92	1115 ± 262
Liver PhIP, (ng/kg wet weight)	6956 ± 1775	6995 ± 1365	13390 ± 5926	9630 ± 877	7320 ± 707
Colon PhIP (ng/kg wet weight)	22590 ± 4872	26785 ± 1549	5625 ± 2114	22255 ± 2114	22055 ± 4646
Prostate PhIP (ng/kg wet weight)	2370 ± 1410	1170 ± 120	3920 ± 2090	1830 ± 340	920 ± 540
Adducts/10^{12} nucleotides in liver DNA	1197 ± 106	859 ± 135	1029 ± 83	1095 ± 99	1162 ± 156
Adducts/10^{12} nucleotides in colon DNA	2839 ± 584	1696 ± 73	2832 ± 549	2335 ± 31	2711 ± 321
Adducts/10^{12} nucleotides in prostate DNA	**5174 ± 1735**	**1945**	4359	3327	4241
Albumin adduct level (pg PhIP/g albumin)	11590 ± 2816	8659 ± 2246	12146 ± 295	15637 ± 2726	13592 ± 721

As proposed in the statement of work, this aim will be completed in years 2 and 3.

Key Research Accomplishments:

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

- PhIP, a prostate carcinogen, is bioavailable to the prostate at dietary levels of exposure and that the levels in the prostate tissue are dose-dependent.
- PhIP damages DNA in the prostate through the formation of DNA adducts. Adduct levels are dose-dependent and are higher in the prostate than in other target and non-target organs.
- PhIP forms dose-dependent levels of albumin adducts in the blood. Albumin adducts may be a good biomarker of exposure to PhIP and of DNA adduct formation in tissues.
- In rats, PhIP is bioactivated to N-OH PhIP, which circulates in the blood and may lead to DNA adduct formation in the prostate.
- PEITC treatment reduces the genotoxic effects of PhIP in the prostate and may be useful in the chemoprevention of PhIP-induced prostate cancer.

Reportable Outcomes:

- Abstracts/poster presentations
  - Posters entitled 'The Effect of Dietary Supplements with Chemopreventive Potential on Metabolism and DNA-Adduct Formation of the Heterocyclic Amine PhIP in Rats' were presented at The UC Davis Cancer Center Research Symposium, October 6-7, 2000, and The Federation of American Societies for Experimental Biology (FASEB) meeting, March 31-April 4, 2001. The abstract was published in FASEB J. (2001),15(4), A618-A618 Part 1.

- Oral presentations
  - This work was presented as an oral presentation as part of DOE Science Days at Lawrence Livermore National Laboratory, March 22-23, 2001.

- Manuscripts
  - Two manuscripts containing work from this grant are currently in preparation. They are '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' and 'The Effect of Dietary Supplements with Chemopreventive Potential on Metabolism and DNA-Adduct Formation of the Heterocyclic Amine PhIP in Rats'.

- Employment/Research Opportunities
  - As a consequence of this grant, we were able to hire a new post-doc, Jason West, from the UC Davis Toxicology Program. This has given him the opportunity to become involved in the field of prostate cancer research.

Conclusions:

During the first year of this grant, we have made significant progress on our specific aims. We have determined that PhIP, a compound formed in meat during cooking, is bioavailable to the prostate tissue following exposure at dietary-relevant doses. Importantly, PhIP exposure also results in DNA adduct formation in the prostate. DNA adducts are a form of DNA damage that is considered an early event in the development of cancer, hence these results imply that PhIP is a cancer risk at dietary levels of exposure. In fact, at low dose, the DNA adduct levels were higher in the prostate than in other target and non-target tissues, implying that exposure may pose a significant prostate cancer risk. Analysis of the blood for protein adduct formation also indicated that albumin adducts may be a useful biomarker of PhIP exposure and adduct formation in the tissues for use in molecular epidemiology studies.

To better understand why PhIP may target the prostate, we have been investigating the pathways of PhIP metabolism in the rat. Our results suggest that PhIP is activated to N-OH-PhIP (probably primarily from the liver), which then circulates in the blood. This metabolite is important, as it is considered to be responsible for the formation of PhIP-DNA adducts. Consequently, N-OH PhIP may reach the prostate via the blood, leading to adduct formation. We are now in the process of determining if PhIP metabolism within the prostate may also contribute to the high adduct levels.

We have started to investigate whether several potential chemopreventive agents may be useful in reducing DNA adduct levels in the prostate following PhIP exposure. PEITC, an isothiocyanate found in cruciferous vegetables, reduced adduct formation in the tissues examined, including the prostate. Therefore, this compound may be a useful in preventing prostate cancer as a result of PhIP exposure.

"So What?"

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

1. Obtained further evidence linking the dietary prostate carcinogen PhIP to the development of human prostate cancer. This complements epidemiological studies that link meat consumption to prostate cancer risk.
3. Validated albumin adduct formation in the blood as a biomarker of dietary PhIP exposure and prostate cancer risk that could be used to identify individuals for prevention and for monitoring the effect of chemoprevention strategies.
4. Identified one candidate chemopreventive agent that could be used to reduce human prostate cancer risk as a result of dietary exposure to PhIP.
5. Started to obtain a mechanistic insight into how dietary factors may be involved in prostate cancer etiology.

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Appendices:

- Copy of abstract entitled 'The Effect of Dietary Supplements with Chemopreventive Potential on Metabolism and DNA-Adduct Formation of the Heterocyclic Amine PhIP in Rats' FASEB J. (2001),15(4), A618-A618 Part 1.

; and Diet Restriction Increase Survival of Rats with N-Methyl-Testosterone-Induced Prostate Cancer

ell Boileau[1], Steven K. Clinton[2], Zhiming Liao[2], John W. Erdman, ois, 901 S. Goodwin Ave., Urbana, IL 61801, [2]The Ohio State

oducts and energy restriction are both hypothesized to reduce the In a factorial design, we tested the ability of whole tomato powder diet providing 13 mg lycopene /kg diet) or lycopene (Hoffman roviding 161 mg lycopene/kg diet) as well as diet restriction (20%) ats with chemically-induced prostate cancer. Five-week old male re randomly assigned to one of 3 AIN-based experimental diets ne beadlets, or tomato powder) and fed for one week prior to the drogen and NMU (50 mg/kg, i.v.), followed by promotion of tumor terone implants. Three days after carcinogen treatment each dietary nly subdivided to either 20% diet restriction or ad libitum feeding. nt 39% increase in the duration of survival in rats fed the tomato 006) by proportional hazards analysis and a 17% increase in survival lets (RR=0.837; P=0.16) as compared to the control AIN diet. Diet tly increased overall survival (23% increase, RR=0.770; P=0.04) as feeding. At necropsy >75% of animals showed histopathologic ıcer. We concluded that the consumption of lycopene beadlets, diet restriction enhance survival in this chemically-induced prostate nore, tomato products may contain compounds, in addition to modulate prostate carcinogenesis. (Supported by the National 72482 and The OSU-CCC Support Grant P30-CA16058).

oxycholate on Colonocytes Expressing Either Mutant or Wild-

lny, Jing Xu: Univ. of North Carolina at Greensboro, Cell. & Molec. Program in Nutrition, Greensboro, NC 27402

and HCT-15 (mutant p53) colonocytes were exposed to 300 μM of ıoter, deoxycholate (DOC), to compare the effects of DOC on the exposure, DNA damage was increased as indicated by the comet l 88±6 in HCT-116 and HCT-15 cells, respectively, but only 38±5 counterparts). After 6 h of DOC exposure, both cell lines exhibited apoptotic bodies. Chromatin condensation and fragmentation were 16 cells and 43±17% of HCT-15 cells, but not seen in control cells. HCT-15 cells had annexin binding scores of 8±3 and 7±3, -treated HCT-116 and HCT-15 cells had annexin binding scores of tively. After 20 h of DOC exposure, 26±6% of HCT-116 cells and vere TUNEL-positive, whereas control cells were TUNEL-negative. OC induced DNA damage that subsequently triggered apoptosis in a

494.10

EFFECT OF DIETARY SUPPLEMENTS WITH CHEMOPREVENTIVE POTENTIAL ON METABOLISM AND DNA-ADDUCT FORMATION OF THE HETEROCYCLIC AMINE PHIP IN RATS

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2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), a heterocyclic amine formed in meat cooked at high temperatures, may be a risk factor for colon and prostate cancers. Physiologic intakes of PhIP are absorbed, and can be detected in tissues such as liver, colon, and prostate, where it damages DNA through the formation of DNA adducts. We used a very sensitive method (Accelerator Mass Spectrometry) to determine if a nutritionally adequate diet fortified with sulforaphane, phenethylisothiocyanate (PEITC), catechin, quercetin, wine solids, or genistein influenced PhIP metabolism, distribution or reduced DNA adduct levels in liver, colon, or prostate. Male F344 rats were fed an amino acid based nutritionally adequate diet that was fortified with each agent for 10 days, then orally administered [^{14}C]PhIP (90 μg/kg body-weight) and euthanized 24 h later. Plasma, liver, colon, and prostate were analyzed for PhIP levels and DNA adduct formation. Quercetin, genistein, and sulforaphane raised the plasma and organ levels of PhIP (or its metabolites), suggesting they altered the kinetic behavior of PhIP. Only PEITC reduced adduct levels in all organs. We conclude that PEITC may be protective of PhIP-induced cancer in rats, but its utility in humans needs further study. Conducted under auspices of US DOE (LLNL W-7405-ENG-48) and supported by USMRMC-PC991395, NIHDK45939 & UCD Cancer Center.

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RACE AND AGE-DEPENDENT ALTERATIONS IN GLOBAL METHYLATION OF DNA IN SQUAMOUS CELL CARCINOMA OF THE LUNG.

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We previously reported that alteration in global DNA methylation is an epigenetic difference in susceptibility for the development of squamous cell cancer (SCC) of the lung. The current study investigated the age and race-dependent alterations in global DNA methylation on the development and progression of SCCs of the lung. Global methylation status was evaluated in SCC and in the associated uninvolved bronchial mucosa and epithelial hyperplasia of 53 Whites and 23 Blacks by using an antibody specific for 5-methyl cytosine (5-mc). A low 5-mc score indicates global hypomethylation of DNA. 5-mc scores of SCC (0.59 ± 0.06) were significantly lower compared to 5-mc scores of uninvolved bronchial mucosa (UBM) (0.94 ± 0.07) and epithelial hyperplasia (EH) 0.99 ± 0.10) in Whites ($p< 0.05$). In Blacks, 5-mc scores of SCC (0.55 ± 0.09) were not significantly different from 5-mc scores of UBM (0.61 ± 0.08) and EH (0.54 ± 0.14), suggesting an involvement of methylation in the development of SCCs in Whites, but not in Blacks. 5-mc scores were lower in younger (< 65-years) subjects compared to older (> 65-years) subjects in Whites, but not in Blacks. Hypomethylation of SCCs in White men was associated with shorter survival from the disease. These preliminary results suggest that the methylation status of DNA may affect the development and prognosis of SCCs in Whites. It is unclear whether the inconsistencies across race and gender subgroups are real or an effect of selection and size of the study groups.