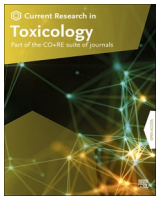
Current Research in Toxicology 6 (2024) 100162

Contents lists available at ScienceDirect

Current Research in Toxicology

journal homepage: www.journals.elsevier.com/current-research-in-toxicology



Acceleration of benzo(a)pyrene-induced colon carcinogenesis by Western diet in a rat model of colon cancer

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ARTICLE INFO

Keywords:
Colon cancer
Benzo(a)pyrene
Polycyclic aromatic hydrocarbons
Western diet
DNA adducts
Lipid peroxidation

ABSTRACT

Colorectal cancer (CRC) is the third leading cause of cancer-related mortalities in the USA and around 52,550 people were expected to die from this disease by December 2023. The objective of this study was to investigate the effect of diet type on benzo(a)pyrene [B(a)P]-induced colon cancer in an adult male rat model, the Polyposis In the Rat Colon (PIRC) kindred type. Groups of PIRC rats (n = 10) were fed with AIN-76A regular diet (RD) or Western diet (WD) and received 25, 50 and 100 μg B(a)P/kg body wt. via oral gavage for 60 days. Rats fed diets alone, but no B(a)P, served as controls. After exposure, rats were euthanized; colon and liver samples were analyzed for activation of drug metabolizing enzymes (DMEs) CYP1A1, CYP1B1, SULT and GST. Plasma and tissue samples were analyzed by reverse phase-HPLC for B(a)P metabolites. In addition to these studies, DNA isolated from colon and liver tissues was analyzed for B(a)P-induced DNA adducts by the ^{32}P-postlabeling method using a thin-layer chromatography system. Western diet consumption resulted in a marked increase in DME expression and B(a)P metabolite concentrations in rats that were administered 100 μg/kg B(a)P + WD ($p < 0.05$) compared to other treatment groups. Our findings demonstrate that WD accelerates the development of colon tumors induced by B(a)P through enhanced biotransformation, and the products of this process (metabolites) were found to bind with DNA and form B(a)P-DNA adducts, which may have given rise to colon polyps characterized by gain in tumor number, sizes, and dysplasia.

Introduction

Colorectal cancer (CRC) is one of the third leading cancers in the Western world. In the United States alone, nearly 153, 020 new cases of CRC have been diagnosed recently and 52,550 deaths were expected to occur in 2023 due to this cancer (American Cancer Society, 2023). Along with genetic mutation, epidemiological studies have shown that environmental factors, and especially diet, play a key role in susceptibility to sporadic colon cancer (Howlader et al., 2011; Ruiz-Saavedra et al., 2022).

Consumption of red meat and saturated dietary fat have been proposed as risk factors that influence susceptibility to CRC (Burnett-Hartman et al., 2012, Fu et al., 2012). There has been increasing epidemiological evidence demonstrating that red meat intake and excessive adiposity increase susceptibility to colorectal neoplasia (Lee et al., 2013, Vongsuvanh,et al., 2013). Individuals that are obese have a

Abbreviations: AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care; Ahr, aryl hydrocarbon receptor; AIN, American Institute of Nutrition; ANOVA, analysis of variance; *Apc*, adenomatous polyposis coli; ARP, aldehyde reactive group; ARRIVE, Animal Research: Reporting In Vitro Experiments; B(a)P, benzo(a)pyrene; BPDE, benzo(a)pyrene 7,8 diol-9,10 epoxide; bw, body weight; BSA, bovine serum globulin; CRC, colorectal cancer; CYP1A1, cytochrome P450 1A1; CYP1B1, cytochrome P450 1B1; dA, deoxyadenosine; dG, deoxyguanosine; DME, drug metabolizing enzymes; FAP, familial adenomatous polyposis; GST, glutathione-S-transferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDL, high density lipoproteins; HPLC, high performance liquid chromatography; HRP, horseradish peroxidase; MDA, malondialdehyde; *Min*, multiple intestinal neoplasia; PAGE, polyacrylamide gel electrophoresis; PAH, polycyclic aromatic hydrocarbon; PVDF, polyvinylidene difluoride; RD, regular diet; RIPA, radioimmunoprecipitation assay; ROS, reactive oxygen species; RLU, relative luminescence units; RT-PCR, reverse transcription-polymerase chain reaction; SDS, sodium dodecyl sulfate; SULT, sulfotransferase; TC, tricaprylin; TEMED, tetramethylethylenediamine; TLC, thin layer chromatography; WD, Western diet.

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https://doi.org/10.1016/j.crtox.2024.100162
Received 14 January 2024; Received in revised form 2 March 2024; Accepted 6 March 2024
Available online 7 March 2024

large amount of adipose tissue that secretes various chemokines and growth factors. Secretion of these molecules plays a role in the low-grade, chronic inflammatory state that is linked to their obesity, as well as to subsequent cancer risk (Birmingham et al. 2009). These studies estimate that diet contributes to up to 80 % of documented colorectal cases.

With diet posing to be a critical contributor in the initiation and progression of CRC, it is no surprise that studies have now focused on the effects of the "Western style diet." Specific components of Western diet (i.e., consumption of red meat and saturated dietary fat) have been proposed as risk factors that influence susceptibility to CRC (Castelló et al., 2022; Benninghoff et al., 2020).

One group of chemicals, the polycyclic aromatic hydrocarbons (PAHs) have evoked a great deal of interest as they are not only formed in red meat cooked at high temperatures (Cheng et al., 2021a,b), but are also released from several environmental sources (Diggs et al., 2011). At least 20 PAHs are detectable in most dietary items at measurable levels (Ramesh et al., 2004),so it is no surprise that they have been reported to have an impact on obesity due to their highly lipophilic nature and presence in foods that are high in saturated fats (Layeghkhavidaki et al., 2014).

Studies have shown that PAHs in high fat diets cause intestinal inflammation (Khalil et al., 2010). As intake of fatty foods is linked to adiposity (Poret et al., 2018) and tumor growth (O'Neill et al., 2016), these studies point out that obese individuals are more likely to sequester these compounds in lipid-rich tissues and therefore are at a higher risk of environmental toxicant-induced ill health effects of which colorectal cancer is one. From the information presented the cause and progression of colorectal cancer is overly complex, especially considering the various factors that have been reported to play a role in its progression (Ajayi et al., 2016; Song and Chan, 2019; Farombi et al., 2020; Zheng et al., 2022; Wan et al., 2020). There has been increasing epidemiological evidence demonstrating that consumption of fatty foods contaminated with B(a)P and excessive adiposity increase susceptibility to colorectal neoplasia (Lee et al., 2013; Vongsuvanh et al., 2013).

It is reported that one-third of adults and 17 % of adolescents in the United State are obese (Ogden et al., 2014). As obese individuals have a higher risk for colon cancer due to the excess energy intake from dietary fat it is imperative that studies are performed on the effects of both environmental toxicants and consumption of high fat diets because the findings can prove to be greatly beneficial in understanding how they both affect the progression of colorectal cancer. Henceforth, formulation of effective detoxification or intervention strategies can be developed to curtail inflammation arising out of signaling pathways involving PAHs.

The long-range goal of this study is to determine the role of Western diet (WD) in the etiology of benzo(a)pyrene ([B(a)P]; a PAH compound) -induced colon carcinogenesis. The central hypothesis is that Western diet exacerbates B(a)P-initiated colon carcinogenesis through altered biotransformation and DNA damage. The rationale underlying this initiative is that the formation and progression of colon tumors depends on altered B(a)P biotransformation by Western diet. Since adenomas are biomarkers of tumor formation, examining the relationship among WD consumption, B(a)P biotransformation, DNA damage, and adenoma development provides an understanding of the extent to which target tissues are susceptible to damage from exposure to B(a)P alone or in combination with WD.

Materials and methods

Animals

Seven-week-old male PIRC rats (purchased from Taconic (Hudson, NY) or bred in house which are genetically altered to mimic sporadic cancer as seen in humans were used for the studies reported below. Animals were cared for and housed in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited animal care facility according to the recommendations established in the NIH Guide for the Care and Use of Laboratory Animals and the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines (NIH, 1981).

Reagents and chemicals

Benzo(a)pyrene (98 % pure), tricaprylin and endoplasmic reticulum isolation kit were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Lithium chloride, urea, sodium phosphate (monobasic and dibasic), methanol, chloroform, ethanol and 10 % formalin, isopropyl alcohol were purchased from Fisher Scientific Company (Kennesaw, GA). Polyethylenimine-cellulose TLC plates were purchased from Bodman Chemical Company (Aston, PA). DNase and alkaline phosphatase were purchased from Worthington Biomedical Corporation (Feehold, NJ). The CYP1A1, CYP1B1, GST, SULT1A1 and GAPDH antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The UGT antibody was purchased from Cell Signaling Technology (Danvers, MA). The rabbit anti-goat IgG-HRP and mouse anti-goat IgG-HRP antibodies were purchased from LiCor (Lincoln, NE). The Quick Start Bradford Protein Assay Kit, ethidium bromide, tetramethylethylenediamine (TEMED), ammonium persulfate (APS), 30 % acrylamide and bis-acrylamide solution, Laemmli sample buffer, and 2-mercaptoethanol (βME) were purchased from Bio-Rad Laboratories (Richmond, CA). RNase/DNase free water and Trizol reagent were both purchased from Invitrogen (Carlsbad, CA). The cDNA synthesis kit was purchased from Bio-Rad Laboratories (Richmond, CA) The CYP1A1 and 1B1 enzyme assay kits for phase I DME analysis were purchased from Promega (Madison, WI). The GST assay kit used to analyze phase II DME was purchased from Biovision Inc. (Mountain View, CA). For DNA isolation and analysis, the DNeasy blood and tissue kit was purchased from Qiagen (Valencia, CA).

Animal husbandry, B(a)P and Western diet exposure

The PIRC rats were maintained on a 12/12-hour light/dark cycle (lights on at 0600 h) and allowed free access to either the AIN-76A diet (regular diet; RD; 5 % fat content) or the Western diet (WD; 58 % fat content) and water. The diets were purchased from the Research Diets, Inc. (Cat# D10001 and Cat# D12079B for RD and WD respectively). The PIRC rats were housed in groups of 2 per cage and allowed a seven-day acclimation period prior to being randomly assigned to the following treatment categories: I-AIN-76 diet (RD) only (n = 10), II-Western diet (WD) only (n = 10), III-B(a)P (25 μg/kg bw) + RD (n = 10), IV-B(a)P (50 μg/kg bw) + RD (n = 10), V-B(a)P (100 μg/kg bw) + RD (n = 10), VI-B(a)P (25 μg/kg bw) + WD (n = 10), VII-B(a)P (50 μg/kg bw) + WD (n = 10), VIII- B(a)P (100 μg/kg) + WD (n = 10).

The number of animals used were determined after performing a "statistical power analyses" using PS software (Dupont and Plummer 1990). Power analysis revealed a minimum of 10 rats is required for each control or treatment group to detect a 20 % change in experimental endpoints with 80 % power and a type I error rate of 5 %. Rats were administered B(a)P (97 % pure, Sigma Chemical Co., St. Louis, MO Cat# B1760), dissolved in research grade tricaprylin (Sigma, Cat# T9126), daily through oral gavage at a volume of 300 μl for 60 days (Fig. 5). As B(a)P is a potential carcinogen, and with this knowledge it was handled in accordance with NIH guidelines (NIH, 1981). At the end of exposure period, rats were euthanized; and tissues of interest such as colon, liver, adipose, and other tissues were retrieved. Rats that were fed with the above mentioned-diets but received no B(a)P (categories I & II) served as controls. All rats in both control and treatment groups were observed twice a day (including holidays and weekends) for morbidity, mortality and overall wellbeing. Rat body weights and food consumption were monitored periodically.

Extraction of tissues for B(a)P/metabolites

A volume of 250 μl of plasma or urine or 250 mg of tissue were each homogenized in two volumes of Tris-sucrose-EDTA buffer (0.25 M; pH 7.4). Ten micro liters of sodium dodecyl sulfate (1 %) were added to the mixture, vortexed for 1 min and the homogenate was subjected to a liquid–liquid extraction using methanol, deionized water, and chloroform. The oil samples used for the preparation of test diets was also analyzed for background levels of B(a)P as described above. The extracts were concentrated, filtered and analyzed as detailed in Ramesh et al. (2001).

HPLC analysis of B(a)P metabolites

Sample analyses were conducted on a High-Performance Liquid Chromatograph, (HPLC; Model 1200, Agilent, Wilmington, DE) equipped with a HP1046 fluorescence detector (Ramesh et al., 2001). The identification and quantification of the metabolites were accomplished by comparing the retention times and peak areas of all samples with that of standards. The standards were supplied to our laboratory by the NCI Chemical Carcinogen Repository within the Midwest Research Institute Repository located in Kansas City, MO.

Isolation of total RNA and cDNA Synthesis

From all treatment groups total RNA was isolated using RNAgents Total RNA Isolation System (Promega, Madison, WI) and stored at −80 °C. cDNA was prepared from total RNA (2 μg) using oligo(dT)20 primers and AMV Reverse Transcriptase. Oligo(dT)20 primers (3.8 μM) were added to 2 μg of RNA and 0.77 mM dNTPs to a final volume of 13 μl. Reactions were incubated at 65 °C for 5 min, then cooled on ice for 2 min. Next, 7 μl of a solution containing 14 mM dithiothreitol, 40 units of RNaseOUT Recombinant RNase inhibitor (Invitrogen, Carlsbad, CA), and 200 units of SuperScript III (Invitrogen) were added to the reaction mixture. The mixture was then incubated at 50 °C for 50 min, followed by 75 °C for 10 min (to inactivate the reverse transcriptase). Distilled water (80 μl) was added to the isolated cDNA and stored at −80 °C.

Polymerase Chain Reaction (PCR)

The amplification of biotransformation enzymes (CYP1A1, 1B1, and GST) were performed by PCR analysis using cDNA from the total RNA of all the previously described treatment groups of PIRC rats. The PCR products were visualized and captured in agarose gels stained with ethidium bromide in IVP Bio-Imaging System. In order to analyze the concentration of PCR products ribosomal protein s18 was used as a control for normalization of the sample groups.

Protein isolation

Colon or liver tissues were washed in PBS and weighed 250 mg for analysis. Radioimmunoprecipitation assay (RIPA) Buffer 9X vol (900 μl) containing a protease inhibitor was added to samples. Tissues were homogenized on ice for 45 s then centrifuged at 12 x 1000 g at 4 °C for 10 min. The supernatant containing protein was transferred to a fresh microcentrifuge tube and stored at −20C for further analysis.

Protein assay

Protein concentrations of liver or colon samples were determined using the Bradford assay. A standard curve was derived from bovine serum albumin (BSA) dilutions ranging from 0 μg to 2 mg/ml. The working reagent, consisted of 4 ml of Bio-Rad Protein Assay Dye Reagent (Bio-Rad, Hercules, CA; Cat #500–0006) containing Coomassie blue and 2 ml of deionized water. Next, 5 μl of each standard and sample was placed into appropriately labeled tubes. Subsequently, 995 μl of working reagent was added to each tube. Samples were vortexed and protein absorbance for each standard was measured at 595 nm using a Smart Spectrophotometer (Bio-Rad, Hercules, CA) to obtain a standard curve. The protein stocks and aliquots were stored at −80 °C and −20 °C, respectively.

Preparation of protein and Western blotting

Protein samples were mixed 1:1 with Laemmle Sample Buffer (Bio-Rad, Hercules, CA) and boiled for 5 min for denaturation. Equivalent amounts of protein (15 μg) were loaded into each lane of a 7.5 % gel (Bio-Rad, Hercules, CA) and separated by SDS-PAGE at 100 V for 1 h. The separated protein bands were next transferred onto a Sequi-Blot polyvinyldiene difluoride (PVDF) membrane (Bio-Rad, Hercules, CA) at 100 V for 1 h. The PVDF membranes were blocked at room temperature for 1 h using the Li-Cor Odyssey Blocking Buffer (Lincoln, NE; Cat #927–40000) containing PBS.

After 1-hour incubation with Li-Cor Odyssey Blocking Buffer the membranes were then incubated with mouse CYP1A1, CYP1B1, GST, SULT1A1 and GAPDH antibodies (Santa Cruz Biotechnology, Inc., Dallas, TX; Cell Signaling Technology, Danvers, MA). Post 1-hour incubation with primary antibodies, membranes underwent extensive washing with phosphate buffered saline containing 1 % tween (TBST). Subsequently, blots were incubated with the corresponding HRP-coupled secondary antibody (1:10,000) for 1 h followed by additional washing in TBST and detected using the Li-Cor Digital Fluorescence Imaging System (Lincoln, NE; Cat #927–40000). Immununodetection was performed with the Odyssey Procedure (Li-Cor Biosciences, Lincoln, NE) using an IRDye800 coupled anti-rabbit IgG secondary antibody and an IRDye680 coupled anti-mouse IgG secondary antibody. Normalization of drug metabolizing enzyme signals with GAPDH was performed to quantify protein expression.

DNA isolation from target tissues

Genomic DNA was isolated from liver and colon tissues of rats post-B(a)P exposure using the Qiagen® DNeasy Blood and Tissue kit. For DNA isolation, 25 mg of target tissues were cut into small pieces and placed in a 1.5 ml microcentrifuge tube. 180 μl of Buffer ATL was added to the microcentrifuge tube. Next, 20 μl of proteinase K was added to the microcentrifuge tube and mixed by vortexing. The samples were incubated at 56 °C until tissue was completely lysed. Once completely lysed the samples were vortexed for 15 s. Following, 200 μl of Buffer AL was added to the samples and mixed thoroughly by vortexing. Next, 200 μl of ethanol (100 %) was added to the samples and the samples were mixed by vortexing. The samples were then pipetted into a DNeasy Mini spin column that was placed in a 2 ml collection tube. The samples were centrifuged at 6000 x g for 1 min. The collection tubes containing the flow-through were discarded.

The DNeasy spin column was placed in a new 2 ml collection tube and 500 μl of Buffer AW1 were added to the membrane of the spin column. The samples were again centrifuged for 1 min at 6,000 x g. The collection tube containing the flow-through was discarded and the spin column was placed in a new 2 ml collection tube and 500 μl of Buffer AW2 was placed on the membrane of the spin column and centrifuged for 3 min at 20,000 x g. The collection tube containing the flow-through was discarded and the spin column was placed in a clean 2 ml microcentrifuge tube. Next, 200 μl of Buffer AE was placed onto the spin column membrane and allowed to incubate for 1 min at room temperature. The samples were centrifuged for 1 min at 6,000 x g to elute the DNA. Isolated DNA allowed for further analysis of DNA segments to determine DNA adduct formation. The concentration of DNA was determined by spectrophotometry.

^{32}P–Postlabeling and Thin Layer Chromatography (TLC)

To observe the extent to which exposure to B(a)P + AIN-76A diet and B(a)P + Western diet contributes to adduct formation, rats were euthanized post exposure. From the colon and liver of these rats, the concentrations of B(a)P -DNA adducts such as 2′-deoxyguanosine (dGuo) and 2′-deoxyadenosine (dAdo) were measured every five days for one-month (days 5, 10, 15, 20, 25, and 30) after the cessation of B(a)P exposure by ^{32}P-postlabeling and four-directional thin layer chromatography system as detailed in the work of Ramesh et al. (2004) and Ramesh and Knuckles (2006). Adduct levels were calculated by relative adduct labeling and represented as fmol/μg DNA.

Identification of B(a)P-DNA adducts

To identify DNA adducts the reactive metabolites generated from the epoxide- [B(a)P 7, 8-diol, 9, 10-epoxide] and quinone pathways [B(a)P 3,6-quione, and B(a)P 6,12-quione] of B(a)P metabolism were incubated with 40 μM DNA and subjected to co-chromatography with unknown adduct sample. Unknown adducts that exhibit equivalent mobility (co-migration) with that of known standards (commercially purchased) were mapped and identified.

Quantification of aldehyde lesions in DNA

The DNA damage was estimated using a DNA damage quantification kit (Biovision, Mountain View, CA). Benzo(a)pyrene-induced DNA damage was determined by quantifying the number of abasic sites per 10^5 base pairs in genomic DNA samples. This estimation of DNA damage provided insight to the extent of the effects of B(a)P in conjunction with the Western diet. Isolated DNA was incubated with 5 μl of Aldehyde Reactive Probe (ARP) solution at 37 °C for 1 h to tag DNA AP sites. Tris and EDTA (TE) buffer and glycogen were then added to incubated samples and mixed well. Samples were then mixed with 70 % ethanol for 10 min at −20 °C and centrifuged at top speed (10,000 rpm) for 10 min to precipitate AP-site tagged DNA. Each pellet was washed three times with 0.5 ml of 70 % ethanol and spun quickly to remove trace amounts of ethanol. The pellet was air dried for 5 min and then dissolved in 1 ml of TE buffer. Samples were mixed with ARP-DNA standards and DNA binding solution in a 96 well plate and allowed to incubate overnight at room temperature (25 °C). The following day, DNA binding solution was discarded and each well was washed with wash buffer 5 times. One hundred microliters of HRP-Streptavidin solution were added to each well and allowed to mix via a rocker for 1 h at room temperature. Each well was washed with wash buffer. Once the wash buffer was discarded, 100 μl of HRP developer solution was added to each well. The samples were then allowed to incubate for 1 h at 37 °C. The absorbance (OD reading) was measured using a spectrometer and the basic AP sites per 10^5 bp in the DNA samples were calculated using a calibration curve.

Statistical treatment of data

Data on polyp counts and biochemical markers were analyzed by two-way analysis of variance (ANOVA) to determine the effect of diet type and B(a)P dose. The differences among means were determined by using Bonferroni's post-hoc test. The criterion for statistical significance was set at $p < 0.05$. Data on concentrations of biotransformation enzyme activities and B(a)P metabolite concentrations were analyzed by two-way ANOVA to determine the effect of diet type and B(a)P dose. The differences among means were determined by using Bonferroni's post-hoc test. The criterion for statistical significance was set at $p < 0.05$. Data on concentrations of B(a)P-DNA adducts and apurinic sites were analyzed by two-way ANOVA to determine the effect of diet type and B (a)P dose. The differences among means were determined by using Bonferroni's post-hoc test. The statistical significance was set at $p < 0.05$.

Results

Benzo(a)pyrene treatment-related effects on B(a)P metabolizing enzyme transcription expression in liver.

To determine RNA levels of the phase I and II biotransformation enzymes, RNA was isolated from liver tissue and subjected to RT-PCR analysis. The mRNA of CYP1A1 in liver showed that B(a)P did not increase CYP1A1 mRNA at the 25 μg B(a)P/kg bw for rats provided the AIN-76A diet nor the WD. There was significant increased mRNA expression in the 50 μg B(a)P/kg bw + RD group in comparison to their counterpart (Fig. 1A). The 100 μg B(a)P/kg bw + WD did exhibit a slight increase in mRNA expression when compared to the rats provided the same B(a)P dosage and the AIN-76A diet, but there is no statistically significant difference. Also, although the 100 μg B(a)P/kg bw + WD group showed a slight increase in the overall mRNA expression compared to 100 μg B(a)P/kg bw + RD, 100 μg B(a)P/kg bw + WD were low when compared to other treatment groups + diets (Fig. 1A). Analysis of CYP1A1 Western Blot in liver samples showed no difference in protein expression via among the control diet (RD and WD) groups and groups receiving 25 μg B(a)P/kg bw + RD or WD (Fig. 1B). For the diets alone and the 25 μg B(a)P/kg bw + RD and WD groups showed very negligible protein expression of CYP1A1. However, as B(a)P doses increased to 50 and 100 μg B(a)P/kg bw, there was an overall increase in CYP1A1 protein expression. When comparing groups that receive 50 and 100 μg B(a)P/kg bw to their counterparts there was slight increase in CYP1A1 expression in rats who received the WD (Fig. 1B).

The mRNA of CYP1B1 in liver revealed comparable results to those of CYP1A1 mRNA expression (Fig. 2A), except for a slight increase in the 50 μg B(a)P/kg bw + RD in comparison to PIRC rats provided the WD. The 100 μg B(a)P/kg bw + WD also exhibited a slight increase in CYP1B1 mRNA expression when compared to its counterpart which received the WD (Fig. 1C). B(a)P was found to increase the CYP1B1 protein expression in the B(a)P treated groups in comparison to their respective counterparts. Significant increased expression was demonstrated in PIRC rats that received 100 μg B(a)P/kg bw + WD compared to those that received the same B(a)P dose along with the RD. On the other hand, PIRC rats that received 25 μg (B(a)P/kg bw + RD or the WD showed no difference in protein expression (Fig. 1D).

Sulfotransferase (SULT1A1) protein expression also occurred in all the treatment groups in the liver (Fig. 2A). Expression among rats given WD alone (no [B(a)P]) as well as treatment groups 25 μg B(a)P/kg bw + WD showed increased expression in SULT1A1 in comparison those that received the RD diet. There was no difference in protein expression for treatment groups that received 50 and 100 μg B(a)P/kg bw + RD or WD (Fig. 2A).

Glutatione-S-transferase (GST) protein expression occurred in all the treatment groups in the liver (Fig. 2B). However, expression among diets alone (no [B(a)P]) showed a slight increase in GST expression in PIRC rats that received RD in comparison to those who received WD. There was a significant increase in both 25 and 50 μg B(a)P/kg bw + WD treatment groups compared to their counterparts. Very little difference was shown in PIRC rats provided with 100 μg B(a)P/kg bw and consumed either RD or WD (Fig. 2B).

Benzo(a)pyrene treatment-related effects on B(a)P metabolizing enzyme transcription expression in colon

In addition to liver, many of the CYP enzymes and detoxification enzymes are also found in extra hepatic tissues, we proceeded with determining the transcription and expression of these enzymes in the colon. To determine the mRNA and protein expression of activation enzymes, CYP1A1 and 1B1, RNA was isolated from colon tissue and subjected to RT-PCR. The mRNA of CYP1A1 in PIRC rat colon samples demonstrated that B(a)P- induced expression were only measurable in 50 μg B(a)P/kg bw + WD and 100 μg B(a)P/kg bw + RD and WD

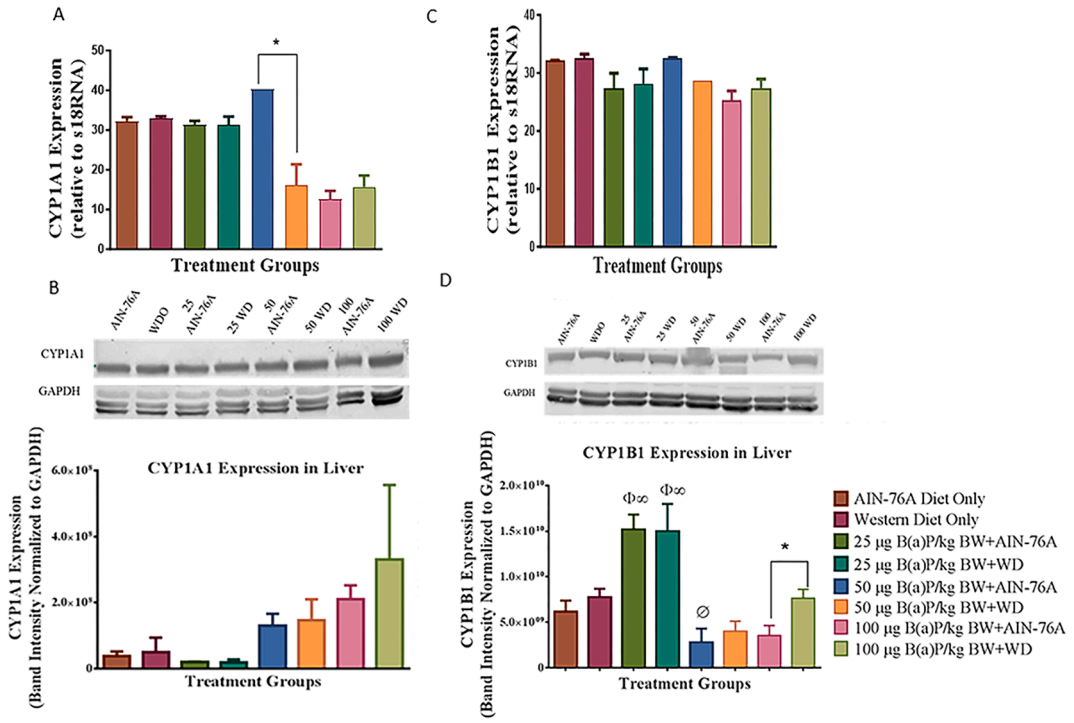

Fig. 1. A. RT-PCR analysis of liver CYP1A1 mRNA levels in PIRC rat liver samples following treatment with B(a)P and consumption of RD or WD. b. CYP1A1 protein expression in liver samples of PIRC rats following treatment with B(a)P and consumption of RD or WD. c. RT-PCR analysis of liver CYP1B1 mRNA levels in PIRC rat liver samples following treatment with B(a)P and consumption of RD or WD. d CYP1B1 protein expression in liver samples of PIRC rats. Annotations denote statistical significance (*$p < 0.05$, $^{\Phi}p < 0.0001$ compared to RD Diet Only, $^{\varnothing}p < 0.05$ compared to WD Only, and $^{\infty}p < 0.001$ compared to WD Only) among diets and B(a)P concentrations used.

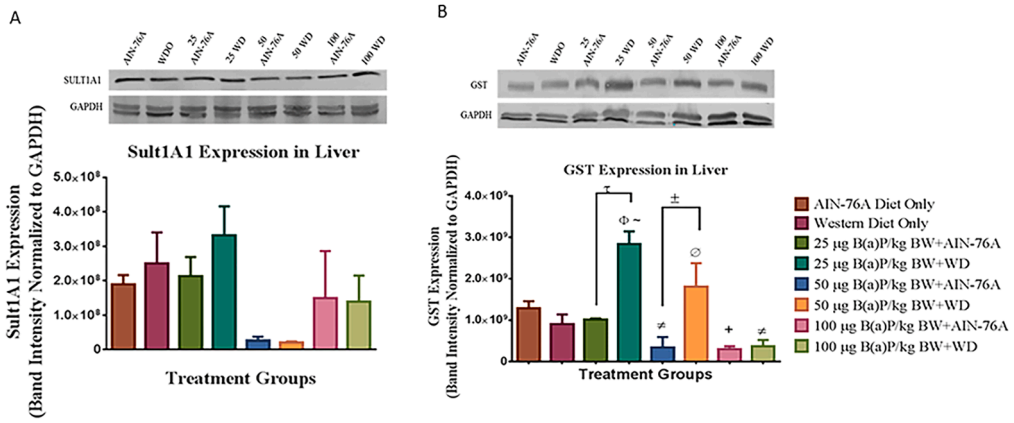

Fig. 2. A. SULT1A1 protein expression in liver samples of PIRC rats following treatment with B(a)P and consumption of RD or WD. b. GST protein expression in liver samples of PIRC rats. Annotations denote statistical significance ($^{\pm}p < 0.001$, $^{t}p < 0.0001$, $^{\neq}p < 0.05$ compared to RD Only, $^{+}p < 0.01$ compared to RD Only, $^{\Phi}p < 0.0001$ compared to RD Only, $^{\varnothing}p < 0.05$ compared to WD Only, and $^{\sim}p < 0.0001$ compared to WD Only) among diets and B(a)P concentrations used.

(Fig. 3A). While increased CYP1A1protein expression observed in hepatic tissues (Fig. 5B) is not surprising, CYP1A1 protein expression was seen in all in the colons of PIRC rats from all treatment groups. However, the 25 µg B(a)p/kg bw + RD and WD showed the greatest expression among all the treatment groups. PIRC rats that received the RD diet regardless of B(a)P exposure showed a slight increase in CYP1A1 protein expression in comparison to rats that were provided the WD. Although there were increases in CYP1A1 protein expression and RD diet consumption the increase showed no statistical significance (Fig. 3B).

The mRNA of CYP1B1 in colon samples revealed no noticeable difference among the diet groups (no [B(a)P]) and the 100 µg B(a)P/kg bw + RD and WD. However, when after B(a)P exposure we see a significant increase in mRNA levels of 25 µg B(a)P/kg bw + RD in comparison to those on the WD (Fig. 6A). However, with the 50 µg B(a)P/kg bw exposure groups we do see that the groups provided the WD showed significant increase in mRNA expression over its counterpart (Fig. 3C).

The results of CYP1B1 mRNA expression are fairly consistent with CYP1B1 protein expression. Analysis of PIRC rats that received 25 µg B (a)P/kg bw and provided the AIN-76A diet showed significant increased protein expression when compared to rats given the same B(a)P dose and provided the WD. Results show that as B(a)P dosing is increased from 25 µg B(a)P/kg bw to 50 and 100 µg CYP1B1 protein expression is increased when comparing 50 and 100 µg B(a)P/kg bw + WD to their counterparts receiving the RD. However, these results showed no significance (Fig. 3D).

Analysis of SULT1A1 expression in the colons of PIRC rats showed

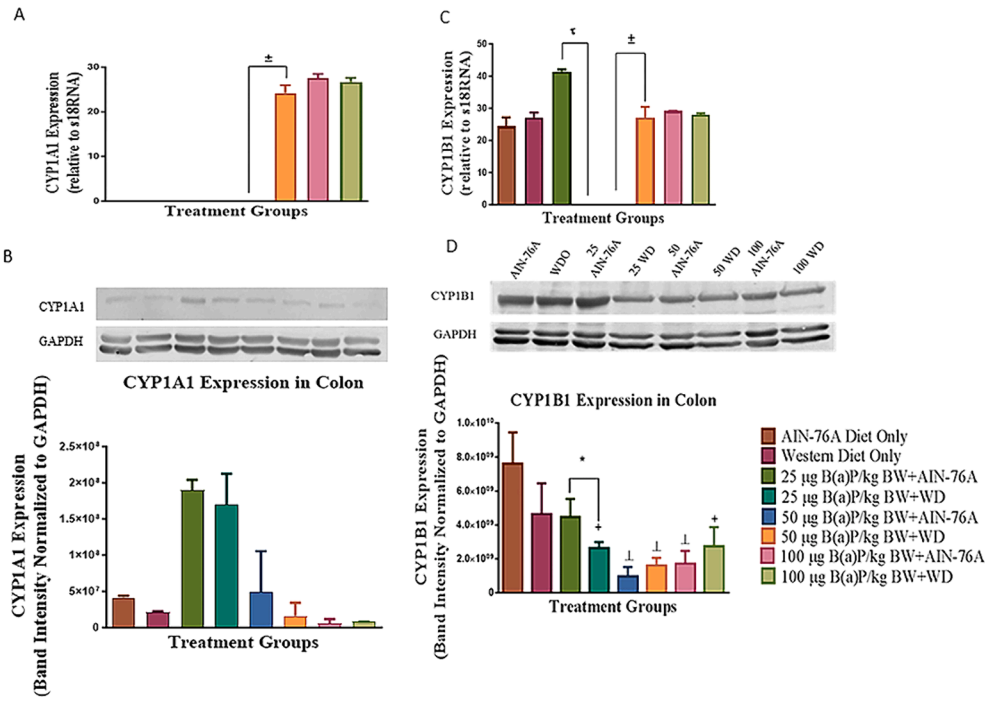

Fig. 3. A. RT-PCR analysis of liver CYP1A1 mRNA levels in PIRC rat liver samples following treatment with B(a)P and consumption of RD or WD. b. CYP1A1 protein expression in colon samples of PIRC rats following treatment with B(a)P and consumption of RD or WD. c. RT-PCR analysis of liver CYP1B1 mRNA levels in PIRC rat liver samples following treatment with B(a)P and consumption of RD or WD. d. CYP1B1 protein expression in colon samples of PIRC rats following treatment with B (a)P and consumption of RD or WD. Annotations denote statistical significance (*$p < 0.05$, $^{\pm}p < 0.001$, $^{+}p < 0.01$ compared to RD Only, $\perp p < 0.001$ compared to RD Only) among diets and B(a)P doses used.

that SULT1A1 expression was influenced with increasing B(a)P doses. SULT1A1 protein expression was negligible in the control groups and 25 µg B(a)P/kg bw + RD and WD (Fig. 4A). However, in the 50 µg B(a)P/kg bw dose groups for both diets we see increased SULT1A1 protein expression although there was no statistical significance between the two 50 µg B(a)P/kg bw dose groups. There were significant findings in the 100 µg B(a)P/kg bw dose groups, where SULT1A1 protein expression in the 100 µg B(a)P/kg bw + WD was significantly increased when compared to its RD counterpart (Fig. 4A).

Glutathione-S-transferase (GST) protein expression in the colon demonstrated surprising results where all treatment groups that received the RD diet showed increased GST expression in comparison to treatment groups that received the WD (Fig. 4B). There was significant increase in expression in the 25 µg B(a)P/kg bw + RD group over the WD group of the same B(a)P dose (Fig. 4B). The 25 µg B(a)P/kg bw + RD had the largest increase in GST expression among all the groups.

Tissue -specific disposition of B(a)P metabolites

Metabolic processing of chemicals by the body is the major force

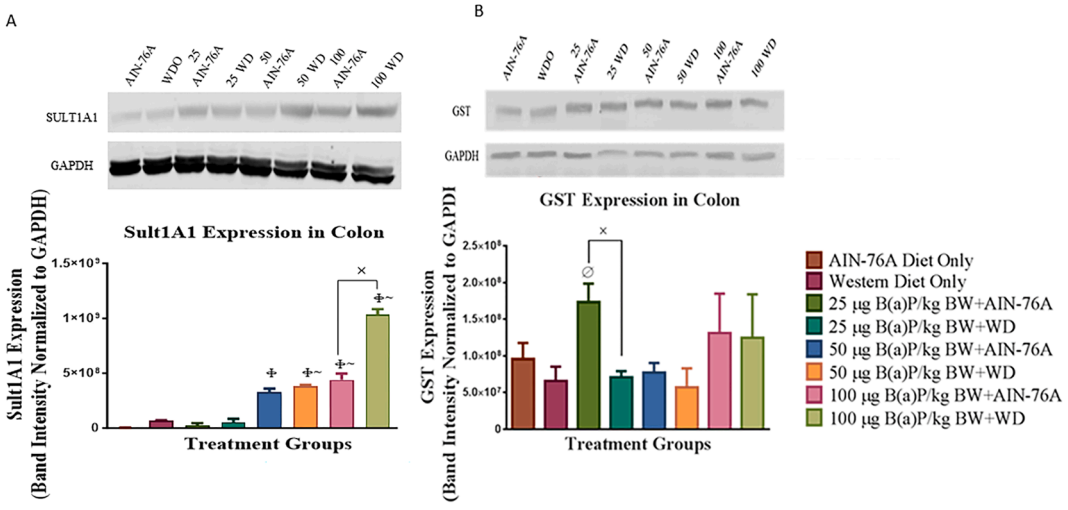

Fig. 4. A. SULT1A1 protein expression in colon samples of PIRC rats following treatment with B(a)P and consumption of RD or WD. Annotations denote statistical significance ($^{x}p < 0.01$, and $^{\Phi}p < 0.0001$ compared to RD Only, and $^{\sim}p < 0.0001$ compared to WD Only) among diets and B(a)P doses used. B. GST protein expression in colon samples of PIRC rats following treatment with B(a)P and consumption of RD or WD. Annotations denote statistical significance ($^{x}p < 0.01$ and $^{\varnothing}p < 0.05$ compared to Western Diet Only) among diets and B(a)P doses used.

behind toxicity or carcinogenesis. Colon, liver tissues and plasma samples were analyzed for B(a)P and its produced metabolites. By measuring B(a)P metabolite concentrations we can answer the question of whether tissue burden of B(a)P reactive metabolites (total metabolite load and specific metabolite types) is greater when B(a)P is administered in conjunction with WD consumption. The results for distribution of B(a)P metabolites in plasma, colon and liver tissues are shown in Fig. 5A–C. Results of the disposition studies revealed no traces of the parent B(a)P compound, indicating that the ingested B(a)P was metabolized and delivered to the target tissues. Rats that ingested B(a)P + WD registered greater amounts of B(a)P metabolites both in liver and colon compared to those that received B(a)P + RD (Fig. 5). The metabolite concentrations were found to be B(a)P dose-dependent (Fig. 5A–C).

Next, we analyzed the B(a)P metabolite profiles and proportions in order to determine the extent to which the WD alters the B(a)P metabolite composition. The B(a)P metabolites identified were as follows: B(a)P 4,5-diol; B(a)P 7,8-diol; B(a)P 9,10- diol; B(a)P 3,6-dione, B(a)P 6,12-dione; 3(OH) B(a)P and 9(OH) B(a)P. The former are reactive metabolites and the latter two are detoxification products. Among the B(a)P metabolite concentrations, the proportion of its reactive metabolites produced such as 7,8-diol-9,10-epoxide; B(a)P-3,6-dione, and B(a)P-6,12-dione were high (Fig. 5D). When comparing B(a)P exposure among the two diets the data shows that the reactive metabolites were produced more in PIRC rats that received B(a)P + WD.

Benzo(a)pyrene treatment-dependent effects on B(a)P-induced oxidative stress and DNA damage

One of the first steps toward adduct formation is the presence of DNA base pair damage. Assessment of the number of damaged DNA base pairs in the presence of B(a)P can indicate the extent to which possible adducts may eventually form. Both liver and colon samples of PIRC rats were analyzed for the presence of abasic sites. As shown in Fig. 6A there was no difference in the number of abasic sites in the livers of PIRC rats treated with B(a)P + WD in liver samples). Observation of colon samples showed a slight increase in abasic site numbers in PIRC rats provided with the WD only and 25 μg B(a)P/kg bw + WD when compared to their counterparts who received the AIN-76A diet (Fig. 6B). Alternatively, the results showed a slight increase in abasic site numbers in PIRC rats that received 50 and 100 μg B(a)P/kg bw + RD when compared to their counterparts that received the same dosage of B(a)P but provided the WD (Fig. 6A).

DNA base pair damage is a very good indicator of the underlying mechanism to tumor polyp formation. However, it is not the only possible route. Oxidative stress and the production of reactive oxygen species are also key player in tumor development. To fully account for all the potential players in the tumor development of the PIRC rats our studies next focused on the production of malondialdehyde (MDA), a naturally occurring product of lipid peroxidation. Results on lipid peroxidation from colon and serum samples are illustrated in Fig. 6C & D. In the colon samples MDA production was increased in PIRC rats that received the WD only as well as B(a)P + WD. On the contrary, there was no difference among the treatment groups when analyzing serum samples for oxidative stress in other areas of the body, except in the case of 100 μg/kg.

Benzo(a)pyrene treatment- and diet type-dependent effects on disposition of B(a)P-DNA adducts in colon and liver tissues

As B(a)P-DNA adduct formation is one of the key steps in colon carcinogenesis, the B(a)P-DNA adducts concentrations in these tissues were measured as these adducts serve as a markers of B(a)P-induced damage to colon cells following B(a)P ingestion. Additionally, the B(a)P-DNA adduct concentrations would also inform us if there is any effect of diet type on adduct formation. No B(a)P-DNA adducts were detected either in colon or liver tissues of control rats. However, B(a)P-

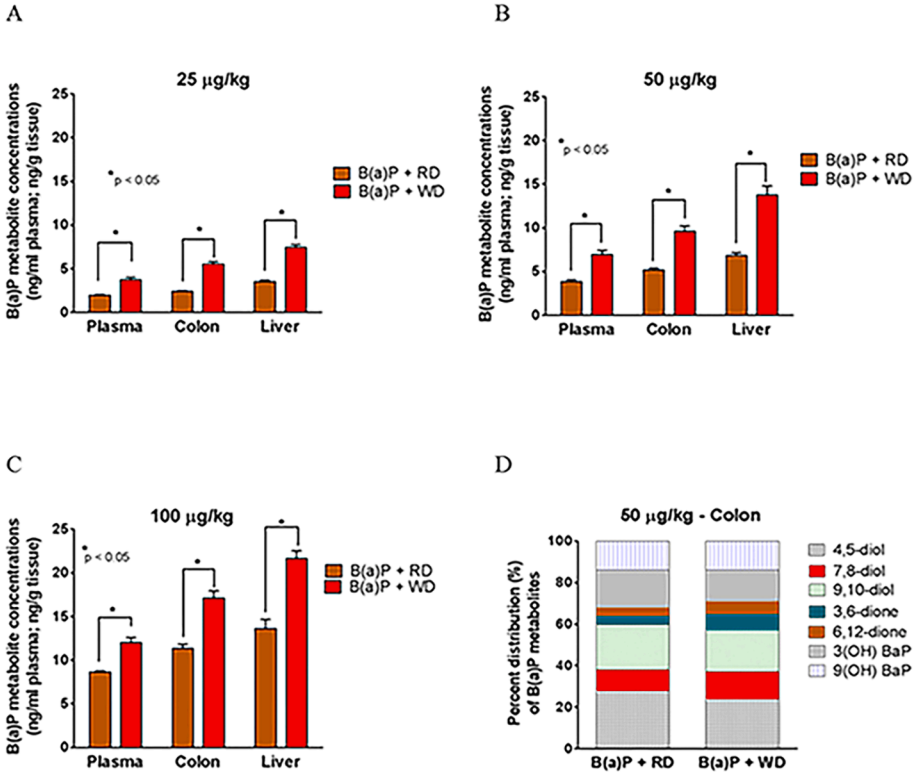

Fig. 5. A–C B(a)P metabolite concentrations in plasma, liver and colon samples from PIRC rats following treatment with 25, 50, and 100 μg B(a)P/kg bw for 60 days via oral gavage and consumption of RD diet or WD. d. Representative analysis of Benzo(a)pyrene metabolism in colon sample of PIRC rat exposed to 50 μg B(a)P/kg bw and provided RD or WD.

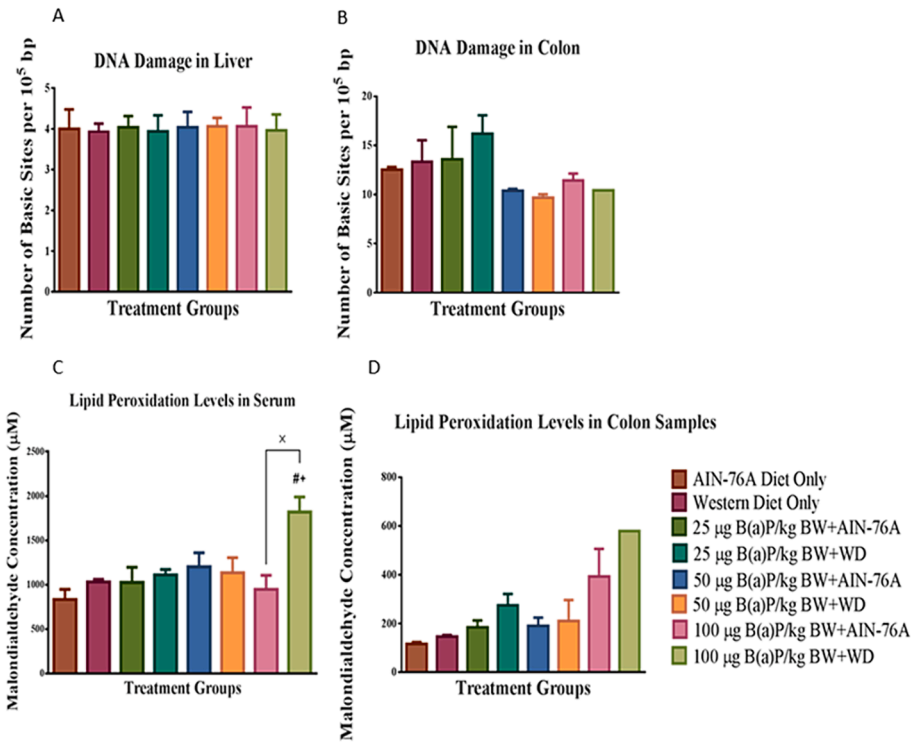

Fig. 6. A–C. DNA strand breaks in liver (A) and colon (B) samples of PIRC rats following treatment with 25, 50, and 100 μg B(a)P/kg bw for 60 days via oral gavage and consumption of RD or WD. c,d. Oxidative stress marker concentrations in serum (C) and colon (D) samples from PIRC rats following treatment with 25, 50, and 100 μg B(a)P/kg bw for 60 days via oral gavage and consumption of RD or WD. Annotations denote statistical significance ($^{x}p < 0.01$, $^{+}p < 0.01$ compared to RD Only, $^{\#}p < 0.01$ compared to WD Only) among diets and B(a)P concentrations used.

DNA adducts were detected in colon, and liver tissues of rats that were fed RD or WD. The adduct concentrations in colon and liver for all three doses employed are shown in Fig. 7. Rats that were fed WD showed the greatest concentration of B(a)P-DNA adducts compared to its RD counterparts and a B(a)P dose–response relationship as well ($p < 0.05$; dose x treatment interaction).

The major adducts identified in colon and liver were derived from the 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE), which are the deoxyguanosine (BPDE- N2-dG-3′P) adducts. On the other hand, the proportion of deoxyadenosine adducts formed from BPDE (BPDE- N2-dA-3′P) were minor in both colon and liver tissues. The 3-OH-B(a)P; 9-OH–B(a)P; B(a)P-3,6-dione, and B(a)P-6,12-dione metabolites were not found to contribute to adduct formation in any of the tissues studied. The distribution of BPDE adduct types in both colon and liver for the three B(a)P treatment groups are shown in Table 1. The B(a)P-DNA adducts identified were the deoxyadenosine (dA) and deoxyguanosine (dG) ones. The dG adducts were predominant in both colon and liver tissues at all the B(a)P doses employed for the diet groups (RD & WD) used. Since there is no major difference in dA/dG ratio of adducts among the three B(a)P doses employed, the distribution of adduct types at 50 μg/kg are shown as a representative example in Table 1.

Table 1
Effect of diet type on major B(a)P-DNA adduct types in colon and liver tissues of PIRC rats.

	Colon		Liver	
Treatment	dA	dG	dA	dG
B(a)P + AIN-76A	20	80	10	90
B(a)P + WD	15	85	10	90

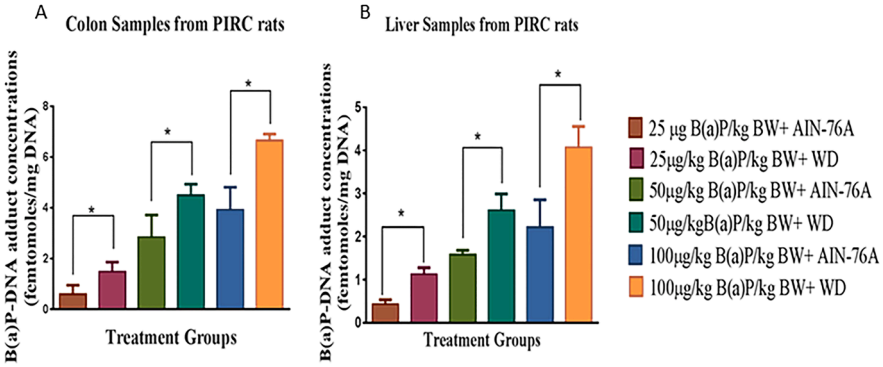

Fig. 7. A-B. Benzo(a)pyrene-DNA adduct total concentrations in colon (A) and liver (B) tissues of PIRC rats that were fed either RD or WD and exposed to 25-,50- and 100 μg B(a)P/kg bw. The bars represent mean ± S.E. for three independent experiments. *$p < 0.05$ in adduct concentrations for the respective B(a)P + RD treatment group compared to B(a)P + WD treatment group.

Discussion

The polyposis in rat colon (PIRC) rat was chosen for this study due to the long lifespan of this model and its amenability to replicate the sporadic colon cancer situation seen in humans (Irving et al., 2014) and widely used in colon cancer prevention and mechanistic studies (Femia et al., 2015; Yun et al., 2020; Tortora et al., 2022). The doses (25, 50, and 100 μg B(a)P/kg bw) chosen for this study are relevant to human exposure scenario in terms of dietary intake and environmental exposure as detailed in our earlier publication (Harris et al., 2016).

Biotransformation plays a pivotal role in carcinogenesis caused by some environmental toxicants including PAHs (Tao et al., 2020; Stipp and Acco, 2021; Guengerich, 2023). The regulation of biotransformation enzyme gene expression both at the transcriptional and post translational levels influences the elimination of carcinogens and alters the pathophysiology of disease (Jennings et al., 2013; Dutta and Jain, 2023). The fact that chemical carcinogen exposure is linked with expression of biotransformation enzymes in target tissues (Shimizu et al., 2000) necessitates how alterations in these enzymes render the colon susceptible to B(a)P exposure. Though the association of dietary PAHs with gastrointestinal tract tumors in epidemiological and animal model studies has already been established (Halberg et al., 2008, Harris et al., 2009, Hakura et al., 2011, Diggs et al., 2011), information on underlying mechanisms is lacking. With this knowledge we next attempted to gain a more mechanistic understanding of how WD potentiates B(a)P-induced polyp formation. Since it is our hypothesis that WD modulates B(a)P-induced colon carcinogenesis through cytochrome P450 mediated metabolic pathways, the protein, mRNA, and enzyme activity of key enzymes proven to be involved in B(a)P biotransformation was assessed.

Studies have revealed that CYP450s are responsible for metabolizing B(a)P in the body (Kundu and Surh, 2008) and two prominent CYPs found both in the liver and colon are CYP1A1 and CYP1B1. Induction of these two proteins by B(a)P promotes its own metabolism in both organs. Findings of our study showed several fold greater inductions of CYP1B1 in liver and colon compared to CYP1A1 following exposure to B(a)P regardless of the type of diet administered. Elevated CYP1B1 expression is a marker for more aggressive colon tumors (Tang et al., 2009) which is evident from the pathology of colon tumors that demonstrated invasive neoplasia after only 60 days of B(a)P exposure. In the liver CYP1B1 expression was elevated by WD when compared to their counterparts, except in the case of the 25 μg/kg B(a)P dose groups (RD [AIN-76A] and WD) in which both diets showed an amplified expression when compared to other treatment groups. While in the liver the controls (AIN-76A and WD only) and low B(a)P dose group showed a greater increase in CYP1B1 expression in PIRC rats that received the RD diet. However, as the B(a)P doses increased we did start to see that the WD caused an elevated expression of CYP1B1 over those provided the normal diet. The role of CYP1B1 in metabolic activation, particularly the conversion of B(a)P-7,8-diol to the ultimate carcinogen BPDE (Harrigan et al., 2006) may play a critical role in the neoplastic adenoma formation in the PIRC rat colon. Reports by Galvan et al (2005) state that aryl hydrocarbon receptor induction upon B(a)P exposure leads to an increase in CYP1B1 induction, leading to the generation of metabolites and cytotoxicity.

Although CYP1A1 expression in liver and colon tissue was less than those of CYP1B1 this is not to disregard the effect of B(a)P on the protein expression. Studies regarding CYP1A1protein expression have reported increased expression in hepatic tissues (Diggs et al., 2013; Stoddard et al., 2021) and results from our Western Blot analysis are consistent with this fact. Results from our studies demonstrated that B(a)P + WD did cause an increase in the protein's expression over their counterparts. Also, it has been alluded to that CYP1A1 plays a role in detoxification and not necessarily in metabolic activation (Uno et al., 2008, Shi et al., 2010a,b). Studies using CYP1A1 (-/-) global knockout mice that were exposed to 1.25 mg/kg/day of B(a)P showed a survival life span of 4 to 5 months, while the CYP1A1 (+/+) wild type mice survived for a year (Shi et al., 2010a). However, when these groups of mice received 125 mg/kg/day of B(a)P, the knockout mice lived for 18 days, and their counterparts survived for one year (Shi et al., 2010b). These studies also revealed that small intestinal CYP1A1 processes much of the toxicant load before it reaches the liver and is induced in liver tissues (Uno et al., 2004; Shi et al., 2010a,b). These studies concluded that CYP1A1 is required for detoxification, while CYP1B1 is needed for metabolic activation. Immunohistochemistry studies performed by Uno et al. (2008) reaffirmed this position. Results from these studies showed the localization of CYP1A1 throughout the small intestines of the mice, and the isozyme was located close to the lumen when compared to CYP1B1, allowing CYP1A1 Zheng et ato process B(a)P in the intestines. This similar localization of CYP1B1 can be expected in the large intestine, in essence, providing the balance between CYP1A1 and 1B1 distinguishing the consequences of metabolic activation versus detoxication processes. With CYP1A1 possibly playing a role in detoxification this may account for the lack of liver damage seen in our studies.

The B(a)P metabolite concentrations in plasma, colon and liver of rats that received the B(a)P doses along with WD consumption were greater when compared to their RD counterparts. Our findings on increase in B(a)P metabolite load with WD ingestion in colon tissues are similar to our lab's earlier findings wherein we have shown that saturated fat contributed to an increase in fluoranthene (a PAH compound) metabolite concentrations in target tissues of F-344 rats (Walker et al., 2007), as well as B(a)P metabolite concentration in Apc^{Min} mice (Diggs et al., 2013) in a subchronic exposure regimen. Zheng et al. (2022) report that subchronic exposure to low doses of B(a)P significantly induces the expression of IL-6, TNF α, NF-kβ, SOD1, and mucin-1 and decreases the expression of p53 is in support of the argument that B(a)P exposure induces the expression of inflammatory and oxidative stress parameters, which is exacerbated by a lipid rich environment is conducive for carcinogenic process.

Our findings could also be explained from the context of bioaccessibility of B(a)P. Bioaccessibility refers to the fraction of B(a)P that is mobilized from the food into the digestive juice (chyme) and represents the maximum amount of this toxicant available for intestinal absorption (Harris et al., 2013). Using Caco-2 cells and the *in vitro* simulation of food digestion in the gastrointestinal tract (INFOGEST) model (Brodkorb et al., 2019), Faria et al. (2020) demonstrated that WD facilitates a higher bioaccessibility of B(a)P.

Our results exhibiting that the highest concentration of B(a)P metabolites being generated in the liver are consistent with reports from Wall et al. (1991) that the liver is the principal organ for metabolism of PAHs. The increased liver weight when compared to the that weight of other organs allows a greater capacity for processing toxic chemicals such as B(a)P (Doherty and Charman, 2002). Our findings also revealed high levels of B(a)P metabolite formation in the colon demonstrating that the intestine also plays a key role in the processing of orally ingested PAHs (Choi et al., 2004).

The production of reactive oxygen species (ROS), such as superoxide anion (O2–), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO•), consist of radical and non-radical oxygen species that are formed by the partial reduction of oxygen. Cellular ROS are generated either in the process of mitochondrial oxidative phosphorylation, or they may be produced from interactions with exogenous sources such as xenobiotic compounds. Reactive oxygen species can overwhelm the cellular antioxidant defense system, either through an increase in ROS levels or a decrease in the cellular antioxidant capacity, causing oxidative stress to occur (Rayet al., 2012). This occurrence results in direct or indirect ROS-mediated damage of nucleic acids, proteins, and lipids, and has been implicated in carcinogenesis, bronchopulmonary dysplasia, and vascular diseases (Trachootham et al., 2009; Stading et al., 2020).

It is assumed that the pathological changes in the target tissues induced by toxicants were associated with production of highly reactive free radicals and initiation of oxidative damage (Cooke et al., 2003). Our

studies did not show any increase in number of DNA base pairs damaged in either the liver or colon tissues. This is not surprising for the liver samples seeing as the potential protective effect CYP1A1 may have regarding the liver tissue. Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and has been used as an indicator of oxidative stress in cells and tissues (Armstrong and Browne, 1994). Lipid peroxides derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds, which include reactive carbonyl compounds, such as MDA (Wang et al., 2001). Although increased number of DNA base pair damage was expected, studies regarding lipid peroxidation showed slight increases in B(a)P + WD groups, providing indication that the production of reactive oxygen species is being modified by WD in the PIRC rat model.

Binding of B(a)P with DNA constitutes a key event by which B(a)P causes cancer (Luch, 2005). The induction of drug metabolizing enzyme mRNA and protein following exposure of PIRC rats to B(a)P in this study also resulted in an increase in B(a)P metabolite formation and a corresponding increase in B(a)P-DNA adduct formation in colon and liver. The adduct concentrations revealed a food matrix (RD or WD)-specific and B(a)P dose- specific disposition. Interestingly, the B(a)P-DNA adduct concentrations in both colon and liver mirrored the B(a)P metabolite concentrations in tissues. The concordance between metabolite and adduct concentrations strongly suggests that B(a)P metabolite load in colon plays an important role in determining the likelihood of causing damage to cellular macromolecules and disrupting the cellular homeostasis.

The low B(a)P-DNA adduct concentrations in colon and liver tissues of rats in the RD group reflect a decreased biotransformation of B(a)P in target tissues. The greater affinity of PAHs and their metabolites for high-density lipoproteins (HDL; Ma et al., 2022), may facilitate the HDL-sequestered B(a)P and its metabolites to undergo cellular internalization (Vondráček and Machala, 2021), which prevents the metabolites from undergoing hydrolysis in rats that received WD. Literature reports also indicate that B(a)P-DNA binding was greater in tissues of rats that received diets containing lard and cod liver oil compared to their counterparts that were fed fat-free diet (Willis, 1983). High-fat cholesterol diet was reported to accelerate DNA damage due to B(a)P exposure in a hyperlipidemic mouse model (Curfs et al., 2003). Earlier studies conducted in our laboratory have also shown a pronounced increase in B(a)P-DNA adduct concentrations in colon and liver tissues of Apc^{Min} mice that received B(a)P through saturated fat compared to unsaturated fat (Diggs et al., 2013). The increased concentration of B(a)P- DNA adducts in colon and liver from WD group in the present study suggest that these organ systems are susceptible to damage from intake of B(a)P. A similar trend was observed in our earlier studies in a fluoranthene (a PAH compound)-administered to the F-344 rat model, wherein we observed association of a saturated fat-elevated DNA damage compared to unsaturated fat (Walker et al., 2007). Our argument gains support from studies reported in human subjects as well. Diet-originated B(a)P has been reported to cause B(a)P-DNA adduct formation in human colon mucosa (Sattar et al., 1999). Also, of interest in this regard was the report that calorie intake, which is associated with lipid-rich diet, showed a strong association with B(a)P-DNA adduct levels in white blood cells isolated from human subjects (Rundle et al., 2007). Taken together, all the above-cited studies reinforce the notion that the carcinogenic effects of ingested B(a)P are governed by the intake of dietary lipid content.

An interesting observation that stemmed from our studies is the strong association of B(a)P-DNA adduct levels with our previously published studies on adenoma levels in colon of PIRC rats (Harris et al., 2016). The adenoma counts displayed a dose–response relationship with the 100 μg/kg dose group registering more adenomas compared to its 25- and 50 μg B(a)P/kg bw counterparts. Our findings reported here agree with findings from our earlier studies wherein we have observed an increased incidence of adenomas in Apc^{Min} mice that received B(a)P through saturated fat compared to unsaturated fat and controls (Diggs et al., 2013). In those studies, also, the adenoma counts displayed a dose–response relationship with the 100 μg/kg dose group registering more adenomas compared to its 50 μg/kg counterpart for B(a)P, B(a)P + unsaturated fat and B(a)P + saturated fat treatment groups. A similar correlation between B(a)P-DNA adducts concentrations and adenomas have been observed for the Apc^{Min} mouse small intestine (Sattar et al., 1999). Additionally, in a clinic-based study of colorectal adenomas, a positive association between leucocyte PAH-DNA adducts concentrations and adenoma prevalence was seen (Gunter et al., 2007). The concordance between B(a)P-DNA adducts concentrations and adenomas strongly suggest that adenoma development is a function of robust bioactivation of B(a)P leading to an enhanced B(a)P-DNA adduct formation, accumulation of mutations and subsequent progression to tumor development.

The preponderance of deoxyguanosine (dG) adducts relative to that of deoxyadenosine (dA) adducts are consistent with the results of studies reported from our laboratory (Walker et al., 2007; Diggs et al., 2013) and those of others (Ross et al., 1995; Dreij et al., 2005). Since the carcinogenicity of B(a)P results from this compound's propensity to form dG adducts (Kramata et al., 2003), the greater incidence of these adducts at 100 μg/kg, compared to 25- and 50 μg/kg, recorded in the present study, indicate the vulnerability of colon tissues to damage resulting from subchronic exposure to high doses of B(a)P and consumption of WD.

To summarize, findings from our present- and our prior (Harris et al., 2016) studies on B(a)P-induced colon carcinogenesis have demonstrated that i) WD results in a greater number of colon tumors in PIRC rats compared to animals that were fed unsaturated fat/normal rodent chow; ii) WD results in accrual of visceral and subcutaneous fat depots, likely reservoirs for B(a)P accumulation; iii) WD does promote increased tumor formation in the rat when treated with B(a)P and iv) treatment with B(a)P and WD causes adenoma progression to high grade dysplasia in the PIRC rat colon. The tumor burden was associated with circulating cholesterol, triglyceride, glucose, insulin, leptin and adiponectin concentrations. The concordance between dietary fat, B(a)P dose, tumor load, invasive nature of adenomas, and the concentrations of the above-mentioned markers provide a compelling rationale that Western diet accelerates B(a)P-induced colon tumor formation and proliferation through enhanced insulin, leptin and other inflammatory molecules, which as a consequence may induce important signaling pathways such as PI3k/Akt and ERK1/2 (Kasdagly et al., 2014; Santarelli et al., 2022; Xu et al., 2020; Zheng et al., 2022).

Future studies will concentrate on the role of glucuronidation in detoxifying B(a)P alone and in combination with Western diet in the rat model. Additional studies will include assessing injury to colon and liver tissues using etheno DNA adducts (markers of lipid peroxidation) and F2-isoprostanes as more definitive biomarkers of oxidative stress.

Declaration of generative artificial intelligence (AI) and AI-assisted technologies in scientific writing.

During the preparation of this work the author(s) used no AI-assisted technologies.

CRediT authorship contribution statement

Kelly L. Harris: Methodology, Investigation, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition. **Kenneth J. Harris:** Investigation, Formal analysis. **Leah D. Banks:** Investigation, Formal analysis. **Samuel E. Adunyah:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Aramandla Ramesh:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Funding

The research reported in this publication work was supported by the National Institutes of Health (NIH) grants 1F31ESO2407901, 1UG3HG013248-01, 5U54CA163069-04, 5U54MD007593-07, G12RR003022, American Cancer Society (ACS) grant DICRIDG-21-071-01, and the US Department of Energy (DOE) grant DE-EM0005266. The content is solely the responsibility of the authors and does not officially represent the views of the NIH or ACS or DOE.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank Dr. James Amos-Landgraf at the University of Missouri-Columbia for his valuable help in maintaining the PIRC rat model. Also, our thanks are due to Drs. Carlos Virgous, Mohammad Niaz, Ms. Katrina Stanford, Mr. Tracey Randolph, and Mr. Nihad Sakic at Meharry Medical College for their assistance in animal handling, care, and management.

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