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Olive oil prevents benzo(a)pyrene [B(a)P]-induced colon carcinogenesis through altered B(a)P metabolism and decreased oxidative damage in Apc^{Min} mouse model

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Abstract

Colon cancer ranks third in cancer related mortalities in the United States. Many studies have investigated factors that contribute to colon cancer in which dietary and environmental factors have been shown to play an integral role in the etiology of this disease. Specifically, human dietary intake of environmental carcinogens such as polycyclic aromatic hydrocarbons (PAHs) has generated interest in looking at how it exerts its effects in gastrointestinal carcinogenesis. Therefore, the objective of this study was to investigate the preventative effects of olive oil on benzo(a)pyrene [B(a)P]-induced colon carcinogenesis in adult Apc^{Min} mice. Mice were assigned to a control (n = 8) or treatment group (n = 8) consisting of 25, 50 and 100 µg B(a)P/kg body weight (bw) dissolved in tricaprylin [B(a)P-only group] or olive oil daily via oral gavage for sixty days. Our studies showed that Apc^{Min} mice exposed to B(a)P developed a significantly higher number (p < 0.05) of larger dysplastic adenomas compared to those exposed to B(a)P + olive oil. Treatment of mice with B(a)P and olive oil significantly altered (p < 0.05) the expression of drug metabolizing enzymes in both the colon and liver tissues. However, only GST activity was significantly higher (p < 0.05) in the liver of mice treated with 50 and 100 µg B(a)P/kg bw + olive oil. Lastly, olive oil promoted rapid detoxification of B(a)P by decreasing its organic metabolite concentrations and also decreasing the extent of DNA damage to colon and liver tissues (p < 0.05). These results suggest that olive oil has a protective effect against B(a)P-induced colon tumors.

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Authors' Contributions

LDB and AR designed the study and applied for Institutional Animal Care & Use Committee approval. LDB, PA, and MSN performed the experiments and collected the data. LDB and AR analyzed the data and prepared draft figures and tables. LDB and AR prepared the manuscript draft with intellectual input from MKW and SEA. All authors approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Keywords

Benzo(a)pyrene; polycyclic aromatic hydrocarbons; olive oil; Apc^{Min} mouse; colon cancer; drug metabolizing enzymes; oxidative DNA damage

1. Introduction

Colorectal cancer (CRC) is one of the most common malignancies in Western populations. In the United States alone, CRC is the third most common cancer amongst males and females and accounts for 136,830 diagnoses and 50,310 deaths annually [1]. While 10% of colon cancer cases are attributed to family history, sporadic gene mutations account for 90% of colon cancer cases. It is alleged that dietary factors as well as exposure to environmental carcinogens may contribute to these sporadic genetic modifications [2-4].

Diet, especially fat intake, is an important nutritional influence on CRC [5]. Studies have suggested that environmental factors including exposure to environmental toxicants play an integral role in the susceptibility of CRC cases [6,7]. One family of such toxicants known as polycyclic aromatic hydrocarbons (PAHs) has been shown to contaminate the environment as a result of release from combustion-related activities (tobacco smoke, coal tar burning, automobile exhausts, refuse burning, forest fires etc.). Additionally, these toxicants are also known to contaminate foodstuffs during cooking, which includes deep frying, barbecuing, grilling etc.[8]. Benzo(a)pyrene [B(a)P], a five ring member of the PAH family, is a widely distributed environmental toxicant that is commonly found in cigarette smoke, red meats, charcoal grilling of food, food rich in fat as well as industrial emissions. Studies have implicated B(a)P as a causative agent in several cancers including colon cancer.

Specifically, studies have found that dietary exposure of B(a)P more than likely leads to CRC cases in humans by dietary contamination [5,9]. Furthermore, evidence pertaining to the dietary intake of PAHs and their role in the development of digestive tract cancers in animal models and humans has also been documented [10]. Therefore, investigating preventive solutions through diet to reduce the number of CRC cases is of great importance.

Dietary interventions through chemopreventive agents have become a priority in recent years [11-15]. There's sufficient evidence from human and laboratory (animal model and cell culture) studies, which have shown that a relationship exists between dietary constituents and disease prevention [16-18]. Specifically epidemiological studies have shown that colon cancer rates were significantly reduced in Mediterranean countries where olive oil is the main ingredient of diet [19,20]. Olive oil is rich in phenolic components that have potent antioxidant properties, which could render a protective effect against several types of cancer [21]. Studies have shown that the bioactive components of olive oil have exerted its effects by inhibiting cell proliferation, and promoting apoptosis in colorectal cancer cell lines [22,23]. This dietary ingredient has also been shown to prevent colon carcinomas in rats [24]. Being safe, inexpensive, and accessible through diet, olive oil holds promise as an ideal preventive agent against colon cancers [25]. However, to date, little is known about the mechanisms by which olive oil regulates colon carcinogenesis induced by dietary carcinogens. Therefore, the main goal of this study was to provide an insight into the

mechanisms by which olive oil reduces/prevents colon cancer. By using an animal model specific for colon cancer, the *Apc^{Min}* mouse, we are able to address the mechanisms by which olive oil can possibly alter the biotransformation of B(a)P and the accompanying damage to colon DNA.

2. Materials and Methods

2.1. Chemicals

Benzo(a)pyrene (CAS No. 50-32-8; 98% pure), olive oil, sodium dodecyl sulfate (SDS), Ponceau S solution and tricaprylin were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Sodium phosphate (monobasic and dibasic), methanol, chloroform, ethanol and 10% formalin, isopropyl alcohol was purchased from Fisher Scientific Company (Kennesaw, GA). Sucrose, EDTA and tris-HCl were purchased from Curtin Matheson Scientific Inc. (Houston, TX). The CYP1A1, CYP 1B1, actin, and rabbit anti-goat IgG-HRP antibodies were purchased from Santa Cruz Biotechnology (Dallas, TX). The GST-P antibody was purchased from Assay Designs (Ann Harbor, MI). The Quick Start Bradford Protein Assay Kit, ethidium bromide, Precision Plus Protein blue standards, tetramethyl-ethylenediamine (TEMED), ammonium persulfate (APS), 30% acrylamide and bis-acrylamide solution, Laemmli sample buffer, agarose, EZ load 100 bp molecular ruler, Immun-Star HRP substrate and 2-Mercaptoethanol (β ME) were purchased from Bio-Rad Laboratories (Richmond, CA). The PVDF membranes were obtained from the Amersham Pharmacia Biotech (Piscataway, NJ). The HyGLO ECL Spray Chemiluminescent HRP antibody detection reagent was purchased from Denville Scientific (Metuchen, NJ). The RNase/DNase free water and Trizol reagent were purchased from Invitrogen (Carlsbad, CA). The magnesium chloride, Go Taq Flexi DNA Polymerase, random primers, reverse transcription buffer, deoxynucleotide triphosphates (dNTPs), RNase inhibitor, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Tris/Borate/EDTA (TBE) buffer, Tris-acetate-EDTA (TAE) buffer, CYP1A1 and 1B1 enzyme assay kits were purchased from Promega (Madison, WI). The DNeasy blood & tissue kit was procured from Qiagen Inc. (Valencia, CA). The DNA damage quantification kit and GST assay kits were purchased from Biovision Inc., (Mountain View, CA). HeLa whole cell lysate (positive control for CYP1A1) and mouse kidney extract (positive control for CYP1B1) were obtained from Santa Cruz Biotechnology. Mouse kidney extract (positive control for GST) was also purchased from Assay Designs. Benzo(a)pyrene metabolite standards were obtained from the National Cancer Institute Chemical Carcinogen Repository (Midwest Research Institute, Kansas City, Missouri, USA). The HPLC & GC columns were purchased from Agilent (Wilmington, DE).

2.2. Olive oil Analysis

Olive oil analysis was based on a procedure that converts fatty acids to fatty acid methyl esters (FAME) through saponification reaction. The olive oil samples (1 ml) were saponified with 100 μ l of KOH (2N) and 10 ml of n-hexane. Post saponification, the samples were washed with HPLC grade water to wash away the KOH and extracted with ethanol. The extracts were then pooled and evaporated to dryness under a stream of N_2 . The residue was dissolved in hexane. The FAME in the sample were analyzed by injecting the sample on to a

gas chromatograph mass spectrometer (GC-MS; Agilent 6890 series) equipped with a mass-selective detector (Agilent 5973N). A capillary column was used (HP88; 60m × 250 μM, 0.2 μM) which had a flow rate of 1 mL/min and helium was used as a carrier gas. The injector and detector temperatures were maintained at 175°C. The oven temperature was programmed as follows: 175°C for 5 min and 5°C/min to 250°C. The individual components (identities of FAME) were identified by their mass spectral characteristics. The GC-MS quadrupole mass analyzer was operated in the select-ion-monitoring (SIM) mode.

In addition to analyzing olive oil for its main constituents, aliquots of olive oil samples drawn from the same lot were analyzed to examine if there are any residual levels of B(a)P in it. One milliliter of olive oil was solubilized in 20 ml of n-heptane and extracted with 10 ml of DMSO in a separate funnel. This step was repeated three times. The DMSO layers (bottom layer) from each one of the extraction steps were collected and pooled. The pooled DMSO extracts were added to 30 ml of cold HPLC grade water and extracted three times with 30 ml of cyclohexane. The hexane extracts were pooled and concentrated to 5 ml. The concentrated extract was passed through a column containing anhydrous sodium sulfate and activated florisil. The elution through the column was performed using 10 ml of cyclohexane and 5 ml of dichloromethane. The eluate was collected and dried under a stream of N₂. The residues were reconstituted in 500 μl of methanol, passed through 0.2 μM Acrodisc filters to remove particulates prior to HPLC analysis.

2.3. Animal Husbandry and Exposure to Benzo(a)pyrene

Seven-week-old male Apc^{Min} mice (Jackson Labs, Bar Harbor, ME, USA) weighing approximately 25 g were used in this study. The animals were housed in groups of 4 per cage and maintained on a 12/12 hour light/dark cycle and allowed free access to rodent chow (NIH-31 open formula diet) and water. The cages were housed in the animal care facility at Meharry Medical College (MMC). The animal care facility is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and is under the oversight of Institutional Animal Care and Use Committee (IACUC) at MMC. The IACUC ensured that animal related experiments adhered to the National Institutes of Health (NIH) guidelines for the humane care and use of laboratory animals. Prior to treatment, the animals were allowed a seven-day acclimation period and then assigned (on the basis of body weight match) to a control group [n=8 per each one of vehicle controls] or treatment group [n=8 per each dose of B(a)P dose groups]. Treatment consisted of the following doses: 25 μg B(a)P/kg body weight (bw), 50 μg B(a)P/kg bw, and 100 μg B(a)P/kg bw of B(a)P (97% pure, Sigma) dissolved in tricapylin [vehicle for B(a)P] or olive oil (Sigma; 300 mg/kg bw). The animals were then administered their daily treatment via a single oral gavage (200μl volume) for 60 days.

Since B(a)P and its metabolites were categorized under human carcinogens Group I, designated by the International Agency for Research on Cancer [26], they were handled according to US Environmental Protection Agency health effects testing guidelines (40CFR 798) and NIH [27] guidelines. In addition to wearing the appropriate protective equipment which includes a laboratory coat, gloves and mask, all handling was conducted in an exhaust

hood and in subdued light to prevent photocatalysis of B(a)P. Any unused B(a)P, and solutions was placed in hazardous waste containers for proper disposal.

2.4. Collection of Tissue Samples and Histopathology Analysis

After 60 days of treatment, mice were fasted overnight, euthanized and the target tissues (liver and colon) preserved in 5% formalin for observation of gross pathological changes. The colon was then opened longitudinally and flushed with physiological saline to remove residues of excreta. The location, size and number of adenomas were assessed using a magnifying glass and adenomas were measured using a digital vernier caliper. Colon tissue was then prepared for histopathology analysis by using the Swiss roll technique [28], fixed in 10% neutral buffered formalin and transferred to 70% ethanol after 24 hours. The H&E staining and sectioning of the colons were performed at Vanderbilt Ingram Cancer Center (VICC) Human Tissue Acquisition and Pathology Core Laboratory. The neoplastic lesions were evaluated by a pathologist for type (adenoma, carcinoma) and degree of dysplasia [28]. The student, who enumerated the tumors; the technician, who processed the samples for histology studies; and the pathologist, who evaluated the slides have no prior knowledge of the control or treatment groups.

2.5. Drug Metabolizing Enzyme Assays

2.5.1. RNA Isolation and reverse transcriptase PCR—Colon and liver tissues were washed in PBS buffer and weighed. Total RNA was isolated from liver and colon tissues using RNeasy Mini Kit following the manufacturer's instructions (Qiagen Inc, Valencia, CA). The concentration of the eluted RNA was determined using a Nanodrop Spectrophotometer (Thermo Scientific, Wilmington, DE), and RNA was stored at -20°C . Equal amounts of the total RNA was used for the first strand synthesis using the iScript cDNA synthesis kit (Biorad, Hercules, CA). Semi-quantitative PCR amplification was then carried out by using SsoAdvanced Universal SYBR Green Supermix and a CFX96 Touch Real-Time PCR Detection System as instructed by the manufacturer (Biorad, Hercules, CA). The primers used for amplification of CYP1A1, CYP1B1, GSTP1 and 18sRNA (Invitrogen, Carlsbad, CA) are listed in Table 1. Amplification of the 18sRNA was used as an internal standard.

2.5.2. Protein Isolation—Colon and liver tissues were washed in PBS and 100 mg of the respective tissues were resuspended in 9 volumes of RIPA buffer (25mM Tris•HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) containing protease inhibitor cocktail (Sigma). Tissues were then homogenized using a Polytron PT 2100 homogenizer (Fisher Scientific Pittsburgh, PA) on ice until the tissue had minced in the buffer. Samples were centrifuged at $12000\times g$ at 4°C for 10 minutes. The supernatant containing the detergent solubilized proteins was then transferred to a fresh microcentrifuge tube and stored at -80°C . Protein concentration was determined using the Quick Start Bradford Protein Assay Kit (Biorad).

2.5.3. Western Blot Analysis—Equal amounts of protein extracts from the indicated tissues were resolved by electrophoresis in 10% SDS-PAGE gels followed by electroblotting onto PVDF membranes (Amersham Pharmacia Biotech, Piscataway, NJ).

Membranes were then blocked in Li-Cor blocking buffer (Li-Cor Biotechnology, Inc., Lincoln, NE) and probed with mouse anti-CYP1A1, CYP1B1, or GST antibodies. Immunodetection was performed with the Odyssey Procedure (Li-Cor) using an IRDye800 coupled anti-rabbit IgG secondary antibody and an IRDye680 coupled anti-mouse IgG secondary antibody. Detection of GAPDH using rabbit anti-GAPDH antibodies (Santa Cruz Biotechnology, Inc., Dallas, TX) was used as the loading control. Protein expression was quantified by densitometry of the detected protein bands.

2.5.4. Drug Metabolizing Enzyme Activity Assays—Microsomes were isolated from colon and liver tissues using the Endoplasmic Reticulum Isolation Kit (Sigma-Aldrich, St. Louis, MO). Using the P450-Glo assay (Promega, Madison, WI) isolated microsomes were then used to assay for CYP1A1 and 1B1 enzyme activity in the liver and colon. For analysis of GST activity, isolated proteins from the colon and liver were used and assayed using the Glutathione S-Transferase Assay Kit (Cayman Chemical, Ann Arbor, MI).

2.6. Pharmacokinetics

To gain a better understanding of B(a)P's pharmacokinetics as well as how olive oil affects B(a)P's kinetics of disposition, one group ($n = 5$) of 7-week-old male *Apc^{Min}* mice received a single oral dose of B(a)P (50 $\mu\text{g/kg}$ bw) dissolved in tricaprylin (vehicle), while another group received B(a)P dissolved in olive oil. Blood was drawn at different time intervals subsequent to B(a)P administration and used for the preparation of plasma. B(a)P pharmacokinetic parameters in plasma and colon tissues were analyzed using PK solutions 2.0 (Summit Research Services, Ashland, OH) software. The biological half-life ($t_{1/2}$) of B(a)P was calculated by a linear regression of the log plasma concentration versus the time curve. The area under the curve (AUC) was calculated by measuring the area under the blood B(a)P concentration time curve. The mean residence time (MRT) was determined as AUC/AUMC where AUMC is the area under the first moment of curve. The volume of distribution (V_d) was calculated by considering the volume of B(a)P in the body assuming if present throughout the body, B(a)P remains at the same concentration as in plasma. The total body clearance (Cl) was computed as the ratio of B(a)P dose and AUC. The elimination rate constant (K_d) was determined as a ratio of Cl and V_d .

2.7. B(a)P Metabolite Analysis

Plasma, colon and liver samples that were harvested from control mice and mice exposed to B(a)P dissolved in tricaprylin or olive oil were processed for analysis of B(a)P metabolites by liquid-liquid extraction and reverse phase high performance liquid chromatography coupled with UV and fluorescence detection methods as described previously in Ramesh et al. [29].

2.8. DNA Damage Studies

2.8.1. DNA Isolation—DNA was isolated from liver and colon tissues by using DNeasy Blood and Tissue Kit using 25 μg of tissue from respective tissues according to the manufacturer (Qiagen). The concentration of DNA was then determined by using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

2.8.2. idative DNA Damage Analysis—Oxidative DNA Damage was determined using the isolated DNA (0.5 µg/reaction) and a DNA Damage Quantification Kit as described by the manufacturer (BioVision). Briefly, 5 µl of purified sample DNA (0.1 µg/µl) was mixed with 5 µl Aldehyde Reactive Probe (ARP) solution in a 1.5 ml microcentrifuge tube and incubated at 37°C for 1 hour to tag the DNA AP site. Samples were then mixed with 88 µl TE and 2 µl glycogen. Three hundred microliters of pure ethanol was then added and mixed and samples were kept at –20°C for 10 minutes. Samples were then centrifuged at 13,000 × g for 10 minutes to precipitate the AP site tagged DNA. The pellet was then washed three times with 0.5 ml 70% ethanol and spun quickly to remove traces of ethanol. The DNA standards were then diluted to generate 200 µl each of the 0, 8, 16, 24, 32, 40 ARP-DNA standard solutions in microcentrifuge tubes. The Biotin-tagged DNA samples were then dissolved in 1 ml TE buffer. Sixty microliters of the ARP-DNA standards and ARP-labeled DNA samples were added into each well. 100 µl of DNA binding solution was added to the standards and samples and the plate was sealed and kept at room temperature overnight to allow the tagged DNA to bind to the plate surface. The next day, the DNA binding solution was discarded from the wells and each well was washed with 250 µl wash buffer 5 times. 100 µl of HRP-Streptavidin was added to each well and the plate was shaken for 1 hour at room temperature. The HRP-Streptavidin was discarded from each well and was washed with 250 µl wash buffer 5 times. One hundred microliters of HRP developer was added to each well and the plate incubated for 1 hour at 37°C. To measure the A/P sites (markers for oxidative DNA damage), the OD for each well was read at 650 nm.

2.9. Statistical Analyses

To evaluate the comparison of two experimental variables [B(a)P doses and B(a)P + olive oil doses] on drug metabolizing enzyme expression for mRNA transcription, protein, enzyme activities, B(a)P metabolites concentrations, and DNA A/P sites, 2-way analysis of variance (ANOVA) was used and the differences among the means were tested by Holm-Sidak tests. A p-value <0.05 was considered statistically significant for interpreting our results.

3.0. Results

3.1. Effects of olive oil on colon tumor phenotypes and histopathology in *Apc^{Min}* mice

To study the effects of olive oil exposure on B(a)P-induced adenoma development in *Apc^{Min}* mice, the number and size of adenomas in mice that ingested tricaprylin (vehicle control), olive oil only, B(a)P only and B(a)P + olive oil were measured. Representative colon tumors in mice treated with 100 µg B(a)P/kg bw only are depicted in Fig. 1A and mice treated with 100 µg B(a)P/kg bw + olive oil is depicted in Fig. 1B. Most of the tumors observed in *Apc^{Min}* mice that received 100 µg B(a)P/kg bw + olive oil were smaller in size (compared to what you need to specify if you use the term “smaller”) and non-polypoid. The few adenomas that were > 5.0 mm were relatively flat and non-exophytic. In contrast, mice that received 100 µg B(a)P/kg bw only developed larger pedunculated exophytic adenomas. Distribution of polyps in the colon and their respective sizes were then analyzed. There was not a significant difference in polyp number and size in mice from tricaprylin [vehicle for B(a)P] and olive oil control groups (Fig. 2). However, it is to be noted that olive oil was

capable of reducing the number and size (< 2.5 mm and >2.5 mm) of polyps in all B(a)P + olive oil-treated mice. Specifically, all B(a)P + olive oil groups significantly ($p < 0.05$) compared to mice that received B(a)P alone. Histopathological examination of colon tissues (Figure 3) showed that while there was a greater amount of polyps that displayed high grade dysplasia in the colon of all B(a)P-treated mice, there were fewer polyps that displayed low grade dysplasia in the colon of mice treated with B(a)P + olive oil.

3.2. Olive oil analysis

Since B(a)P is an ubiquitous environmental toxicant and likely to contaminate air, soil and also plants, we examined whether olive oil used in our study was free from B(a)P contamination. When aliquots of olive oil were analyzed by HPLC, no detectable traces of B(a)P were found. In an effort to determine the principal ingredients of olive oil, samples of olive oil were saponified and the FAMES were analyzed by the GC-MS. Oleic acid was found to be the main ingredient followed by linoleic and palmitic acids (Table 2).

3.3. Effects of olive oil on drug metabolism enzyme protein, mRNA expression and activity in the colon of *Apc^{Min}* mice

To have a better understanding of how olive oil modulates B(a)P-induced polyp phenotypes in the colon of *Apc^{Min}* mice, studies were conducted to assess mRNA expression, protein expression and enzyme activity of enzymes involved in the metabolism of B(a)P in the colon. As shown in Fig. 4A B(a)P induced CYP1A1 mRNA levels in all B(a)P treatment groups compared to their respective controls. The mRNA expression of CYP1A1 was significantly upregulated in mice that were treated with 50 μg B(a)P/kg bw + olive oil compared to mice treated with 50 μg B(a)P/kg bw alone ($p < 0.05$). Conversely, although CYP1A1 mRNA expression was decreased in the 100 μg B(a)P/kg bw and 100 μg B(a)P/kg bw + olive oil groups, the CYP1A1 expression was significantly lower in mice treated with 100 μg B(a)P/kg bw + olive oil compared to mice treated with 100 μg B(a)P/kg bw alone. As shown in Fig. 4B CYP1A1 protein expression was significantly decreased in mice treated with 25 μg B(a)P/kg bw + olive oil compared to mice treated with 25 μg B(a)P/kg bw alone. At 50 and 100 μg B(a)P/kg bw treatment there was no difference in protein expression between B(a)P and B(a)P + olive oil groups. Consistent with the protein expression data there was no significant difference in activity of CYP1A1 between mice treated with B(a)P alone or B(a)P + olive oil groups as shown in Fig. 4C.

Fig. 5A shows that although there was no difference in the expression of CYP1B1 mRNA in mice treated with 25 or 50 μg B(a)P/kg bw with or without olive oil, the expression of this gene was significantly lower in mice treated with 100 μg B(a)P/kg bw + olive oil compared to mice treated with 100 μg B(a)P/kg bw ($p < 0.05$). Meanwhile, CYP1B1 protein expression was induced in all B(a)P treatment groups compared to their controls and the expression of this enzyme was significantly decreased in all B(a)P + olive oil treatment groups compared to B(a)P only treatment groups all ($p < 0.05$) (Fig. 5B). However, Fig. 5C shows that there was no significant change in activity of CYP1B1 in B(a)P + olive oil treatment groups compared to mice treated with B(a)P alone.

Next, we examined the detoxification enzyme, GST. There was a significant increase in GST mRNA expression in mice treated with 50 and 100 µg B(a)P/kg bw + olive oil compared to mice treated with 50 and 100 µg B(a)P/kg bw alone respectively (Fig. 6A). Interestingly, the maximum difference was observed in mice treated with 50 µg B(a)P/kg bw with or without olive oil. The GST protein expression was also observed to be significantly higher ($p < 0.05$) in mice treated with 50 µg B(a)P/kg bw + olive oil compared to mice that were treated with 50 µg B(a)P/kg bw alone (Fig. 6B). However, only modest increases in GST activity were detected in mice treated with 25 and 50 µg B(a)P/kg bw + olive oil compared to 25 and 50 µg B(a)P/kg bw treated mice respectively (Fig. 6C), but the findings were not significant.

3.4. Effects of olive oil on drug metabolism enzyme proteins, mRNA expression and activity in the liver of Apc^{Min} mice

In the liver, CYP1A1 mRNA expression levels were significantly lower ($p < 0.05$) in all B(a)P + olive oil B(a)P treatment groups compared to mice treated with B(a)P alone (Fig. 7A) and this was correspondingly associated with a significant decrease in CYP1A1 protein expression (Fig. 7B). However, consistent with data in Fig. 4C, the activity of CYP1A1 did not significantly change in the liver of mice treated with 25 and 50 µg B(a)P/kg bw + olive oil compared to mice treated with 25 and 50 µg B(a)P/kg bw alone (Fig. 7C).

As depicted in Fig. 8A, mice treated with B(a)P + olive oil demonstrated a reduced expression of CYP1B1 at all concentrations compared to B(a)P alone. Similarly, CYP1B1 protein expression in the liver was significantly decreased ($p < 0.05$) in mice treated with 25 µg B(a)P/kg bw + olive oil compared to mice treated with 25 µg B(a)P/kg bw alone (Fig. 8B). Again, these treatments did not lead to significant changes in the activity of CYP1B1 (Fig. 8C).

Regarding the mRNA expression for GST in the liver, Fig 9A shows a significant decrease ($p < 0.05$) in mice treated with 50 µg B(a)P/kg bw + olive oil and 100 µg B(a)P/kg bw + olive oil compared to their respective B(a)P alone treatment counterparts. Although GST protein expression was not altered by treatments (Fig. 9B), GST activity was significantly increased in the liver of mice treated with 50 and 100 µg B(a)P/kg bw + olive oil compared to 50 and 100 µg B(a)P/kg bw treatment groups (Fig. 9C; $p < 0.05$).

3.5. Effect of olive oil on B(a)P pharmacokinetics in the Apc^{Min} mice

In pharmacokinetics, processes such as absorption, distribution, metabolism, and excretion (ADME) govern the distribution of a toxicant within an organism. These steps impact the kinetics of drug disposition in plasma, tissues and affect the pharmacological activity of the compound of interest. Benzo(a)pyrene suspended in tricaprylin, when administered through oral gavage registered a presence in the body for a longer period of time compared to it being suspended in olive oil. Pharmacokinetic parameters such as volume of distribution, biological half-life, mean residence time and clearance were significantly different ($p < 0.05$) between B(a)P + olive oil compared to B(a)P alone (Table 3).

3.6. Effects of olive oil on B(a)P metabolite disposition in the plasma, colon and liver of Apc^{Min} mice

In order for B(a)P to exert its toxicity in the gastrointestinal (GI) tract, it must undergo biotransformation to form reactive metabolites. To better understand if olive oil reduces the tumor burden of B(a)P-treated mice by reducing the total metabolite load and the specific metabolite types, B(a)P metabolites were extracted from plasma, colon and liver tissues of B(a)P and B(a)P + olive oil-treated mice. The concentrations of identified metabolites and the proportions of individual metabolite types among total metabolites were shown in Figs. 10 A and B respectively. Metabolite disposition studies revealed that mice which received 25, 50 & 100 µg B(a)P/kg bw + olive oil registered significantly lower concentrations of B(a)P metabolites in the plasma, colon and liver samples compared to mice treated with B(a)P alone at these doses ($p < 0.05$). While an increase in proportion of metabolites that were generated through B(a)P activation such as B(a)P 4,5-diol, B(a)P 7,8-diol, B(a)P 9,10-diol, B(a)P 3,6-dione and B(a)P 6,12-dione were found in mice that were treated with 50 µg B(a)P/kg bw alone, the picture was different in mice that were treated with 50 µg B(a)P/kg bw + olive oil. In the B(a)P + olive oil mice the most abundant metabolites were the 3(OH) B(a)P and 9(OH) B(a)P. This suggests that detoxification of B(a)P was more common in mice which received B(a)P + olive oil compared to those that received B(a)P alone.

3.7. Effects of olive oil on B(a)P-induced oxidative damage in the colon and liver of Apc^{Min} mice

The B(a)P metabolites formed through biotransformation undergo a redox cycle resulting in formation of reactive oxygen species (ROS), which ultimately interact with DNA and form lesions. These lesions known as apurinic/apyrimidinic (AP) sites, which are the major types of DNA damage, were measured to determine oxidative DNA damage caused by B(a)P. Fig. 11 shows that oxidative DNA damage was common in the colon (Fig. 11A) or liver (Fig. 11B) of the mice treated with 25, 50, and 100 µg B(a)P/kg bw alone compared to mice treated with 25, 50 and 100 µg B(a)P/kg bw + olive oil ($p < 0.05$). The extent of DNA damage was significantly reduced ($p < 0.05$) in the liver or colon of all B(a)P + olive oil treatment groups, compared to mice treated with B(a)P alone.

4.0 Discussion

Epidemiological studies have shown that in the Western world, diet is one of the most significant factors associated with diseases such as breast cancer, prostate cancer, coronary heart disease and colon cancer [30]. It is estimated that 80% of known colon cancer cases are attributed to diet. In addition, dietary intake of an environmental toxicant such as B(a)P has been linked to the development of colon tumors [10]. Studies exploring dietary interventions to prevent colon cancer have become of interest [31-33]. Investigations have shown that polyphenols, which constitute phytochemical ingredients of diet play a major role in disease prevention [18]. Olive oil, an important component of Mediterranean diet, is composed of polyphenols and lignans and has shown promise in preventing colon carcinomas in rats as well as inhibiting cell proliferation and promoting apoptosis in colon cancer cell lines [22,23,25]. However, there is a paucity of information evaluating the efficacy of olive oil in toxicant-induced colon cancers. Therefore, the goal of this study was

to explore the mechanisms through which olive oil exerts its anticancer activities by modulating B(a)P metabolism and reducing colon tumor formation in *Apc^{Min}* mouse, a model we have been using in our laboratory to study environmental toxicant-induced colorectal tumors and chemoprevention aspects [33,34].

Since the major emphasis of this study was to investigate how olive oil could act as a preventive agent against environmental and dietary toxicants such as B(a)P, questions naturally arise about the human exposure relevance of doses of the test chemicals that we used in this study. The rationale for choosing B(a)P doses was based on human dietary intake. While dietary exposure of humans to B(a)P has been reported to vary from 8.4 µg/person/day [35] to 17 µg/person/day [36], that of total PAHs [including B(a)P] has been reported to range from 14 µg/person/day [37] to 59.2 µg/person/day [38]. To calculate the B(a)P dose that was to be administered to mice, we chose the highest reported daily exposure of 17 µg/person/day for an average male who weighs 70 kg. Using this value, the human intake of B(a)P translates to 0.24 µg/kg bw/day. While we are cognizant of the fact that the doses of 25, 50 and 100 µg B(a)P/kg bw given to *Apc^{Min}* mice in our study are at least 200 times the highest average daily exposure reported for humans, because of the increasing environmental contamination by B(a)P, and reported inconsistencies in computing the daily human intake of B(a)P [39], high doses of B(a)P were chosen for this study. As regards olive oil and/or its products, dietary intake ranged from 8-10g/person/day [in the United States; 40] to 30-70 g/person/day [countries around the Mediterranean Sea; 41]. However, the dose of olive oil given to mice in the present study (300 mg/kg bw) was somewhat high. The reason being that recent years have seen a surge in world olive oil consumption (1.7 fold in the past 15 years; [42]) owing to its protective role against chronic diseases [43]. If this trend continues, the doses used for mice could easily fall in the dietary range for humans.

To gain an insight into how olive oil affects B(a)P-induced colon tumors, adenoma numbers and degree of dysplasia in tumor bearing mice treated with various doses of B(a)P alone or B(a)P + olive oil were evaluated. Studies conducted in our laboratory [33,34, 44] and those of others [45,46] have documented that B(a)P and other PAHs administered to animal models, contribute to colon tumor development. It is to be noted that our studies have also found that B(a)P exacerbated the development of polyps in mice treated with B(a)P only. Conversely, in our current study, our findings clearly demonstrated that olive oil could not only reduce the tumor burden, but also the tumor progression. We observed that there were fewer polyps in mice treated with B(a)P + olive oil. There were no differences in polyp number and size in control [tricaprylin-vehicle for B(a)P and olive oil alone] mice. Similar to our observation, Barone et al [47] also found that olive oil-enriched diet reduced the spontaneously developed tumors in the colon to a minor extent. Overall, our findings demonstrate that mice that were treated with B(a)P + olive oil resulted in a decrease in the incidence, size and number of adenomas formed in the colon. Histopathological analysis of the colon further allowed us to assess B(a)P-induced pathological manifestations in the presence and absence of olive oil. Mice that received only B(a)P demonstrated high grade dysplasia with invasive adenocarcinomas in the submucosa of the colon, whereas mice that received B(a)P + olive oil displayed low grade adenomas with minimal invasion of adenocarcinomas in the colonic submucosa. Gross pathological analysis of the colon of

Apc^{Min} mice treated with B(a)P + olive oil were in agreement with Bartoli et al [24], who noted that dietary olive oil prevented the development of aberrant crypt foci and colon carcinomas in rats that were treated with azoxymethane. In other studies, Sanchez-Fidalgo et al [48] observed that extra virgin olive oil showed less incidence, multiplicity of colon tumors and minor dysplastic lesions in a colitis-associated colon cancer mouse model. Additional corroborative evidence on olive oil prevention of colon tumors comes from the studies of Hashim et al [49]. Olive oil polyphenols were found to inhibit invasion of HT115 human colon cancer cells *in vitro* and also *in vivo* when transplanted in the Severe Combined Immuno Deficiency [SCID] Balb-C mouse model [49]. Linoleic acid, one of the main ingredients of olive oil, was shown to inhibit the forestomach neoplasms induced by B(a)P in Kunming mice [50,51].

Next, we wanted to gain a more mechanistic understanding as to how olive oil is exerting its effects to reduce B(a)P-induced polyp formation. As the carcinogen-induced tumors in the gastrointestinal tract are inextricably linked to the expression of drug metabolizing enzymes [52], pharmacological intervention strategies target these enzymes to assess the magnitude of induction or inhibition by carcinogens in the presence or absence of chemopreventive agents [53]. Towards this end, we investigated how olive oil modulates the metabolism of B(a)P by analyzing the cytochrome P450 biotransformation enzyme expression. It is well established that biotransformation of toxicants is the driving force of carcinogenesis [54,55], and in the case of B(a)P the CYP450s are responsible for its metabolism [56]. The two major CYPs found both in the liver and colon are CYP1A1 and CYP1B1, which convert B(a)P into metabolites of toxicological relevance such as B(a)P 7,8 diol 9,10 epoxide (BPDE), and quinones [57]. Additionally, we have also investigated how glutathione-S-transferase, a phase II enzyme that is involved in detoxification of electrophilic toxicants such as B(a)P and render cellular protection [58] was modulated by olive oil. Towards this end, the mRNA, protein expression and enzyme activities of CYP1A1, CYP1B1 and GST were assayed in colon and liver tissues.

Our analysis of CYP1A1, CYP1B1 and GST levels in the liver and colon suggests that there is differential regulation of these enzymes in mice treated with B(a)P alone or B(a)P + olive oil. In addition to there being differential regulation in these enzymes among the different treatment groups, we also observed differential regulation of these enzymes in the colon and liver tissues. In contrast to the colon, in the liver we saw a significant increase in CYP1A1 mRNA as well as protein expression in all B(a)P treated mice compared to mice treated with B(a)P + olive oil. CYP1B1 mRNA expression was increased in all B(a)P treated mice compared to B(a)P + olive oil treated mice, but there was only an increase in CYP1B1 protein expression observed in mice treated with 25 µg B(a)P/kg bw compared to mice treated with 25 µg B(a)P/kg bw + olive oil. These findings are in agreement with previous studies conducted by Harrigan et al. [59] who found that CYP1A1 had a greater fold induction in liver compared to CYP1B1. Several studies have elucidated that CYP1A1 and CYP1B1 are required for metabolic activation of B(a)P [10,60]. However, there have been studies that have further analyzed the enzymatic role of CYP1A1 and have found that it also plays a role in detoxification of toxicants [61,62]. In the present study, in the colon we observed a significant increase in CYP1B1 protein expression in mice treated with B(a)P

alone compared to mice treated with B(a)P + olive oil. In contrast, results from mRNA and protein expression analysis of CYP1A1 were not as consistent as what we observed for CYP1B1 analysis. Based on our findings, it is possible that CYP1B1 is the major inducible enzyme of B(a)P metabolism in the colon. To corroborate our findings of CYP1B1 as being important in inducing the metabolism of B(a)P in the colon, there have been studies that have found elevated expression of CYP1B1 in crypts and stroma of the intestine adjacent to tumors in the Apc^{Min} mouse [45]. Additionally, studies have shown that CYP1B1 induction is increased upon B(a)P exposure and results in a generation of metabolites and cytotoxicity in mice and rat tissues [8,29,63, 64]. In contrast, CYP1A1 appears to be the major inducible enzyme of B(a)P metabolism in the liver compared to CYP1B1. Variations among the mRNA, protein and enzyme activity analysis of CYP1A1, CYP1B1 in the colon could be attributed to several factors such as the rate of transcription initiation, mRNA stability and/or protein stability [65]. Epigenetic factors such as methylation or phosphorylation of enzymes that could be affecting the transcriptional rates cannot be ruled out.

Even though, olive oil was reported to induce GST expression in liver cells [19], there was no significant difference in GST mRNA and protein expression in liver among B(a)P treated and B(a)P + olive oil-treated mice. These results clearly suggest that olive oil is not capable of inducing a significant change in GST mRNA and protein expression in the liver. There may be alternative detoxification enzymes such as sulfotransferase or UDP-glucuronosyltransferase that may be aiding in the detoxification of B(a)P in the liver.

The pharmacokinetics of B(a)P in the presence of olive oil makes it amenable for rapid absorption and detoxification. Our current findings suggest that olive oil is more than likely assisting with an increase in bioavailability of B(a)P due to its monounsaturated nature. Our earlier studies have shown that polyunsaturated fats (a class to which olive oil belongs) favor increased bioavailability of PAHs [66,67]. Laher et al [68] first demonstrated that olive oil facilitates the lymphatic transport of B(a)P through rapid absorption in the enterocytes. Specifically, studies have observed that the long chain fatty acids of olive oil may have absorbed B(a)P and incorporated it into chylomicrons for transport through the lymphatic system or peripheral circulation [69]. An enhanced bioavailability of B(a)P in the presence of olive oil was demonstrated in rat and mouse models [70,71]. Additionally oleic acid, an important ingredient of olive oil, was reported to facilitate the accumulation of B(a)P within the cell membrane of lung adenocarcinoma cells [72]. In line with these published reports are our current findings where the pharmacokinetic behavior of B(a)P is different when administered through olive oil compared to tricaprylin. The fact that pharmacokinetic parameters for B(a)P were significantly altered in the presence of olive oil suggests that olive oil itself is bioavailable and also facilitates a rapid uptake of B(a)P and clearance from the body. Reviews of published literature on uptake of olive oil in animal models, humans and cell cultures support the notion that olive oil polyphenols are concentrated more in the gastrointestinal tract [73,74] and rapidly metabolized. Since the residence time of B(a)P is shorter in the presence of olive oil, the net amount of B(a)P and/or its metabolite fraction available to cause damage to target tissues is also low.

Measuring the disposition of metabolized B(a)P and identification of metabolites arising from bioactivation and detoxification of B(a)P is critical in order to better understand factors

that underlie colon tumor formation and the role of olive oil in alleviating the colon tumor burden. Mice that were treated with 50 B(a)P/kg bw + olive oil had lower concentration of metabolites in the plasma, colon and liver compared to their counterparts treated with 50 µg B(a)P/kg bw alone. The metabolite types that were identified in our study are in agreement with previous studies conducted which concluded that orally administered B(a)P goes through an extensive biotransformation generating an array of metabolites [75]. Mice that were treated with 50 µg B(a)P/kg bw only displayed a greater amount of reactive metabolites such as B(a)P 7,8 diol; 3,6- and 6,12-B(a)P diones compared to mice treated with 50 µg B(a)P/kg bw + olive oil that had a smaller percentage of these metabolites. These findings clearly illustrate that olive oil reduces the concentration of B(a)P metabolites that were generated. Conversely, it was observed that mice that were treated with 50 µg B(a)P/kg bw + olive oil had an increase of hydroxy metabolites such as 3(OH) B(a)P and 9(OH) B(a)P which possibly could lead to pathways that favor B(a)P detoxification.

Lastly, this study investigated the effects of olive oil on B(a)P-induced oxidative DNA damage. Pathological changes in target tissues induced by toxicants were associated with production of highly reactive free radicals that ultimately lead to oxidative damage [76]. Studies have explored how B(a)P metabolites bind to DNA and proteins and cause damage as a result of the B(a)P hydroxylase enzymes [77-79]. The antioxidant properties of dietary lipids have proven to be effective in ameliorating the effects of carcinogens by protecting against lipid peroxidation and scavenge oxygen derived free radicals [80]. One study [81] examined how olive oil can protect rat liver microsomes against B(a)P-induced oxidative damage. In their study, microsomes were exposed to benzo(a)pyrene [B(a)P] and olive oil individually and in combination. A decrease in B(a)P hydroxylase activity and metabolite concentrations were found to be generated by the microsomal system in the presence of olive oil compared to B(a)P exposure. These researchers observed that antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were increased in rats that were exposed to B(a)P and olive oil whereas protein carbonyl content (PCC) and lipid peroxidation products were increased when rats were exposed to B(a)P alone. These findings indicated that olive oil is a promising agent to treat B(a)P-induced toxicity and cancer. Components of olive oil have also been shown to induce apoptosis in human colon cancer cells through ROS generation as observed by Sun et al. [82]. Our results stand in agreement with the aforementioned findings in that olive oil was able to reduce the amount of oxidative damage induced by B(a)P not only in the colon, but the liver as well. This was observed in all B(a)P + olive oil treatment groups compared to B(a)P only treatment groups.

In addition to its antioxidant activity, there are other mechanisms through which olive oil could exert its protective effect by altering and dysregulating specific pathways that aid in cell growth, inflammation and cell cycle arrest. Studies by Cardeno-Sanchez et al. [83] in a mouse model of colitis found that olive oil suppressed the nuclear factor kappa-light-chain-enhancer of activated B cells-interaction with p65 (NFκB-p65) pathway. Since NFκB is required for binding to the promoter region of proinflammatory genes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX2), the reduced expression of iNOS and COX2 observed indicates that olive oil inhibits tumor growth. Studies by this group also revealed that olive oil reduced the activation of p38 which is major contributor of Mitogen-activated protein kinases (MAPK) signaling. The same research group followed up

the in vivo studies by conducting in vitro experiments to explore the effects that olive oil would have on HT-29 human colon adenocarcinoma cells [83]. They observed that olive oil induced apoptosis in HT-29 cells by modulating COX2 via peroxisome proliferator-activated receptor gamma (PPAR- γ) and NF κ B signaling pathways, specifically upregulating caspase 9 and poly ADP ribose polymerase (PARP) cleavage. In other in vitro experiments, Guichard et al. [84] observed that hydroxytyrosol (HT), an ingredient of olive oil was capable of inducing cell growth arrest and apoptosis in HT-29 cells by modulating stress signaling pathways involving Ire1/JNK/c-jun/AP-1/N α 4. Additionally, this group found that HT was also capable of altering phosphorylated extracellular-signal-regulated kinases (ERK 1/2) and phosphatidylinositol 3-kinases/serine/threonine kinases (PI3K/Akt) pathways by enhancing protein phosphatase 2 and preventing the activation of NF κ B by tumor necrosis factor alpha (TNF- α). In this context, it is of interest to note that B(a)P has been reported to induce signaling molecules such as MAPK [85], p53 and c-Jun N-terminal kinases (JNK; [86]) in different cell systems. In the light of the above-mentioned reports, it is likely that olive oil renders a protective effect by modulating B(a)P-induced oncogenic pathways and inhibiting contributing factors such as inflammation and cell growth that favor colon carcinogenesis.

Taken together the findings of our study suggest that olive oil alters B(a)P-induced colon tumor formation by modulating the biotransformation of B(a)P. As a result of the altered B(a)P biotransformation, the disposition of metabolites was affected as well. There were greater concentrations of more reactive metabolites in mice treated with B(a)P only compared to its olive oil counterparts, which eludes to the fact that olive oil favors the formation of B(a)P hydroxy metabolites that will be excreted. Lastly, our oxidative DNA damage analysis studies further proved that olive oil was effective in reducing the DNA damage caused by reactive oxygen species (ROS) generated during B(a)P metabolism.

This study has some limitations in that though it covered most of the phase I metabolism and oxidative damage to DNA induced by B(a)P is altered by olive oil, it has not explored the phase II metabolism except for GST. As most chemopreventive agents are known to be involved in detoxification, activities and expression of other phase II enzymes such as UDP-glucuronosyltransferases, sulfotransferases, and N-acetyltransferases are necessary to determine whether the B(a)P administered to the Apc^{Min} mice through olive oil gets detoxified through these pathways. Additionally, studies on the B(a)P-DNA adducts in the experimental regimen used are necessary to determine whether the persistence of B(a)P-DNA adducts is reduced in the presence of olive oil compared to B(a)P exposure alone. In this regard, investigations on DNA repair enzymes such as 8-oxo-guanine glycosylase are also needed to assess if olive oil favors DNA repair enzyme expression in eradicating damage caused by ROS. Also worthwhile will be attempts to examine which component of olive oil has the high antioxidant potential and a greatest impact on modulating the metabolism of B(a)P.

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Figure 1.
Representative pictures of polyps in the colon of Apc^{Min} mice exposed to (A.) 100 $\mu\text{g/kg}$ B(a)P/kg bw or (B.) 100 $\mu\text{g/kg}$ B(a)P+ olive oil for sixty days via oral gavage.

Figure 2.

Distribution and size of polyps in colon of *Apc^{Min}* mice treated with No B(a)P (vehicle controls), 25 µg B(a)P/kg bw, 50 µg B(a)P/kg bw and 100 µg B(a)P/kg bw only or 25 µg B(a)P/kg bw, 50 µg B(a)P/kg bw and 100 µg B(a)P/kg bw + olive oil. The bars represent mean $\pm$ SD for three independent animals. * $P < 0.05$ in polyp size between B(a)P+ olive oil and B(a)P alone.

Figure 3.

Histopathology of colon of *Apc^{Min}* mice. (A.) Normal colonic mucosa without adenomatous change obtained from control (no B(a)P) mouse (B.) Adenoma with high grade dysplasia (arrows) in a mouse treated with 50 µg B(a)P/kg bw only (C.) Normal colonic mucosa in a mouse treated with 50 µg B(a)P/kg bw + olive oil (D.) Adenoma with high grade dysplasia (arrows) in mouse treated with 100 µg B(a)P/kg bw only (E.) Adenoma with low grade dysplasia (arrows) in mouse treated with 100 µg B(a)P/kg bw + olive oil.

Figure 4.

(A.) RT-PCR analysis of colon CYP1A1 mRNA expression in *Apc^{Min}* mice treated with control vehicles (tricaprylin or olive oil), 25 µg B(a)P/kg bw, 50 µg B(a)P/kg bw and 100 µg B(a)P/kg bw dissolved in tricaprylin [B(a)P-only group] or olive oil. CYP1A1 mRNA was normalized to 18S RNA that was used as the internal control. (B.) Western blot analysis of colon CYP1A1 protein expression in the colon of *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P+ olive oil. CYP1A1 relative expression was quantified by fluorescence intensity of its respective bands and was normalized to GAPDH that was used as the loading control. (C.) Enzyme activity of colon CYP1A1 in the colon of *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P + olive oil. CYP1A1 activity was determined using luminescence. The bars represent mean ± SD for three independent animals. **P* < 0.05 in drug metabolizing enzyme expression between B(a)P+ olive oil and B(a)P alone.

Figure 5.

(A.) RT-PCR analysis of colon CYP1B1 mRNA expression in *Apc^{Min}* mice treated with control vehicles (tricaprylin or olive oil), 25 µg B(a)P/kg bw, 50 µg B(a)P/kg bw and 100 µg B(a)P/kg bw dissolved in tricaprylin [B(a)P-only group] or olive oil. CYP1B1 mRNA was normalized to 18S rRNA that was used as the internal control. (B.) Western blot analysis of colon CYP1B1 protein expression in the colon of *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P+ olive oil. CYP1B1 relative expression was quantified by fluorescence intensity of its respective bands and was normalized to GAPDH that was used as the loading control. (C.) Enzyme activity of colon CYP1B1 in the colon of *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P + olive oil. CYP1B1 activity was determined using luminescence. The bars represent mean ± SD for three independent animals. **P* < 0.05 in drug metabolizing enzyme expression between B(a)P+ olive oil and B(a)P alone.

Figure 6.

(A.) RT-PCR analysis of colon GST mRNA expression in *Apc^{Min}* mice treated with control vehicles (tricaprylin or olive oil), 25 µg B(a)P/kg bw, 50 µg B(a)P/kg bw and 100 µg B(a)P/kg bw dissolved in tricaprylin [B(a)P-only group] or olive oil. GST mRNA was normalized to 18S rRNA that was used as the internal control. (B.) Western blot analysis of colon GST protein expression in the colon of *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P+ olive oil. GST relative expression was quantified by fluorescence intensity of its respective bands and was normalized to GAPDH that was used as the loading control. (C.) Enzyme activity of colon GST in the colon of *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P + olive oil. GST activity was determined using a spectrophotometer. The bars represent mean ± SD for three independent animals. *P < 0.05 in drug metabolizing enzyme expression between B(a)P+ olive oil and B(a)P alone.

Figure 7.

(A.) RT-PCR analysis of liver CYP1A1 mRNA expression in *Apc^{Min}* mice treated with control vehicles (tricaprylin or olive oil), 25 µg B(a)P/kg bw, 50 µg B(a)P/kg bw and 100 µg B(a)P/kg bw dissolved in tricaprylin [B(a)P-only group] or olive oil. CYP1A1, mRNA was normalized to 18sRNA that was used as the internal control. (B.) Western blot analysis of liver CYP1A1 protein expression in *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P+ olive oil. CYP1A1 relative expression was quantified by fluorescence intensity of its respective bands and was normalized to GAPDH that was used as the loading control. (C.) Enzyme activity of liver CYP1A1 in *Apc^{Min}* mice treated with no B(a)P, B(a)P only or B(a)P + olive oil. CYP1A1 activity was determined using luminescence. The bars represent mean ± SD for three independent animals. *P < 0.05 in drug metabolizing enzyme expression between B(a)P+ olive oil and B(a)P alone.

Figure 8.

(A.) RT-PCR analysis of liver CYP1B1 mRNA expression in *Apc^{Min}* mice treated with control vehicles (tricaprylin or olive oil), 25 μ g B(a)P/kg bw, 50 μ g B(a)P/kg bw and 100 μ g B(a)P/kg bw dissolved in tricaprylin [B(a)P-only group] or olive oil. CYP1B1 mRNA was normalized to 18S RNA that was used as the internal control. (B.) Western blot analysis of live CYP1B1 protein expression in *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P + olive oil. CYP1B1 relative expression was quantified by fluorescence intensity of its respective bands and was normalized to GAPDH that was used as the loading control. (C.) Enzyme activity of liver CYP1B1 in *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P + olive oil. CYP1B1 activity was determined using luminescence. The bars represent mean $\pm$ SD for three independent animals. * P < 0.05 in drug metabolizing enzyme expression between B(a)P + olive oil and B(a)P alone.

Figure 9.

(A.) RT-PCR analysis of liver GST mRNA expression in *Apc^{Min}* mice treated with control vehicles (tricaprylin or olive oil), 25 µg B(a)P/kg bw, 50 µg B(a)P/kg bw and 100 µg B(a)P/kg bw dissolved in tricaprylin [B(a)P-only group] or olive oil. GST mRNA was normalized to 18sRNA that was used as the internal control. (B.) Western blot analysis of live GST protein expression in *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P+ olive oil. GST relative expression was quantified by fluorescence intensity of its respective bands and was normalized to GAPDH that was used as the loading control. (C.) Enzyme activity of liver GST in *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P + olive oil. GST activity was determined using a spectrophotometer. The bars represent mean ± SD for three independent animals. *P < 0.05 in drug metabolizing enzyme expression between B(a)P+ olive oil and B(a)P alone.

Figure 10.

Effect of olive oil on B(a)P metabolite concentration in plasma, colon and liver of *Apc^{Min}* mice treated with 50 µg B(a)P/kg bw + olive oil or 50 µg B(a)P/kg bw alone (A.) and effect of olive oil on B(a)P metabolite distribution in plasma, colon and liver of *Apc^{Min}* mice treated with 50 µg B(a)P/kg bw + olive oil or 50 µg B(a)P/kg bw alone (B.). The bars represent mean ± SD for three independent animals. *P < 0.05 in metabolite concentrations between B(a)P+ olive oil and B(a)P alone.

Figure 11.

Abrogation of B(a)P-induced DNA base pair damage by olive oil in colon (A) and liver (B) of *Apc^{Min}* mice treated with No B(a)P, B(a)P only or B(a)P + olive oil. The bars represent mean $\pm$ SD for three independent animals. * $P < 0.05$ DNA base damage between B(a)P+olive oil and B(a)P alone.

Table 1

Primers used for amplification of CYP1A1, CYP1B1, GST and 18sRNA.

Gene	Primer	Length (BP)	NCBI Reference Sequence
CYP1A1	5'-GGCCACTTTGACCCTTACAA-3' Forward 5'-CAGGTAACGGAGGACAGGAA-3' Reverse	186	NM_09992.4
CYP1B1	5'-TTCTCCAGCTTTTGCCTGT-3' Forward 5'-TAATGGAAGCCGTCCTTCTCC-3' Reverse	187	NM_009994.1
GSTP1	5'-GGCATCTGAAGCCTTTTGAG-3' Forward 5'-GAGCCACATAGGCAGAGAGC-3' Reverse	174	NM_013541.1
18s	5'-CGCGGTTCTATTTTGTGTTGGT-3' Forward 5'-AGTCGGCATCGTTTATGGTC-3' Reverse	219	NR_003278.3

Table 2

Constituents of olive oil.

Constituent	% Methyl Esters
Oleic acid (C18:1)	67
Linoleic acid (C18:2)	13
Palmitic acid (C16:0)	10
Stearic acid (C18:4)	6
Arachidic acid (C20:0)	1
Others (Hydroxytyrosol, oleocanthal, pinorelinol chlorophyll, and tocopherol)	<1

Olive oil samples were saponified and subsequently extracted with hexane and methanol. The methyl esters were analyzed by a GC-MS equipment as outlined in the methods section. Numbers in parenthesis indicate carbon and double bond notation of the fatty acids. Fatty acids were arranged in order of relative magnitude of methylesters (%).

Table 3

Pharmacokinetics of B(a)P (50 ug/kgbw dose; alone and in the presence of olive oil) orally administered to Apc^{Min} male mice. Values represent mean $\pm$ standard error.

Parameter	Plasma	
	B(a)P alone	B(a)P + olive oil
Area under curve (AUC; mg/hr/ml)	0.15 $\pm$ 0.005	0.08 $\pm$ 0.004
Biological half-life (t _{1/2} ; hrs)	2.0 $\pm$ 0.021	1.0 $\pm$ 0.015*
Vol. of distribution (V _d ; ml/kg)	0.64 $\pm$ 0.050	0.25 $\pm$ 0.064
Clearance (Cl; ml/hr/kg)	0.12 $\pm$ 0.01	0.04 $\pm$ 0.008*
Mean residence time (MRT; hr)	2.6 $\pm$ 0.015	1.4 $\pm$ 0.005*
Elimination rate (K _d ; hr)	0.19 $\pm$ 0.05	0.16 $\pm$ 0.02

* $P < 0.05$ compared to B(a)P alone treatment group