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Differential modulation of dibenzo[*def*,*p*]chrysene transplacental carcinogenesis: Maternal diets rich in indole-3-carbinol versus sulforaphane

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ABSTRACT

Cruciferous vegetable components have been documented to exhibit anticancer properties. Targets of action span multiple mechanisms deregulated during cancer progression, ranging from altered carcinogen metabolism to the restoration of epigenetic machinery. Furthermore, the developing fetus is highly susceptible to changes in nutritional status and to environmental toxicants. Thus, we have exploited a mouse model of transplacental carcinogenesis to assess the impact of maternal dietary supplementation on cancer risk in offspring. In this study, transplacental and lactational exposure to a maternal dose of 15 mg/Kg B.W. of dibenzo[*def*,*p*]chrysene (DBC) resulted in significant morbidity of offspring due to an aggressive T-cell lymphoblastic lymphoma. As in previous studies, indole-3-carbinol (I3C, feed to the dam at 100, 500 or 1000 ppm), derived from cruciferous vegetables, dose-dependently reduced lung tumor multiplicity and also increased offspring survival. Brussels sprout and broccoli sprout powders, selected for their relative abundance of I3C and the bioactive component sulforaphane (SFN), respectively, surprisingly enhanced DBC-induced morbidity and tumorigenesis when incorporated into the maternal diet at 10% wt/wt. Purified SFN, incorporated in the maternal diet at 400 ppm, also decreased the latency of DBC-dependent morbidity. Interestingly, I3C abrogated the effect of SFN when the two purified compounds were administered in equimolar combination (500 ppm I3C and 600 ppm SFN). SFN metabolites measured in the plasma of neonates positively correlated with exposure levels via the maternal diet but not with offspring mortality. These findings provide justification for further study of the safety and bioactivity of cruciferous vegetable phytochemicals at supplemental concentrations during the perinatal period.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are produced from the combustion of fossil fuels, and within this chemical class, DBC is among the most potent as a carcinogen in animal models (Cavalieri et al., 1991; Platt et al., 2004; Prahalad et al., 1997; Siddens et al., 2012) and a probable human carcinogen (IARC, 2010). The relative tissue concentration in neonates exposed transplacentally to PAHs is expected to be approximately 1% of the maternal burden (Shorey et al., 2012), yet Whyatt et al. (2001) demonstrated that levels of PAH-DNA adducts were higher in newborn white blood cells than in paired maternal samples, demonstrating the increased sensitivity of the neonate to environmental carcinogens. Various PAHs have been evaluated in preclinical rodent models as transplacental carcinogens including 3-methylcholanthrene (3-MC), 7,12-dimethylbenz[a]anthracene (DMBA), and benzo[*a*]pyrene (BaP) (Anderson et al., 1985, 1995; Miller et al., 1998). Our laboratory previously established that transplacental and/or lactational exposure to DBC in a murine model produces T-cell lymphoblastic lymphomas during early adulthood, in addition to lung tumors and liver tumors (predominantly in males) later in life (Castro et al., 2008a, 2008b, 2008c, 2009; Yu et al., 2006a, 2006b).

Although the etiology for acute lymphoblastic leukemia/lymphoma is not precisely known, it remains the most prevalent childhood cancer (NCI, 2011). Furthermore, polymorphisms in *CYP1A1* and *GSTM1*, two enzymes involved in the bioactivation and elimination of PAHs, have been associated with increased risk of childhood leukemia (Swinney

Abbreviations: I3C, indole-3-carbinol; SFN, sulforaphane; DBC, dibenzo[*def*,*p*]chrysene; GFN, glucoraphanin; GSH, glutathione; CG, cysteine–glycine; Cys, cysteine; NAC, N-acetylcysteine; 3-MC, 3-methylcholanthrene; DMBA, 7,12-dimethylbenz[a]anthracene; BaP, benzo[a]pyrene; PND, post natal day.

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et al., 2011; Vijayakrishnan and Houlston, 2010). Multiple lymphatic tissues, including bone marrow, spleen, and thymus, constitutively express *CYP1B1* during both fetal development and adulthood, the CYP isoform primarily responsible for immunotoxic and carcinogenic effects of specific PAHs (Choudhary et al., 2005; Miyata et al., 2001; Uno et al., 2006). For example, the preleukemic effects of DMBA are almost completely ablated in *Cyp1b1* null animals, whereas DBC-induced lymphomas in our transplacental model depend on *Cyp1b1* gene dosage (Castro et al., 2008a; Heidel et al., 2000).

High-heat cooking and preserving practices such as grilling, smoking, drying, or broiling introduce PAHs to foods, as does growth of vegetables in soils contaminated from atmospheric sources. Therefore, the diet is a primary route of exposure to PAHs among non-smokers, especially PAHs of large molecular weight (5–6 rings) having mutagenic properties (Ramesh et al., 2004). Conversely, evidence from epidemiological and animal studies suggests that modification of the diet to increase consumption of cruciferous vegetables reduces the occurrence of some common cancers (Bosetti et al., 2012; Davis et al., 2002; Keum et al., 2009; Richman et al., 2012). Broccoli, Brussels sprouts, mustard, kale, cabbage, horseradish and arugula are vegetables in the Brassicaceae (Cruciferae) plant family and are rich sources of glucosinolates, substituted β-thioglucoside N-hydroxysulfates (Hayes et al., 2008). Breakdown products of glucosinolates, known as "indoles" and "isothiocyanates", are widely accepted as contributing to the beneficial properties of crucifers. For example, indole-3-carbinol (I3C) is derived from the glucosinolate, glucobrassicin, abundant in Brussels sprouts, kale, and cabbage varietals, and has been widely studied for its anticancer properties (reviewed in Bradlow, 2008; Sarkar et al., 2009; Weng et al., 2008). Moreover, I3C supplemented to the maternal diet at 2000 ppm in our transplacental model of carcinogenesis reduces T-cell lymphoblastic lymphoma mortality and decreases lung tumor multiplicity in surviving offspring, and I3C is transplacentally bioavailable to the developing fetus (Yu et al., 2006a).

Interest in isothiocyanates as antineoplastic agents has been growing since the 1960s, and more recently, sulforaphane (SFN), derived from glucoraphanin and abundant in broccoli and broccoli sprouts, has become the most studied isothiocyanate due to its potency for induction of phase II enzymes. SFN has been demonstrated to inhibit CYP1A1 and CYP1A2 activity induced by the prototypic PAH, BaP, in MCF-7 cells at low micromolar concentrations achievable in vivo (Skupinska et al., 2009a, 2009b). SFN also decreased BaP-mediated AHR activation and CYP content while inducing phase II enzyme systems in the lungs of animals exposed to BaP (Kalpana Deepa Priya et al., 2011). These changes were associated with restored mitochondrial glutathione levels, reduced lipid peroxidation, and lessened alveolar hyperplasia (Priya et al., 2011a, 2011b).

Both I3C and SFN modulate a wide-range of biological targets and consequently may influence risk at all stages of cancer from initiation through metastasis. For example, I3C and SFN alter the expression and activity of phase I CYP enzymes and phase II detoxifying enzymes, cell signaling kinases (i.e. *MAPK*, *NF-κB*), and histone deacetylase (HDAC) expression and activity (Aronchik et al, 2010; Clarke et al., 2008; Ho et al., 2009; Saw et al., 2011). Based on these findings and others, we hypothesize that I3C, at lower concentrations than previously tested, SFN, and their whole food sources will protect against DBC-induced transplacental carcinogenesis. To this end, we conducted a large preclinical animal study with multiple dietary regimens to compare the efficacy of these purified phytochemical components to whole food sources (Brussels sprouts and broccoli sprouts) and to one another.

Materials and methods

Chemicals and diet

DBC was obtained from the NCI carcinogen repository at the Midwest Research Institute (Kansas City, MO) and was confirmed as >98% pure by HPLC. Custom diets and semi-purified control diets, AIN93G and AIN93M, were purchased from Research Diets (New Brunswick, NJ). Dietary additives were purchased from the following suppliers: I3C, Cat # 17256, Sigma Aldrich (St. Louis, MO); SFN, Cat # S699115, Toronto Research Chemicals (North York, Ontario); Brussels sprout powder, Cat # N54 and broccoli sprout powder, Cat # N216, Future Ceuticals (Momence, IL). Custom diets were prepared with AIN93G diet base, adjusted for macronutrient content between diets by Research Diets (Table 1). Diets were stored protected from light at −20 °C throughout the feeding phase of the trial. Whole food powders were analyzed by American Analytical Chemistry Laboratories Corporation for I3C content and by Van Drunen Farms/Future Ceuticals for glucoraphanin content, and subsequently in-house as described below.

Glucoraphanin extraction and analysis from freeze-dried powders

Approximately 30 mg of powder (broccoli or Brussels sprout), in triplicate, was weighed into 2 mL vials. Glucotropaeolin (GTP) (Applichem cat # A5300,0020) was used as an internal standard, and 100% MeOH (375 μl) was added to each powder sample and sonicated for 10 min before centrifuging (Spectrafuge 24D at 16,300 ×*g* for 5 min) and transferring supernatant to a 15-mL conical tube. Three additional MeOH extractions were collected similarly and the four extracts pooled. Supernatants were centrifuged through 0.22-μm nylon Spin-X® filter columns (VWR, Radnor, PA), diluted with 0.1% (v/v) formic acid in H_2O, and stored at −80 °C until analysis by HPLC–MS/MS. Ten μl was injected in duplicate and HPLC conditions were as follows: Phenomenex Synergi Hydro-RP column (80 Å, 150 × 2 mm, 4 μm pore size), mobile phases of 0.1% (v/v) formic acid in acetonitrile and 0.1% (v/v) formic acid in H_2O, 0.25 mL/min flow rate at 25 °C. An Applied Biosystems MDX Sciex 3200™ triple quadrupole mass spectrometer was used in negative ion mode to detect GFN (436 > 97) and GTP (408 > 97). Quantification was performed based on a 6-point calibration curve that spanned the extract concentrations and showed excellent linearity ($r^2 = 0.999$).

Sulforaphane extraction and analysis from freeze-dried powders

Six samples of approximately 30 mg were weighed into 2 mL vials and the weights recorded for each powder (broccoli or Brussels sprout). Three samples per powder type were supplemented with 0.64 units of exogenous Sinapis alba (white mustard) myrosinase (Sigma-Aldrich, St. Louis, MO) before incubation in 1 mL dH_2O at 60 °C, in the dark, for 2 h. Hydrolyzed samples were centrifuged at 16,300 ×*g* for 5 min to pellet powder, and supernatants were filtered through 0.22-μm nylon Spin-X® filter columns, diluted with 0.1% (v/v) formic acid in H_2O, and stored at −80 °C until analysis by HPLC–MS/MS. Deuterated SFN-NAC was used as an internal standard. Ten μl of extract was injected in duplicate and HPLC conditions were as follows: Phenomenex® Kinetex PFP column (100 Å, 100 × 2.1 mm, 2.6 μm pore size), mobile phases of 0.1% (v/v) formic acid in acetonitrile and 0.1% (v/v) formic acid in H_2O, 0.25 mL/min flow rate at 40 °C. An Applied Biosystems MDX Sciex 3200 ™ triple quadrupole mass spectrometer was used in positive ion mode to detect SFN (178 > 114) and deuterated SFN-NAC (344.1 > 114). Quantification was performed based on a 7-point calibration curve that spanned the extract concentrations and showed excellent linearity ($r^2 = 0.999$).

Indole-3-carbinol extraction and analysis from freeze-dried powders

Broccoli sprout/Brussels sprout powders were analyzed for I3C content with slight modification of a previously published method (Liu et al., 2009). In brief, powders were hydrolyzed in triplicate in H_2O (10 mg/mL) for 4 h at room temperature away from light, with periodic vortexing. Solutions were centrifuged at 3600 ×*g* for 5 min to pellet sprout powder, and supernatant was extracted twice with 5 mL of dichloromethane. Extracts were pooled and evaporated under a stream