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Journal of Nutritional Biochemistry 24 (2013) 713–724

Journal of Nutritional Biochemistry

Chemoprevention of benzo(a)pyrene-induced colon polyps in Apc^{Min} mice by resveratrol

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Received 16 March 2012; received in revised form 31 March 2012; accepted 4 April 2012

Abstract

Human dietary exposure to benzo(a)pyrene (BaP) has generated interest with regard to the association of BaP with gastrointestinal carcinogenesis. Since colon cancer ranks third among cancer-related mortalities, it is necessary to evaluate the effect of phytochemicals on colon cancer initiation and progression. In this study, we investigated the preventive effects of resveratrol (RVT) on BaP-induced colon carcinogenesis in Apc^{Min} mouse model. For the first group of mice, 100 μg BaP/kg body weight was administered to mice in peanut oil via oral gavage over a 60-day period. For the second group, RVT was coadministered with BaP at a dose of 45 μg/kg. For the third group, RVT was administered for 1 week prior to BaP exposure for 60 days. Jejunum, colon and liver were collected at 60 days post BaP and RVT exposure; adenomas in jejunum and colon were counted and subjected to histopathology. RVT reduced the number of colon adenomas in BaP+RVT-treated mice significantly compared to that in mice that received BaP alone. While dysplasia of varying degrees was noted in colon of BaP-treated mice, the dysplasias were of limited occurrence in RVT-treated mice. To ascertain whether the tumor inhibition is a result of altered BaP-induced toxicity of tumor cells, growth, apoptosis and proliferation of adenocarcinoma cells were assessed posttreatment with RVT and BaP. Cotreatment with RVT increased apoptosis and decreased cell proliferation to a greater extent than with BaP alone. Overall, our observations reveal that RVT inhibits colon tumorigenesis when given together with BaP and holds promise as a therapeutic agent.

Keywords: Benzo(a)pyrene; Polycyclic aromatic hydrocarbons; Resveratrol; Colon carcinogenesis; Apc^{Min} mouse

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers in the Western world. In the United States alone, nearly 150,000 new cases of CRC are reported every year, and 56,000 deaths are attributed to this cancer. The overall incidence of CRC is higher in men (58.9/100,000 in 1987–1991) than in women (40.4/100,000), and this holds for all age groups [1]. In 90% of the colon cancer cases, there is no familial history of colon cancer. Sporadic gene damage seems to play an important role in the development of tumors in the colon. It has been postulated that dietary and environmental factors might contribute to the sporadic gene mutations and therefore be involved in the induction of sporadic colon carcinomas [2].

One environmental compound which has been linked to dietary intake leading to the development of colon tumors is benzo(a)pyrene (BaP). BaP is a prototypical representative of the family of polycyclic aromatic hydrocarbons (PAHs) chemicals. Cigarette smoke, automobile exhausts, charcoal-broiled meat and industrial emissions contain considerable amount of BaP. When inhaled or ingested through water and diet, BaP becomes activated in biological systems to reactive metabolites and as a consequence can lead to the development of cancer [3]. A study conducted by Kazerouni et al. [4] revealed substantial amounts of BaP in bread, cereals, grains, vegetables and fruits. These authors also found elevated levels of BaP in meats. Contamination of a variety of foods, the average daily intake and the contribution of BaP dietary intake to toxicity and carcinogenesis from a risk assessment standpoint were reviewed by Ramesh et al. [5]. All these studies highlight that sustained dietary exposure of humans to BaP most likely leads to the development of CRC. Evidence pertaining to the dietary intake of PAHs and their role in the development of digestive tract cancers in animal models and humans has recently been reviewed by our research group [6].

Epidemiological and animal model studies have shown that phytochemical ingredients of diet play a major role in disease prevention [7,8]. Nutritional prevention has been suggested to reduce the occurrence of colon cancer by ~60% [9]. Just as there are many carcinogenic chemicals of environmental origin or cooking-generated ones in human diet, the diet also contains chemicals, which are biologically active and proven to be effective against tumors in animal

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http://dx.doi.org/10.1016/j.jnutbio.2012.04.005

models and cell culture studies [10,11]. One such promising compound is resveratrol (RVT; 3,5,4'-trihydroxystilbene). RVT is a phytoalexin and a polyphenolic compound present in grapes, peanuts and mulberries [12]. Because of its anticarcinogenic and chemotherapeutic activities, studies have been undertaken to test its ability to block tumor initiation, promotion and progression [13]. A great majority of the available studies on RVT's chemopreventive and anticarcinogenic effects on toxicity of environmental chemicals were conducted using liver microsomal preparations, mammary cell cultures of rodents, liver, hepatoma cells and bronchial epithelial cells of humans. These studies showed decreases in tumor cell proliferation, increased apoptosis and decreases in cell proliferation [12,14].

Animal models have increasingly been used in cancer prevention research as they are useful to developing biomarkers for early detection and surrogate endpoint biomarkers and also serve as screening tools to test the efficacy of anticarcinogenic compounds [15]. Towards this end, transgenic mouse models are developed through germ line manipulation by overexpressing or deleting certain genes with the sole objective of generating mice that are more prone to developing cancer and mimic human cancer paradigms. In most patients with CRC, whether sporadic or inherited, there is a mutation in the adenomatous polyposis coli (APC) tumor suppressor gene. The APC protein interacts with β-catenin in a multiprotein complex to regulate the level of expression of β-catenin [16]. Loss of normal APC protein function can lead to an accumulation of β-catenin in the cytosol and the nucleus. This loss of function is associated with biallelic mutations of the *APC* gene [17]. These mutations are signatures of sporadic CRC and colorectal tumors that develop in familial adenomatous polyposis (FAP) patients. FAP is a dominantly inherited disease that manifests itself by the development of polyps in the colon and the upper gastrointestinal (GI) tract, which ultimately evolve into fatal aggressive tumors when left untreated. The Apc^{Min} mouse model has a mutated adenomatous polyposis coli (Apc) gene, similar to that in patients with familial adenomatous polyposis. The Apc^{Min} mice are born with a large number of small polyps of the upper GI tract but fewer polyps in colon and have an average lifespan of 120 days [18]. This model is most promising as it mimics the rapid development of adenomatous polyps that affect humans and sporadic CRCs and hence is widely used to elucidate the cellular and molecular mechanisms that underlie GI tract cancers [19]. This model is ideal to evaluate the effects of diets and chemopreventive compounds on the rate and extent of colon cancer initiation and progression.

The purpose of the current study was to investigate the chemopreventive effects of RVT on BaP-induced adenomas and pathology of the colon in Apc^{Min} mouse model. Since adenomas are biomarkers of tumor formation, examining the relationship between RVT exposure and adenoma development provides an understanding of the extent to which the target tissues are susceptible to damage from exposure to BaP alone and BaP in combination with RVT. In this study, we show that RVT treatment caused a decrease in the incidence, size and number of adenomas formed in the colon of mice exposed to BaP compared to mice exposure to BaP alone.

2. Materials and methods

2.1. Animal husbandry and BaP and RVT exposure

Five-week-old male Apc^{Min} mice (Jackson Labs, Bar Harbor, ME, USA) weighing approximately 30 g were housed in groups of two to three per cage, maintained on a 12/12-h light/dark cycle and allowed free access to rodent chow (NIH-31 open formula diet) and water. All animals were allowed a 7-day acclimation period prior to being randomly assigned to a control ($n=10$ per each time point) or treatment group ($n=10$ per each time point). Treatment consisted of a single dose [100 μg/kg body weight (bw)] of BaP (97% pure, Sigma Chemical Co., St. Louis, MO, USA) dissolved in research-grade peanut oil (Sigma). RVT (45 μg/kg bw; Sigma), dissolved in 10% ethanol and 90% deionized water, was given concurrently with BaP (for 60 days) or prior (daily for 1 week) to BaP exposure (for 60 days). The test chemicals (BaP and RVT) were administered through oral gavage (200 μl volume). All animal studies carried out were in conformity with the policies of Institutional Animal Care and Use Committee of Meharry Medical College. As BaP is a potential carcinogen, it was handled in accordance with National Institutes of Health guidelines [20].

All the mice from control and treatment groups were observed twice a day (including holidays and weekends) for moribundity and mortality. Mice body weight and food consumption were monitored periodically.

2.2. BaP and RVT dose relevance

Dietary exposures of humans to BaP vary. While some studies reported BaP intake of 2.8 μg/person/day [21], others reported 8.4 μg/person/day [22] and 17 μg/person/day [23]. Because of the increasing environmental contamination by BaP, allowance was made for exceptionally high dietary exposures. For computing dietary intake of BaP by mice, which approximates the human dietary intake of BaP, the highest daily exposure of 17 μg/person/day for an average male weighing 70 kg was chosen. Using this value, the human intake of BaP translates to 0.24 μg/kg bw/day. Thus, the dose of 100 μg BaP / kg bw when given to Apc^{Min} mice was equivalent to the human dietary intake of BaP for a period of 12 months.

The dose of RVT given to mice was also within the range of dietary levels of this compound in humans. Wang et al. [24] have shown that RVT levels range from 1.6 to 1040 nmol/g in grape products, and are 1.1 and 1.6 nmol/g in cranberry and grape juice, respectively. Thus, consumption of an 8-oz portion of cranberry or grape juice by a healthy human will result in an intake of 0.24 to 0.37 μmol/g RVT; consumption of a 230-g portion of grape products will result an intake of 0.24 mmol/g RVT. Thus, the dose of 45 μg BaP/kg bw when given to Apc^{Min} mice was equivalent to human dietary intake of RVT for a period of 6 months.

2.3. Sample collection

At the end of 60 days of exposure, mice were sacrificed, and target tissues (liver, small intestine and large intestine) were retrieved following the guidelines of Ruehl-

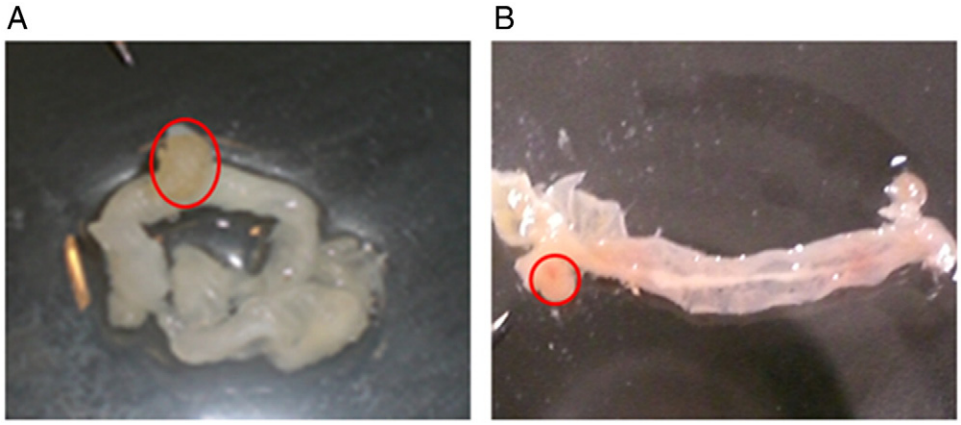

Fig. 1. Representative pictures of colon polyps of Apc^{Min} mouse exposed to (A)100 μg/kg BaP/kg bw or (B)100 μg/kg BaP+45 μg/kg RVT.