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Effects of di(2-ethylhexyl)phthalate exposure on 1,2-dimethyhydrazine-induced colon tumor promotion in rats

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ABSTRACT

Di(2-ethylhexyl)phthalate (DEHP) may cause carcinogenicity in the liver; however, few have detailed on the potential effects of DEHP exposure on colorectal cancer. Male Sprague-Dawley rats received i.p. injections of 1,2-dimethylhydrazine (DMH) once-a-week for the first 4 weeks, and rats in each group were treated with DEHP through oral gavage daily for either 7, 10 or 15 weeks; after which, all rats were euthanized and their colons were assessed (a) morphologically for aberrant crypt foci (ACF) or tumors, (b) cytologically for mitotic index (MI), and (c) immunohistochemically for the expression of β-catenin, cyclooygenase (COX)-2, vascular endothelial growth factor (VEGF), proliferating cell nuclear antigen (PCNA), cyclin D1, and c-myc. Our results indicated that the mean total ACF, tumor incidence, and MI were significantly higher in the DEHP-treated DMH compared to control and the DEHP-alone groups. The level of β-catenin and cyclin D1 was increased in DEHP-exposed rats. Expression of β-catenin, COX-2, VEGF, and cyclin D1 was significantly higher in the combined DMH and DEHP-treated rats by comparison to that of the DMH group. In conclusion, this study indicates that exposure to DEHP may exacerbate DMH-induced colon tumorigenesis and provides impetus to evaluate the effect of DEHP in conjunction with other carcinogens.

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide; it is also the most common malignancy in Taiwan, with an age-standardized incidence rate of 22.9 per 100 000 individuals in 1995, which rapidly increased to 49.7 per 100 000 individuals in 2012 (CRAR, 2012). According to the World Health Organization data, Taiwan has the highest CRC incidence worldwide.

Cancer is caused by genetic and environmental factors: the majority of cancers, approximately 90%–95% of the cases, are caused by environmental factor alone, whereas the remaining 5%–10% occur because of inherited genetics (Anand et al., 2008). Environmental carcinogens include chemicals, oncogenic viruses, and high-energy radiation. Of these, diet and chemical carcinogens are factors most likely associated with the high incidence rate of CRC in Taiwan. Refined red meat foods, such as bacon and ham, have been defined as first class carcinogens. However, in Taiwan, people do not consume these foods as frequently as people in Western countries do; therefore, food additives may be having the key role in colorectal carcinogenesis in Taiwan.

Di(2-ethylhexyl)phthalate (DEHP), a phthalate derivative and a well-known environmental endocrine disruptor with anti-androgenic properties, is widely used as a plasticizer in the manufacture of polyvinylchloride plastics. In Taiwan, plastic bags are usually used to store hot food; therefore, DEHP exposure is common among people in Taiwan. Human environmental or occupational exposure to DEHP is associated with sperm DNA damage (Hauser et al., 2007; Huang et al., 2011, 2014). Experimental animal studies have demonstrated that DEHP might be associated with antiandrogenic activity and reproductive toxicity (Tanaka,

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Abbreviations

15-PGDH	15-hydroxyprostaglandin dehydrogenase
ACF	aberrant crypt foci
ANOVA	analysis of variance
BHA	butylated hydroxyanisole
COX-2	cyclooygenase-2
CRC	colorectal cancer
DBP	dibutyl phthalate
DEHP	di(2-ethylhexyl) phthalate
DMH	1,2 dimethylhydrazine
HSD	honestly significant difference
MI	mitotic index
NOAEL	no-Observed-Adverse-Effect-Level
PBS	phosphate-buffered saline
PCNA	proliferating cell nuclear antigen
PGE2	prostaglandin E2
TFDA	Taiwan Food and Drug Administration
VEGF	vascular endothelial growth factor

2005; Ge et al., 2007; Hsu et al., 2016).

In vitro exposure of human cells or tissues to DEHP may induce DNA damage; alter mitotic rate, proliferation, and apoptosis; increase mobility and invasiveness of tumor cells; and activate nuclear receptor numbers (Caldwell, 2012). Several studies have demonstrated that animal exposure to DEHP may cause extensive carcinogenicity through genotoxicity in the livers of rats, mice, and hamster in U.S. National Toxicology Program carcinogenesis bioassays (Kluwe et al., 1982; Popp et al., 1987; James et al., 1998; Isenberg et al., 2000). Because the liver is a potential target for cancer in humans and rodents exposed to DEHP, the effect of DEHP on the rodent liver may be associated with the peroxisome proliferator function of DEHP (Klaunig et al., 2003). However, few studies have detailed the potential effects of DEHP exposure on colon tumorigenesis promotion in rats.

Among the chemically induced animal models of CRC, 1,2-dimethylhydrazine (DMH) model of colon carcinogenesis is an ideal model, widely used for experimental colon carcinogenesis (Perše and Cerar, 2005). The use of the aberrant crypt foci (ACF) system as a tool to study colon carcinogenesis has been reviewed elsewhere (Bird, 1995). Sporadic CRCs generally originate from an initiating genetic event in a normal colonic stem cell, involving overactivation of Wnt signaling (gatekeeper lesion), enabling this cell to outgrow surrounding cells and form a dysplastic ACF. Abnormal levels of β-catenin may contribute to neoplastic transformation by resulting in accumulation of cyclin D1 in colon carcinoma cells (Tetsu and McCormick, 1999). Constitutively activated β-catenin signaling—caused by adenomatous polyposis coli deficiency or β-catenin degradation-preventing mutations—leads to excessive stem cell renewal or proliferation that predisposes cells to tumorigenesis (MacDonald et al., 2009).

Aberrant cyclooygenase-2 (COX-2) expression may have a crucial role during CRC development (Sinicrope and Gill, 2004). COX-2 is overexpressed in CRC partly because of mutant oncogenic PIK3CA-driven incessant PI3K signaling. Angiogenesis also has a critical role in CRC progression. Evidence from preclinical and clinical studies indicates that vascular endothelial growth factor (VEGF) is the predominant angiogenic factor in CRC (Guba et al., 2004). Moreover, proliferating cell nuclear antigen (PCNA), an inhibitor of apoptosis protein, is correlated with more aggressive tumor growth in CRC (Ioachim, 2008). The objective of this study is to investigate whether DEHP may exacerbate DMH-induced colon tumorigenesis and to explore the possible molecular mechanism using a well-established animal model.

2. Materials and methods

2.1. Study design

Male Sprague-Dawley rats were randomized to receive i.p. injections of DMH or sodium acetate (vehicle) once-a-week for the first 4 weeks, and rats in each group were conjunctly treated with DEHP or corn oil (vehicle) through oral gavage daily for either 7, 10 or 15 wk; after which, all rats were euthanized and their colons were assessed (a) morphologically for prenoplastic ACF or tumors, (b) cytologically for mitotic index (MI) in the crypts, and (c) immunohistochemically for the spatial expression of β-catenin, COX-2, VEGF, PCNA, cyclin D1, and c-myc (Fig. 1).

2.2. Reagents and antibodies

DEHP, DMH, and corn oil were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mouse monoclonal antibodies against β-catenin, VEGF, PCNA, and cyclin D1 were purchased from Cell Signaling Technology (Beverly, MA, USA). COX-2 was purchased from BD Transduction Laboratories (Lexington, KY, USA). c-Myc was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.3. Animals and treatment

The male SD rats were obtained from BioLASCO Experimental Animal Center (Taiwan Co., Ltd., Taipei, Taiwan) after weaning. The animal chamber was supplied with UV-sterilized air and maintained at 24 °C–26 °C, the humidity was typically 55%–60%, and a 12:12-h light/dark cycle. Solid-bottomed polycarbonate cages with stainless steel wire-bar lids were used to house 2 rats per cage. The rats were provided food (Laboratory Rodent Diet 5001, LabDiet, Richmond, IN, USA) and distilled water *ad libitum*. All experimental animal care and treatment were in accordance with the Institutional Animal Care and Use Committee of the National Kaohsiung First University of Science and Technology. At the age of 4 wk, the rats of the control group were administered with intraperitoneal injections of 40 mg/kg body weight/wk sodium acetate for 4 wk with daily oral gavage of corn oil. The rats of the DEHP group were treated with intraperitoneal injections of 40 mg/kg body weight/wk sodium acetate for 4 wk and orally gavaged 500 mg/kg/d DEHP (99% pure, CAS no. 117-81-7) for 7, 10, or 15 wk. The rats in the DMH group were administered intraperitoneal injections of 40 mg/kg body weight/wk DMH for 4 wk and gavaged with corn oil for 7, 10, or 15 wk daily. The rats in the DEHP + DMH group were administered intraperitoneal injections of 40 mg/kg body weight/wk DMH for 4 wk and gavaged with 500 mg/kg/d DEHP for 7, 10, or 15 wk (Fig. 1).

The rationale of utilizing a single dose of DEHP at a dose of 500 mg/kg/day was based on the 100-fold greater than the No-Observed-Adverse-Effect-Level (NOAEL) of 5 mg DEHP/kg/day used by the European Union (EFSA, 2005). Treatment with DMH (40 mg/kg) and experimental period was designed to induce colon carcinogenesis in rats (Akagi et al., 1995).

2.4. Animal termination

After DEHP exposure for 7, 10, and 15 wk, the animals were weighed and anesthetized using CO_2 followed by removal and weighing of their livers. Relative liver weight was calculated using the ratio of liver and body weights.