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Three components of cigarette smoke altered the growth and apoptosis of metastatic colon cancer cells via inducing the synthesis of reactive oxygen species and endoplasmic reticulum stress

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ABSTRACT

Cigarette smoke (CS) is a well-known risk factor for carcinogenesis and has been found to be related to the occurrence and development of colon cancer. In this study, the effect of formaldehyde (FA), benzene (Bz), and isoprene (IP), which are included in main components of CS, on cell viability and apoptosis of SW620 colorectal cancer cells was examined to identify the connection between CS components and colon cancer. In cell viability assay, FA, Bz, and IP decreased cell viability of SW620 cells in a dose dependent manner. In Western blot assay, the protein expression of cell cycle related genes, cyclin D1 & E1, was decreased by FA, Bz, and IP, which corresponded to their inhibitory effect on cell viability. In addition, FA, Bz, and IP increased the protein expression of pro-apoptotic genes, C/EBP homologous protein (CHOP) and Bax, and reduced the protein expression of anti-apoptotic gene, Bcl-2. In reactive oxygen species (ROS) assay using dichlorofluorescin diacetate (DCFH-DA), FA, Bz, and IP increased the ROS production in SW620 cells. In the measurement of apoptotic cells, the numbers of apoptotic cells were increased by the treatment of FA, Bz, and IP. As CHOP is an endoplasmic reticulum (ER)-stress related apoptosis marker of which production is induced by ROS, it was considered that these CS components induce apoptosis of SW620 cells by increasing ROS synthesis and ER-stress. Taken together, these results showed that CS components, i.e., FA, Bz, and IP, inhibited the cell viability of SW620 cells by down-regulating the protein expression of cyclin D1 & E1 and induced apoptosis of SW620 cells by increasing ROS production and simultaneously activating ER-stress.

1. Introduction

Cigarette smoke (CS) has been known to be one of the largest risk factors to cause heart, pulmonary, and gastrointestinal diseases and many other serious diseases including cancer via a variety of mechanisms (Bak et al., 2015; McBride, 1992; Newcomb and Carbone, 1992; Not-listed, 1997; Sherman, 1992; Sobus and Warren, 2014). CS is usually consisted of about 5000 different chemicals, of which approximately 150 chemicals are considered to be toxicants (Bartal, 2001; Eldridge et al., 2015). However, yet not much is known about which of toxicants in CS play important roles in causing bad impact on human health in comparison with the whole effect of CS or the individual effect of several main components including nicotine and tar. Meanwhile, formaldehyde (FA), benzene (Bz), and isoprene (IP) were reported to be included in the typical materials belonging to CS (Adams et al., 1987; Adlkofer et al., 1990).

FA is a common organic compound belonging to aldehyde family. FA exposure is closely related with activation of the oxidative stress to normal cells, leading to cytotoxicity and potential carcinogenesis (Attia et al., 2016). Bz is the simplest aromatic compound consisted of a cyclic hydrocarbon. CS is the most frequent source of exposure to Bz (Wallace, 1996, 1989), which has been known to affect human health by disturbing normal functions of various hormones in reproductive system (Reutman et al., 2002). In addition, IP is the 2-methyl structural analogue of butadiene. IP was reported to affect reproductive and developmental processes in animals. Swiss CD-1 mice and Sprague-Dawley rats exposed to IP resulted in decreased maternal weight gain and reduced fetal body weight (Melnick et al., 1994). For female B6C3F mice exposed to IP, ovarian primordial follicle counts were reduced 24%, and growing primary to preantral follicle counts were reduced by 54% (Doerr et al., 1995).

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In relation with the risk to human cancer, the International Agency for Research on Cancer (IARC) have classified FA and Bz as human carcinogens (group 1), and IP as a possible human carcinogen (group 2). Generally, lung cancer is reported to be the most common form of cancer caused by cigarette smoking (Adams et al., 1987). The causes of laryngeal, esophagus, and gastric cancers are also associated with smoking (Saffiotti and Kaufman, 1975; Toh et al., 2010). However, etiology of CS to colorectal cancer is a more recent concern. Colorectal cancer also known as colon cancer is the development of cancer in the colon or rectum, parts of the large intestine. Risk factors for colorectal cancer usually include lifestyle, older age, and inherited genetic disorders, and other factors such as diet, smoking, and alcohol are also addressed (Baena and Salinas, 2015). According to previous studies, CS was associated with small and large colorectal adenomas, which are generally accepted as being precursor lesions for colorectal cancer (Peppone et al., 2010), and CS induced human colon tumor growth in mice (Wong et al., 2009) and proliferation of colon cancer cells (Liu et al., 2005). As one of main components of CS, nicotine showed the dual effects on colon cancer cells, growth inducing or preventive effects (Pelissier-Rota et al., 2015), but research on risk of other CS components on colon cancer is still lacking.

In the present study, the connection between CS components and colon cancer was examined to elucidate the harmful effect of CS on colon cancer and its relevant mechanism. Specifically, we selected FA, Bz, and IP described above as major components of CS and investigated their effects on SW620 colon cancer cells to provide information about the roles of CS components. We focused on the influences each exposure of FA, Bz, and IP exerted on the proliferation and apoptosis of SW620 cells and also tried to explain the mechanisms underlying altered cell proliferation and apoptosis of SW620 cells induced by FA, Bz, and IP.

To elucidate the mode of actions of FA, Bz, and IP in relation with cell proliferation and apoptosis, their impacts on cell cycle progression and oxidative stress in SW620 cells were investigated by measuring the alterations in expression of cell cycle related genes, reactive oxygen species (ROS) synthesis, and endoplasmic reticulum-stress (ER-stress) (Kim et al., 2015). ROS which are chemically reactive molecules containing oxygen increase dramatically during times of environmental stress and can cause significant damage to cell structures such as ER (Kaufman, 1999). ER is an eukaryotic organelle that has a crucial role in sensing cellular homeostasis and generating suitable signals and responses. ER-stress is known to be activated by DNA damage and ROS numbers and to be associated with cellular events such as autophagy, apoptosis, and cell cycle arrest (Holczer et al., 2015; Xu et al., 2005). Therefore, the identification of the effects of FA, Bz, and IP on the synthesis of ROS and ER-stress may clarify their roles in cellular events including apoptosis and cell proliferation.

2. Materials and methods

2.1. Reagents and chemicals

Formaldehyde (FA), benzene (Bz), isoprene (IP) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). All chemicals were dissolved in 100% dimethyl sulfoxide (DMSO; Junsei Chemical Co., Tokyo, Japan).

2.2. Cell culture and media

The human colon cancer cell line, SW620, was purchased from Korean cell line bank (KCLB, Seoul, Korea). SW620 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; HyClone Laboratories Inc., Logan, UT, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; HyClone Laboratories Inc.), 2% penicillin G and streptomycin (Cellgro; Mediatech, Inc., Manassas, VA, USA), and 1% HEPES (Invitrogen Life Technologies, Carlsbad, CA, USA) at 37 °C in a humidified atmosphere with 5% CO_2-95% air as previously described (Kim et al., 2014).

2.3. Cell viability assay

SW 620 cells were seeded at a density 5×10^3 cells per well in 96well plates (SPL Life Science, Seoul, Republic of Korea) in a humidified atmosphere of 5% CO_2 at 37 °C. After the cells were incubated with phenol red-free DMEM with 5% CD-FBS medium for 48 h, they were treated with various concentrations of FA, Bz, or IP (FA: 10^{-14}–10^{-8} M, Bz: 10^{-12}–10^{-8} M, IP: 10^{-12}–10^{-8} M) in phenol red-free DMEM with 5% CD-FBS supplemented with 0.1% DMSO for 9 days. During this period, the media were changed to the same new media every third day. DMSO was used as a vehicle to carry the chemicals to the media. Cell viability was detected with the addition of 3-(4-5-dimethylthiazol-2-yl)-2.5-dyphenyltetrazolium bromide (MTT; Sigma-Aldrich) solution. MTT (10 ml of 5-mg/ml solution) was added to each well of 96 well-plates and the plates were incubated for 3 h at 37 °C in a humidified atmosphere of 5% CO_2. Supernatants were removed, and 100 μl DMSO was added to each well to dissolve resultant formazen crystals. The optical density (OD) of each well was measured at 540 nm using an ELISA reader (VERSA man, Corp., Molecular Devices, Sunnyvale, CA, USA) and used to calculate the number of viable cells.

2.4. Protein extraction and Western blot assay

To measure protein expression of cyclin D1, cyclin E1, C/EBP homologous protein (CHOP), Bcl-2, Bax, and GAPDH, SW620 colon cancer cells were cultured to a density of 1×10^6 cells (SPL Life Sciences, Corp.) and then incubated with FA, Bz or IP at a concentration of 10^{-11} M or 10^{-8} M (in DMSO) for 24 or 72 h. 0.1% DMSO was used as a control. After treatment, whole cell lysates of SW620 cells were prepared in 80 μl 1X RIPA buffer (50 mM Tris-HCl; pH 8, 150 mM NaCl, 1% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA), 0.5% deoxycholic acid (Sigma-Aldrich, St. Louis, MO, USA), and 0.1% SDS). Total protein concentrations were determined by bicinchoninic acid (BCA; Sigma-Aldrich, Corp.). Fifty μg of total protein was separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to a polyvinylidenedifluoride (PVDF) membrane (Bio-Rad Laboratories, Corp.), and the membranes were blocked with 5% bovine serum albumin (BSA; Sigma-Aldrich, Corp.) for 90 min at room temperature. The membrane was incubated at room temperature with mouse monoclonal anti-GAPDH antibody (Abcam plc.), mouse monoclonal anti-cyclin D1 antibody (Abcam plc.), mouse monoclonal anti-cyclin E1 antibody (Abcam plc.), mouse monoclonal anti-Bax (1:1000 dilution in 5% BSA buffer; Santa Cruz Biotechnology), mouse monoclonal anti-Bcl-2 (1:200; Cell Signaling Technology, Inc., Danvers, MA, USA), mouse monoclonal anti-CHOP (1:1000 dilution in 5% BSA buffer; Cell Signaling Technology, Inc., Danvers, MA, USA) for overnight at 4 °C. Primary antibody binding was detected with horse radish peroxidase (HRP)-conjugated anti-rabbit IgG or anti-mouse IgG (1:2000, Thermo Scientific, Corp, Rockford, IL, USA) for 2 h at room temperature. Target proteins were detected by used the West-Q Chemiluminescent Substrate Plus kit (GenDEPOT, Barker, TX, USA). All of the aforementioned protein expression levels were normalized by GAPDH protein.

2.5. Determination of ROS production

ROS as chemically reactive molecules containing oxygen can cause ER-stress and further apoptosis and cell cycle arrest (Zhu