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Detoxification of chlorella supplement on heterocyclic amines in Korean young adults

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ABSTRACT

Heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) have been established as carcinogenic chemicals in Western diet. This study was performed to estimate HCA exposure levels in Korean daily life and to assess the ability of *Chlorella vulgaris* to detoxify carcinogenic HCAs in a randomized, double blind, placebo-controlled crossover study with chlorella supplement ($N = 6$, all females, age: 27.17 ± 7.73 yr) for 2 weeks. We analyzed HCAs in hydrolyzed urine specimens using LC/TOF-MS. As results, urinary levels of MeIQx, PhIP, and IQx-8-COOH were 323.36 ± 220.11 ng/L, 351.59 ± 254.93 ng/L, and 130.85 ± 83.22 ng/L, respectively. Effects of chlorella to reduce urinary MeIQx were marginally significant (before, 430 ± 226.86 pg/mL vs. after, 174.45 ± 101.65 pg/mL: $0.05 < p < 0.1$). However, urinary levels of PhIP or IQx-8-COOH, a major metabolite of MeIQx, were not changed by chlorella supplementation. In conclusion, our study demonstrates that current daily levels of HCA exposure in Korean young adults are not lower than those in the Western world. In addition, the effects of chlorella's to detoxify HCAs likely occur by interfering e with absorption or metabolism.

1. Introduction

Many epidemiological studies, e.g. immigrant or twin studies have demonstrated a strong association between environment and diseases (Khan et al., 2010). Diet has been suspected as a major risk factor for gastrointestinal tract-related cancers. For example, westernized dietary habits in Asian countries have been suspected as the reason for increasing numbers of colorectal cancer in Asian populations (Chan et al., 2011). Increased consumption of meat in Asian population showed an association with increased number of common cancers such as breast, colorectal, and prostate cancers in many epidemiological studies (Zheng and Lee, 2009). Among various food-related carcinogens, HCAs and polycyclic aromatic hydrocarbons (PAHs) have been the focus of research when

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looking at risk factors in cancers related to meat consumption (US Department of Health and Human Services, 2011). HCAs are formed when amino acids, sugars, and creatine in muscle meats including beef, pork, fish, and poultry, react with one another when cooked at high-temperatures (Knize et al., 1994), as in pan-frying or grilling directly over an open flame (Cross and Sinha, 2004). Carcinogenic PAHs such as benzo(a)pyrene are also formed when meat is cooked smoked. Therefore, improvements in both dietary habits and cooking methods have been suggested to prevent exposure to carcinogenic heterocyclic amines (HCAs) and PAHs (Santarelli et al., 2008).

Functional food can work by detoxifying carcinogenic HCAs via pharmaco- or toxicokinetic pathways via absorption, distribution, metabolism or elimination. A chlorella supplement is used as a popular functional food and can be taken as a powder, extract liquid, capsule, or food additive (Kim et al., 2009). *Chlorella vulgaris*, the main component of chlorella, is a species of unicellular algae, which contains chlorophyll, proteins, minerals, dietary fibers, nucleic acids, and vitamins (Morita et al., 1999). Like other food with intense colors, for example, blueberries with anthocyanin, tomatoes with lycopene, etc., chlorella has chlorophyll, a well-known family of green coloring compounds. The basic structure of a chlorophyll molecule is a porphyrin ring coordinated to a central magnesium atom, similar in structure to the heme group found in globins or cytochromes but with a different coordinating metal (Higdon, 2005). Several studies have shown that chlorella has various biological effects, such as lowering blood sugar, and detoxifying cadmium and dioxin (Morita et al., 1999), stimulating growth, modulating lipid metabolism and immunomyelopoietic activity, enhancing tumor resistance (Ramos et al., 2010) and preventing cancers (Jeong et al., 2009; Shim et al., 2009). In addition, chlorophyllin, a semi-synthetic form of chlorophyll, forms stable complexes with carcinogenic HCAs, such as 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx) or 2-amino-3-methyl-imidazo[4,5-f]-quinoxaline (IQx) (Shaughnessy et al., 2011). Some studies have suggested that chlorophyllin can act as an interceptor molecule through the formation of tight molecular complexes with carcinogens such as aflatoxin B (Kensler et al., 2004).

Dietary supplements for chemoprevention are very popular in the market, even though their efficacy in humans is still controversial. Recently, probiotics showed chemopreventive effects against genotoxicity due to HCA exposure via in vitro and in animal models (Nowak and Slizewska, 2014; Klewicka et al., 2012). However, human studies are very few. Therefore, we performed the present study to estimate HCA-exposure in the daily lives of in Koreans and to assess the chemopreventive potential of chlorella supplements against carcinogenic HCAs in a clinical trial.

2. Materials and methods

2.1. Materials

2-Amino-1-trideutromethyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3-trideutromethyl-8-methylimidazo[4,5-f]quinoxaline-d_3 (MeIQx) were purchased from Toronto Research Chemicals (Ontario, Canada). MeIQx, 2-amino-3-trideutromethylimidazo[4,5-f]quinoxaline-8-carboxylic acid (IQx), and PhIP were graciously supplied by Dr. Robert J. Turesky (New York State Department of Health, Albany, NY).

2.2. Clinical trial

All of the study protocols were approved by Institutional Review Board of Sookmyung Women's University (SM-IRB-10-0920-006). All participants filled out the informed consent form before starting this study. This trial was designed as a randomized, double blind, placebo-controlled crossover study (Fig. 1). Recruited subjects [$N = 6$, all females, age: 27.17 ± 7.73 yr, body mass index (BMI): 19.81 kg/m^2] were randomly advised to take 4 chlorella supplement tablets with each meal (12 tablets/day) (100 mg of chlorella/day) (Daesang, Seoul, S. Korea) or placebo tablets for 2 weeks. To control diet for the whole trial, we asked them record what and how much they ate for every meal. We calculated the quantities of nutrients from foods using the Can Pro version 4.0 (Computer Aided Nutritional analysis Program, The Korean Nutrition Society, 2011).

Urine specimens were collected before and after the trial and stored at −20 °C until analysis.

2.3. Preparation of urine samples

Before analysis, we performed solid-phase extraction (SPE) to separate HCAs from urine. In detail, 1.0 mL of each urine sample was used for each solid-phase extraction (SPE) well (Strata^{TM}-X-C 33 μm Polymeric Strong Cation 96-well plate, Phenomenex, Torrance, CA). Each SPE well plate was conditioned with 1.0 mL of methanol and equilibrated with 1.0 mL of water containing 2% formic acid. Each urine sample (1.0 mL) containing deuterated internal standards (500 pg) was hydrolyzed with 50 μL of formic acid, vortexed for 30 s, then centrifuged for 2 min to pellet particulates before loading supernatant on SPE well plates. With an approximate pressure of 2 bar, a vacuum manifold facilitated flow through the SPE resin during the sample loading and adsorption of the analytes. The SPE well plates were then rinsed sequentially under acidic conditions with (1) H_2O containing 0.1 N HCl (pH ~ 1.68) and (2) 0.1 N HCl in 10:90 (v/v) MeOH/H_2O (pH ~ 1.65) again under ~5 mm Hg to remove adsorbed cations leaving analytes in their protonated forms. HCAs were finally eluted from the SPE well plates under basic conditions (pH ~ 12.98) with three 500 μL aliquots of 50:50 (v/v) acetonitrile (CH_3CN)/ammonium hydroxide (NH_4OH) and collected in 2.0 mL tubes. The resultant 1.5 mL samples were placed in a ventilated hood for NH_3 evaporation (~30 min), and then vacuum centrifuged at 45 °C to dryness (~3 h). The residues were reconstituted in 50 μL of 50:50 (v/v) water/methanol and transferred into polypropylene vials for subsequent LC–MS analysis.

2.4. Analysis of urinary HCAs by LC/TOF-MS

Following Turesky et al.'s method (Gu et al., 2010) with some modification, we analyzed urinary HCAs by LC/TOF-MS (G6230A, Agilent Technologies, Palo Alto, CA) with an Agilent