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Lack of preventive effects of dietary fibers or chlorophyllin against acrylamide toxicity in rats

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Abstract

Dietary fibers and chlorophyllin have shown to exert anti-carcinogenic effects against co-administered carcinogens. To test the possibility of chemoprevention by such dietary supplements on subacutely induced acrylamide (ACR) toxicity, Sprague–Dawley male rats were administered 2.5% sodium alginate, 5% glucomannan, 5% digestion resistant maltodextrin, 2.5% chitin or 1% chlorophyllin in the diet, and starting one week later, co-administered 0.02% ACR in the drinking water for 4 weeks. For comparison, untreated control animals given basal diet and tap water were also included. Neurotoxicity was examined with reference to gait abnormalities and by quantitative assessment of histopathological changes in the sciatic and trigeminal nerves, as well as aberrant dot-like immunoreactivity for synaptophysin in the cerebellar molecular layer. Testicular toxicity was assessed by quantitation of seminiferous tubules with exfoliation of germ cells into the lumen and cell debris in the ducts of the epididymides. Development of testicular toxicity as well as neurotoxicity was evident with ACR-treatment, but was not suppressed by dietary addition of fibers or chlorophyllin, suggesting no apparent beneficial influence of these dietary supplements on experimentally induced subacute ACR toxicity.

Keywords: Acrylamide toxicity; Sodium alginate; Glucomannan; Digestion resistant maltodextrin; Chitin; Chlorophyllin

1. Introduction

Acrylamide (ACR), a well-recognized neurotoxicant, is a water-soluble vinyl monomer. Recently, exposure to ACR in foodstuffs has become a worldwide concern because of its generation in a variety of fried and oven-baked foods during cooking through Maillard reactions of sugars with asparagine residues (Mottram et al., 2002). Earlier studies indicated exposure of humans and laboratory animals to ACR produced neurotoxicity characterized by ataxia and skeletal muscle weakness (Spencer and Schaumburg, 1974; Le Quesne, 1985). The initial target of ACR appears to be nerve terminals in both the central and peripheral nervous systems, resulting in autonomic, behavioral, sensory, and motor disturbances (LoPachin et al., 2003), although the mechanisms underlying the diverse neurotoxicity remain controversial.

Genotoxic potential is another concern of ACR through its reactive metabolite, glycidamide, generated by catalysis with cytochrome P450 (CYP) 2E1 (Sumner et al., 1999). Glycidamide is regarded as a cancer-initiating species, reacting with DNA to cause mutagenicity and clastogenicity (Adler et al., 2000), and eventually carcinogenicity as reported in rodents (Johnson et al., 1986; Friedman et al., 1995). The clastogenic potential is also responsible for dominant lethality targeting male gonadal spermatocytes to result in reproductive toxicity in rodents (Sakamoto et al., 1988; Sublet et al., 1989; Lähdetie et al., 1994; Xiao and Tates, 1994; Adler et al., 2000).

Abbreviations: ACR, acrylamide; CYP, cytochrome P450; DRMD, digestion resistant maltodextrin; NOEL, no-observed-effect level; SYP, synaptophysin.

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At the 64th Joint FAO/WHO Expert Committee on Food Additives (WHO/IPCS, 2006), the Committee concluded that an intake of 1 μg/kg body weight/day of ACR could be taken to represent the average for the general population. Based on the study results of Burek et al. (1980), however, the Committee determined the no-observed-effect level (NOEL) of ACR for neurotoxicity to be 0.2 mg/kg body weight/day (WHO/IPCS, 2006). Also, the overall NOEL for reproductive and developmental effects was determined to be 2 mg/kg body weight/day (Tyl et al., 2000; WHO/IPCS, 2006). Based on these estimates, the Committee concluded that adverse effects on neurotoxicity and male reproductive toxicity are unlikely at the estimated mean intakes. Nevertheless, the Committee noted that genotoxic and carcinogenic potential of ACR may indicate a human health concern. As described earlier, effects of ACR on male reproduction are related to its genotoxic potential. Regarding neurotoxicity, the question of its link with genotoxicity remains unclear. Therefore, although the exposure risk for neurotoxicity and reproductive toxicity is regarded to be extremely low, it is still reasonable to establish approaches to reduce any deleterious influence.

Dietary fiber has been shown to be effective for prevention of colorectal cancers in man (McKeown-Eyssen and Bright-See, 1984; Howe et al., 1992). Mechanisms by which dietary fiber could act against toxicity and carcinogenesis include the reduction of chemical adsorption in relation with physicochemical capacity to bind chemicals, physiologic and mechanical effects to enhance faecal passage and bulking in the gastrointestinal tract (Jacobs, 1986; Jacobs, 1988). Another potential mechanism involves the role of dietary fiber as a substrate for bacterial fermentation to increase the production of bioactive volatile fatty acids (Jacobs, 1986). Chemical adsorption properties with respect to mutagens have been shown with chlorophyllin (Dashwood et al., 1998; Breinholt et al., 1999; Sugiyama et al., 2002), a semisynthetic anti-carcinogen consisting of water-soluble sodium–copper salts of chlorophylls (Dashwood et al., 1991) found to be effective for chemoprevention of heterocyclic amine-induced experimental tumors (Xu and Dashwood, 1999; Hirose et al., 1999, 2002).

Recently, we established an in vivo assay system of chemoprevention of ACR-induced neurotoxicity and male reproductive toxicity using rats and examined effects of additional exposure to antioxidative agents, *N*-acetylcysteine, phenylethyl isothiocyanate (PEITC) and 1-*O*-hexyl-2,3,5-trimethylhydroquinone (Lee et al., 2005) and found a partial inhibition of ACR testicular toxicity on co-administration of PEITC. In the present study, we focused on potential beneficial properties of dietary fibers and chlorophyllin with reference to parameters of neurotoxicity and testicular toxicity of ACR. As dietary fibers, we chose sodium (Na) alginate, a sea kelp-derived water-soluble fiber that produces highly viscous gels in the presence of multivalent cations such as Ca^{2+} (Grant et al., 1973), glucomannan, a soluble, fermentable, and highly viscous dietary fiber derived from the root of the elephant yam or konjac plant, which is native to Asia (reviewed in Keithley and Swanson, 2005), digestion resistant maltodextrin (DRMD), a nondigestible, low viscosity, water-soluble oligosaccharides obtained by heating and enzyme treatment of potato starch (Wakabayashi et al., 1995), and chitin, the *N*-acetylaminocellulose (poly [1 → 4]- β-*N*-acetyl-D-glucosamine) present in the exoskeleton of arthropods and therefore a fiber of animal origin (van Bennekum et al., 2005).

2. Materials and methods

2.1. Chemicals

ACR obtained from Sigma (St. Louis, MO) as a white powder with a purity over 98% was mixed at a concentration of 0.02% (w/v; corresponding to 2.8 mM) into distilled water for administration according to an experimental protocol inducing clear neurotoxicity within 22 days (LoPachin et al., 1992). Sodium (Na) alginate (Wako Pure Chemical Industries, Ltd., Osaka, Japan), glucomannan (Wako Pure Chemical Industries, Ltd.), digestion resistant maltodextrin (abbreviated here as DRMD that is commercially available as fibersol-2™; Matsutani Chemical Industry Co., Ltd., Hyogo, Japan), chitin (Kyowa Technos Co., Ltd., Chiba, Japan), and chlorophyllin (Tama Biochemical Co., Ltd., Tokyo, Japan) were mixed into powdered basal diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) at concentrations of 2.5%, 5%, 5%, 2.5% and 1%, respectively. The dose level of each compound except for DRMD was determined judging from the results of previous studies using rats that have shown apparent chemopreventive effects on certain endpoints without assuming toxic outcome by itself or simply no apparent toxicity after repeated oral doses (Na-alginate, Mouecoucou et al., 1990; Seal and Mathers, 2001; glucomannan, Hou et al., 1990; Yamada et al., 1999; Gallaher et al., 2000; chitin, Niho et al., 1999; chlorophyllin, Hasegawa et al., 1995). The dietary concentration of DRMD at 5% was selected on the basis of previous study results showing very slight body weight reduction after 10% dietary administration for 7 weeks (Dr. Toshio Imai et al., unpublished data).

2.2. Animals

A total of 95 male CD(SD)IGS rats at 5 weeks of age, purchased from Charles River Japan Inc. (Atsugi, Japan), were housed three to four animals per polycarbonate cage with sterilized softwood chips as bedding in a barrier-sustained animal room conditioned at 24 ± 1 °C and 55 ± 5% humidity, with a 12-h light/ dark cycle, and were given a pelleted basal diet (CRF-1) and water *ad libitum* during one week of acclimation.

2.3. Experimental design

At six weeks of age, animals were randomly allocated to 12 groups. Group 1, consisting of 10 animals, served as an untreated control, receiving basal diet and tap water *ad libitum* without any supplement for 5 weeks. Animals in groups 2, 3, 4, 5 and 6, consisting of five animals in each group, received Na-alginate, glucomannan, DRMD, chitin and chlorophyllin, respectively in the diet, and tap water *ad libitum* for 5 weeks. In group 7 (ACR-alone), 10 animals were given tap water without ACR during the initial one week and then treated with 0.02% ACR in the drinking water for 4 weeks. Basal diet without supplement was given to this group throughout the experiment. Animals in groups 8, 9, 10, 11 and 12, consisting of 10 animals in each group, received Na-alginate, glucomannan, DRMD, chitin and chlorophyllin, respectively, in the diet for 5 weeks starting 1 week prior to ACR. During the animal experiment, food consumption and body weight were recorded weekly in all groups, and