Journal of Health Science, **46**(1) 56—58 (2000)

Evaluation of Hepatotoxicity and Nephrotoxicity of Natural Food Colorants in Mice Depleted of Glutathione by DL-Buthionine Sulfoximine

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(Received July 21, 1999; Accepted August 31, 1999)

Natural colorants are widely used as additives to various foods. However, the basic data concerning the safety of these colorants are insufficient. Seven natural food colorants were assessed for their hepato- and nephrotoxicity in mice depleted of glutathione by pretreatment with buthionine sulfoximine (BSO). Oral administration of red cabbage color (4500 mg/kg), gardenia yellow (4500 mg/kg), cochineal extract (3000 mg/kg), beet red (6000 mg/kg), carthamus yellow (6000 mg/kg), purple corn color (4500 mg/kg), and lac color (1400 mg/kg) to mice in combination with BSO (4 mmol/kg, i.p.) produced no significant changes in serum glutamic pyruvic transaminase (SGPT) activity and serum urea nitrogen (SUN) concentration, although eugenol as the positive control for hepatotoxicity and thiabendazole as the positive control for nephrotoxicity produced significant increases in SGPT activity and SUN concentration, respectively.

Key words —— natural food colorant, DL-buthionine sulfoximine, glutathione depletion, hepatotoxicity, nephrotoxicity, mouse

INTRODUCTION

It is recognized that many xenobiotics require metabolic activation to form electrophilic intermediates before they exert their toxic effects. Glutathione (GSH) is important in the detoxification of the toxic intermediates.[1] Actually the toxic effects of xenobiotics, such as acetaminophen,[2] bromobenzene,[3] and 4-ipomeanol[4] often are markedly enhanced in animals depleted of tissue GSH. Furthermore, our previous studies have shown that even chemicals with no obvious acute toxicity in normal animals, *e.g.*, thiabendazole (TBZ),[5] eugenol,[6] and *p*-dichlorobenzene,[7] can sometimes cause severe organ injury when they are administered to mice depleted of GSH by pretreatment with DL-buthionine sulfoximine (BSO), an inhibitor of GSH synthesis. Taking into account these results, the possibility that unexpected toxic reactions may be encountered in cases of tissue GSH depletion should be emphasized in assessing the toxic potential of xenobiotics. Our previous studies also showed that the GSH-depleted animal system by treatment with BSO can be a useful tool for providing evidence for the formation of electrophilic intermediates which, in normal animal, may be conjugated with GSH and fail to develop its intrinsic toxicity.

Natural colorants are widely used as additives to various foods. The acute toxicity of natural food colorants is generally believed to be rather low. However, the basic data concerning the safety of these colorants are insufficient. In the present study, seven natural food colorants were assessed for their hepato- and nephrotoxicity in mice depleted of GSH by pretreatment with BSO.

MATERIALS AND METHODS

Chemicals —— Eugenol was purchased from Wako Pure Chemical Industries (Osaka, Japan). TBZ was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). BSO was synthesized according to the published procedure.[8] Red cabbage color, gardenia yellow, cochineal extract, beet red, carthamus yellow, purple corn color, and lac color were kindly provided by San-Ei Gen F.F.I., Inc. (Osaka, Japan).

Animals —— 6 weeks old male ddY mice were obtained from Japan SLC, Inc. (Hamamatsu, Japan) and acclimatized to our laboratory conditions for 1 week before being used. Mice were housed in aluminum boxes on a wood chip bedding (White Flake, Charles River Japan, Inc., Kanagawa, Japan) at a constant temperature (23 ± 2℃) and under a 12 h

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light/dark cycle. Except when stated otherwise, mice received food (Funabashi F-2, Funabashi Farms, Chiba Japan) and water *ad libitum*.

Assessment of Hepato- and Nephrotoxicity —— Mice were treated i.p. with BSO (4 mmol/kg body wt) dissolved in water (20 ml/kg body wt) (8.00—9.00 h). One hour later, the treated animals received *p.o.* doses of red cabbage color (4500 mg/kg body wt), gardenia yellow (4500 mg/kg body wt), cochineal extract (3000 mg/kg body wt), beet red (6000 mg/kg body wt), carthamus yellow (6000 mg/kg body wt), and purple corn color (4500 mg/kg body wt) in water (5 ml/kg body wt) or *p.o.* doses lac color (1400 mg/kg body wt), eugenol (600 mg/kg body wt), and TBZ (200 mg/kg body wt) in olive oil (5 ml/kg body wt). The animals were fasted for 16 h before dosing with test compounds and then for 2 h after administration. Blood was collected by cardiac puncture under pentobarbital anesthesia 24 h after administration of test compounds. The blood was allowed to clot at 37℃ for 1 h, and serum was prepared by centrifugation. Serum glutamic pyruvic transaminase (SGPT) activities and serum urea nitrogen (SUN) concentrations were measured with commercial kits, GPT-UV test Wako and Urea-NB test Wako (Wako Pure Chemical Industries), respectively.

Statistical Analysis —— Data from the SGPT activity assay were analyzed by the Mann–Whitney's *U* test. Data from the SUN concentration assay were analyzed by the Dunnett's test. Differences were considered significant if $p<0.05$.

RESULTS AND DISCUSSION

Table 1 shows the effects of natural food colorants given in combination with BSO on SGPT activity (as an indication of hepatotoxicity) and SUN concentration (as an indication of nephrotoxicity) 24 h after dosing with the test colorants. Our previous studies have shown that treatments of normal mice with eugenol (up to 600 mg/kg) or TBZ (up to 1200 mg/kg) do not produce any increases in either SGPT activity or SUN concentration.[5,6] However, eugenol (400 mg/kg) and TBZ (200 mg/kg) produce marked increases in SGPT activity and SUN concentration, respectively, when administered in combination with BSO.[5,6] In the present study, therefore, eugenol and TBZ were used as the positive controls for hepato– and nephrotoxicity, respectively. The dose levels of food colorants were selected primarily on the basis of solubility limits of the colorants in the vehicles. Eugenol as the positive control compound for hepatotoxicity produced a significant increase in SGPT activity. In contrast, red cabbage color, gardenia yellow, cochineal extract, beet red, carthamus yellow, purple corn color, and lac color did not produce any significant changes in SGPT activity. TBZ as the positive control compound for nephrotoxicity produced a significant increase in SUN concentration. However, neither of the colorants produced significant changes in SUN concentration.

Table 1. SGPT Activity and SUN Concentration 24 h after Administration of Natural Food Colorants in Mice Depleted of GSH by Treatment with BSO

Test compound	Dose (mg/kg body wt)	SGPT (Karmen unit/ml)	SUN (mg %)
Control		16.9 ± 2.0	19.1 ± 2.0
Red cabbage color	4500	16.3 ± 2.2	17.7 ± 1.4
Gardenia yellow	4500	18.2 ± 4.7	19.7 ± 1.5
Cochineal extract	3000	14.0 ± 1.4	16.3 ± 1.0
Beet red	6000	18.0 ± 3.4	21.2 ± 2.0
Carthamus yellow	6000	22.6 ± 5.3	19.4 ± 1.8
Purple corn color	4500	13.2 ± 0.8	16.7 ± 1.0
Lac color	1400	29.6 ± 9.1	16.7 ± 1.4
Eugenol	600	2160 ± 901*	—
TBZ	200	—	159 ±11.4*

Control mice were treated with saline instead of BSO and then received olive oil 1 h later. Values are means ± S.E. * Significantly different from controls ($p<0.05$).

Eugenol caused liver toxicity at a dose of 600 mg/kg, one-fifth of the oral LD_{50} (3000 mg/kg),[9] and TBZ also caused kidney toxicity at a dose of 200 mg/kg, one-twelfth of the oral LD_{50} (3600 mg/kg).[10] The oral LD_{50}'s in mice are >15000 mg/kg for gardenia yellow, 8900 mg/kg for cochineal extract, >10000 mg/kg for beet red, >20000 mg/kg for carthamus yellow, and >3300 mg/kg for lac color[11] (So far the oral LD_{50}'s of red cabbage color and purple corn color have not been reported.). Therefore, the doses employed in this study for gardenia yellow (4500 mg/kg), cochineal extract (3000 mg/kg), beet red (6000 mg/kg), carthamus yellow (6000 mg/kg), and lac color (1400 mg/kg) correspond to approximately one-third to half of the oral LD_{50}'s of these colorants. However, these natural food colorants given in combination with BSO had no effect on the liver and kidney in mice. These results suggest that

natural food colorants tested in the present study do not produce significant amounts of hepato- or nephrotoxic metabolites which are detoxified by tissue GSH.

Acknowledgements We thank Dr. Mikio Nakamura, San-Ei Gen F.F.I., Inc., for supplying natural colorants. This work was supported partly by Grant-in-Aid 05780011 for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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