Effect of Buthionine Sulfoximine, an Inhibitor of Glutathione Biosynthesis, on the Selenium-Induced Lethality in Mice

Masaaki ISHIKAWA, Giichi TAKAYANAGI and Ken-ichi SASAKI
Cancer Research Institute, Tohoku College of Pharmacy, Sendai 983, Japan
Accepted July 12, 1988

Abstract—The effect of buthionine sulfoximine (BSO), an inhibitor of glutathione (GSH) biosynthesis, on the selenium induced-lethality was examined in mice. A single injection of BSO (500 mg/kg, i.p.) markedly decreased the concentration of GSH in the liver after 5 hr. The acute lethality induced by selenium was greatly increased in BSO-treated mice. In contrast, the selenium induced-lethality decreased by pre- and post-treatments of cysteine (100 mg/kg, i.p.) in mice.

Selenium has recently been identified as an essential trace element in mammals, being an integral component of enzymes such as glutathione peroxidase, formate dehydrogenase, glycine reductase, thiolase, xanthine dehydrogenase and nicotinic acid dehydrogenase (1, 2). Recent interest in selenium has centered on the ability of this metal, which is present in some foods, to serve as a natural protectant against the toxicity of antitumor agents such as cis-diammine dichloro-platinum (3, 4). There have been additional reports that selenium can inhibit or delay the formation of tumors in animals exposed to chemical carcinogens (5).

However, upon administration to animals this selenium has been found to induce a wide variety of toxic manifestations including growth retardation, gastrointestinal disorders, liver and spleen damage, and hepatocellular carcinomas and adenomas (6). Selenium toxicity can be acutely induced in laboratory animals, but the mechanism by which selenium exerts its toxic effects has not been elucidated.

When selenium is administered in the form of sodium selenite at quantities which exceed nutritional requirements, selenite is processed for pulmonary and urinary elimination by interaction with the tripeptide glutathione (7). Moreover, early studies by DuBois et al. (8) reported that the lethalities in 80% of male rats receiving the minimal lethal dose of selenium were prevented by administration of glutathione in a 10-fold molar ratio 2 hr before administration of selenium. If cellular amounts of glutathione are depleted, selenites are hypothesized to bind covalently to tissue macromolecules, leading to cytotoxicity. Alterations in hepatic reduction of glutathione amounts were investigated in mice as possible factors by which buthionine sulfoximine (BSO), a glutathione depletor, attenuates selenium lethality.

Male mice of the ddY strain, weighing 16–18 g, were purchased and housed as a group of 10 animals in plastic cages. They were kept in a room maintained at an ambient temperature of 23±1 °C and given normal laboratory diet and tap water ad libitum. After reaching 23–25 g of body weight, they were employed for the experiments. BSO, sodium selenite (selenium) and cysteine were dissolved in double-distilled, deionized water prior to injection. Control animals were treated with physiological saline. All injections were given by the intraperitoneal route.

Acute lethality was calculated over a period of 7 days. Hepatic GSH content was measured by the method of Ellman (9) as described by Sedlak and Lindsay (10). Statistical analyses were performed by Student's t-test.

Figure 1 shows the change of hepatic GSH levels at indicated time intervals after injection of BSO (500 mg/kg, i.p.). The GSH levels of liver decreased progressively until it reached a minimal value at 5 hr after BSO adminis-
Fig. 1. Time-course of hepatic glutathione content in buthionine sulfoximine-treated mice. Mice were sacrificed at the indicated time after injection of buthionine sulfoximine (500 mg/kg, i.p., BSO), and 10% homogenates in 0.02 M EDTA solution were prepared from their livers. Control mice was administered the solvent solution for BSO. Values plotted are means (±S.E.M.) of determinations on groups of six animals each. Open symbols are significantly different from the control, P<0.05. Symbols: ——— Control, — O — BSO-treated mice.

Fig. 2. Effect of buthionine sulfoximine on the selenium-induced lethality in mice. Each value is expressed as the percent of lethality in mice obtained from the cumulative data for 7 days after administration of selenium. Buthionine sulfoximine (BSO), 500 mg/kg, i.p., 5 hr before selenium; cysteine, 100 mg/kg, i.p., twice: 5 min before and 20 min after selenium. Control animals were treated with physiological saline. Symbols: ——— Control mice, —— BSO+Selenium, —— Cysteine+Selenium.
tration. This effect of BSO was completely reversed at 48 hr after administration.

The lethality was observed for 7 days in those animal groups treated with selenium in doses of 10, 12.5, 15 and 17.5 mg/kg, being 10%, 60%, 90% and 100%, respectively. In the BSO-treated mice, the lethality of selenium in smaller doses of 2.5, 5.0, 7.5 and 100 mg/kg were 10%, 50%, 80% and 100%, respectively.

In contrast, no lethality was observed in the groups of mice treated with selenium alone in a dose of 7.5 mg/kg or with BSO alone in a dose of 500 mg/kg (Fig. 2).

However, concomitant administration of cysteine (100 mg/kg) decreased the selenium-induced lethality.

Recent studies have shown that BSO is useful as a specific agent for depleting tissue glutathione levels (11). Depletion of cellular glutathione by BSC has been shown to sensitize tumor cells to irradiation (12) or enhanced host toxicity may occur when systemic glutathione depletion is combined with drugs such as doxorubicin (13) or acetaminophen (14), since the adverse side effects of these drugs are caused in part by reducing glutathione content in the target organs. The effects of decreasing cellular thiols in vivo on the lethality of selenium have not been studied.

The metabolic disposition of selenium remains obscure, but elegant studies by Ganther et al. suggested that the conversions of selenite or selenate to demethylelenide exhibits an absolute requirement for glutathione (15–17). The underlying basis for the increased selenium lethality in BSO-treated animals is most likely related to the role of glutathione in the intoxication scheme for selenium as elaborated by Ganther et al. (15–17). In this metabolic scheme, selenite combines nonenzymatically with reduced glutathione to form selenodiglutathione. This compound, in turn, is further metabolized stepwise by glutathione reductase to remove the glutathione groups, leaving hydrogen selenide. This product is then methylated by an S-methyltransferase using S-adenosylmethionine as a donor or methyl radical. Thus, from the data presented, it would appear that hepatic glutathione may interrupt the normal toxification process for selenium and the enhancement of its lethality.

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