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Taurine as a protective agent for 5-fluorouracil-induced hepatic damage related to oxidative stress

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5-Fluorouracil (5-FU) is a pyrimidine analog widely used for the treatment of various cancers, but often causes hepatic damage in clinical practice. In this study, we examined the influence of taurine on 5-FU-induced hepatotoxicity in mice with respect to changes in oxidative stress. Elevations in the aspartate aminotransferase and alanine aminotransferase serum levels after 5-FU administration were significantly suppressed in a dose-dependent manner by concurrent treatment with taurine. The activity of superoxide dismutase and reduced glutathione content in the liver were significantly decreased following treatment with 5-FU alone, but these changes were markedly inhibited by the administration of taurine. Our findings suggest that taurine protects against 5-FU-induced hepatotoxicity by suppressing oxidative stress.

1. Introduction

5-Fluorouracil (5-FU) is a pyrimidine analog widely used for the treatment of different types of solid tumors such as breast, colorectal, gastric, head, neck, and pancreatic cancers. However, this agent often causes hepatic impairment in clinical practice (Moertel et al. 1993; Oettle et al. 1999; Li et al. 2014). Impairment of hepatic function is a clinically significant adverse complication that frequently appears in patients receiving chemotherapy, and occasionally requires withdrawal or dose reduction of administered agents, thereby increasing the risk of therapeutic failure. This drawback narrows the scope of clinical applications of anticancer agents despite contributing to an improved survival rate. Therefore, an attempt to suppress hepatic toxicity of 5-FU may be an important strategy for improving chemotherapeutic outcomes. *In vitro* and *in vivo* studies suggested that administration of 5-FU generates oxidative stress in the liver and subsequently leads to structural and functional deterioration in hepatocytes (Ray et al. 2007; El-Hoseany 2012).

Taurine (2-amino ethanesulfonic acid), a semi-principal amino acid, exists abundantly in mollusca, and is a major constituent of bile in animals. This agent is generally used for therapeutic purposes for liver dysfunction and congestive heart failure, and is also known as an antioxidant. The beneficial properties of taurine have been confirmed in damaged organ repair involving increased oxidative stress caused by the administration of anticancer drugs (Tabassum et al. 2006; Wang et al. 2015; Nagai et al. 2016). In a recent study, it was shown that taurine inhibited histological abnormalities of rat livers exposed to 5-FU (Al-Asmari et al. 2016a), but it is still unclear whether the protective effects of taurine on 5-FU-induced hepatotoxicity can be attributed to the suppression of oxidative stress. Therefore, in the present study, we examined the influence of taurine on 5-FU-induced hepatotoxicity by with respect to changes in oxidative stress using mice.

2. Investigations, results and discussion

First, we investigated the changes in serum activities of biomarkers of hepatic impairment after administration of 5-FU and taurine (Fig. 1). Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were not altered by the 200 mg/kg 5-FU administration (data not shown), but they were significantly increased by administration of 400 mg/kg 5-FU. Elevations in the serum enzyme levels by 5-FU administration were significantly suppressed by treatment with taurine at either dose of 1 mg/kg, 5 mg/kg or 10 mg/kg, but the degree of suppression showed dose-dependency. When 10 mg/kg of taurine was given in combination with 5-FU, serum enzymes remained almost at the control levels, while the treatment with 10 mg/kg taurine alone to mice had no significant effect on the levels of AST or ALT in serum. This finding strongly suggests that taurine has a protective effect on 5-FU-induced hepatotoxicity because the changes in AST and ALT levels in serum are likely to reflect membrane fragility and cellular injury of hepatocytes.

We next examined the changes in the activity of superoxide dismutase (SOD) and the content of reduced glutathione (GSH) in the liver of mice treated with 5-FU in combination with taurine in order to elucidate the mechanism underlying the protection of taurine against 5-FU-induced hepatotoxicity. It is generally believed that the increased production of reactive oxygen species (ROS) responsible for oxidative stress is involved in the onset and progression of liver disorders. This notion is supported by clinical and fundamental evidence that various types of antioxidants exhibit therapeutic effects on liver diseases, including those caused by exposure to anticancer drugs (Li et al. 2015). Implication of ROS in the development of 5-FU-related hepatotoxicity is also presumable in light of a recent report which describes that captopril, acting as a free radical scavenger, protects the liver by suppressing tissular lipid peroxidation estimated as malondialdehyde concentration in rats (El-Hoseany 2012). In addition, ascorbic acid, a well-known antioxidant, also ameliorated the structural alteration of the mouse liver after 5-FU administration (Abou-Zeid 2014), and the attenuation of oxidative stress in the presence of ascorbic acid was demonstrated by an *in vitro* experiment using a goat liver homogenate incubated with 5-FU (Ray et al. 2007). In agreement with these observations, we found that the hepatic SOD activity, as well as GSH content, was markedly reduced by 5-FU exposure (Fig. 2). However, these aggravating alterations were significantly suppressed by the concurrent treatment with taurine (Fig. 2). SOD and GSH are antioxidants present in the body and play an essential role in the removal of ROS and other reactive intermediate metabolites. As these substances work as a defense system against oxidative stress (Pompella et al. 2003), decreases in their activity and content are considered to be due to accelerated consumption by an increase in ROS. Considering the biological behavior of these antioxidative substances, our findings strongly suggest that the preventive effects of taurine on 5-FU-induced hepatotoxicity are attributed to a reduction of oxida-

tive stress. It has been reported that taurine suppresses histological abnormalities of the liver tissue accompanied by massive infiltration of inflammatory cells following enhancement of myeloperoxidase activity, an important marker of inflammation, in rats given 5-FU (Al-Asmari et al. 2016a). This observation is supported by the present results because inflammation is a pathological event that develops secondary to excessive oxidative stress in organ tissues.

Taurine is an ingredient in commercial functional foods and drinks, and many products contain more than 1,000 mg taurine per serving. The presumed daily dose of taurine is much higher than that used in the present study (Rosa et al. 2014). Orally administered taurine is rapidly absorbed from the gastrointestinal tract, with bioavailability being about 90%. From this aspect, taurine may be effective in routine care to suppress 5-FU-induced hepatotoxicity because of the absence of significant toxicity as seen by the lack of changes in AST and ALT levels.

According to a recent report (Al-Asmari et al. 2016b), modulation of redox-sensitive transcription factors and relevant molecules may be associated with the onset of hepatotoxicity after 5-FU administration. Furthermore, there is no information regarding therapeutic interference by taurine on the anticancer potency of 5-FU, and thus, a great concern about the reduction in the pharmacological response of neoplasm to 5-FU is conversely raised, despite the reduced cytotoxic effect on normal cells. Therefore, further examination using cell lines and tumor-bearing mice are required to evaluate whether taurine is a useful biomedical modulator for promoting the use of 5-FU, including investigation of the molecular mechanisms.

In conclusion, the present study demonstrated that taurine protected against 5-FU-induced hepatotoxicity by suppressing oxidative stress.

3. Experimental

3.1. Chemicals

5-FU and taurine were purchased from Wako Pure Chemical Ind. (Osaka, Japan). 5-FU and taurine were dissolved in physiological saline and used for injections into mice. All other reagents were commercial or analytical grade requiring no further purification.

3.2. Animal treatment

Male B6D2F mice aged 5 weeks were obtained from Japan SLC, Inc. (Hamamatsu, Japan). Mice were acclimatized for at least 2 days before assignment to their experi-

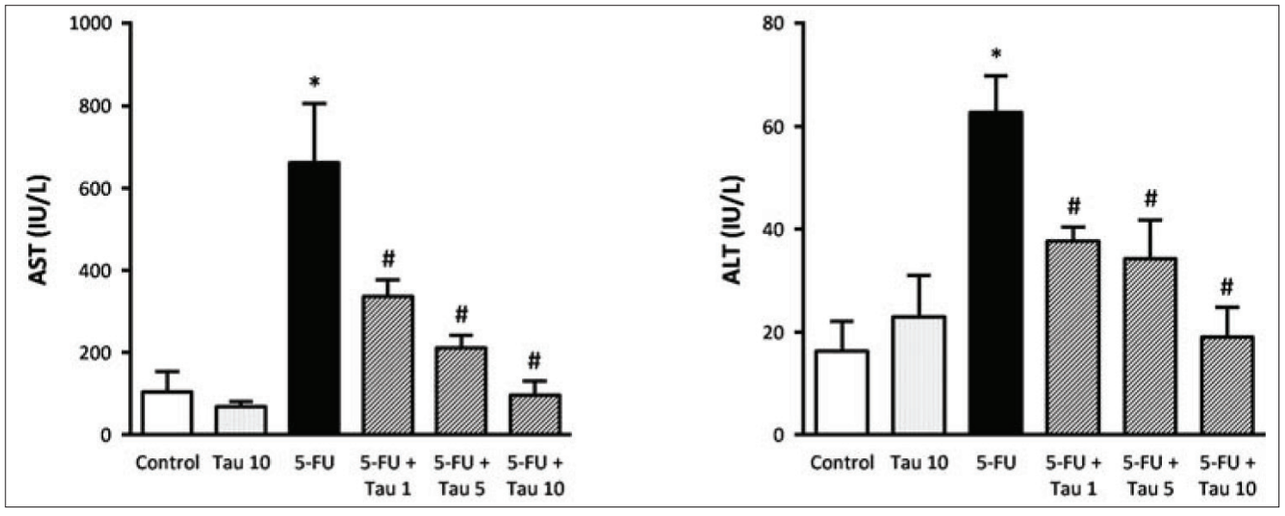

Fig. 1: Effects of taurine on 5-FU-induced changes in blood chemistry. Mice were treated for four days with 400 mg/kg 5-FU and/or 1, 5, 10 mg/kg taurine (Tau 1, 5 or 10, respectively). The activities of AST and ALT were examined on the 5th day. Results are shown as means ± S.D. of four mice per group. *: Significantly different from the mean value of the control group. #: Significantly different from the mean value with the 5-FU group.

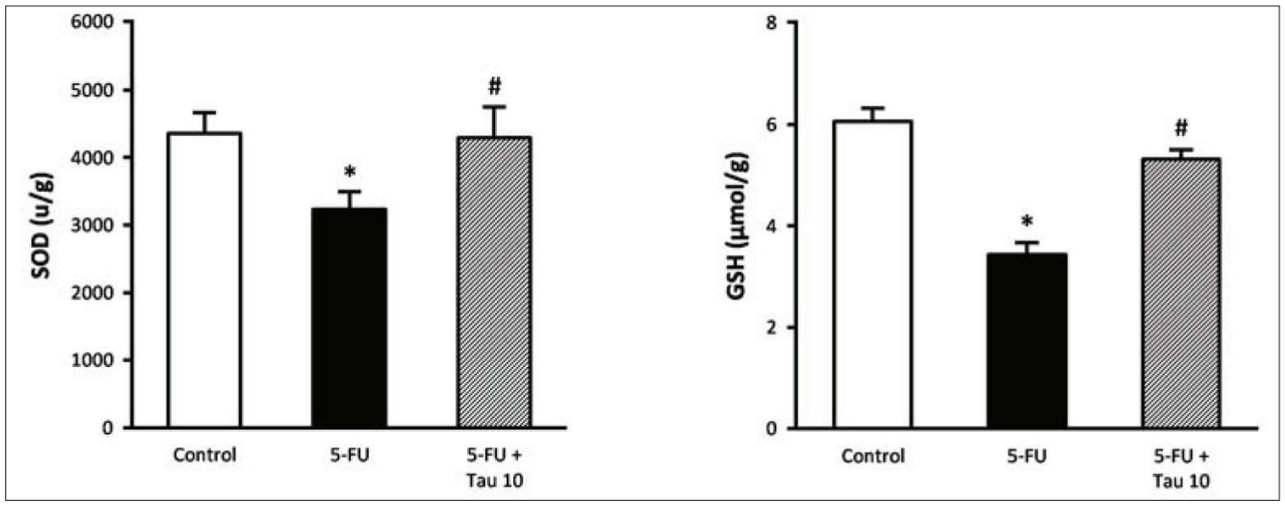

Fig. 2: Effects of taurine on 5-FU-induced changes in hepatic molecular markers related to oxidative stress. Mice were treated with 400 mg/kg 5-FU in combination with or without 10 mg/kg taurine for four days, and the suppressive effects of taurine on decreased SOD activity and GSH content after the 5-FU treatment was examined. Results are shown as means ± S.D. of four mice per group. *: Significantly different from the mean value of the control group. #: Significantly different from the mean value with the 5-FU group.

mental groups, and housed in a clean room maintained at 23±2 °C with a relative humidity of 55±10% and 12-h light/dark cycle. They had free access to a regular animal diet and tap water.

Mice were divided into four treatment groups, designated as 5-FU, taurine, 5-FU plus taurine, and control groups. In the 5-FU group, 5-FU was injected intraperitoneally at doses of 200 or 400 mg/kg once daily for 4 consecutive days. In the taurine group, 10 mg/kg taurine was injected intraperitoneally once daily from the 1st to the 4th day. The 5-FU plus taurine group was divided into three subgroups, designated as the 1, 5 and 10 mg/kg taurine-concurrent treatment subgroups (5-FU + Tau 1, 5-FU + Tau 5 and 5-FU + Tau 10). In these subgroups, 5-FU (400 mg/kg) and taurine (1 mg/kg, 5 mg/kg or 10 mg/kg) were concurrently administered *via* the intraperitoneal route once daily from the 1st to the 4th day. Control mice received only physiological saline at the same time. The volume of vehicle was fixed at 0.025 mL, which was based on the body weight (g) of the mouse. Biochemical examinations using serum samples and liver tissues obtained from mice were conducted on the 5th day as described below. The experimental protocols and animal care methods in the present study were approved by the Animal Experiment Committee at Osaka Ohtani University.

3.3. Biochemical determination

Blood was collected by cardiac puncture under anesthesia, and the serum fraction was separated by centrifugation. The activities of AST and ALT were measured by enzyme-based colorimetric methods using commercial reagent kits run on a biochemistry analyzer (SpotochemTM EZsp-4430 analyzer; ARKRAY Inc., Kyoto, Japan).

3.4. Measurement of superoxide dismutase activity and glutathione content

Liver tissue was dissected immediately and homogenized in ice-cold phosphate buffer (pH 8.0) (0.1/3, w/v). The homogenate was centrifuged, and the supernatant was used in the assay of SOD activity. SOD activity was estimated based on the inhibition of nitroblue terazolium reduction to formazan dye by the xanthine/xanthine oxidase system as a superoxide generator. To determine GSH content, the dissected liver tissue was immediately homogenized in ice-cold 5% 5-sulfosalicylic acid solution (0.1/3, w/v). The homogenate was centrifuged, and the deproteinized supernatant was used for measurement samples. The contents of total glutathione and its oxidative form (GSSG) were measured using a kinetic assay based on a 5,5-dithiobis-2-nitrobenzoic acid/5-mercapto-2-nitrobenzoic acid recycling method using glutathione reductase. The content of GSH was calculated by subtracting that of GSSG from that of total glutathione. SOD activity and GSH content were measured using commercially available kits (Wako Pure Chemical Ind. and Dojindo Molecular Technology, Inc. (Kumamoto, Japan), respectively).

3.5. Statistical analysis

Data were represented as means±S.D. Comparisons among groups were made by analysis of variance (ANOVA) followed by Tukey's test. Differences with a p value of 0.05 or less were considered significant.

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