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Taurine Possesses *In vitro* Antimutagenic Activity Comparable to Major Antioxidants

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Abstract

Taurine is known to suppress oxidant-induced tissue injury by stabilizing biomembrane and scavanging free radicals. The purpose of this study was to determine the antioxidative and antimutagenic activities of taurine, and to compare those activities with major antioxidants. For the measurement of antioxidative capacity, 0.05, 0.1, 0.5 and 1.0 mg/ml of taurine, L-ascorbic acid, alpha-tocopherol, and BHT (dibutyl hydroxy toluene) were prepared and tested for their ability to donate electrons to DPPH (1,1-diphenyl-2-picryl-hydrazyl). Antimutagenic activity was examined using the Ames salmonella test system at concentrations of 600, 900, and 1200 μg/ml. Results indicated that taurine possesses electron-donating capacity, however, the degree of donation was very weak compared to the major antioxidants tested. However, taurine was evaluated as a potent mutation suppressor. Antimutagenic capacity was in increasing order BHT>taurine>L-ascorbic acid>alpha-tocopherol at concentrations of 600 and 900 μg/ml. There was a dose-dependent increase in antimutagenicity of these compounds, however, antimutagenicity of the 900 μg taurine/plate was not significantly different from that of 1200 μg taurine/plate. These results indicate that taurine effectively suppresses the mutagenicity of AFB_1 without noticeable electron donating ability.

Key words: taurine, antioxidants, antimutagenicity

INTRODUCTION

Taurine, 2-aminoethane sulfonic acid, is one of the most abundant free amino acid in a variety of animal tissues. It is formed from cystein by removal of the carboxylic group and oxidation of the sulfur to form a sulfonic acid group. The carboxylic, sulfonic, sulfhydryl, and amino groups all undergo ionization, and the dominant species at physiologic pH is shown in Fig. 1. Taurine possesses many biological activities related to the pathophysiology of cardiovascular and nervous systems (1,2). Recently, antioxidative and antiinflammatory effects of taurine have been reported (3,4), and one of the possible expianations for its antioxidative capacity is to scavange free radicals including reactive oxygen species (ROS).

The ROS are generated during normal metabolic processes such as the reduction of oxygen to water by an enzyme catalized reaction (5). One of the most destructive effects of ROS is the initiation of lipid peroxidation causing cell membrane damage. Membranes of retinal rod outer segments are rich in polyunsaturated fatty acids and vulnerable to peroxidative damage (6,7), and taurine was shown to have protective actions on the functions of retinal rod outer segments (8). Bleomycin, carbontetrachloride, paraquat, NO_2 and ozone produce oxygen free radicals, and taurine protects tissues from these oxidative agents (9). Free radicals including ROS are produced during normal metabolic conversion of chemical carcinogens such as benzo[a]pyrene, N-methyl-N'-nitro-N-nitrosoguanidine, and aflatoxin B_1 (10). These oxidation products are capable of damaging DNA and producing mutagenicity. Oxidative damage to DNA, protein, lipid and other macromolecules accumulates over time, and the body's ability to repair this damage decreases with age resulting in degenerative diseases. Therefore, radical-scavanging activity of a compound may well be correlated to their antimutagenicity. Recent studies have indicated that anti-carcinogenic properties of many dietary antioxidants including vitamin E, vitamin C, and beta-carotene are derived from their inhibitory effects on radical formation (11). In the present study, antioxidative and antimutagenic activities of taurine were examined, and compared to those of known antioxidants, such as vitamin C, alpha-tocopherol, and BHT.

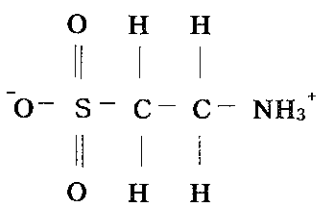

Fig. 1. Structure of taurine.

MATERIALS AND METHODS

Reagents

L-ascorbic acid, DL-α-tocopherol, taurine, DPPH (1,1-diphenyl-2-picryl hydrazyl), aflatoxin B_1, DMSO (dimethyl sulfoxide), NADP, L-histidine · HCl, biotin, sodium ammonium

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phosphate were purchased from Sigma Chemical Co. (St. Louis, USA). Bacto agar and nutrient broth were purchased from Difco Co. (Grand Island, NY).

Measurement of electron donating ability

Electron donating ability (EDA) was measured based on the method of Blios (12) which measures EDA of taurine and major antioxidants to DPPH. Briefly, 20 mg of DPPH was dissolved in 150 ml ethanol, and taurine, ascorbic acid, alpha-tocopherol and BHT were dissolved in methanol to give concentrations of 0.05, 0.1, 0.5, 1.0 mg/ml. A half ml of each sample solution was added to vials containing 0.5 ml of DPPH solution, and mixed throughly for 5 seconds. After 30 minutes of room temperature incubation, absorbance was measured at 570 nm. Absorbance of the vial containing each compound was compared to that of the vial containing DPPH only. Percent EDA was calculated by the following formula.

EDA (%)=(1-sample absorbance/control absorbance)×100

Mutagenicity test

Salmonella mutagenicity test was performed as described by Maron and Ames (13) using Salmonella typhimurium TA 100. To confirm the genotype of tester organism, histidine requirement test, *rfa* mutation test, *uvr*B mutation test, R factor test were performed. As a metabolic activation system, S9 mix was prepared from the liver of Sprague Dawley rats injected with Aroclor 1254 before sacrifice, based on the method of Ames et al. (14). For the experiment, 0.5 ml of 4% S-9 mix, 0.1 ml of bacterial cell suspension ($1\sim2\times10^9$ cells/ml), 50 μg/ml AFB_1, and 0.5 ml of each sample solution were mixed in a cap tube and incubated for 30 minutes at 37°C. Two ml of top agar (45°C) were added to each tube, mixed, and poured on minimal glucose agar plate. Plates were incubated for 48 hours at 37°C and the number of revertant colonies were counted. The concentrations of AFB_1 and sample solutions were determined by pilot tests. Antimutagenic activity of each compound was expressed as % inhibition compared to the positive control.

Statistical analysis

Values of antioxidative and antimutagenic activities of taurine and major antioxidants were compared by the ANOVA and Duncan's multiple range test. Analyses were performed using the Statistical Analysis System (SAS/STAT version 6, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Electron-donating ability (EDA) of taurine and major antioxidants

Electron donating abilities (EDA) of taurine and major antioxidants to DPPH (1,1-diphenyl-2-picryl-hydrazyl) were measured. The nitrogen atom in DPPH easily accepts an electron and loses its purple color. Taurine possessed a weak electron donating ability. EDA of test compounds were in increasing order L-ascorbic acid>alpha-tocopherol> BHT> taurine (Fig. 2).

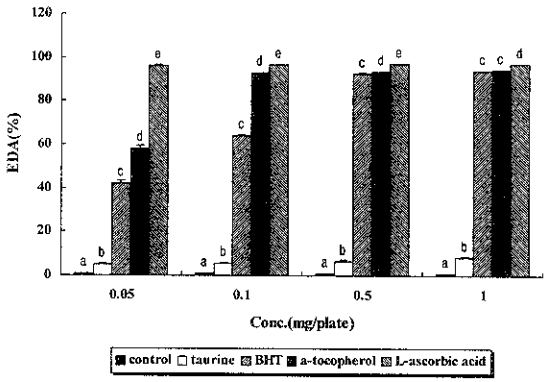

Fig. 2. Electron donating abilities (EDA) of major antioxidants to DPPH radicals. Bars with different letters (a,b,c,d) are singnificantly different from each other at $p<0.01$ as determined by Duncan's multiple range test.

Taurine possessed 5 to 8% EDA at the concentration range used, while vitamin C showed more than 96% of EDA at all concentrations. Alpha-tocopherol at concentrations above 0.1 mg/ml, and BHT at concentrations above 0.5 mg/ml revealed more than 90% EDA. There was a significant dose-dependent EDA increases in all compounds tested ($p<0.01$).

Taurine has been shown to inhibit oxidant-induced lung damages *in vivo*. In rats, when pneumocytes were exposed to ozone, cell membrane leakage was evident with increased appearance of malondialdehyde in the medium (15), while taurine treatment prevented this membrane damage. Taurine in coordination with niacin inhibited the bleomycin induced pulmonary fibrosis by decreasing lung malondialdehyde contents and superoxide dimutase activity (16). Lung injury initiated by cyclophospharnide administration were significantly reduced by taurine/niacin treatment (17). These effects were accompanied by decreased inflammatory reactions, less lipid peroxidation and increased restoration of antioxidants. Lung injury caused by paraquat in hamsters was also inhibited by taurine (18). Since major effect of paraquat appears to be lipid peroxidation of plasma membranes, it is assumed that taurine acted on the membrane. All of these *in vivo* studies confirm that oxidative tissue damages might be prevented by taurine administration through two possible mechanisms. As a direct antioxidant, taurine would act to quench radicals derived from the interaction of oxidants with membrane lipids. As an indirect antioxidant, taurine would act to stabilize the plasma membrane and thus prevent oxidant-induced increases in membrane damage. However, the results from this study showed taurine to be a very weak electron donor, implying that its action might be mediated through an indirect mechanism, possibly a membrane stabilizing activity. A previous study (19) on rats fed diet supplemented with cholesterol or cholesterol plus taurine for 5 weeks indicated that taurine supplementation resulted in modifications in hepatic total lipid and phospholipid fatty acid composition. Animals fed taurine-supplemented diet showed elevated percentage of total saturated fatty acids and decreased

percentage of total monounsaturated fatty acids compared to the values for controls. Such changes in hepatic phospholipid fatty acid composition might be associated with the modulation of physical characteristics and stability of the membrane. Also, a recent study indicated that taurine is ineffective in decreasing Fe^{2+} oxidation and lipid hydroperoxide dependent lipid peroxidation *in vitro* (20). In liver carcinogenesis models, hepatic microsomal TBARS were not decreased by taurine supplements (21). These results imply that taurine exerts its antioxidative effect on membranes by a mechanism other than direct scavanging. The free energy difference between a sulfonate group at oxidation state +4 and a sulfate group at oxidation state of +6 is around 260 kJ/mol (22). Therefore, theoretically, the sulfonate group of taurine can serve as reducing agent by undergoing oxidation to sulfate. However, taurine was shown to react poorly with superoxide, peroxide, and the hydroxyl radical (23). This study showed that taurine has a very weak ability to donate an electron to DPPH which implies that the conversion of sulfonate group of taurine to sulfate may not be favored. However, taurine reacts with hypochlorite which is a powerful oxidant generated from leukocytes and neutrophils (9). Also, hypotaurine which is enzymatically oxidized to taurine has been observed as an effective radical scavanger (9).

Effects of taurine and major antioxidants on aflatoxin B_1-induced mutagenicity in *Salmonella typhimurium* TA 100

Aflatoxin B_1 is a mycotoxin produced by *A. Flavus* and *A. parasiticus*. In the body AFB_1 is metabolized to AFB_1-8,9-epoxide and binds to cellular DNA causing mutagenicity (24). Inhibitory effects of taurine and major antioxidants on AFB_1 induced mutagenicity in Salmonella typhimurium TA 100 which is shown in Table 1. Supplemention of taurine as well as major antioxidants significantly reduced the mutagenic activity of AFB_1 ($p<0.01$). Taurine at a concentration of 600 μg/plate inhibited 51.3% of AFB_1 mutagenicity, and at a concentration of 900 μg/plate, its antimutagenic activity was increased to 62.4%. No significant difference in antimutagenic activity was observed between 900 and 1200 μg taurine/plate supplementation. Antimutagenic activity of taurine was significantly higher than those of ascorbic acid when 600 and 900 μg/plate were used. Among compounds tested, BHT was most effective in suppressing AFB_1 mutagenicity ($p<0.01$).

Table 1. Inhibition rate (%) of various antioxidants on the mutagenicity induced by aflatoxin B_1 (AFB_1, 1 μg/plate) in *Salmonella typhimurium* TA 100

Treatment	Inhibition rate (%)			
Conc. (μg/plate)	Ascorbic acid	α-Tocopherol	BHT	Taurine
Positive control		0[z]		
600	32.1±6.9[1)cy]	39.4±4.9[bcy]	77.7±5.8[ay]	51.3±2.8[by]
900	43.0±3.5[cy]	53.6±5.0[bx]	83.6±0.4[ay]	62.4±2.3[bx]
1200	62.6±3.7[cx]	73.5±3.3[bw]	94.4±2.2[ax]	67.7±3.2[bcx]

[1)]Values are mean±S.D.

[a~c]Numbers with different superscripts within the same row are significantly different at $p<0.01$.

[w~z]Numbers with different superscripts within the same column are significantly different at $p<0.01$.

DNA mutation occurs when electrophilic compounds such as oxidative intermediates bind to cellular nucleophilic compartments including proteins, lipids and DNA (25). These electrophilic compounds are derived from many different sources including chemicals and natural toxins. *In vivo*, these compounds are metabolized to very reactive derivatives by mixed function oxidase system. Antimutagenic effects of most known antioxidants are mediated through their electron-donating ability to these reactive compounds (11). However, in this study, taurine exhibited antimutagenic activity comparable to those of major antioxidants, without a noticeable electron donating ability to DPPH. Therefore, the answer may lie in mechanisms other than their electron donating capacity. Possible explanations are: 1) taurine may bind directly to AFB_1; 2) it may alter the enzyme activities involved in the metabolism of AFB_1; 3) membrane stabilizing ability of taurine may be involved. Previous reports (15) indicated taurine acts as a membrane stabilizer by reducing oxidative stress-induced membrane permeability which may control the passage of intra- and extracellular compounds such as mutagens.

Results from this study imply taurine is a potent antimutagen and might also be a chemopreventive agent. Further investigations are required to clarify mechanisms of taurine's antimutagenic activity and possible roles in carcinogenesis.

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