The Effect of Taurine, a Novel Biochemical Modulator, on the Antitumor Activity of Doxorubicin

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Taurine is contained in seafood and has been studied extensively on life-style related diseases. Theanine increased the effects of the doxorubicin (DOX) as an antitumor agent in some tumors and enhanced the DOX level in tumor cells. It is expected that the advanced effect of food uptake in cancer chemotherapy may be effective from the viewpoint of quality of life (QOL) improvement, although this approach has not been investigated in detail. In this study, the effect of taurine as a functional amino acid was examined. Taurine did not change the DOX influx into M5076 cells, whereas it significantly inhibited DOX efflux, which maintained the DOX level in tumor cells. Furthermore, experiments with taurine decreased tumor weight by 40%, compared to the DOX-alone group and significantly increased its antitumor effect. Moreover, as taurine did not increase DOX concentration in normal tissue, it is suggested that it increased the antitumor effect without enhancing DOX-induced adverse effects. DOX efflux is inhibited by β-alanine as a taurine transporter inhibitor, therefore, enhancement of the DOX level by taurine was suggested to act via taurine transport. Namely, it was clarified that taurine was useful as a modulator to enhance the therapeutic index of cancer patients and improve QOL.

Key words taurine; doxorubicin; M5076 ovarian sarcoma; taurine transporter; permeability

In cancer therapy, novel chemotherapeutic agents are being developed continually,1) however, cancer-induced lethality is still high in many countries because of recalcitrant tumors and the appearance of resistant tumors. Furthermore, adverse reactions by antitumor drugs not only inhibit the therapeutic index but also require the use of combined agents to decrease these adverse reactions and the quality of life (QOL) of patients can worsen. While the development of novel antitumor drugs is a long and costly process, increasing the effectiveness of current therapies is also very important and should be addressed in clinical trials. As an example combined medicines, such as p-glycoprotein inhibitor in resistant tumors, have been used to increase the effectiveness of current antitumor agents.2–4)

From the viewpoint of biochemical modulation, combined medicines such as Tegaful have been applied in clinical therapy. However, it has been reported that some combined therapies increase the adverse effects of antitumor drugs as the antitumor effect increases,5) and effective enhancement of the therapeutic index is not achieved. Furthermore, the QOL of patients can be affected negatively by many medicines. It is hoped to increase antitumor agent activity by combining antitumor therapies with foods that do not show adverse reactions or decrease QOL.

From such a viewpoint, it was reported previously that theanine, an amino acid peculiar to green tea, has properties to increase the efficacy of doxorubicin (DOX) as an antitumor drug.6)

This action is due to the glutamate transporter expressed in the tumor cell membrane and is dependent on the influx inhibition of glutamate by theanine inhibition of this transporter and the resulting decrease in glutathione (GSH) synthesis.7) Theanine did not increase DOX-induced adverse reactions in normal tissue, whereas reduced its adverse effects, and was therefore effective in enhancing the antitumor effect while decreasing the adverse effects of antitumor drugs. It is expected that there are other compounds with similar effectiveness, such as an amino acids or peptides, in other foods.

Taurine (2-amino ethanesulfonic acid) is a major amino acid in fish and is found in all mammalian tissues. In clinical therapy, taurine is used to protect the liver and improve function with congestive cardiac failure. This study investigated the effect of taurine on the tumor cell permeability of DOX in vitro, studied its action on the antitumor effect of DOX in vivo, and elucidated the novel effect of taurine to increase DOX-induced antitumor activity.

MATERIALS AND METHODS

Chemicals Taurine, β-alanine was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). DOX 10 mg/vial (Adriacin), was purchased from Kyowa Fermentation, Inc. (Tokyo, Japan). RPMI 1640 medium was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). [3H]-Taurine (9.5 MBq/μmol) was purchased from Amersham (Tokyo, Japan). Scintillation cocktail Scientisol Ex-H®, β-alanine, and γ-amino butyric acid (GABA) were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

The other chemicals used in this study were of the highest purity available.

Animals Male C57BL/6 and BDF 1 mice (5 weeks old, weighing 20—25 g) were obtained from Japan SLC, Inc. (Hamamatsu, Japan). The animals were housed in a room maintained at 25±1°C with 55±5% relative humidity and were given free access to regular chow pellets and water.

Effects of Taurine or Taurine Transporter Inhibitors on the DOX Concentration in Tumor Cells in Vitro M5076 ovarian sarcoma cells (1×106 cells/animal) were transplanted intraperitoneally into male C57BL/6 mice. Ascites fluid was collected on the 14th day after transplantation. Sarcoma cells were washed twice and then resuspended in RPMI 1640 medium containing 10% fetal bovine serum.

To examine the influx of DOX into M5076 ovarian sarcoma cells, cells (5×105 cells/ml medium) were incubated...
with 9.0 nmol/ml of DOX at 37 °C for 60 min in the presence or absence of taurine (100 or 500 μM) or taurine transporter inhibitors.

To examine the effect of taurine on DOX efflux from M5076 ovarian sarcoma cells, the cells were preincubated with 9.0 nmol/ml of DOX in medium at 37 °C for 30 min. After incubation, the medium was cooled on ice and then centrifuged at 150 g for 3 min. The cells were washed and then resuspended in fresh medium. This cell suspension (5 × 10^5 cells/ml) was incubated at 37 °C for 120 min in the presence or absence of taurine (100 or 500 μM) or taurine transporter inhibitors.

In both systems, after incubation, the medium was cooled on ice and then centrifuged at 150 g for 3 min. The cells were washed and resuspended in ice-cold phosphate buffer (10 mM, pH 7.8). The suspension was mixed for 30 s with 5.0 ml of chloroform–methanol (4:1, v/v) and then centrifuged (1200 g, 15 min). The concentration of DOX in the organic phase was determined using a fluorescence spectrophotometer (excitation wavelength, 470 nm; emission wavelength, 585 nm).

Identical examinations were performed using Ehrlich ascites carcinoma cells.

**Effect of Taurine on the Antitumor Activity Induced by DOX in Vivo** M5076 ovarian sarcoma cells (5 × 10^5 cells/animal) were transplanted into the backs of BDF1 mice. DOX (2.0 mg/kg/d for 4 d) was administered intraperitoneally at 18, 20, 22, and 24 d post-inoculation. Taurine (10 mg/kg/d for 4 d) was injected intraperitoneally at 19, 21, 23, and 25 d post-inoculation. The mice were sacrificed by cervical dislocation on the 26th day, and then the solid tumors, livers, and hearts were removed immediately and weighed. Tissue samples were homogenized in 10 volumes (w/v) of 10 mM phosphate buffer (pH 7.8). Each suspension was mixed for 30 s with 5.0 ml of chloroform–methanol (4:1, v/v) and then centrifuged (1200 g, 15 min). The DOX concentration was determined as described above.

**[^3H]-Taurine Intracellular Transport Activity Assay** The [^3H]-taurine transport activity in tumor cells followed the method of Takeuchi et al. [12]. M5076 cells or Ehrlich cells were suspended in incubation buffer (125 mM NaCl, 4.5 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 5 mM glucose, pH 7.4) and preincubated at 25 °C for 5 min. The reaction medium with taurine (0.1 μM [^3H]-taurine) was incubated at 25 °C. In addition, β-alanine (100 μM) or GABA (100 μM) was added to the cell suspension with [^3H]-taurine. After centrifugation at 200 × g for 5 min, and washing twice, the sample was added 0.25 ml NaOH (200 μl) after and the cells were resuspended in ice-cold phosphate-buffered saline (pH 7.0: PBS). Aliquots (100 μl) were resuspended in scintillation cocktail Scintisol Ex-H® (5.0 ml) and radioactivity was measured using aliquid scintillation counter.

**Statistical Analysis** Statistical analysis was performed using Student's t-test and ANOVA.

**RESULTS**

The effect of taurine on DOX influx into M5076 ovarian sarcoma cells is shown in Fig. 1. The DOX concentration in the tumor cells increased gradually, whereas the cellular concentration of DOX did not change with increasing taurine concentration. For DOX efflux, the intracellular DOX concentration decreased transiently but the addition of taurine depressed DOX efflux over a 60 min incubation period. In particular, the level of DOX efflux with 500 μM taurine was 5% and 45% of that in DOX-alone group at 60 and 120 min, respectively (Fig. 2). In Ehrlich ascites carcinoma cells, taurine had the same effect as in M5076 ovarian sarcoma cells (data not shown).

**Effect of Taurine on the Antitumor Activity Induced by DOX in vitro** The effect of taurine on DOX uptake into M5076 ovarian sarcoma cells is shown in Fig. 3. M5076 sarcoma cells were incubated with DOX (9.0 nmol/ml) in the presence or absence of taurine (100 or 500 μM) at 37 °C for 30 or 60 min. Each point is the mean ± S.D. (n=4).

**Fig. 1. Effects of Taurine on DOX Influx into M5076 Ovarian Sarcoma Cells**

M5076 cells were incubated with DOX (9.0 nmol/ml) in the presence or absence of taurine (100 or 500 μM) at 37 °C for 30 or 60 min. Each point is the mean±S.D. (n=4).

**Fig. 2. Effects of Taurine on DOX Efflux from M5076 Ovarian Sarcoma Cells**

M5076 cells were incubated with DOX (9.0 nmol/ml) in the presence or absence of taurine (100 or 500 μM) at 37 °C for 60 or 120 min. Each point is the mean±S.D. (n=4). Significant differences from the level of the DOX-alone group (control) are indicated by a) *p*<0.05 and b) *p*<0.01.

**Fig. 3. Effects of Taurine on the Antitumor Activity of DOX against M5076 Ovarian Sarcoma**

M5076 ovarian sarcoma cells (5 × 10^5 cells/animal) were transplanted into the backs of BDF1 mice. DOX (2.0 mg/kg/d for 4 d) was administered intraperitoneally at 19, 21, 23, and 25 d post-inoculation. Each column is the mean±S.D. (n=4). Significant differences from the level of the DOX-alone group are indicated by a) *p*<0.001.
In Ehrlich ascites carcinoma cells, the intracellular level of [3H]-taurine decreased by 31.3% (p<0.01) at 5 and 30 min, respectively, compared to the control level. The addition of taurine (100 μg/kg) had the same effect on tumor weight, and the DOX concentration in the tumor in the taurine group was significantly inhibited by 23.2% (p<0.01) compared to that in the control group. The presence of β-alanine or GABA as taurine transporter inhibitors significantly promoted DOX efflux compared to that in the taurine group.

**DISCUSSION**

The consumption of beneficial foods after cancer chemotherapy may be effective from the viewpoint of improvements in QOL, although this approach has not been investigated extensively. In our previous reports, theanine has shown to increase antitumor effect by DOX. This study examined the effect of taurine as a functional amino acid.

Taurine is an aminosulfonic acid, which comprises 1—2% in seafood, including top shell, cuttlefish, and sea plum, but only low levels are found in meat. Taurine has been used as a medicine to improve hepatic function in congested heart failure and hyperbilirubinemia. Taurine has a regulatory effect on calcium in cardiac muscle, lowers blood pressure, and increases the excretion of bile acid. Taurine is widely used as a supplement to help recovery from stress.

The combined effects of taurine with antitumor agents have not been examined previously. In this study, taurine did not change the DOX influx into M5076 cells, whereas it significantly inhibited DOX efflux, which resulted in maintaining DOX levels once reached in tumor cells. In vivo experiments, taurine administration decreased tumor weight by 40%, compared to the DOX-alone group and significantly increased its antitumor effect. Moreover, as taurine did not increase DOX concentrations in normal tissues, it is suggested that it increased the antitumor effect without enhancing DOX-induced adverse effects. Tissue distribution of taurine is important factor in its effect. We expect that taurine distributed in tumor in vivo, and directly or indirectly affected on DOX concentration in tumor.
Taurine is transported with Na\(^+\) and Cl\(^-\) by taurine transporter. Furthermore, taurine transport is changed osmotic pressure in cell. Namely, these changes by taurine were considered to induce the changes of other transporters on DOX transport in tumor cells. It was very useful as a modulator to enhance the therapeutic index of cancer patient QOL.

To elucidate the enhancement mechanism of the DOX antitumor effect by taurine, a focus was made on DOX transport across the tumor cell membrane. Taurine is transported by a taurine transporter,\(^{11,12)}\) but it was not indicated that taurine transporters are connected with the membrane transport of DOX.

The taurine transporter belongs to the SLC6A6 family, which contains various nerve transmitter substances, has over ten subtypes, have a characteristic twelve-loop transmembrane domain, and are symporters that carry transport substances with Na\(^+\) and Cl\(^-\). Taurine is recognized as a substrate of the taurine transporter, and GABA and \(\beta\)-alanine are taurine transporter inhibitors.\(^{11,12)}\) The chemical structure of these inhibitors is similar to that of taurine and they are competitive substrates. \(\beta\)-Alanine and GABA are known as inhibitors of taurine transporter. \(\beta\)-Alanine and taurine are \(\beta\)-amino acids, and have high affinity for taurine transporter. Taurine transporter may contribute to taurine uptake in keratinocyte. \((K_i\) value of inhibitor for the taurine uptake in keratinocyte, taurine\(=5\) \(\mu\)M, \(\beta\)-alanine\(=40\) \(\mu\)M, GABA\(=330\) \(\mu\)M).\(^{13)}\) There are not a report on the expression of SLC6A6 transporter in M5076 ovarian sarcoma cells. However, we confirmed the intracellular uptake of \[^{3}\text{H}]\)-taurine in keratinocyte, taurine\(=5\) \(\mu\)M, \(\beta\)-alanine\(=40\) \(\mu\)M, GABA\(=330\) \(\mu\)M).\(^{13)}\)

**REFERENCES**