Basic Research Paper

Synergism and attenuation effects of taurine on cyclophosphamide

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Key words: sarcoma, S-180 cell line, taurine, cyclophosphamide, synergism, attenuation, mouse

Background and Objective: Cyclophosphamide (CTX) is a commonly used clinical antitumor drug with severe side effects. Therefore, it is important to find ancillary drugs which have synergism and attenuation effects on CTX. This study was to investigate the synergism and attenuation effects of taurine (Tau) on CTX via different administration methods. Methods: S_{180}-bearing mice were given Tau combined with CTX via either intravenous injection or intragastric administration. The tumor inhibition rate, the count of bone marrow nucleate cells and white blood cells, the spleen index, the thymus index, lymphocyte proliferation and the phagocytic activity of peritoneal macrophages were calculated. Results: The tumor inhibition rates of intravenous injection of 40 mg·kg⁻¹, 80 mg·kg⁻¹ and 160 mg·kg⁻¹ Tau combined with CTX (20 mg·kg⁻¹) were 66.4%, 74.5% and 84.6%, while those of intragastric administration of 160 mg·kg⁻¹, 320 mg·kg⁻¹ and 640 mg·kg⁻¹ Tau with CTX (20 mg·kg⁻¹) were 60.1%, 69.7% and 81.2%, all of which were higher than that of administration of CTX (20 mg·kg⁻¹) alone (55.8%). Compared to the CTX group, the count of bone marrow nucleate cells and the white blood cells, the spleen index, the thymus index, lymphocyte proliferation and the phagocytic activity of peritoneal macrophages were elevated in all Tau and CTX combination groups. Conclusion: Tau has synergism and attenuation effects on CTX via both intravenous and intragastric administration.

Cyclophosphamide (CTX), a common alkylating agent with a wide anti-tumor spectrum, is clinically used for the treatment of malignant tumors, including malignant lymphoma, multiple myeloma, leukemia and breast cancer. However, the effect of CTX lacks tumor specificity, which damages normal cells when it kills tumor cells. In particular, it inhibits hematopoietic cells with high proliferative potential and the immune system, leading to peripheral leukopenia and suppressed immune function. Therefore, it is imminent to find ancillary drugs to antagonize myelosuppression and increase immune functions during chemotherapy. Taurine (Tau) is a conditional essential amino acid of the human body, which is extensively distributed in all cells of mammals.¹ Tau is rich in neutrophils, lymphocytes and monocytes, accounting for 50–70% of all free amino acids. Many studies have revealed that Tau can promote immunological functions, which can be used as a good immunological adjuvant.²,³ Moreover, some reports show that Tau exerts certain inhibitory effects on tumors.⁴,⁵ In this study, the S_{180}-bearing mouse model was used to investigate the synergism and attenuation effects of Tau on CTX via intravenous (IV) and intragastric (IG) administration, in order to provide experimental evidence for developing Tau into an ancillary drug for chemotherapy.

Materials and Methods

Animals and cell line. Swiss mice (clean grade II) weighing 20 ± 2 g, half males and half females, were provided by Henan Provincial Center of Experimental Animals (certificate No. 410117). The mouse sarcoma cell line (S_{180}) was provided by Union Medical College and was passaged as peritoneal effusion in our laboratory.

Drugs and reagents. Tau was purchased from Tianjin Rgent Chemical Co., Ltd. (Lot number: Jin Q/HX0016-2005). CTX was purchased from Jiangsu Hengrui Medicine Co., Ltd. (Lot number: 06043021). Concanavalin A, lipopolysaccharide and MTT were all the products of Sigma Company, and RPMI-1640 was the product of Gibco biotechnology Company.

Instruments and equipments. The multifunction microscope was manufactured by Olympus, Japan. The CO₂ incubator was the product of Heraeus, Germany. The microplate reader was the product of BIO-RAD, USA.

Establishment of the mouse xenograft model. Seven days after inoculation with S_{180} cells, mice with well-grown peritoneal effusion were selected. Skin of the abdomen was sterilized using alcohol and peritoneal effusion, ivory white thick fluid, was extracted and collected. Cell viability was confirmed to be more than 95% by the trypan blue method. The cells were diluted into 1 × 10⁷ cells/mL suspension using normal saline (NS). Then, 0.2 mL of S_{180} cell suspension (2 × 10⁶ cells) was subcutaneously inoculated into each mouse via the right axillary fossa.

Grouping and administration. S_{180}-bearing mice were divided into 12 groups with 10 in each group (Tables 1–4) The model group was administered with 0.2 mL NS.
The drug was administered to each group 24 h after inoculation, once a day for eight consecutive days. The animals were housed routinely. Water and food were provided ad libitum. All indices were detected 24 h after the last administration of the drug.

**Determination of the tumor inhibition rate, spleen index and thymus index.** The mice were sacrificed by dislocation of the neck 24 h after the last administration of the drug. The tumor, spleen and thymus of the mice in each group were dissected and weighed. The tumor inhibition rate, spleen index and thymus index were calculated respectively. Tumor inhibition rate = \[\frac{\text{average tumor weight of control group (g) – average tumor weight of treatment group (g)}}{\text{average tumor weight of control group (g)}} \times 100\%\]. Thymus index = thymus weight (g) / body weight (g). Spleen index = spleen weight (g) / body weight (g).

**Count of peripheral white blood cells and bone marrow nucleated cells.** Blood was drawn by cutting off the tail 24 h after the last administration of the drug, and peripheral white blood cells were counted. The mice were sacrificed by dislocation of the neck and the right thigh bone was isolated. Bone marrow was rinsed repeatedly with RPMI-1640 using a syringe needle, and the number of bone marrow nucleated cells in the rinsing solution was determined.

**Determination of spleen T and B lymphocyte transformation rates.** The mice were sacrificed by dislocation of the neck 24 h after the last administration of the drug. The transformation rates of spleen T and B lymphocytes were determined as previously described.7,8

**Determination of phagocytic activity of peritoneal macrophages.** The mice were sacrificed by dislocation of the neck 24 h after the last administration of the drug. Phagocytic activity of peritoneal macrophages was determined as previously described.9

**Statistical analyses.** Experimental data were expressed as mean ± SD. Statistical analysis was performed using SPSS11.5 software and single factor variance analysis was adopted. The Tukey HSD test was utilized for inter-group comparison. p < 0.05 was considered statistically significant.
model group, the proliferation rates of T and B lymphocytes were decreased in the CTX group (p < 0.01), while were increased in all doses of Tau (IV and IG) groups (p < 0.01). Compared with the CTX group, the proliferation rates of T and B lymphocytes were increased in all doses of Tau (IV and IG) combined with CTX groups (p < 0.01) (Table 4).

Discussion

In the present study, the S180-bearing mouse model was used to investigate the synergism and attenuation effect of Tau on CTX via intravenous and intragastic administration. The results showed that Tau enhanced the tumor growth inhibition effect and attenuated the toxicities of CTX, including myelosuppression and immunodepression.

Many researches have studied the ancillary effect of traditional Chinese medicines on improving the immune function. However, there are only a few reports on attenuating myelosuppression. Although some Western medicines can increase the count of white blood cells, they do not obviously improve immunosuppression. Tau, a conditional essential amino acid of the human body that is extensively distributed in all kinds of cells in mammals, is involved in maintaining homeostasis and plays an active role in the central nervous system, cardiovascular system, genital system, digestive system, endocrine system and immune system.\(^1^0,1^1\) The molecular weight of Tau is small and it has no antigenicity. Therefore, Tau can be easily absorbed by all routes of administration. In addition, Tau has no toxicity; its source is extensive, and can be obtained by natural extraction, microorganism fermentation and chemical synthesis.\(^1^2\) Currently, Tau is used in clinic for the treatment of many diseases. So far, there is no report on Tau as an ancillary drug for chemotherapy.

It was reported that Tau exerted a chemo-protective effect on liver cancer induced by diethylinitrosamine and phenobarbital,\(^1^3\) decreased the incidence rate of infective colorectal adenocarcinoma\(^1^4\) and decreased the tumor numbe. In this study, the tumor growth inhibition rate was increased in Tau combined with CTX groups in S180-bearing mice compared with administration of CTX alone, indicating that combination with Tau could enhance the tumor inhibition effect of CTX.

The count of bone marrow nucleated cells is an index which directly reflects hematopoiesis. A large number of bone marrow nucleated cells represent a large number of immature blood cells, which indicates good bone marrow hematopoiesis.\(^1^5,1^6\) The count

![Table 2 Effects of taurine combined with cyclophosphamide on the count of bone marrow nucleate cells and white blood cells of S180-bearing mice (mean ± SD)](attachment)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg·kg⁻¹)</th>
<th>Mouse number</th>
<th>WBC (×10³·mL⁻¹)</th>
<th>Bone marrow nucleate cell (×10²·mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>begin</td>
<td>end</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>8.00±0.08</td>
</tr>
<tr>
<td>CTX</td>
<td>20</td>
<td>10</td>
<td>9</td>
<td>6.00±0.10</td>
</tr>
<tr>
<td>Tau (IV)</td>
<td>40</td>
<td>10</td>
<td>9</td>
<td>3.30±0.07</td>
</tr>
<tr>
<td>Tau (IV)</td>
<td>160</td>
<td>10</td>
<td>10</td>
<td>8.59±0.08</td>
</tr>
<tr>
<td>Tau (IV)+CTX</td>
<td>40+20</td>
<td>10</td>
<td>10</td>
<td>6.49±0.05</td>
</tr>
<tr>
<td>Tau (IV)+CTX</td>
<td>80+20</td>
<td>10</td>
<td>10</td>
<td>6.71±0.06</td>
</tr>
<tr>
<td>Tau (IV)+CTX</td>
<td>160+20</td>
<td>10</td>
<td>9</td>
<td>6.89±0.09</td>
</tr>
<tr>
<td>Tau (IG)</td>
<td>160</td>
<td>10</td>
<td>10</td>
<td>8.10±0.09</td>
</tr>
<tr>
<td>Tau (IG)</td>
<td>640</td>
<td>10</td>
<td>10</td>
<td>8.40±0.07</td>
</tr>
<tr>
<td>Tau (IG)+CTX</td>
<td>160+20</td>
<td>10</td>
<td>9</td>
<td>6.30±0.06</td>
</tr>
<tr>
<td>Tau (IG)+CTX</td>
<td>320+20</td>
<td>10</td>
<td>9</td>
<td>6.65±0.07</td>
</tr>
<tr>
<td>Tau (IG)+CTX</td>
<td>640+20</td>
<td>10</td>
<td>10</td>
<td>6.79±0.11</td>
</tr>
</tbody>
</table>

\(^a\) p < 0.01 vs. model group; \(^b\) p < 0.01 vs. CTX group; \(^c\) p < 0.01 vs. Tau (40 mg·kg⁻¹, IV) + CTX group; \(^d\) p < 0.01 vs. Tau (80 mg·kg⁻¹, IV) + CTX group; \(^e\) p < 0.01 vs. Tau (160 mg·kg⁻¹, IG) + CTX group; \(^f\) p < 0.05, \(^g\) p < 0.01 vs. Tau (320 mg·kg⁻¹, IG) + CTX group.

![Table 3 Effects of taurine combined with cyclophosphamide on the thymus index and the spleen index of S180-bearing mice (mean ± SD)](attachment)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg·kg⁻¹)</th>
<th>Mouse number</th>
<th>Spleen index</th>
<th>Thymus index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>begin</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>5.47±0.12</td>
</tr>
<tr>
<td>CTX</td>
<td>20</td>
<td>10</td>
<td>9</td>
<td>4.48±0.13</td>
</tr>
<tr>
<td>Tau (IV)</td>
<td>40</td>
<td>10</td>
<td>9</td>
<td>6.00±0.17</td>
</tr>
<tr>
<td>Tau (IV)</td>
<td>160</td>
<td>10</td>
<td>10</td>
<td>6.48±0.13</td>
</tr>
<tr>
<td>Tau (IV)+CTX</td>
<td>40+20</td>
<td>10</td>
<td>10</td>
<td>5.01±0.17</td>
</tr>
<tr>
<td>Tau (IV)+CTX</td>
<td>80+20</td>
<td>10</td>
<td>10</td>
<td>5.99±0.13</td>
</tr>
<tr>
<td>Tau (IV)+CTX</td>
<td>160+20</td>
<td>10</td>
<td>9</td>
<td>6.29±0.17</td>
</tr>
<tr>
<td>Tau (IG)</td>
<td>160</td>
<td>10</td>
<td>10</td>
<td>5.78±0.13</td>
</tr>
<tr>
<td>Tau (IG)</td>
<td>640</td>
<td>10</td>
<td>10</td>
<td>6.28±0.13</td>
</tr>
<tr>
<td>Tau (IG)+CTX</td>
<td>160+20</td>
<td>10</td>
<td>9</td>
<td>4.76±0.11</td>
</tr>
<tr>
<td>Tau (IG)+CTX</td>
<td>320+20</td>
<td>10</td>
<td>9</td>
<td>5.79±0.13</td>
</tr>
<tr>
<td>Tau (IG)+CTX</td>
<td>640+20</td>
<td>10</td>
<td>10</td>
<td>6.03±0.20</td>
</tr>
</tbody>
</table>

\(^a\) p < 0.01 vs. model group; \(^b\) p < 0.01 vs. CTX group; \(^c\) p < 0.01 vs. Tau (40 mg·kg⁻¹, IV) + CTX group; \(^d\) p < 0.01 vs. Tau (80 mg·kg⁻¹, IV) + CTX group; \(^e\) p < 0.01 vs. Tau (160 mg·kg⁻¹, IG) + CTX group; \(^f\) p < 0.01 vs. Tau (320 mg·kg⁻¹, IG) + CTX group.
of peripheral white blood cells directly reflects the condition of blood cells and indirectly reflects bone marrow hematopoiesis. Tau can promote hematopoietic recovery to some extent. The count of peripheral white blood cells and bone marrow nucleated cells were decreased after the administration of CTX, while were significantly increased after combination administration of Tau and CTX, suggesting that Tau could improve the myelosuppression caused by CTX.

Thymus and spleen are important immune organs. Thymus is mainly involved in cellular immunity, while spleen has a close relationship with humoral immunity. Thymus and spleen indices are important to evaluate the immune state of the body. Tian et al. reported that Tau could promote the growth of immune organs. T and B lymphocytes mediate specific cellular immunity and humoral immunity respectively, thus proliferation of the cells can reflect the functional status of immunocytes. Immunocyte is the functional agent of human body, providing immunological surveillance on tumors. Enhancement of cellular immune function, especially the function of T cells, is beneficial for the treatment of tumors. Tau has protective effects on lymphocytes, and can promote the proliferation of lymphocytes in human body in a dose-dependent manner. The reticuloendothelial system (RES, mononuclear phagocytic system) is an important defense system. Macrophages can also kill tumor cells. Tau has been shown to enhance the phagocytic activity of the reticuloendothelial system.

In the present study, the thymus index, spleen index, splenic T and B lymphocyte proliferation and phagocytic activity of peritoneal macrophages were all increased after combined administration of Tau (IV and IG) and CTX, suggesting that Tau could improve the immunodepression of CTX.

In summary, Tau can not only enhance the tumor growth inhibition effect of CTX, but also attenuate the inhibition effect of CTX on bone marrow and immune functions. These effects may be achieved through the enhancement of immune functions. Further studies need to be performed to explore the exact mechanisms of Tau used as an ancillary drug for radiotherapy.

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