Experimental Study of Counteractive Effect of Kanglaite Injection on Cancer Cachexia

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Abstract

The study was conducted to investigate the effect of Kanglaite (KLT) in the treatment of cancer cachexia through animal cancer cachexia model established by inoculating mice T739 with tumor LA795 (lung adenocarcinoma). Observations were made on the improvement of physiological conditions (body weight, food intake, etc.) of the cachetic mice following treatment and also of the continuing changes in levels of serum cytokines such as TNF-2, IL-1 and IL-6 which were considered to correlate with significant inhibition of body weight loss, increased food intake, prolonged life span, inhibited tumor growth and in several mice liquefaction and necrosis of tumor. The serum levels of TNF-2 and IL-1 also declined substantially in comparison with normal -saline treated tumor bearing mice. All above findings strongly suggested that KLT had significantly counteractive effect against cancer cachexia.

Keywords: Kanglaite, cancer cachexia, TNF-2, IL-1, IL-6, body weight, food intake

Cancer Cachexia (CC) is the major complication of cancer patients. The main symptoms include jaded appetite, extreme maceration, anemia, weakness and exhaustion, etc. More than 50% of cancer patients in late stage will die of the disease. It is, therefore, very necessary to discover its genetic mechanism and develop an ideal way to fight against the disease. The experiment was conducted to apply KLT in treating mouse cancer cachexia and through observing the physiological variations and analyzing serum cytokines which were currently considered to correlate with cancer cachexia to investigate the curative action of KLT in the treatment of cancer cachexia.

Materials and methods

1.1 Materials

(1) Animal for experiment: T739 mice, male and female, half and half, weight: 22-26g, inbred strain, purchased from the Tumor Institute of Chinese Academy of Medicine, Beijing;
(2) Carcinoma strain: LA795 (mouse lung adenocarcinoma) offered by the Tumor Institute of Chinese Academy of Medicine, Beijing;
(3) Drug and reagents: 1) Kanglaite Injection supplied by Zhejiang Kanglaite Pharmaceutical Co., Ltd. 2) TNF-2 RIA Reagent Box purchased from East Asia Immunotechnical Institute of PLA General Hospital. 3) IL-1, IL-2 ELISA Reagent Box purchased from Genzyme Co., Ltd., U.S.A.
1.2 Methods

(1) Establishing animal cancer cachexia model: Fresh LA 795 carcinoma lump was cut into pieces, grounded and filtered through a 200m filternet and thus prepared into a unicellular suspension. Then the suspension was diluted into a 6x106/ml carcinoma cell stock solution with physiological saline. Total 160 mice were divided randomly into 4 groups (n=40): Non-tumor-bearing group, as blank control (NTB), early tumor-bearing group. (TB), cancer cachexia group. (CC) and Kanglaite treatment group. (KT). 0.1ml of stock solution was inoculated at right abdomen of the mice in TB, CC and KT group by the routine method. Subcutaneously the equivalent of physiological saline was injected at the same position of the mice in NTB group. On the 14th day after inoculation by then tumor-bearing mice entered the cachetic conditions - food and water intake, body weight declined evidently). The mice in KLT group were administered with KLT 50ml/kg (body weight) through abdominal injection for continual 1 week while other groups were administered with equivalent of physiological saline abdominally as controls.

(2) Observation Indexes: 1) Body weight: The body weight of mice was measured once every 2 days from 8:00-10:00 AM which should be as accurate as 0.1g. The body weight of the mouse in each group was defined as the gross weight after removing tumor away (the tumor weight was calculated based on tumor size). 2) Food and water intake: The mice in each group were enclosed in the same group, and fed with same forage and tube water. The total food and water intake of each group of mice was recorded daily from 8:00-10:00 AM. 3) Tumor size: On the 8th day after subcutaneous inoculation the three diameters of tumor were measured by vernier caliper. The tumor volume was calculated based on the Equation: Volume of tumor \( V = \frac{\pi}{6} L \times W \times D \), wherein L, W, D stand for length, width and depth of tumor respectively. 4) Collecting mouse serum specimen: Vein blood of mouse eyelid which had been laid aside for about 1 hr till coagulated, was centrifuged at 2500/min for 15 min. The serum obtained then was stored at -20 °C. 5) Detection of TNF, IL-1 and IL-6 was conducted according to the instruction of the Reagent Boxes.

1.3 Statistic processing

T-Test and variance analysis were applied in the statistic processing.

Results

2.1 Variation on body weight

Body weight variation of the mice in the 4 groups on the 4th, 14th, 22nd and 28th day after inoculation with carcinoma cells was quite defined based on Variance Analysis (Table 1).

Table 1. Body weight variation among the four groups of mice in different periods

<table>
<thead>
<tr>
<th>Group</th>
<th>4th day</th>
<th>14th day</th>
<th>22nd day</th>
<th>28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTB (1)</td>
<td>23.4 ± 1.02</td>
<td>28.65 ± 1.12</td>
<td>29.12 ± 1.43</td>
<td>29.20 ± 1.28</td>
</tr>
<tr>
<td>T-B (2)</td>
<td>22.9 ± 1.14</td>
<td>26.12 ± 1.34*</td>
<td>Killed</td>
<td>Killed</td>
</tr>
<tr>
<td>CC (3)</td>
<td>23.3 ± 1.06</td>
<td>25.82 ± 0.48</td>
<td>21.75 ± 1.52</td>
<td>20.12 ± 1.36</td>
</tr>
<tr>
<td>KLT (4)</td>
<td>22.7 ± 1.23</td>
<td>25.13 ± 1.21</td>
<td>24.41 ± 1.22</td>
<td>24.21 ± 1.08**</td>
</tr>
</tbody>
</table>

*Comparing with (1) P<0.05, **comparing with (3) P<0.01
Table 1 showed that body weight, declining on the 14th day after inoculation, was more
evident among the mice in tumor-bearing group than those in NTB group. Statistic difference
was remarkable (P<0.05). After treatment the mice in KLT group began to gain their weight on
the 22nd and 28th day after inoculation. The statistic difference was extremely remarkable
comparing with the results of CC group. (P<0.01).

2.2 Variation on food intake (Table 2)

*Comparing with (1) P<0.05,  **comparing with (3) P<0.01

Table 2 showed that food intake of the mice in CC group was the lowest while this index of
KLT group was remarkably improved (P<0.05) comparing with that of CC group in the same
period.

2.3 Variation on tumor volume (Table 3)

*Comparing with CC group P<0.01

Table 3 showed that the tumor growth almost was ceased after treatment with KLT and
liquefaction and necrosis of tumor could be observed among several mice.

2.4 Detection results of serum cytokines (Table 4)

TNF-2comparing (1) and (2) , (4), P<0.05, comparing (1) and (2), P<0.01
IL-1comparing (1) and (2), (4), P<0.05, comparing (l) and (2), P<0.01
IL- VI comparing (1) and (2) , (3), P<0.05, comparing (1) and (4), P<0.01
Table 4 showed that the levels of TNF, IL-1 and IL-2 rose markedly comparing with those of NTB group. In KLT group the levels of the former two were declined while the level of IL-2 unexpectedly rose. The average life span was extended to 32 days after treating with KLT.

Discussion

Cancer cachexia is the major cause of death yet its genetic mechanism is still indefinite. So far there is no ideal way in treating the disease. Our former researches and reports from some foreign scholars estimated that TNF-2, IL-1, IL-2 and IL-6 might act to induce occurrence of cachexia. The experiment indicated that KLT could markedly improve cachetic condition of tumor-bearing mice. Although routine therapeutic ways (surgery, chemotherapy, radiotherapy) are widely used in treating tumors yet they usually exacerbate cachexia and could result the treatment fail to proceed normally. Many foreign scholars, therefore, are pursuing an ideal drug which is counteractive on both cancer and cachexia. Corticosteriod may stimulate appetite of late cancer patients yet unable to increase their body weight. Indometacin may improve common condition of cachexia yet fail to prolong life span. Hydrazine sulfate may exert its disease-resistant role through inhibiting phosphoenol or pyruvate kinase and reduce host glycongenesis yet itself can not counteract tumor.

KLT is a dual function, broad-spectrum drug against cancer. it can be used not only to improve immunity but fight against cancer as well. Its inhibitory and killing effects on carcinoma cells are clinically confirmed. It could strengthen body immunity, release toxic and adverse effects resulted from chemotherapy and radiotherapy and could also supply body with high-energy nutrients and release ache resulted from carcinoma and improve living quality of late cancer patients.

Our experimental results showed that food intake of the cachetic mice after being injected with KLT was increased substantially comparing with CC group (P<0.05). Body weight loss was not very evident and the tumor growth was effectively postponed. It was particularly worth to be mentioned: Liquefaction and necrosis of tumor were evident among some mice after being administered with KLT and their life span was prolonged. The detection result of serum cytokines which were considered to correlate with occurrence of cancer cachexia declared that KLT may decline TMF-2, IL-1 level in serum (P<0.05). However IL-6 level did not decline. Instead, it rose markedly (P<0.01). The major cause, the role IL-6 plays in cancer cachexia and relation between KLT and rising IL-6 level are all worthwhile for further study. Nevertheless the inhibitory action of KLT on cancer cachexia was undoubtable.