
**In vivo and in vitro effects of Bletilla striata polysaccharide-loaded paclitaxel nanoparticles on human gastric cancer cells**

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Sent for review: 24 October 2018 Revised accepted: 27 December 2018

**Abstract**

**Purpose:** To investigate the in vivo and in vitro effects of paclitaxel nanoparticle (PTX)-loaded Bletilla striata polysaccharide (BSP) on human gastric cancer cells.

**Methods:** Mice weighing 13 to 17 g and aged 4 to 6 weeks, were inoculated with human gastric gland cancer cell line (MKN45), and randomly assigned to five groups: control group, PTX-1 (10 mg/kg) group; PTX-2 (15 mg/kg) group, BSP-PTX-1 (10 mg/kg) group, and BSP-PTX-2 (15 mg/kg) group. The anti-proliferative influence of BSP-PTX and its cellular target were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and fluorescence microscopy, respectively.

**Results:** Inhibition of MKN45 cells was significantly higher in BSP-PTX group (88.24 %) than in PTX group (76.74 %, p < 0.05). More BSP-PTX entered the cells than PTX. Tumor inhibition was significantly low in PTX-1 group (37.58 %), relative to the BSP-PTX-1 group (45.00 %, p < 0.5). In addition, tumor inhibition was significantly lower in PTX-2 group (52.35 %) than in BSP-PTX-2 group (69.80 %, p < 0.5). The weight gain of mice was lower in the PTX or BSP-PTX groups than in control mice, while the weight gain of mice in BSP-PTX-2 group (26.35 %) was significantly higher than that of PTX-2 group (19.43 %, p < 0.5).

**Conclusion:** Bletilla striata polysaccharide-loaded paclitaxel nanoparticles enhance drug delivery, and effectively and safely exert anti-proliferative effect on MKN45 cells and in mice. Thus, these nanoparticles have good potential for development into anti-gastric cancer agents for clinical application.

**Keywords:** Bletilla striata polysaccharide, Paclitaxel nanoparticles, Human gastric cancer cells, Tumor target, Liver cancer

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**INTRODUCTION**

Since the beginning of this century, studies on carrier materials for functional anti-tumor drugs have received huge attention [1]. Bletilla striata polysaccharide (BSP), a compound isolated from the rhizome of B. striata, confers protection on gastric mucosa. It can exert anti-tumor effects by inhibiting the growth and cell cycle arrest of human gastric cancer cells, human ovarian...
cancer cells (A2780) and liver cancer cells (HepG2) and possesses great biocompatibility and biodegradability properties [2,3]. As a medical carrier material, it has great potential in clinical applications. Structurally, BSP contains numerous hydrophilic hydroxyl groups. However, it can be made into an amphipathic material with increased capability to trap insoluble anti-cancer drugs, and improved cellular targeting by the introduction of cholesteryl units [4]. Although paclitaxel (PTX) exhibits good anti-cancer activity, its therapeutic effectiveness is poor due to its relatively poor water solubility. This study was aimed at investigating the in vivo and in vitro effects of paclitaxel nanoparticles loaded with Bletilla striata polysaccharide (BSP-PTX) on human gastric cancer cells.

EXPERIMENTAL

Materials

Human gastric gland cancer cell line (MKN45) was bought from Shanghai Cell Institute of Chinese Academy of Sciences. Dulbecco’s Modified Eagle’s Medium (DMEM) was product of American Gibco Company, USA; Trypsin-EDTA cell dissociation solution was purchased from Beijing Neuronbc Technological Development Co. Ltd, China. Fetal bovine serum (FBS) was a product of American ThermoFisher Company, USA. The MTT kit was purchased from German Sigma Company. Penicillin and streptomycin were obtained from Shandong Medical Incorporated Company, China, while dimethyl sulfoxide (DMSO) was a product of Jiangxi Lanchun Biological Reagents Co. Ltd, China. Paclitaxel injection was purchased from Sichuan Taiji Pharmaceutical Co. Ltd, China. Thermostated water bath (HWA-50D) was a product of Korean Autonics Company, Korea. Cell culture incubator was purchased from Japanese Sanyo Electric Appliance, Japan, while clean bench directs HEPA-filtered laminar flow was obtained from Suzhou Puaide Purifying Equipment Technological Co. Ltd., China. Inverted optical microscope was a product of Japanese Olympus Company, Japan. High-speed table top refrigerated centrifuge (GTR16-2) was obtained from Beijing Era Beili Centrifuge Co. Ltd, China. Fluorescence microscope was a product of American Veeco Wyko Company, USA.

This study received approval from the Ethical Committee of our hospital (approval number: 20187891), and carried out according to the guidelines of the Declaration of Helsinki promulgated in 1964 as amended in 1996 [5].

Cell culture

Poorly-differentiated human gastric gland cancer cell line (MKN45) was cultured at 37 °C on DMEM in a 5 % CO₂ incubator. The cells were placed in thermostated water bath at 37 °C for 20 min to completely defrost, and then centrifuged. The supernatant was discarded, while DMEM was added to the pellets. The packed cells were dissociated into single cells and inoculated into culture bottles using a sterile sucker. The culture medium was further incubated at 37 °C in an atmosphere containing 5 % CO₂, after which the cells were examined under an optical microscope. The culture medium was changed every 24 to 48 h.

MTT assay

This was carried out to assay the inhibitory effect of BSP-PTX on proliferation of MKN45 cells. After washing thrice in PBS, the cells were trypsinized and subsequently examined under inverted optical microscope within 1 min. After digestion of connective proteins among the cells, the pancreatic enzymes were discarded, followed by the addition of DMEM. Smears of the cells were prepared, mounted on a cover slide, viewed under a light microscope, and counted. The cells were maintained at a density of 2 × 10³/mL and seeded in 96-well plates.

The medium was removed, following incubation for 24 h, and different concentrations of the drugs ranging from 1.0 to 16.0 ng/mL were added. The culture medium was incubated for 96 h, after which the medium was discarded. A solution of MTT (100 uL) was added to each well and the wells were further subjected to incubation for 4 h under the same conditions as before, after which the supernatant was discarded. The medium in each well was replaced with 200 uL of DMSO and placed on an oscillator for 5 min, and subsequently examined under the microscope. The content of each well was read at 570 nm in a microplate reader, while the reference was read at 450 nm. The extent of inhibition (H) of cells was calculated as in Eq 1.

\[
H (%) = \left\{ \frac{Ac - At}{Ac} \right\} \times 100 \quad \text{(1)}
\]

where Ac and At are the absorbance of control and treatment groups, respectively.

Determination of BSP-PTX target

The MKN45 cells were seeded in 96-well plates at a density 2 × 10⁴ cell/well, and subjected to incubation for 24 h in a 5 % CO₂ incubator at 37 °C, after which the supernatant was discarded.
The BSP-PTX and PTX were labelled with fluorescein isothiocyanate (FITC) injection for 3 h. The cells were thereafter rinsed thrice with PBS and viewed under a fluorescence microscope.

Transplantation of MKN45 cell and grouping

Mice weighing 13 to 17 g (mean weight = 15.2 g), aged 4 to 6 weeks (mean age = 5.1 weeks) were used. The MKN45 cells (2 × 10^7) were directly inoculated into the abdominal cavity of each mouse. After 3 weeks, the abdominal injection sites of mice were disinfected and their ascites excised with the aid of a 5-mL syringe. The mice were subsequently sacrificed by cervical dislocation and the excised ascites diluted with sterile saline solution (1:2 volume ratio) to a final concentration of 5 × 10^8 cells/mL. A portion (0.2 mL) was subcutaneously inoculated into the armpit of each mouse.

After 24 h of inoculation, the mice were allotted to 5 groups: control group, PTX-1 (10 mg/kg bwt) group, PTX-2 (15 mg/kg bwt) group, BSP-PTX-1 (10 mg/kg bwt) group, and BSP-PTX-2 (15 mg/kg bwt) group. The route of administration was intravenous (i.v.) injection, and administration was done 5 times at different time points: 1, 3, 5, 7, 9 days following inoculation. Weight, activity, capability and diet status were recorded on daily basis for 12 days. Then, following sacrifice via cervical dislocation, the mice were dissected, and the tumor tissues were excised and their weights were recorded. The extent of inhibition of tumor (TI) was calculated as in Eq 2.

\[ TI (%) = (1 - W)100 \quad \text{(2)} \]

where W is mean weight of tumor in treatment group.

Statistical analysis

The results are presented as mean ± SEM. Comparison between groups was done with \( \chi^2 \) test and Student's t-test. All analyses were done with SPSS ver. 19. Values of \( p < 0.05 \) were taken as indicative of statistical significance.

RESULTS

Inhibition of cell proliferation

Higher inhibition of MKN45 cells was seen in BSP-PTX group (88.24 %), relative to PTX group (76.74 %) \( (p < 0.05) \). These results are shown in Table 1.

### Table 1: Anti-proliferative effect of different concentrations of TAX in the five groups

<table>
<thead>
<tr>
<th>Group</th>
<th>TAX concentration (ng/ml)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4.90</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>12.75</td>
</tr>
<tr>
<td>PTX</td>
<td>4.0</td>
<td>35.29</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>68.63</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>76.47</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>12.75</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>21.57</td>
</tr>
<tr>
<td>BSP-PTX</td>
<td>1.0</td>
<td>12.75</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>21.57</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>51.96</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>83.33</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>88.24</td>
</tr>
</tbody>
</table>

Results of fluorescence microscopic examination showed that the drugs labelled with FITC entered the MKN45 cells. The amount of BSP-PTX that entered the cells was significantly higher than that of PTX.

Tumor inhibitory effects of PTX and BSP-PTX

Tumor Inhibition was significantly lower in PTX-I group (37.58 %) than in the BSP-PTX-I group (45.00 %) \( (p < 0.5) \). Tumor inhibition was also significantly lower in PTX-2 (52.35 %) than in BSP-PTX-2 (69.80 %) \( (p < 0.5) \). Significant reductions in mice weight were evident in the PTX/BSP-PTX groups, when compared with the control group, and weight change in mice in BSP-PTX-2 group (26.35 %) was significantly higher than that in the PTX-2 group (19.43 %) \( (p < 0.5) \). These results are shown in Tables 2 and 3.

**DISCUSSION**

The anti-cancer activity of TAX has become quite popular in recent times, and it is currently one of the anti-cancer drugs readily available in clinics [6]. In 1992, the Food and Drug Administration (FDA) approved that TAX injection should be used for the treatment of metastatic ovarian cancer. Due to the poor solubility of PTX, polyoxyethylated castor oil is usually added to its solution as co-solvent [7].
Polyoxyethyiated castor oil causes allergic reactions and hyperlipidemia. Thus, it has become important to develop new formulations of PTX [8]. In 2005, FDA approved that a combination of albumin and BSP-PTX injection should be used for the treatment of metastatic or recurrent breast cancer within 6 months after adjuvant chemotherapy [9]. Studies have shown that this combination has higher bioavailability and safety than PTX [10].

Bletilla striata polysaccharide is a pectic polymer isolated from the rhizome of Bletillae and it confers protection on gastric mucosa [11]. After fluorouracil white and polysaccharide microspheres, patients with primary liver cancer can release 5-Fu in the body after hepatic artery embolization, which has long-acting, high-efficiency and low toxicity [12]. Compared with gelatin sponge, white and polysaccharide can significantly reduce the size of the tumor, the average time of recanalization and the half-year survival rate [13]. It has advantages such as slow release, self-degradation and non-stimulatory effect, which make it a potential drug carrier material. Several studies have shown that the use of nanoparticles prepared with BSP can serve as good drug targets with features such as slow release, biocompatibility, self-degradation and reduced toxicity [14]. Therefore, in the present study, the inhibitory effect BSP-derived taxol nanoparticles on human gastric cancer MKN45 cell growth was investigated. Taxol belongs to a class of insoluble anti-cancer drugs. Modification of BSP by dewatering converts it to an amphipathic carrier material which increases solvation of drugs, transport of drugs to target cells, and delay in drug release [15]. In addition, BSP protects the gastric mucosa and enhances the release of prostaglandins. Some studies have suggested that BSP can be used for treating gastric cancer [16].

In this study, inhibition of tumor was significantly lower in PTX-I group than in the BSP-PTX-I group. The inhibition was also significantly lower in PTX-2 than in BSP-PTX-2. Lower weight gains were obtained in the PTX/BSX-PTX groups than that in control group, and weight gain was significantly higher in BSP-PTX-2 group than in the PTX-2 group. These results suggest that BSP-PTX may be less toxic to mice than PTX. The PTX-loaded nanoparticles appear to enter cells more easily than single PTX. Thus, PTX-loaded nanoparticles have enhanced pharmacological functions. In addition, BSP has good biosafety. The polyoxyethylated castor oil contained in PTX injection easily causes multiple toxic reactions. Therefore, in terms of effectiveness and safety, compared with TAX, injection, BSP-PTX has treatment advantages.

### Table 2: Inhibition of tumor by PTX and BSP-PTX (mean ± SD, n = 10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mk/kg)</th>
<th>Number of deaths</th>
<th>Tumor weight (g)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>1.49 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>PTX-1</td>
<td>10</td>
<td>0</td>
<td>0.93 ± 0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.58</td>
</tr>
<tr>
<td>PTX-2</td>
<td>15</td>
<td>1</td>
<td>0.71 ± 0.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.35</td>
</tr>
<tr>
<td>BSP-PTX-1</td>
<td>10</td>
<td>0</td>
<td>0.82 ± 0.51&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>45.00</td>
</tr>
<tr>
<td>BSP-PTX-2</td>
<td>15</td>
<td>0</td>
<td>0.45 ± 0.38&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>69.80</td>
</tr>
</tbody>
</table>
<sup>a</sup>P < 0.05, relative to control; <sup>b</sup>p < 0.05, relative to PTX-1 group; <sup>c</sup>p < 0.05, relative to PTX-2 group

### Table 3: Changes in weight of mice following treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mk/kg)</th>
<th>Weight before treatment (g)</th>
<th>Weight after treatment (g)</th>
<th>Increase in weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>19.24 ± 2.28</td>
<td>28.32 ± 3.37</td>
<td>47.19</td>
</tr>
<tr>
<td>PTX-1</td>
<td>10</td>
<td>18.69 ± 2.46</td>
<td>24.14 ± 3.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.16</td>
</tr>
<tr>
<td>PTX-2</td>
<td>15</td>
<td>19.15 ± 1.99</td>
<td>22.87 ± 2.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.43</td>
</tr>
<tr>
<td>BSP-PTX-1</td>
<td>10</td>
<td>19.21 ± 2.04</td>
<td>25.38 ± 2.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.12</td>
</tr>
<tr>
<td>BSP-PTX-2</td>
<td>15</td>
<td>19.20 ± 1.62</td>
<td>24.26 ± 3.02&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>26.35</td>
</tr>
</tbody>
</table>
<sup>a</sup>P < 0.05, relative to control; <sup>c</sup>p < 0.05, relative to PTX-2 group
CONCLUSION

These results suggest that Bletilla striata polysaccharide-loaded paclitaxel nanoparticles provide enhanced drug delivery, and effectively and safely exert anti-proliferative effect on MKN45 in vivo and in vitro. Thus, these nanoparticles have good potential for development into newer and more effective anti-gastric cancer agents.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Bai Guang conceived and designed the study, Li Xuchen, Bai Guang collected and analyzed the data, while Li Xuchen wrote the manuscript.

REFERENCES