Tropical Journal of Pharmaceutical Research January 2019; 18 (1): 13-17
ISSN: 1596-5996 (print); 1596-9827 (electronic)
© Pharmacotherapy Group, Faculty of Pharmacy, University of Benin, Benin City, 300001 Nigeria.

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v18i1.2

Original Research Article

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

Li Xuchen, Bai Guang*
General Surgery, The First Affiliated Hospital of Jinzhou Medical University, No 2, Section 5, Renmin Street, Guta District, Jinzhou City, Liaoning Province 121000, China

For correspondence:* ***Email:* *pr1181@163.com*

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 mk/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-I 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*

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

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

© 2019 The authors. This work is licensed under the Creative Commons Attribution 4.0 International License

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 Appliances, Japan, while clean bench direct 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 ℃ on DMEM in a 5 % CO_2 incubator. The cells were placed in thermostated water bath at 37 ℃ 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 ^{o}C in an atmosphere containing 5 % CO_2, 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 2×10^4/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.

$$H (\%) = \{(Ac\ At)/Ac\}100 \quad \ldots\ldots\ldots (1)$$

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 2×10^4 cell/well, and subjected to incubation for 24 h in a 5 % CO_2 incubator at 37 ℃, 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.

$$TI\ (\%) = (1 - W)100 \quad \ldots\ldots\ldots (2)$$

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 χ^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

Group	TAX concentration (ng/ml)	Inhibition (%)
Control	0	-
PTX	1.0	4.90
	2.0	12.75
	4.0	35.29
	8.0	68.63
	16.0	76.47
BSP-PTX	1.0	12.75
	2.0	21.57
	4.0	51.96
	8.0	83.33
	16.0	88.24

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.

Figure 1: Photomicrographs of MKN45 cells showing the targets of PTX and BSP-PTX. A: Control (no green fluorescence in the cells); B: PTX group (presence of light green fluorescence); C: BSP-PTX group (presence of dark green fluorescence)

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].

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

Group	Dose (mk/kg)	Number of deaths	Tumor weight (g)	Inhibition (%)
Control	0	0	1.49 ± 0.76	
PTX-1	10	0	0.93 ± 0.62[a]	37.58
PTX-2	15	1	0.71 ± 0.60[a]	52.35
BSP-PTX-1	10	0	0.82 ± 0.51[ab]	45.00
BSP-PTX-2	15	0	0.45 ± 0.38[ac]	69.80

[a]$P < 0.05$, relative to control; [b]$p < 0.05$, relative to PTX-1 group; [c]$p < 0.05$, relative to PTX-2 group

Table 3: Changes in weight of mice following treatment

Group	Dose (mk/kg)	Weight before treatment (g)	Weight after treatment (g)	Increase in weight (%)
Control	0	19.24 ± 2.28	28.32 ± 3.37	47.19
PTX-1	10	18.69 ± 2.46	24.14 ± 3.09 [a]	29.16
PTX-2	15	19.15 ± 1.99	22.87 ± 2.81 [a]	19.43
BSP-PTX-1	10	19.21 ± 2.04	25.38 ± 2.94 [a]	32.12
BSP-PTX-2	15	19.20 ± 1.62	24.26 ± 3.02 [ac]	26.35

[a]$P < 0.05$, relative to control; [c] $p < 0.05$, relative to PTX-2 group

Polyoxyethylated 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 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 the present investigation, higher inhibition of MKN45 cells was seen in BSP-PTX group, relative to the PTX group. This result suggests that BSP may change the pharmacological activity of PTX thereby conferring better anti-tumor activity on it. This property may be related to the packaging effect of BSP on PTX, which makes it easy for the nanoparticle form to enter cells. The amount of BSP-PTX that entered the cells was significantly higher than that of PTX. This may be an indication that BSP-PTX has a readier cellular target than PTX.

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/BSP-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 polyoxy-ethylated 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.

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

1. *Guan Q, Zhang G, Sun D, Wang Y, Liu K, Wang M, Sun C, Zhang Z, Li B, Lv J. In vitro and in vivo evaluation of docetaxel-loaded stearic acid-modified Bletilla striata polysaccharide copolymer micelles. PLoS One 2017; 12(3): 0173172.*
2. *Yang AiZ, Li J, Xu HJ. A Study on Antitumor Effect of Liposome Encapsulated Paclitaxel in Vivo and in Vitro. Bull Chin Cancer 2006; 15(12): 862-864.*
3. *Guan QX, Zhang GY, Sun DD, Sun SL, Sun C, Liu X, Han B. Investigation on Synthesis of Bletilla Striata Polysaccharides Amphiphilic Polymer and Drug-loaded Delivery System. Chem J Chin Univ 2016; 37(10): 16-19.*
4. *Bi X, Zhang LN, Zhang XB. Study on BSP-PTX. J Shenyang Pharm Univ 2015; 32(5): 335-342.*
5. *World Health Organization. Declaration of Helsinki. Br Med J 1996; 313(7070): 1448-1449.*
6. *He X, Wang X, Fang J, Zhao Z, Huang L, Guo H, Zheng X. Bletilla striata: Medicinal uses, phytochemistry and pharmacological activities. J Ethnopharmacol 2017; 195: 20-38.*
7. *Luo L, Yang F, Wang Y. BSP trail and application study. Hubei J Tradit Chin Med 2016; 38(11): 69-72.*
8. *Shi S, Huang Z, Luo Y, Chen JN, Zhang JF. Bletilla striata polysaccharide for the treatment of ulcerative colitis in mice. J China Pharm Univ 2012; 43(6): 535-540.*
9. *Ma Y, He S, Ma X, Hong T, Li Z, Park K, Wang W. Silymarin-loaded nanoparticles based on stearic acid-modified bletilla striata polysaccharide for hepatic targeting. Mol 2016; 21(3): 265.*
10. *Deng LD, Dong AJ, Zhang YT, Huo JZ, Yuan YJ, Sun DX. Paclitaxel-Loaded Amphiphilic Copolymer Nanoparticles. J Tianjin Univ 2004; 37(1): 15-18.*
11. *Zhang M, Sun L, Zhao W, Peng X, Liu F, Wang Y, Bi Y, Zhang H, Zhou Y. Cholesteryl-modification of a glucomannan from Bletilla striata and its hydrogel properties. Mol 2014; 19(7): 9089-100.*
12. *Weiyong L, Gansheng F, Chuansheng Z. Pharmacokinetics of Fu Bletilla Microspheres after hepatic arterial embolization in patients with primary liver carcer. Chin Hosp Pharm 2001; 21 (11): 643-644.*
13. *Shihua L, Chuansheng Z, Weiyong L. Experimental study on anti-tumor effect of white and polysaccharide in vitro. Chin Tradit Pat Med 2014; 36 (1): 165-168.*
14. *Dong CC, Zhong L, Zhang GY, Wang ZR, Ran FL, Chen X. Influences and mechanism of suberin on proliferation and apoptosis of human gastric cancer cell line MKN45. Shandong Med 2017; 57(7): 5-8.*
15. *Liu JW, Wei XD, Tang YP. Influences of BSP and its 5-Fu on human gastric cancer MKN45 cell lines. Inner mongol J Tradit Chin Med 2014; 2: 46-48.*
16. *Ge Z, Ma R, Xu G, Zhang D, Wang Q, Hei L, Ma W. Development and In Vitro Release of Isoniazid and Rifampicin-Loaded Bovine Serum Albumin Nanoparticles. Med Sci Monit 2018; 24: 473-478.*