Inhibition of five xenografted human cancers and two murine cancers by the tripeptide tyroservatide

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The tripeptide tyroservatide (tyrosyl-seryl-valine, pTyr-Ser-Val-NH_2) has been shown to have antitumor effects on experimental hepatocarcinoma. This study aimed to observe the effects of tyroservatide on other five human carcinomas: A549 (nonsmall cell lung carcinoma), BGC-823 (gastric cancer), MCF-7 (breast cancer), K562 (leukemia), A375 (melanoma) and two murine cancers: Lewis lung cancer and B16 (melanoma) *in vivo*. *In vivo* nude mice bearing xenografts of five different human tumors or C57BL/6 mice bearing xenografts of two different murine tumors were given daily intraperitoneal injections of tyroservatide or saline as controls, after tumor implantation. The inhibition of xenografts was determined by calculating the tumor volume and measuring tumor weight. Tyroservatide could significantly inhibit the growth of human lung carcinoma A549, human leukemia K562 and human melanoma A375 in nude mice ($P<0.05$). In addition, tyroservatide significantly inhibited the subcutaneous tumor growth of Lewis lung carcinoma and B16 melanoma ($P<0.05$). Tyroservatide, however, could not significantly suppress xenografts of BGC-823 and MCF-7 in nude mice ($P>0.05$). The results obtained indicate that tyroservatide exhibits different effects on different tumors, which will provide clinical applications guidance of tyroservatide as an anticancer drug. *Anti-Cancer Drugs* 18:467–470 © 2007 Lippincott Williams & Wilkins.

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Introduction

In our previous studies, the tripeptide tyroservatide (YSV, tyrosyl-seryl-valine, pTyr-Ser-Val-NH_2) from spleen of pigs showed obvious antitumor effects on human hepatocarcinoma *in vitro* and *in vivo* [1,2]. Its chemical structural formula is L-tyrosyl-L-seryl-L-valine, its molecular formula is $C_{17}H_{25}N_3O_6$, and its molecular weight is 367.40 Da. We deduced that the anticancer effects of YSV might result from its inhibition of tumor cell proliferation and the induction of apoptosis [3]. In this study, we studied its effects on other human tumors and murine tumors *in vivo*.

Lung cancer, stomach cancer and liver cancer are the three leading cancer killer worldwide. Leukemia is a common cancer in the western world and breast cancer is the most common cancer form in women [4]. Melanoma is a kind of cutaneous cancer with a high degree of malignancy, which is not very sensitive to chemotherapeutics. We have chosen five human cancer cell lines and two murine cancer cell lines from these kinds of cancer mentioned above in this study. They are human lung cancer cell A549, human gastric cancer cell BGC-823, human breast cancer cell MCF-7, human erythroleukemia cell K562 and human melanoma cell A375, and murine Lewis lung cancer (LLC) and mice melanoma B16, respectively.

Materials and methods

Reagents and cell lines

The YSV used in this study was custom manufactured by Shenzhen Kangzhe Pharmaceutical (Shenzhen, China). RPMI-1640 cell culture medium was purchased from Gibco/BRL (New York, USA). Fetal bovine serum was provided by Hyclone (Hyclone, Logan, Utah, USA).

Human nonsmall cell lung carcinoma A549 and human melanoma A375 were purchased from the Cancer Research Department, China Medical Science Institute. Human gastric cancer BGC-823 and human breast cancer MCF-7 were provided by the Biology Research Department, China Science Institute. Human leukemia K562 was from the Hematology Research Department, China Medical Science Institute. The B16 melanoma cell line and LLC cell lines were obtained from the China Center for Type Culture Collection (Wuhan, China). All the cells were routinely examined and found to be free of mycoplasma contamination. Cells were cultured in

RPMI-1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37°C.

Animals

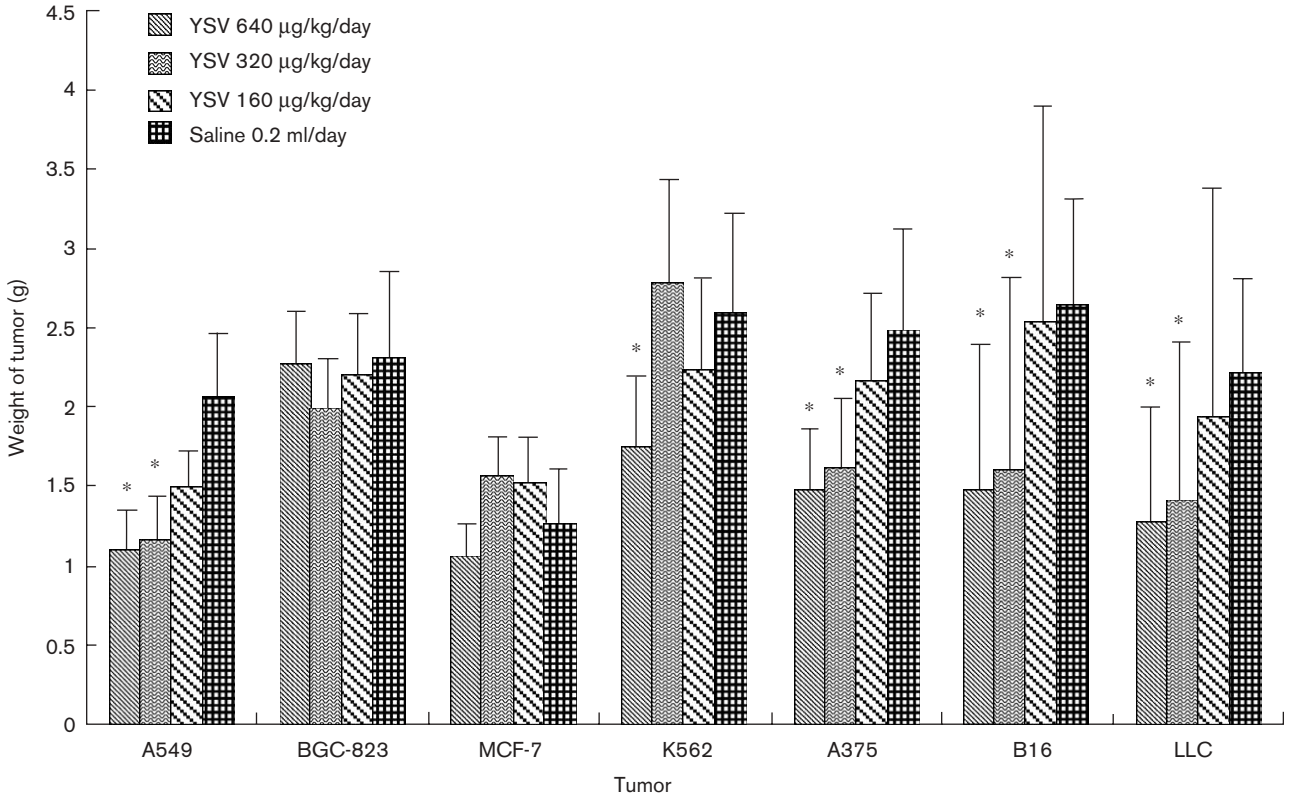
Healthy, female BALB/c *nu/nu* nude mice (specific pathogen-free grade, 4–5 weeks, 18–22 g) and healthy C57BL/6 female mice (4–5 weeks, 18–22 g) were obtained from the Chinese Medical Academy of Science (Beijing, China). The animals were maintained at our university under specific pathogen-free conditions using a laminar airflow rack, and had continuous access to sterilized food and autoclaved water with a controlled light–dark cycle, temperature (25 ± 2°C), and humidity (50–70%). Experiments commenced after 1 week of acclimatization. Animal studies were conducted in accordance with the standards established by the Guidelines for the Care and Use of Laboratory Animal Center of Tianjin Medical University.

Implantation of the human carcinomas in nude mice and mice carcinomas in C57BL/6 mice

Intraperitoneal injection of 2.5 mg cyclophosphamide once a day for 3 days in per nude mice was used to establish the human leukemia K562 nude mice model [5–7]. Tumor implanting was performed from the next day of the last injection. Tumor was induced in the right flank of nude mice by subcutaneous injection of 1×10^7 cultured, log-phase K562 cells in 0.1 ml of RPMI-1640. Other tumors were relatively established through subcutaneous injection of log-phase tumor cells A549 (2×10^8/ml), BGC-823 (1.6×10^8/ml), MCF-7 (2×10^7/ml) and A375 (1×10^7/ml) in 0.1 ml of RPMI-1640 in nude mice, and B16 melanoma cell line (5×10^7/ml) and LLC (2.5×10^7/ml) cell lines in C57BL/6 mice.

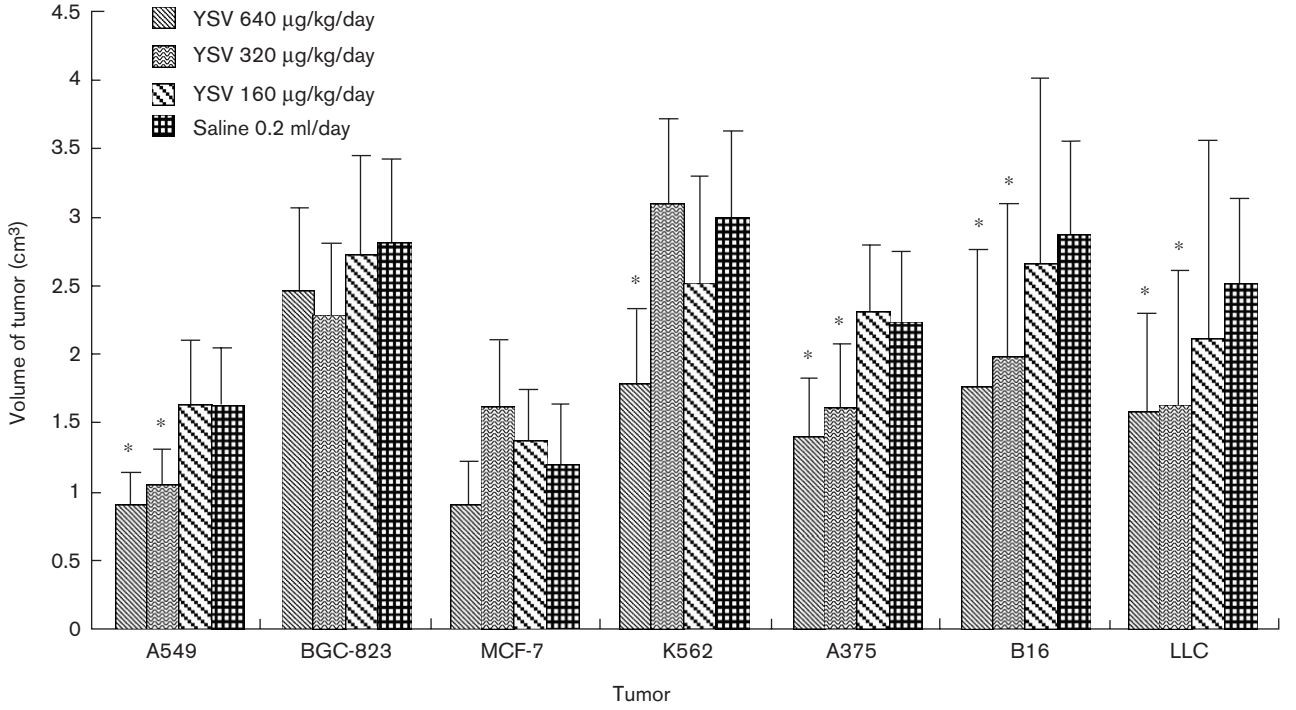
When the tumors had reached an average volume of 100 mm^3, the tumor-bearing nude mice or tumor-bearing C57BL/6 mice were randomized into YSV groups (160, 320 or 640 μg/kg/day) and a saline group (0.2 ml/day). Drug administration commenced on the day following tumor implantation, and was delivered by intraperitoneal injection once per day for 25 days (in B16 melanoma and LLC models), 30 days (in BGC-823, MCF-7 and K562 tumor models) or 60 days (in A549 and A375 tumor models). The day after the last drug administration, the tumor of each mouse was dissected and its weight recorded. Three diameters of the tumor (*A*, *B*, *C*) were

Fig. 1



Inhibition of tyroservatide (YSV) on tumor weights of five transplanted human tumors in nude mice, including human nonsmall cell lung carcinoma A549, human melanoma A375 human gastric cancer BGC-823, human breast cancer MCF-7, human leukemia K562, murine B16 melanoma and Lewis lung carcinoma (LLC). $n = 10$, mean ± SD; $^*P < 0.05$. Bars indicate SD.

Fig. 2



Inhibition of tyroservatide (YSV) on tumor volumes of five transplanted human tumors in nude mice, including human nonsmall cell lung carcinoma A549, human melanoma A375 human gastric cancer BGC-823, human breast cancer MCF-7, human leukemia K562, murine B16 melanoma and Lewis lung carcinoma (LLC). $n=10$, mean ± SD; $^*P<0.05$. Bars indicate SD.

measured by caliper. The volume of the tumor was calculated as $V = 1/6\pi ABC$. The tumor growth inhibition index was calculated according to the following formula:

$$\text{Tumor growth inhibition index } (\%) = \frac{(\text{mean tumor weight of control group} - \text{mean tumor weight of treatment group})}{\text{mean tumor weight of control group}} \times 100\%$$

Statistical methods

All experimental data were expressed as mean value ± standard deviation (SD). Experimental data were analyzed using one-way analysis of variance and the significant differences between two groups were assessed by Student Newman–Keuls. Data were considered statistically significant if P values were 0.05 or lower.

Results

Effects of tyroservatide on transplanted human tumors in nude mice and two murine tumors in C57BL/6 mice (Figs 1 and 2)

At the dosage of 640 and 320 μg/kg/day YSV significantly inhibited the growth of human lung carcinoma A549 and human melanoma A375 xenografts in nude mice compared with the saline control ($P < 0.05$), at 46.97 and 45.97% (inhibition in A549) and 50.95 and 45.97% (inhibition in A375). At a dosage of 640 μg/kg/day, YSV could significantly inhibit the growth of human leukemia K562 in nude mice by an inhibition rate of 32.39%, significantly lower than that of the saline control ($P < 0.05$). YSV, however, could not significantly suppress xenografts of BGC-823 and MCF-7 in nude mice ($P > 0.05$). Moreover, 640 and 320 μg/kg/day YSV significantly inhibited the growth of murine LLC and murine melanoma B16 tumors in C57BL/6 mice compared with the saline control ($P < 0.05$), at 42.25 and 36.77% (inhibition in LLC) and 44.54 and 39.57% (inhibition in B16) (Table 1). During the period of administration, mice of the YSL group were in better physical condition and showed weight gain. In addition, there were obvious gross alterations in the internal organs of mice, such as heart, liver and kidney.

Discussion

In the year 2000, malignant tumors were responsible for 12% of the nearly 56 million deaths worldwide from all causes. In many countries, more than a quarter of deaths are attributable to cancer. From a global perspective, cancer incidence has increased year by year, and

Table 1 Effects of YSV on transplanted murine LLC and mice melanoma B16 in C57BL/6 mice

Group	*n*	LLC		Melanoma B16	
		Tumor weight (g)	Tumor volume (cm^3)	Tumor weight (g)	Tumor volume (cm^3)
YSV 640 μg/kg/day	12	1.2775 ± 0.7177*	1.5776 ± 0.7245*	1.4665 ± 0.9177*	1.7645 ± 1.0023*
YSV 320 μg/kg/day	12	1.3988 ± 1.0131*	1.6234 ± 1.0023*	1.5982 ± 1.2131*	1.9876 ± 1.1123*
YSV 160 μg/kg/day	12	1.9339 ± 1.4588	2.1176 ± 1.4654	2.5339 ± 1.3588	2.6766 ± 1.3443
Saline 0.2 ml/day	12	2.2122 ± 0.5998	2.5223 ± 0.6234	2.6446 ± 0.677	2.8769 ± 0.6898

ANOVA, analysis of variance; LLC, Lewis lung cancer; SNK, Student Newman–Keuls; YSV, tyroservatide.
*Compared with the saline group, $P < 0.05$, one-way ANOVA, SNK.

conquering cancer has become an urgent task. Recently, a small peptide of six amino acids has been identified that inhibits the growth of several malignant tumors such as lung carcinoma, gastric cancer and intestine carcinoma [8]. More and more scientists focus on the research of peptides as anticancer drugs, antibiotics and inhibitors of enzymes because of their small molecular weights, no immunogenicity, simple structures, fewer side effects, easy synthesis, and lower production costs and high purity [9]. In our previous research, two tripeptide, tyroserleutide [10] and YSV [2,3], exhibited significant inhibition on human hepatocarcinoma BEL-7402 in nude mice.

In our studies, we established an implantation model of the human carcinomas to nude mice for lung cancer A549, stomach cancer BGC-823, breast cancer MCF-7, erythroleukemia K562 and melanoma A375, and murine B16 melanoma and LLC in C57BL/6 mice to investigate the anticancer effect of YSV *in vivo*. YSV could inhibit human lung cancer A549 and melanoma A375 growth in nude mice, and murine B16 melanoma and LLC in C57BL/6 mice. Also YSV inhibited growth of erythroleukemia K562 transplanted in nude mice with the best inhibition rate of 32.39%. We, however, did not observe the inhibiting effect of YSV on stomach cancer BGC-823 and breast cancer MCF-7 *in vivo*.

In conclusion, YSV shows different effects on different experimental tumors, which is vital for the success of clinical applications of YSV as anticancer drug. The mechanism of action of YSV might be multistrata and we will investigate its anticancer mechanism in a further study.

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