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The effect of tripeptide tyroserleutide (YSL) on animal models of hepatocarcinoma

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ABSTRACT

This study aimed to observe the effects of tyroserleutide (tyrosyl-seryl-leucine, YSL) on the survival time of mice transplanted with the ascitic fluid-type hepatocarcinoma H22, as well as the inhibitory effect of tyroserleutide on the human hepatocarcinoma Bel-7402 that was transplanted into nude mice. At doses of 80, 20 and 5 μg/kg/d, tyroserleutide significantly prolonged the survival of mice transplanted with H22 tumor cells, producing survival rates of 89%, 39% and 49%, respectively, which were statistically significantly different from the saline group ($P < 0.05$). YSL, at doses of 80, 160 and 320 μg/kg/d significantly inhibited the growth of the human hepatocarcinoma Bel-7402 tumor in nude mice, producing inhibition of 40%, 64% and 59%, respectively; this inhibition was significantly greater than that by saline ($P < 0.05$). HE staining and electron microscopy of the pathological changes of the tumor in nude mice showed that YSL changed the structure Bel-7402 tumor cells that were transplanted into nude mice, and also induced tumor cell apoptosis and necrosis, which could be a mechanism by which YSL inhibits the tumor growth in animal models.

1. Introduction

The World Health Organization (WHO) has documented that every year there are more than 1,000,000 humans diagnosed with cancer and that 600,000 die of the cancer annually, which accounts for 12% of all deaths through the world [4]. Hepatocarcinoma is one tumor with a particularly high incidence and mortality rate [1]. In China, the mortality of hepatocarcinoma is the second and third most common cause of cancer-related deaths in males and females, respectively [17]. In the United States, hepatocarcinoma is the eighth cancer-related killer of males [1]. There are currently no effective therapies for hepatocarcinoma. Although the chemotherapeutic agents are the main therapeutic approach for hepatocarcinoma, they are relatively ineffective. Accordingly, screening compounds for potential use as effective therapeutic agents for hepatocarcinoma is an important undertaking.

Tyrosyl-seryl-leucine (or tyroserleutide as approved by the Chinese pharmacopoeia committee, YSL) is a tripeptide compound that was developed in China. It consists of three natural amino acids, L-tyrosine, L-serine and L-leucine with a

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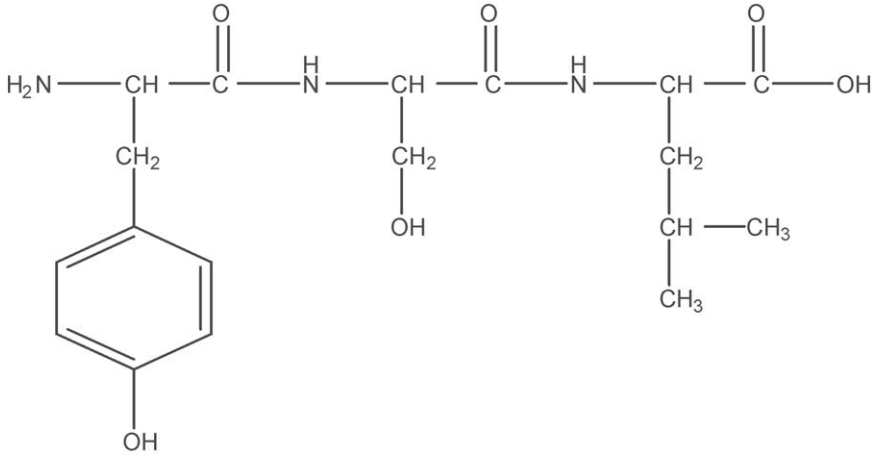

Fig. 1 – Structural formula of the YSL.

corresponding chemical structure $C_{18}H_{27}N_3O_6$ (Fig. 1). The molecular weight of YSL is 381.42. YSL has been documented to inhibit tumor growth both in vitro and in vivo [20]. However its efficacy in hepatocarcinoma is not well documented. So in the current study, we examined the effects of YSL on the survival rate of mice transplanted with an ascitic-type hepatocarcinoma cell line H22, as well as the growth of the human hepatocarcinoma Bel-7402 transplanted in nude mice.

2. Materials and methods

2.1. *Reagents and cell lines*

The YSL used in this study were custom manufactured by Shenzhen Kangzhe Pharmaceutical Co. Ltd., Shenzhen, China. Cyclophosphamide was purchased from Jiangsu Henrui Pharmaceutical Co. Ltd., Jiangsu, China. Saline was purchased from China OTSUKA Pharmaceutical Co. Ltd., Tianjin, China.

H22 is a hepatocarcinoma of the mouse. This model comes from a chemically induced hepatocarcinoma [21]. Bel-7402 is a human hepatocarcinoma cell line established in 1975 by Chen [2]. When transplanted into nude mice, the Bel-7402 cell line preserves its inherent traits and grows stably.

2.2. *Animals*

Healthy BALB/c mice (CLA grade, 6–8 weeks, weight 18–22 g) were purchased from the Animal Center of Military Medical Academy of Science (Beijing, China). All protocols were approved by the Tianjin Medical University Animal Care and Use Committee. Mice bearing the ascitic-type hepatocarcinoma H22 were purchased from the tumor department of the Medical Institute of the Chinese Medical Academy of Science (Beijing, China). All the mice were maintained at our university under clear conditions and were fed with clear foods and water.

Healthy male BALB/c nu/nu nude mice (SPF grade, 4–5 weeks, 18–22 g) and nude mice bearing the human Bel-7402 hepatocarcinoma were obtained from the Chinese Medical Academy of Science (Beijing, China). The animals were maintained at our university under specific pathogen free (SPF) conditions using a laminar air-flow rack, and had continuous access to sterilized food and autoclaved water with a controlled light/dark cycle, temperature, and humidity. Experiments commenced after 1 week of acclimatization.

2.3. *Administration of ascitic type hepatic carcinoma H22 mice model [19]*

The mice bearing the ascetic-type hepatocarcinoma were sacrificed 6–8 days after tumor implant. The skin was sterilized and removed and the ascitic fluid was drawn through the muscles of the abdominal wall using a sterile syringe, and then diluted to a concentration of 1×10^7 cells/ml in Hanks solution. We injected 0.2 ml of diluted solution into the abdominal cavity of each BALB/c mouse in each group, except for the healthy control groups. Before injection of tumor cells the animals were randomized into groups, i.e. groups receiving different doses of YSL (80, 20, 5 μg/kg/d), a cyclophosphamide group (20 mg/kg/d), a saline group (0.2 ml/mouse/d), and a model control group and normal group, with 18 mice in each group. For groups receiving either YSL or saline, administration was started 5 days before the tumor transplantation, while cyclophosphamide commenced on the day after tumor transplantation. All drugs were dissolved in 0.2 ml saline, and administered i.p., once a day, for 60 days or until death of the mouse. General reactions were observed every day after drug administration, with the time of death recorded and survival time prolongation calculated. Prolonged survival rate (%) was calculated as ((median survival days of experimental group − median survival days of control group)/median survival days of control group) × 100%. The mice that survived for 60 days after transplantation were considered long-term survivors.

2.4. *Implantation of the human Bel-7402 hepatocarcinoma [7]*

Nude mice bearing a tumor with a diameter >1 cm were selected for withdrawal of tumor cells. The tumor was removed aseptically and cut into 2–4 mm^3 pieces in RPMI1640