Tanshinone IIA inhibits human hepatocellular carcinoma J5 cell growth by increasing Bax and caspase 3 and decreasing CD31 expression in vivo

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Abstract. Tanshinone IIA (Tan-IIA) decreases the viability of human hepatocellular carcinoma (HCC) cells through the induction of apoptosis in vitro. However, there are no reports that Tan-IIA is capable of inhibiting J5 HCC cell growth in vivo. In this study, J5 cells were implanted directly into nude SCID mice which were divided randomly into four groups to be treated with vehicle, Tan-IIA (30 mg/kg of body weight, Q.week days 3 and 5), 5-FU (30 mg/kg of body weight, Q.week day 1) or Tan-IIA (30 mg/kg of body weight, Q.week days 3 and 5) plus 5-FU (30 mg/kg of body weight, Q.week day 1). Each agent was injected intraperitoneally, with treatment starting 4 weeks after inoculation with J5 cells. Treatment with Tan-IIA 30 mg/kg or with 30 mg/kg of 5-FU resulted in a reduction in tumor size and weight compared with the control group. The protein expression of Bax and caspase-3 in the J5 xenograft tumors treated with Tan-IIA 30 mg/kg or with 30 mg/kg of 5-FU was upregulated, whereas that of CD31 was downregulated compared with the control group. These findings indicate that Tan-IIA may inhibit tumor growth in a J5 xenograft animal model by increasing Bax and caspase 3 and decreasing CD31 expression in vivo.

Introduction

Hepatocellular carcinoma is the leading cause of cancer-related death in men (41.4/100,000 deaths) in Taiwan (1). HCC is chemoresistant to a number of available chemotherapeutic agents (2). Numerous prescription drugs in use for cancer treatment are derived from plants (3). Tanshinone IIA (Tan-IIA) is an extract from Salviae Miltiorrhizae radix (4,5), and its anti-inflammatory activities (6,7), antitumor activity in numerous human cancer cell types (8-10) and anti-oxidant properties (11,12) have been well documented. In addition, it has been extensively reported that Tan-II A inhibits cell proliferation and induces apoptosis in hepatocellular carcinoma cell lines (13-15). Tan-IIA effectively inhibited the invasion and metastasis of HCC cells in vitro and in vivo (16). Our previous study revealed that Tan-IIA inhibited hep-J5 cell growth in vitro via induction of apoptosis and increase in protein expression of calreticulin, caspase 12 and GADD153 (17). However, the anticancer effects of Tan-IIA on hep-J5 cells in vivo are not yet fully understood. The present study focused on the anticancer effect of Tan-IIA in vivo in SCID mice, using a human hepatocellular carcinoma xenograft model of J5 cells. Additionally, since 5-fluorouracil (5-FU) is one of the chemotherapeutic agents for HCC, we also examined whether there were any synergistic effects between Tan-IIA and 5-FU in this study.

Materials and methods

Chemicals. Tan-IIA (molecular formula: C19H18O3, purity >96% by HPLC) was purchased from Herbasin Co. (Shenyang, China). Corn oil, aprotinin, antipain, sodium deoxycholate, leupeptin, propidium iodide (PI), sodium orthovanadate, Triton X-100, Tris-HCl, ribonuclease-A and trypan blue were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Cell culture. The human hepatocellular carcinoma J5 cell line was kindly provided by Dr H.J. Harn (Graduate Institute of Cancer Biology and Center for Molecular Medicine, China Medical University and Hospital, Taichung, Taiwan).

The J5 cells were maintained in RPMI-1640 medium containing 10% FBS, 1% penicillin-streptomycin (10,000 U/ml penicillin; 10 mg/ml streptomycin) in a 37˚C humidified atmosphere containing 5% CO2.

In vivo J5 cell tumor xenograft model. A total of 24 3-week-old male nude SCID mice (NOD.CB 17-Prkdcscid/Tcu) were
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Figure 1. Experimental design of the xenograft animal model. J5 cell tumor xenograft in NOD.CB 17-Prkdcscid/Tcu mice. J5 cells were implanted directly into nude SCID mice, which were divided randomly into four groups and treated with vehicle, Tan-IIA (30 mg/kg of body weight, Q.week days 3 and 5), 5-FU (30 mg/kg of body weight, Q.week day 1) or Tan-IIA (30 mg/kg of body weight, Q.week days 3 and 5) plus 5-FU (30 mg/kg of body weight, Q.week day 1). Each agent was injected intraperitoneally, with treatment starting 4 weeks after inoculation with the J5 cells, and the hep-J5 cell xenograft tumor volumes were measured. Mice were sacrificed by CO₂ inhalation 35 days later, after which hep-J5 cell xenograft tumors were dissected and individually weighed.

Figure 2. SCID mice bearing J5 cell xenograft tumors were treated with vehicle (control group), Tan-IIA [30 mg/kg of body weight, Q.week day 3, 5 (C3T group)], 5-FU [30 mg/kg of body weight, Q.week day 1 (C3F group)] or Tan-IIA (30 mg/kg of body weight, Q.week days 3 and 5) plus 5-FU (30 mg/kg of body weight, Q.week day 1) (3F3T group) for 4 weeks and then sacrificed with CO₂ inhalation. Xenograft tumors were subsequently dissected.

xenografted with human hepatocellular carcinoma (HCC) J5 cells (3x10⁶/0.2 ml) and maintained in a pathogen-free environment (Laboratory Animal Center of Tzu Chi University, Hualien, Taiwan). On day 28, the mice were divided randomly into four groups (6 mice/group) to be treated with Tan-IIA (30 mg/kg of body weight, dissolved in corn oil, Q.week days 3 and 5), 5-FU (30 mg/kg of body weight, dissolved in normal saline, Q.week day 1) or Tan-IIA (30 mg/kg of body weight, Q.week days 3 and 5) plus 5-FU (30 mg/kg of body weight, Q.week day 1). As a control, xenografted tumors were separately treated with normal saline (0.1 ml/10 g body weight, Q.week day 1) and corn oil (0.1 ml/10 g body weight, Q.week days 3 and 5). Each agent was injected intraperitoneally. At the end of the 4-week dosing schedule, the SCID mice
were sacrificed by CO₂ inhalation on day 35. Following xenograft transplantation, mice exhibiting tumors were monitored, and tumor size was measured once every 2 days using calipers. The tumor volume for each animal was estimated according to the following formula: Tumor volume (mm³) = L x W²/2 (where L is the length and W is the width) with the final measurement taken 5 weeks after tumor cell inoculation. Mice were treated for 28 days, then sacrificed by CO₂ inhalation. The hep-J5 cell xenograft tumors were dissected and individually weighed. (A-C) Treatment with Tan-IIA 30 mg/kg or with 30 mg/kg of 5-FU resulted in a reduction in tumor size and (D) weight compared with the control group. *P<0.05

Results and discussion

The results indicated that Tan-IIA and 5-FU significantly reduced hep-J5 cell xenograft tumor size. Representative animals treated with Tan-IIA relative to the control are shown in Fig. 2. Tan-IIA treatment significantly decreased tumor volume (Fig. 3A-C) and weight (Fig. 3D) compared with the control. The percent inhibition of tumor weight is shown in Table I. None of the treatments altered the body weight significantly (data not shown). Tumors were only observed at the inoculation sites. Based on these in vivo experiments, it can be seen that Tan-IIA at 30 mg/kg can inhibit tumor growth in a J5 xenograft model. In the present study, tumors in mice that received Tan-IIA alone were approximately 55% smaller than those of the control group (Fig. 3A). In the 5-FU treatment and Tan-IIA plus 5-FU groups, tumors continued to grow slowly compared with the control group, indicating that complete regression of J5 cell xenografts was not achieved using a single treatment agent, and therefore that multiple treatments may be required to achieve a complete response.
However, our results revealed that Tan-IIA possesses therapeutic potential in HCC J5 cells in vivo. This is in agreement with other studies (18). However, the combination of Tan-IIA and 5-FU was not capable of producing any synergistic effects in J5 cells in vivo (Fig. 3C and D). Our results also revealed that the protein expression of Bax (Fig. 4A) and caspase 3 (Fig. 4B) in xenograft tumors treated with Tan-IIA and/or 5-FU was upregulated when compared to the control group. This is in agreement with our previous in vitro study and others (13,17). The protein expression of CD31 (Fig. 4C) in xenograft tumors treated with Tan-IIA (C3T group), 5-FU (C3F group) or their combination (3F3T group) was downregulated when compared to the control group. This result indicated that Tan-IIA and/or 5-FU did not increase the vascularity in hep-J5 xenograft tumors. The present study provides the first report of the efficacy of Tan-IIA against tumors in an in vivo xenograft of human HCC J5 cells in SCID mice.

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References