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• *BRIEF REPORTS* •

Antitumor and synergistic effect of Chinese medicine "Bushen huayu jiedu recipe" and chemotherapy on transplanted animal hepatocarcinoma

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Abstract

AIM: To investigate the antitumor and synergistic effect of Chinese medicine "Bushen huayu jiedu recipe" (recipe for invigorating the kidney, removing blood stasis and toxic substances) and chemotherapy on mice hepatocarcinoma.

METHODS: Bushen huayu jiedu recipe (BSHYJDR) consisting of Chinese Cassia Bark, Psoralea, Zedoary, Rhubarb, *etc.* is equal to 1.5 g/mL liquid of originated herbs after being decocted, filtered, and concentrated. Kunming mice, weighing 18-22 g, were injected with 0.2 mL ascitic hepatocarcinoma H_{22} containing 1×10^7 cells/mL into armpit of the right forelimb of mice. After 24 h, the mice were weighed and randomly divided into tumor-bearing model control group, cisplatin (DDP) group, BSHYJDR high dosage group, low dosage BSHYJDR group, DDP combined with high and low dosage BSHYJDR group, 10 mice in each group. DDP group received injection intraperitoneally (ip) at the dosage of 1 mg/kg (equal to 1/10 LD_{50}), once a day for 4 d. High and low dosage BSHYJDR groups received intragastric BSHYJDR at the dosages of 26.6 and 13.3 g/kg (20 and 10 times each of clinical adult dosage) respectively, while tumor-bearing model group received the equal volume of distilled water once a day for 10 d. On the 11th d, the mice were weighed and killed, then the tumor was dissected and weighed, the repression rate (RR) was calculated according to the mean weight of tumor (MWT).

RESULTS: Compared to the model group (MWT: 1.30±0.73), DDP group (MWT: 0.41±0.09, RR: 68.46%) had a significant difference in the inhibition of hepatocarcinoma H_{22} (P<0.01). High dosage BSHYJDR group (MWT: 0.69±0.29, RR: 46.92%) also had a significant difference in inhibition (P<0.05), while no difference was found in low dosage BSHYJDR group (MWT: 0.85±0.34, RR: 34.62%) (P>0.05). When DDP was combined with high dosage BSHYJDR (MWT: 0.29±0.17, RR: 77.69%) and low dosage BSHYJDR (MWT: 0.38±0.21, RR: 70.77%) respectively, we could see improvement of the inhibition effect of DDP on transplanted hepatocarcinoma H_{22}. DDP combined with high dosage BSHYJDR had a significant difference (P<0.001) compared to DDP, while DDP combined with low dosage BSHYJDR only had a little improvement that is not remarkable.

CONCLUSION: Chinese medicine BSHYJDR in combination with chemotherapy can inhibit transplanted hepatocarcinoma in mice.

Key words: Bushen huayu jiedu recipe; Mice hepatocarcinoma H_{22}; Antitumor effects; Synergy

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INTRODUCTION

Bushen huayu jiedu recipe (BSHYJDR) is used in treatment of many kinds of cancer, especially digestive tract and respiratory system cancer. We have discovered that BSHYJDR could obviously improve clinical symptoms, quality of life and prolong the life span of cancer patients. BSHYJDR could restrain transplanted lung cancer cells, and has a synergistic effect, when combined with chemotherapy. After animals received intragastric BSHYJDR, it could inhibit the expression of drug-resistance gene, increase the level of DDP in drug-resistant cells, and regulate the activity of calcium channel of resistant cells. Meanwhile, BSHYJDR could also restrain transplanted Lewis lung cancer[1,2]. In order to investigate whether BSHYJDR in combination with chemotherapy could synergistically inhibit hepatocarcinoma, we selected transplanted mice hepatocarcinoma H_{22}, and investigated the inhibitory effect of BSHYJDR on transplanted hepatocarcinoma.

MATERIALS AND METHODS

Drugs

To prepare BSHYJDR decoction, Chinese Cassia Bark, Psoralea Fruit, Zedoary, Rhubarb, etc., were put into a container, cold water of about 5-7 times as the herbs was added. After being soaked for 1-2 h and boiled for 30 min, the decoction was filtered. Once again, water of about 3-5

times as the herbs was added, decocted and boiled for 20 min before the second filtration. The two decoctions were mixed. Then the mixture was concentrated into medical fluid (equals 1.5 g/mL originate herbs in water bath). The fluid was poured into a sterilized vial after cooling and stored at 4 ℃ for use. DDP was produced by Qilu Pharmacy Factory in Jinan, Shandong Province, China.

Animals

Kunming mice were supplied by Experimental Animal Center of Shandong University of traditional Chinese medicine.

Tumor strains

Hepatocarcinoma H_{22} was provided by Tumor Pharmacology Laboratory of Tumor Prevention and Treatment Center in Shandong University of traditional Chinese medicine.

Main instruments

Ultra-clean worktable was from Suzhou Yuhua Equipment Co. Electronic balance (Type FA2004) was purchased from Shanghai Balance Instrument Factory.

Inhibition of BSHYJDR on mice hepatocarcinoma H_{22}

Experimental animal model and grouping Ascites was drawn from hepatocarcinoma mice H_{22} under aseptic condition, and diluted in normal saline at 1:4. Concentration of tumor cells was 1×10^7 cells/mL. Then 40 healthy Kunming mice weighing 18-22 g were selected, 0.2 mL tumor cells was injected into the armpit of right forelimb of each mouse. After 24 h, they were weighed and randomly divided into model control group, high dosage BSHYJDR group, and low dosage BSHYJDR group and DDP group, 10 mice in each group.

Drug dosages and methods DDP group received intraperitoneal injection of DDP at the dosage of 1 mg/kg (equals to 1/10 LD50), once a day for 4 d. High and low dosage BSHYJDR groups received intragastric BSHYJDR decoction at the dosages of 26.6 and 13.3 g/kg (20 and 10 times each of clinical adult dosage) respectively, while tumor-bearing model group received the same dosage of distilled water, once a day for 10 d. On the 11th d, all the mice were weighed and killed, and then the tumor was dissected and weighed. The repression rate (RR) was calculated, according to the mean weight of tumor (MWT).

Synergistic effect of BSHYJDR and DDP on mice hepatocarcinoma H_{22}

Experimental animals model and grouping The mice were divided into tumor-bearing model control group, DDP group, DDP combined with high dosage BSHYJDR group, DDP combined with low dosage BSHYJDR group, 10 mice in each group.

Drug dosages and methods After grouping, DDP group received intraperitoneal injection DDP (1 mg/kg). DDP combined with high dosage BSHYJDR group received intraperitoneal injection of DDP (1 mg/kg) and intragastric BSHYJDR (26.6 g/kg). DDP combined with low dosage BSHYJDR group received intraperitoneal injection of DDP (1 mg/kg) and intragastric BSHYJDR (13.3 g/kg). Model control group received an equal volume of distilled water. DDP was injected once a day for 4 d, while Chinese medicine and distilled water were lavaged once a day for 10 d. Then on the 11th d, all the mice were weighed and killed, and then the tumor was dissected and weighed. The RR was calculated according to the MWT and the formula of calculating the RR. RR of the tumor (%) = tumor weight of the control group-tumor weight of the experimental group/tumor weight of the control group×100%.

Statistical analysis

The data were expressed as mean±SD. Student's *t*-test was used to assess the significant differences among groups. All statistical analyses were performed with the PEMS statistical software package.

RESULTS

Inhibition of BSHYJDR on mice hepatocarcinoma H_{22} (Table 1)

Table 1 shows that compared to the control group, significant inhibitory effect of BSHYJDR was found in DDP group ($P<0.01$) and high dosage BSHYJDR group ($P<0.05$). But no significant difference of inhibitory effect was found in low dosage BSHYJDR group ($P>0.05$). The inhibitory effect in high and low dosage BSHYJDR groups was lower than that in DDP group ($P<0.01$).

Table 1 Inhibition of BSHYJDR on hepatocarcinoma H_{22}

Group	Dose (mg/kg)	Mice (*n*) (before/after)	Tumor weight (g) (mean±SD)	RR (%)
Model	–	10/10	1.30±0.73	–
DDP	1×4	10/10	0.41±0.09[b]	68.46
BSHYJDR	26 600×10	10/10	0.69±0.29[a,d]	46.92
BSHYJDR	13 300×10	10/10	0.85±0.34[d]	34.62

[a]$P<0.05$, [b]$P<0.01$ *vs* control group, [d]$P<0.01$ *vs* DDP group.

Synergistic effect of BSHYJDR and DDP on mice hepatocarcinoma H_{22} (Table 2)

Table 2 shows that compared to control group, DDP exhibited significant inhibition on H_{22} ($P<0.01$). Both high and low dosage BSHYJDR could significantly improve the inhibition of DDP when used in combination with DDP ($P<0.001$). But low dosage BSHYJDR had no such effects compared to DDP ($P<0.05$).

Table 2 Synergistic effect of BSHYJDR and DDP on hepatocarcinoma H_{22}

Group	Doses (mg/kg)	Mice (*n*) (before/after)	Tumor weight (g) (mean±SD)	RR (%)
Model	–	10/10	1.30±0.73	–
DDP	1×4	10/10	0.41±0.09[b]	68.46
DDP+BSHYJDR	1×4+26 600×10	10/10	0.29±0.17[d]	77.69
DDP+BSHYJDR	1×4+13 300×10	10/10	0.38±0.21[b]	70.77

[b]$P<0.01$, [d]$P<0.001$ *vs* control group.

DISCUSSION

Hepatocarcinoma is one of the malignant tumors, and has high death rate in the world. Most patients in the middle or

late period are diagnosed of having the tumor. Therefore, it is very important to find a new therapy with Chinese medicine for middle and late hepatocarcinoma.

The present study has indicated that extractions from some herbs can restrain hepatocarcinoma. Alkaloids have cellulotoxic effect on hepatocarcinoma cells[3]. The herbal extractions of flavonoid and arsenic trioxide can not only restrain proliferation of hepatocarcinoma cells, but also induce apoptosis of hepatocarcinoma cells[4,5]. It was reported that Chinese medicine can induce cytodifferentiation of hepatocarcinoma[6].

Toxic side effect of chemotherapy is an important influencing factor in treatment of hepatocarcinoma. Chinese medicine combined with chemotherapy can reduce the toxic side effects and improve inhibition of chemotherapy on hepatocarcinoma cells, reduce the metastasis, enhance the quality of life and prolong the life span[7]. The use of Xuefu Zhuyu Tang (recipe for removing blood stasis in the vessels) combined with chemotherapy can enhance the effect of chemotherapy and prolong the life span of mice bearing hepatocarcinoma[8].

According to the theory of traditional Chinese medicine, the occurrence of tumor is closely related with stagnation of qi and blood and combination of blood stasis and poison, which are caused by deficiency of the kidney and invasion of poisonous pathogenic factors. BSHYJDR can reinforce the kidney and support yang, remove blood stasis and toxic substances.

It has been proved that cinnamic acid in Cassia Bark and its derivatives could induce apoptosis of cancer cells, promote the breakage of DNA, inhibit the proliferation of A549 cells in adenocarcinoma and promote its differentiation[9,10]. The psoralen in psoralea fruit has cellulotoxic effects on hepatocarcinoma cells, promotes the adherence and excursion of melanoma cells *in vitro*[11,12] by preventing cancer cells from metastasis. The extractions of Zedoary could improve immunogenicity of tumor cells and enhance the killer effect of the cells. Moreover, Zedoary could strengthen the antitumor effect of tumor necrosis factor. Zedoary preparation is mainly used to treat hepatocarcinoma. The main components of Rhubarb are Rhubarb substance and Rhubarb acid. Rhubarb substance could restrain proliferation of hepatocarcinoma cells, induce apoptosis, and promote gene expression of P53 and P21[13-16]. Rhubarb acid could restrain cell proliferation of various tumors, such as mastocarcinoma, lung cancer, hepatocarcinoma and colonocarcinoma, and has synergistic effects when used in combination with mutamycin. It could inhibit synthesis of DNA, promote apoptosis of cancer cells. It also has been found that the inhibitory effect of repression of Rhubarb on cancer cells is closely related with cell signal transduction[17].

Our research showed that BSHYJDR decoction could significantly restrain transplanted hepatocarcinoma H_{22} in mice, enhance the effect of DDP on transplanted hepatocarcinoma H_{22}, indicating that BSHYJDR restrains tumor and has synergistic effects when used in combination with chemotherapy. But the mechanisms still remain unknown and need further studies.

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