Basic Investigation

The Effect-enhancing and Toxicity-reducing Action of the Extract of Herba Scutellariae Barbatae for Chemotherapy in Hepatoma H22 Tumor-bearing Mice

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Objective: To investigate the effect-enchancing and toxicity-reducing action of the extract of Ban Zhi Lian (半枝莲 Herba Scutellariae Barbatae, EHSB) for chemotherapy in hepatoma H22 tumor-bearing mice. **Methods:** The tumor-bearing mice were divided into 6 groups randomly: a model group, a high dose EHSB group, a low dose EHSB group, a 5-fluorouracil (5-FU) group, a 5-FU+high dose EHSB group and a 5-FU+low dose EHSB group, and with a normal group set as the controls. All the groups were treated for 10 days. The life prolongation rate, toxic reactions of chemotherapy, WBC count, the body weight, tumor weight, thymus index and spleen index, and phagocytic function of intra-abdominal macrophages were investigated in the H22 tumor-bearing mice. **Results:** The increase of the body weight in both the 5-FU+EHSB groups was significantly higher than that in the 5-FU group, with the toxic reactions such as anorexia, abdominal distension and emaciation significantly alleviated. Growth of the tumor was significantly inhibited in the high dose EHSB group, the 5-FU group, the 5-FU+high dose EHSB group, and the 5-FU+low dose EHSB group. The survival time in the 5-FU+high dose EHSB group and the 5-FU+low dose EHSB group was significantly prolonged as compared with that of the 5-FU group. The life prolongation rate was 98.72% in the 5-FU+high dose EHSB group and 52.11% in the 5-FU+low dose EHSB group. Growth of the transplanted tumor was significantly inhibited in the high dose EHSB group, the 5-FU group, the 5-FU+high dose EHSB group, the 5-FU+low dose EHSB group. The tumor inhibition rate in the high dose EHSB group, the 5-FU group, the 5-FU+high dose EHSB group and the 5-FU+low dose EHSB group was 36.98%, 42.26%, 65.28% and 52.45%, respectively. 5-FU combined with a high-dose EHSB could significantly enhance the tumor inhibition rate (P<0.05). The thymus index and the spleen index significantly increased in the high dose EHSB group, and atrophy of the immunological organs induced by chemotherapy was improved in the 5-FU+high dose EHSB group and in the 5-FU+low dose EHSB group. The WBC count decreased significantly in the 5-FU group, but increased in both the 5-FU+EHSB groups. The phagocytic function of intra-abdominal macrophages was increased in both the 5-FU+EHSB groups, with the phagocytic rate and the phagocytic index increased by 78.55% and 81.63% in the 5-FU+high dose EHSB group and by 43.97% and 44.90% in the 5-FU+low dose EHSB group. **Conclusions:** EHSB can significantly enhance the tumor inhibition rate of 5-FU, reduce the toxic effects, prolong the survival time, and improve immune function in the H22 tumor-bearing mice.

Recent studies have demonstrated that Ban Zhi Lian (半枝莲 Herba Scutellariae Barbatae, HSB) has a good anti-tumor effect, which can be used for treatment of primary liver cancer, lung cancer and carcinoma of the uterine cervix, and when in combination with other Chinese medicine compounds it is indicated for more kinds of cancers.[1-3] At present, the toxicity-reducing and effect-enhancing action of

Chinese medicine for radiotherapy and chemotherapy of tumors have been confirmed. [4-6] The present experiment was designed to investigate the anti-tumor effect of EHSB combined with 5-fluorouracil (5-FU), so as to probe the effect-enhancing and toxicity-reducing action for chemotherapy in H22 tumor-bearing mice.

MATERIALS AND METHODS

Extract of Herba Scutellariae Barbatae (EHSB, Xi'an Zhongxin Biotechnology Co. Ltd.); 5-fluorouracil (5-FU, Shanghai Xudong Haipu Pharmaceutical Co. Ltd); the other reagents are analytical pure made in China. ICR mice, weighing 20±2 g, male and female in half, supplied by Animal Center of Xi'an Jiaotong University (No.08-004). The mouse hepatoma H22 cell strain was preserved in College of Life Sciences, Shanxi Normal University.

Modelling of the tumor-bearing mice

Ascites was taken from hepatoma H22 mice passing on from generation to generation for 6-7 days under aseptic condition, which was diluted with normal saline into a suspended solution in a concentration of $1x10^7$ cells/ml. For the solid tumor experiment, 0.2 ml of the cell suspension was injected into the subcutaneous part of the right axillary fossa in each mouse, and for the ascites tumor experiment, 0.2 ml of the cell suspension was injected into the abdominal cavity in each mouse.

Grouping and administration

Twenty-four hours after injection of the H22 cells, the mice were randomly divided into 6 groups, i.e. a model group, a high dose EHSB group, a low dose EHSB group, a 5-fluorouracil (5-FU) group, a 5-FU+high dose EHSB group and a 5-FU+low dose EHSB group, 10 mice in each group, and with a normal group set as the controls (0.2 ml saline was injected into the right axillary fossa for each mouse of this group). The mice in both the normal control group and the model group were given intra-gastric perfusion of 0.4ml saline, once a day. In the 5-FU group, 5-FU (0.02 g/kg) was injected into the abdominal cavity, once daily. The high and low dose EHSB groups received intra-gastric perfusion of EHSB at the dose of 12 g and 3 g/kg respectively once daily. The above treatments lasted 10 days. 24 h after the last administration, all the mice were killed. The blood sample was taken for count of WBC, and the tumor was separated and weighed. The tumor inhibition rate was calculated: (the tumor weight of the model group − the tumor weight of the treatment group) / the tumor weight of the model group x 100%.

Determination of life prolongation rate in the H22-bearing mice

The grouping and drug administration for the ascites tumor experiment were the same as the solid tumor experiment. The survival time of the mice was recorded for 35 days after the drug was withdrawn. The experiment would be invalid if the mortality rate in the control group during the experiment was ≥ 20% or the survival time of 20% mice was longer than 4 weeks. The life prolongation rate = (the mean living days of the mice in the treatment group / the mean living days in the control group −1) x 100%.

Determination of the thymus index and spleen index

After the tumor inhibition experiment in the mice with solid tumor was ended, the mice in each group were sacrificed. The thymus and spleen were taken and weighted, and the thymus index and the spleen index were calculated. The thymus index = the thymus weight (mg) / body weight (g); while the spleen index = the spleen weight (mg) / body weight (g).

Assessment of the phagocytic function of intra-abdominal macrophages

One day before the last administration, 1 ml of 5% chicken erythrocyte suspension was injected into the abdominal cavity of each mouse. 24 hours later, all the mice were sacrificed, and 2 ml normal saline was injected into the abdominal cavity of each mouse through the peritoneum with the median abdominal skin incised. After that, the abdomen of each mouse was kneaded, the abdominal liquid was sucked and dropped on a slide, and incubated at 37℃ for 30 min,

then washed with saline, fixed with acetone-methanol solution (1:1) for 5 min, stained with 4% giemsa for 3 min, rinsed with running water and dried. Ten consecutive high power fields were taken for calculating the phagocytic rate and the phagocytic index. The phagocytic rate (%) = (The macrophages phagocytizing RBC / the total macrophages) x 100%; and the phagocytic index = the phagocytized chicken erythrocytes / the total macrophages.

Statistical analysis

The data were expressed as mean ± standard deviation (SD). SPSS11.0 software was used for *q* test in single factor analysis of variance to evaluate the differences between two groups, with $\alpha = 0.05$ regarded as a significant difference.

RESULTS

Effect of EHSB on body weight and general conditions in H22 tumor-bearing mice

The body weight of the tumor-bearing mice in all the groups increased in varying degrees. The body weight increase was significantly higher in the high dose EHSB group than that of the model group ($P<0.05$); but the increase in the 5-FU group was significantly lower than those of the other groups. The increase in the 5-FU+EHSB groups was significantly higher than that of the 5-FU group (see Table 1).

The movement gradually became slower in the mice of the model group, with trichoxerosis, less eating and drinking and slow increase of body weight; and the mice in the 5-FU group even died in the course of administration. In contrast, the mice in the EHSB groups were vigorous with flourishing hair, rapid increase of the body weight, showing a good general condition. The toxic responses in the 5-FU group were also serious, manifested by anorexia, abdominal distention, listlessness and emaciation, etc.; while the mice in the EHSB groups showed no such the symptoms, and the mice in the 5-FU+EHSB groups showed milder adverse effects with no obvious anorexia, abdominal distention and emaciation, etc.

Table 1. Effect of EHSB on body weight in H22 tumor-bearing mice ($\bar{x} \pm s$, n=10)

Group	Dosage (g/kg/d)	Body weight (g)		Increase of body weight (g)
		Pre-treatment	Post-treatment	
Model	-	20.03±2.07	23.50±3.24	3.47±3.82$^{\#}$
EHSB	3.00	20.00±1.94	24.52±1.82	4.52±3.93$^{\#}$
	12.00	20.01±2.10	25.15±2.13	5.14±3.76*$^{\#}$
5-FU	0.02	19.96±2.03	20.74±1.86	0.78±1.93*
5-FU+EHSB	0.02+3.00	20.00±2.13	22.46±1.92	2.46±1.62$^{\#}$
	0.02+12.00	19.98±2.05	20.74±2.05	4.35±3.25*$^{\#}$
Control	-	20.03±1.94	24.12±2.02	4.09±2.32$^{\#}$

Note: *$P<0.05$, vs the model grouo; $^{\#}P<0.05$, vs the 5-FU group.

Effects of EHSB on the life prolongation rate and the tumor inhibition rate in the H22 tumor-bearing mice

The survival time of the ascites tumor mice in the high dose EHSB group was significantly higher than those of the model group and the 5-FU group ($P<0.05$); and the survival time of the 5-FU group was little shorter than that of the model group with no significant difference between the two groups. The life prolongation rates in the 5-FU+high dose EHSB group and the 5-FU+low dose group were 98.72% and 52.11% respectively, which were significantly higher than that of the 5-FU group (see Table 2).

The tumor weight in the high dose EHSB group and the 5-FU group was significantly lower than that of the model group ($P<0.05$), with a tumor inhibition rate of 36.98% and 42.26%, respectively. The tumor inhibition rate was 14.34% in the low dose EHSB group, with no significant difference as compared to the model group ($P>0.05$); and the rat was 65.28% in

the 5-FU+high dose EHSB group, which was significantly higher than that of the 5-FU group (P<0.05). No significant difference was found between the 5-FU+low dose EHSB group and the 5-FU group in the tumor inhibition rate (P>0.05), as showed in the Table 2.

Table 2. Effects of EHSB on the life prolongation rate and the tumor inhibition rate in H22 tumor-bearing mice ($\bar{x} \pm s$, n=10)

Group	Dosage (g/kg/d)	Survival time (d)	Life prolongation rate(%)		Tumor weight (g)	Tumor inhibition rate (%)
			vs model group	vs 5-FU group		
Model	-	16.32±4.92	-	-	2.65±1.12#	-
EHSB	3	18.47±5.23#	13.17	39.29	2.27±0.93#	14.34
	12	24.65±5.80*#	51.04	85.90	1.67±0.76*	36.98
5-FU	0.02	13.26±4.15	-	-	1.53±0.64*	42.26
5-FU+EHSB	0.02+3	20.17±4.54#	23.59	52.11	1.26±0.45*	52.45
	0.02+12	26.35±5.65*#	61.46	98.72	0.92±0.58*#	65.28

Note: *P<0.05, vs the model group; #P<0.05, vs the 5-FU group.

Effects of EHSB on the thymus index, spleen index and WBC count in H22 tumor-bearing mice

As compared with the model group, the thymus index and the spleen index increased significantly in the high dose EHSB group (P<0.01), but decreased significantly in the 5-FU group. As compared with the normal control group, the thymus index and the spleen index were significantly lower in the model group and further decreased in the 5-FU group. In the 5-FU+high dose EHSB group and the 5-FU+low dose EHSB group, atrophy of the immune organs induced by chemotherapy were improved. As compared with the model group, the WBC count showed no significant difference in both the EHSB groups (P>0.05), a significant decrease in the 5-FU group, and an increase tendency in the 5-FU+EHSB groups (see Table 3).

Table 3. Effects of EHSB on the thymus index, spleen index and WBC count in H22 tumor-bearing mice ($\bar{x} \pm s$, n=10)

Group	Dosage (g/kg/d)	Thymus index (mg/g)	Spleen index (mg/g)	WBC count (10^9/L)
Model	-	2.47±0.68#	3.82±2.32#	8.78±0.68
EHSB	3	2.62±1.54#	4.25±1.92#	8.82±0.59
	12	3.65±2.36*	5.44±3.06*	9.16±0.70
5-FU	0.02	1.43±0.56*#	2.92±1.73*#	5.15±0.52*
5-FU+EHSB	0.02+3	2.55±1.32#	3.96±1.65#	7.62±0.64#
	0.02+12	3.16±2.15#	4.72±2.02#	8.47±0.58#
Control	-	3.64±2.03*#	5.67±2.37*#	9.08±0.74

Note: *P<0.05, vs the model group; #P<0.05, vs the 5-FU group.

Effect of EHSB on phagocytic function of intra-abdominal macrophages in H22 tumor-bearing mice

The phagocytic function of the intra-abdominal macrophages was significantly enhanced in the high dose EHSB group. The lowered phagocytic function of the intra-abdominal macrophages that may be induced by 5-FU treatment was improved in the 5-FU+high dose EHSB group and the 5-FU+low dose EHSB group. As compared with the 5-FU group, the phagocytic rate was increased by 78.55% and 43.97%, and the phagocytic index was increased by 81.63% and 44.90% respectively in the 5-FU+high dose EHSB group and in the 5-Fu+low dose EHSB group (see Table 4).

Table 4. Effect of EHSB on phagocytic function of intra-abdominal macrophages in H22 tumor-bearing mice ($\bar{x} \pm s$, n=10)

Group	Dosage (g/kg/d)	Phagocytic rate (%)	Phagocytic index
Model	-	38.52±3.16#	0.73±0.04#
EHSB	3	41.32±3.05#	0.79±0.05#
	12	47.46±3.55*#	0.93±0.06*#
5-Fu	0.02	22.47±2.12*	0.49±0.03*
5-Fu+EHSB	0.02+3	32.35±2.53#	0.71±0.06#
	0.02+12	40.12±3.68#	0.89±0.05#

Note: *P<0.05, vs the model group; #P<0.05, vs the 5-FU group.

DISCUSION

At present, chemotherapy is usually adopted as one of the main measures for a comprehensive treatment of tumors. However, the adequate dosage in a complete course may induce such adverse effects as bone marrow suppression, gastrointestinal reactions and a lowered immunological function. This may possibly limit the adequate dosage, and decrease the therapeutic effects.[4, 5] Now, the immuneoregulation, the body resistance-strengthening and anti-cancer effects of Chinese herbal medicines have attracted more and more attention. [7, 8]

Traditional Chinese medicine holds that primary liver cancer should be attributed to the damp-heat pathogen protractedly attacking the human body, leading to a weakened body resistance and resulting in a lingering pathogenic course with stagnancy of *qi* and blood, imbalance of *yin* and *yang*, stagnation of liver-*qi*, deficiency of spleen, and with blood stasis blocking the channels and collaterals, thus, forming tumor in the hypochondrium.[7] Herba Scutellariae Barbatae may show the effects of clearing away heat and toxic materials, removing blood stasis and promoting urination. It has been demonstrated that EHSB possesses an obvious inhibitory action on the transplanted tumors (S180 and H22), and it can promote proliferation of the spleen cells in mice with a good dose-effect relation; [9] and the experiment in vitro shows that EHSB can inhibit proliferation of the hepatic tumor cells and induce the apoptosis.[2] The present study indicates that in the high dose EHSB group, the growth of H22 transplanted tumor is significantly inhibited; and that the tumor inhibition rate in the 5-FU+high dose EHSB group is significantly higher than that of the 5-FU group, while no such significant difference was found in the low dose EHSB group.

How to increase the sensitivity of tumor to chemotherapeutic drugs and how to reduce the side effects have attracted more and more attention for the cancer treatment. In this study, the increase of body weight in both the 5-FU+EHSB groups is significantly higher than that of the 5-FU group. The adverse effects, such as anorexia, abdominal distention, listlessness and emaciation, were found in the 5-FU group, but no such side effects were shown in both the EHSB groups; and the toxic reactions were markedly reduced in both the 5-FU+EHSB groups. The life prolongation rate is 61.46% in the 5-Fu+high dose EHSB group and 23.59% in the 5-Fu+low dose EHSB group; and as compared with that of the 5-Fu group, the rates were 98.72% and 52.11% respectively, showing the importance of supporting treatment in chemotherapy.

With development and deterioration of the malignant tumor, the immune function of the patient may be decreased, which will become more severer in the course of radiotherapy and chemotherapy, lower the therapeutic effect, or even discontinue the treatment.[4,8] In the present study, the thymus index

and the spleen index obviously decreased in the mice of the model group, and even more decreased in the 5-FU group; but they were significantly increased in the high dose EHSB group and in both the 5-FU+ EHSB groups, indicating that EHSB can improve the condition of immune organs, and decrease the immune response in the tumor-bearing mice induced by chemotherapy. And for the tumor-bearing mice given 5-FU, EHSB can elevate WBC count and enhance the phagocytic function of intra-abdominal macrophages, indicating that EHSB can effectively antagonize the immunosuppression caused by tumor. Therefore, EHSB may probably be used as a biological response modifier in chemotherapy.

The present study shows that a high dose of EHSB (12g/kg/d) has a tutor-inhibiting action, show a certain synergism with 5-FU, and can reduce the toxic side effects; while the low dose of EHSB (3g/kg/d) can obvious reduce the toxic reactions and improve the immunosuppression though it can not elevate the tumor inhibition rate. The effect-enhancing and toxicity-reducing action of EHSB may be possibly due to the anti-tumor effect displayed by flovonoids and diterpenes contained in EHSB;[10] and its components, such as alkaloid, steroid and poly-saccharide, may also activate the immune action of immune cells on the tumor cells.

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