

Original Research Article

Hemodynamic changes and tissue expressions of MVD and VEGF in rabbits before and after treatment of VX2 liver implantation tumor with β -elemene

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Abstract

Purpose: To investigate hemodynamic changes, tissue microvessel density (MVD), and expression of vascular endothelial growth factor (VEGF) in rabbits before and after treatment of VX2 liver implantation tumor with β -elemene.

Methods: Forty New Zealand white rabbits of VX2 tumor strain provided by Xi'an Jiaotong University were used for establishment of VX2 liver implantation tumor model. Two groups of rabbits were used: control and study groups (20 rabbits per group). Control group was treated with saline infusion (2mL/10min), while rabbits in the study group were treated with 50 mg β -elemene perfusion for 10 min. Changes in hemodynamic indices, i.e., hepatic artery perfusion volume (AP), hepatic artery perfusion index (HPI), blood flow (BF), blood volume (BV) and hepatic portal vein blood flow (PVP) were determined using CT perfusion scanning. Following the sacrifice of the rabbits, tumor tissues and normal tissues around the tumor were subjected to immunohistochemical staining for assessment of MVD (CD31) and expression of VEGF.

Results: Post-treatment levels of hepatic arterial perfusion (HAP), BF and BV in rabbits in each group changed significantly, and PVP level was increased, but was markedly reduced in the study group, relative to control ($p < 0.05$). The pre- and post-treatment levels of HPI were comparable ($p > 0.05$). However, in the study group, there was a significant reduction in MVD and VEGF, when compared with control ($p < 0.01$).

Conclusion: β -elemene causes significant changes in the hemodynamic parameters of VX2 liver transplantation tumor in rabbits. The treatment significantly decreases the levels of HAP, BV and BF, while increasing that of PVP. Moreover, β -elemene significantly decreases VEGF expression and MVD.

Keywords: β -Elemene, VX2 liver implantation tumor, Hemodynamics, MVD, VEGF

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INTRODUCTION

Liver cancer is one of the common malignant tumors in China. Based on the location and causes, it is classified into two types: primary liver cancer and secondary liver cancer. Primary

liver cancer originates from the epithelial or interlobar tissue of the liver, and its incidence ranks fifth in the world [1]. With advancements in social economy, living standards have improved tremendously, leading to accentuated incidence of obesity, hepatitis and liver cirrhosis.

The early symptoms of liver cancer are not usually obvious. Thus, by the time obvious symptoms appear, more than one-third of liver cancer patients are already in advanced stage of the disease [2]. At present, surgery is the main method for treating hepatocellular cancer, but the probability of recurrence in patients with advanced stage is high. Therefore, there is need to develop newer methods for the clinical treatment of liver cancer [3].

The strategy of transhepatic artery chemoembolization (TACE) blocks the blood supply of the tumor, and results in tumor ischemia and hypoxia, thereby inhibiting tumor growth by promoting tumor cell death and apoptosis [4]. Some studies have found that arterial injection of chemotherapeutic drugs increases the local drug concentration in the tumor, and also reduces the toxic and side effects of the drug on normal tissues [5].

β -Elemene is a major bioactive component responsible for the anti-tumor effect of *Curcuma aromatica* Salisb. It is associated with a wide spectrum of anti-tumor and curative effects and low toxicity/side effects, and it significantly improves the quality of life of patients, without compromising liver and kidney functions [6]. The purpose of this study was to investigate the effects of β -elemene on hemodynamics, and tissue MVD and VEGF expressions in rabbits before and after implantation of VX2 liver tumor.

EXPERIMENTAL

Animals and tumor strains

Forty healthy New Zealand White rabbits aged 4 months (mean weight = 2.7 ± 0.3 kg) were obtained from the Experimental Animal Center of Xi'an Jiaotong university. The rabbits were raised in a single cage. The experimental tumor strain (VX2 tumor strain) was provided FFOurth Military Medical University.

This research was approved by the Animal Ethical Committee of Xi'an Gaoxin Hospital (approval no. 20183187), and was conducted according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [7].

Instruments and reagents

The major instruments and reagents used, and their sources (indicated in brackets) were: fetal bovine serum (Shanghai Hengyuan Biotechnology Co. Ltd.); pentobarbital sodium (Beijing HuayeHuanyu Chemical Co. Ltd.,

specification: 5 g); phosphate buffered saline (PBS; Wuhan Punosai Life Technology Co. Ltd.); β -elemene (Jiangsu Fiya Biotechnology Co. Ltd.), and centrifuge (Hunan Kaida Scientific instrument Co. Ltd., Model: KL04A). The others were cryogenic refrigerator (Zhongke Meiling Biomedical Co. Ltd., Model: DW-YL450); DMEM medium (Shanghai Thermo Fisher Scientific Co. Ltd.), and electronic balance (Sartorius Scientific Instruments Co. Ltd.).

Establishment of rabbit model of VX2 tumor

The VX2 tumor was inoculated into the lateral muscle of the hind leg of each rabbit. After 7 days, the nodular mass attained a diameter larger than 1cm. Then, the tumor tissue with active growth at the edge of the tumor mass was cut into fine grain chippings of volume 1 mm^3 . The rabbits were anesthetized, and the abdominal cavity was opened to expose the liver. Then, the VX2 tumor was injected into the liver parenchyma. The surface of the liver at the puncture site was covered with gelatin sponge and sutured. After 3 weeks of implantation, when the diameter of tumor grew to about 2 to 3cm, it was used in subsequent experiments.

Two groups of rabbits were used: control and study groups, with 20 rabbits per group. Rabbits in the control group were infused with saline at the rate of 2 mL/10 min, while those in the study group received β -elemene perfusion at the rate of 50 mg/10 min.

Study indices

Changes in hemodynamic indices, i.e., hepatic artery perfusion volume (AP), hepatic artery perfusion index (HPI), blood flow (BF), blood volume (BV) and hepatic portal vein blood flow (PVP) were recorded using CT perfusion scanning technique. Then, the rabbits were sacrificed. Tumor tissue and the normal tissue around the tumor were excised and preserved in 4 % formaldehyde solution. After routine dehydration, the tissue specimens were embedded in paraffin blocks. Using a microtome, the tissue specimens were cut into $4\text{-}\mu\text{m}$ slices which were fixed on slides and oven-dried at 60°C . Thereafter, the tissue specimens were rinsed thrice with PBS phosphate buffer (each rinse for 3 min). The slides were then stained using S-P staining kit, and DAB coloring was carried out for 3 to 5 min.

The slides were counterstained with hematoxylin, followed by dehydration, transparency and sealing. The expressions of MVD (CD31) and VEGF was determined using immunohisto-

chemical method. For VEGF, the degree of positive cell staining was indicated in the cell membrane or cytoplasm as brownish yellow and brown colors. The percentage of positive cells in each visual field was calculated by selecting 6 visual fields under each high magnification field. For MVD (CD31), a brownish yellow stain in tumor cells or other connective tissues or isolated endothelial cells or endothelial cell clusters in the tumor indicated the presence of a microvessel. The tumor areas rich in microvessels were selected and 6 high density areas of microvessels were designated and observed under high power microscope. The number of microvessels in each visual field and its average value were calculated.

Statistical analysis

Measurement data were compared with independent sample *t*-test, while counting data were compared using χ^2 test. Ridit test was used to compare grade data. All statistical analyses were done with SPSS21.0 software package. Statistical significance was fixed at $p < 0.05$.

RESULTS

Effect of treatment on hemodynamic changes in rabbits

As shown in Table 1, compared with values before treatment, the levels of HAP, BF and BV in rabbits in each group were significantly changed, and PVP level was increased after treatment, but was markedly reduced in the study group ($p < 0.05$). The pre- and post-treatment levels of HPI were comparable ($p > 0.05$).

MVD (CD31) and VEGF expressions

There were significant decreases in the levels of MVD and VEGF in the study group, when compared to control group ($p < 0.01$). These results are shown in Table 2 and Figure 2.

Table 2: MVD (CD31) and VEGF expressions

| Group | MVD | VEGF |
|----------|--------------|-------------|
| Study | 18.73 ± 1.28 | 1.01 ± 0.64 |
| Control | 31.59 ± 1.11 | 3.02 ± 1.28 |
| <i>T</i> | 33.945 | 6.281 |
| <i>P</i> | < 0.001 | < 0.01 |

Values are mean ± SD

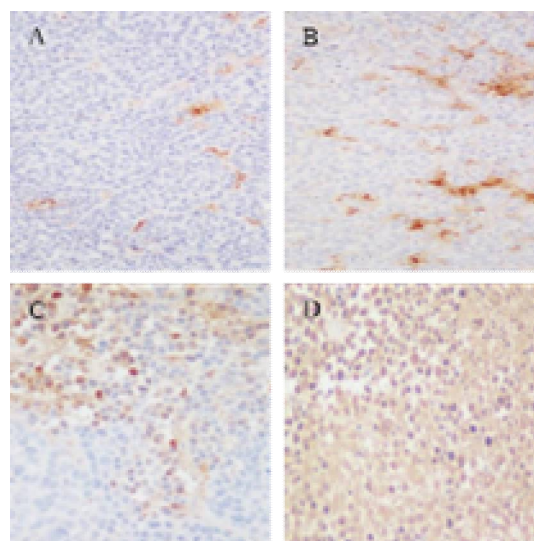


Figure 2: MVD (CD31) and VEGF expressions in the rabbits. A: expression of MVD (CD31) in the study group; B: expression of MVD (CD31) in control group; C: expression of VEGF in the study group, and D: expression of VEGF in control group

Table 1: Hemodynamic changes in the rabbits (n = 20)

| Group | Time | HAP (ml/100 ml/min) | HPI | BF (ml/100 ml/min) |
|---------|------------------|----------------------------|----------------------------|-----------------------------|
| Study | Before treatment | 51.84 ± 5.22 | 99.76 ± 0.47 | 54.93 ± 11.94 |
| | After treatment | 27.99 ± 3.14 ^{ab} | 96.15 ± 5.69 ^{ab} | 28.88 ± 12.88 ^{ab} |
| Control | Before treatment | 51.18 ± 2.58 | 99.98 ± 0.04 | 55.32 ± 10.55 |
| | After treatment | 58.42 ± 7.38 ^a | 99.27 ± 0.55 ^a | 46.47 ± 14.52 ^a |

| Group | Time | BV (ml/100ml) | PVP (ml/100ml/min) |
|-------------|------------------|---------------------------|---------------------------|
| Observation | Before treatment | 9.24 ± 0.53 | 0.22 ± 0.13 |
| | After treatment | 4.41 ± 2.33 ^{ab} | 2.02 ± 1.35 ^{ab} |
| Control | Before treatment | 9.05 ± 1.23 | 0.21 ± 0.11 |
| | After treatment | 10.14 ± 2.11 ^a | 1.38 ± 0.52 ^a |

^a $P < 0.05$, vs same group before treatment; ^b $p < 0.05$, vs control. Values are mean ± SD

DISCUSSION

Primary liver cancer occurs frequently in China. In recent years, the incidence of liver cancer has been on the increase. The early symptoms of liver cancer are not usually obvious. Thus, most of the patients are already in the middle and late stages at the time of diagnosis and treatment, thereby missing the best period for operation. This results in poor prognosis. Therefore, the search for strategies for early diagnosis of liver cancer has become a key clinical problem.

At present, TACE is the recognized and preferred method. The technique is recognized as a non-surgical method for the treatment of primary lung cancer. It selectively blocks the arterial blood supply of liver cancer and plays an anticancer role [8]. Some studies have shown that chemoembolization induces apoptosis in tumor cells through its influence on apoptosis-related genes, as well as inhibition of the proliferation of cancer cells [9].

β -Elemene is an effective bioactive monomer extracted from the root and stem of *Rhizoma curcumae*. It is one of the three antitumor components of elemene [10]. Many studies have shown that β -elemene exerts its anti-tumor effect through several mechanisms including induction of apoptosis, interference with cancer cell growth and metabolism, inhibition of the development of cancer cells, and suppression of synthesis and metabolism of DNA, RNA and protein [11,12].

The killing effect of β -Elemene on tumor requires effective concentration and continuous action. The purpose of this study was to investigate the hemodynamic changes, MVD and the expression of VEGF in rabbit tissues before and after treatment of VX2 liver implantation tumor with β -elemene.

It has been suggested that early evaluation of hemodynamic changes in tumor therapy can be effectively used for clinical evaluation of the therapeutic effect of drugs on tumor [13]. Studies have shown that hemodynamic parameters change significantly after drug treatment [14]. In the present study, the level of HAP, BF and BV in rabbits in each group were significantly changed by β -elemene. The PVP level was increased after β -elemene treatment, but was markedly lower in the study group. The level of HPI was not significantly affected by the treatment. These results indicate that the levels of HAP, BF, BV, PVP and other parameters reflect the perfusion of rabbit VX2 liver implantation tumor tissue before and after treatment. Thus, they may be used for effective detection of hemodynamic

changes, and for early evaluation of the curative effect of tumor treatment strategies. The results also indicate that β -elemene significantly changes hemodynamic indices and reduces blood flow in rabbits.

Many studies have found that although TACE causes coagulation necrosis in most tumors due to collateral circulation, portal vein blood supply and neovascularization, it does not result in complete necrosis. The presence of residual tumors with blood supply is a critical factor involved in recurrence, metastasis and prognosis of liver cancer post-TACE. The expression of VEGF and neovascularization are among the most important of these factors [15,16]. It has been reported that the levels of VEGF and MVD in cancer tissues are significantly higher than those in normal tissues, and that there is a significant positive correlation between VEGF and MVD [17].

Vascular endothelial growth factor (VEGF) is a highly glycosylated basic protein secreted by normal cells and tumor cells. It has been found that VEGF is closely related to the occurrence and development of tumor blood vessels, and is also directly involved in tumor invasion and metastasis [18]. It is an important vascular permeability factor which can increase the permeability of small vessels such as capillaries and small veins to macromolecules. Increases in microvascular permeability cause plasma protein exudation into the vascular space and promotes the growth of new blood vessels. Microvessel density (MVD) refers to the number of microvessels per unit area of biological tissue such as skin, muscle and organ. It is an important index for assessing the current angiogenesis activity of tumors, and is closely related to tumor invasion, metastasis and prognosis [19]. It has been revealed in this study that, compared with the control group, the levels of MVD and VEGF in the observation group were significantly lower than those in the control group. The results suggest that β -elemene effectively destroys the microvessels of tumor tissues, reduces MVD, and inhibits VEGF expression.

CONCLUSION

The results obtained indicate that β -elemene significantly changes the hemodynamic indices of rabbit VX2 liver implantation tumor by decreasing the levels of HAP, BV and BF, while increasing PVP level. Besides, β -elemene inhibits the expression of VEGF and decreases MVD in tumor tissues.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

This work was done by the authors named in this article and the authors accept all liabilities resulting from claims which relate to this article and its contents. Liangshan Lv designed the study and interpreted the results. Jingtao Gu, Liangshan Lv collected data and drafted the manuscript. Jingtao Gu performed the experiments.

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