EXPERIMENTAL STUDY OF THE EFFECT OF ANGIOGENESIS INHIBITOR TNP-470 ON THE GROWTH AND METASTASIS OF GASTRIC CANCER *IN VIVO*

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Abstract

Objective: To study the effect of angiogenesis inhibitor TNP-470 on the growth and metastasis of gastric cancer *in vivo*. Methods: Metastatic model simulating human gastric cancer was established by orthotopic implantation of histologically intact tumor tissue into gastric wall of nude mice. TNP-470 was administrated S.C. at doses of 0 mg/kg, 15 mg/kg, 30 mg/kg, 60 mg/kg every other day for eight weeks. Ten weeks after implantation, the mice were sacrificed and the tumor size measured and the presence of metastasis recorded. The microvascular density was examined by immunohistochemical staining with anti-human factor VIII antibody. Results: Compared to the untreated controls, growth of the orthotopically implanted tumor was significantly reduced in size in the mice treated with TNP-470 with an inhibition rate of 59.9%, 77.0% and 84.9% at the dosage of 15 mg/kg, 30 mg/kg and 60 mg/kg, respectively. Tumor metastasis to the liver and peritoneum was also significantly inhibited in a dose-dependent manner. The microvascular density was also decreased significantly in the treated mice. Conclusion: Angiogenesis inhibitor TNP-470 has strong inhibitory effect both on tumor growth and metastasis of human gastric cancer in nude mice.

Key words: Gastric cancer, TNP-470, Angiogenesis, Liver metastasis, Tumor growth, Inhibition.

Recently, more and more attention has been paid to the anti-tumor effects of the anti-angiogenesis. TNP-470, a synthetic analogue of fumagillin, which is a naturally secreted antibiotic of Aspergillus fumigatus Fresenius,[1] can inhibit tumor angiogenesis by inhibiting endothelial division. In this study, we treated the metastatic model of gastric cancer with TNP-470 and detected the microvascular count in both treated and untreated mice to determine its anti-tumor effects.

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MATERIALS AND METHODS

Animals

Six-week-old male BABL/C nu/nu nude mice (provided by Shanghai Institute of Tumor) specific pathogenic free degree. Body weight was about 20 g.

Drugs

TNP-470, a synthetic analogue of fumogillium (kindly provided by Dr. M. Yamaoka, Takeda chemistry Co. Osak, Japan). Its structure to see the reference.[1] TNP-470 was dissolved in 1% ethanol, 5% gum Arabic in saline solution.

Metastatic Model of Gastric Cancer

SGC-7901 human gastric cancer cells 2×10^6 were injected subcutaneously to nude mice. Transplant the tumor to the next generation when tumor formed. In this experiment, the tumors were the sixth generation. Tumors at the exponential growth phase in nude mice were resected aseptically, necrotic tissues were cut away, and the remaining healthy tumor tissues were scissors minced into pieces about 3 mm in diameter in normal saline solution. Each piece of tumors was weighted, and adjusted to be 150 mg with scissors.

Mice were anesthetized with 45 mg/kg of pentobarbital sodium, and an incision was made through the pararectal line and peritoneum. The stomach wall was carefully exposed, and a part of the serosal membrane, about 3 mm in diameter, in the middle of the greater curvature of the glandular stomach was mechanically injured by scissors. Tumor pieces of 150 mg were then fixed on each injured site of the surface with a 5–0 Dexon transmural suture. The stomach was returned to the peritoneal cavity. The abdominal wall and skin were closed with 3–0 Dexon suture. Animals were kept in a sterile environment.

Assay of Tumor Growth and Metastasis

Animals were given TNP-470 S.C. at doses of 15, 30, 60 mg/kg everyday from a week after implantation. As a control, the same volume of 1% ethanol, 5% gum Arabic in saline solution for 8 weeks.

Mice were sacrificed 10 weeks after implantation. Autopsy was performed immediately, and the tumors growing on gastric wall were removed and the length (a) and the width (b) were measured. Tumor volume was calculated by a standard formula. Tumor volume $(v)=ab^2/2$;

Inhibition ratio = (control group volume-experimental group volume)/control group volume×100%

Microvesscular Density

Tumor tissues were embedded in paraffin according to standard histological procedures. Sections (5 μm thick) were permeabilized with 0.05% trypsin at 37C° for 30 min, and washed in PBS. Endogenous peroxidase activity was quenched by incubation with 0.3% H_2O_2 in PBS for 15 min followed by three PBS washes. Sections were incubated with rabbit against human VIII factor monoclonal antibody. Antibody binding was detected by sequential incubation for the section with peroxidase-anti-peroxidase complex. Positive staining was detected by substrate reaction with diaminobenzidine.

RESULTS

Inhibition Effect on Tumor Growth

All implanted tumors grew up on the stomach wall. The effect of TNP-470 on tumor growth at three different concentrations (15, 30, 60 mg/kg) was compared with the results of administration of a vehicle of 1% ethanol and 5% gum Arabic in saline solution.

The mean volume of experimental groups was smaller than that of control group. The tumor growths were inhibited by 59.5%, 77.0% and 84.9% respectively at the dose of 15, 30 and 60 mg/kg TNP-470 (Table 1).

Inhibition Effect on Tumor Metastasis

In the control group, the metastatic model of gastric cancer established by orthotopic implantation histological-intact tumor tissues, local lymph node metastasis occurred in 100% mice, tumor metastasis to peritoneum in 89.3% mice (25/28), and metastasis to liver occurred in 83.1% (23/28) mice in addition. There were 4 of 28 mice with ascites and other site metastasis. Tumor metastases were inhibited significantly by TNP-470. 51.3, 87.8% and 87.8% liver metastases were inhibited, and metastases to the peritoneum were inhibited by 32.8%, 66.4% and 77.6% at the dosage of 15 mg/kg, 30 mg/kg and 60 mg/kg, respectively.

Table 1. The inhibition effect of TNP-470 on the metastasis and growth of gastric cnacer

Dosage (mg/kg)	Tumor volume (cm^3)	Peritoneal metastases		Liver metastases		Ascites	Other site metastases	Microvascular Density*
		Case	%	Case	%			
0	1.52±0.59	25/28	89.3**	23/28	82.1**	4/28	10/28	11.35±3.71
15	0.61±0.44	6/10	60.0	4/10	40.0	0/10	3/10	5.84±2.15
30	0.35±0.30	3/10	30.0	1/10	10.0	0/10	0/10	3.23±1.33
60	0.23±0.17	2/10	20.0	1/10	10.0	0/10	0/10	2.23±0.83

* Microvascular count per view **Metastasis rate = Metortases case/experimental case ×100%

Microvascular Density

The microvascular density in treated group was significantly decreased than that of in the control group. The inhibition of microvascular density was associated with the dose of TNP-470. The most effective dosage for microvascular density was 60 mg/kg, which inhibited about 80%. But there was no significant different between 30 mg/kg and 60 mg/kg group ($P>0.05$).

DISCUSSION

Tumorigenesis and development is dependent on angiogenesis. Tumorigenesis proceeds through two distinct preneoplastic stages. The first involves a switch from quiescence to hyperproliferation of the oncogene expression of the growth/survival factor. The second step is induction of angiogenesis, where in the normally quiescent vasculature is activated to proliferate and form new capillaries. Parangi et al.[2] treated the transgenic mouse model of β islet cell carcinomas with angiogenesis inhibitor TNP-470, minocyclin and INF-α/β to block the tumor angiogenesis. Their study demonstrated that the treatment regimen markedly attenuated tumor growth, tumor volume was reduced to 11% and capillary density to 40% of control. Antiangiogenesis therapy with angiogenesis inhibitor may be a new way to inhibit tumor growth.

An increasing number of angiogenesis inhibitors are candidates for therapeutic studies. These include the following: endogenous endothelial inhibitors, such as thrombospondin, the 16 KD fragment of prolactin, and angiostatin, a fragment of plasminogen; and regulatory cytokines, such as INF-α/β, synthetic derivatives of fungal and bacterial compounds, such as TNP-470 and the antibiotic minocyclin. Currently, the latter is expected to be a new anticancer agent. Fumagillin is a naturally secreted antibiotic of Aspergillus fumigatus Fresenices and inhibits human umbilical vein endothelial cell proliferation and tumor-induced angiogenesis at a concentration of 2.5 ng/ml. However, because of its side effect, prolonged administration is limited. TNP-470 is one of the synthetic analogues of fumagillin and shows more potent antiangiogenic activity and less toxicity than fumagillin. Ingber et al.[1] demonstrated that TNP-470 exhibited inhibitory activity on endothelial cells and solid tumor growth.

In this report, we treated the metastatic model settled by orthotopic implantation of histologically intact human tumor tissue into gastric wall of nude mice with TNP-470, the tumor growth were inhibited significantly.

Tumor growth and dissemination of both the primary and secondary depend on tumor angiogenesis. Tumor angiogenesis is the neovascularization permits the shedding of cells from the primary lesion. Secondary tumor growth depends on the development of an adequate blood supply through angiogenesis. In this opinion, angiogenesis inhibitors should have an inhibitory effect on tumor growth *in vivo*. We treated the metastatic model of gastric cancer with angiogenesis inhibitor TNP-470, and got perfect inhibition effect both on tumor growth and metastasis. Tanaka et al.[3] reported that the liver metastasis of colon cancer had inhibited by 55.0% and 91.3% at the dosage of 20 mg/kg and 30 mg/kg TNP-470. Kanai[4] treated the metastatic model of gastric cancer with 30 mg/kg TNP-470, metastasis to the liver and the peritoneum were 57% (4/7) and 0% (0/7) respectively, while in the untreated group, that were 100% (10/10) and 60% (6/10) respectively. So, long term less toxicity angiogenesis inhibitor will be one of valuable anticancer therapy.

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