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ORIGINAL ARTICLE

Bevacizumab followed by chemotherapy is potential therapy for gastric cancer

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Summary

Purpose: *To investigate the antitumor effects of the angiogenesis inhibitor bevacizumab combined with chemotherapy, and the application of in vivo imaging technology of growth of fluorescence-labelled gastric cancer (GC) in nude mice.*

Methods: *Twenty-five nude mice were randomly divided into 5 groups (A-E). Subcutaneous xenograft of human MGC803 cells was transplanted to nude mice, followed by different treatments for the groups, including A (bevacizumab combined with chemotherapy), B (24-h chemotherapy with FP followed by bevacizumab), C (bevacizumab 24-h followed by FP chemotherapy), D (bevacizumab only) and E (normal saline). Then, dynamic variation of tumor growth during 4 weeks was evaluated by calculating the tumor inhibition rate and fluorescence signal strength by in vivo imaging system.*

Results: *After 28-day treatment, fluorescence signal strength in the groups A-D changed significantly compared with the E (control) group, while tumor inhibition rate in C group was highest (68.42%). Furthermore, on the 4th week, the fluorescence signal value in C group was lowest.*

Conclusions: *Administration of bevacizumab followed by chemotherapy was more effective therapeutic method for GC. The in vivo imaging could show off dynamic variation of tumors and was a sensitive and objective detection method.*

Key words: *bevacizumab, chemotherapy, gastric cancer, in vivo imaging*

Introduction

GC is the third most common carcinoma and the second leading cause of death worldwide [1]. So far, surgery remains the only curative option for GC patients, while it is restricted to early or locally advanced disease [2]. At present, with low early-stage diagnosis rate, most patients suffer of advanced or metastatic GC when diagnosed [3]. Then chemotherapy is the only treatment option for advanced GC, despite its limited efficacy [4].

A recent study confirmed that angiogenesis inhibitors could inhibit GC growth through antiangiogenesis therapy [5]. How to evaluate the effect of targeted therapy combined with chemotherapy in GC and the investigation of the best protocol of chemotherapy drug administration have become researchers' hotspots [6,7]. The present research dealt with an intervention to treatment to study tumor growth in nude mice with subcutaneous xenograft of human GC using bevacizumab combined with chemotherapy (5-fluorouracil and cisplatin) and investigate the best drug administration protocol. Tumor growth was also dynamically observed by *in vivo* imaging system.

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Methods

Conditions

Male BALB/c-nu nude mice aged 28-35 d with body weights of 20-22 g were provided by Beijing HFK Bioscience (permission number SCXK (Jing) 2009-0004). The nude mice used in this experiment were housed in a laminar air flow room with a specific pathogen free (SPF) facility. In the laminar air flow room, everything that came into contact with the nude mice was sterilised and staff entering the laminar air flow room had to wear sterile surgical masks, caps, gloves, and gowns. Room temperature of the laminar air flow room was controlled within 26-28 °C, humidity was maintained within 40-60%, and the ventilation exchange was 10-15 times per hr. Feed was made into a monolayer on a small plate and was autoclaved for 45 min at 120 °C. Drinking water was filtered through a 0.2 microporous membrane. The pressure per square inch did not exceed 10 pounds. Water bottles were changed every day.

Subcutaneous xenograft nude mice model

The fluorescence-labelled human GC MGC803 cells in logarithmic phase (provided by Shanghai Science light Biology Science & Technology) were collected and adjusted to a concentration of 2.5×10^7/L. Each nude mouse was inoculated subcutaneously on the back with 0.2 ml of the cell suspension. Xenograft modules <1 cm were considered to be successfully established.

Grouping

Twenty-five subcutaneously xenografted nude mice were randomly divided into 5 groups, including experimental groups (A, B, C and D) and control group (E). Bevacizumab (5 mg/kg/d) was administered twice a week on Tuesday and Friday. Chemotherapy was intraperitoneal injection of 5-FU (40 mg/kg/d) with cisplatin (4 mg/kg/d) 4 times a week on Monday, Tuesday, Thursday, and Friday. Group A was administered bevacizumab combined with chemotherapy. Group B was treated with 24-h chemotherapy followed by bevacizumab administration. Group C was administered 24-h bevacizumab followed by chemotherapy. Group D was administered of bevacizumab only. Group E was the control group with intraperitoneal injection of 1 mL normal saline on Monday, Tuesday, Thursday, and Friday. After 28-day treatment, all nude mice were sacrificed.

Detection methods

The body weight of nude mice was recorded every 7 days. The growth and distribution of tumors in nude mice were investigated and recorded every 7 days by *in vivo* imaging, and tumor volume was measured according to the following formula: $V = 1/2 \times a \times b^2$ mm^3, where a and b were length and width, respectively. Tumor inhibition rate was calculated as follows: tumor inhibition rate=1-(tumor volume in the control group-tumor volume in the experimental group)/tumor volume in the control group×l00%. Before detection of fluorescence signals of nude mice tumors, each mouse was subcutaneously injected with 0.4 g/kg chloral hydrate (4%) and 150 mg/kg luciferase substrate. Ten min after the injection, *in vivo* imaging was performed and the fluorescence signal strength was recorded.

Statistics

Data are presented as mean±standard error of the mean (SEM). Comparisons of homogeneity between different groups before the experiment were performed using one-way analysis of variance (ANOVA). Comparison of body weight, tumor volume and fluorescence signal strength between different time points were performed with repeated ANOVA measurements as well. $P < 0.05$ indicated that the differences were statistically significant. All data were analysed using SPSS 16.0 software.

Results

Confirmation of homogeneous nude mice

There was no significant difference ($p<0.05$) in body weight, tumor volume and fluorescence signal strength among the groups, indicating that nude mice in the 5 groups were homogeneous before treatment (Table 1).

Table 1. The body weight, tumor volume, and fluorescence signal strength of the nude mice in each group were homogeneous before treatment (mean±SEM)

Groups	*Weight (g)*	*Tumor volume (mm^3)*	*Fluorescence signal strength*
A	21.86±0.50	225.71±116.83	31131±11345
B	20.84±0.95	199.61±44.00	68614±44212
C	21.22±1.30	221.22±39.67	77956±5779
D	21.48±0.75	248.51±81.21	26741±10828
E	22.16±0.39	170.15±43.46	47236±4760
F	1.893	0.854	3.377
P	0.151	0.508	0.054

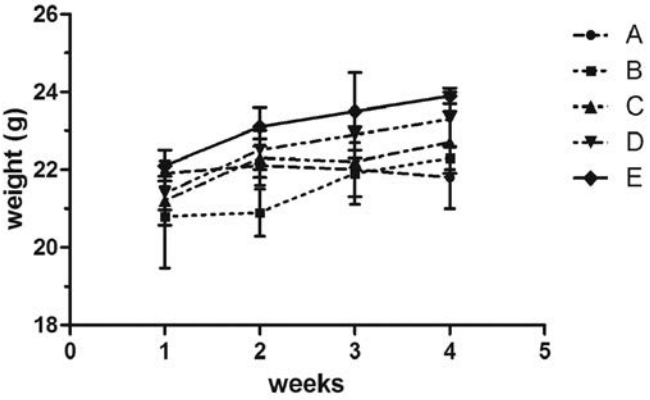

Figure 1. The changes in body weight of nude mice in different groups. There was no significant difference (F=1.578, p=0.219) in body weight variation in nude mice among the different groups. Further group-wise comparison showed that the body weight variation at different time points of the nude mice in group **A** did not exhibit significant difference (F=0.375, p=0.773), whereas the body weight in the **B**, **C**, **D**, and **E** groups showed an increasing trend over time (p<0.05)

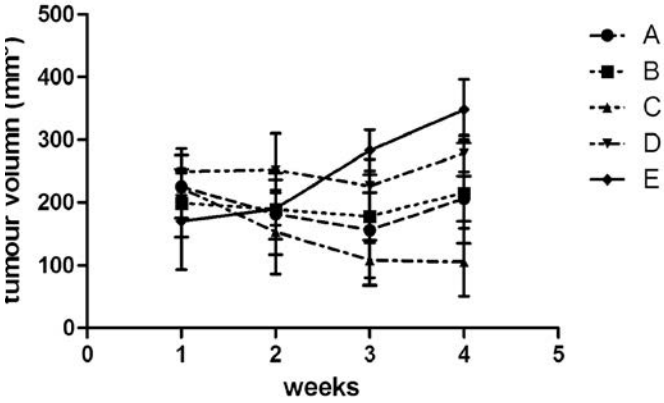

Figure 2. The effects of different sequence times of administration of bevacizumab combined with chemotherapy on the xenograft tumor volume of nude mice. Tumor volume variation of nude mice among the different groups did not exhibit significant difference (f=1.812, p=0.166). The volume in group **C** was significantly smaller compared with groups **D** and **E** (p<0.05). The tumor volume of nude mice in group **C** exhibited a significantly decreasing trend over time (p<0.05), while that in group **E** exhibited a significantly increasing trend over time (p<0.05)

Evaluation of body weight of nude mice

There was no significant difference (F=1.578, p=0.219) in body weight variation in nude mice among the different groups. Further group-wise comparison showed that the body weight variation at different time points of the nude mice in group A did not exhibit significant difference (F=0.375, p=0.773), whereas the body weight in the B, C, D, and E groups showed an increasing trend over time (p<0.05) (Figure 1).

Evaluation of the xenograft tumor volume of nude mice

After 28-day treatment, tumor inhibition rates were 40.95% in group A, 37.57% in group B, 68.42% in group C, and 19.84% in group D, respectively. Tumor volume variation of nude mice among the different groups did not exhibit significant difference (F=1.812, p=0.166). The mean tumor volume of different groups after treatment from small to large was as follows: C, A, B, D, and E. The volume in group C was significantly smaller compared with groups D and E (p<0.05). The tumor volume of nude mice in group C exhibited a significantly decreasing trend over time (p<0.05), while that in group E exhibited a significantly increasing trend over time (p<0.05) (Figure 2).

Evaluation of the fluorescence signals of nude mice

Variation in the fluorescence signals of tumors were significantly different among groups (F=18.219, p<0.001). Further group-wise comparisons indicated that the changes in different groups before treatment were not significantly different (F=2.979, p=0.074). After treatment, groups were listed according to fluorescence signals from low to high as follows: C, A, B, D, and E. Fluorescence signals of the nude mice tumors in groups A and C at the 4th week were lower than those before treatment (p<0.05). The fluorescence signals of the tumors in group E showed a dramatically increasing trend over time (p<0.05) (Figures 3 and 4).

Discussion

The synergistic effect of the combination of bevacizumab and chemotherapy

In addition to killing tumor cells, traditional chemotherapeutic drugs also have a certain cytotoxic effect on cells in normal tissues and can display strong toxic side effects [8]. Therefore, their efficacy in treating GC is limited. With the appearance of anti-tumor targeted drugs, targeted therapy is more advantageous [9]. The treatment effect of bevacizumab alone, a macromolecular anti-angiogenic drug, is minimal [10]; however, when combined with chemotherapy, bevacizumab has shown some positive results in clinical studies [11], suggesting that these two have synergistic effects.

The present study indicated that the tumor inhibition rates of combined targeted drugs and chemotherapy were significantly higher than in the bevacizumab-alone group. Treatment with

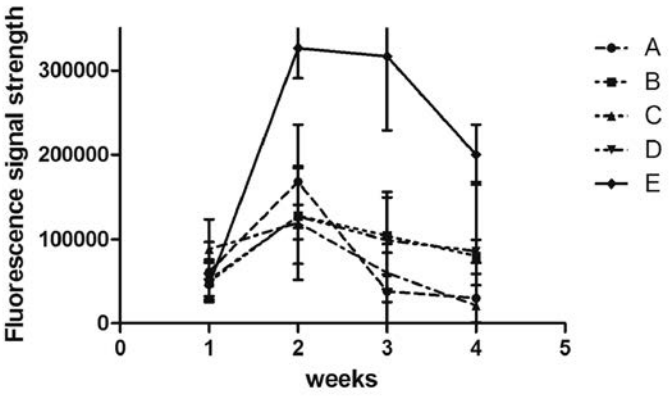

Figure 3. The effects of different sequence times of administration of bevacizumab combined with chemotherapy on fluorescence signals in the xenograft tumors of nude mice. Variation in the fluorescence signals of tumors were significantly different among groups (f=18.219, p<0.001). Further group-wise comparisons indicated that the changes in different groups before treatment were not significantly different (f=2.979, p=0.074). Fluorescence signals of the nude mice tumors in groups **A** and **C** at the 4th week were lower than those before treatment (p<0.05). The fluorescence signals of the tumors in group **E** showed a dramatically increasing trend over time (p<0.05).

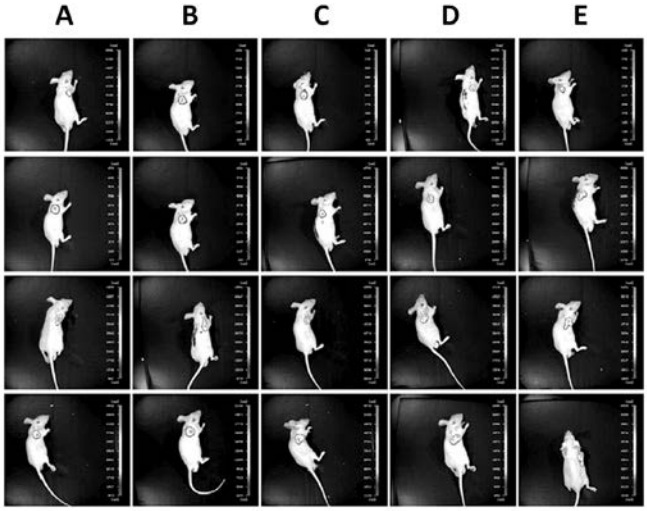

Figure 4. *In vivo* imaging of fluorescence signals in the xenograft tumors of nude mice. Variation in the fluorescence signals of tumors were significantly different among groups (F=18.219, p<0.001). Further group-wise comparisons indicated that the changes in different groups before treatment were not significantly different (F=2.979, p=0.074). Fluorescence signals of the nude mice tumors in groups **A** and **C** at the 4th week were lower than those before treatment (p<0.05). The fluorescence signals of the tumors in group **E** showed a dramatically increasing trend over time (p<0.05).

bevacizumab for 24 hrs, followed by chemotherapy (group C) showed the highest rate of tumor inhibition (68.42%) in nude mice, treatment of bevacizumab combined with FP chemotherapy showed 40.95% tumor inhibition rate, treatment of 24-h FP chemotherapy followed by bevacizumab showed 37.57% tumor inhibition rate, while bevacizumab-alone (group D) showed only 19.84% tumor inhibition rate.

Recently, a study investigated the administration sequences of another anti-angiogenic drug (endostar) and chemotherapy and indicated that treatment with endostar followed by chemotherapy could significantly induce apoptosis of transplanted human lung carcinoma [12]. The administration of targeted drugs combined with different chemotherapeutic agents with different sequence times may have opposite results [13], which may influence the cell cycle. This mechanism will be addressed in a follow-up study.

Changes in fluorescence strength could intuitively reflect treatment effects

Current methods for the evaluation of anti-tumor drug efficacy generally include animal sacrificing after drug administration, weighing the tumor, and obtaining experimental data through calculation of tumor inhibition rates using different formulas [14], which bears hazard of a certain human manipulation error. However, *in vivo* imaging system can trace the tumor changes in the same subjects (labelled cells) by recording the experimental subjects in different groups at different time points [15]. Because this imaging system can perform continuous detection on the same animal to dynamically obtain the long and short tumor diameters and their fluorescence signal strength, the effects of errors caused by the traditional detection method on experimental results can be minimized to a maximum extent [16,17].

The results of the present study confirmed that this imaging system could evaluate whether an established cell line was tumorigenic in animals, dynamically provide variations of tumors to calculate tumor volume, and the dynamic fluorescence signal strength of tumors to reflect tumor cell activities. The tumor volume in group C showed no change after 3 weeks of drug administration, but the fluorescence signal strength continuously decreased, indicating that using tumor cell activity to determine treatment effects is more sensitive and more objective.

In summary, different time sequences of targeted drugs combined with chemotherapy had different anti-tumor effects. The results showed that administration of anti-angiogenesis target-

ed drugs before chemotherapy had significantly stronger anti-tumor effects. Monitoring anti-tumor effects with *in vivo* imaging system could perform continuous and dynamic observations and could accurately provide tumors' variations, which was a new intuitive and sensitive method for the investigation of anti-tumor drug activities.

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Conflict of interests

The authors declare no confict of interests.

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