The Effect of Bevacizumab on Intratumoral Angiogenesis of Malignant Fibrous Histiocytoma in Animal Model.

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Introduction

Vascular endothelial growth factor (VEGF) is considered to be a key mediator among the angiogenic growth factors causing tumor growth and metastasis, hence the development of anticancer drugs targeting angiogenesis and clinical trials have been widely conducted. Bevacizumab (Avastin ○?R), one of the specific inhibitor for angiogenesis and a neutralizing antibody against VEGF, has recently been used as a drug against human malignancies such as colorectal cancer, lung cancer, breast cancer, and renal cell carcinoma (1, 2). We previously demonstrated that bevacizumab inhibited tumor growth of malignant fibrous histiocytoma (MFH) in vivo. In this study, we examined the effect of bevacizumab on intratumoral angiogenesis.

Materials & Methods

Animals. Male athymic BALB/c nude mice (6 week-old) were maintained in pathogen free conditions and in accordance with institutional principals. All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals at our institution.

Implantation of tumor cells. Human MFH cell line, Nara H, was used in this study. We injected Nara H cells (1.2×10^7) subcutaneously to the dorsal area of mice. After implantation, we measured body weight and tumor dimensions twice a week. Tumor volume was calculated according to the formula $V=\pi/6\times a^2\times b$, where a and b represent the shorter and the longer dimension of the tumor.

Effect of bevacizumab on tumor growth. We examined whether bevacizumab affects tumor volume and survival rate. We randomly divided fifty mice into two groups, treatment group (n=25) and control group (n=25) and gave them intraperitoneal injection twice a week for 8 weeks (2mg/kg of bevacizumab to treatment group or PBS only to control group). We measured body weight and calculated tumor volume and survival rate at the indicated time.

Immunohistochemical analysis. To evaluate intratumoral angiogenesis of tumor, we performed immunohistochemical staining of both VEGF and Factor VIII. Nineteen mice received intraperitoneal injection with bevacizumab or PBS twice a week (treatment group (n=10) and control group (n=9)). After 18 days, all tumors were removed and immunohistochemical staining of both VEGF and Factor VIII were performed. To estimate the expression of VEGF, we used the IHC score, which is a semi-quantitative evaluation system to evaluate the level of antigen expression. Micro vessel density (MVD) was determined with expression of Factor VIII.

Statistical analysis. The statistical significance were evaluated by the Mann-Whiteny U-test, Student's T-test and Spearman's rank correlation. $P<0.05$ was considered as statistically significant.

Results

Tumor growth was significantly inhibited by bevacizumab (Figure 1). We did not find a difference in body weight between treatment group and control group. Tumor volume was significantly decreased in treatment group compared with control group after 16 days treatment. At the end of experimental period, the mean tumor volume of treatment group and control group were $2.7\times10^{-5}m^3$ and $1.4\times10^{-5}m^3$, respectively. There was no significant difference in survival rate between two groups, however survival rate of treatment group was higher than that of control group (76.6% with treatment group and 59.7% with control group).

Bevacizumab significantly decreased MVD but not VEGF expression (Figure 2, Table 1). There was no significant difference in IHC score of VEGF expression between two groups. MVD which was determined by immunohistochemical staining of Factor VIII was significantly decreased in treatment group. The mean MVD value was 4.2 in treatment group and 7.2 in control group (p=0.005). We also found a significant correlation between tumor volume and MVD in treatment group (p=0.02, r=0.53).

Discussion

Recent studies have revealed a critical role of VEGF in regulation of physiological and pathological angiogenesis. We previously demonstrated that VEGF was often expressed in bone and soft tissue tumors as well as in various solid tumors (3). Bevacizumab is an anti-angiogenic drug by binding specifically with VEGF, and its effectiveness has been reported for the adjuvant therapy of colorectal cancer as well as lung cancer and breast cancer. In this study, bevacizumab significantly inhibited tumor volume and intratumoral MVD of MFH in vivo, and there was a significant correlation between tumor volume and MVD in treatment group. These results suggest that bevacizumab may suppress tumor growth of MFH via inhibiting intratumoral micro vessel formation and that bevacizumab may be a novel therapeutic agent for MFH.

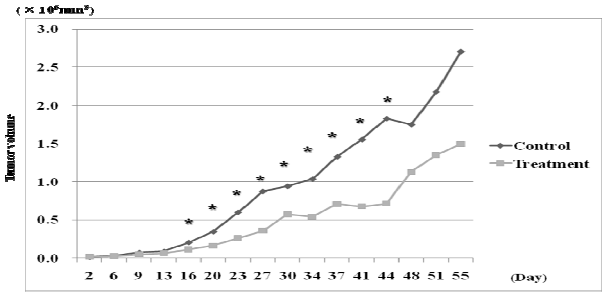
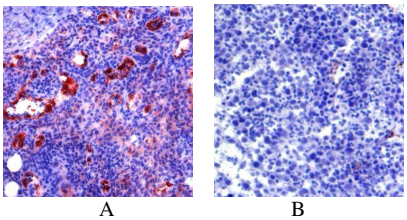


Figure 1. Inhibition of tumor growth by bevacizumab.
After day 16 of treatment, tumor volume was significantly inhibited in treatment group compared with that in control group. * $P<0.05$



A B

Figure 2. Factor VIII expression.
Sections were stained immunohistochemically using Factor VIII antibody. A, control group; B, treatment group.

	IHC score	MVD
Control group	**4.8±1.1**	**7.2±2.5**
Treatment group	**4.1±1.2**	**4.2±1.4**

Table 1. Immunohistochemical analysis.
There was no significant difference in IHC score of VEGF expression between two groups. The mean MVD value was significantly decreased in treatment group compared with control group (P=0.005).

References

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