ANTITUMOR EFFECT OF AN ANTI-ENDOTHELIAL CELL MONOCLONAL ANTIBODY BVE-1 ON SOLID TUMOR XENOGRAFT IN NUDE MICE

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ABSTRCT

Objective: To study the possibility of treatment for solid tumors by targeting vascular endothelial cells with a monoclonal antibody (MoAb) BVE-1. Methods: Leiomyosarcoma cell line SK-LMS-1, liver cancer cell line 7721 and pancreatic cancer cell line SW1990 xenografts in nude mice were treated ip with an anti-endothelial cell monoclonal antibody BVE-1 or ^{131}I labeled BVE-1, with normal mouse IgG or ^{131} I labeled IgG as controls. The tumor volume was measured at regular intervals following treatment. After sacrificing of the mice, the tumors were histologically examined and the intra-tumoral microvessel density (TMVD) recorded. Results: The inhibition effects of tumor growth in mice treated with BVE-1 were 49.8% in SK-LMS-1, 48.7% in SW1990 and 70.5 in 7721 respectively. Metastasis of leiomyosarcoma was also inhibited by the antibody treatment, leading to a decrease in the death rate. This effect was enhanced when treated with ^{131}I-labeled BVE-1 as the inhibition rate of tumor growth increased to 82.2–86.6%. Pathologically, vascular endothelial cells degeneration, occlusion of blood vessels and massive tumor cells necrosis around the degenerated vessels were observed in the BVE-1 treated mice. TMVD was significantly lower in the BVE-1 treated mice than that in mice treated with normal mouse IgG and in the untreated mice. Conclusion: The monoclonal antibody against vascular endothelial cells BVE-1 is effective in the treatment of human cancer xeno-grafted in nude mice by the induction of vascular endothelial degeneration and vascular occlusion inside the tumor. It may be used as a novel strategic approach in the treatment of human solid tumors.

Key words: Liver neoplasms, Pancreatic neoplasms, Leiomyosarcoma, Monoclonal antibody, Angiogenesis

The development of new blood vessels is important in tumor growth and metastasis. Targeting vascular endothelial cells to inhibit angiogenesis or occlude the blood vessels, block the passage of blood elements to tumor cells, resulting in irreversible tumor cells death, may be a novel strategy of tumor therapy. Burrows FJ, et al. was the first who proposed the approach of antibody derives targeting vascular endothelial cells in solid tumors. He developed an animal model expressing tumor vascular endothelium by transfection of the tumor cell with IFN-γ gene. When anti-MHC-II immunotoxin was given to these mice, it resulted in complete thrombosis of the tumor vasculature, widespread infarction and dramatic regressions of large solid tumors.[1] We report here the experimental antitumor effect of an anti-neovascular endothelium monoclonal antibody prepared by our laboratory on a murine model of solid tumor xenografts.

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MATERIALS AND METHODS

Animals

Female BALB/c nu/nu mice were obtained from Chinese Academy of Military Medical Sciences and

used at 4 or 6 weeks of age, each weighing 20 to 24 grams.

Antibody and ^{131}I-labeled Antibody

MoAb BVE-1 prepared by our laboratory was an anti-neovascular endothelium monoclonal antibody. Intact murine MoAb BVE-1 which was the IgG_{2a} isotype was reactive with a 150KD molecular weight lipo-glucoprotein containing sialic acids.[2] Ascitic MoAb BVE-1 was purified by protein A column. Iodination of MoAbs was performed by standard techniques. MoAb BVE-1 preparations were labeled with ^{131}I using the Iodogen method and then purified by Sephadex G-50 column. Labeling efficiency and specific activity (Mbq/μg) were determined using paper and column chromatography methods. The immunoreactivity of radiolabeled MoAb preparations was measured using an *in vitro* live cell-binding assay.

Cell Lines and Murine Model of Tumors

Hepatocarcinoma cell line (human) 7721 was provided by Second Military Medical College of PLA. Pancreatocarcinoma cell line (Human) SW1990 and leiomyosarcoma cell line (human) SK-LMS-1 was purchased from ATCC. Tumor cells 7721(1.0×10^7/body), SW1990 (2.0×10^6/body) and SK-LMS-1(0.5×10^6/body) in 0.1 ml saline were inoculated subcutaneously into the right flank of nude mice respectively.

Treatment Schedules

Treatment schedules were described in the Table 1–3. Drugs were administered intraperitoneally.

Table 1. Treatment of mice xenografted with hepatocarcinoma

Groups	No. of mice	Tumor size before treatment (mm^3)	Dosages of MoAb (μg/per time/per week)
MoAb BVE-1	10	0	200/per time ×2
NaCl solution	5	0	*

Treatment began three days after xenografted tumor cells.
* 0.9% NaCl solution was administered with the same volume and times as MoAb BVE-1 solution.

Table 2. Treatment of xenografted with leiomyosarcoma

Groups	No. of mice	Tumor size before treatment (mm^3)	Dosages of MoAb(μg/per time/per week)
MoAb BVE-1	5	23.8 ± 6.4	200/per time×3
Murine IgG	5	22.9 ± 6.2	200/per time×3
NaCl solution	5	22.7 ± 5.0	*

* 0.9% NaCl solution was administered with the same volume and times as MoAb BE-1 solution

Table 3. Treatment of xenografted with pancreatocarcinoma

Groups	No. of mice	Tumor size before treatment (mm^3)	Dosages of ^{131}I (Mbq)	Dosages of MoAb (μg)
^{131}I-BVE-1	4	120.4 ± 21.3	18.5	97×1
^{131}I-murine IgG	4	119.8 ± 27.8	18.5	98.5×1
MoAb BE-1	4	122.9 ± 37.5	0	200×6
Murine IgG	4	118.7 ± 31.1	0	200×6
NaCl solution	4	122.9 ± 31.0	0	*

* 0.9% NaCl solution was administered with the same volume and times as MoAb BVE-1 solution

Observations

Tumor volume. Tumors were measured every other day following treatment using a sliding caliper and the tumor size was calculated as: Tumor volume (mm^3) = (long diameter × short diameter^2) ×π/6.

Histology and Immunohistochemistry. After the mice died naturally or were sacrificed, primary tumors and metastatic tumors were observed for gross and microscopic appearance. Intratumor microvessel density (TMVD) was assessed by light microscopic analysis for areas of the tumor that contained the most capillaries and small venules. Areas of highest neovascularization were found by scanning the tumor sections at × 100 magnification and selecting those areas of tumor with the greatest density of distinct factor VIII antigen staining microvessels. (Using immunoperoxidase technique, Anti-factor VIII antibody was purchased from Zhongshan Reagent Corporation, Beijing)[3]

Statistical Analysis

The significance of differences of tumor volume

and MTVD between the experimental groups was calculated by Student's t test. $P< 0.05$ was considered significant.

RESULTS

Preparation of Antibody

After ascitic MoAb BVE-1 preparation was purified, it was up to electrophoresis's purity and its immunoreactivity by immunoperoxidase analysis was 1: 10,000. The MoAb reacted with vascular endothelium in leiomyosarcoma frozen sections. Labeling efficiency of ^{131}I-BVE-1 and ^{131}I-murine IgG was 97.3%, 96.2% and their specific activity was 189.3 and 187.9 kBq/μg respectively. Reactivity of ^{131}I-BVE-1 with endothelium and controlling cell lines determined by *in vitro* live cell banding assay was 24.5% and 2.2% respectively, whereas reactivity of ^{131}I-murine IgG with endothelial cell lines was 3.5%.

Inhibitory Effect of Treatment on Tumor Growth

Changes of tumor volume and tumor inhibition rate were described in the Table 4–7. In the MoAb BVE-1 treated group, more times MoAb BVE-1 was administrated, more inhibitory effect on tumors was caused. Inhibition rates of leiomyosarcoma, pancreatocarcinoma were 49.8% on day 24, 48.7% on day 18 respectively. The inhibitory effect on them was very close. However, inhibition rate of hepatocarcinoma was up to 74.6% on day 28. In the ^{131}I-BVE-1 treated group of pancreatocarcinoma, inhibitory effect on tumor occurred on day 4 following treatment, reached the top on day 10, and sustained until day 18, of which inhibition rate was 82.2 – 86.6%. In the course of treatment, administration of BVE-1 and ^{131}I-BVE-1 made no significant alteration on the life of mice; mice lived normally. In the IgG treated and untreated group, however, mice gradually had a poor appetite, low spirits and eventually died.

Table 4. Changes of tumor volume in the treatment group of leiomyosarcoma

Groups	Tumor volume after treatment (mm^3)		
	Day 6	Day 12	Day 24
NaCl solution	97.6± 12.3	239.0± 32.4	802.5± 60.4
Murine IgG	97.4± 16.8	252.3± 34.0	807.3± 101.1
MoAb BVE-1	81.4± 16.1	160.9± 18.7	403.1±43.3

Table 5. Changes of tumor volume in the treatment group of hepatocarcinoma

Groups	Tumor volume after treatment (mm^3)			
	Day 7	Day 14	Day 21	Day 28
NaCl solution	40.0± 10.2	119.2± 34.1	343.0± 56.5	556.7±66.6
MoAb BVE–1	27.4± 9.0	40.0± 1.0	105.8± 27.1	141.7±35.6

Table 6. Changes of tumor volume in the treatment group of pancreatocarcinoma

Groups	Tumor volume after treatment (mm^3)		
	Day 4	Day 10	Day 18
NaCl solution	333.6± 118.7	1101.9±245.1	2289.0±506.1
Murine IgG	337.6± 123.7	1054.9± 330.0	1983.9± 666.3
MoAb BVE-1	332.0± 82.2	736.0± 107.1	1173.7± 249.0
^{131}I-murine-IgG	407.1± 90.4	664.9±184.4	1628.4± 393.4
^{131}I-BVE-1	107.5± 19.4	147.3± 25.9	407.1± 90.4

Effect of MoAb BVE-1 and ^{131}I-BVE-1 on Tumor Metastasis and Mortality

In the MoAb BVE-1 treated group of leiomyosarcoma, there was no tumor metastasis nor mice death, whereas there were 4/5, 3/5 tumor metastasis and 3/5, 3/5 mice death respectively in the murine IgG treated and untreated group of leiomyosarcoma. In the group of hepatocarcinoma and pancreatocarcinoma, no matter whether mice were

treated or not, there were no tumor metastasis nor mice death until the experiment ended on day 28 and day 18 following treatment.

Histological Examination

When the mice died naturally or were sacrificed at the end of treatment, the gross and microscopic appearance of tumors from experimental mice was examined. In the group of leiomyosarcoma, MoAb BVE-1 treated mice had no metastatic tumors, whereas untreated and murine IgG treated mice had subcutaneous, celiac, thoracic, pulmonary and hepatic metastatic tumors. In the group of hepatocarcinoma and pancreatocarcinoma, all experimental mice had no metastatic tumors. Also, sections of tumor were examined for pathological changes of tumor microvessels. In the leiomyosarcoma, pancreatocarcinoma tissues from MoAb BVE-1 treated mice, many blood vessels became occluded by thrombosis, endothelial cells of vessels became denaturation and surrounding tumor cells became necrosis. Whereas, no pathological changes were found in microvessels of tumors from untreated and murine IgG treated mice.

Table 7. Comparison of tumor inhibition rate among treatment groups

Groups	Inhibitory rate			
	Day 4	day 10	day 18	day 24
HPC treated by BVE-1	12.4± 2.0	38.7± 3.1	65.4± 4.1	70.5± 3.8
LMS treated by BVE-1	9.1± 1.2	36.7± 3.1	44.8± 4.2	49.8±4.1
PCC treated by BVE-1	1.1± 0.1	33.2± 2.1	48.7± 3.5	*
PCC treated by ^{131}I-BVE-1	68.0± 5.2	86.6± 7.7	82.2± 7.2	*
PCC treated by ^{131}I-BVE-1	1.3± 0.1	39.7± 5.3	20.1± 3.3	*
LMS treated by Murine IgG	0	0	0	0
PCC treated by Murine IgG	0	0	0	*

* Treatment for murine model of pancreatocarcinoma ended on day 21.
HPC: hepatocarcinoma; LMS: leiomyosarcinoma; PCC: Pancreatocarcinoma.

Assessment for Intratumor Micro-vessel Density

TMVD from mice xenografted pancreatocarcinoma cells of each experimental group is described in Table 8. TMVD from MoAb BVE-1 and ^{131}I-BVE-1 treated mice was much lower than that from untreated and murine IgG treated mice. The difference of TMVD was very significant at $P<0.001$.

Table 8. TMVD from mice xenografted pancreatocarcinoma cells of each group

Groups	TMVD
MoAb BVE-1	3.5 ± 2.4
^{131}I-BVE-1	7.3 ± 3.2
^{131}I-murine IgG	20.3± 1.9
Murine IgG	29.7± 4.5
NaC1 solution	30.8± 4.6

DISCUSSION

Based on the rapid development of blood vessels in the process of tumor growth and close relation between blood vessels and tumor metastasis, a new strategy was proposed to treat tumors by targeting tumor vasculature. Many kinds of drugs that inhibit, kill or damage blood vessels have been reported, but they always have many side effects. Recently, it has been reported that inhibitory effect on tumor growth has been achieved in treatment of solid tumors in murine model with anti-VEGF monoclonal antibody.[4,5] Herein, we report an experimental study of treating solid tumors by targeting endothelial cells of new blood vessels with anti-endothelial cells MoAb BVE-1. The result shows that MoAb can inhibit and kill endothelial cells and induce blood vessels occlusion to cause tumor cells necrosis and decrease tumor metastasis. ^{131}I labeled MoAb BVE-1 can enhance the inhibitory effect on tumor growth.

The effect of MoAb BVE-1 inhibiting tumor growth and decreasing tumor metastasis *in vivo* is consistent with its effect on inhibiting endothelial cell proliferation, migration, sprout, lumen formation and its cytotoxic effect mediated with complement *in vitro*.[2] Its effect is enhanced as its administration is increased. Higher inhibition rate is achieved by MoAb BVE-1 treatment in hepatocarcinoma group than in leiomyosarcoma, and pancreatocarcinoma groups contributed probably due to administration beginning at an earlier time (3 days after tumor cells inoculation) before solid tumor formation. This indicates that early administration can achieve better effects.

Once MoAb BVE-1 is labeled with radioisotope ^{131}I, one injection can cause an inhibitory effect on

tumor growth rapidly. The inhibition rate of tumor growth increases by up to 68.6% on day 4, and reaches the top at 82.2% on day 10. The effect sustains with inhibition rate 82.2% at day 18. In comparison to the effect of ^{131}I labeled MoAb targeting tumor cells, inhibition rate of tumor is 67% on days 6–15, and decreases to 47.2% on day 16 by applying the same doses of radioisotope using the same method of administration.[6] This primary result suggests that tumor treatment by targeting vasculature is superior to tumor treatment by targeting tumor cells. It has been reported that the binding between endothelial cells and antibody reaches is peak about 1 hour following injection, all vessels are thrombotic and surrounding tumor cells begin necrosis about 12 hours and widespread tumor cells become necrosis about 48 hours.[7,8] The experiment result here also verifies pathological changes of blood vessels in tumors.

In general, our experimental results suggest that treatment for solid tumor by targeting vascular endothelial cells have many advantages over targeting tumor cells. First, one strain of monoclonal antibody can be applicable to treatment for all types of solid tumors, without preparation of different antibody for different types of tumors. Second, the vascular endothelial cells are directly accessible to the circulating antibody, thus rapidly taking effect and overcoming the problem of antibody access to tumor cells. Third, endothelial cells are genetically normal cells, making the outgrowth of resistant mutation unlikely. Lastly, the approach has a built-in amplification mechanism. Because thousands of tumor cells rely on one capillary for nutrients and oxygen, only a proportion of endothelial cells killed or damaged by an antibody could completely cause occlusion of vasculature and result in a massive tumor cells death. Whereas, treatment of tumors by targeting tumor cells needs therapeutic agents to bind to each tumor cell and kill it.

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