

Adeno-associated virus 2 mediated gene transfer of vascular endothelial growth factor Trap: a new treatment option for glioma

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ABSTRACT

Background: Adeno-associated virus 2 mediated gene transfer of vascular endothelial growth factor Trap (AAV2-VEGF-Trap) has been reported to inhibit the growth of primary tumor as well as distant metastasis in 4T1 metastatic breast cancer models. The aim of this study was to investigate the inhibiting efficacy of AAV2-VEGF-Trap for glioma.

Methods: The intracranial transplanted model of glioma in rats was established. They were treated with AAV2-VEGF-Trap, bevacizumab (BEV), temozolomide (TMZ), TMZ combined with AAV2-VEGF-Trap, TMZ combined with BEV and the control group, respectively. A 7.0 Tesla magnetic resonance (MR) was used to assess the tumor volumes and obtain the apparent diffusion coefficient (ADC) values. Immunohistochemical and terminal dextranucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) staining were used to evaluate the effects on tumor angiogenesis, proliferation and apoptosis.

Results: The combination of TMZ with AAV2-VEGF-Trap or BEV showed greater tumor growth inhibition than the other groups, and the ADC values in these two groups were larger than that of the control group. The decreased microvessel density in treatment groups which contain AAV2-VEGF-Trap or BEV was observed. The reduced proliferation activity in groups containing TMZ and increased apoptotic tumor cells in TMZ combined with AAV2-VEGF-Trap group and TMZ combined with BEV group were detected. In addition, there were no differences in antitumor effect, ADC values, Ki-67 and CD31 staining and apoptosis analysis between the two combined therapy groups.

Conclusion: AAV2-VEGF-Trap has an obvious anti-angiogenic effect and inhibits the growth of glioma just by a single intravenous injection, which is similar to BEV. Moreover, there is a synergistic antitumor effect between AAV2-VEGF-Trap and TMZ.

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Introduction



Glioma is the most frequent and lethal primary tumor of the brain in adults. Safe and maximal resection followed by concurrent radiotherapy of daily temozolomide (TMZ) plus 6 cycles of adjuvant chemotherapy with TMZ has become the standard first-line treatment for high-grade gliomas. Despite recent improvements in surgery, radiotherapy and chemotherapy, the overall survival (OS) of patients with this disease has remained strikingly poor^{1,2}.

Angiogenesis is a key process that contributes to solid-tumor growth, survival and metastasis, include glioma, which makes anti-angiogenic therapy a promising strategy for antitumor treatment^{3–6}. At present, it is clear that vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) plays a predominant role in angiogenesis, which makes the inhibition of VEGF or VEGFR a cornerstone of anti-angiogenic therapies^{7,8}.

Bevacizumab (BEV), a monoclonal antibody against VEGF, showed increased progression-free survival (PFS) and durable

objective tumor response rate as a single agent in recurrent glioblastoma^{9–11}, which has been approved by the FDA for the treatment of recurrent glioblastoma. Recently, a phase III study demonstrated that the addition of BEV to standard radiotherapy-TMZ as first-line treatment for glioblastoma lead to a 4.4-month improvement in median PFS, with quality of life and functional status maintained¹².

Adeno-associated virus 2 mediated gene transfer of VEGF-Trap (AAV2-VEGF-Trap) is a new drug independently developed by the State Key Laboratory of Biotherapy, West China Hospital of Sichuan University and has been reported long-term expression of VEGF-Trap *in vivo* via a single intravenous injection, which can simultaneously suppress the growth of primary tumor and lung metastasis in 4T1 metastatic breast cancer models¹³. This suggested that AAV2-VEGF-Trap may be a new approach to the control of malignant growth. We therefore hypothesized that AAV2-VEGF-Trap can inhibit the growth of glioma. Additionally, a large number of studies have shown that the efficacy of anti-angiogenic therapy alone was limited and the

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better therapeutic effects could be achieved in association with chemotherapy. This may be due to that the anti-angiogenic drugs can reestablish the tumor vasculature, normalize the tumor vessels and improve the delivery of drugs within the tumor^{14,15}. Thus, we also combined AAV2-VEGF-Trap with TMZ to explore the antitumor effects and determine whether there is a synergistic effect.

In the present study, the antitumor effect of AAV2-VEGF-Trap alone or combined with TMZ on rat C6 glioma models was assessed by a 7.0 Tesla magnetic resonance (MR) scanner. The effect of BEV on C6 glioma models was also evaluated to compare with AAV2-VEGF-Trap.

Materials and methods

Cells and reagents

C6 cell line was purchased from the cell bank of Chinese Academy of Science (Shanghai, China), stored according to supplier's requirement. AAV2-VEGF-Trap was constructed in the State Key Laboratory of Biotherapy, West China Hospital of Sichuan University. BEV was obtained from Roche. TMZ capsules were purchased from MSD Inc. (USA), contrast agent (Gadopentetic Acid Dimeglumine Salt Injection) from Bayer (Germany). Terminal dextrynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) apoptosis detection kit (In Situ Cell Death Detection Kit, Fluorescein) from Roche Diagnostics GmbH (Germany). Anti-Ki-67 antibody and anti-CD31 antibody are rabbit polyclonal and purchased from Abcam (Shanghai, China).

The C6 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% antibiotics (100IU/ml penicillin and 100µg/ml streptomycin) at 37°C in a 5% CO₂ atmosphere.

Animals and xenograft model

All the animal studies were approved by the ethics committee of West China Hospital of Sichuan University and compliance with the regulation on the administration of experimental animals. Male SD rats (body weight 220-280g) were purchased from Chengdu Dashuo Experimental Animal CO.Ltd (Chengdu, China) and housed in a specific pathogen-free grade environment with access to chow and water ad libitum at the Animal Research Center of West China Hospital. For each rat, a total amount of 10µL C6 glioma cell suspension (1×10^6 cells) was injected into the right caudate nucleus at the rate of 1µL/min under stereotaxic apparatus. The day of C6 cells administration was designated as day 0, and observation continued until day 21. The rats were scanned by MR on day 7 after implantation to screen out 36 rats with basically identical tumor size and then divided into 6 groups randomly to receive different treatments. TMZ group (group 1), received intragastrical (IG) administration of TMZ solution (50mg/kg, once daily for 5 days). AAV2-VEGF-Trap group (group 2), received intravenous (IV) injection of AAV2-VEGF-Trap through vena caudalis (1×10^{12} vg, only once). BEV group (group 3), received intraperitoneal (IP) injection of BEV (5mg/kg, three times a week). TMZ combined with AAV2-VEGF-Trap group (TMZ+ AAV2-VEGF-Trap group,

group 4), received TMZ and AAV2-VEGF-Trap (dose and dosing schedule the same as in group 1 plus group 2). TMZ combined with BEV (TMZ+BEV group, group 5), received TMZ and BEV (dose and dosing schedule the same as in group 1 plus group 3). Control group (group 6), received IG administration of physiological saline (50mg/kg, once daily for 5 days) and IV injection of physiological saline (1×10^{12} vg, only once) through vena caudalis.

MR imaging

The MR scan was performed in 6 groups at day 7, day 14 and day 21 respectively after the implantation. All MR images were obtained with a 7.0 Tesla MR scanner (Bruker BioSpec 70/30, Ettlingen, Germany). Multislice multiecho (MSME) T1-weighted images were obtained with the use of following parameters: field of view (FOV) = 30 × 30mm, repetition time (TR) = 561 msec, echo time (TE) = 14 msec, number of excitations (NEX) = 4, matrix size = 256 × 256, Flip angle (FA) = 180°, slice thickness = 1mm, slice gap = 1mm, and acquisition time = 9 minutes, 34 seconds and 502 msec. Turbo Rapid Acquisition Relaxation Enhancement (RARE) T2-weighted images were obtained with the use of following parameters: FOV = 30 × 30mm, TR = 3000 msec, TE = 45 msec, NEX = 4, matrix size = 256 × 256, FA = 180°, slice thickness = 1mm, slice gap = 1mm, acquisition time = 6 minutes 24 seconds. Diffusion-weighted images (DWIs) were collected with following sequence parameters: FOV = 30 × 30mm, TR = 3000 msec, TE = 45 msec, matrix size = 256 × 256, slice thickness = 1mm, slice gap = 1mm, b values = 0, 200, 400, 600, 800, 1000s/mm². After the completion of scan, rats were injected with contrast agents through vena caudalis and T1-weighted sequences were repeated after 5 minutes.

Apparent diffusion coefficient (ADC) maps were generated automatically by software, and then regions of interest (ROIs) were drawn in the solid and central part of the tumor by two experienced radiologists who did not know the grouping information. The ADC values of 5 ROIs in each rat were measured and the mean value was recorded.

Tumor size was measured for three-dimensional length, width and height, and tumor volume calculated as $\pi/6 \times \text{length} \times \text{width} \times \text{height}$.

Immunohistochemical and TUNEL staining

After the completion of MR scan at day 21, rats were sacrificed and brain tumor tissues were fixed in 4% paraformaldehyde, embedded in paraffin and then cut into sections. The tumor tissue sections were stained for blood vessels using anti-CD31 antibody, for proliferative cells using anti-Ki-67 antibody. Apoptosis was determined by TUNEL staining.

Scanned the slides at 100× to find out the areas with the highest microvessel density and then randomly selected 5 fields at 200× to count the number of CD31 stained positive vessels. Similarly, 5 different fields were selected randomly at 200×, counting 100 tumor cells and calculating the percentage of Ki-67 stained positive cells. And the percentage of TUNEL stained positive cells was calculated the same as Ki-67 staining.

Statistical analysis

SPSS 16.0 was used for statistical analysis. All quantitative data were expressed as mean values \pm SD. One-way analysis of variance and Bonferroni correction were used for comparisons among multiple groups and between two groups, respectively. *P* values less than 0.05 was considered statistically significant.

Results

Antitumor effect of AAV2-VEGF-Trap

We successfully formed tumors in the right caudate nucleus regions on day 7 after implantation. On day 7, no significant difference was found in tumor volume among six groups ($P > 0.05$). On day 14 and 21, the tumor growth rate of TMZ + AAV2-VEGF-Trap group and TMZ+ BEV group was significantly slower than that of the other four groups ($P < 0.01$). AAV2-VEGF-Trap monotherapy could slow down the tumor growth compared with control group ($P < 0.05$), which was similar to that of BEV group and TMZ group (Table 1 and Figure 1).

Changes in ADC values of tumor

The mean ADC values of ROIs in each group were recorded at 7d, 14d and 21d to analyze the changing tendency and scopes (Table 2 and Figure 2). No significant difference was found in baseline tumor ADC values among six groups ($P > 0.05$). Over time, the ADC values of treatment groups increased in different degrees, while that of the control group decreased gradually. The combination of TMZ with AAV2-VEGF-Trap or BEV showed an apparent increase of ADC values compared with control group ($P < 0.05$). However, there was no significant difference in ADC values among monotherapy groups and the control group ($P > 0.05$).

Immunohistochemistry and apoptosis analysis

In order to investigate whether the decreased tumor growth was related to a reduction of tumor angiogenesis or cell proliferation, tumor sections were stained with anti-CD31 antibody and anti-Ki-67 antibody (Figure 3A). The treatment groups which contain AAV2-VEGF-Trap or BEV exhibited

obvious inhibition of CD31 expression compared with TMZ group ($P < 0.05$) and control group ($P < 0.01$) (Figure 3B). And in the groups containing TMZ, the Ki-67 expression was significantly lower than that of the control group ($P < 0.05$) (Figure 3C).

To assess the apoptosis of tumor cells, TUNEL staining was conducted (Figure 4A). Obviously, there were more apoptotic cells in the groups treated with TMZ + AAV2-VEGF-Trap or TMZ+ BEV compared with TMZ group ($P < 0.05$), AAV2-VEGF-Trap group ($P < 0.01$), BEV group ($P < 0.01$) and control group ($P < 0.01$). However, monotherapy of AAV2-VEGF-Trap or BEV did not promote tumor cell apoptosis compared with control group ($P > 0.05$) (Figure 4B).

Discussion

Angiogenesis plays an important role in the process of growth, survival and metastasis of glioma. And VEGF is the critical factor in tumor angiogenesis, which makes it a potent target for anti-angiogenic therapy. Therefore, scientists have developed a variety of anti-VEGF or VEGFR drugs, of which two classes of drugs were most frequently used in clinical practice: monoclonal antibodies and tyrosine kinase inhibitors (TKIs).

BEV, a humanized monoclonal antibody targeting VEGF with a long elimination half-life (approximately 20 days), received an accelerated approval from FDA in 2009 for the treatment of recurrent glioblastoma based on two phase II studies. One is a single-arm study to evaluate single-agent activity of BEV in 48 patients with recurrent glioblastoma⁹. Results showed that the median PFS was 16 weeks, the 6-month PFS rate was 29% and the 6-month OS rate was 57%. The other is a multicenter, open-label, noncomparative trial to evaluate the efficacy of BEV alone and in combination with irinotecan in 167 patients with recurrent glioblastoma¹⁰. The 6-month PFS and objective response rate (ORR) in BEV-alone and BEV-plus-irinotecan groups were 42.6% vs. 50.3%, and 28.2% vs. 37.8%, respectively. These two studies showed increased PFS and durable ORR in recurrent glioblastoma, facilitates BEV as an important part of the treatment of glioma.

TKIs are small molecule drugs that inhibit the activity of tyrosine kinases. They can bind tyrosine kinases of VEGFR and compete with ATP to block the downstream signals. It is clear that TKIs have shown potent antitumor effects on many solid tumors. However, its efficacy on glioma seems to be unsatisfactory. A variety of clinical trials of TKIs have demonstrated a limited antitumor effect on glioma¹⁶⁻¹⁹. Recently, several studies have shown good results, which was encouraging. A randomized phase II study comparing axitinib with the combination of axitinib and lomustine was conducted in 79 patients with recurrent glioblastoma²⁰. The results showed that overall response rate was 28% vs. 38%, 6-month PFS rate was 26% vs. 17% and median OS was 29 weeks vs. 27.4 weeks for patients treated with axitinib versus axitinib combined with lomustine, respectively. This suggested that axitinib improves response rate and PFS in patients with recurrent glioblastoma compared to historical controls. Results of a pilot clinical study evaluating apatinib plus irinotecan in

Table 1. Tumor volume changes in each group at 7d, 14d and 21d.

Groups	Tumor size (mm ³)		
	7d	14d	21d
TMZ+AAV2-VEGF-Trap	15.31 \pm 2.45	34.11 \pm 3.43**	79.31 \pm 4.05**
TMZ+BEV	16.48 \pm 3.28	34.09 \pm 3.42**	76.37 \pm 4.03**
TMZ	16.40 \pm 1.73	46.82 \pm 3.53*	93.86 \pm 1.85*
AAV2-VEGF-Trap	15.53 \pm 2.42	46.51 \pm 2.75*	94.17 \pm 3.61*
BEV	14.99 \pm 2.88	46.82 \pm 3.08*	94.02 \pm 1.07*
Control	15.78 \pm 3.58	57.55 \pm 2.52	104.73 \pm 0.72

Abbreviations: TMZ, temozolomide; AAV2-VEGF-Trap, adeno-associated virus 2 mediated gene transfer of vascular endothelial growth factor Trap; BEV, bevacizumab.

P values:

** : TMZ+AAV2-VEGF-Trap group and TMZ+BEV group vs. TMZ group, AAV2-VEGF-Trap group, BEV group and control group, < 0.01 ;

* : TMZ group, AAV2-VEGF-Trap group and BEV group vs. control group, < 0.05 .

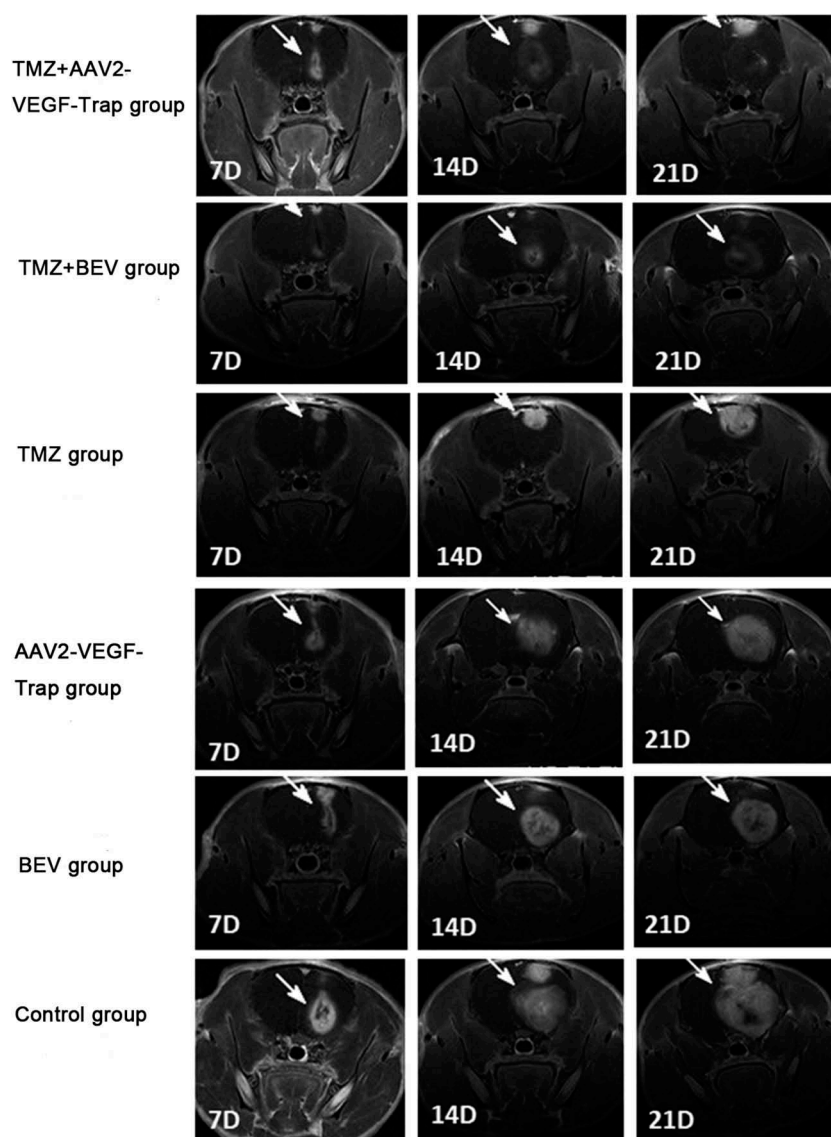


Figure 1. Contrast enhanced T1-weighted images: the tumor areas were obviously enhanced (white arrow) with significant space-occupying effect.

patients with recurrent high-grade glioma were reported²¹. Among 9 patients available for the efficacy evaluation, 5 with partial response, 2 with stable disease and 2 with progressive disease. The ORR was 55% and median PFS was 8.3 months. This indicated that apatinib combined with irinotecan seems to be an effective therapeutic option for

recurrent malignant glioma. A phase II study of cabozantinib was conducted in 152 patients with refractory or recurrent glioblastoma²². Although the study did not meet its primary end point of ORR improvement, cabozantinib showed evidence of clinical activity in patients with refractory or recurrent glioblastoma naive to anti-angiogenic therapy. The results of the above studies need to be further validated in more large-scale clinical trials.

Table 2. Changes in ADC values of tumor in each group at 7d, 14d and 21d.

Groups	ADC values of tumor ($\times 10^{-3} \text{mm}^2/\text{s}$)		
	7d	14d	21d
TMZ+AAV2-VEGF-Trap	1.02 ± 0.11	1.35 ± 0.18**	1.46 ± 0.19**
TMZ+BEV	0.93 ± 0.19	1.31 ± 0.13**	1.38 ± 0.10**
TMZ	0.93 ± 0.14	1.02 ± 0.14*	1.16 ± 0.21*
AAV2-VEGF-Trap	1.06 ± 0.14	1.16 ± 0.12*	1.17 ± 0.13*
BEV	1.04 ± 0.18	1.15 ± 0.20*	1.19 ± 0.18*
Control	1.07 ± 0.14	0.85 ± 0.13	0.74 ± 0.13

Abbreviations: TMZ, temozolomide; AAV2-VEGF-Trap, adeno-associated virus 2 mediated gene transfer of vascular endothelial growth factor Trap; BEV, bevacizumab.

P values:

** : TMZ+AAV2-VEGF-Trap group and TMZ+BEV group vs. control group, < 0.05;

* : TMZ group, AAV2-VEGF-Trap group and BEV group vs. control group, > 0.05.

VEGF-Trap, a high-affinity soluble decoy receptor for VEGF, which has been demonstrated one of the most potent and efficacious VEGF blocker²³. Compared with BEV, VEGF-Trap binds the VEGF ligand with about 800-fold higher affinity and maintains similar pharmacokinetic properties²⁴. Moreover, VEGF-Trap can bind not only multiple isoforms of VEGF-A like BEV, but also the related VEGFR1 ligands, VEGF-B and placental growth factor (PIGF), which are important factors in promoting pathological angiogenesis²⁵⁻²⁸. Due to its significant anti-angiogenic effect, it has been approved by the FDA for the treatment of wet macular degeneration and metastatic colorectal cancer that is resistant to or has

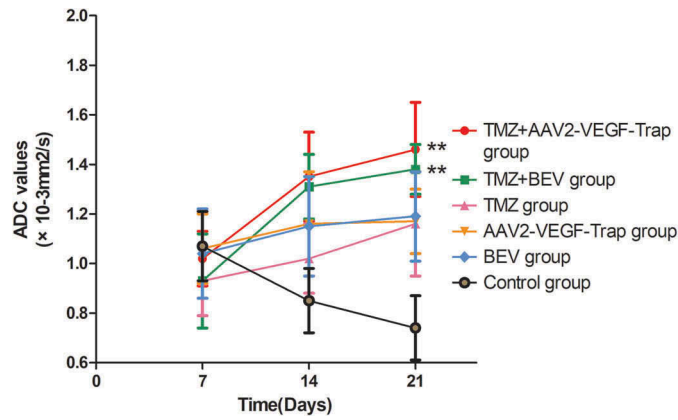


Figure 2. Changes in ADC values of tumor in each group at 7d, 14d and 21d.

Abbreviations: TMZ, temozolomide; AAV2-VEGF-Trap, adeno-associated virus 2 mediated gene transfer of vascular endothelial growth factor Trap; BEV, bevacizumab. On day 14 and 21, the ADC values of TMZ+AAV2-VEGF-Trap group and TMZ+BEV group were larger than that of the control group (** $P < 0.05$). There was no significant difference among TMZ group, AAV2-VEGF-Trap group, BEV group and the control group ($P > 0.05$).

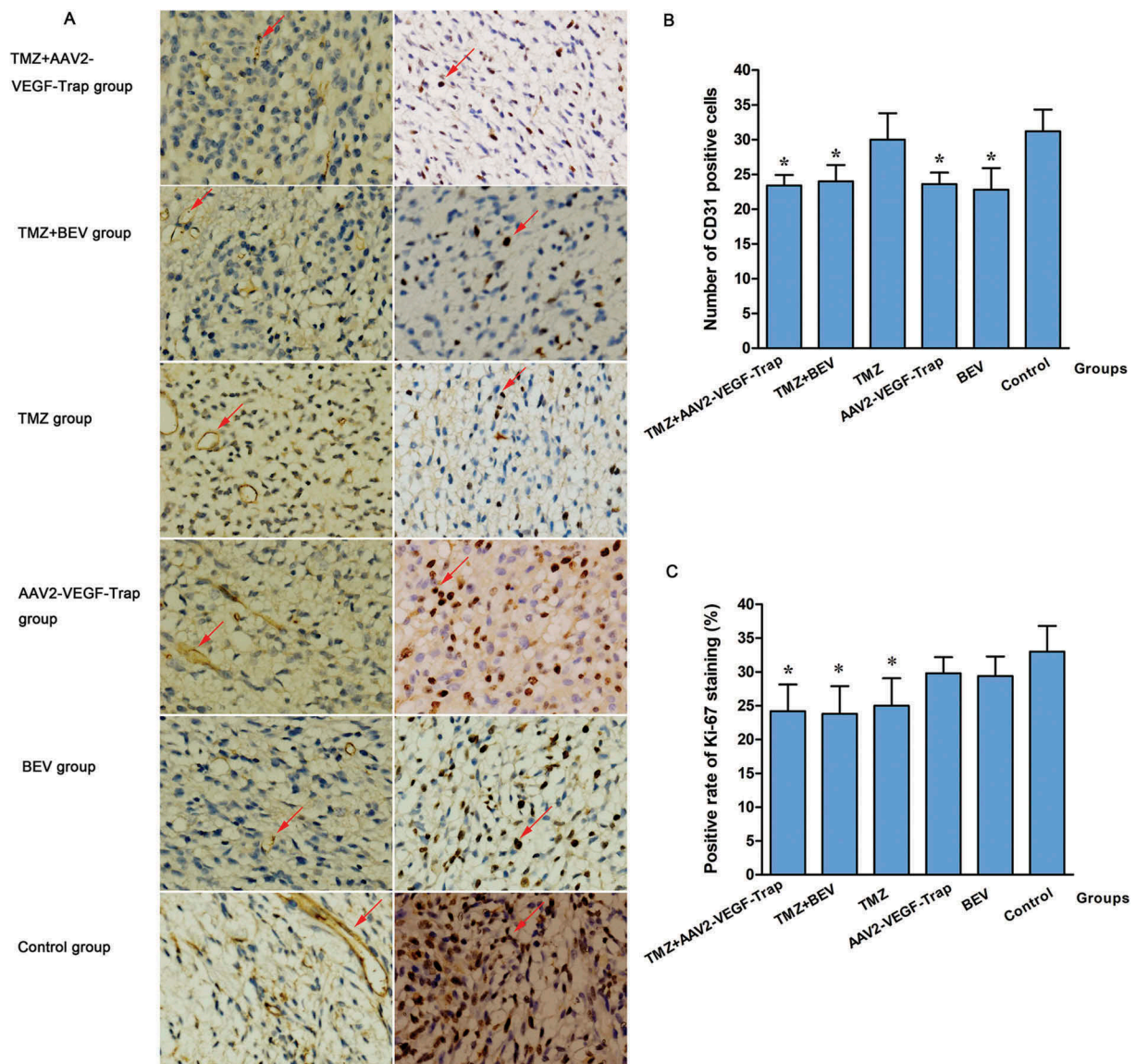


Figure 3. Inhibition of tumor angiogenesis and cell proliferation in tumor sections.

Abbreviations: TMZ, temozolomide; AAV2-VEGF-Trap, adeno-associated virus 2 mediated gene transfer of vascular endothelial growth factor Trap; BEV, bevacizumab. (A) CD31 and Ki-67 staining in each group (magnification, $\times 200$). (B) The number of CD31 stained positive cells in each group.

P values: *: TMZ+AAV2-VEGF-Trap group, TMZ+BEV group, AAV2-VEGF-Trap group and BEV group vs. TMZ group (< 0.05) and control group (< 0.01); TMZ group vs. control group, > 0.05 . (C) The positive rate of Ki-67 staining in each group.

P values: *: TMZ+AAV2-VEGF-Trap group, TMZ+BEV group and TMZ group vs. control group, < 0.05 ; No significant difference was found among AAV2-VEGF-Trap group, BEV group and control group ($P > 0.05$).

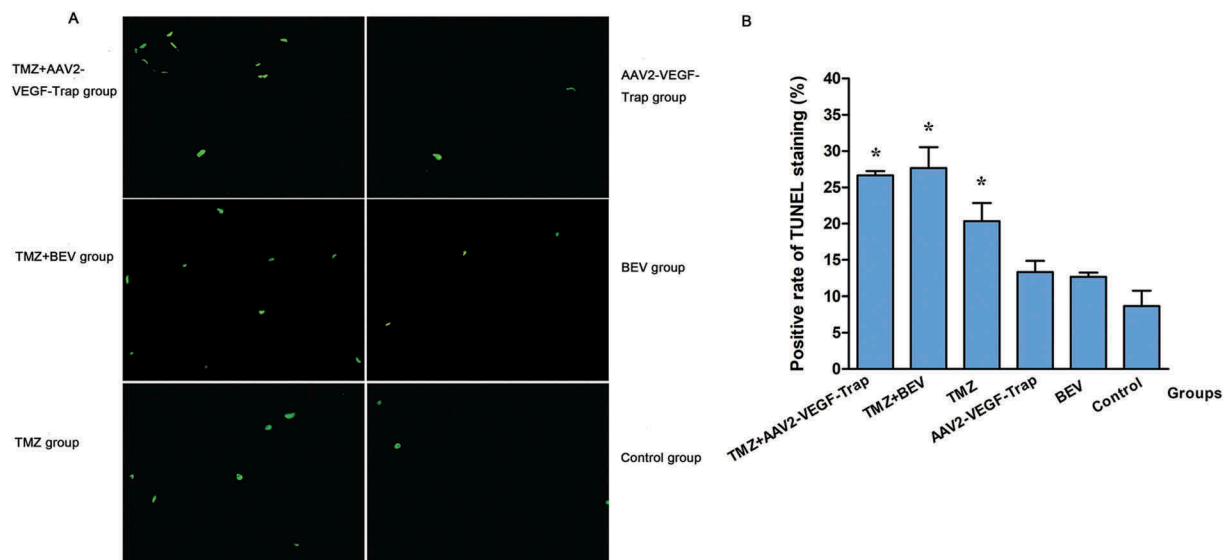


Figure 4. Detection of tumor cell apoptosis by TUNEL staining.

Abbreviations: TMZ, temozolomide; AAV2-VEGF-Trap, adeno-associated virus 2 mediated gene transfer of vascular endothelial growth factor Trap; BEV, bevacizumab.

(A) TUNEL staining in each group (magnification, $\times 200$).

(B) The positive rate of TUNEL staining in each group.

P values: *: TMZ+AAV2-VEGF-Trap group and TMZ+BEV group vs. TMZ group, < 0.05 ; TMZ+AAV2-VEGF-Trap group and TMZ+BEV group vs. AAV2-VEGF-Trap group, BEV group and control group, < 0.01 . And there was no difference between TMZ+AAV2-VEGF-Trap group and TMZ+BEV group ($P > 0.05$), among AAV2-VEGF-Trap group, BEV group and the control group ($P > 0.05$).

progressed following an oxaliplatin-containing regimen. A study has shown that VEGF-Trap is effective for both early and advanced gliomas, and can significantly prolong the OS of patients²⁹. However, it has a short half-life, which may increase costs and lead to patients discomfort because of repeated administration.

AAV2-VEGF-Trap, constructed by our lab, State Key Laboratory of Biotherapy, West China Hospital of Sichuan University, has overcome the deficiency of short half-life of VEGF-Trap, which can sustain long-term expression of VEGF Trap via a single intravenous injection and inhibit the growth of primary tumor and pulmonary metastases in 4T1 metastatic breast cancer models¹³. Furthermore, the study found that AAV2-VEGF-Trap can not only decrease the concentration of VEGF, but also inhibit other angiogenic factors' function, such as acid fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF) and angiopoietin-1. In the present study, our results demonstrated that AAV2-VEGF-Trap could inhibit the growth of glioma via a single intravenous administration, and the inhibiting efficacy was similar to that of BEV.

DWI is one of the important imaging methods of magnetic resonance imaging (MRI), which can explore the abnormalities of tissue structure earlier than other traditional imaging techniques by detecting the microscopic changes in the motion of water molecules^{30,31}. ADC is a key parameter derived from DWI, negatively correlated with cell density of tumor and tissue. Because of the superiority of early detection, DWI has been used in many types of tumors to predict and detect the early therapeutic response, including glioma, bone and soft-tissue sarcoma, breast cancer, cervical cancer, head and neck squamous cell carcinoma and rectal cancer^{30,32–37}. Successful treatment in most malignancies is usually associated with an increase of ADC values, so is the

glioma^{32,38–42}. In this study, the ADC values of TMZ+AAV2-VEGF-Trap group and TMZ+BEV group increased significantly. We speculated that the treatment of these two groups may induce ischemia, necrosis and apoptosis of tumor cells, reduce cell density, and then result in an increase of ADC values. To confirm our hypothesis, we further performed immunohistochemical and TUNEL staining.

CD31 staining is mainly used to determine microvessel density and assess tumor angiogenesis. The greater the expression of CD31 in tumor tissues, the greater the microvessel density and the smaller the effect of anti-angiogenesis⁴³. In the present study, the expression of CD31 was obviously inhibited in groups containing AAV2-VEGF-Trap or BEV. This indicated that AAV2-VEGF-Trap and BEV have similar and definite anti-angiogenic effects. Moreover, AAV2-VEGF-Trap can achieve the same effect as multiple injections of BEV with a single administration, which is more convenient. Ki-67 staining was used to evaluate the proliferation activity of tumor cell. Our finds showed that monotherapy of AAV2-VEGF-Trap or BEV did not inhibit the proliferative activity of tumor. It was TMZ that exerts an anti-proliferative effect in this study.

Otherwise, TUNEL staining was conducted to assess the apoptosis of tumor cells. Monotherapy of AAV2-VEGF-Trap or BEV did not increase apoptosis. And monotherapy of TMZ promoted apoptosis compared with AAV2-VEGF-Trap group, BEV group and control group. Interestingly, when combined TMZ with AAV2-VEGF-Trap or BEV, a stronger pro-apoptotic effect than TMZ monotherapy was observed. This suggested that there was a synergistic antitumor effect between AAV2-VEGF-Trap, BEV and TMZ. A number of studies on tumor transplantation models have found that, compared with monotherapy of VEGF Trap, the combination of VEGF Trap and cytotoxic drugs, or radiotherapy

can produce a stronger inhibiting efficacy on tumor growth and angiogenesis^{44,45}, which is consistent with our research. There may be a lot of possible mechanisms accountable for this effect. One of the most important explanation is “Normalization of tumor vasculature”, the VEGF Trap and BEV can improve the tumor microenvironment of interstitial hypertension, hypoxia and acidosis, leading to more efficient delivery of TMZ and oxygen to tumor cells^{14,15}. A second possible mechanism is that by reducing vascular permeability, the VEGF Trap and BEV can prevent many serum-derived growth factors that promote tumor cell survival from reaching the tumor parenchyma, thus slowing down tumor cell growth. Thirdly, in addition to its cytotoxic effect, TMZ has a direct anti-angiogenic effect as well as other cytotoxic drugs, and genetically stable endothelial cells of newly forming tumor vessels are considered to be sensitive to chemotherapeutic drugs⁴⁶⁻⁴⁸, thereby the combination of anti-angiogenic agents and TMZ can enhance the antitumor effect. Further experiments will be needed to confirm whether any or all of these mechanisms contribute to this synergistic antitumor effect.

As to the Normalization of tumor vasculature, some scholars believe that there is a “normalization window” in anti-angiogenic therapy, a period during which the addition of chemotherapy and radiotherapy achieves the best therapeutic outcome⁴⁹. It means that the antitumor efficacy of anti-angiogenic therapy combined with cytotoxic drugs depends on the dose and delivery schedule of each drug. Consequently, the optimal dose and schedule of AAV2-VEGF-Trap needs to be further studied to develop optimal combination therapies and yield the best therapeutic effects.

There is a limitation in this study. The overall survival curves of each groups were not drawn to confirm whether there was a survival benefit. Further studies are necessary to validate our findings and determine whether AAV2-VEGF-Trap monotherapy or combined with TMZ may eventually provide a survival benefit.

Conclusion

The results of this study showed that AAV2-VEGF-Trap can achieve the same anti-angiogenic effect as BEV just by a single intravenous injection, which is more convenient. MRI is a good method for early and dynamically evaluation of antitumor effect and is worthy of application. Furthermore, combined application of AAV2-VEGF-Trap and TMZ can produce a better antitumor effect.

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Conflict of interest

There are no potential conflicts of interest to disclose by any of the authors.

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