Original Article

Effect of Hyperbaric Oxygen on the Growth of Intracranial Glioma in Rats

Jian-Bo Ding, Jun-Rui Chen, Hong-Zhi Xu, Zhi-Yong Qin

Department of Neurosurgery, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai 200040, China

Abstract

Background: Numerous studies have confirmed that hyperbaric oxygen (HBO) in combination with radiotherapy or chemotherapy may increase the efficacy of radiotherapy or chemotherapy in patients with glioma. However, whether HBO therapy alone may inhibit or promote the growth of malignant tumors remains controversial. This study aimed to investigate the effect of HBO on the growth of glioma in rats, and the impact of HBO on the expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1-alpha (HIF-1α), angiogenesis, and apoptosis of glioma cells.

Methods: Male Sprague–Dawley rats were treated with or without HBO after glioma cell inoculation and followed for up to 16 days postinoculation. Rats were randomized to receive bilateral forelimb function tests (n = 20 per group) and head magnetic resonance imaging (n = 5 per group). Differences between HBO and control groups were tested using 2-sample independent t-tests and changes over time within treatment groups were analyzed using a repeated measurement analysis of variance with Bonferroni correction. The effect of HBO on the expression of VEGF, HIF-1α, von Willebrand factor, angiogenesis, and tumor cell apoptosis were also examined (n = 5 per group).

Results: Forelimb function scores were reduced in both HBO-treated and control groups. HBO-treated rats had significantly larger tumor volume and more water in the cerebellum compared with control rats. The intratumoral expression of VEGF was significantly higher in HBO-treated rats compared with control rats (23.2% vs. 13.3%, P = 0.002). HIF-1α was significantly increased in HBO-treated rats compared with controls in the expression of both intratumoral (72.7% vs. 54.9%, P = 0.001) and peritumoral (2.6% vs. 1.9%, P = 0.003) cells. The intratumoral microvessel density (MVD) was significantly higher in the HBO group (15.6 vessels/field vs. 4.4 vessels/field, P < 0.001), and the peritumoral MVD was not significantly different between the two groups (P > 0.05). Apoptosis was significantly lower in HBO-treated rats compared with controls (44.4% vs. 82.8% for intratumoral; 10.1% vs. 77.5% for peritumoral, both P < 0.001).

Conclusions: The current results demonstrate that HBO alone may promote tumor growth, and is therefore not suitable to treat patients with gliomas with neurological deficits or disorders with HBO alone. If HBO must be used as a mean of rehabilitation, it is recommended that HBO should be combined with radiotherapy or chemotherapy.

Key words: Angiogenesis; Apoptosis; Glioma; Hyperbaric Oxygen; Rat

Introduction

Hyperbaric oxygen (HBO) therapy has been used as a component of care in a wide variety of medical conditions, including cancer, over the past 50 years.[1] HBO consists of breathing pure oxygen in a pressurized chamber. In a typical HBO therapy chamber the air pressure is increased up to three times higher than the normal air pressure; consequently, up to three times more oxygen is then available than would be possible at normal air pressure.[2] HBO therapy has been widely used for the treatment of decompression sickness, serious infections, and wounds that will not heal as a result of diabetes or radiation injury. Specifically, HBO has demonstrated positive outcomes when used to treat chronic radiation tissue injury, including osteoradionecrosis of the jaw, curaneous radionecrosis that caused open wounds, laryngeal radionecrosis, radiation cystitis, gastrointestinal

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Address for correspondence: Dr. Zhi-Yong Qin, Department of Neurosurgery, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai 200040, China
E-Mail: wisdomqin@vip.163.com

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radionecrosis, and in conjunction with oral surgery in a previously irradiated jaw.[3]

Increased oxygen can stimulate the release and activity of several molecules associated with tumor growth, including vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1-alpha (HIF-1α), and von Willebrand factor (vWF), in patients with brain glioma. The growth and prognosis of brain glioma have been demonstrated to be closely associated with the expression of VEGF and HIF-1α. VEGF contributes directly to angiogenesis and plays an important role in the pathological angiogenesis of cancers.[4] HIF-1α is a transcriptional factor that may be associated with the response to hypoxia in cancers.[5,6] In general, increased expression of VEGF and HIF-1α is associated with rapid glioma growth and poorer prognosis.[7-9] vWF is a marker of endothelial cells and is involved not only in blood clotting and thrombosis, but also in the synthesis and expression of adhesion molecules. It has been demonstrated that vWF plays an important role in the angiogenesis of cancers and the invasion of cancer cells.[10]

HBO therapy has also been used to augment the therapeutic effects of radiotherapy and/or chemotherapy and may improve therapeutic efficacy in patients receiving either of these treatments.[2,11] Numerous studies have confirmed that HBO in combination with radiotherapy or chemotherapy (such as temozolomide) may increase the efficacy of radiotherapy or chemotherapy in patients with glioma,[12,13] different from the effects of HBO alone. Clinically, patients with gliomas have neurological deficits in some functional areas, therefore, need HBO for rehabilitation purposes. However, whether HBO therapy alone may inhibit or promote the growth of malignant tumors remains controversial.[14] HBO has been associated with a significant reduction in tumor growth in an extracranial glioma model (60% reduction vs. control in a 9-day treatment model).[15] This result was characterized by enhanced cell death, reduced vascular density, and changes in gene expression.

In clinical practice, when patients with gliomas require HBO as part of their rehabilitation to treat neurological deficits or disorders, clinicians may be unsure of the patient suitability. To date, few studies have been conducted to investigate the effects of HBO on intracranial glioma. This study aimed to investigate the effect of HBO on the growth of rat glioma; as well as, the impact of HBO on the expression of VEGF and HIF-1α, angiogenesis, and apoptosis of glioma cells.

Methods

Rat basal ganglia glioma model

Male Sprague–Dawley rats (n = 60, aged 12 weeks and weighing 250–280 g) were purchased from the Experimental Animal Center of Fudan University and housed in the Experimental Animal Center of Fudan University in a pathogen-free environment. Animals were provided water and food *ad libitum* and were exposed to a 12:12 h light–dark cycle (humidity: 55%, temperature: 22°C). This study was approved by the Institutional Animal Care and Use Committee of Fudan University.

Rats were anesthetized using 10% chloral hydrate (3.6 ml/kg) via intraperitoneal injection. A stereotactic instrument (Shanghai Alcbio Co., Ltd., China) was used to stabilize the head for the experimental procedure. A midline incision was made on the head exposing the coronal and sagittal sutures. Next, a 1-mm hole was drilled on the right coronal suture 3 mm away from the middle line. A microinjector (Shanghai Gaoge Industry and Trade Co., Ltd., China) containing 1×10^6 rat C6 cells (10 μl; Cell Bank of Chinese Academy of Science, Shanghai, China) was inserted along the bone edge (depth: 6 mm). The microinjector was then withdrawn approximately 1 mm and cells were injected into right caudate nucleus of each rat at a rate of 1 μl/min with a microinfusion pump (Shanghai Alcbio Co., Ltd.) within 10 min. Following cell injection, the microinjector remained in the brain for 5 min and then slowly withdrawn. Bone wax (Johnson and Johnson, New Brunswick, NJ, USA) was used to seal the hole in the skull, and skin wounds were sutured. Following surgery, all rats received 5% glucose in normal saline (2 ml) via intraperitoneal injection.

Experimental design

Rats (n = 40) were randomly assigned to receive HBO therapy (n = 20) or not (control; n = 20). HBO therapy consisted of exposure to 100% oxygen at 3.0 ATA (1 ATA = 0.1 Mpa) for 1 h in an animal hyperbaric chamber (DWC150/300; Shanghai 701 Institute Yangyuan Hyperabric Chamber Co., Ltd., China). The chamber was pressurized to 3.0 ATA within 15 min. After 1 h, decompression to 1 ATA was accomplished within 15 min. Rats in the HBO therapy group were treated with HBO at 2, 4, 6, 8, 10, and 12 days postsurgery. For controls, rats were placed in a chamber and exposed to room air for 1 h. All the rats were subjected to a bilateral forelimb function via tentacle test at 1, 3, 5, 7, 9, 11, and 13 days postsurgery. At 16 days postsurgery, all rats were weighed and 5 rats from each group had a magnetic resonance imaging (MRI) of the brain to determine tumor volume. In addition, 10 rats were randomly selected from each group for the quantification of water content. The remaining 10 rats in each group were observed until they died (overall survival).

Expression of vascular endothelial growth factor/hypoxia-inducible factor 1-alpha, angiogenesis, and tumor cell apoptosis

Rats (n = 20) were randomly assigned to receive HBO therapy (n = 10) or not (control; n = 10). Rats in the HBO therapy group were treated with HBO at 2, 4, 6, 8, 10, and 12 days after surgery. At 16 days after surgery, rats were sacrificed and brain sections were obtained for hematoxylin and eosin (H and E) staining and immunohistochemistry (IHC). The expression of vWF, VEGF, and HIF-1α in the tumors and adjacent normal tissue was also evaluated. The number of vWF positive blood vessels was determined as microvessel density (MVD), and

transferase dUTP nick end labeling (TUNEL) staining was done to detect the apoptotic cells in the tumors and adjacent normal tissue.

Evaluation of neurological functions

The vibrissae stimulated forelimb placing test was employed to evaluate the neurofunction of rats at 1, 3, 5, 7, 9, 11, and 13 days after surgery and to assess the influence of surgery and cancer growth on neurofunction. The bilateral forelimbs were assessed 10 times for each rat; the highest score was 10 and the lowest score was 0. Scoring was done by investigators blind to the study design and treatments.

Magnetic resonance imaging scanning

Rats were anesthetized using 10% chloral hydrate (3.6 ml/kg; Shanghai Sinopharm Chemical Reagent Co., Ltd., China) via intraperitoneal injection. A Sigma 1.5T magnetic resonance machine was used for MRI analysis (GE, USA). The following parameters were used: 3 in (1 in = 2.54 cm) surface coil; field: 6 cm × 6 cm; matrix: 512 × 256; scanning sequence: SE; T1-weighted (T1WI): TR = 440 ms, TE = 14 ms, stimulating: 3 times; T2WI: TR = 3000 ms, TE = 78 ms, stimulating: 3 times; and horizontal and coronary scanning was performed. The coronary and cross-sectional slices with maximal area were selected from T2WI images, and the maximal anteroposterior diameter (L), transverse diameter (W), and height (H) were measured, and the tumor volume (V)[16] was calculated as follow: $V = (4/3 \times \pi \times L \times W \times H)/8$ (mm^3).

Detection of brain water content

Brain tissues 2–12 mm behind the frontal pole were preserved and weighed. Then, the brain tissues were placed in an oven at 100°C and dried for 24 h. The tissues were weighed again. The brain water content was calculated: Brain water content (%) = (wet weight – dry weight)/wet weight × 100%.[17] The water content of the cerebellum served as a control.

Cardiac perfusion, brain collection, paraffin embedding, and sectioning

At 16 days after surgery, rats were intraperitoneally anesthetized with 10% chloral hydrate, and transcardially perfused with normal saline (about 300 ml) and then with 4% paraformaldehyde (400 ml). Following decapitation, brains were removed for fixation (4% paraformaldehyde for 4–6 h). Following dehydration and transparentization, tissues were embedded in paraffin. Then, 4-μm sections were obtained.

Immunohistochemistry

Brain sections were treated with rabbit anti-rat VEGF antibody (1:100; Santa Cruz Biotechnology, TX, USA) at 4°C overnight. Sections were then incubated with goat anti-rabbit fluorescein isothiocyanate IgG (1:500, Invitrogen, Carlsbad, CA, USA) in phosphate buffered saline and 1% bovine serum albumin at room temperature for 2 h in dark. Cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (1:1500) at 37°C for 30 min.

In addition, IHC for HIF-1α (Wuhan Boster, Hubei, China) and vWF (Abcam, Cambrigde, MA, USA) in the brain were performed per manufacturer's instructions. Briefly, sections were treated with rabbit anti-rat antibodies against HIF-1α (1:100) or vWF (1:400) at 4°C overnight. Sections were incubated with goat anti-rabbit biotin conjugated secondary antibody (Santa Cruz) at 37°C for 20 min. Sections were treated with SABC at 37°C for 20 min. Sections were visualized with 0.04% 3,3'-diaminobenzidine tetrachloride (DAB)-H_2O_2 for 5 min. Secondary antibodies: Goat anti-rabbit IgG (Jackson, USA) were used in this study. DAPI was purchased from Sigma, USA.

VEGF positive, HIF-1α positive, and apoptotic cells were quantified by using five randomly selected fields from the area of interest at a magnification of × 400, a total of 200 cells were counted in each field, and averages were obtained. For MVD, five fields were randomly selected from the area of interest at a magnification of × 400, and vWF positive cells per field were counted as the MVD. Detection of apoptotic cells was determined using terminal deoxynucleotidyl TUNEL staining per manufacturer instructions (Roche, Basel, Switzerland). Apoptotic cells were visualized using DAB.

Statistical analysis

Data are presented as mean ± standard deviation (SD) by treatment group. Differences between HBO and control groups were tested using 2-sample independent *t*-tests. Changes over time within treatment groups were analyzed using a repeated measurement analysis of variance (ANOVA) with Bonferroni correction. Differences between various time points or left and right side within the group were tested using a paired *t*-test. A two-tailed $P < 0.05$ was considered statistically significant. Statistical analyses were assessed using SPSS 15.0 statistics software (SPSS Inc., Chicago, Illinois, USA).

Results

Neurological function

Overall, the surgical intervention was successful in introducing intracranial glioma. Two days postsurgery, all rats demonstrated normal activities, including eating and drinking. Rats demonstrated progressively increased apathy, weight loss, and disordered hair. At 16 days postsurgery, cranial MRI showed tumors in the brains of all rats [Figure 1a and b].

No significant neurological differences were observed between treatment groups 1-day postsurgery [Figure 1c]. By day 3 and through day 11, left limb neurological function scores were significantly higher in rats in the HBO-therapy group ($P \leq 0.003$). However, considering left hemiplegia was seen in all animals' 1-day postsurgery, it is likely that this was a result of operative injury and not tumor growth. At 3 days after surgery, significant differences were observed in left limb scores, suggesting the rehabilitative effects of HBO. At 5 days postsurgery both groups showed reduced

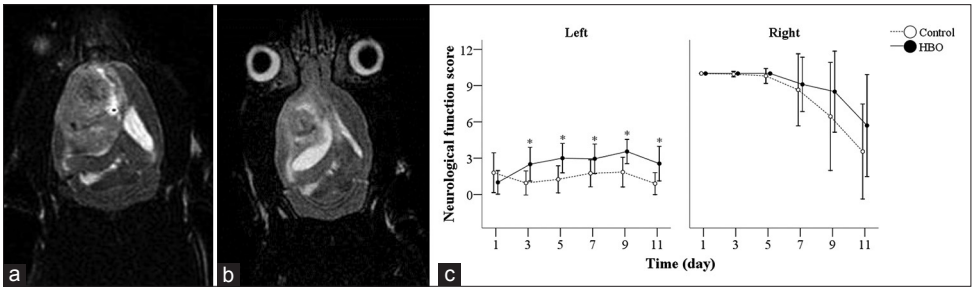

Figure 1: The effects of hyperbaric oxygen on glioma formation and neurological function in an orthotopic glioblastoma model. The representative functional magnetic resonance imaging brain images demonstrating the presence of glioma at the basal ganglia. (a) Hyperbaric oxygen group, cross section and (b) control group, cross section. (c) Neurological function with or without hyperbaric oxygen ($n = 20$). Data are presented as a mean ± standard deviation. *$P < 0.05$ indicates a significant difference between groups. Due to missing data, the data on day 13 were not shown.

right forelimb function scores, with no statistical differences observed between groups. Taken together, neurological differences were not different between groups.

Body weight, tumor volume, and water content of brain on the 16th day

Changes in body weight were similar between groups; there was no difference at baseline or 16-day postsurgery. Within the control group, a significant increase in body weight was observed (from 252.9 g at baseline to 280.7 g 16-day postsurgery, $P < 0.001$). No significant increase in body weight was observed in the HBO group. Tumor volume in HBO-treated rats was significantly larger compared with control rats, 0.410 ± 0.018 cm^3 versus 0.272 ± 0.027 cm^3, respectively ($P < 0.001$). The water content of the right hemisphere was significantly higher than that of the left hemisphere in both groups (control: 79.7% vs. 78.3%, $P = 0.014$; HBO: 80.6% vs. 78.4%, $P = 0.011$). The HBO-treated group had significantly more water in the cerebellum than control rats (77.7% vs. 77.0%, $P = 0.015$). There were no significant differences in the water content of either left or right hemispheres between two groups [$P > 0.05$; Table 1].

The limb function of rats was influenced by both cancer growth and HBO, two factors interacting with each other. However, the large tumor volumes observed in HBO group at 16 days suggested that the reduce score [Figure 1c] was not ascribed to the slow cancer growth in the HBO group.

Overall survival

Control rats survived for 25–36 days; whereas, HBO-treated rats survived for 24–32 days. There was no significant difference in overall survival between the two groups [$P > 0.05$; Table 1].

Tumor cell growth and angiogenesis

Glioma was observed in both groups at the basal ganglia as visualized using HE staining [Figure 2]. Tumor cells were spindle-shaped with dark and large nuclei and pathological karyokinesis. Glioma had swirling cancer nests, tumor cells had invaded surrounding tissues with clear boundary, and capsular structure was observed in some tumor samples. Tumor cells demonstrated higher density with an increased

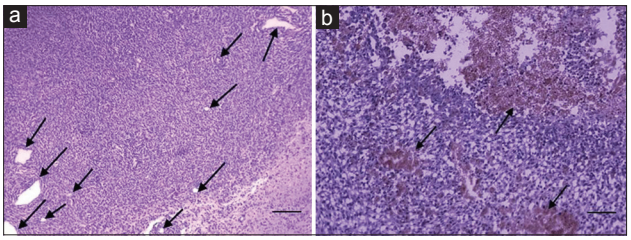

Figure 2: HE staining of tumors and surrounding tissues in hyperbaric oxygen-treated rats (a) and control rats (b). In the hyperbaric oxygen-treated group, an increase in the number of microvessels was observed (arrows). In the control group, evidence of hemorrhage and necrosis were observed in tumors (arrows). Scale bar: 100 μm.

Table 1: Body weight, tumor volume, brain water content, and overall survival between control and HBO groups

Variables	Control	HBO	*P*
Body weight (g)			
Baseline	252.9 ± 24.8	246.5 ± 20.8	0.385
16-day postsurgery	280.7 ± 33.0*	261.6 ± 52.8	0.179
MRI tumor volume (cm^3)	0.272 ± 0.027	0.410 ± 0.018	<0.001†
Water content (%)			
Left hemisphere	78.3 ± 0.5	78.4 ± 0.6	0.679
Right hemisphere	79.7 ± 1.4‡	80.6 ± 2.4‡	0.296
Cerebellum	77.0 ± 0.7	77.7 ± 0.3	0.015†
Overall survival (days)	29.5 ± 3.9	28.7 ± 2.6	0.599

Data are presented as mean ± SD. Body weight ($n = 20$ for each group); MRI tumor volume ($n = 5$ for each group); water content and overall survival ($n = 10$ for each group). *Significant change compared to baseline within the group; †Significant difference between HBO and control group; ‡Significant difference between left and right hemispheres within the group. SD: Standard deviation; MRI: Magnetic resonance imaging; HBO: Hyperbaric oxygen.

number of microvessels in the HBO-treated group compared with controls. In the control group, tumor cell division was active, tumor cell necrosis was observed, and hemorrhage was also noted [Figure 2].

The intratumoral MVD was significantly higher in the HBO-treated group (15.6 vessels/field vs. 4.4 vessels/field, $P < 0.001$), and the peritumoral MVD was not significantly different between groups [$P > 0.05$; Figure 3a and b]. The representative vWF staining images are shown in

Figure 3c-f, in which vWF was highly expressed by vascular endothelial cells.

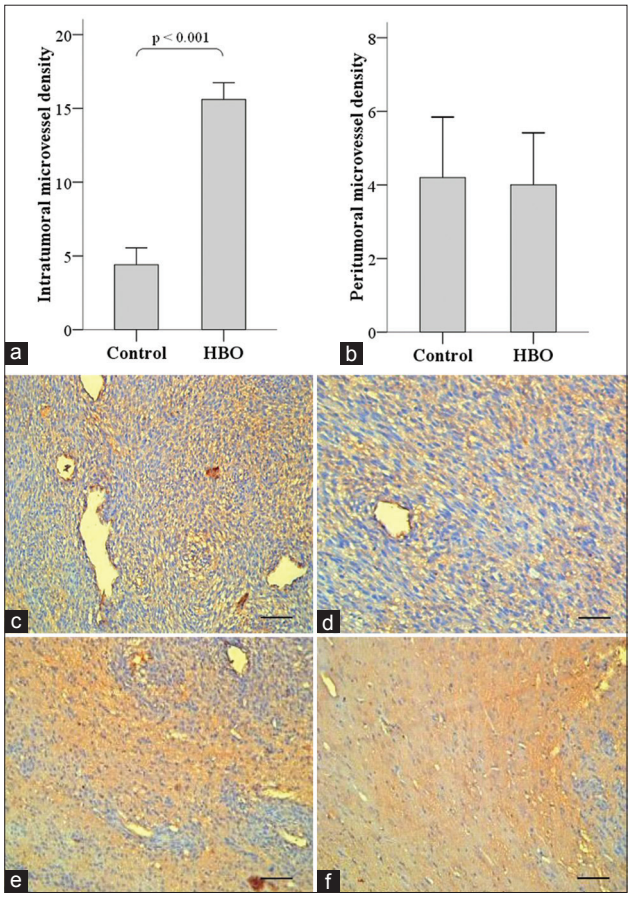

Figure 3: The effect of hyperbaric oxygen on microvessel density. (a) Intratumoral microvessel density. (b) Peritumoral microvessel density. Data are presented as a mean ± standard deviation (n = 5 for each group). von Willebrand factor protein expression in glioma and surrounding normal brain tissue. (c) Glioma in the hyperbaric oxygen group; (d) Glioma in the control group; (e) Surrounding normal brain tissue in the hyperbaric oxygen group; (f) Surrounding normal brain tissue in the control group. Scale bar: 100 μm.

Taken together, tumor cells in the HBO-treated group demonstrated greater evidence of growth compared with controls.

Vascular endothelial growth factor and hypoxia-inducible factor 1-alpha expression by immunohistochemistry

The positive percentage of intratumoral VEGF expression was significantly higher in HBO-treated rats compared with control rats (23.2% vs. 13.3%, P = 0.002). Peritumoral VEGF expression was not observed in either group. The positive percentage of HIF-1α expression was significantly increased in HBO-treated rats compared with controls in the expression of both intratumoral (72.7% vs. 54.9%, P = 0.001) and peritumoral (2.6% vs. 1.9%, P = 0.003) cells [Figure 4].

Therefore, HBO-treated rats' demonstrated elevated protein expression of VEGF and HIF-1α, two molecules that associated with the growth of brain glioma.

Apoptosis

Apoptosis was significantly lower in HBO-treated rats compared with controls (44.4% vs. 82.8% for intratumoral; 10.1% vs. 77.5% for peritumoral, both P < 0.001); [Figure 5a and b]. Brown apoptotic cells were observed in the representative TUNEL staining images [Figure 5c].

Discussion

This study aimed to examine the effects of HBO on the growth of intracranial glioma in rats and the expression of VEGF and HIF-1α, angiogenesis, and apoptosis of glioma cells. Briefly, the current results suggest HBO may play a role in promoting tumor growth using a C6 rat intracranial glioma model. In addition, HBO may improve neurological deficits caused by intracerebral tumor growth and operative injury, as well as up-regulating the expression of vWF, HIF-1α, and VEGF, while inhibiting apoptosis of glioma cells and peritumoral cells.

There are hypoxic areas in the glioma, and the more malignant the glioma, the more severe the hypoxia. David

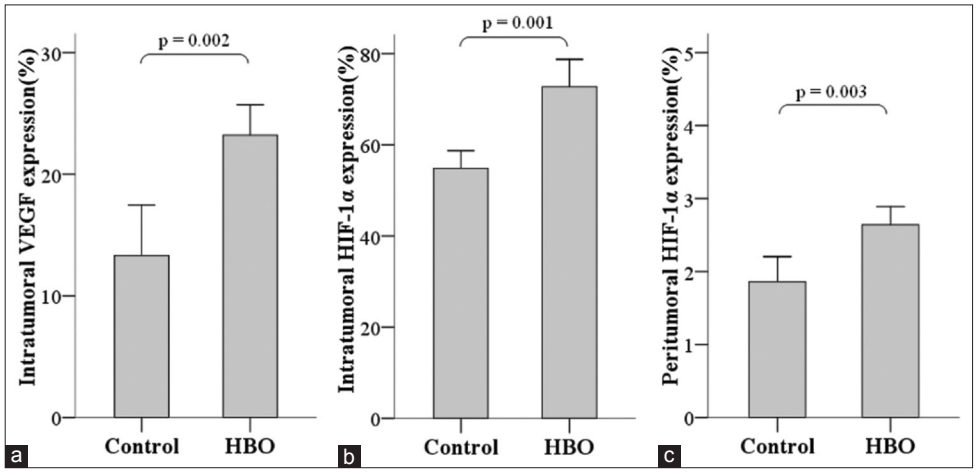

Figure 4: Positive percentage of intratumoral vascular endothelial growth factor (a), intratumoral hypoxia-inducible factor 1-alpha (b), and peritumoral hypoxia-inducible factor 1-alpha (c) expression. Data are presented as mean ± standard deviation (n = 5 for each group).

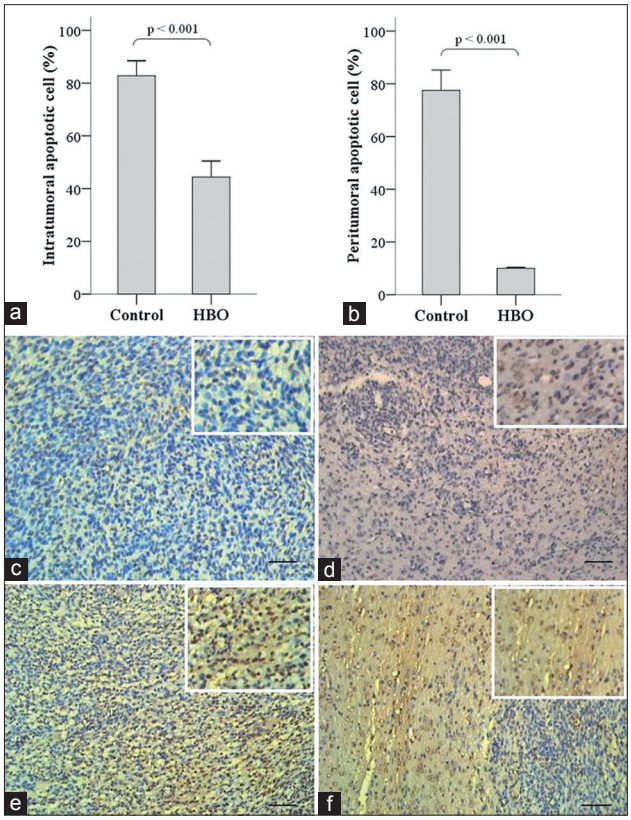

Figure 5: Apoptosis with or without hyperbaric oxygen therapy. (a) Intratumoral apoptosis; (b) peritumoral apoptosis. Data are presented as a mean ± standard deviation (n = 5 for each group). The representative images of transferase dUTP nick end labeling staining. (c) Glioma in the hyperbaric oxygen group; (d) surrounding brain tissue in the hyperbaric oxygen group; (e) Glioma in the control group; (f) surrounding brain tissues in the control group. Scale bar: 100 μm.

et al. reported that the hypoxia in advanced glioma was more severe than that in well-differentiated glioma.[13] HBO can increase the blood oxygen partial pressure, promote cell metabolism, and inhibit the release of inflammatory cytokines.[18,19] Thus, HBO might exert trophic effects on glioma cells with rapid proliferation and relative hypoxia, thereby promoting the growth of glioma. This is evidenced by reports showing that HIF-1α transcription in cancers under hypoxic conditions is increased, thereby elevating cellular tolerance to hypoxia and promoting cancer growth.[20] Although HBO can improve hypoxia, it also simultaneously elevates the production of nitric oxide and free radicals.[21] The accumulation of these mediators may up-regulate HIF-1α expression and further promote the transcription of downstream genes. Our results showed the proliferation of cancer cells was more active, and HIF-1 expression was higher in the HBO group, suggesting a hypoxic area in cancer. The potential mechanisms remain unclear regarding the effect of HBO on glioma growth, as observed in this study, or if an anti-cancer effect is possible. Further study is warranted to investigate the impact of HBO on improving ischemia/hypoxia, and thus the promotion of cancer growth.

The upregulated expression of VEGF resulting from HBO may facilitate angiogenesis resulting in a more invasive cancer. In this study, detection of MVD following vWF staining was associated with angiogenesis in the glioma in HBO-treated rats and was increased compared with controls. VEGF expression in HBO-treated rats was also significantly higher than that observed in controls. These results may indicate that HBO promotes the growth of glioma via upregulation of VEGF and increased tumor angiogenesis.

Various protocols have been used to examine hyperbaric and hyperoxic treatments and their impact on various cancers and glioma models. In contrast with this results, previous reports suggest alternative mechanisms and results under different experimental conditions. Stuhr *et al*. conducted a study similar to ours that explored the biological effects of hyperoxic treatment on BT4C rat glioma xenografts *in vivo*.[15] Results demonstrated that hyperoxic treatment caused an approximately 60% reduction in tumor growth compared to the control group after 9 days ($P < 0.01$), although glioma cells were inoculated into the skin. In contrast, the current results suggest that HBO may play a role in promoting tumor growth on the C6 rat intracranial glioma model. Although the discrepancy in results may be due to distinct assay systems (skin vs. intracranial injections, cancer cell types, HBO procedures, etc.), the overall effect of HBO on tumor growth is not consistent.

Lu *et al*. found that, compared with temozolomide or HBO alone, combined treatment inhibited growth and induced apoptosis of cultured glioma U251 cells, which was accompanied by a significant reduction in VEGF and MRP-1 expression.[22] Results that are consistent with those reported by Dagistan *et al*., where combined HBO + temozolomide reduced glioma growth in a C6 rat glioma model.[12] In contrast, the current study reports treatment of HBO alone was associated with the upregulation of VEGF in rat brain glioma models, and inhibition of apoptosis of glioma cells.

In this study, HBO was used to treat rats with a basal ganglia model, and promotive effects were observed in glioma. Of note, our findings conflicted with previously published reports in which nude mice were inoculated with glioma cells. The discrepancy may be due to differences in cell lines and inoculation sites. Furthermore, in an attempt to put forth a clinically relevant treatment schedule, additional treatment sessions and an increased duration of HBO therapy were used in the current study.

Several limitations should be considered when evaluating the current results. First, forelimb function scores were not evaluated at 13 days or later after surgery; as a result, there is no follow-up to the current 5-day postsurgery score which showed no statistical differences between treatment groups. Second, the magnification of all images was at ×200 (higher and lower magnification was not available); therefore, distinct histological differences are not ideally visualized. Finally, this study did not investigate the effect of HBO on

glioma cell proliferation which warrants further study based on the current results.

In conclusion, the current results demonstrate that HBO alone may promote tumor growth, so it is not suitable to treat patients with gliomas with neurological deficits or disorders with HBO alone. If HBO must be used as a mean of rehabilitation, it is recommended that HBO should be combined with radiotherapy or chemotherapy.

Acknowledgment

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Conflicts of interest

There are no conflicts of interest.

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