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Research paper

Temozolomide/PLGA microparticles plus vatalanib inhibits tumor growth and angiogenesis in an orthotopic glioma model

Yu-Hui Zhang [a], Zhi-Jian Yue [a], He Zhang [b], Gu-Sheng Tang [c], Yang Wang [d], Jian-Min Liu [a,*]

[a] Department of Neurosurgery, Second Military Medical University, Shanghai, China
[b] Department of Pharmaceutics, Second Military Medical University, China
[c] Medical Laboratory Research Center, Second Military Medical University, Shanghai, China
[d] Department of Pathology, Second Military Medical University, Shanghai, China

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ABSTRACT

Temozolomide (TM) has anti-tumor activity in patients with malignant glioma. Implantable poly (d,l-lactide-co-glycolide) (PLGA) microparticles of TM (TM–MS) have been developed, enhancing the cytotoxicity of TM to Glioma C6 cells [1]. Vatalanib, as anti-angiogenic agent, has also shown anti-tumor activity with malignant gliomas [1,2]. We examined the combined effects of TM–MS and vatalanib in a rat orthotopic glioma model and found TM–MS offered a greater tumor inhibition than TM, and combination treatment with both of them improved the survival time versus single agent therapy. The combination treatment also demonstrated an inhibition to rat glioma tumors, a significant decrease in cell proliferation, an increase in apoptosis, and a lower microvessel density within the glioma tumors. The results suggest that TM–MS can more effectively inhibit tumor than TM, and combination treatment with TM–MS and vatalanib inhibits tumor growth and angiogenesis and may prove to be a promising therapy for malignant gliomas.

1. Introduction

Malignant gliomas comprise the most common types of primary central nervous system tumors, having an incidence of 5–8 per 100,000. Gliomas are non-metastasizing, locally infiltrating, highly invasive and hypervascularized tumors with a poor prognosis [3–5]. Despite important advances in surgery, neuroimaging, radiation therapy, and chemotherapy, the prognosis of patients with malignant gliomas remains poor, with a median survival rate of less than 1 year [6–9]. Chemotherapy, even with the most active regimens currently available, achieves only modest improvement in overall survival, and even tumors initially responsive to chemotherapy generally develop chemo-resistance upon recurrence of the disease [10–12].

Temozolomide (TM), an imidazotetrazine-derived DNA alkylating agent, exhibits broad-spectrum anti-tumor activity and has shown significant penetration into the CSF and brain tissue [13] . Large-scale clinical trials have demonstrated improved survival in patients receiving intratumoral chemotherapy with biodegradable implants compared with patients receiving control implants [14]. Malignant gliomas recur locally, and in order to decrease local recurrences, recent efforts have focused on designing polymer devices that deliver anti-tumor drugs into the surgical resection cavity. Macroscopic non-biodegradable devices and more recently, biodegradable wafers have been used for local chemotherapy of human brain tumors. TM-loaded PLGA microparticles (TM–MS) developed by Zhang et al. were shown to have anti-tumor activity against Glioma C6 cancer cells in vitro [1].

Recent new achievements in the study of molecular mechanisms in tumor progression have led to the re-evaluation of chemotherapies and development of new drugs. Many agents are now being tested in phase I and II clinical trials and have shown some promising results [13,15].

Vatalanib, an inhibitor of VEGF receptor tyrosine kinases, decreases tumor growth and vascularization in a variety of tumors [16–19]. High-grade gliomas are highly vascularized, apparently through the acquisition of a number of genetic alterations that enable them to overexpress inducers of angiogenesis or to down-regulate natural angiogenesis inhibitors [20–22]. Vatalanib has effects on tumor growth in patients with recurrent high-grade gliomas and may provide additive or synergistic anti-tumor effects when given concurrently with temozolomide (TM) [20–22]. Such synergy between anti-angiogenic and cytotoxic agents has been reported [23]. A preclinical study suggested that the anti-tumor activity of TM and vatalanib combination was significantly more potent than that of either agent alone [24].

* Corresponding author. Department of Neurosurgery, Changhai Hospital, Second Military Medical University, Changhai Street 168, Shanghai, China. Tel.: +86 021 81873450; fax: +86 021 81873449.
E-mail address: liu_jm_cn@126.com (J.-M. Liu).

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In the present study, we evaluated the effects of TM–MS or vatalanib alone or in combination on tumor growth and angiogenesis in a rat orthotopic brain tumor model using C6 glioma cell line.

2. Materials and methods

2.1. Cell culture

The rat C6 glioma cell line was obtained from the Shanghai Institutes for Biological Sciences and cultured in DMEM medium with 10% FBS, penicillin (100 units/ml), and streptomycin (100 μg/ml). Cells were grown as monolayers in a humidified atmosphere of 5% CO_2, 37 °C.

2.2. Animals and orthotopic implantation of tumor cells

The animal study was performed in accordance to the experimental animal rules of Shanghai Science and Technology Committee. The protocol included the using intra-cerebral tumor models objective endpoints (e.g. ataxia, massive loss of body weight of more than 20%, social deprivation, and isolation). Before all surgical procedures, male (200–250 g) Sprague Dawley rats were anesthetized by i.p. injection of a mixture of ketamine (55 mg/ml) and xylazine (9 mg/ml) at a dose of 1 ml/kg. The head of the anesthetized rat was shaved and disinfected with 70% ethyl alcohol and povidone iodine, and the animal was secured in a rodent stereotactic frame. A midline incision was made on the dorsal aspect of the head, and the pericranium was moved laterally to expose bregma. A 1-mm hole was made exactly 1 mm anterior and 3 mm left lateral to the bregma; 2 × 106 C6 cells (in Hanks balanced salt solution) were injected into the white matter to a 5-mm depth through a 50-μl Hamilton syringe connected to the manipulating arm of the stereotactic device. The exact injection volume in which the two million C6 cells were suspended is 10 μL, and the velocity of the injection is 1 μL/min; after the complete injection, the syringe was held still in the original position for 5 min to gain a ball-pattern of tumor growth.

2.3. Drug administration

TM-loaded PLGA microparticles (TM–MS) were obtained from Department of Pharmaceutics, School of Pharmacy, Second Military Medical University (Shanghai, China). The release rates, antitumor activity against glioma C6 cells, and others were studied in vitro [1]. Vatalanib (PTK787/ZK 212583) was obtained from Novartis AG (Basel, Switzerland).

One day after intracranial tumor implantation in all animals, 120 rats were equally randomized into six groups: Group 1 served as an untreated control; Group 2 served as sham operated untreated control; Group 3 was treated with oral Temozolomide (TM, 50 mg/kg) on postoperative day (POD) 6–10; Group 4 was treated with TM–MS (equivalent to 4 mg TM/kg) as interstitial chemotherapy on POD 6; Group 5 was treated with oral vatalanib (100 mg/kg) on POD 1–12; and Group 6 was treated with oral vatalanib on POD 1–12, in addition to interstitial chemotherapy (TM–MS) on POD 6.

On POD 6, all C6 tumor-bearing rats of Groups 2, 4, 5, and 6 were anesthetized as described above. A small craniectomy was performed around the burr hole made previously. A cruciate incision was made into the dura. The underlying brain tissue over the tumor was aspirated gently until the surface of the tumor was exposed. In Groups 4 and 6, the surface of C6 tumor was covered with 16 mg TM–MS containing 1 mg TM. In Groups 2 and 5, blank microparticles were added.

From POD 1–12, Groups 5 and 6 were orally administered with vatalanib dissolved freshly in PEG300 once a day at a dose of 100 mg/kg of body weight.

2.4. Harvesting of specimens

Of the 20 rats in each group, 10 were treated without sacrifice for survival time. On the 12th POD, the rats were killed. The brain was removed, sectioned axially, fixed in 10% buffered formalin, and paraffin embedded. The tumor volume was calculated by measuring the section with the largest tumor portion and applying the formula: $\text{width}^2 \times \text{length} \times 0.5$.

2.5. Immunohistochemistry

Paraffin-embedded tissues were used to assay expression of proliferation cell nuclear antigen (PCNA), TUNEL, CD31, and vascular endothelial growth factor (VEGF). Tissue sections (3 μm thick) were mounted on silanized glass slides, dried overnight, deparaffinized in xylene, treated with a graded series of alcohol [100%, 95%, and 80% ethanol/dd H_2O (vol/vol)], and rehydrated in PBS (pH 7.5). For PCNA, the sections were stained with mouse anti-PCNA, clone Mab4078 (Chemicon, USA). The DeadEnd fluorometric TUNEL system (Promega, USA) was used to assay apoptosis. Mouse anti-rat CD31 (clone TLD-3A12, Acris Antibodies, Germany) and mouse anti-VEGF (clone JH121, Upstate, USA) were used to stain their respective antigens.

2.6. Quantification of immunostaining

For quantification of immunostaining for PCNA assay, the number of stained cells was counted in 10 random fields at 400× magnification, and the number of stained cells was counted in 10 random fields at 100× magnification for TUNEL. For CD31, the number of stained cells was counted in 10 random fields at 200× magnification. To quantify immunohistochemical reaction intensity for VEGF, the absorbance of stained cells was measured using the Optima image analysis software.

2.7. Data analysis and statistics

Values are presented as mean ± SD. Kaplan and Meier method was used for survival time in statistical comparison. ANOVA method and LSD test were used for tumor volume, cell proliferation, apoptosis, and angiogenesis. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Combination treatment with TM–MS and vatalanib improves survival time

The median survival of Groups 1, 2, 3, 4, 5, and 6 was 20, 21.5, 26.5, 37, 23.5, and 40.5 days, respectively. Treatment with TM–MS at 4 mg/kg improved the survival of Group 1. The median survival time was the longest in Group 6 ($P = 0.032$, Kaplan and Meier method) (Fig. 1).

3.2. Combination treatment with TM–MS and vatalanib inhibits tumor growth

To determine the effects of combination treatment with TM–MS and vatalanib, we monitored tumor size after treatment in an orthotopic rat glioma model (Fig. 2). There was no sign of drug toxicity, such as weight loss upon treatment over the course of the

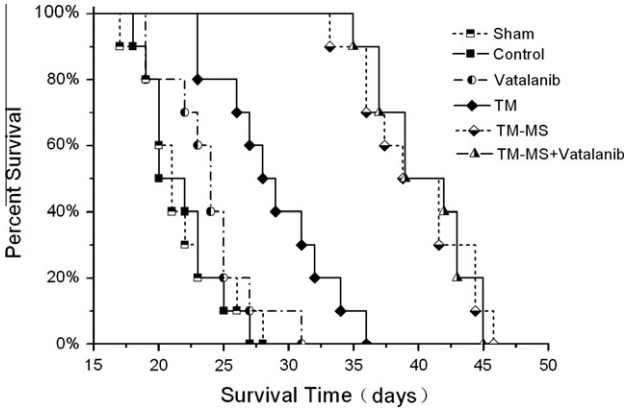

Fig. 1. Combination treatment with TM–MS and vatalanib improves the survival time of rats bearing orthotopic gliomas. Median survival times were 20, 21.5, 26.5, 37, 23.5, and 40.5 days, respectively. Combination treatment with TM–MS and vatalanib significantly increased median survival compared with control group ($P = 0.0002$).

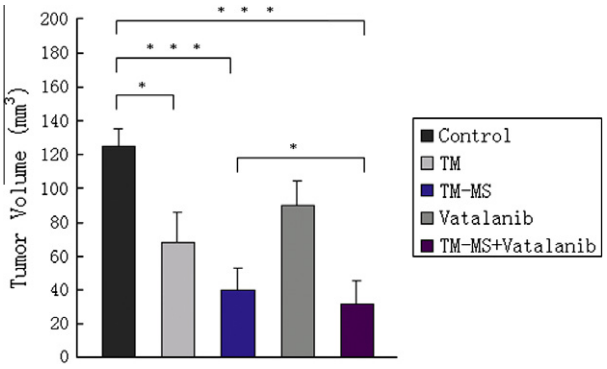

Fig. 2. Combination treatment with TM–MS and vatalanib inhibits tumor growth in an orthotopic rat glioma model. C6 rat glioma cells were orthotopically inoculated into the brains of rats; 50 mg/kg TM was orally administered from POD 6 to POD 10. TM–MS (equivalent to 4 mg/kg) was peritumorally administered on the 6th day after tumor cell inoculation. Oral vatalanib (100 mg/kg) was administered daily for 12 days, starting on the day after intracranial implantation. $^{*}P < 0.05$ and $^{***}P < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

experiment (data not shown). Compared with Group 1, the mean tumor volume decreased by 45.6% in Group 3 ($P = 0.021$, ANOVA method and LSD test), 68.0% in Group 4 ($P = 0.001$), 28.1% in Group 5 ($P = 0.010$), and 74.4% in Group 6 ($P = 0.001$) (Fig. 2). The tumor volume in Group 6 significantly reduced by 52.9% compared with Group 3 ($P = 0.002$), 20% compared with Group 4 ($P = 0.021$), and 64.4% compared with Group 5 ($P = 0.001$), respectively.

3.3. Combination treatment of TM–MS and vatalanib inhibits cell proliferation and induces apoptosis

To understand how TM–MS and vatalanib affected tumor pathology, cell proliferation was examined immunohistochemically by counting strongly stained PCNA-positive cells (Fig. 3A). Compared with untreated control tumors, cell proliferation was reduced in TM, TM–MS, and vatalanib group, by 45.4% ($P = 0.002$, ANOVA method and LSD test), 35.5% ($P = 0.021$), and 63.3% ($P = 0.045$), respectively. TM–MS + vatalanib combination therapy decreased the number of PCNA-positive cells by 79.3% ($P = 0.001$) (Fig. 3B). Cell proliferation was reduced in TM–MS group by 39.4% ($P = 0.003$) compared with TM group.

Compared with the untreated controls, the percent of TUNEL stained apoptotic cells in all cells per section increased 3-fold for TM group ($P = 0.007$, ANOVA method and LSD test), 4.7-fold for TM–MS group ($P = 0.006$), 2-fold for vatalanib group ($P = 0.03$), and 8.3-fold for TM–MS + vatalanib combination group ($P = 0.002$) (Fig. 4).

3.4. TM–MS + vatalanib combination treatment inhibits angiogenesis

Knowing that gliomas are hypervascularized brain tumors and dependent upon angiogenesis for their growth, anti-angiogenic potential of TM–MS + vatalanib combination treatment was examined by immunohistological staining of CD31 for vessel detection (Fig. 5). In vatalanib group, the number of CD31-stained vessels decreased by 46.5% compared with the untreated controls ($P = 0.003$, ANOVA method and LSD test). In TM–MS + vatalanib group, the number of blood vessels stained with CD31 decreased by 76.4% ($P = 0.001$).

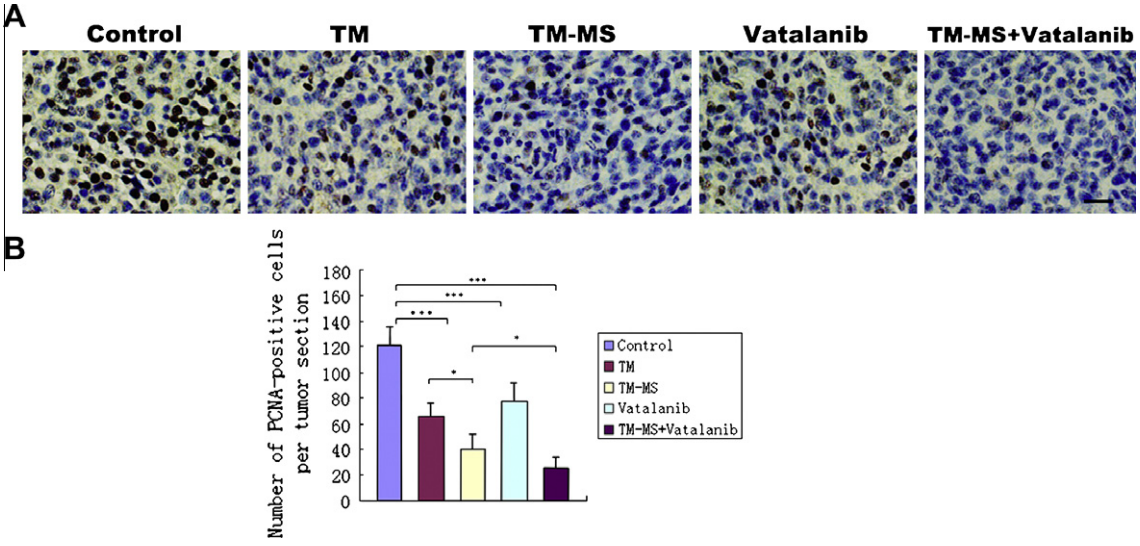

Fig. 3. Combination treatment with TM–MS and vatalanib inhibits cell proliferation in glioma xenografts. (A) Paraffin sections of tumors stained for proliferating cells using an anti-PCNA antibody. Scale bar: 50 μm. (B) Mean numbers of PCNA-positive cells are shown ($n = 10$). $^{***}P < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

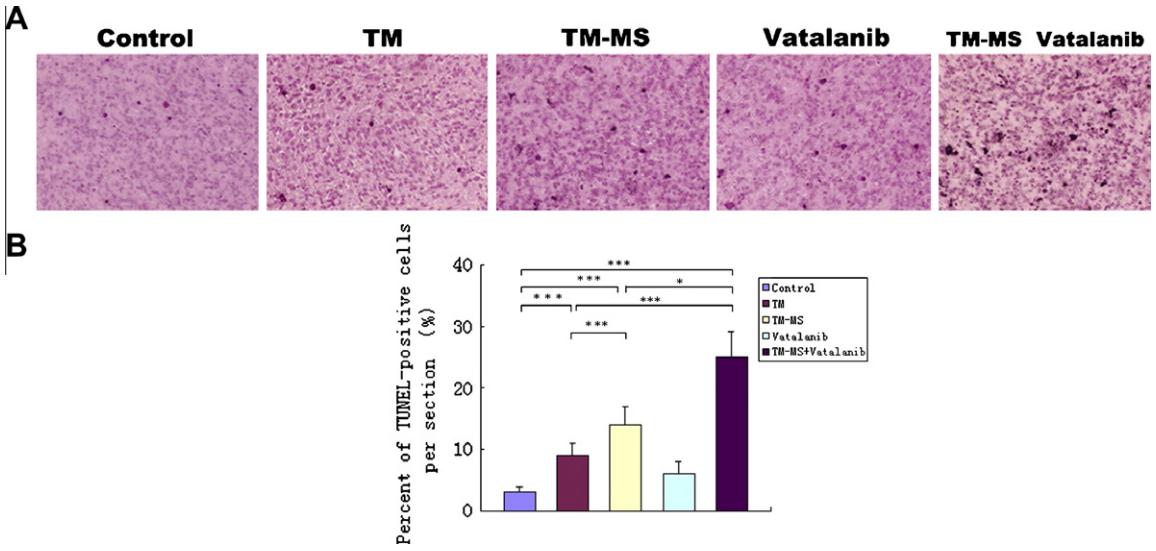

Fig. 4. Combination treatment with TM–MS and vatalanib induces cell apoptosis in glioma xenografts. (A) Paraffin sections of tumors stained for proliferating cells by TUNEL method at 100 × magnification. (B) Percent of TUNEL-positive cells in all cells per section are shown ($n = 10$). $^{***}P < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Data obtained from the rat orthotopic glioma model of this study indicate that TM–MS can inhibit cell proliferation and induce the stimulation of apoptosis. Vatalanib can inhibit vascularization of malignant glioma by inhibiting VEGF-mediated effects. We hypothesized that combination of TM–MS and vatalanib could more significantly inhibit tumor growth.

We killed the rats on the 12th day after C6 injections, because it is the early period of tumor, and the tumor grew with round shape in this period. As the tumor growing, it was preferred to growing through the pin hole, which was used to inject the C6 cells, and grew diffusely subcutaneously. Our data showed the therapeutic effect of TM–MS and vatalanib during the early period of tumor growing.

TM has be proven to be an effective chemotherapeutic agent for the treatment of patients with malignant glioma [13,25,26]. Polymeric devices implanted into the brain can locally release neuroactive substances for extended periods of time, and TM–MS was developed to get better therapeutic effect. Then, the vitro and vivo studies were performed to evaluate the effect of TM–MS. In vitro study, TM–MS has shown anti-tumor activity against Glioma C6 cancer cells [1]. In this vivo study, we found that TM–MS alone significantly improved the survival time, inhibited tumor volume, decreased the number of PCNA-positive proliferating cells, and induced more cell apoptosis, probably because of the following reasons: local drug delivery could avoid permeability problems of the blood brain barrier, thus achieving high local drug concentrations. Furthermore, PLGA microspheres have been shown to continuously release TM for up to one month in vitro [1], and therefore, TM–MS is predicted to be sufficient for tumor inhibition in vivo levels. Our results confirmed that TM–MS prolonged survival time, decreased cell proliferation, and increased cell apoptosis as compared with TM group. TM–MS alone differs from vatalanib in that it inhibited tumor growth without inhibiting angiogenesis. These results are consistent with those of others [1,27,28]. However, frequent administration of TM at a low dose (metronomic treatment) markedly inhibited angiogenesis in a TM-resistant C6/LacZ rat glioma model as well as tumor growth [29].

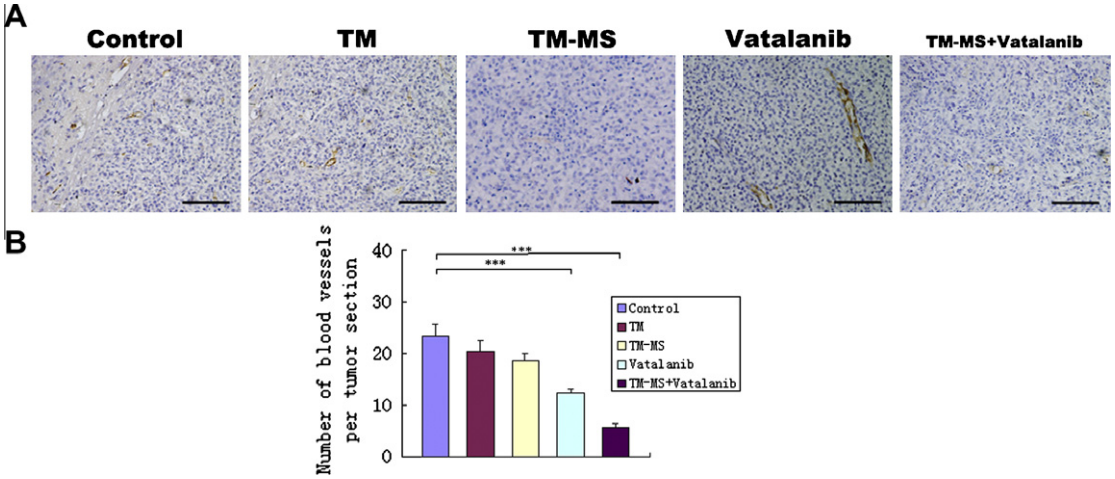

Fig. 5. Combination treatment with TM–MS and vatalanib inhibits microvessel density in glioma xenografts. (A) Paraffin sections of tumors stained for CD31. Scale bar: 100 μm. (B) Mean numbers of blood vessels are shown ($n = 10$). $^{***}P < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Vatalanib is able to suppress vascularization and growth of malignant brain tumors, and the main effect of vatalanib is its inhibition on VEGF receptor tyrosine kinases. We found that vatalanib inhibited tumor angiogenesis as other studies have found [16].

Thus, the combination of cytotoxic therapy and anti-angiogenic treatment during the vascular normalization would be more effective than either of them. The present findings are consistent with Jain's concept [30]. TM–MS + vatalaninb combination treatment not only significantly improved the survival time but also inhibited the tumor volume, decreased the number of PCNA-positive proliferating cells, increased the number of apoptotic tumor cells, and inhibited tumor angiogenesis.

Thus, TM–MS and vatalanib may act through different anti-tumor mechanisms: TM–MS as a cytotoxic agent inhibiting tumor growth, and vatalanib as a maintenance agent to hinder new vessel formation and perhaps induce tumor vascular normalization. These results provide an experimental basis for clinical study in combination with the use of TM–MS and vatalanib, which may be a promising strategy for the management of malignant gliomas.

5. Conclusions

TM–MS can more effectively inhibit tumor cells than TM. Combination treatment with TM–MS and vatalanib offered a greater tumor inhibitory effect. The results of the present study may provide an experimental basis for further clinical studies of TM–MS and vatalanib as a promising strategy for the treatment of malignant gliomas.

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References

[1] H. Zhang, S. Gao, Temozolomide/PLGA microparticles and antitumor activity against glioma C6 cancer cells in vitro, Int. J. Pharm. 329 (2007) 122–128.
[2] C. Conrad, H. Friedman, D. Reardon, J. Provenzale, E. Jackson, H. Serajuddin, D. Laurent, B. Chen, W.K.A. Yung, A phase I/II trial of a single agent PTK787/ZK 222584 (PTK/ZK), a novel, oral angiogenesis inhibitor, in patients with recurrent glioblastoma multiforme (GBM), Am. Soc. Clin. Oncol. Annu. Meet. 22 (2004) 1512.
[3] E.A. Maher, F.B. Furnari, R.M. Bachoo, D.H. Rowitch, D.N. Louis, W.K. Cavenee, R.A. DePinho, Malignant glioma: genetics and biology of a grave matter, Genes Dev. 15 (2001) 1311–1333.
[4] H. Colman, K. Aldape, Molecular predictors in glioblastoma: toward personalized therapy, Arch. Neurol. 65 (2008) 877–883.
[5] J.A. Benitez, G. Dominguez-Monzon, J. Segovia, Conventional and gene therapy strategies for the treatment of brain tumors, Curr. Med. Chem. 15 (2008) 729–742.
[6] H.A. Fine, K.B. Dear, J.S. Loeffler, P.M. Black, G.P. Canellos, Meta-analysis of radiation therapy with and without adjuvant chemotherapy for malignant gliomas in adults, Cancer 71 (1993) 2585–2597.
[7] F.J. Attenello, D. Mukherjee, G. Datoo, M.J. McGirt, E. Bohan, J.D. Weingart, A. Olivi, A. Quinones-Hinojosa, H. Brem, Use of Gliadel (BCNU) wafer in the surgical treatment of malignant glioma: a 10-year institutional experience, Ann. Surg. Oncol. 15 (2008) 2887–2893.
[8] P.Y. Wen, S. Kesari, Malignant gliomas in adults, New Engl. J. Med. 359 (2008) 492–507.
[9] S.E. Combs, J. Wagner, M. Bischof, T. Welzel, L. Edler, R. Rausch, F. Wagner, A. Zabel-du Bois, J. Debus, D. Schulz-Ertner, Radiochemotherapy in patients with primary glioblastoma comparing two temozolomide dose regimens, Int. J. Radiat. Oncol. Biol. Phys. 71 (2008) 999–1005.
[10] A.A. Brandes, State-of-the-art treatment of high-grade brain tumors, Semin. Oncol. 30 (2003) 4–9.
[11] I. Zawlik, S. Vaccarella, D. Kita, M. Mittelbronn, S. Franceschi, H. Ohgaki, Promoter methylation and polymorphisms of the MGMT gene in glioblastomas: a population-based study, Neuroepidemiology 32 (2009) 21–29.
[12] J.Y. Wu, J. Wang, J.C. Lai, Y.W. Cheng, K.T. Yeh, T.C. Wu, C.Y. Chen, H. Lee, Association of O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation with p53 mutation occurrence in non-small cell lung cancer with different histology, gender, and smoking status, Ann. Surg. Oncol. 15 (2008) 3272–3277.
[13] S.M. Chang, K.R. Lamborn, M. Malec, D. Larson, W. Wara, P. Sneed, J. Rabbitt, M. Page, M.K. Nicholas, M.D. Prados, Phase II study of temozolomide and thalidomide with radiation therapy for newly diagnosed glioblastoma multiforme, Int. J. Radiat. Oncol. Biol. Phys. 60 (2004) 353–357.
[14] H. Brem, S. Piantadosi, P.C. Burger, M. Walker, R. Selker, N.A. Vick, K. Black, M. Sisti, S. Brem, G. Mohr, P. Muller, R. Morawetz, et al., Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The polymer-brain tumor treatment group, Lancet 345 (1995) 1008–1012.
[15] H.A. Fine, P.Y. Wen, E.A. Maher, E. Viscosi, T. Batchelor, N. Lakhani, W.D. Figg, B.W. Purow, C.B. Borkowf, Phase II trial of thalidomide and carmustine for patients with recurrent high-grade gliomas, J. Clin. Oncol. 21 (2003) 2299–2304.
[16] R.H. Goldbrunner, M. Bendszus, J. Wood, M. Kiderlen, M. Sasaki, J.C. Tonn, PTK787/ZK222584, an inhibitor of vascular endothelial growth factor receptor tyrosine kinases, decreases glioma growth and vascularization, Neurosurgery 55 (2004) 426–432.
[17] S. Sathornsumetee, J.N. Rich, Antiangiogenic therapy in malignant glioma: promise and challenge, Curr. Pharm. Des. 13 (2007) 3545–3558.
[18] J. Drevs, R. Muller-Driver, C. Wittig, S. Fuxius, N. Esser, H. Hugenschmidt, M.A. Konerding, P.R. Allegrini, J. Wood, J. Hennig, C. Unger, D. Marme, PTK787/ZK 222584, a specific vascular endothelial growth factor-receptor tyrosine kinase inhibitor, affects the anatomy of the tumor vascular bed and the functional vascular properties as detected by dynamic enhanced magnetic resonance imaging, Cancer Res. (2002) 4015–4022.
[19] C. Hess, V. Vuong, I. Hegyi, O. Riesterer, J. Wood, D. Fabbro, C. Glanzmann, S. Bodis, M. Pruschy, Effect of VEGF receptor inhibitor PTK787/ZK222584 [correction of ZK222548] combined with ionizing radiation on endothelial cells and tumour growth, Br. J. Cancer 85 (2001) 2010–2016.
[20] K.H. Plate, G. Breier, H.A. Weich, W. Risau, Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo, Nature 359 (1992) 845–848.
[21] A.D. Norden, J. Drappatz, P.Y. Wen, Novel anti-angiogenic therapies for malignant gliomas, Lancet Neurol. 7 (2008) 1152–1160.
[22] A. Chi, A.D. Norden, P.Y. Wen, Inhibition of angiogenesis and invasion in malignant gliomas, Expert Rev. Anticancer Ther. 7 (2007) 1537–1560.
[23] H.J. Mauceri, N.N. Hanna, M.A. Beckett, D.H. Gorski, M.J. Staba, K.A. Stellato, K. Bigelow, R. Heimann, S. Gately, M. Dhanabal, G.A. Soff, V.P. Sukhatme, et al., Combined effects of angiostatin and ionizing radiation in antitumour therapy, Nature 394 (1998) 287–291.
[24] D. Reardon, H. Friedman, N. Brada, C. Conrad, J. Provenzale, E. Jackson, H. Serajuddin, B. Chen, A phase I/II trial of PTK787/ZK 222584 (PTK/ZK), a multi-VEGF receptor tyrosine kinase inhibitor, in combination with either temozolomide or lomustine for patients with recurrent glioblastoma multiforme (GBM), Neuro-oncology 6 (2004) 381.
[25] M. Ziobro, J. Rolski, A. Grela-Wojewoda, A. Zygulska, M. Niemiec, Effects of palliative treatment with temozolomide in patients with high-grade gliomas, Neurol. Neurochir. Pol. 42 (2008) 210–215.
[26] M.E. Hegi, A.C. Diserens, T. Gorlia, M.F. Hamou, N. de Tribolet, M. Weller, J.M. Kros, J.A. Hainfellner, W. Mason, L. Mariani, J.E. Bromberg, P. Hau, et al., MGMT gene silencing and benefit from temozolomide in glioblastoma, New Engl. J. Med. 352 (2005) 997–1003.
[27] S.G. Kang, J.S. Kim, K. Park, J.S. Kim, M.D. Groves, D.H. Nam, Combination celecoxib and temozolomide in C6 rat glioma orthotopic model, Oncol. Rep. 15 (2009) 7–13.
[28] D. Devineni, A. Klein-Szanto, J.M. Gallo, Uptake of temozolomide in a rat glioma model in the presence and absence of the angiogenesis inhibitor TNP-470, Cancer Res. 56 (1996) 1983–1987.
[29] J.T. Kim, J.S. Kim, K.W. Ko, D.S. Kong, C.M. Kang, M.H. Kim, M.J. Son, H.S. Song, H.J. Shin, D.S. Lee, W. Eoh, D.H. Nam, Metronomic treatment of temozolomide inhibits tumor cell growth through reduction of angiogenesis and augmentation of apoptosis in orthotopic models of gliomas, Oncol. Rep. 16 (2006) 33–39.
[30] R.K. Jain, Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy, Science 307 (2005) 58–62.