Local Delivery of Minocycline and Vorinostat Act Synergistically to Inhibit Recurrence of Gliomas

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Research

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Abstract

Background: Postoperative recurrence is the main reason of poor clinical consequences in glioma patients, so preventing recurrence of tumors is crucial in management of gliomas.

Methods: In this study, the expression of matrix metalloproteinases (MMPs) in tissues from normal were detected by using RNA-seq analysis. Glioma cases from the public databases (*The Cancer Genome Atlas (TCGA)*, *The Chinese Glioma Genome Atlas (CGGA)*, *Betastasis*) were included in this study. The hydrogel contains minocycline (Mino) and vorinostat (Vor) (G/Mino + Vor) was formed under 365 nm when photoinitiator was added in. High Performance Liquid Chromatography (HPLC) assay was used to assess the release of drugs in G/Mino + Vor hydrogel. MTT assay was used to explore the biosecurity of GelMA. Immunohistochemistry assay, ELISA assay, TUNEL assay were used to demonstrate the antitumor effect of G/Mino + Vor hydrogel.

Results: We developed G/Mino + Vor hydrogel successfully. Then the experiment in vitro and in vivo confirmed MMPs-responsive delivery of minocycline and vorinostat in hydrogel and the anti-glioma effect on incomplete tumor operation model, which indicated that G/Mino + Vor hydrogel effectively inhibited the recurrence of glioma after surgery.

Conclusions: In summary, G/Mino + Vor hydrogel could continuously release minocycline and vorinostat in surgical cavity for inhibiting local recurrence of glioma after operation.

Background

Gliomas are recognized as the most aggressive tumor in central nervous system (CNS) (1, 2). Despite standard therapy including surgery, chemo- and radio- therapy have been widely adopted, the average survival time is only about 15 months, whereas the survival rate for 5 years is only 4–5% (3–5). Irrespective of the current aggressive and multi-modal first-line approaches at diagnosis and treatment, recurrence in glioblastoma (GBM) is practically inevitable with average time to recurrence is less than 10 months (2, 6–8). Various treatment modalities are utilized including repeat resection, irradiation and systemic agents, yet prognosis for these patients remains discouraging. The need for more innovative therapeutic strategies to expedite recurrence glioma therapeutics is imperative.

Clinical studies have found 80–90% of recurrent gliomas occur near the primary site, suggesting that residual tumor cells caused most recurrence gliomas (9–11). To break through the physiological barrier, glioma secretes extracellular matrix (ECM) degrading matrix metalloproteinases (MMPs) highly (12–14), which are also upregulated after tissue injury. So, it may play an important role on the recurrence of gliomas after surgery. One agent showing promise is the tetracycline antibiotics targeting MMPs, minocycline. Researchers found that minocycline can reduce the MMPs expression from microglia via blocking toll like receptor 2 (TLR2) signaling and p38 mitogen-activated protein kinase (MAPK) pathway (15, 16). Unfortunately, minocycline can cross the blood-brain barrier (BBB), which restricts the

concentrations of drugs delivered within the glioma microenvironment(17), still remain a limiting factor for efficacy. Higher doses of minocycline are proscribed due to severe liver and kidney toxicity(18).

The immune response may be suppressed by polarization of macrophages within TME into M2 macrophages, which hamper immune responses by releasing anti-inflammatory cytokines and angiogenic factors(19–22). Thus, repolarizing M2 to proinflammatory M1 could improve the immune response in the tumor microenvironment(23, 24). Previous studies have shown the ability of vorinostat to have anti-tumor effects by manipulating macrophages(25–27). But due to poor solubility and stability, vorinostat were found to be difficult to be absorbed into body, thus resulted in poor bioavailability in vivo. Moreover, vorinostat has significant adverse effects including nausea, fatigue, and thrombocytopenia(28, 29).

Because of these problems in clinical current application of minocycline and vorinostat. We use GelMA to developed a drug-loaded hydrogel. It allows for delivery of high local drug doses to the residual infiltrative cells with minimized systemic toxicity. Due to the presence of methacrylamide groups, the mixture solution was immediately cross-linked under UV light when photoinitiator was added in, and a hydrogel with good thermal stability was formed(30–32). GelMA could release drugs owing to its biodegradability in tumor microenvironment(33, 34) (Sch.1A). Then minocycline would hamper the MMPs expression from microglia and vorinostat help repolarizing M2 to proinflammatory M1 (Sch.1B). Minocycline could synergize with vorinostat that improve the efficiency of anti-tumor therapy.

Methods

Tissue samples

Twelve primary malignant glioma tissues and adjacent normal tissues (ANT) were collected from the glioma patients in the Affiliated Hospital of Xuzhou Medical University, and informed consent was obtained from all patients.. The pathological diagnosis was made independently by two pathologists. The public database of glioma from *The Cancer Genome Atlas (TCGA)*, *the Chinese Glioma Genome Atlas (CGGA)*, *Betastasis*, *Gene Expression Omnibus (GEO)* #GSE122556 were also included in this study.

Materials

GelMA was purchased from Tissueink Biotechnology Co., LTD (Wenzhou, China). Minocycline, vorinostat were purchased from Dalian Meilun Biotechnology Co., LTD. (Dalian, China). MTT kit was bought from Shanghai Biyuntian Co., LTD (Shanghai, China). ELISA kit came from Wuhan Boster Biological Technology Co. LTD (Wuhan, China).

Cells And Mice

Cell lines were obtained from the Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China) and the National Experimental Cell Resource Sharing Platform (Beijing, China). Male mice (C57BL/6 male at 6–8 weeks of age) were purchased from Beijing HFK Bioscience Co., LTD. (Beijing, China). All animal experiments were conducted in accordance with protocols approved by Experimental Animal Ethics Committee of Xuzhou Medical University.

Synthesis And Drug Release Of G/mino + vor Hydrogels

GelMA (50 mg) and UV photoinitiator (5 mg) were mixed, followed by the addition of ultrapure sterile water to dissolve it, then minocycline (10 mg) and vorinostat (4 mg) were added to the mixture solution. Put 100 μ L G/Min + Vor hydrogel was taken in a dialysis tube, and then put it into a 15 mL centrifuge tube containing 10 mL Phosphate Buffered Saline (PBS) buffer, and add recombinant human MMP2 (100 ng/mL), MMP9 (100 ng/mL) and Lipase (5000 U/mL) at 37 °C. 50 μ L of dialysate was taken at each time point, and its content was measured by high performance liquid chromatography, and the release curve was drawn according to the regulations. The column of minocycline is Thermo ODS-C18, and the mobile phase is 0.2 mol/L ammonium oxalate- dimethylformamide-0.01 mol/L disodium EDTA (550: 200: 250), the detection wavelength is 280 nm, the injection volume is 20 μ L, flow rate is 1.5 mL/min. Vorinostat detection conditions: Chromatographic column is Thermo ODS-C18, mobile phase is 20 mmol/L potassium dihydrogen phosphate solution-acetonitrile (700: 300), detection wavelength is 242 nm, injection volume is 20 μ L, flow rate is 1.5 mL/min.

The standard curve for minocycline. $Y = 0.1423x - 0.1431$, $R^2 = 0.9997$.

The standard curve for vorinostat. $Y = 0.2517x + 0.0233$, $R^2 = 0.9995$

Cytotoxicity Assay

Detection of cytotoxicity of hydrogel carriers using MTT method. In briefly, GL261 and HA1800 cells were trypsinized and cultured at a density of $5 \cdot 10^3$ per well and spread on 96-well plate containing 10% Fetal Bovine Serum (FBS) high-sugar dulbecco's modified eagle medium (DMEM) medium for 24 hours. Then replaced with FBS-free high-sugar medium. PBS buffer or GelMA hydrogel soaking solution was incubated. After 24 hours, 20 μ L 0.5 mg/mL MTT solution was added in each well and incubated for 4 h. Thereafter, all solution was carefully removed and 150 μ L DMSO was added to each well to dissolve the formazan (precipitate). The optical density (OD) was measured by absorbance at 570 nm with a microplate reader.

Anti-tumor efficacy in vivo and toxicity evaluation

Briefly, $5 \cdot 10^5$ GL261 glioma cells were resuspended and injected into the right forebrain of 8-week-old mice. On day 10 after tumor implantation, mice bearing tumors were randomly divided into 5 groups.

Mice were gas anesthetized and fixed in a stereotaxic apparatus. A 1.0 cm incision was made along the midline of the previous surgical scar, and a 1.0 cm circular skull window was subsequently drilled with a drill to expose brain tissue. A biopsy punch with a diameter of 2 mm was inserted to 5 mm depth. The biopsy punch was rotated to cut the glioma tissue for 15 s. And aspirate tumor and brain tissue using a vacuum pump. After the hemostasis was compressed, hydrogels containing different drugs (PBS, GelMA, G/Mino, G/Vor, G/Mino + Vor) were injected into the tumor cavity after surgery. Later suture the wound. Then record the weight of mice and the survival rate after administration. On day 28 after glioma transplantation, mice in each group (n = 3) were euthanized. The main organs and brain tissues of dead mice were collected and fixed with 10% formalin. The fixed organs and brain tissue were embedded in paraffin and stained with H&E, further observed and photographed with a microscope.

Immunohistochemistry

To assess the expression of MMPs of tumor tissues. The glioma tissues sections were dewaxed in xylene and rehydrated with gradient ethanol. Next, the sections were incubated in 4% H₂O₂ for 20 min and then in 3% albumin from bovine serum (BSA) for 60 min. The sections were incubated with rabbit primary antibody against MMP2 (1:100, Abcam, Cambridge, MA) or MMP9 (1: 100) at 4 °C overnight. Then incubate with anti-rabbit secondary antibody (Zhongshan Golden Bridge, Beijing, China) for 30 min. After counterstaining with DAB and hematoxylin, the sections were dehydrated according to gradient ethanol, and then the coverslips were observed and quantified by microscope. The images were analyzed using Image Pro Plus 6.0 software.

Enzyme-linked Immunosorbent Assay (elisa)

In order to confirm the repolarize effect of M2 to M1, the markers of M1 (tumor necrosis factor (TNF) - α , interleukin (IL) -6) and the markers of M2 (IL-4 and IL-10) were assessed with enzyme-linked immunosorbent assay (ELISA). Briefly, the mice were anesthetized using overdose of 4% chloral hydrate and killed. Brain tissues were collected, then homogenized in PBS, and high speed centrifuged (4 °C, 8000 rpm, 10 min). The supernatant was collected and diluted with ELISA diluent buffer. The protein content of each homogenate sample was normalized to total protein levels per sample with BCA kit.

Statistical analysis

All statistical analysis results were analyzed the differences between the two groups using the SPSS 19.0 software. When $P < 0.05$, it has statistical significance.

Results

High MMPs expression correlates with poor glioma patient survival.

To clarify the clinicopathological significance of MMP2 and MMP9, we analyzed the expression of MMP2 and MMP9 in the patients with different grade gliomas. We performed immunohistochemistry for MMP2 and MMP9 using the samples from WHO grade II–III gliomas and glioblastomas (GBM, WHO grade IV), and samples from normal brain served as a control. Compared with normal group, the expression of MMP2 and MMP9 was obviously high level in both grade II–III gliomas and GBM (Fig. 1A). Then we studied MMP2 and MMP9 expression in human glioma specimens using betastasis dataportal (<http://www.betastasis.com>). When we explored MMP2 and MMP9 expression in different histological glioma subclasses, significantly more MMP2 and MMP9 in all types of glioma patients compared to the normal people (Fig. 1B and C). Simultaneously, we investigated MMP2 and MMP9 expression in The Cancer Genome Atlas (TCGA) RNA seq datasets for GBM. In the TCGA dataset, high MMP2 expression and MMP9 associated with poor survival compared with low MMP2 expression (Fig. 1D and E). The datasets from The Chinese Glioma Genome Atlas (CGGA) was also assessed. When we analyzed MMP2 and MMP9 expression in different grade gliomas (grade II–IV), significantly more MMP2 and MMP9 was expressed in GBM samples compared to those from lower grade gliomas (Fig. 1F and G). All these results show that high MMP2 and MMP9 expression is associated with tumor progression and poor glioma patient survival.

M2 macrophages constitute the main immune cell population in GBM.

As well known, the tumor microenvironment is composed of multiple components including parenchyma cells, soluble factors, blood vessels, extracellular matrix and infiltrating immune cells(19, 35, 36). M2 macrophages are often found huge proportion in the malignant tumor microenvironment, which could suppress on immune response by reducing CD4⁺ and CD8⁺ T cells proliferation, enhancing tumor cell invasion and intravasation into blood vessels(37). Therefore, we assessed M1 and M2 macrophages in GBM with the data obtained from TCGA database. As expected, macrophages account for the highest proportion of all immune cells in tumor tissues, which is not obvious in normal tissues (Fig. 2A). Moreover, M2 macrophages were more highly expressed in GBM compared to M1. In contrast, the difference in the expression of M1 and M2 in normal tissues is not statistically significant (Fig. 2B).

Preparation of MMPs responsive G/Mino + Vor hydrogel for localized delivery drugs

In this study, we make fully use of the characteristics of GelMA, which could delivery minocycline and vorinostat directly to the remnant tumor tissues and release them owing to its biodegradability. Initially, we added minocycline and vorinostat to the GelMA aqueous solution at 50 °C. The resultant solution, which also contained the photoinitiator, was then irradiated with 365 nm UV light to form G/Mino + Vor hydrogel (Fig. 3A). The surface of G/Mino + Vor hydrogel was imaged by SEM (Fig. 3B). GelMA had been reported to have a MMPs-responsive property(38). Thus, we evaluated the ability of the G/Mino + Vor hydrogel to release the encapsulated minocycline and vorinostat in response to MMPs in vitro (Fig. 3C). As expected, the addition of MMP2 and MMP9 caused an obviously increased release of drugs from G/Mino + Vor hydrogel (65.6%, 68.8%) compared with PBS group (23.6%) (Fig. 3D). These results demonstrated G/Mino + Vor hydrogel had good ability to release agents under MMPs conditions.

Meanwhile, the cytotoxicity of GelMA was tested to ensure biosecurity. As shown in Fig. 3E and F, GelMA was not toxic in both GL261 glioma cells and HA1800 cells.

G/mino + vor Treatments Reduced The Expression Of Mmps

Then we evaluated the effect of in situ injection of G/Mino + Vor hydrogel in lower expression of MMPs in mice bearing glioma. Above all, we performed immunohistochemistry staining using MMP2 and MMP9 antibody on the sections from the tumors (Fig. 4A). The MMP2 and MMP9 expression were assessed semi quantitatively by integral optical density (IOD) analysis. Compared with the PBS group, the G/Mino group and the G/Mino + Vor group showed visible improvement in MMP2 and MMP9 expression, but there was no difference between the GelMA and the G/Vor groups (Fig. 4B and C).

G/mino + vor Caused Hydrogel M2 Macrophage Reprogramming

Next to determine the effects of G/Mino + Vor on the repolarization of M2 to M1, we assessed markers for M1 and M2 macrophages with ELISA kit. Compared with the PBS group, both G/Vor and G/Mino + Vor treatment drastically increased TNF- α expression in TAMs and changed their activity to produce IL-4, consistent with the M1 phenotype, which was not observed after treatment with GelMA and G/Mino (Fig. 5A and B). Meanwhile the expression levels of M2 macrophages markers IL-4 and IL-10 decreased, and no significant changes happened in the group GelMA and G/Mino, too (Fig. 5C and D). Taken together, beside reducing the expression of MMP2 and MMP9, G/Mino + Vor hydrogel could also lead to M2 repolarization. density (IOD) analysis.

In vivo anti-glioma recurrence of G/Mino + Vor hydrogel

To further explored the synergistic effect of minocycline and vorinostat released from G/Mino + Vor hydrogel, we established an incomplete resection of orthotopic glioma model. Briefly, $5 \cdot 10^5$ GL261 glioma cells were resuspended and injected into the right frontal lobe. The incomplete operation was done and the drug-loaded hydrogels were injected into the cavity to validate the therapeutic effects of G/Mino + Vor hydrogel at day 10 post-tumor implantation. H&E staining was used to stain the coronal section of main organs of the mice at 28 days after implantation (Fig. 6A). As expected, G/Mino + Vor hydrogel potently retarded recurrence tumor growth, while neither PBS nor GelMA treatment alone affected tumor growth, both G/Mino and G/Vor treatment partially slowed down tumor growth (Fig. 6B). Similarly, the G/Mino + Vor hydrogel-based therapy showed best performance in extending the median survival times (38.5 days). While the median survival times of group PBS, GelMA, G/Mino, G/Vor were 22, 24, 27.5 and 28.5 days, respectively (Fig. 6C). Changes in body weight were also evaluated. Not surprisingly, the body weights of mice of group G/Mino + Vor decreased slowly compared to the other treatments (Fig. 6D).

Simultaneously, the results of TUNEL staining indicated that apoptosis was significantly increased following G/Mino + Vor treatment compared with cells in other groups (Fig. 6E).

Subsequently, the slices of major organs were stained with H&E staining to evaluate toxicity *in vivo*. All groups did not exhibit distinguishable abnormality (Fig. 7). It demonstrated that G/Mino + Vor hydrogel induced negligible systemic toxicity. Taken together, these findings showed that G/Mino + Vor hydrogel had potential value for clinical use.

Discussion

In recent years, the postoperative recurrence was recognized as the main reason for glioma difficult to cure(39). And tumor microenvironment, which was constituted with stromal cells, immune cells, and vasculatures, plays a critical role in the process of tumor recurrence(40, 41). Thus, the tumor microenvironment is becoming an emerging source of novel therapeutic targets in cancer. MMPs could help tumor cells break through the ECM and were highly expressed in glioma tissues(42). And it may play a pivotal role in glioma recurrence because of its abnormal increase after surgery(42). As these reasons, hampering the expression of MMPs has its significance in the clinical practice. Minocycline, which has been approved for over 30 years by the FDA, has been demonstrated to block the p38 MAPK pathway in microglial cells(13). So it could blunt the expression of microglia dependent MMPs. Current therapies of minocycline, however, have many negative effects and technical barriers, which limit its application in the clinic.

On the other hand, TAM, up to 70% of them can be M2 macrophages, have been shown to promote tumor development by inducing immunosuppression, angiogenesis, and metastasis(43). Macrophage density in the tumor tissues within the tumor microenvironment has become an important predictor of patient's survival time based on analysis of multivariate Cox proportional hazards(44). Thus, repolarization of TAMs from M2 toward M1 phenotype would enhance the antitumor efficacy of cancer therapeutics for gliomas. At present, various approaches such as exhaustion of TAMs as well as repolarization of M1 with cytokines, decoy complexes, small molecules, and nucleic acids have been evaluated as antitumor therapies(35). Vorinostat is a representative drug could repolarize M2 toward M1. Unfortunately, the various shortcomings of vorinostat also limit its clinical application.

In this study, we explored the significance of MMPs and the quantity of M2 macrophages among immune cells in gliomas. Simultaneously, we developed injectable G/Mino + Vor hydrogel which could induce the adjustment of tumor microenvironment. G/Mino + Vor hydrogel represented such a strategy, which can be injected at the time of incomplete surgical resection to deliver agents directly to the remnant tumor tissues. So that the hydrogel could avoid the systemic side effects while maximizing the antitumor effects. More importantly, compared with a single drug, G/Mino + Vor hydrogel achieved a great improvement in the anti-cancer effect through the synergy of the two drugs. This phenomenon was consistent with our observation on the stasis about survival of mice bearing tumor.

In short, we achieved the synergistic effect of minocycline and vorinostat by developing G/Mino + Vor hydrogel. And our experiment results demonstrated that G/Mino + Vor hydrogel provided a potential local delivery approach for restricting recurrence glioma after surgery. In fact, the expression of MMPs was also reported to be associated with the amount of M2 macrophages, but the exact underlying molecular mechanism remains to be further investigated. Therefore, to clarify the mechanism of it would be our future research direction.

Conclusion

In this research, an injectable biodegradable G/Mino + Vor hydrogel has been successfully developed, which can be used to treat the recurrence of postoperative glioma. *In vitro* release study showed that the release of MMPs gradually degraded the hydrogel therefore drugs loaded in it, minocycline and vorinostat had a combined effect. *In vivo* experiments showed that the G/Mino + Vor hydrogel inhibits recurrence glioma cancer progression. Additionally, the secretion of MMP2 and MMP9 decreased and M2 to M1 repolarization was shown. In summary, it suggested that G/Mino + Vor hydrogel may be utilized as a potential formulation strategy to restrain the recurrence of residual glioma after surgery. Huber et al. have reported that expression of MMPs is associated with immune cells such as M2 macrophages. Therefore, to detect the expression level of MMPs in tumor tissues and explore its value in the clinical diagnosis and treatment of patients with glioma is our future research direction.

List Of Abbreviations

gelatin methacryloyl (GelMA)

minocycline (Mino)

vorinostat (Vor)

central nervous system (CNS)

glioblastoma (GBM)

extracellular matrix (ECM)

matrix metalloproteinases (MMPs)

blood-brain barrier (BBB)

The Cancer Genome Atlas (TCGA)

the Chinese Glioma Genome Atlas (CGGA)

integral optical density (IOD)

optical density (OD)

enzyme linked immunosorbent assay (ELISA)

TdT-mediated dUTP Nick-End Labeling (TUNEL)

hematoxylin-eosin (H&E)

Phosphate Buffered Saline (PBS)

Toll Like Receptor 2 (TLR2)

mitogen-activated protein kinase (MAPK)

analysis of variance (ANOVA)

scanning electron microscope (SEM)

tumor necrosis factor (TNF)

interleukin (IL)

adjacent normal tissues (ANT)

dulbecco's modified eagle medium (DMEM)

Fetal Bovine Serum(FBS)

albumin from bovine serum (BSA)

Declarations

Ethics approval and consent to participate

All experimental protocols were approved by the Ethics Committee of the First Affiliated Hospital of Xuzhou Medical University (Xuzhou, China) and performed in accordance with the relevant guidelines and regulations. Written informed consent was obtained from all patients. All animal experiments were approved by the Experimental Animal Ethics Committee of Xuzhou Medical University (Xuzhou, China).

Consent for publication

Not applicable

Availability of data and materials

The dataset supporting the conclusions of this article was retrieved from TCGA, CGGA, GEO and Betastasis.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

GZ and JJ conceived and designed the experiments. GZ, JJ, LSW, YKZ, HY, YL performed the experiments. HML, RTY and YFZ provided the technical support. GZ, JJ and LSW analyzed and interpreted the data. GZ wrote the manuscript. HML and JJ revised the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Association of MMP2 and MMP9 expression with patient survival. (A) Glioma tissue samples from patients were stained to visualize MMP2 and MMP9 (brown). Scale bar, 50 μ m. (B and C) The difference in MMP2 and MMP9 expression among different histological types of anaplastic glioma patients and normal people, one-way ANOVA. (D and E) Kaplan-Meier survival curves for the glioma patients with low MMP2 and low MMP9, respectively. (F and G) MMP2 and MMP9 expression across WHO grades (II, III, IV).

expression (blue) compared to those with high MMP2 expression (red), Mantel-Cox Log-rank test. (F and G) Expression of MMP2 protein level in different grade glioma, one-way ANOVA.

Figure 2

M1 and M2 macrophages expression in GBM. (A) Proportion of immune cells in normal and tumor tissues (1-4, normal; 5-12, GBM). (B) Expression of M1 and M2 level in normal and GBM, T test.

Figure 3

Preparation and characterization of G/Mino+Vor hydrogels. (A)Gelation of G/Mino + Vor hydrogel before and after UV irradiation. (B)SEM images of G/Mino+Vor hydrogel. Scale bar,2 μ m. (C)Schematic illustration of G/Mino+Vor hydrogel degradable process under MMPs conditions. (D)The drug release profile of G/Mino+Vor hydrogels under different conditions.(E and F) Toxicity detection of GelMA on GL261 and HA1800 cell lines.

Figure 4

Reduced MMPs expression in response toG/Mino+Vor treatments. (A) Immunohistochemistry staining of representative of tumor sections using MMP2 and MMP9 antibodies on the 28th day after tumor implantation. Scale bar,50 μ m. (B)Immunohistochemical slides were analyzed quantitatively using integral optical density (IOD) analysis,

Figure 5

Repolarization of M2 macrophages caused by G/Mino+Vor treatments. (A-D) Levels of TNF- α , IL-4, IL-6 and IL-10 in glioma tissue by ELISA on the 21th day after tumor implantation. One-way ANOVA.

Figure 6

Therapeutic efficacy of G/Mino+Vor hydrogel in the GL261 glioma tumor model. (A) Schematic outline shows the in vivo experimental design for treatments. (B) Representative micrographs of H&E stained tumors in orthotopic models. (C) Kaplan-Meier survival curves for the mice (n = 6), one-way ANOVA. (D) Body weight change. Data expressed as the mean $\pm$ SEM (n = 6). (E) TUNEL staining of coronal sections from mouse brains with orthotopic tumors. Scale bar, 50 μ m.

Figure 7

Toxicity analysis of G/Mino+Vor treatments. HE staining of heart, liver, spleen, lung and kidney from mice treated from different groups. Scale bar, 200 μ m.