Original Research

Biocompatibility study of glycofurol in rat brains

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Abstract

Glycofurol (GF) has been used clinically as a solvent for parenteral drug delivery systems. However, the application and toxicity of GF in the brain have not been reported. This study was carried out to assess the systemic and neurologic reactions of GF in rats upon intracranial injection. Hematological and neuropathological assessments of rats were performed during the acute, subacute and chronic period after the injection. Injection of the GF solution (GF 25 μL + PBS 25 μL) into the brain cortex showed that it did not cause any deaths or clinical neurobehavioral abnormalities. At the same volume as phosphate-buffered saline (PBS) injection, it had mild effects on all hematological data and histopathology of brain tissues. Nevertheless, histomorphologic assessments of the brain tissues treated with PBS 70 μL revealed different tissue responses compared with those of 70 μL GF solution (30 μL + PBS 40 μL) where tissues around the administration site showed elevated polymorphonuclear leukocytes, macrophages and gliosis. These results demonstrated that the GF solution (GF 25 μL + PBS 25 μL) administration was well tolerated and caused minor inflammatory responses of cerebral cortex. This suggests possibilities of GF for drug delivery systems in the brain parenchymal tissues.

Keywords: glycofurol, biocompatibility, brain, stereotactic surgery, drug delivery, histopathology

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Introduction

Delivery of drugs to the central nervous system (CNS) remains one of the most challenging tasks in the drug delivery field owing to the presence of the blood–brain barrier (BBB). Many drug delivery systems have been designed and developed to overcome the selective permeability and low transportation across the BBB, which is present as tight junctions of endothelial cells in the CNS.[1,2] Although systemic delivery of drugs at high doses may result in drug accumulation in the therapeutic level, undesirable side-effects are the major concern of drug administration. Intense efforts have been made to enhance the transportation of therapeutic agents across the BBB as alternative strategies for drug delivery to the CNS such as modulation of the BBB,[3,4,] nanocarriers[5,6] and drug conjugates.[7] A number of devices are currently employed for treatments of human CNS diseases, for example, intrathecal infusion of baclofen into the cerebrospinal fluid which was used for the treatment of spasticity.[8] In addition, subcutaneously implanted Ommaya reservoirs were used to deliver bleomycin into cystic benign brain tumors via a catheter to treat patients with craniopharyngioma.[9,10]

Interstitial drug delivery to the CNS has also gained considerable attention in the past few decades. As a result, the development of drug-impregnated implants has been the main focus for the delivery of therapeutic agents directly to the brain with improved efficacy and reduced side-effects.[11–13] In 1996, disc-shaped polymer wafers were approved by the US Food and Drug Administration (FDA) for the treatment of brain tumors.[14–16] The wafers, 1.4 cm in diameter and 1 mm in thickness, can be placed inside the brain cavity after surgery and have a mechanism to release drugs for 2–3 weeks. An analysis of data involving patients with malignant glioma over a 10-y span revealed the favorable survival in patients who received Gliadel wafer implants.[17] However, cautions have recently been raised regarding the implantation of the wafers after the lateral ventricles had been opened, resulting in fatal consequences.[18]

Glycofurol (tetrahydrofurfuryl alcohol polyethylene glycol ether, described herein as GF) has been used as a solvent for drug-impregnated formulations such as skin depigmentation, creams and ointments,[19] and intranasal doses of benzodiazepine.[20] A variety of small-molecule drugs such as diazepam and phenytoin,[21] genes[22] and proteins,[22,23] can be formulated into injectable solutions using GF as a solvent. Recently, bioavailability and tolerability of supersaturated intranasal formulations of diazepam in a mixture of GF and water has been evaluated in humans. Results showed 75% bioavailability of the drug.[24] A high bioavailability in humans of methadone formulation in 20% GF was observed upon rectal administration, which can be used as an alternative route for cancer pain and palliative care besides intravenous and oral administration.[25] GF was also one of several solvents tested as potentially precipitating liquid embolics in swine rete mirabile. It was found that GF induced less severe vasospasm which resolved faster than commonly used dimethyl sulfoxide.[26] Given its excellent biocompatibility, GF presents as an excellent alternative solvent for the development of drug-impregnated formulations or implants. In our laboratory, we aim to develop biodegradable brain implants that can be formed *in situ* upon an injection of polymer-drug solution in GF. Unlike solid implants, these implants can form small or irregular shapes after solidification depending on the injection site or cavity. This will increase the surface area between the implant and surrounding tissues which enhances the uptake of therapeutic agents. Moreover, these implants do not require large incisions for implantation because of the *in situ* solidification process. Although the recommended maximum human dose per day of GF has been mandated at less than 70 μL/kg,[27] the use of GF for drug delivery applications and its toxicology in the brain have not been previously explored. In this study, we investigated the systemic and brain tissue reactions of GF after intracranial injection in rats. GF is a good solvent for several pharmaceutical agents and an understanding of its biocompatibility will certainly be very useful for the development of drug-impregnated brain implants.

Materials and methods

Materials

Glycofurol and phosphate-buffered saline (PBS, pH = 7.4) were purchased from Sigma-Aldrich (St Louis, MO, USA).

Animals

Adult male Sprague–Dawley rats (200–250 g) were obtained from the National Animal Center, Mahidol University and housed in the Laboratory Animal Center, National Institute of Health, Ministry of Public Health. Rats were kept separately in an individually pressure-ventilated cage at a positive airflow of 75 air changes per hour. The facility was on a 12-h light–dark cycle with an average temperature of 25°C, 55% humidity and atmospheric air pressure. Rats were fed with CP082 pellets (Charoenpokapan Co, Bangkok, Thailand) and provided with sterile water *ad libitum* and woodchip bedding (Lignocel®, Germany). The animals were acclimatized for two weeks prior to the experiment. All procedures were approved by the Institutional Animal Care and Use Committee of the National Institute of Health (DMSc-IACUC), Thailand.

Administration of chemicals to rat brains

Rats ($n = 48$) were anesthetized by an intraperitoneal injection of xylazine (10 mg/kg) and tiletamine-zolazepam (40 mg/kg), then randomly assigned to receive different treatments. The scalp of the animal was shaved, disinfected and the rat's brain was stereotactically targeted at the right frontal cortex using a stereotactic device (Kopf Instruments, Miami Lakes, FL, USA). A burr hole was made at 3 mm posterior to the bregma and 3 mm lateral to the midline. A tuberculin syringe containing an injecting solution was attached to the vertical stereotactic arm. Then the solution was slowly injected 3 mm deep into the cerebral cortex. Animals in the control group were injected with either 50 μL ($n = 12$) or 70 μL ($n = 12$) of PBS (pH 7.4). In the study group ($n = 24$), 12 animals were injected with a solution of 25 μL GF and 25 μL PBS, while the other 12 animals were injected with a solution of 40 μL GF and 30 μL PBS. The injections were administered at a rate of 5 μL per min. The animal's body temperature was stabilized using a temperature-controlled blanket during the procedure. The scalp was sutured with silk 4-0 continuously. The animals were kept in the incubator during the recovery period. Their body weights and activities were monitored during the experiment.

Daily evaluation

After the injection, the animals were monitored daily to evaluate their physical and behavioral conditions. Animals' response to the treatment was graded according to the following scale: 0 – no sign of toxicity; 1 – weight loss; 2 – partial paresis; 3 – total paresis; 4 – premoribund; and 5 – death. The animals were monitored for 3, 6, 14 or 30 d, after which they were sacrificed for further analyses.

Hematological analysis

During euthanasia of animals, blood samples (~5–7 mL) were collected by heart puncture using EDTA-containing vacutainer tubes (BD Vacutainer, Plymouth, UK). Hematological analysis includes total white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin concentration (HGB), hematocrit (HCT), platelet (PLT) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The analyses were carried out within two hours of collection using a Sysmex hematology analyzer (PocH-100iV Diff, Sysmex, France). *P* value was calculated by the SPSS Statistics 17.0 software. A value of less than 0.05 was considered statistically significant.

Histopathological analysis

After the animals were sacrificed, brains were harvested and fixed in 10% formalin for 24 h. The brain tissues were sectioned at 5 μm and then stained with hematoxylin and eosin (H&E). The coronal brain section from the targeted coordinates[28] was used to compare the histopathology between the two groups. The histopathological slides were blinded to the neuropathologist (NL). All procedures relating to animals in this study were approved by the Institutional Animal Care and Use Committee of the National Institute of Health (DMSc-IACUC), Thailand.

Results and discussion

GF has been widely used as a biocompatible solvent for a variety of pharmaceutical agents such as antiepileptic drugs,[21] proteins[24] and genes[22] because of its excellent biocompatibility and solvent property. With the strong interest in the development of drug-impregnated implants for treatments of brain diseases, GF's role in such applications could become more pronounced. An understanding of GF's biocompatibility in the brain upon administration is therefore necessary. In this study, we investigated the biocompatibility of GF in rat brains after intracranial injection. GF was stereotactically administered into the cortex of rat brains. Sterile solutions of GF or PBS were administered through a burr hole at 3 mm posterior to the bregma, 3 mm lateral from the sagittal suture and 3 mm deep from the cortex. Animal behavior and histopathology were evaluated during and after the procedure for any signs of toxicity corresponding to the chemicals. All animals survived throughout the course of the experiment and did not show any behavioral abnormalities caused by local toxicity of GF. Animals were sacrificed at days 3, 6, 14 and 30. The brains were then removed and fixed in formalin buffer for histopathological evaluations.

All animals injected with 50 μL of either PBS or GF survived throughout the experiment without significant weight loss as a sign of toxicity (Figure 1). The animals receiving 50 μL of GF showed a slight weight loss (2.1%) within two days of the operation. However, the animals regained their original weights, and subsequently showed a gradual weight gain. There was no weight loss in the animals injected with PBS. No abnormal behaviors or neurological deficits were observed during the course of the experiment. Increasing the injection volume to 70 μL drastically affected the weight change profiles of animals in both PBS and GF groups. Interestingly, animals treated with PBS showed a lower weight average (the highest weight loss = 3.16%) than that of animals injected with GF (the highest weight loss = 1.93%). The average weight of animals in both groups started to increase slightly after five days.

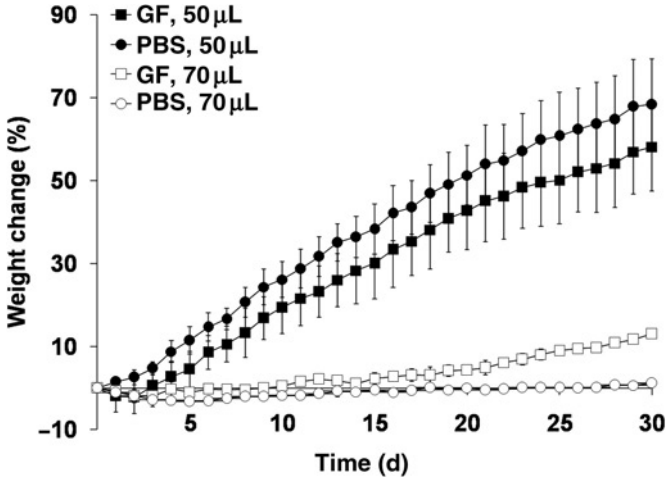

Figure 1 Animal weight change after administration of PBS and GF. GF, glycofural; PBS, phosphate-buffered saline

Hematological analysis (Table 1) demonstrated that PBS and GF treatments at the injection volume of 50 and 70 μL did not cause significant effects on the values of RBC, HGB, HCT, MCV, MCH and MCHC. These hematological results from the animals injected with PBS were not significantly different from those of animals injected with GF ($P > 0.05$). However, the WBC counts of animals injected with 50 μL of PBS at day 14 was statistically different ($P = 0.008$) from those of the GF treatment (44.2% reduction) as shown in Figure 2. The PLT counts of animals injected with 50 μL of GF at day 30 also showed a statistically significant change ($P = 0.048$) from PBS (12.2% reduction). Lower injection volume (50 μL) had much less systemic toxicity on the animals as confirmed by relatively higher WBC and PLT counts for both PBS and GF injections. Although the WBC count of GF administration (4.93×10^6 μL^{-1}) was below the normal value 14 d after the injection, the value increased and reached the normal value at day 30 (7.57×10^6 μL^{-1}). PLT counts of animals at days 14 and 30 slightly dropped to 86.15% and 83.89% of the normal value, respectively. It should be noted that the RBC counts in both PBS and GF (50 and 70 μL) injections were above the average number at days 14 and 30.

Increase in the injection volume from 50 to 70 μL led to lower hematological values regardless of the type of injected solution. For example, WBC, RBC and PLT counts at day 30 of animals treated with 70 μL of PBS compared with those receiving 50 μL were decreased by 22.73%, 7.74% and 16.83%, respectively. The same trend was observed in the GF group where the WBC, RBC and PLT counts of animals injected with 70 μL were 23.38%, 3.04% and 21.14% lower than the values of animals injected with 50 μL. The more pronounced systemic toxicities in animals injected with 70 μL of solutions prevented the animals from gaining weight at a normal rate as observed in the 50 μL group. It should be noted that the WBC counts of animals receiving 70 μL of PBS and GF were higher than normal values at days 3 and 6 (data not shown). However, lower values were found when the counts were performed again at days 14 and 30. Reduction in the WBC count can occur as a result of a number of conditions. The cause of low WBC counts in this study was probably owing to the mobilization of WBC from circulation, leading to the accumulative pool of WBC at local sites. Nevertheless, the extent of reduction as mentioned above was at an acceptable level and did not cause the toxicity.

Table 1 Hematological data of rats treated with phosphate-buffered saline or glycofurol solution. The injection volume is either 50 or 70 μL

	Chemical			Time (d)			
Hematological data	**Total volume (μL)**	**PBS**	**GF**	**14***	***P* value†**	**30***	***P* value†**
HGB (g/dL)	50	50	–	16.93 ± 0.25	0.185	17.07 ± 0.31	0.193
		25	25	15.70 ± 1.31		16.73 ± 0.21	
	70	70	–	15.50 ± 0.71	0.784	*14.65 ± 0.49*	0.204
		40	30	15.70 ± 0.57		15.30 ± 0.00	
HCT (%)	50	50	–	51.57 ± 1.01	0.369	51.23 ± 1.02	0.478
		25	25	48.57 ± 5.03		50.60 ± 0.96	
	70	70	–	47.55 ± 2.76	1.000	*44.90 ± 1.70*	0.292
		40	30	47.55 ± 0.78		46.80 ± 0.85	
MCV (fL)	50	50	–	58.43 ± 1.23	0.687	56.67 ± 0.12	0.414
		25	25	58.10 ± 0.50		56.93 ± 0.49	
	70	70	–	*55.35 ± 1.20*	0.542	*53.85 ± 0.21*	0.636
		40	30	*56.05 ± 0.64*		*54.30 ± 1.13*	
MCH (pg)	50	50	–	19.20 ± 0.53	0.421	18.90 ± 0.10	0.866
		25	25	18.83 ± 0.47		18.87 ± 0.31	
	70	70	–	18.05 ± 0.64	0.432	*17.60 ± 0.00*	0.728
		40	30	18.50 ± 0.14		*17.80 ± 0.71*	
MCHC (g/dL)	50	50	–	32.87 ± 0.21	0.274	33.33 ± 0.15	0.203
		25	25	32.37 ± 0.65		33.03 ± 0.31	
	70	70	–	32.60 ± 0.42	0.493	32.60 ± 0.14	0.831
		40	30	33.05 ± 0.64		32.70 ± 0.57	

PBS, phosphate-buffered saline; GF, glycofurol

*Numbers that are lower than the normal value are shown in italic

†*P* values were calculated by paired comparison between the PBS and GF groups. A *P* value of $\leq$0.05 was considered statistically significant

RBC counts of animals in both groups were also low after the injection (below normal values for the PBS group), but the counts increased above the average numbers after 14 days of the injection. Interestingly, the PLT counts of animals in both groups were lower than the normal value over the course of the experiment.

Brain tissues were collected and processed for histopathological evaluation on days 3 and 6 (acute phase), day 14 (subacute phase) and day 30 (chronic phase) after the injection of PBS or GF at 50 and 70 μL which is summarized in Table 2. In the acute inflammatory response (day 3), PBS and GF induced mild inflammatory reactions as evident by increased number of macrophages and polymononuclear leukocytes (PMN) (Figures 3a–d). In animals treated with PBS, macrophages and neurons were found within the disorganized cortex where no PMN were observed in these animals (Figures 3a and b). Minimal coagulative necrosis (approximately 0.2 mm in radius) at the cortical surface

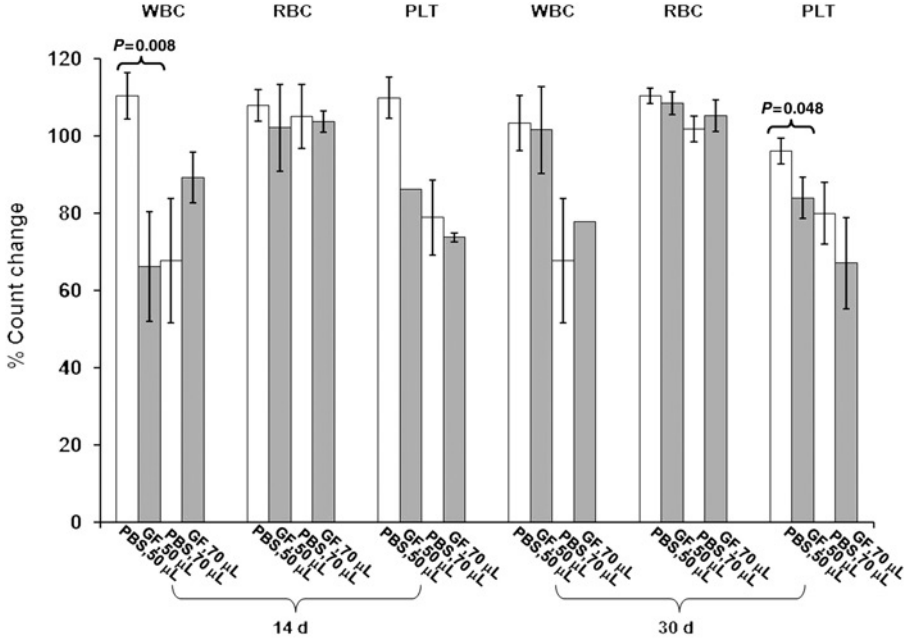

Figure 2 Percent count changes of WBC, RBC and PLT from animals treated with 50 and 70 μL of PBS or glycofurol solution at day 14 and 30. The percent count changes were calculated by comparing with the normal values of WBC, RBC and PLT in healthy rats. PBS, phosphate-buffered saline; WBC, white blood corpuscles; RBC, red blood corpuscles; PLT, platelets

Table 2 Histopathology analysis

		Histopathological analysis	
Chemical	**Days**	**Injection volume = 50 μL**	**Injection volume = 70 μL**
PBS	3	- Few macrophages - No PMN - Disorganized cortex	- Few macrophages - Minimal coagulative necrosis (0.4 mm in diameter) at cortical surface
Glycofurol	3	- Few PMN - Few macrophages - Subpial infiltration (found RBC)	- Mild gliosis (0.25 mm in radius) - Few macrophages - Few lymphocytes
PBS	6	- Few macrophages (more than day 3) - Cavity 1 mm in radius - Dying PMN - Neovasculature - Neutrophils	- Few macrophages
Glycofurol	6	- Mild gliosis - Moderate mononuclear leukocytes - Few PMN - Plasma leak cavity (surrounded by gliosis)	- Macrophages (more than PBS) - Mild gliosis - Early formation of fibrovascular granulation tissues
PBS	14	- No inflammation - Subpial infiltration of inflammatory cells	- Mild gliosis - Cavity (1 mm in diameter)
Glycofurol	14	- Normal neurons - Few macrophages - Gliosis (0.4 mm in diameter) - Well-established granulation tissue, new blood vessels and collagen deposition - RBC with hemosiderin - Subpial infiltration (higher than PBS)	- Mild gliosis - Cavity (1 mm in diameter)
PBS	30	- Normal	- Mild inflammation
Glycofurol	30	- Mild gliosis (0.3–0.6 mm in radius and 2–3 mm in depth) - Few macrophages - Normal neurons - New RBC (less than 3 days) - Hemosiderin	- Mild gliosis - Mild inflammation - Hemosiderin

PMN, polymorphonuclear leukocytes; RBC, red blood cells; PBS, phosphate-buffered saline

was observed when the injection volume was increased to 70 μL. Figures 3c and d demonstrates the neuropathology of the brains treated with GF. The subpial layer was found to be heavily infiltrated by red blood cells along with mild PMN and macrophages. Increasing the injection volume from 50 to 70 μL produced more severe damages to the brains as can be seen from the necrosis in the PBS group and early mild gliosis (0.25 mm in radius) in the GF group (Figure 3d). Interestingly, gliosis indications were not observed in the brain tissues of animals injected with 50 μL of PBS or GF. An evaluation at day 6 showed an increase in the number of macrophages and mononuclear leukocytes compared with those of day 3 in both groups. Moreover, animals treated with GF solution (50 μL) started to show signs of mild gliosis around plasma cavity from the middle to the inner layer of the cortex and glial cells were observed as indicated by the small dark-blue color in the area (Figure 3g). Gliosis is a common finding of non-specific reaction which is most likely caused by post-inflammatory reaction in this experiment. Even though the persistence of gliosis generally leads to the formation of glial scars, the animal health was not considerably affected as can be seen from their normal behavior, body weight gain pattern and hematological data. Histological examination revealed that the acute inflammatory responses resolved over 14 days after the treatment, where no abnormality indicating the inflammatory response was found in the animals treated with 50 μL PBS. At this time point, PMN, which are a characteristic of acute inflammatory disappeared and gliosis, became the predominant response. Unlike the other three groups, the animals treated with 50 μL of PBS showed an absence of gliosis and brain tissues appeared to be completely healed. Increasing the injection volume of PBS from 50 to 70 μL caused more damages to the brains as can be seen from the earlier onset of gliosis. Interestingly, tissues of the animals injected with 70 μL of the solutions showed the same size of cavities (approximately $1 \times 1\ mm^2$) around the administration area which were space-occupying lesions as a result of large injection volume. These cavities, which were absent in the animals injected with 50 μL of the solutions, are the most prominent histopathological sign affecting the animal health such as low WBC, PLT counts and unusual body weight. Hemosiderin was also observed as a result of old hemorrhage.

An evaluation at 30 days after the injection showed mild gliosis and hemosiderin in the GF group. The tissues of animals injected with 50 μL of PBS revealed a normal histopathological condition where the mild inflammation was observed at the injection volume of 70 μL. Animal weight gradually increased after day 14 corresponding to the absence of cavity in both groups. These data provided direct evidence of the cavity formation on the animal health. Although a sign of mild disorganized cortex is noticeable in both groups, no clinical significance was observed during the daily housing and there was no evidence of neuronal cell death or tissue necrosis (data not shown).

At the injection volume of 70 μL for both groups, all rats experienced dose-limiting toxicity (DLT) as can be observed by abnormal body weight, low WBC and PLT counts and cavity formation. Animal health, hematological and histopathological data confirmed the maximal tolerated dose of GF solution up to 50 μL (25 μL of GF and 25 μL of PBS) in rat brains. The injection volume and the amount of GF used in this experiment were relatively high compared with the average brain (~2 g) and body weight (200–250 g) of rats. According to the Eliaz and Szoka[22] formulation, 25 μL of GF could be used to form a poly(lactide-*co*-glycolide) (PLGA) implant at the polymer weight of 6.8 mg. The amount of polymer can be increased further using more

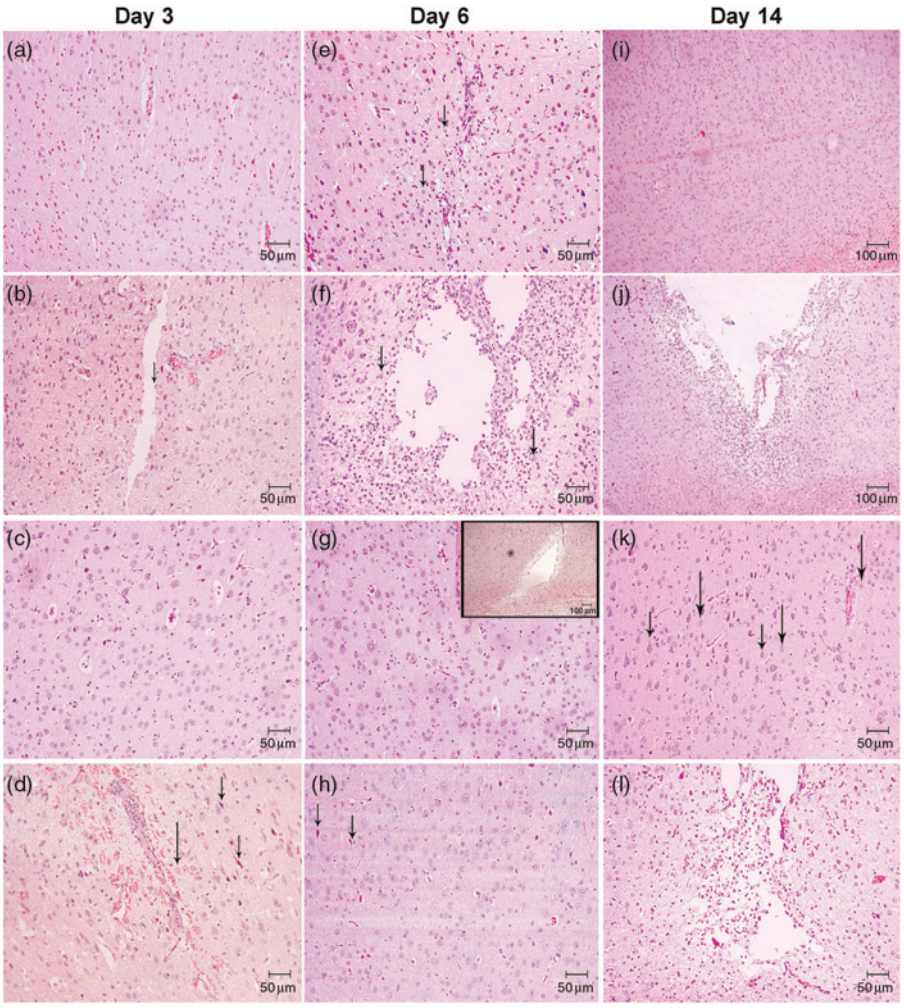

Figure 3 H&E histological sections of tissues. **(a)** Day 3, PBS, 20×, 50 µL; **(b)** Day 3, PBS, 20×, 70 µL: the arrows indicate the macrophage within disorganized cortex; **(c)** Day 3, GF, 20×, 50 µL; **(d)** Day 3, GF, 20×, 70 µL: the small arrows indicate mild gliosis where a big arrow shows few macrophages; **(e)** Day 6, PBS, 20×, 50 µL: the accumulation of macrophages is shown by arrows. The newly formed blood vessels are found as can be observed by the pink lines throughout the photo; **(f)** Day 6, PBS, 20×, 70 µL: macrophages are shown by arrows; **(g)**: Day 6, GF, 20×, 50 µL: the inset shows a lower magnification image (10×) indicating plasma cavity; **(h)** Day 6, GF, 20×, 70 µL: a mild gliosis was shown by arrows; **(i)** Day 14, PBS, 10×, 50 µL; **(j)** Day 14, PBS, 10×, 70 µL; **(k)** Day 14, GF, 20×, 50 µL: gliosis (shown by small arrows) and macrophages (shown by big arrows); **(l)** Day 14, GF, 20×, 70 µL. PBS, phosphate-buffered saline; H&E, hematoxylin and eosin stain; GF, glycofural (A color version of this figure is available in the online journal)

concentrated polymer solutions, which is a window of opportunity for the use of GF in sustained and local delivery of a variety of therapeutic agents to the CNS.

Conclusion

This study has demonstrated the brain biocompatibility of GF upon intracranial injection. GF was successfully administered into the rat brains by stereotactic surgery. All animals survived the course of the experiment and did not show any behavioral abnormalities. It has been shown that administration of GF solution at 50 µL (GF 25 µL + PBS 25 µL) in the rat brains was feasible without causing any systemic or neurological toxicities. The inflammatory responses to GF did not lead to severe tissue damage as shown by histopathological assessments compared with the control group (PBS). The results from this study suggest that GF could be used for interstitial delivery of therapeutic agents to the brains for the treatment of neurological diseases.

Author contributions: All authors participated in the interpretation and analysis of the data, conducted the experiments and reviewed the manuscript. AB and NN wrote the manuscript.

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REFERENCES

1 Huynh GH, Deen DF, Szoka JFC. Barriers to carrier mediated drug and gene delivery to brain tumors. *J Control Release* 2006;**110**:236–59

2 Gabathuler R. Blood–brain barrier transport of drugs for the treatment of brain diseases. *CNS Neurol Disord – Drug Targets* 2009;**8**:195–204
3 Abbott NJ. Inflammatory mediators and modulation of blood–brain barrier permeability. *Cell Mol Neurobiol* 2000;**20**:131–47
4 Bart J, Groen HJM, Hendrikse NH, van der Graaf WTA, Vaalburg W, de Vries EGE. The blood–brain barrier and oncology: new insights into function and modulation. *Cancer Treat Rev* 2000;**26**:449–62
5 Wu D, Pardridge WM. Blood–brain barrier transport of reduced folic acid. *Pharm Res* 1999;**16**:415–9
6 Steiniger SC, Kreuter J, Khalansky AS, Skidan IN, Bobruskin AI, Smirnova ZS, *et al.* Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles. *Int J Cancer* 2004;**109**:759–67
7 Laske DW, Youle RJ, Oldfield EH. Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors. *Nat Med* 1997;**3**:1362–8
8 Taira T, Steven L, Nicholas D. S, Adrian M. O. Intrathecal administration of GABA agonists in the vegetative state. *Progr Brain Res* 2009;**177**:317–28
9 Mottolese C, Stan H, Hermier M, Berlier P, Convert J, Frappaz D, *et al.* Intracystic chemotherapy with bleomycin in the treatment of craniopharyngiomas. *Childs Nerv Syst* 2001;**17**:724–30
10 Takahashi H, Yamaguchi F, Teramoto A. Long-term outcome and reconsideration of intracystic chemotherapy with bleomycin for craniopharyngioma in children. *Childs Nerv Syst* 2005;**21**:701–4
11 Halliday AJ, Cook MJ. Polymer-based drug delivery devices for neurological disorders. *CNS Neurol Disord Drug Targets* 2009;**8**(3):205–21
12 Dang W, Daviau T, Brem H. Morphological characterization of polyanhydride biodegradable implant gliadela during *in vitro* and *in vivo* erosion using scanning electron microscopy. *Pharm Res* 1996;**13**:683–91
13 Dang W, Daviau T, Ying P, Zhao Y, Nowotnik D, Clow CS, *et al.* Effects of GLIADEL@ wafer initial molecular weight on the erosion of wafer and release of BCNU. *J Control Release* 1996;**42**:83–92
14 Mary PW, Janet AT, Henry B, Robert L. *In vivo* versus *in vitro* degradation of controlled release polymers for intracranial surgical therapy. *J Biomed Mater Res* 1994;**28**:387–95
15 Madrid Y, Langer LF, Brem H, Langer R. New directions in the delivery of drugs and other substances to the central nervous system. *Adv Pharmacol* 1991;**22**:299–324
16 Brem H, Langer R. Polymer-based drug delivery to the brain. *Sci Med* 1996;**3**:52–61
17 Attenello F, Mukherjee D, Datoo G, McGirt M, Bohan E, Weingart J, *et al.* Use of Gliadel (BCNU) Wafer in the surgical treatment of malignant glioma: a 10-year institutional experience. *Ann Surg Oncol* 2008;**15**:2887–93.
18 Gallego JM, Barcia JA, Barcia-Mariño C. Fatal outcome related to carmustine implants in glioblastoma multiforme. *Acta Neurochir* 2007;**149**:261–5
19 Kim S, Jung S-H, Cho C-W. Physicochemical studies of a newly synthesized molecule, 6-methyl-3-phenethyl-3,4-dihydro-1*H*-quinazoline-2-thione (JSH18) for topical formulations. *Arch Pharmacol Res* 2008;**31**:1363–8
20 Hjortkjaer RK, Bechgaard E, Gizurarson S, Suzdak C, McDonald P, Greenough RJ. Single- and repeated-dose local toxicity in the nasal cavity of rabbits after intranasal administration of different glycols for formulations containing benzodiazepines. *J Pharm Pharmacol* 1999;**51**:377–83
21 Ivaturi VD, Riss JR, Kriel RL, Siegel RA, Cloyd JC. Bioavailability and tolerability of intranasal diazepam in healthy adult volunteers. *Epilepsy Res* 2009;**84**(2–3):120–6
22 Eliaz R, Szoka F. Robust and prolonged gene expression from injectable polymeric implants. *Gene Ther* 2002;**9**:1230–7
23 Bagger MA, Nielsen HW, Bechgaard E. Nasal bioavailability of peptide T in rabbits: absorption enhancement by sodium glycocholate and glycofurol. *Eur J Pharm Sci* 2001;**14**:69–74
24 Rom EE, Joseph K. Characterization of a polymeric PLGA-injectable implant delivery system for the controlled release of proteins. *J Biomed Mater Res Part A* 2000;**50**:388–96
25 Ola D, Pamela S, Evan DK. Bioavailabilities of rectal and oral methadone in healthy subjects. *Br J Clin Pharmacol* 2004;**58**:156–62
26 Dudeck O, Jordan O, Hoffmann KT, Okuducu AF, Tesmer K, Kreuzer-Nagy T, *et al.* Organic solvents as vehicles for precipitating liquid embolics: a comparative angiotoxicity study with superselective injections of swine rete mirabile. *AJNR Am J Neuroradiol* 2006;**27**:1900–6
27 Wade A, Weller PJ, eds. *Handbook of Pharmaceutical Excipients*. 2nd edn. Washington, DC: American Pharmaceutical Association, 1994
28 Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. 5th edn. Burlington: Elsevier Academic Press, 2005

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