Intranasal Administration of Temozolomide Delayed the Development of Brain Tumors Initiated by Human Glioma Stem-Like Cell in Nude Mice

Pineda JR1,6*, Jeitany M1, Andrieux A2, Junier MP3–5#, Chneiweiss H3–5# and François D Boussin1*†

1CEA DSM IRM CSCR, INSERM, Laboratory of Radiopathology, UMR967, F-92265 Fontenay-aux-Roses, France
2CEA, IRITV-GPC, INSERM, U036-GIN I Health and Beauty Health in La Tronche, BP170, 38042 Grenoble, Cedex 9, France
3CNRS UMR8246 Neuroscience Paris Seine-IBPS, Team Glial Plasticity, 7 Quai Saint-Bernard, 75005 Paris, France
4INSERM U1130, Neuroscience Paris Seine-IBPS, Team Glial Plasticity, 7 Quai Saint-Bernard, 75005 Paris, France
5University Pierre and Marie Curie UMR18, Neuroscience Paris Seine-IBPS, Team Glial Plasticity, 7 Quai Saint-Bernard, 75005 Paris, France
6Achucarro Basque Center for Neuroscience Fundazioa, Sede Building 3rd Floor, Barrio Sarriera s/n. 48940 Leioa, Vizcaya, Spain

Contributed equally to this work, #Contributed equally to this work.

Abstract

Objective: Intranasal route is an emerging option for brain cancer treatment to infuse directly telomerase inhibitors and/or viruses into the brain. Paradoxically, the standard chemotherapeutic Temozolomide (TMZ) widely used to treat glioma tumors is orally given. Here, we tested for the first time the intranasal administration of TMZ in nude mice xenograft models carrying human glioblastoma tumors generated from the human glioma stem-like cells TG16, TG1N and TG20.

Methods: The resistance to TMZ of the different glioma stem-like cells was determined by WST-1 cell proliferation and cell viability assay. Tumour cells were stereotaxically injected intrastriatally and one month after graft, mice were anaesthetized using Isoflurane and TMZ was infused into the nostrils three times a week during two weeks with a nano-injector using Hamilton syringe coupled to a cannula. Buried food pellet test was carried out to check the sense of smell. Animals were weighted and surveilled once a week and separated into two cohorts, one for histopathological analysis and the other for Kaplan-Meier survival analysis.

Results: Intranasal administration of TMZ did not induce major adverse effects on the sense of smell of the animals. TMZ administered intranasally delayed tumour growth and significantly extended the lifespan of mice engrafted with TG16 and TG1N cells, which are sensitive in vitro to TMZ. By contrast, TMZ at the dose tested had no effects on the tumors generated by TG20 cells that are resistant to TMZ in vitro.

Conclusion: Our results demonstrate that the intranasal route should be further considered as an option for TMZ delivery into the brain to treat intrastriatal brain tumours. Moreover, it consists of an easy, fast, and cost-less method to gain direct access to the brain.

Keywords: Nasal absorption; Cancer stem-like cells; Nasal drug delivery; Alkylators; Stereotaxia; Cancer chemotherapy; Drug effects

Intranasal route became recently a center of attention as an alternative way to treat brain solid tumours [5]. The intranasal route has been previously used to deliver hormones, Perillyl alcohol, telomerase inhibitors, neurupetides and viruses into the brain [6–11]. Recent works described the feasibility of the route to direct lipid-based nanoparticles of TMZ or treat glioma in rats [12,13]. The molecules intranasally infused are driven through the trigeminal and olfactory nerves anatomic connection between the nose and the brain. Intranasal delivery could thus increase the dose effectively delivered in the brain and reduce the adverse effects associated with systemic delivery.

In the present study, we have assessed for the first time, the effects of intranasal administration of TMZ on slow-progressing tumors generated in immunodeficient mice by intracerebral grafts of three human glioma stem cell lines (GSC), two of which being sensitive to TMZ in vitro and one being resistant [14].

Material and Methods

Intracerebral grafts of glioma stem cells

The human glioma cell lines TG16, TG1N and TG20 have been previously described [15,16]. GSC were intrastriatally injected in nude mice with the following schedule:

1. Heterotopic xenografts: Tumors Initiated by Human Glioma Stem-Like Cell in Nude Mice. J Cancer Sci Ther 9: 374-378. doi: 10.4172/1948-5956.1000445

Copyright: © 2017 Pineda JR, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Jose R Pineda, Achucarro Basque Center for Neuroscience Fundazioa, Sede Building 3rd Floor, Barrio Sarriera s/n. 48940 Leioa, Vizcaya, Spain, Tel: 34946018139; E-mail: jr.pineda@achucarro.org

Received March 17, 2017; Accepted March 29, 2017; Published March 31, 2017

Citation: Pineda JR, Jeitany M, Andrieux A, Junier MP, Chneiweiss H, et al. (2017) Intranasal Administration of Temozolomide Delayed the Development of Brain Tumors Initiated by Human Glioma Stem-Like Cell in Nude Mice. J Cancer Sci Ther 9: 374-378. doi: 10.4172/1948-5956.1000445

*Corresponding authors: François D Boussin, CEA, DSM, IRICM, CSCR, Laboratory of Radiopathology, BP n°6 F-92265 Fontenay-aux-Roses, Cedex, France, Tel: 33146549791; Fax: 33146549180; E-mail: boussin@cea.fr
Intranasal Administration and Kaplan-Meier survival analysis

One month after graft, mice were anesthetized using Isoflurane. Ten µL of TMZ (100 mM, Sigma-Aldrich, France, to ensure a delivery of 7 mg/Kg) or 10 µL of vehicle (DMSO, Sigma-Aldrich, France) were infused into the nostrils at the rate of 12 µL/minute with a nanoinjector (KOPFT, KD Scientific, Holliston, MA) using a 33-gauge stainless Hamilton syringe at the following coordinates from bregma: anteroposterior= +0.5 mm, lateral ± 1.5 mm, dorsoventral -3 mm. Paracetamol 1.64 mg/mL (Doliprane, Sanofi, France) was administered in drinking water for 5 days following the graft to ensure post-operative analgesia.

Histopathological analysis

Brains were post-fixed for 2 h in 4% PFA, cryoprotected in increasing concentration of sucrose (10% to 30% in PBS). Serial coronal cryostat sections of 20 µm (200 µm apart) were made and mounted on polysine slides (Thermo Scientific, Braunschweig, Germany) for Haematoxylin-Eosin staining. Tumour volumes were calculated by multiplying the sum of all sectional areas (square millimeters) by the distance between successive sections as described previously [19]. Images acquisition were obtained using a UPlanFl 4X dry objective of 0.13 numerical aperture using a motorized microscope Olympus BX51 equipped with a Sony ExwaveHAD 3CCD DSP camera and the software Histolab 7.6.1 (Microvision Instruments, Evry France).

Buried food pellet test

Olfactory functions of mice were assessed as previously described [20]. Animals were food starved for 12 hours prior to being transferred into a clean cage containing an approximately 4 g pellet of rodent food buried under the mouse bedding. The time (in seconds) taken by mice to grab the food was then measured. The trial was repeated 3 times, the food being each time hidden into different positions.

WST-1 assay

TG16, TG1N or TG20 cells were plated on laminin-coated 96-well plates at 7 000 cells/well. Temozolomide was added the next day at different concentrations and the WST-1 assay (11644807001, Roche) was performed 72 hours later according to the manufacturer’s instructions.

Statistical Analysis

Log-rank and non-parametric tests, Mann-Whitney, Kruskal-Wallis, Student’s T and Scheffe’s test for pair comparisons were conducted using StatView5 software (SAS Institute Inc., Cary, NC). Statistical significance was set at p<0.05.

Results

The sensitivity of the GSC lines to TMZ was first determined in vitro using the WST-1 assay. As shown Figure 1B, TG16 and TG1N cells were sensitive to high concentration of this alkylating agent, whereas TG20 cells were resistant (Figure 1B).

Mice engrafted with TG16 were sacrificed 142 days post graft, revealing a significant reduction of tumour volume in TMZ-treated mice versus controls consistent with the sensitivity of these cells to TMZ observed in vitro (p=0.05, n=3 per groups, Figure 1C). By contrast, no effect of TMZ on TG20 tumor growth was observed in animals sacrificed at 171 days post graft, confirming the resistance to TMZ of TG20 cells (Figure 1D).

Kaplan-Meier survival analysis of animals grafted with TG16 cells determined a median of survival of 184 days for controls. TMZ significantly increased the survival of the animals since the median of survival in the TMZ-treated group was more than 625 days for TMZ-treated mice. Indeed sixty six percent of TMZ-treated mice were still alive at 625 days corresponding to the end of the experiment (Figure 1D, p=0.0002 Log-rank test). Similar results were obtained in mice engrafted with TG1N cells: the median survival for the DMSO control group was 266 days post graft and more than 357 days for TMZ-treated mice since 85% of TMZ-treated animals were still surviving at that time.

Since the DMSO was used to dissolve TMZ, we have assessed the damage its administration may induce to the olfactive mucosa. For this purpose, we used the buried food pellet test which relies on the sense of smell to locate food. Ungrafted mice that were treated with either DMSO or saline solution were tested 24 hours after the last treatment. The results showed that mice treated with DMSO needed more time to locate buried food than mice treated with saline solution (372 s vs. 157 s respectively, p= 0.0078, Mann-Whitney, test Figure 2B). However, 7 and 21 days after the last treatment, no difference remained between the two groups (Figures 2C and 2D), indicating that intranasal infusions of DMSO had only temporary short-term side effects on the sense of smell of the animals.

Finally, we assessed olfactory functions of mice engrafted with TG16 that survived at 195 days post graft. We did not find any statistical differences between control and TMZ-treated mice for the time spent to locate buried food (177 ± 139 s vs.157 ± 133 s respectively, p=0.6905, Figure 2E). These data indicate therefore that intranasal treatments with TMZ did not induce a long-term impairment of the olfactory function.

Discussion

We report here for the first time, that intranasal administration of TMZ delayed tumour growth and significantly extended the lifespan of mice engrafted with two human GSC lines. Importantly, we have also shown that intranasal administration of TMZ did not induce major adverse effects on the sense of smell of the animals, confirming previous reports of minimal side effects related to intranasal administration of other compounds like Perillyl alcohol [14].
Figure 1: A) Experimental design for the Kaplan-Meier assays. Intranasal treatments with TMZ were delivered 30 days after cell injection. Mice were sacrificed at 142 or 171 days for histological staining. B) In vitro WST-1 proliferation assay for TG20, TG1N and TG16 cells, 72 hours after treatment with increasing concentrations of Temozolomide. Values were calculated relative to control cells treated with DMSO. Error bars represent the SEM from 6 replicates (*p<0.05, **p<0.01, ***p<0.001, Kruskal-Wallis test). C) The site of injection of cells is represented on the left. On the right, the graphs represent the mean percentages ± SD of TG16 or TG20 tumor volumes in DMSO or TMZ-treated mice. In the middle, representative Hematoxilin-eosin stained brain sections are shown for each condition (scale bars=2 mm) (*p<0.05, Anova’s test). D-E) Kaplan Meier survival plots showing overall survival of mice grafted with TG16 or TG1N cells and treated with either DMSO or TMZ. Statistical analysis was performed using log rank Mantel-Cox test.
GBM are associated with high rates of relapse, particularly due the emergence of glioma cells resistant to TMZ. We showed that intranasal administration of TMZ was efficient only on tumors generated by TG1N and TG16 cells, two GSC lines that have been isolated from samples of untreated GBM tumors. By contrast, intranasal administration of TMZ was efficient only on tumors generated by TG1N cells, which have been isolated from a second-relapse GBM in a patient that received the Stupp protocol associating surgery, radiation and TMZ [15,16]. These results are in accordance with in vitro data showing that TG20 cells were resistant to high concentrations of TMZ in vitro unlike TG1N and TG16. Therefore our study indicates that the intranasal route does not allow TMZ to overcome the resistance already acquired by tumor cells at the dose tested. Future studies should thus determine if increasing the dose delivered by this alternative route to reach the brain is able to reduce or delay the development of resistance to TMZ by glioma cells in comparison to systemic routes.

More generally, this work not only corroborates the feasibility of the use of intranasal route for the administration of anti-cancer drugs in preclinical models of glioma, but suggests that the intranasal route could be considered as an alternative method to deliver easily, fast and direct to brain, the standard anticancer drug TMZ in patients suffering of incurable glioblastoma.

Acknowledgement

We are indebted to C. Joubert, V. Neuville and all of the staff of the animal facilities. O. Etienne and A. Gouret for help in mice weight and to T. Kortulewski and G. Livera for imaging facility. This study was supported by grants from Inca (Tetratips, PLBIO10-030), Fondation de France (N° Engt 2013-00042632), J.R.P. and G. Livera for help in imaging facility. O. Etienne and A. Gouret for help in mice weight and to T. Kortulewski and G. Livera for imaging facility.

Grants/Financial supporters

Inca (Tetratips, PLBIO10-030), Fondation de France (N° Engt 2013-00042632), Ramon y Cajal program (RYC-2013-13450), Ligue Nationale contre le Cancer and IRTELIS-CEA.

Disclosures

None.

References

1. Holland EC (2001) Gliomagenesis: genetic alterations and mouse models. Nat Rev Genet 2: 120-129.
2. Stupp R, Dietrich PY, Ostermann KS, Pica A, Maillard I (2002) Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide. J Clin Oncol 20: 1375-1382.
3. Zhang J, Stevens MF, Bradshaw TD (2012) Temozolomide: Mechanisms of action, repair and resistance, Curr Mol Pharmacol 5: 102-114.
4. Jackson M, Hassiotou F, Nowak A (2015) Glioblastoma stem-like cells: At the root of tumor recurrence and a therapeutic target. Cancers. 2: 177-195.
5. Peterson A, Bansal A, Hofman F, Chen TC, Zada G (2014) A systematic review of inhaled intranasal therapy for central nervous system neoplasms: An emerging therapeutic option. J Neurooncol 116: 437-446.
6. Shahiwala A, Misra A (2004) Nasal delivery of levonorgestrel for contraception: An experimental study in rats. Fertil Steril 81: 893-898.
7. Thorne RG, Pronk GJ, Padmanabhan V, Frey WH (2004) Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience 127: 481-496.
8. da Fonseca CO, Landeiro JA, Clark SS, Quirico-Santos T, da Costa Carvalho Mda G (2006) Recent advances in the molecular genetics of malignant gliomas disclose targets for antitumor agent perillyl alcohol, Surg Neurol 1: 8.
9. Hashizume R, Ozawa T, Gryaznov SM, Boltan AW, Lamborn KR (2008) New therapeutic approach for brain tumors: Intranasal delivery of telomerase inhibitor GRN163. Neuro Oncol 10: 112-120.
10. Kanazawa T, Taki H, Tanaka K, Takashima Y, Okada H (2011) Cell-penetrating peptide-modified block copolymer micelles promote direct brain delivery via intranasal administration. Pharm Res 28: 2130-2139.
11. Kiprianova I, Thomas N, Ayache A, Fischer M, Leuchs B (2011) Regression of glioma in rat models by intranasal application of parvovirus h-1. Clin Cancer Res 17: 5333-5342.
12. Li Y, Gao Y, Liu G, Zhou X, Wang Y, Wang Y, Ma L (2014) Intranasal administration of temozolomide for brain-targeting delivery: therapeutic effect on glioma in rats. Nan Fang Yi Ke Da Xue Xue Bao. 34(5):631-5.
13. Khan A, Imam SS, Anjil M, Adad A, Sultana Y, Ali A, Khan K. (2016) Brain Targeting of Temozolomide via the Intranasal Route Using Lipid-Based Nanoparticles: Brain Pharmacokinetic and Scintigraphic Analyses. Mol Pharm. 13:3773-3782.
14. Cho HY, Wang W, Shaver N, Torres S, Tseng J (2012) Perillyl alcohol for the treatment of temozolomide-resistant gliomas. Mol Cancer Ther 11: 2462-2472.
15. Patru C, Romao L, Varlet P, Coulombel L, Lapointe E (2010) CD133+, CD15- SSEA-1, CD34 or side populations do not resume tumor-initiating properties of long-term cultured cancer stem cells from human malignant glio-neural tumors. BMC Cancer 10: 66.
16. Silvestre DC, Pineda JR, Hoffschir F, Studler JM, Mouton MA (2011) Alternative lengthening of telomeres in human glioma stem cells. Stem Cells 29: 440-451.

17. Pineda JR, Dayna M, Chicheportiche A, Cabrian-Silla A, Sii Felice K (2013) Vascular-derived TGF-beta increases in the stem cell niche and perturbs neurogenesis during aging and following irradiation in the adult mouse brain. EMBO Mol Med 5: 548-562.

18. Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G (2010) Guidelines for the welfare and use of animals in cancer research. Br J Cancer 102: 1555-1577.

19. Canals JM, Pineda JR, Torres-Peraza JF, Bosch M, Martin-Ibanez R (2004) Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. J Neurosci 24: 7727-7739.

20. Duncan-Lewis CA, Lukman RL, Banks RK (2011) Effects of zinc gluconate and 2 other divalent cationic compounds on olfactory function in mice. Comp Med 61: 361-365.