Title page

Radio-neuroprotective effect of L-alpha-glycerylphosphorylcholine (GPC) in an experimental rat model

Imola Plangár[1], Emília Rita Szabó[2], Tünde Tőkés[3], Imola Mán[2], Kitti Brinyiczki[4], Gábor Fekete[2], István Németh[5], Miklós Ghyczy[3], Mihály Boros[3], Katalin Hideghéty[2,*]

Author's Affiliation:

[1] Department of Neurology, Albert Szent-Györgyi Clinical Center, University of Szeged, Hungary

[2] Department of Oncotherapy, Albert Szent-Györgyi Clinical Center, University of Szeged, Hungary

[3] Institute of Surgical Research University of Szeged, Hungary

[4] Institute of Pathology, University of Szeged, Hungary

[5] Department of Dermatology and Allergology, University of Szeged, Hungary

Running title: Radio-neuroprotective effect of GPC in rats

[*]**Correspondence:** Katalin Hideghéty, MD, PhD, Department of Oncotherapy, Albert Szent-Györgyi Clinical Center, University of Szeged, Korányi fasor 12., H-6720 Szeged, Hungary; Phone: +36 62 545 405; Fax: +36 62 545 922; E-mail: katalin.hideghety@gmail.com

Grant support: This study was supported by the Országos Tudományos Kutatási Alapprogram (OTKA; Hungarian Science Research Fund) through OTKA 75833 and OTKA K104656, by the Társadalmi Megújulás Operatív Program (TÁMOP; Social Renewal Operational Program) through TÁMOP-4.2.2/B-10/1-2010-0012 and TÁMOP-4.2.1/B-09/1/KONV-2010-0005, and by the Társadalmi Megújulás Operatív Program Konvergencia Régió (TÁMOP-KONV; Social Renewal Operational Program - Regional Convergence) through TÁMOP-4.2.2A-11/1-KONV-2012-0035. The project "TÁMOP-4.2.1/B-09/1/KONV-2010-0005 – Creating the Center of Excellence at the University of Szeged" is supported by the European Union and co-financed by the European Regional Development Fund.

Disclosure of Potential Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Word count: 3347

Total number of Figures: 4

Statement of translational relevance

Radiation-induced brain injury is a sincere complication which is frequently associated with appreciable functional morbidity and declined quality of life in patients treated with brain radiotherapy. In case of primary central nervous system tumors as well as the palliation of metastatic tumors, the brain is one of the most generally irradiated sites for curing. Radiation dose-escalations are the most expansively applied dose schemes in recent years. With these disturbances, severe damage to the parenchyma of normal brain has become one of the inevitable consequences. Therefore, the development of alternative therapeutic tools is required. We previously developed an effective radiation model that results in dose-dependent focal brain damage in rats. The present study has provided experimental evidence of the changes in cognitive function and histological deterioration after the irradiation of one hemispherical hippocampus and clearly illustrates the protective effects of L-alpha-glycerylphosphorylcholine (GPC) at both functional and morphological level in this model.

Abstract

Purpose: Ionizing radiation plays a major role in the treatment of brain tumors, but side-effects may restrict the efficiency of therapy. In the present study, our goals were to establish whether the administration of L-alpha-glycerylphosphorylcholine (GPC) can moderate or prevent any of the irradiation-induced functional and morphological changes in a rodent model of hippocampus irradiation.

Material and methods: Anesthetized Sprague-Dawley rats were subjected to 40-Gy irradiation of one hemisphere of the brain, without or with GPC treatment (50 mg/kg bw by gavage), the GPC treatment continuing during the 4-month observation period. The effects of this partial rat brain irradiation on the spatial orientation and learning ability of the rats were assessed with the repeated Morris water maze (MWM) test. Histopathologic (HP) evaluation based on hematoxylin-eosin and Luxol blue staining was performed 4 months after irradiation.

Results: The 40-Gy irradiation resulted in a moderate neurological deficit at the levels of both cognitive function and morphology 4 months after the intervention. The MWM test proved to be a highly sensitive tool for the detection of neurofunctional impairment. The site navigation of the rats was impaired by the irradiation, but the GPC treatment markedly decreased the cognitive deterioration. HP examination revealed a pronounced protective effect of GPC as concerns the macrophage density, reactive gliosis, calcification and extent of demyelination.

Conclusion: Four months after the delivery of a single dose of 40-Gy, significant focal brain irradiation-induced functional and morphological changes were observed in the central nervous system. However, GPC supplementation provided significant protection against the cognitive disturbances and cellular damage.

Keywords: partial brain irradiation, radioprotection, cognitive function, morphological changes, L-alpha-glycerylphosphorylcholine

Introduction

Radiation therapy plays an important role in the complex management of primary and secondary brain tumors. However, the side-effects of radiation therapy, among them acute and chronic brain injury, are a major concern, limiting its clinical application and preventing the effective treatment of brain tumors (1-3). The damage induced by radiation is generally observed from 3 months to more than a year after the completion of the provision of chemoradiation, often in the form of histopathological changes including endothelial apoptosis, increased vascular permeability, edema, gliosis and demyelination, together with white matter and cell necrosis (4-10). The extent, location and severity of such damage depend on the radiation dose administered (11). The late effects, occurring after recovery from the early-onset syndromes from 3 months to 10 years after irradiation (70% of the cases occur in the first 2 years) are irreversible and most devastating (12).

An important brain structure, the hippocampus, is involved in a number of processes that are essential for the creation of new memories. Injuries to the hippocampus have been demonstrated to impair learning and memory in a variety of behavioral paradigms (13-14), and it has been suggested that ionizing radiation may induce damage to the hippocampus which can result in hippocampal-related behavioral alterations (15). Clinical studies have revealed that radiation-induced damage to the hippocampus makes a significant contribution to the cognitive deficit (16-18). It has been postulated that the major cause of the cognitive dysfunction after irradiation is the impairment of neurogenesis in the dentate subgranular zone of the hippocampus (19).

Considerable effort has been devoted to attaining a reduction in the risk of dose-dependent minor-to-severe neurocognitive deficits and focal necrosis, with its consequences of progressive deterioration and death. The emerging external beam techniques, such as stereotactic radiosurgery, conformal and intensity-modulated teletherapy, intensity-modulated

brachytherapy, volumetric arc therapy and proton therapy permit an increase in the physical selectivity of the dose delivery. However, the involvement of the surrounding normal brain tissue in the clinical target volume can not be avoided, because of the potential tumor cell content.

A different approach, in which the radiation tolerance of the healthy brain cells is enhanced, is therefore of great importance. In order to be able to investigate the potential protective effects of different drugs, a reproducible, reliable experimental model of partial brain irradiation in a small animal, and detection of the resultant functional and morphological changes at a reasonable time point is required.

An active neuroprotective agent could theoretically increase the therapeutic index, providing the prospective of an improved outcome of radiation or combined chemoradiation. It might well lead to ameliorated local control and consequently to prolonged survival for patients with high-grade astrocytoma or glioblastoma multiforme.

In an earlier study, the peripheral plasma levels of key pro-inflammatory mediators were successfully modulated when irradiation was preceded by the administration of L-alpha-glycerylphosphorylcholine (GPC), a water-soluble, deacylated phosphatidylcholine derivative (20). It has been demonstrated that GPC, which is the precursor of the common neurotransmitter acetylcholine (ACh), can improve both the cognitive functions and the learning and memory capacity via Ach-ergic pathways (21-22). It has therefore emerged that GPC, which is effective against loss of the membrane function in central nervous system (CNS) injuries (23-24), may be a potential protective agent in this experimental system. The primary aim of the present study was to set up a small animal model of late changes evoked in the CNS by partial brain irradiation. An additional aim was to define an appropriate experimental system for research on a selective radio-neuroprotective agent.

Materials and methods

Animals

A total of 24 male Sprague-Dawley (SPRD) rats (purchased from the Animal House of the University of Szeged) were used in these experiments. They were housed together, 3-4 animals per cage, under standard laboratory conditions, with *ad libitum* access to tap water and food. They were kept under natural light in 12-h cycles. The experimental protocol was approved by the Ethical Committee for the Protection of Animals in Scientific Research at the University of Szeged and followed the National Institutes of Health (Bethesda, MD, USA) guidelines on the care and use of laboratory animals. The animals were allocated randomly to the study groups.

Irradiation

After separate experiments to determine the dose-effect relationship (dose-effect curves), of single fraction radiation doses to the partial rat brain, and the resultant morphological and biological changes (25), the 40-Gy dose level (which was found to be appropriate for the detection of brain injury in a reasonable time period) was selected for the investigation of neuroprotection. Male SPRD rats (weighing from 180 to 220 g) were anesthetized (4% chloral hydrate (Fluka Analytical, Buchs, Switzerland), 1 ml/100 g, intraperitoneally) and placed in the prone position, using laser alignment. After earpin fixation, they were imaged in the Emotion 6 CT scanner (Siemens AG, Erlangen, Germany) in order to plan the radiation geometry. Treatment planning and dosimetry of the special electron insert had been performed. A 6-MeV lateral electron beam at a 100-cm source-to-skin distance was chosen because it has a sharp dose fall-off with depth, limiting the radiation dose delivery to the defined volume of the hippocampus, including the corpus callosum of the ipsilateral hemisphere, while sparing the skin, eyes, ears, cerebellum, frontal lobe and contralateral half of the brain. The planned dose was delivered as a single fraction, using a linear accelerator

(Primus IMRT, Siemens, Germany) at a dose rate of 300-900 monitor units (MU)/min, with six 10-mm diameter apertures in a 20-mm-thick Newton metal insert placed into the 15x15-cm electron applicator for the following groups of animals: a sham-irradiated control (CO) group (n=6), an only GPC-treated (GPC) group (n=6), an irradiated (RT) group (n=6), and a both GPC-treated and irradiated (GPC+RT) group (n=6). Positioning to the beam was achieved with the laser optical system installed in the treatment room and the light field. Irradiation was carried out on 6 animals at the same time (described in detail by Hideghéty et al., 2013) at a dose rate of 300/900 MU/min under TV-chain control. The 12,000 MU (40-Gy) applied for the irradiation was derived from the previous small field dosimetry. The radiation geometry was verified prior to the irradiation, and documented by control imaging on film after it. The CO animals were anesthetized and treated similarly, but received sham-irradiation. Following treatment, the animals were transferred to their home cages and kept under the standard conditions, with weekly weight measurements, descriptive behavior observations and skin checks.

Treatment

Beginning from the day of irradiation, the SPRD rats received GPC (Lipoid GmbH, Ludwigshafen, Germany; 50 mg/kg bw, dissolved in 0.5 ml sterile saline, administered by gavage) or the vehicle at the same time every second day (on Mondays, Wednesdays and Fridays) for 4 months.

Morris water maze test

The apparatus was a large circular water tank (185 cm in diameter and 60 cm in depth). The tank was filled to a height of 40 cm with water at room temperature and was made opaque by the addition of a non-toxic white dye. The pool was divided into four equal quadrants, and a removable transparent Plexiglas platform (10 cm in diameter) that could not be seen by the swimming rats was hidden at the center of one of the quadrants, with its top 1 cm below the

surface. The platform provided the only escape from the water. Many extra-maze cues, such as a door, a table, and pictures on the walls of the room in which the water maze was housed, helped the rat to locate the hidden escape platform. The animals performed four consecutive trials, all beginning at a fixed starting point. Trials ended either when the platform was found or when 120 s had elapsed. If the rats did not find the platform within 120 s, they were guided to it and left there for 15 s. They were then removed from the pool and returned to their home cages after being dried with a towel. This site navigation test was performed once before the irradiation and after that once in the third and once in the fourth month.

Histopathology

Rats were deeply anesthetized with 4% chloral hydrate and perfused transcardially with 100 ml 0.1 M phosphate-buffered saline (PBS, pH 7.0-7.4) to flush out the blood, followed by 500 ml 4% paraformaldehyde in 0.1 M phosphate-buffer (PB) at 4 ºC. The brains were dissected out and fixed in paraformaldehyde for one day, before being cut into 6 equal pieces, which were then embedded in paraffin. Serial 30-µm sections were cut with a vibratome. Multiple sections were processed with hematoxylin and eosin (H&E) for histological evaluation; for the demonstration of demyelination, Luxol fast blue (LFB) staining was applied. Sections were analyzed under an Axio Imager.Z1 (EC Plan Neofluar 40x/0.75 M27, Freiburg, Germany) light microscope, and photomicrographs were taken with AxioCam MR5 camera equipment. Digital photos were analyzed with the aid of Image-Pro® Plus 6.1 software (MediaCybernetics Inc., Bethesda, MD, USA). All analyses were performed blindly, using coded sections. Evaluations were carried out by two experienced histopathologists, independently, with a semiquantitative method, scoring each examined parameter (necrosis, macrophage density, reactive gliosis, calcification and demyelination) on a semiquantitative scale from 1 to 4, or 'can not be assessed'. As concerns necrosis, at low magnification (N=50x), the scores were as follows: 1: not detected; 2: necrosis detected in less than 50% of

the field of vision; 3: necrosis detected in more than 50% of the field of vision, but in not more than 100%; 4: necrosis detected that exceeds the field of vision, or affects both hemispheres of the brain. Macrophage density was examined under high magnification (HM) (N=400x) with the following scoring system: 1: no foamy macrophages detected; 2: fewer than 5 foamy macrophages/HM; 3: 5-10 macrophages/HM; 4: more than 10 macrophages/HM. The system for reactive gliosis (N=200x): 1: none; 2: mild; 3: moderate; 4: severe reactive gliosis detected in the brain. Calcification (N=50x): 1: no calcification; 2: a single small calcified focus detected; 3: multiple small, or a single larger focus detected; 4: multiple large calcification detected. Demyelination (N=50x): 1: none; 2: mild; 3: moderate demyelination, but fibers detected; 4: severe demyelination, with the destruction of the fibers.

Statistical analysis

All of the data, expressed as mean ± standard error of the mean (S.E.M.), were analyzed with SigmaStat (Jandel Scientific, Erkrath, Germany) or StatView 4.53 for Windows software (Abacus Concept Inc., Berkely, CA, USA). Nonparametric methods were used. In the behavioral experiments, differences between groups were subjected to Kruskal-Wallis one-way ANOVA on ranks, followed by Fisher's PLSD method for pairwise multiple comparison. One-way ANOVA and Fisher's PLSD post hoc tests were also used for the histology. Values of $p<0.05$ were considered to be statistically significant.

Results

The 40-Gy RT group exhibited a body weight deficit; their body weight remaining under the normal throughout the difference between the RT and CO groups did not reach the level of statistical significance.

Water maze test

The Morris water maze test was used to assess the acquisition and retention of a spatial working memory. Healthy rats improve their performance during such place navigation by using their spatial working memory. After the 40-Gy irradiation, significant, time-related changes in learning ability were detected in both the RT and GPC+RT groups, but these changes were significantly reduced in the GPC+RT group (Figure 1). The first sign of deterioration was detected 90 days post-irradiation and the difference relative to the CO animals was more pronounced after 120 days ($p<0.001$). A relevant memory impairment was detected in the RT group after 120 days, and a significant cognitive deficit was also observed in the GPC+RT group relative to the CO group ($p=0.0025$). Despite this, there was a significant amelioration after GPC management, which reduced the latency of target finding relative to the RT group ($p=0.012$). The GPC ameliorated the memory of the animals and shortened the latency time of platform finding.

Histopathology

The H&E-stained slides of the CO animals and the non-irradiated regions of the brain of the treated animals exhibited no signs of necrosis, i.e. neither reactive astrogliosis, nor any of the other examined histopathological categories. In the irradiated region of the brain, the following parameters correlated closely with the 40-Gy dose level: necrosis, macrophage density, reactive gliosis, calcification and demyelination. The RT group displayed moderate necrosis that reached the gray and white matter, causing demyelination, with destruction of

the fibers (Figure 2). The grades of reactive astrogliosis and calcification, the density of the foamy macrophages and the degree of demyelination were all significantly elevated in the RT group as compared with the CO animals. Marked protective effects of GPC were detected as concerns the macrophage density ($p<0.001$), reactive astrogliosis ($p<0.001$), calcification ($p=0.012$) and the extent of demyelination ($p=0.035$). The scores in the RT group were as follows: necrosis 3.33, macrophage density 2.83, reactive astrogliosis 3.50, calcification 3.0 and demyelination 3.17. In the GPC+RT group mild-to-moderate necrosis was seen, with mild-to-moderate demyelination, but the fibers could mostly be detected. In comparison with the CO group, significant correlations were detected in the following categories: necrosis (Figure 2), macrophage density (Figure 3), reactive gliosis (Figure 4), calcification (Figure 5) and demyelination (Figure 6). The scores were as follows: necrosis 2.33, macrophage density 1.50, reactive astrogliosis 1.83, calcification 2.0 and demyelination 2.17.

Discussion

The various new radiation techniques encourage escalation of the dose in the treatment of primary and secondary brain tumors, though this is accompanied by an increase in the probability of complications in the healthy regions of the brain, while the concurrent chemotherapy applied in cases of glioblastoma results in a rise in the number of treatment-related injuries (26-28). If the level of cognitive reduction could be lessened, the QOL of the survivors would improve and the social and economic strain would be reduced. There is therefore a great need for a potent radioprotector which could decrease the extent of damage to the healthy brain.

We have developed a special technique for partial brain irradiation restricted to a defined area, including the hippocampus and corpus callosum, in one hemisphere in small animals, similarly to human brain tumor radiotherapy, as recommended by others (29). The hippocampus is the major brain area that plays a crucial role in the processes of learning and memory (30), and numerous data clearly confirm that irradiation causes a deterioration of these functions (31, 19, 32). Inside the hippocampus, the dentate gyrus (DG) is the region most susceptible to radiation (33); moreover, this is the site of neurogenesis (34-35). An earlier study of the dose-response relationship indicated that the chosen model is relevant for studying various aspects of healthy brain protection (25).

In our present investigation, this method revealed a promising protective effect of GPC, which can be explained by its role in preserving the cell membranes and cognitive functions in the CNS. Choline and choline-containing phospholipids such as GPC are responsible for maintaining the cell membrane integrity and are also precursors of the neurotransmitter ACh, which is involved in a number of brain processes, including learning and memory. It has previously been studied as a centrally acting parasympathomimetic drug in acute cerebrovascular diseases and dementia disorders (23, 36). After oral administration, GPC can

cross the blood-brain barrier and reach the CNS, where it can exert beneficial effects in the treatment of the sequelae of cognitive disorders and cerebrovascular accidents. It can incorporate into the phospholipid fraction of the neuronal plasma membrane and can also increase the levels of production and release of ACh in the brain (37-39). A trial of the clinical efficacy and tolerability of GPC on 2044 patients after stroke or transient ischemic attacks confirmed the therapeutic efficacy of GPC in the cognitive recovery, the presumed mechanism involving the provision of a high level of choline for the nervous cells, which protects their cell membranes (23).

Our study clearly illustrates the protective effects of GPC at both functional and morphological level. The cognitive dysfunction resulting from irradiation can be examined by different methods. The MWM has been found to be a highly sensitive tool for the detection of a neurofunctional impairment (40-46). The MWM task clearly demonstrated the effects of GPC on the working memory and long-lasting reference memory of rats after irradiation at a 40-Gy dose level, the differences in learning ability between the RT and CO groups becoming more pronounced as time passed.

An earlier analysis of the histological changes led to the finding that brain irradiation modified the spine density and also the proportions of the morphological subtypes in the dendrites of the DG granule cells and the basal dendrites of the CA1 pyramidal neurons, in a time-dependent manner (47). Pathological disturbances such as vascular damage and demyelination are late consequences of irradiation that are likewise revealed by histological examination (48). The primary targets of radiation damage include the oligodendrocytes and the white matter, which suffer necrosis (49-50). In our study, the levels of such histopathological deterioration, scored semiquantitatively, were ameliorated significantly by GPC treatment. The changes in cognitive ability correlated closely with the histopathological findings indicative of the radio-neuroprotecive action of GPC.

Conclusion

Our data have provided experimental evidence of the changes in cognitive function and histological deterioration after the irradiation of one hemispherical hippocampus and the potential for GPC treatment to exert a favorable influence on such events. The previously established model proved appropriate for the investigation of agents modifying the effects of irradiation in the CNS. This study warrants further research on the protective or mitigating effects of GPC on radiation injuries.

Acknowledgments:

The authors are grateful to Boda Gyuláné and Erika Szigeti for their valuable assistance and to Károly Tóth and Kálmán Vas for their excellent work.

References

1. Coderre JA, Morris GM, Micca PL, Hopewell JW, Verhagen I, Kleiboer BJ, et al. Late effects of radiation on the central nervous system: role of vascular endothelial damage and glial stem cell survival. Radiat Res 2006;166:495–503.
2. Douw L, Klein M, Fagel SS, van den Heuvel J, Taphoorn MJ, Aaronson NK, et al. Cognitive and radiological effects of radiotherapy in patients with low-grade glioma: long-term follow-up. Lancet Neurol 2009;8:810-818.
3. Laack NN, Brown PD, Ivnik RJ, Furth AF, Ballman KV, Hammack JE, et al. Cognitive function after radiotherapy for supratentorial low-grade glioma: a North Central Cancer Treatment Group prospective study. Int J Radiat Oncol Biol Phys 2005;63:1175–1183.
4. Ansari R, Gaber MW, Wang B, Pattillo CB, Miyamoto C, Kiani MF. Anti-TNFA (TNF-alpha) treatment abrogates radiation-induced changes in vascular density and tissue oxygenation. Radiat Res 2007;167:80–86.
5. Belka C, Budach W, Kortmann RD, Bamberg M. Radiation-induced CNS toxicity–molecular and cellular mechanisms. Br J Cancer 2001;85:1233–1239.
6. Li YQ, Chen P, Haimovitz-Friedman A, Reilly RM, Wong CS. Endothelial apoptosis initiates acute blood-brain barrier disruption after ionizing radiation. Cancer Res 2003;63:5950–5956.
7. Monje ML, Mizumatsu S, Fike JR, Palmer TD. Irradiation induces neural precursor-cell dysfunction. Nat Med 2002;8:955–962.
8. Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal neurogenesis. Science 2003;302:1760–1765.
9. Tofilon PJ, Fike JR. The radioresponse of the central nervous system: a dynamic process. Radiat Res 2000;153:357–370.

10. Zhao W, Robbins ME. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. Curr Med Chem 2009;16:130–143.
11. Calvo W, Hopewell JW, Reinhold HS, Van Den Berg AP, Yeung TK. Dose-dependent and time-dependent changes in the choroids plexus of the irradiated rat brain. Br J Radiol 1987;60:1109–1117.
12. Rabin BM, Meyer JR, Berlin JW, Marymount MH, Palka PS, Russell EJ. Radiation-induced changes in the central nervous system and head and neck. Radiographics 1996;16:1055–1072.
13. Jarrard LE, Davidson TL, Bowring, B. Functional differentiation within the medial temporal lobe in the rat. Hippocampus 2004;14:434–449.
14. Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia AS, McNamara JO et al. Clinical cases that reveal the anatomical substrate of declarative memories. In: Neuroscience, Third Edition. Sinauer Associates, Inc, Sunderland, MA, U.S.A. 2004. p.742–750.
15. Caceres LG, Aon Bertolino L, Saraceno GE, Zorrilla Zubilete MA, Uran SL, Capani F, et al. Hippocampal-related memory deficits and histological damage induced by neonatal ionizing radiation exposure. Role of oxidative status. Brain Res 2010;1312:67–78.
16. Abayomi OK. Pathogenesis of irradiation-induced cognitive dysfunction. Acta Oncol 1996;35:659–663.
17. Abayomi OK. Pathogenesis of cognitive decline following therapeutic irradiation for head and neck tumors. Acta Oncol 2002;41:346–351.
18. Roman DD, Sperduto PW. Neuropsychological effects of cranial radiation: current knowledge and future directions. Int J Radiat Oncol Biol Phys 1995;31:983–998.

19. Rola R, Raber J, Rizk A, Otsuka S, Van den Berg SR, Morhardt DR, et al. Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. Exp Neurol 2004;188:316–330.
20. Tőkés T, Varga G, Garab D, Nagy Z, Fekete G, Tuboly E, et al. Peripheral inflammatory activation after hippocampus irradiation in the rat. Int J Radiat Biol 2013 (ahead of publication).
21. Drago F, Mauceri F, Nardo L, Valerio C, Lauria N, Rampello L, et al. Behavioral effects of L-alpha-glycerylphosphorylcholine: influence on cognitive mechanisms in the rat. Pharmacol Biochem Behav 1992;41:445–448.
22. Sigala S, Imperato A, Rizzonelli P, Casolini P, Missale C, Spano P. L-alpha-glycerylphosphorylcholine antagonizes scopolamine-induced amnesia and enhances hippocampal cholinergic transmission in the rat. Eur J Pharmacol 1992;211:351–358.
23. Barbagallo Sangiorgi G, Barbagallo M, Giordano M, Meli M, Panzarasa R. Alpha-glycerophosphocholine in the mental recovery of cerebral ischemic attacks. An Italian multicenter clinical trial. Ann N Y Acad Sci 1994;717:253–269.
24. Schettini G, Ventra C, Florio T, Grimaldi M, Meucci O, Scorziello A, et al. Molecular mechanisms mediating the effects of L-alpha-glycerylphosphorylcholine, a new cognition-enhancing drug, on behavioral and biochemical parameters in young and aged rats. Pharmacol Biochem Behav 1992;43:139–151.
25. Hideghéty K, Plangár I, Mán I, Fekete G, Nagy Z, Volford G, et al. Development of a small-animal focal brain irradiation model to study radiation injury and radiation-injury modifiers. Int J Radiat Biol 2013;89:645–655.
26. Brandsma D, Stalpers L, Taal W, Sminia P, van den Bent MJ. Clinical features, mechanisms and management of pseudoprogression in malignant gliomas. Lancet Oncol 2008;9:453–461.

27. Chamberlain MC, Glantz MJ, Chalmers L, Van Horn A, Sloan AE. Early necrosis following concurrent Temodar and radiotherapy in patients with glioblastoma. J Neurooncol 2007;82:81–83.
28. de Wit MC, de Bruin HG, Eijkenboom W, Sillevis PA, van den Bent MJ. Immediate post-radiotherapy changes in malignant glioma can mimic tumor progression. Neurology 2004;63:535–537.
29. Kalm M, Karlsson N, Nilsson MK, Blomgren K. Loss of hippocampal neurogenesis, increased novelty-induced activity, decreased home cage activity, and impaired reversal learning one year irradiation of the young mouse brain. Exp Neurol 2013 (Epub ahead of print).
30. Izquierdo I, Medina JH. Memory formation: The sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiol Learn Mem 1997;68:285–316.
31. Raber J. Unitended effects of cranial irradiation on cognitive function. Toxicol Pathol 2010;38:198–202.
32. Yazlovitskaya EM, Edwards E, Thotala D, Fu A, Osusky KL, Whetsell WO Jr, et al. Lithium treatment prevents neurocognitive deficit resulting from cranial irradiation. Cancer Res 2006;66:11179–11186.
33. Monje M. Cranial radiation therapy and damage to hippocampal neurogenesis. Dev Disabil Res Rev 2008;14:238–242.
34. Monje ML, Palmer T. Radiation injury and neurogenesis. Curr Opin Neurol 2003;16:129–134.
35. Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. Cell 2008;132:645–660.

36. Parnetti L, Amenta F, Gallai V. Choline alphoscerate in cognitive decline and in acute cerebrovascular disease: an analysis of published clinical data. Mech Ageing Dev 2001;122:2041–2055.
37. Amenta F, Tayebati SK. Pathways of acetylcholine synthesis, transport and release as targets for treatment of adult-onset cognitive dysfunction. Curr Med Chem 2008;15:488–498.
38. Tayebati SK, Tomassoni D, Di Stefano A, Sozio P, Cerasa LS, Amenta F. Effect of choline-containing phospholipids on brain cholinergic transporters in the rat. J Neurol Sci 2011;302:49–57.
39. Tayebati SK, Tomassoni D, Nwankwo IE, Di Stefano A, Sozio P, Cerasa LS, et al. Modulation of monoaminergic transporters by choline-containing phospholipids in rat brain. CNS Neurol Disord Drug Targets 2013;12:94–103.
40. Akiyama K, Tanaka R, Sato M, Takeda N. Cognitive dysfunction and histological findings in adult rats one year after whole brain irradiation. Neurologia Medico-Chirurgica 2001;41:590–598.
41. Jirák D, Náměstková K, Herynek V, Liscák R, Vymazal J, Mares V, et al. Lesion evolution after gamma knife irradiation observed by magnetic resonance imaging. Int J Radiat Biol 2007;83:237–244.
42. Justino L, Welner SA, Tannenbaum GS, Schipper HM. Long-term effects of cysteamine on cognitive and locomotor behaviour in rats: relationship to hippocampal glial pathology and somatostatin levels. Brain Research 1997;761:127–134.
43. Liscák R, Vladyka V, Novotný J Jr, Brozek G, Naměstkova K, Mares V, et al. Leksell gamma knife lesioning of the rat hippocampus: the relationship between radiation dose and functional and structural damage. J Neurosurg 2002;97:666–673.

44. Shi L, Olson J, D'Agostino R Jr, Linville C, Nicolle MM, Robbins ME, et al. Aging masks detection of radiation-induced brain injury. Brain Res 2011;1385:307–316.
45. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nature Protocols 2006;1:848–858.
46. Yoneoka Y, Satoh M, Akiyama K, Sano K, Fujii Y, Tanaka R. An experimental study of radiation-induced cognitive dysfunction in an adult rat model. Br J Radiol 1999;72:1196–1201.
47. Chakraborti A, Allen A, Allen B, Rosi S, Fike JR. Cranial irradiation alters dendritic spine density and morphology in the hippocampus. PLoS One 2012;7:e40844.
48. Brown WR, Thore CR, Moody DM, Robbins ME, Wheeler KT. Vascular damage after fractionated whole-brain irradiation in rats. Radiat Res 2005;164:662–668.
49. Shen C, Bao W, Yang B, Xie R, Cao X, Luan S, et al. Cognitive deficits in patients with brain tumor. Chin Med J 2012;125:2610–2617.
50. Valk PE, Dillon WP. Radiation injury of the brain. AJNR Am J Neuroradiol 1991;12:45–62.

Figure 1.

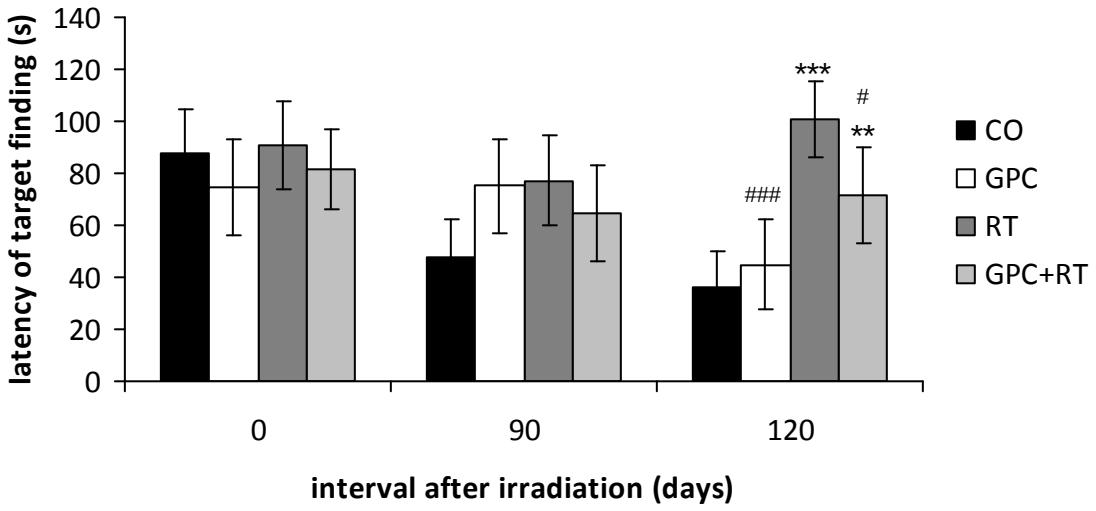

Figure 2.

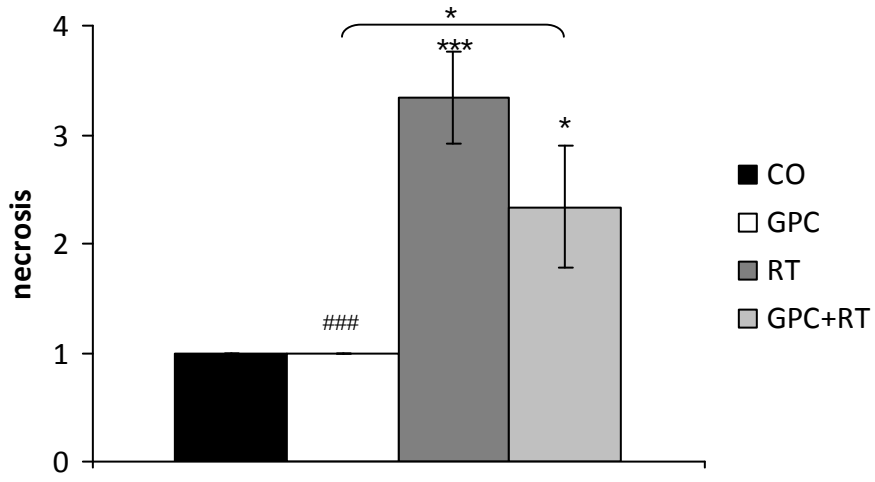

Figure 3.

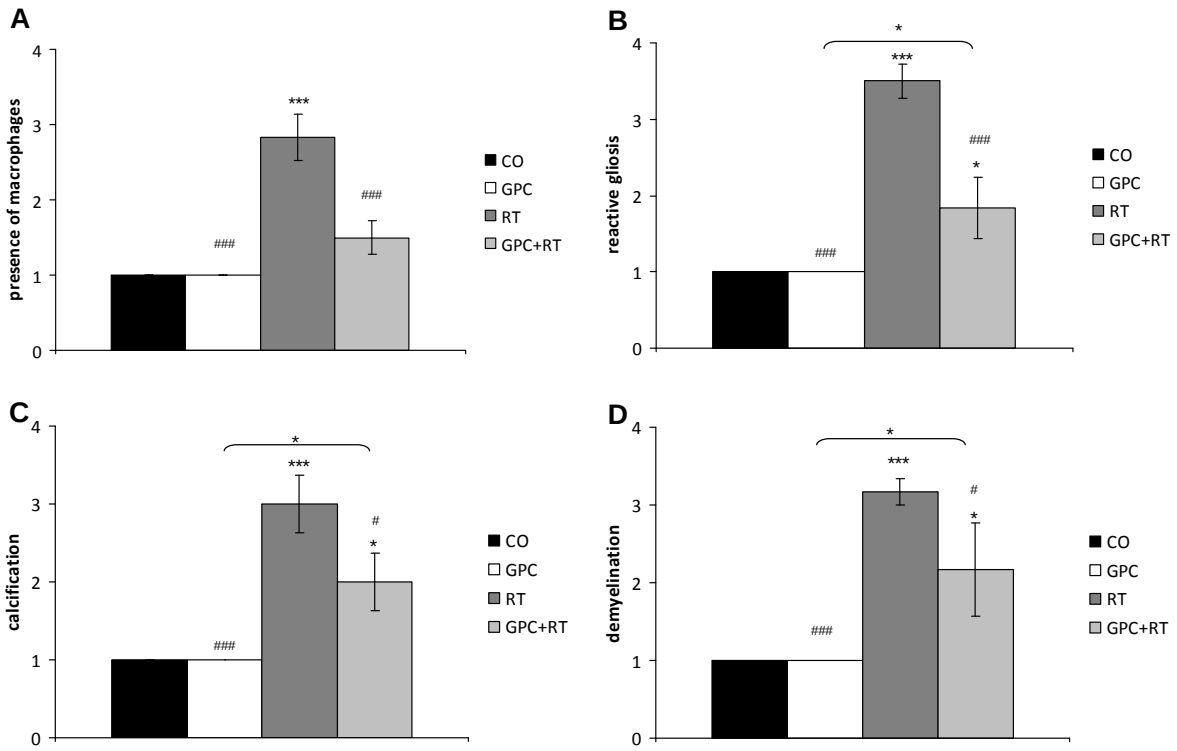
A
presence of macrophages
0
1
2
3
4
***
###
###
CO
GPC
RT
GPC+RT
B
reactive gliosis
0
1
2
3
4
*
***
###
###
*
CO
GPC
RT
GPC+RT
C
calcification
0
1
2
3
4
*
***
###
#
*
CO
GPC
RT
GPC+RT
D
demyelination
0
1
2
3
4
*
***
###
#
*
CO
GPC
RT
GPC+RT

Figure 4.