Int J Radiat Oncol Biol Phys. Author manuscript; available in PMC 2013 November 14.

Published in final edited form as:

Int J Radiat Oncol Biol Phys. 2012 September 01; 84(1): 244–249. doi:10.1016/j.ijrobp.2011.10.054.

Glioblastoma Treatment: Bypassing the Toxicity of Platinum Compounds by Using Liposomal Formulation and Increasing Treatment Efficiency With Concomitant Radiotherapy

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Abstract

PURPOSE—Treatments of glioblastoma (GBM) with cisplatin or oxaliplatin only marginally improve the overall survival of patients and cause important side effects. To prevent adverse effects, improve delivery and optimize the tumor response to treatment in combination with radiotherapy, a potential approach consists in incorporating the platinum agent in a liposome.

METHODS AND MATERIALS—In this study, cisplatin, oxaliplatin, carboplatin, Lipoplatin™ and Lipoxal™, the liposomal formulations of cisplatin and oxaliplatin respectively, were tested on F98 glioma orthotopically implanted in Fischer rats. The platinum compounds were administered by intracarotid infusion and were assessed for the ability to reduce toxicity, improve cancer cell uptake and increase survival of animals when combined or not with radiotherapy.

RESULTS—The tumor uptake was 2.4-fold more important for Lipoxal™ than the liposome-free oxaliplatin. Lipoxal™ also improved the specificity of oxaliplatin as shown by a higher ratio of tumor/right hemisphere uptake. Surprisingly, Lipoplatin™ led to lower tumor uptake compare to cisplatin. However, Lipoplatin™ had the advantage of largely reducing the toxicity of cisplatin and allowed to capitalize on the anti-cancer activity of this agent.

CONCLUSION—Among the five platinum compounds tested, carboplatin showed the best increase in survival when combined with radiation for treatment of glioma implanted in Fischer rats.

Keywords

Platinum compounds; liposomes; gamma radiation; F98 glioma; Fischer rats

Introduction

Glioblastoma multiforme (GBM) is the most aggressive primary brain neoplasm, taking the lives of patients within a median of 12 to 14 months after diagnosis (1). Standard treatment typically consists of optimal surgical resection of the tumor followed by concomitant radiation and chemotherapy. Radiation therapy remains the most effective single treatment

modality, resulting in a transient disease control in most patients. The addition of chemotherapy also produces a slight survival benefit for GBM patients (2). However, despite decades of research, malignant gliomas ultimately become resistant to radiation and chemotherapeutic drugs, contributing to the poor prognosis associated with these tumors (3). One potential way to improve the efficacy of radiation would be by coupling it with a potent radiosensitizer such as platinum compounds. Numerous platinum analogs were evaluated in preclinical and clinical studies, but so far only cisplatin, oxaliplatin and carboplatin have been approved for clinical use (4).

In numerous cell lines, combining radiotherapy and platinum compounds was found to increase cell death, possibly by enhancing the production of DNA single and double-strand breaks (5). To explain the radiosensitizing properties of platinum compounds, our group has demonstrated that the efficiency of low energy electrons produced by ionizing radiation to induce DNA strand breaks is significantly increased in presence of cisplatin (6). However, important adverse effects caused by cisplatin frequently hinder the use of higher doses which limits its antineoplastic effects in clinic (7) and (8). With regard to oxaliplatin, no such trial was undertaken for the treatment of GBM, due to its neurotoxicity.

To prevent the adverse effects caused by cisplatin and oxaliplatin, increase their delivery and thus optimize the tumor response, a potential approach consists in incorporating the platinum agent in a liposome and combining it with radiation to obtain a synergistic effect. A potential benefit for using liposomal drugs in the treatment of GBM patients includes a more selective crossing of the blood-brain barrier derived from their lipid nature, which would lead to higher drug accumulation in brain lesions. Lipoplatin™ and Lipoxal™ were developed in order to reduce the systemic toxicity of these platinum compounds, while simultaneously improving the delivery of the drug to the primary tumor (9) and (10).

As platinum compounds do not reach sufficient concentration to the CNS with i.v. administration, intra-arterial (i.a.) infusion was used in the present study. Although i.a. administration is not a standard procedure in clinic, it is a much more effective route to deliver chemotherapeutic agents in brain tumor compared to drug administration by intra-venous (i.v.) (11).

In the present study we evaluated the efficiency of the i.a. administration of cisplatin, oxaliplatin, Lipoplatin™, Lipoxal™ and carboplatin. These drugs were combined, or not, with radiation and tested in Fischer rats bearing F98 glioma implanted orthotopically.

Materials and Methods

Chemicals

Cisplatin was purchased from Sigma-Aldrich (Oakville, Ontario, Canada). Carboplatin and oxaliplatin were obtained respectively from NOVOPHARM (Canada) and Sanofi-Avantis (Canada). Lipoplatin™ and Lipoxal™ were generously provided by Regulon, Inc. (Athena, Greece).

Cell line and culture conditions

The rat F98 Fischer glioma model was chosen since it was shown to adequately reproduce the behaviour of human glioblastoma (12) and (13). The F98 cell line was obtained from ATCC and tested negative for the MAP assay by Charles River Laboratories (Wilmington, MA, USA). Cells preparation and maintenance are described by Blanchard et *al.* (14).

Animal experiments

The experimental protocol was approved by the institutional ethical committee and conformed to regulations of the Canadian Council on Animal Care. For all procedures (implantation, chemotherapy, radiotherapy and euthanasia) male Fischer rats (Charles River Laboratories) were anesthetised with an intraperitoneal injection of ketamin/xylazin (87/13 mg/ml) at 1 ml/kg.

F98 glial cells implantation in Fischer rat brain

For the implantation procedure, confluent F98 cells were suspended in non-supplemented warm MEM at a concentration of 2000 cells/µl. The implantation (10 000 cells in 5µl) was performed as described by Blanchard et *al.* (14).

Intracarotid infusion of the platinum compounds

Ten days after F98 glioma cells implantation, platinum compounds were infused in the right internal carotid artery in a retrograde manner via the external carotid as described by Fortin et al (15). In order to administer a platinum compound dose equivalent to doses administered in human, the dose was established in respect to the body surface area (BSA), which is determined as 0.04 m^2 for rats weighting 250 g. Platinum doses used in this study were thus as follows: carboplatin 5 mg, oxaliplatin 3 mg, cisplatin 3 mg, Lipoplatin™ 3mg (of cisplatin) and Lipoxal™ 3 mg (of oxaliplatin). Free platinum was diluted in 1 ml of 5% dextrose solution (Baxter, Toronto, Canada). Lipoplatin™ and Lipoxal™ were used without dilution at a concentration of 3 mg platinum/ml. Solution of 1 ml of platinum formulation was injected over 20 min. After i.a. infusion, the external carotid was sacrificed and the neck of the animal was closed by sutures.

Platinum uptake in tumor and brain tissue

Animals (n= 3 to 4 animals per group) were implanted with the F98 glioma cells at day zero, injected with platinum compounds at day 10 and euthanized 24 hours later. Brains were removed by craniotomy and promptly cut in three sections (Fig. 1) with a brain matrix (WPI, RBMA-300C, Sarasota, FL). Tumor section of a thickness of 3.5 mm was standardized in the right hemisphere between slots 2 and 4 of the brain matrix (starting from frontal position). The tumor implantation point is situated in the middle of slot 3. The left hemisphere (contralateral section) and healthy right hemisphere (adjacent tissue) were also isolated. Fresh tissue samples were rapidly weighted and solubilised in 10% nitric acid, 30% hydrogen peroxide and sonicated until homogenization. Samples were then analysed for platinum concentration by Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (ELAN DRC-II, PerkinElmer, Woodbridge, ON).

Gamma Knife irradiation

Twenty four hours after chemotherapeutic treatments (platinum compounds and sham), rats (n= 8–12 animals per group, except for cisplatin where n = 4 animals) were anesthetised and positioned in our home made stereotactic frame (16) designed for the Gamma Knife 4C (Elekta Instruments AB). The 8 mm collimators were used to deliver the radiation treatment (15 Gy with a dose rate of approximately 2.8 Gy/min) at predetermined coordinates targeting the tumor which has a diameter of about 4 mm. (14). A daily radiation dose of 2 Gy cannot be done since such protocol in animal required repetitive anaesthesia, which progressively lead to important toxic effects. Therefore, the brain tumors were irradiated with a single dose of 15 Gy which is equivalent to a typical protocol of 25 fractions of 2 Gy.

Control animals (n = 7) received the same surgical procedures as treated animals and 1ml of 5% dextrose (vehicle for platinum drugs) was infused in the carotid as performed for animals treated with platinum compounds.

Experimental end points (survival)

Monitoring included weight measurement, mobility, coordination, loss of self-grooming (periocular secretion accumulation) and landing ability was performed on a on a daily basis. In agreement with the ethical committee regulations, the experimental endpoint for survival was established as a complete lethargy (and apathy) of the animals. At this point, animals were anesthetised and 4% paraformaldehyde (PFA) was infused by intracardiac route to fix the brain tissue. The brain was removed by craniotomy to corroborate the presence of tumor and kept in PFA for future analysis.

Statistics

Data of brain tissue accumulation were analysed by a Student's t-test for intra group and by ANOVA for inter groups. For the survival study, data were analysed by the Quartile method before doing Kaplan-Meier survival curves which were analysed by Log-Rank test. P values under 0.05 were considered statistically significant.

Results

Platinum uptake in tumor and brain tissue

All platinum compounds preferentially accumulated in brain tumor (Fig. 2). The highest uptake was measured with cisplatin. Liposomal cisplatin (Lipoplatin™) accumulated significantly less in brain tumor; however, this reduction could not be solely attributed to the liposomal formulation. Indeed, 2.4-fold more liposomal oxaliplatin (Lipoxal™), which has a similar liposomal formulation than Lipoplatin™, reached the tumor comparatively to the liposome-free oxaliplatin. Carboplatin behaved like Lipoplatin™ and Lipoxal™ as its distribution in the brain tumor was similar to the liposomal formulations.

The highest ratio of tumor / right hemisphere uptake was obtained with Lipoxal™ (ratio of 4.64). This represented a large improvement compared to the other platinum compounds which reached a ratio ranging from 2.5 to 2.9.

When excluding the tumor section, the infusion by i.a. resulted in a 2.3 to 3.2 fold more accumulation of platinum compounds in the right hemisphere compared to the left one, thus lending credence to the i.a. infusion as an appropriate strategy to improve drug delivery.

Toxicity and tumor response to platinum compounds

The average survival time after tumor implantation was 21 days for sham animals (Table 1, Fig. 4). For this study, drugs were administrated 10 days after implantation of the F98 tumour in the animals. An important toxicity was observed after injecting cisplatin, resulting in the death of all animals three days after drug injection, for an overall survival of 13.3 days (Table 1, Fig. 3A). Because of the high toxicity observed with cisplatin, this group was limited to 4 animals. The liposomal cisplatin (Lipoplatin™) formulation markedly reduced this toxicity. The survival time of rats treated with Lipoplatin™ improved to 30.2 days compared to 13.3 days for cisplatin (Fig. 3B, 4). This result supports that liposomal cisplatin has an anti-cancer activity against F98 cancer cells while decreasing toxicity for the animal. Carboplatin depicted a similar anti-cancer activity than Lipoplatin™ (Fig. 3D, 4).

Treatment with oxaliplatin did not significantly modify the survival time compared to the control group (sham) (Table 1, Fig. 3C, 4). Peripheral toxicity was observed, such as a reduction of the microvasculature in ears, fingers and eyes. Also, hairs bristled and vasomotor spasms were observed in a few animals. It is noteworthy that its liposomal formulation (Lipoxal™) prevented all form of toxicity and largely improved the anti-tumor activity of oxaliplatin, as evidenced by an improved survival time of 29.6 days, the equivalent to carboplatin and Lipoplatin™ (Table 1, Fig. 3D, 4).

Concomitant treatment with platinum compounds and radiation

Irradiation of the F98 brain tumor without chemotherapy increased the mean survival time of Fischer rats from 22 to 34 days, thus supporting that a radiation dose of 15 Gy resulted in a partial transient tumor response (Table 1, Fig. 3F, 4).

The anti-cancer efficiency of cisplatin combined to radiotherapy was not evaluated since this platinum compound led to an important toxicity in Fischer rats bearing the F98 brain tumor. The liposome-free oxaliplatin was also excluded since it was associated to peripheral toxicity and no clear improvement of tumor response was measured when tested without radiotherapy (Table 1).

Infusion of carboplatin followed by radiation treatment resulted in the longest survival time (mean survival of 46.8 days). Among the liposomal formulations tested, the highest concomitant response was obtained with Lipoxal™ resulting in a mean life time of 37.9 days (Fig. 3C and F), compared to 31.2 days for radiation with Lipoplatin™. However, it is noteworthy that the concomitant treatment with Lipoplatin™ resulted in an inferior survival time than obtained after treating solely with radiotherapy (34 days, Table 1).

Discussion

Our preclinical study supports the lack of efficacy of cisplatin to treat GBM tumor implanted in Fischer rats. Severe toxicity leading to a much shorter mean survival time than the control

F98-Fischer rats was observed. This toxicity is not associated to the intracarotid infusion of 5% dextrose used as vehicle, since the average survival time for sham animals was similar than the one reported for non-treated Fischer animals (14). To overcome the toxicity of free cisplatin, we used a formulation of cisplatin encapsulated in a liposomal formulation, Lipoplatin™. Although we cannot state that the toxicity of cisplatin to healthy brain tissue was completely eliminated after liposomal encapsulation, our results nonetheless demonstrated an important improvement in mean survival without obvious signs of toxicity. We could also capitalize on the anti-cancer potential of liposomal cisplatin which resulted in longer mean survival time of Fischer rats than the control animals. It is noteworthy to mention that Lipoplatin™ did not increase the accumulation of cisplatin in brain tumor. Our results suggest that the better anti-cancer potential of Lipoplatin™ was associated with a more favorable selective distribution of cisplatin between tumor and healthy brain tissue than obtained with liposomal-free cisplatin, thereby contributing to spare healthy tissue while ‘hitting’ tumor cells.

The impact of the liposomal formulation on the *in vitro* accumulation of cisplatin in the F98 glioma cells was previously reported. The direct incubation of F98 cells with Lipoplatin™ increased by 3-fold the accumulation of cisplatin (17). This is in contrast to the lower tumor uptake of the liposomal cisplatin measured *in vivo* in the F98 tumor implanted in the Fischer rats. This discrepancy could be caused by the limited diffusion of the liposome through the blood brain tumor barrier.

Accumulation of Lipoplatin™ or Lipoxal™ in the glioma tumor was found to be 2.3 and 4.6-fold higher, respectively, compared to accumulation in the healthy adjacent brain. The specific accumulation of these liposomes in brain tumor was inferior than previously reported (18) with other solids tumors which might be caused by the BBB. It is to be noted that the tumor section samples also contained some surrounding healthy brain tissue, thus potentially underestimating the real tumor uptake (Fig. 1).

Under the conditions used with the F98 model of GBM in Fischer rats, limited migration of F98 cells in surrounding brain occurs (manuscript in preparation), compared to what is observed in clinic with human GBM. Our data support that drug uptake in the tumor core where the BBB is already disrupted would probably not benefit of a transitory opening of the BBB. On the other hand, a way to improve drug uptake in cancer cells scattered in the brain without increasing the injected dose of drugs could still be to transitory open the BBB, which would also avoid systemic toxicity.

Antitumoral activity of oxaliplatin has been observed during clinical phase 1 for some cancers, as well as in glioma patients (19). However, acute neurotoxicity has been observed following oxaliplatin infusion and resolves within days, while chronic neuropathy develops progressively with higher cumulative doses (20). This neurotoxicity has limited the testing of this platinum compound in glioma patients. In our study, the anti-tumor efficacy of the liposomal formulation of oxaliplatin, Lipoxal™, was evaluated. No severe toxicity leading to early death of the animals was observed with Lipoxal™ compared to oxaliplatin. More so, the anti-cancer efficiency of Lipoxal™ was considerably better. While the liposome-free oxaliplatin failed to increase the mean survival time of rats implanted with the F98 tumor,

treatment with Lipoxal™ prolonged the mean survival time to 29.6 days, compared to 22 days for the non-treated animals. This improvement could be associated with a larger tumor uptake of this platinum agent when incorporated in Lipoxal™, without significantly modifying its distribution in adjacent brain tissue.

The concomitant treatment of Lipoplatin™ and radiation led to the same mean survival than obtained with Lipoplatin™ alone or radiotherapy alone. The reason for the lack of additive effect of cisplatin encapsulated in liposome for treatment of brain tumor remains unclear., The combination of radiation and Lipoxal™ showed a tendency to be additive but it's not statically significant compared to radiation alone. Carboplatin, which accumulated at similar levels as Lipoxal™ and Lipoplatin™ in the brain tumors, have retained the additive effect with radiotherapy, as it was previously observed in the *in vitro* study (17).

Interestingly, the best additive effect with radiation was obtained with carboplatin. In the absence of radiation, the anti-cancer potential of carboplatin was similar to Lipoplatin™ and Lipoxal™. However, when combined with radiation, carboplatin was a much more potent agent than the two others platinum agents, even though the accumulation and distribution of carboplatin in the brain were similar to Lipoplatin™ and Lipoxal™. It remains to be determined why carboplatin led to a large improvement of life span when administered in concomitance with radiation compared to Lipoplatin™ and Lipoxal™, while their anti-tumor response without radiation was similar.

Conclusion

A potential advantage of encapsulating platinum compounds in a liposomal formulation is to reduce their systemic and local toxicity, which is particularly important when treating brain tumors. Lipoplatin™ and Lipoxal™ largely reduce the toxicity observed with free cisplatin and oxaliplatin and allowed to capitalize on the anti-cancer activity of these platinum agents. However, no additive effect of Lipoplatin™ or Lipoxal™ was measured when combined with radiotherapy to treat the F98 tumor implanted in the brain of Fischer rats. An additive effect with radiation was observed only with carboplatin which led to the best improvement of mean life time of the animals. Moreover, it remains to be determined if a higher drug uptake in the tumor core would improve the tumor control and therefore increase the additive effect with radiation. A transient opening of the BBB before chemotherapy administration could further improve the efficiency of Lipoxal™, Lipoplatin™ or carboplatin allowing no additive systemic toxicity, increasing the tumor uptake and allows to reaches a larger brain tumor region. A larger area of drug delivery by transient BBB disruption could allow reaching the tumor extensions which show a more intact BBB (characteristic of glioblastoma) that could be far from the deficient leaky vasculature of the tumor core.

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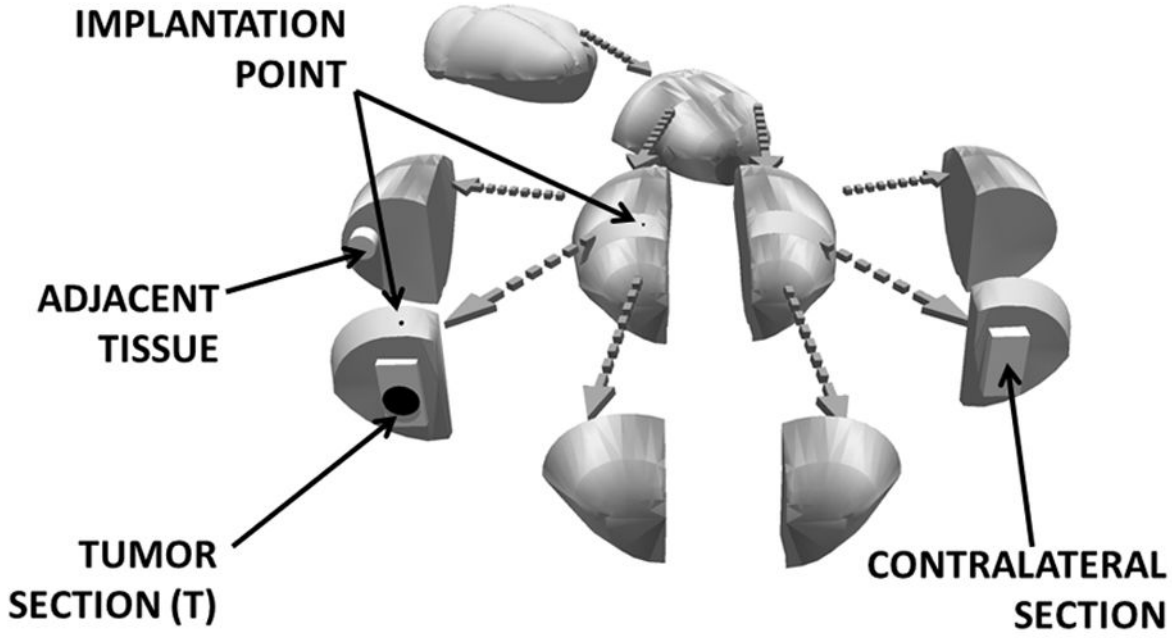

Figure 1.

3-D schematic representation of standardized dissection of the rat brain in three sections. Tumor sections were standardised as a section between slots 2 and 4 of the rat brain matrix (see Materials and Methods). The tumor is represented on the diagram by a black circle. The model was initially based from perpendicular side and top views of rat brain and modeled with Google SketchUp software version 6.4.112.

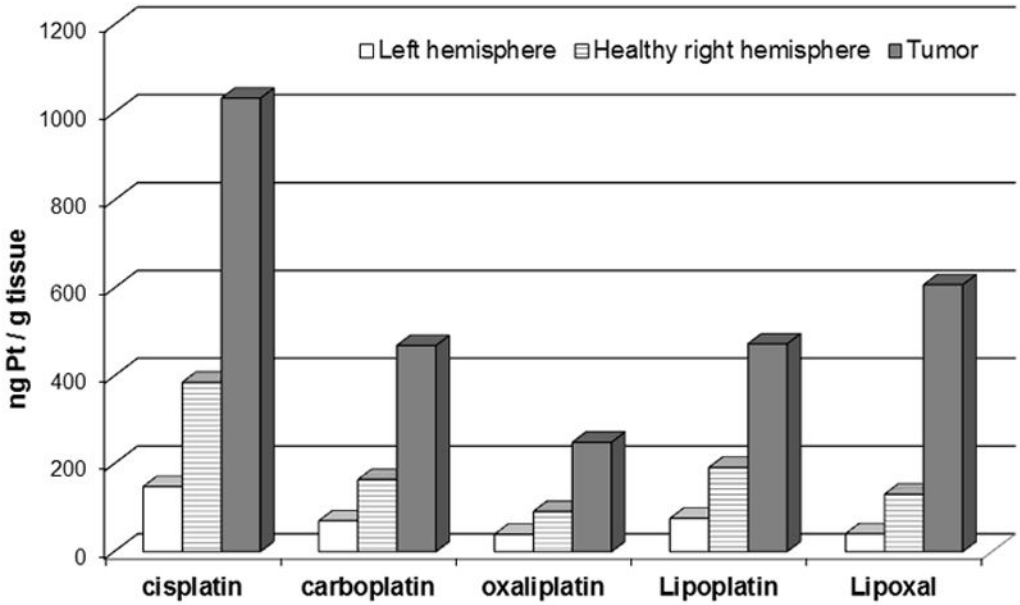

Figure 2.

Platinum compounds accumulation in F98 brain tumor and healthy brain tissues. The tumor implanted in the right hemisphere and the healthy brain tissues of the two hemispheres were isolated 24 h later. The concentration of platinum was determined by an Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

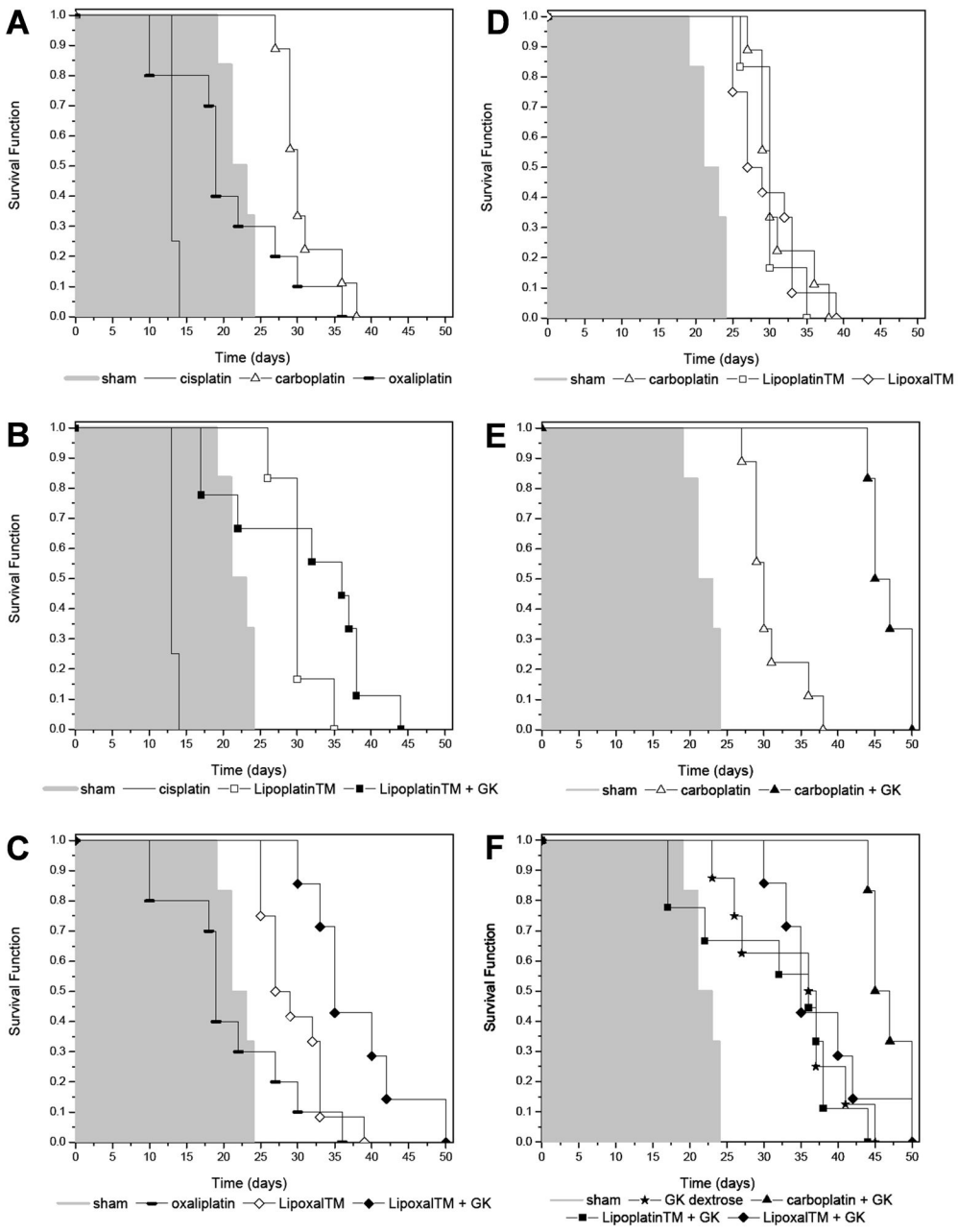

Figure 3.

Kaplan-Meier survival graph for F98 glioma-bearing rats. Rats were implanted at day zero. Chemotherapy was given at day ten. Radiation treatment by Gamma Knife (GK) was performed at day eleven. Sham (control animals) curves are filled in gray. Figure 3A and 3C: each groups were significantly different except sham/oxaliplatin (3A, 3C: $P = 0.934$). Figure 3B: each groups were significantly different except Lipoplatin™/Lipoplatin™-GK ($P = 0.126$). Figure 3D: groups of carboplatin, Lipoplatin™ and Lipoxal™ each were significantly difference compared to sham but not between them. Figure 3E: All groups have $P < 0.05$. Figure 3F: every treatment was different to the sham curve, but only the carboplatin group showed a difference compared to each other groups.

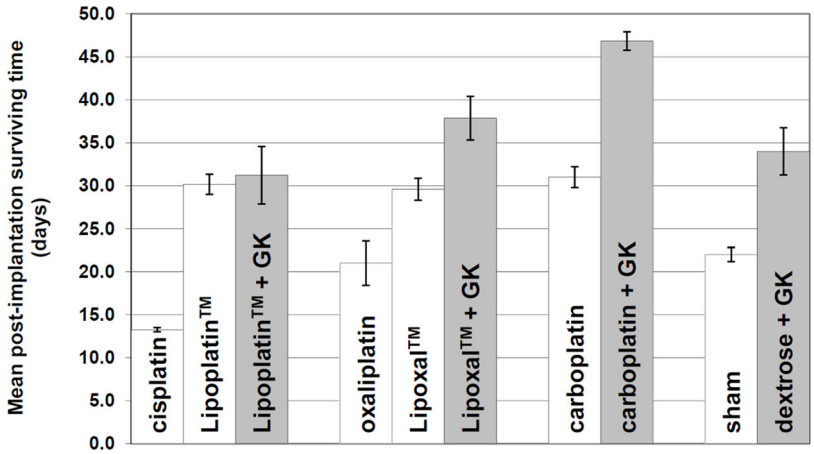

Figure 4.

Mean survival time of F98 glioma-bearing rats after platinum compounds treatment with or without radiation. Experiments were divided in four groups. Group of cisplatin: cisplatin 3 mg, Lipoplatin™ (3 mg of cisplatin) and Lipoplatin™ (3 mg of cisplatin) combined with 15 Gy. Group of oxaliplatin: oxaliplatin 3mg, Lipoxal™ (3 mg of oxaliplatin) and Lipoxal™ (3 mg of oxaliplatin) combined with 15 Gy. Group of carboplatin: carboplatin 5 mg, carboplatin combined with 15 Gy. Control group: sham (glioma-bearing animals treated with 1 ml of 5% dextrose solution) with or without 15 Gy. Dashed line indicates the mean survival time of control animals (sham). Grey columns highlight treatments with radiation. GK = ionizing radiation of 15 Gy by Gamma Knife.

Table 1

Mean survival time of Fischer rats implanted with the F98 brain tumor after treatment with platinum compounds.

Treatment	Mean surviving time (days ± stdev)
cisplatin 3mg	13.3 ± 0.3
oxaliplatin 3mg	21.0 ± 2.6
sham	22.0 ± 0.8
Lipoxal™ 3mg	29.6 ± 1.3
Lipoplatin™ 3mg	30.2 ± 1.2
carboplatin 5mg	31.0 ± 1.2
Lipoplatin™ 3mg + GK	31.2 ± 3.3
GK	34.0 ± 2.8
Lipoxal™ 3mg + GK	37.9 ± 2.5
carboplatin 5mg + GK	46.8 ± 1.1

GK = Gamma Knife

Standard deviations were calculated by using the Kaplan-Meier curves.