

Clinical Study

Neoadjuvant gemcitabine/treosulfan chemotherapy for newly diagnosed glioblastoma

A phase II study

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Summary

The median survival for patients with glioblastoma is 12 months. The authors evaluated whether preirradiation gemcitabine/treosulfan (GeT) chemotherapy followed by standard radiotherapy improved outcome in patients with glioblastoma. Seventeen patients with newly diagnosed glioblastoma were enrolled in a prospective, unicenter trial of preirradiation GeT chemotherapy. Chemotherapy included up to 4 cycles of intravenous gemcitabine (1000 mg/m² body surface) and treosulfan (3500 mg/m² body surface) on days 1 and 8 of 28 days treatment cycles. Involved field radiotherapy (60 Gy in 30 fractions) was given after chemotherapy or earlier in the case of disease progression or drug intolerance. There was no specific treatment-related neurotoxicity reported, but in 3 of 17 patients (18%) chemotherapy was stopped because of World Health Organization (WHO) IV hematological toxicity. With GeT chemotherapy alone, there was a median progression-free survival of 12 weeks and a progression-free survival rate at 4 months of 29%. In 16 of 17 patients who subsequently received a full course of radiotherapy, the median progression-free survival from the time of diagnosis was 8 months, and the progression-free survival rate at 12 months was 25% (4 of 16 patients). The median overall survival was 12 months. Neither age nor extent of the residual postoperative tumor predicted the duration of progression-free survival after chemotherapy alone or after chemotherapy followed by radiotherapy. The combination of gemcitabine and treosulfan produced significant hematological toxicity in patients with newly diagnosed glioblastoma. The schedule used in the present study did not confer any significant survival advantage compared with standard involved field radiotherapy alone.

Introduction

The search for more efficient therapeutic strategies for glioblastoma has led to the evaluation of different schemes of preirradiation chemotherapy in recent years. Thus, we conducted a phase II study of gemcitabine chemotherapy (1000 mg/m² at days 1, 8 and 15 of maximal 4 monthly cycles) prior to conventional involved field radiotherapy with 21 patients. Gemcitabine (LY188011, 2',2'-difluorodeoxycytidine), a cytosine arabinoside analog, is a pyrimidine antimetabolite. It is deaminated by deoxycytidine deaminase to difluorodeoxyuridine or activated by deoxycytidine kinase to difluorodeoxy-CMP. In addition, gemcitabine inhibits ribonucleotide reductase.

The regimen of gemcitabine alone was safe but, with a median progression-free survival of 11 weeks and a progression-free survival rate at 4 months of 24%, not effective [1]. There was an apparent association between the number of completed gemcitabine cycles and overall survival. Importantly, the response to irradiation after gemcitabine was not diminished by prior chemotherapy and the strong radiosensitizing properties of gemcitabine treatment did not result in enhanced neurotoxicity after irradiation.

To improve the outcome for patients with recurrent malignant glioma, we treated 16 patients with malignant glioma as a second line chemotherapy with treosulfan (L-threitol-1,4-bis-methanesulfonate). Treosulfan is a prodrug of a bifunctional alkylating

cytotoxic agent. Its mechanism of action is based on a non-enzymatic, pH- and temperature-dependent formation of mono- and diepoxybutane derivatives. These derivatives are responsible for DNA alkylation and DNA interstrand cross linking, followed by DNA fragmentation and cell death [2]. Treosulfan (Ovastat™) is registered as an oral and intravenous agent for the treatment of advanced ovarian cancer in several European countries [3]. Our study indicated good tolerability at 6–8 g/m² in 4-weekly intervals and moderate activity of the drug with a progression-free survival of 30% at 6 months [4].

Ex vivo analyses of the efficacy of single agent therapy (treosulfan, gemcitabine, paclitaxel, mitoxantrone or cytosine arabinoside) versus the combination of treosulfan and gemcitabine in choroidal melanoma, a chemoresistant tumor, demonstrated a benefit of the combination with activity in 86% of the cases compared to 33–47% with the single substances [5]. A phase I trial of gemcitabine/treosulfan (GeT) combination chemotherapy for ocular melanoma, renal cell cancer, non-small cell lung cancer, breast cancer, ovarian cancer and colorectal cancer showed good palliative effects but also responded without major toxicity to this combination [6].

Given the minimal toxicities of gemcitabine or treosulfan in our studies, with the apparent lack of efficacy of neoadjuvant gemcitabine monotherapy [1,7], but a good correlation between number of gemcitabine treatments and overall survival, our experience with treosulfan and the promising data of the phase I trial of gemcitabine and treosulfan combination chemotherapy in various types of cancer, we evaluated preirradiation GeT combination chemotherapy as a first step in the delineation of a possible role for gemcitabine and treosulfan in the adjuvant treatment of glioblastoma. The initial phase I trials identified the recommended dose of treosulfan in combination with gemcitabine (1000 mg/m² at days 1 and 8) with 3500 mg/m² on days 1 and 8 [6]. The objective of the current study was to determine whether GeT can prevent glioblastoma progression for 4 months and whether GeT followed by radiotherapy provides a survival advantage over radiotherapy alone.

Materials and methods

Study design

This was a non-randomized phase II study of gemcitabine and treosulfan combination therapy in patients

with newly diagnosed glioblastoma who had not received prior radiotherapy or chemotherapy. Treosulfan and gemcitabine were administered consecutively intravenously once each week for 2 weeks, followed by a 3-week rest period (days 1 and 8; 3500 mg/m² treosulfan and 1000 mg/m² gemcitabine; each injection within 30 min). Due to the longer serum half-life of treosulfan (2.2 h) compared with gemcitabine (0.7 h) we administered treosulfan first. This 4-week schedule defined 1 cycle of treatment. Upto 4 cycles were administered. All patients were to receive standard radiotherapy restricted to the tumor site with a 2-cm safety margin (59.6–60 Gy, 5 × 1.8–2.0 Gy fractionated dose per week) after the completion of 4 cycles. Patients were to receive radiotherapy earlier if tumor progression was documented. To avoid increased toxicity from radiotherapy related to gemcitabine, radiotherapy could not be administered earlier than 2 weeks after the last dose of gemcitabine. This protocol was reviewed and approved by the Ethics Committee at the University of Tübingen Medical School (182/99). Informed consent was obtained from all patients.

Inclusion and exclusion criteria

Patients were male or female and were at least 18 years of age. Inclusion criteria were a histologic diagnosis of glioblastoma, residual tumor on postoperative (<72 h) computed tomography (CT) or magnetic resonance imaging (MRI) scans, surgery no longer than 21 days ago, no prior radiotherapy or chemotherapy, a Karnofsky performance status of ≥70, compliance and geographic proximity to allow adequate follow-up, adequate bone marrow reserve (leukocyte count ≥2 × 10⁹/l, granulocyte count >1 × 10⁹/l, platelet count ≥100 × 10⁹/l), and informed consent. If necessary, female and male patients were advised to use an approved contraceptive method (intrauterine device, birth control pills, or barrier device) during and for 3 months after the trial. Exclusion criteria were: active infection, inadequate liver function (bilirubin >3× above upper normal range); alanine aminotransferase (ALT) and aspartate aminotransferase (AST) >3× normal (ALT and AST could be elevated due to phenytoin or ranitidine therapy, which was not considered a criterion of exclusion), inadequate renal function (creatinine >3× above upper normal limit), pregnancy, serious concomitant systemic disorders incompatible with the study, and the use of any investigational agent in the month before enrollment into the study.

Dose adjustments

Dose was adjusted only for treosulfan. Within a cycle, treosulfan was reduced to 75% when WBC were $>1.5 \times 10^9/l$ and $<2 \times 10^9/l$ or platelets were $>50 \times 10^9/l$ and $<100 \times 10^9/l$, to 50% when WBC were $>1.5 \times 10^9/l$ and $<2 \times 10^9/l$ and platelets were $>50 \times 10^9/l$ and $<100 \times 10^9/l$, and was held when WBC were $<1.5 \times 10^9/l$ or platelets $<50 \times 10^9/l$. If the second application within a cycle had to be held, the next cycle was given 3 weeks after the first application with a treosulfan dose adjustment to 75%. The beginning of the next cycle would be postponed for a maximum of 4 weeks until WBC had recovered to $\geq 3 \times 10^9/l$ and platelets to $\geq 100 \times 10^9/l$. Failure of recovery of WBC and platelets for longer than 4 weeks resulted in initiation of radiotherapy. For the ensuing cycle, patients who sustained either febrile neutropenia or World Health Organization (WHO) grade 4 thrombocytopenia or bleeding associated with any grade thrombocytopenia were to have a dose reduction to 50% of the starting dose of the previous cycle for both injections of treosulfan of the cycle. Subsequent dose escalation in 25% steps to the original dose would be allowed providing the patient tolerated the doses given at the 50% level. For hematological toxicity between 2 cycles treosulfan for the next cycle was reduced to 75% when WBC were $>1 \times 10^9/l$ and $<2 \times 10^9/l$ or platelets were $>25 \times 10^9/l$ and $<50 \times 10^9/l$, to 50% when WBC were $>1 \times 10^9/l$ and $<2 \times 10^9/l$ and platelets were $>25 \times 10^9/l$ and $<50 \times 10^9/l$, and was held when WBC were $<1 \times 10^9/l$ or platelets $<25 \times 10^9/l$. Subsequent dose escalation in 25% steps to the original dose would be allowed providing the patient tolerated the doses given at the 50% level.

Concomitant therapy and monitoring

No other chemotherapy or experimental medications were permitted while patients were on study. Disease progression requiring *de novo* treatment with steroids was considered a treatment failure. Anticonvulsants were given as deemed necessary. Patients received full supportive care. Patients were not to be treated with growth factors unless there was evidence of prolonged myelosuppression. Before enrolling in the study, the disease status of each patient was assessed by medical history, physical and neurological examination, evaluation of performance status, postoperative (<72 h) CT or MRI, and chest X-ray. Patients had a full blood count with differential and platelet counts on days of

treatment, blood chemistry and urinalysis at the start of each new cycle, and coagulation studies as appropriate. Before each dose of GeT, there was a performance status evaluation. Before every cycle, patients had medical history assessed, physical and neurological examination, and a repeat MRI scan. The criteria of MacDonald [8] were used to assess response based on cranial MRI scans: complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). Progression-free survival with GeT plus radiotherapy and overall survival as shown in Table 1 were measured from the date of surgery.

Statistical considerations

The enrolment of patient depended on the optimal two-stage design for phase II clinical trials [9] which is suitable for early termination when a drug has low activity.

The calculation of sample size depends on the primary objective of this study, that is, the number of patients without progression at 4 months of treatment with GeT. The rate is given by $R = (CR + PR + SD)/N_r$. N_r describes the number of enrolled patients meeting the criteria for evaluation of tumor response. For the regarded population of patients, a rate of 25% without progression after 4 months defines low drug activity, that is, an activity previously achieved with gemcitabine alone [1]. The assumed rate without

Table 1. Patient characteristics

No.	Age/ Gender	Cycles of GeT chemotherapy	PFS after GeT plus radiotherapy	Overall survival
1	64 f	4	6	17
2	61 m	4	13	24+
3	55 m	1	3	8
4	64 m	2	8	14
5	55 f	3	3	6
6	60 m	3	8	12
7	59 m	2	5	9
8	31 m	4	7	11
9	33 m	3	13	13
10	42 m	1	5	9
11	57 m	3	7	12
12	56 f	1	15	17
13	41 m	1	9	14
14	65 f	3	15	16
15	54 m	4	8	15
16	56 m	4	7	9
17	56 m	2	no RT	5

The table shows patient number, age and gender, number of completed GeT cycles, progression-free survival (PFS) with GeT plus radiotherapy in months, and overall survival in months (RT, radiotherapy; + indicates patient who is alive).

progression after 4 months defining substantial drug activity of GeT is assumed to be 50%. Therefore, $n_1 = 17$ patients were enrolled on the first stage. Termination on the first stage was necessary if less than 6 patients showed no progression after 4 months. Continuation with $n_2 = 20$ patients on the second stage if 6 or more patients showed no progression after 4 months. Therefore, $n = 37 = 17 + 20$ patients would be enrolled. Rule of decision: If the number of $n = 37$ patients showing no progression after 4 months was not greater than 13, the regimen was not worthy of further study. If more than 13 of the 37 patients showed no progression after 4 months, the therapy was worthy of further study. Therefore, $n = 37$ patients meeting criteria for efficacy evaluation would be enrolled. Assuming a rate of 10% dropouts (patients who were entered into the study, but failed to meet criteria for enrolment or failed to meet criteria for efficacy evaluation), 41 patients would be entered into the study.

Results

Toxicity

GeT chemotherapy was generally well-tolerated. Chemotherapy had to be stopped after 1 cycle in 2 patients and after 3 cycles in 1 patient and irradiation commenced because of WHO grade 4 myelosuppression. In the first cycle, 5 patients had WHO grade 1 myelotoxicity, 1 had grade 2 myelotoxicity, and 1 had grade 3 myelotoxicity. In the second cycle, of the remaining 13 patients, 5 had grade 1, and 1 had grade 2 myelotoxicity. In the third cycle, of the remaining 10 patients, 3 had grade 1 myelotoxicity and 1 had grade 2 myelotoxicity. There was no dose reduction in any patient. One patient with grade 4 myelotoxicity suffered atypical pneumonia after the first cycle of GeT and was consecutively switched to radiotherapy. There was a high number of deep venous thromboses during GeT cycles (4/17) with another 2 patients developing deep venous thromboses after GeT and radiotherapy. There was no other organ toxicity. Follow-up after GeT and radiotherapy did not reveal neurotoxicity of this regimen either clinically or by MRI.

Response

Seventeen patients were enrolled (Table 1). There were 13 males and 4 females. The mean ages were 53 ± 8 (mean \pm SD) for all patients, 51 ± 9 for male patients and 60 ± 5 for female patients. Five patients completed

4 cycles of GeT chemotherapy, 5 patients completed 3 cycles, 3 completed 2 cycles, and 4 patients completed at least 1 cycle. Of the 12 patients who did not complete 4 cycles of chemotherapy, 9 discontinued chemotherapy because of radiologically confirmed tumor progression. The condition of one patient deteriorated so rapidly that he was not eligible for radiotherapy with PD after 2 cycles of GeT (P17). Overall initial response rates in all 17 patients with residual tumor, determined from completion of the first cycle, were 0 CR or PR, 15 SD and 2 PD. There were a median progression-free survival of 12 weeks and a progression-free survival at 4 months of 29% with GeT chemotherapy alone. According to the primary objective of the study, the trial was stopped after the recruitment of 17 patients.

Sixteen of 17 patients received the full course of involved-field radiotherapy. On an intent-to-treat analysis, GeT followed by radiotherapy resulted in a median progression-free survival of 8 months and a progression-free survival rate at 12 months of 24% (4/17). For the 16 of 17 patients who received a full course of radiotherapy, median progression-free survival was 8 months, and progression-free survival at 12 months 25% (4/16). The median overall survival was 12 months.

Patients 1, 2, 4, 5, 6, 8, 10, 11, 14, 15 and 16 received second-line procarbazine, lomustine and vincristine (PCV) chemotherapy. One patient received 3 cycles, 3 patients received 2 cycles and 8 patients received 1 cycle before further progression was documented. Two patients were treated with 1 cycle of temozolomide after progression under PCV. One patient progressed and 1 patient suffered prolonged myelosuppression after one cycle.

There was no association between age and response to GeT as assessed by the number of completed cycles and no correlation between age and overall survival. Further, there was no association between the number of completed GeT cycles and overall survival.

Discussion

The concept of preirradiation chemotherapy for glioblastoma is not new [10] but has not been rigorously followed and improved despite some promising results, e.g., with intra-arterial cisplatin [11]. More recently, taxol [12], temozolomide [13] and gemcitabine [1] have been evaluated as a first-line therapy for glioblastoma. There was tumor progression with taxol in 27 of 34 patients (79%) within 9 weeks [12] and with gemcitabine in 12 of 21 patients (57%) within 8 weeks with

a median progression-free survival with gemcitabine alone of 11 weeks [1]. Probably the best results have been achieved with preirradiation temozolomide, this is, a progression-free survival at 4 months of 55% [13]. The consensus of these studies was that delayed radiotherapy did not adversely affect outcome for patients enrolled in these studies. Further, these studies did not allow single patients who were in remission to be further treated with chemotherapy until progression occurred. Thus, prolonged responses to chemotherapy in a subset of glioma patients may have been missed. Recently, experimental data of synergistic activity of gemcitabine and treosulfan in choroidal melanoma with effective tumor kill in up to 86% of cases could be demonstrated [5]. Further, a phase I trial in various cancer types showed minimal toxicity and beneficial palliative effects of GeT combination chemotherapy with the same regimen used in our study. These data prompted us to evaluate GeT in the treatment of newly diagnosed glioblastoma.

Here, we report the results of a phase II clinical trial of preirradiation GeT chemotherapy for patients with newly diagnosed glioblastoma. Five of 17 patients (29%) were progression-free during the chemotherapy phase of this protocol, suggesting that GeT alone has only modest antiglioma activity at the dose and schedule used here. Three patients had to be discontinued on chemotherapy because of major, reversible hematological toxicity, indicating the regimen administered was within a therapeutic range. On intention-to-treat analysis, median progression-free survival of GeT chemotherapy followed by radiotherapy for all patients was 8 months, and progression-free survival with this regimen was 24% at 12 months. The overall median survival of 12 months is within the range of most large glioblastoma trials [14]. The number of deep venous thrombosis (35%) in this study over the median follow-up of 12 months and DVT in 4 of 17 (24%) during GeT is within the expected range of deep venous thrombosis in the course of malignant glioma [15] and may thus not be specifically attributed to GeT chemotherapy.

In summary, the current regimen of GeT followed by radiotherapy provided no advantage over radiotherapy alone in the treatment of glioblastoma.

References

1. Weller M, Streffer J, Wick W, Kortmann RD, Heiss E, Küker W: Preirradiation gemcitabine chemotherapy for newly diagnosed glioblastoma. A phase II study. *Cancer* 91: 423–427, 2001
2. Feit PW, Rastrup-Andersen N, Matagne R: Studies on epoxide formation from (2S,3S)-threitol-1,4-bis(methanesulfonate). The preparation and biological activity of (2S,3S)-1,2-epoxy-3,4-butanediol 4-methanesulfonate. *J Med Chem* 13: 1173–1175, 1970
3. Gropp M, Meier W, Hepp H: Treosulfan as an effective second-line therapy in ovarian cancer. *Gynecol Oncol* 71: 94–98, 1998
4. Schmidt F, Wick W, Herrlinger U, Dichgans J, Weller M: Treosulfan for recurrent malignant glioma. *J Neuro-Oncol* 49: 231–234, 2000
5. Neale MH, Myatt N, Cree IA, Kurbacher CM, Foss AJ, Hungerford JL: Combination chemotherapy for choroidal melanoma: *ex vivo* sensitivity to treosulfan with gemcitabine or cytosine arabinoside. *Br J Cancer* 79: 1487–1493, 1999
6. Szelenyi H, Thiel E, Niederle N, Keilholz U: A phase I trial of gemcitabine and treosulfan (GeT) to overcome multidrug resistance in patients with advanced solid tumors. *Proc Am Soc Clin Oncol* 18: 228a, 1999
7. Gertler SZ, MacDonald D, Goodyear M, Forsyth P, Stewart DJ, Belanger K: NCIC-CTG phase II study of gemcitabine in patients with malignant glioma (IND.94). *Ann Oncol* 11: 315–318, 2000
8. MacDonald DR, Cascino TL, Schold SC, Cairncross JG: Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol* 8: 1277–1280, 1990
9. Simon R: Designs for efficient clinical trials. *Oncology* 3: 43–49, 1989
10. Watne K, Nome O, Hager B, Hirschberg H: Pre-radiation chemotherapy in glioma patients with poor prognostic factors. *J Neuro-Oncol* 13: 261–264, 1990
11. Dropcho EJ, Rosenfeld SS, Morawetz RB, Vitek J, Brothers M, Gorum T: Preirradiation intracarotid cisplatin treatment of newly diagnosed anaplastic gliomas. *J Clin Oncol* 10: 452–458, 1992
12. Fetell MR, Grossman SA, Fisher JD, Erlanger B, Rowinsky E, Stockel J: The new approaches to brain tumor therapy central nervous system consortium: preirradiation paclitaxel in glioblastoma multiforme: efficacy, pharmacology, and drug interactions. *J Clin Oncol* 15: 3121–3128, 1997
13. Friedman HS, McLendon RE, Kerby T, Dugan M, Bigner SH, Henry AJ: DNA mismatch repair and O⁶-alkylguanine-DNA alkyltransferase analysis and response to temodal in newly diagnosed malignant glioma. *J Clin Oncol* 16: 3851–3857, 1998
14. Fine HA, Dear KB, Loeffler JS, Black PM, Canellos GP: Meta-analysis of radiation therapy with and without adjuvant chemotherapy for malignant gliomas in adults. *Cancer* 72: 2585–2597, 1993
15. Marras LC, Geerts WH, Perry JR: The risk of venous thrombembolism is increased throughout the course of malignant glioma: an evidence based-review. *Cancer* 89: 640–646, 2000

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