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Original Research Article

Effect of temozolomide combined with radiotherapy on survival and MGMT protein expression in recurrent malignant glioma patients

Feng Yun, Huang Honghui, Gao Jing, Liu Shizhu*
Medical College of Jiangsu University, Nanjing City, Jiangsu Province 212000, China

For correspondence:* ***Email:* *ds1293@163.com*

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Abstract

Purpose: *To investigate the effect of temozolomide (TMZ) combined with radiotherapy (RT) on O-6-methylguanine-DNA methyltransferase (MGMT) protein and survival of recurrent malignant glioma patients.*
Methods: *Ninety-two patients with malignant glioma in our hospital from January 2014 to January 2015 were assigned to study and control groups using the random table method. Subjects in the control group received radiotherapy (total dose in the range of 60 – 75 Gy), while those in the study group were given TMZ orally (75 mg/m2) daily in addition to radiotherapy, as well as TMZ at 150 – 200 mg/m2. After treatment, clinical effectiveness was compared for the two groups. Changes in methylation of MGMT gene were determined in the two groups. The patients were followed up for 3 years, and the degrees of survival and recurrence were recorded.*
Results: *Total effectiveness of clinical treatment was markedly higher in the study group (76.09 %) than in the control group (45.65 %; $p < 0.05$). One month after radiotherapy, significant decrease in MGMT gene methylation was seen in patients in the study group, relative to control patients ($p < 0.05$). Patients in the study group had lower median recurrence but higher degree of survival in the 2nd and 3rd years, relative to control patients ($p < 0.05$).*
Conclusion: *The combination of temozolomide and radiotherapy is more effective than radiotherapy in the treatment of recurrent malignant glioma. The combined treatment significantly inhibits tumor recurrence in patients, and improves their prognosis and standard of life.*

Keywords: *Temozolomide, Radiotherapy, Recurrent malignant glioma, Survival rate, MGMT protein*

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INTRODUCTION

Gliomas are tumors derived from neuro-epithelial tissue, and the most common primary intracranial tumors. The World Health Organization (WHO) classified gliomas into grades (1 –IV). Grades III and IV are malignant gliomas which account for 40 – 50 % of all gliomas [1, 2]. Malignant glioma is invasive. Thus, it has no obvious boundary with normal brain tissue, and it easily relapses after surgical resection. Therefore, it is usually treated with a combination of radiotherapy and chemotherapy. Traditional alkylating agents and chemotherapeutic drugs are the first choices for

clinical treatment, but most of these drugs cannot penetrate the blood-brain barrier. In addition, they have lots of side effects, and their effectiveness is poor [3]. Studies have shown that TMZ, a new anti-tumor activity and alkylating agent, can get through the blood-brain barrier, is highly effective, and is associated with very few adverse reactions [4]. In this study, TMZ was combined with radiotherapy and used to treat recurrent malignant glioma. The degree of survival, and the impact of the combined therapy on MGMT protein were determined.

METHODS

General clinical information on patients

Ninety-two malignant glioma patients were used as subjects in this study.

Inclusion criteria: Patients in the following categories were included: patients with malignant glioma recurrence confirmed by biopsy or postoperative pathology; glioma patients in grade III or grade IV consistent with WHO central nervous system tumor grade; glioma patients aged 18 years or above, with estimated survival time ≥ 3 months; patients with Cartesian function score > 60 points; glioma patients who had not received other radiotherapy and chemotherapy within the month preceding the study, and patients who signed informed consent with their family members for the treatment.

***Exclusion* criteria:** Patients with a history of other malignant tumors, glioma patients with heart, hepatic and renal dysfunctions, and cardiovascular and hematopoietic diseases; and patients with contraindications for radiotherapy and chemotherapy, were excluded. Other excluded patients included those with severe allergies, especially those who are allergic to TMZ; pregnant and lactating patients, patients who did not follow doctor's advice on the treatment regimen, and those who refused to be followed up. The patients comprised 52 men and 40 women in the age range of 20 - 76 years (mean age = 51.9 ± 3.5 years). There were 50 cases of grade III glioma, and 42 cases of grade IV glioma. Two equal groups of patients were used: observation and control groups (46 patients /group). The observation and control groups had no appreciable differences in age, gender and pathological grade (p > 0.05). The general clinical data of the patients are shown in Table 1.

This research received approval from the Ethical Committee of Affiliated Hospital of Medical College of Jiangsu University (approval no. 20187879), and was performed according to the guidelines of Declaration of Helsinki promulgated in 1964 as amended in 1996 [5].

Table 1: Baseline profile of the patients

Variable	Study group	Control group	*P* value
Sex: Male/Female	27/19	25/21	0.547
Mean age (years)	52.4 ± 3.1	51.3 ± 3.5	0.982
KPS score	69.3 ± 12.1	68.5 ± 12.8	0.701
Pathological grade III	26	24	0.679
IV	24	18	
Surgical resection under microscope	6	10	0.897
Most/near full resection	30	25	
Biopsy only/not removed	10	14	

Treatment

All patients received chemo-radiotherapy 2 to 4 weeks after surgery. The subjects in the control group received radiotherapy alone. Using CT-simulated three-dimensional conformal radiotherapy (3D-CRT) technique, each of the patients was placed in the supine position for CT and MRI positioning, and the head was fixed with skull fixing plate. The 3 mm thin layer continuous enhanced CT was coplanar or non-coplanar irradiation, and the clinical target area included the surgical margin as tumor target or the area 2 – 3 cm outside the edema. The planned target area was 0.5 - 1.0 cm outside the clinical target area. The target area specification was delineated, and the radiotherapy dose was 2 Gy/time/day after conventional segmentation, and 5 times a week. The total dose was 60 Gy. The study group received oral TMZ (75 mg/m^2) along with radiotherapy, with continuous administration for 5 days, and discontinuation for 23 days (one treatment cycle was 28 days). Thereafter, TMZ (150 – 200 mg/m^2) was given as adjuvant chemotherapy once and 5 days before each radiotherapy. According to the patient's reaction, mannitol and dexamethasone were given to reduce cerebral edema reaction.

Study indicators

Clinical efficacy

Clinical effectiveness (TE) was assessed using the following criteria according to the WHO effectiveness evaluation criteria [6]: (a) Complete remission (CR): complete disappearance of tumor sustained for at least 4 weeks; (b) Partial remission (PR): reduction in tumor lesion volume

by more than 50 %, at least for 4 weeks; (c) Stable (SD) : tumor lesion volume reduction < 50 % or tumor volume increase not exceeding 25 % for at least 4 weeks; (d) Progression (PD): 25 % increase in at least one lesion.

$$TE = \frac{(CR + PR)}{N} \times 100 \ldots\ldots\ldots\ldots (1)$$

where TE is total effectiveness and N is total number of cases.

Quality of life

This was based on the pre-treatment Kelvin score (KPS). An increase of 10 points in the patient's KPS score indicated improvement in the quality of life, and a reduction by 10 points indicated reduced quality of life. An increase less than 10 points or the decrease not more than 10 indicated stable quality of life.

Plasma MGMT protein

The degree of methylation of MGMT gene was assessed using nested methylation-specific PCR.

Long-term efficacy

The extent of survival of patients and the degree of recurrence in the first, second and third years were determined through regular follow-up of patients after treatment.

Adverse reactions

Routine blood tests and liver and kidney function tests were performed weekly during treatment to evaluate the adverse drug reactions and drug resistance.

Statistical analysis

Numeric data are presented as mean ± SD, and were statistically analyzed with *t*-test. Count data are expressed as n (%), and χ^2 test was used for analysis. Statistical Package for the Social Sciences, version 19, was used for statistical analyses. Statistical significance was fixed at $p < 0.05$.

RESULTS

Clinical effects post-treatment

Table 2 shows that after treatment, the study group had higher total effectiveness (76.09 %) than control group (45.65 %, $p < 0.05$).

Quality of life scores

As shown in Table 3, after treatment, KPS score was higher in the observation group (80.16 ± 4.52 than the corresponding control value (71.56 ± 2.35; $p < 0.05$). Improvement in quality of life in the observation patients was markedly higher than that in control patients ($p < 0.05$).

Differences in positive methylation of MGMT genes

As shown in Table 4, before radiotherapy, MGMT methylation in both groups were comparable ($p > 0.05$). However, one month after the start of radiotherapy, higher degree of MGMT gene methylation was seen in the control group patients (30.43 %) than in the observation group patients (13.04 %) ($p < 0.05$). After the treatment, MGMT gene methylation in both groups were comparable ($p > 0.05$).

Table 2: Clinical efficacy of treatments {n (%)}

Group	CR	PR	SD	PD	ORR
Study (46)	12n26.09)	23(50.00)	6(13.04)	5(10.87)	35(76.09)
Control (46)	9(19.57)	12(26.09)	11(23.91)	14(30.43)	21(45.65)
χ^2	2.674	4.592	-2.154	-3.027	6.813
p	0.032	0.013	0.037	0.029	0.004

n = 46

Table 3: Quality of life improvement {n (%)}

Group	Improvement in quality of life	Reduced quality of life	Stable quality of life
Study	26 (56.52)	9 (19.57)	11 (23.91)
Control	19 (41.30)	15 (32.61)	12 (26.09)
χ^2	2.674	-2.154	4.592
p	0.032	0.037	0.013

Table 4: Methylation of MGMT genes {n (%)}

Group	Methylation cases {n (%)}		
	Before treatment	***1 month after treatment***	***3 years after treatment***
Study	17(36.95)	6(13.04)	3(6.52)
Control	16(34.78)	14(30.43)	8(17.39)
χ^2	0.043	4.574	2.210
p	0.836	0.032	0.137

Survival rate and recurrence

Table 5 shows that the percentage survival values in the two groups in the first year were comparable ($p > 0.05$). However, in year two and year three, higher percentage survival and median time of recurrence were seen in the observation group patients, relative to control patients *(*$p < 0.05$).

Adverse reactions

As shown in Table 6, the side effects seen in the two groups were myelosuppression, nausea and vomiting; and fever and anemia, mostly in 1st and 2nd degrees. There were no significant differences in adverse reactions between the two groups ($p > 0.05$).

DISCUSSION

Gliomas are common central nervous system tumors. Patients with gliomas often have high intracranial pressure symptoms, and some patients have seizures. Generally, it is thought that the symptoms are related to genetic, environmental, immune dysfunction and other factors [7]. Studies have shown that residual tumor cells are the main cause of recurrence of malignant gliomas. Once malignant gliomas recur, the growth rate is very rapid, and it is very invasive [8,9]. Therefore, postoperative adjuvant radiotherapy and chemotherapy are the main treatment options against malignant glioma.

However, traditional chemotherapeutic drugs have limited effectiveness because they bind to plasma proteins, and so cannot pass the blood-brain barrier. They also inhibit hematopoietic function of the bone marrow. Temozolomide (TMZ), a highly effective and low-toxicity imidazole derivative, is rapidly absorbed orally and can directly penetrate the blood-brain barrier and enter the cerebrospinal fluid to act on brain tumor tissues. It is very effective against primary and recurrent malignant gliomas [10].

Studies have shown that TMZ has a synergistic effect when combined with radiotherapy for treatment of malignant glioma: the combined treatment significantly improves long-term survival, and exerts mild adverse reactions [11]. This study found that the combination of TMZ and radiotherapy was significantly more effective in treating malignant glioma patients than radiotherapy alone. Total efficacy in the study group was 76.09 %, which was markedly superior to that in control group (45.65 %). Moreover, improvement in quality of life was appreciably higher in the study group than in the control. These findings suggest that TMZ combined with radiotherapy is clinically effective in patients with malignant glioma and improves their quality of life. These results are consistent with the reports of Zhang and Cui [12].

The main adverse reaction of TMZ is myelosuppression. In this study, there were 9 cases of myelosuppression in the observation group, accounting for 19.57 %, and 8 cases in the control group, accounting for 17.39 %.

Table 5: Survival rate and recurrence time for patients {n (%)]

Group	1st year survival {n (%)}	2nd year survival {n (%)}	3rd year survival {n (%)}	Median recurrence time (months)
Study	40 (86.95)	30 (65.22)	17 (39.53)	21.65 ± 9.32
Control	33 (71.73)	15 (32.61)	7 (15.22)	14.36 ± 8.56
P-value	> 0.05	< 0.05	< 0.05	< 0.05

Table 6: Incidence of adverse reactions {n (%)}

Adverse reaction	Study group (n=46)	Control group (n=46)	χ^2	*P*-value
Reduced white blood cell count	8 (17.39)	5 (10.86)	1.013	0.092
Radioactive brain edema	7 (15.21)	5 (10.86)	0.692	0.346
Nausea/vomiting	8 (17.39)	7 (15.21)	0.958	0.087
Fever	3 (6.52)	2 (4.34)	0.263	0.739
Myelosuppression	9 (19.57)	8 (17.39)	1.171	0.068
Anemia	7 (15.21)	5 (1086)	0.703	0.294

This study has revealed that TMZ combined with radiotherapy for treating malignant glioma has high safety and low adverse reactions, and bone marrow suppression did not accumulate. In a 3-year follow-up of the patients, first-year survival in the two groups were comparable, but the second and third-year survival and median recurrence times were markedly higher in the observation group. These findings confirm that TMZ combined with radiotherapy is effective in inhibiting tumor cells, reducing recurrence and prolonging survival of patients. This is in agreement with previous reports [13].

Studies have shown that MGMT expression is implicated in resistance to TMZ in malignant glioma. The MGMT gene is a DNA repair gene which is important for tumor repair, and can transfer alkyl groups from guanine to itself. On the cysteine residue, the alkylating agent guanine on the DNA is reduced, and MGMT becomes the inactive alkylating group. This eliminates the toxic effect of the alkylating agent on the tumor cells, resulting in resistance to TMZ [14]. Therefore, before the treatment of malignant glioma, the positive expression of MGMT protein in the tumor tissue is determined, and different chemotherapy regimens are then formulated according to the sensitivity of the tumor to the alkylating agent.

This study showed that the positive methylation of MGMT in both groups were comparable prior to treatment. However, one month after the start of radiotherapy, the methylation of MGMT gene in study group was markedly decreased (13.04 %), relative to corresponding control value (30.43 %). This reflects the re-inhibition of tumor proliferation by chemotherapy. The reason is that TMZ is rapidly converted to methyltriazepen-imidamide under the certain hydrogen ion concentration in the stage of tumor cell division. It exerts its cytotoxic effect by enhancing DNA alkylation in tumor cells. Temozolomide increases sensitivity to radiotherapy and promotes its anti-carcinogenic properties [15,16].

Limitations of the study

Few participants took part in this study. The subjects were all from Asia i.e. only from one area. So, the application of the conclusion drawn from this work is limited.

CONCLUSION

The combination of TMZ and radiotherapy is clinically effective, and significantly improves the quality of life of malignant glioma patients by reducing the positive expression of plasma MGMT protein. It inhibits tumor proliferation and recurrence, prolongs survival time of patients, and is associated with reduced adverse reactions. Thus, the combination treatment appears safe and easily tolerated by patients.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Liu SZ conceived and designed the study. Feng Yun, Huang Honghui, Gao Jing and Liu SZ collected and analyzed the data, while Feng Yun wrote the manuscript.

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