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Patients treatment with neuroglioma by teniposide and semustine and its influence on Twist and E-cadherin expression

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Abstract This study focuses on curative effects of teniposide combining with semustine on patients with neuroglioma and the influences on the expression of Twist and E-cadherin in tissue. Sixty-eight patients with neuroglioma taking operation in our hospital were divided into two groups randomly. Single radiotherapy was given to 34 patients in group A, and teniposide (VM-26) and semustine (Me-CCUN) were added to radiotherapy for 34 patients in group B. Then, curative effects, survival rate, living quality and adverse reaction rate after operation were compared between two groups. Moreover, the difference in positive expression rate of Twist and E-cadherin before and after treatment between two groups was analyzed by immunohistochemistry. Results: In group B, the effective rate of treatment was 88.2%, and the disease control rate was 70.6%, higher than 52.9% and 32.4% in group A with statistical significance ($P < 0.05$). Moreover, the survival rate in three years of group B was 44.1%, and the score of living quality was 67.11 ± 4.32, and also higher than 23.5% and 63.79 ± 4.53 in group A with statistical significance ($P < 0.05$). However, the difference between two groups in adverse reaction rate has no statistical significance ($P > 0.05$). In addition, the difference in positive expression rate of Twist and E-cadherin between group A and group B has no statistical significance before treatment ($P > 0.05$). After treatment, however, the positive rate of Twist in group B is lower than that in group A, while the positive rate of E-cadherin is higher. Both differences have statistical significance ($P < 0.05$). Chemotherapy of VM-26 combining with Me-CCNU can inhibit Twist expression and improve the expression rate of E-cadherin to help improving the curative effects and living quality and increasing survival rate.

1. Introduction

Neuroglioma, also called gliocytoma, is one of the common malignant tumors in central nervous system at present. Neuroglioma can make aggressive growth around brain tissue, so complete radical treatment can be realized by single excision. Therefore, radiotherapy and chemotherapy are always

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combined in clinical. However, the curative effects of different chemotherapeutics are always greatly different for its various varieties and combination with different drugs (Johnson et al., 2014; Rogers, 2013). Therefore, the reasonable combination with chemotherapeutics becomes the key to the postoperative researches on patients with neuroglioma. As the current research hotspots, Twist, the anti-apoptosis protein and E-cadherin, the mark of epithelial–mesenchymal transition have been generally accepted for their role playing in carcinogenesis and transfer (Ding et al., 2012; Min et al., 2013). Currently, there has no report on the influences of chemotherapeutics on such protein expression. However, the curative effect will be more persuasive if the role of chemotherapeutics can be explained in aspect of protein level. Therefore, this work further analyzed the difference in Twist and E-cadherin protein expression before and after chemotherapy when studying the curative effects of teniposide with semustine on patients with neuroglioma. The research achievements are presented as follows:

2. Materials and methods

2.1. General data

68 patients taking therapy in neurosurgery of our hospital from March 2009 to June 2012 were selected. The inclusion criteria are as follows: (1) taking craniotomy in our hospital and being diagnosed by pathology with the complete follow-up materials after operation; (2) age in 20–60 years old, Karnofsky life quality score (Karnofsky, KPS) $\geqslant 60$, and survival period $\geqslant 3$ months; (3) no radiotherapy and chemotherapy contraindication and no severe injury in organs of heart, lung, liver and kidney; and (4) marrow: white blood cell $\geqslant 4 * 10^9$/L, $100 * 10^9$/L blood platelet, and hemoglobin $\geqslant 80 * 10^9$/L. Patients were divided into group A of single radiotherapy and group B of radiotherapy with chemotherapy with 34 patients for each group. In group A, there were 18 male patients and 16 female patients in age of 26–53 years old with average age of (38.5 ± 5.6) years old. Tumor site: 13 patients in frontal lobe, 9 patients in temporal lobe, 7 patients in occipital lobe and 5 patients in parietal lobe. Pathological grading: 8 patients in grade II, 14 patients in grade III and 12 patients in grade IV. In group B, there were 17 male patients and 17 female patients in age of 26–56 years old with average year of (39.1 ± 5.5) years old. Tumor site: 12 patients in frontal lobe, 10 patients in temporal lobe, 8 patients in occipital lobe and 4 patients in parietal lobe. Pathological grading: 9 patients in grade II, 13 patients in grade III and 12 patients in grade IV. According to statistical analysis, there is no statistical difference between two groups in aspects of sex, age, tumor site, pathological grading, etc.

2.2. Therapeutic method

All the patients took operative treatment, and tumor tissue was excised as much as possible without affecting the neurological function of patients. In this work, 34 patients took full excision, 19 patients took sub-excision and 15 patients took partial excision. In group A, patients took single radiotherapy after operation with total dose of 50–60 Gy. Based on the treatment in group B, patients in group B took combined chemotherapy. The chemotherapy plan is as follows: the first chemotherapy was conducted for patients 7–10 days after radiotherapy with VM-26 by intravenous administration in dose of 60–8060–80 mg/m^2, $d_{1\text{-}2}$ and with Me-CCUN by oral administration in dose of 60–80 mg/m^2, $d_{3\text{-}4}$. The administration of both medicines was repeated every 8–12 weeks. According to the tolerance condition by patients, 2–7 courses can be given. Before chemotherapy, 125 ml of 20% mannitol was administrated by intravenous drip to improve the blood–brain barrier permeability.

2.3. Immunohistochemistry

Surgery tissue was embedded by paraffin and sectioned (4 μm) after being fixed with 10% formalin fixation. Then, immunohistochemical staining was conducted by SP method. Primary antibodies of Twist polyclonal antibody and E-cadherin monoclonal antibody and secondary antibodies were all purchased from R&D Co. Immunohistochemical kit was purchased from Shanghai Zemaisheng Biotechnology Co., Ltd.

2.4. Evaluation criteria

According to RECIST (Schramm et al., 2013) complete remission (CR): focus disappears for more than 4 weeks; partial remission (PR): focus shrinks by $\geqslant 50\%$ for 4 weeks; stable disease (SD): focus shrinks by $< 50\%$ or enlarges by $< 25\%$; progression disease (PD): focus enlarges $\geqslant 25\%$ or new focus appears. Response rate (RR) = (CR + PR)/total patients in this group * 100%; disease control rate (DCR) = (CR + PR + SD)/total patients in this group * 100%. After patients were discharged from hospital, follow-up visit was conducted by telephone, outpatient service, etc., and the living condition of patients 1, 2 and 3 years after operation was recorded. Before and after therapy, KPS was used to evaluate the living quality of patients. In addition, drug safety was evaluated according to the grading standard of adverse drug reaction regulated by American National Cancer Institute.

2.4.1. Result judgment

Positive cell rates in 5 random views under 400× microscope were calculated. Twist is positive after staining cell nucleus or cytoplasm around cell nucleus. The score was counted as follows: no color (score 0), light yellow (score 1), yellow (score 2) and brown (score 3); positive cell amount 1–10% (score 1), 11–25% (score 2), 26–50% (score 3) and above 50% (score 4). By adding above two items, if the positive cell amount $< 1\%$ (−), the score is 1–3 scores (+), 4–5 scores (++) and 6–7 scores (+++). E-cadherin is positive if the cytomembrane and cytoplasm near cytomembrane are yellow or claybank, and the positive rate can be calculated by the percentage of staining positive cells accounting for total cells. The rate can be recorded as follows: positive rate $< 10\%$ (−), 10–25% (+), 26–50% (++) and $> 50\%$ (+++). The glioma tissue of patients was detected 1 year after operation and chemotherapy, respectively.

2.5. Statistical method

SPSS statistical analysis software was used to do chi-square test with patient number as numerical data and independent

or matching sample t-test with ($\bar{x} \pm s$) as numerical data. Then, survival rates of both groups were compared with single-factor survivorship curve (Kaplan–Meier) for Log rank test. When $P < 0.05$, the difference has statistical significance.

3. Results

3.1. Comparison of recent curative effects

No death occurred during treatment in both groups. RR and DCR distribution of group A are 32.4% and 52.9%, respectively, lower than 70.6% and 88.2% in group B. The difference has statistical significance (Table 1).

3.2. Survival condition

Kaplan–Meier method was used to calculate the survival condition in both groups. The survival rates in three years of group A and group B are 23.5% and 44.1%, respectively. Log rank test shows that the difference between two groups has statistical significance ($X^2 = 8.344$, $P = 0.003$) (as shown in Fig. 1).

3.3. KPS score in both groups

Before treatment, the difference of KPS score between two groups has no statistical significance. After treatment by different schemes, KPS score of group B is significantly higher than that of group A. Moreover, the difference has statistical significance (Table 2).

3.4. Adverse reaction

Two patients with I-grade myelosuppression in group A and 5 patients receiving I-grade bone marrow transplantation in group B recovered without long-term marrow inhibition after taking treatment with leukogenic drugs. In addition, 10 patients in group A and 14 patients in group B had different degrees of digestive symptoms, including diarrhea, nausea and emesis. After taking expectant treatment, these patients turned better. Statistical analysis shows that the difference of adverse reaction rate between two groups has no statistical significance ($X^2 = 4.870$, $P = 0.300$).

3.5. Twist expression condition in two groups before and after treatment

Before treatment, the positive rate of Twist expression in two groups has no significant difference ($Z = -4.342$, $P = 0.463$). After treatment, however, the positive difference of Twist expression in two groups has statistical significance ($Z = -8.852$, $P = 0.032$) (as shown in Figs. 2 and 3).

Table 1 Comparison treatment effect of two groups.

Groups	n	CR	PR	SD	PD	RR (%)	DCR (%)
Group A	34	2	9	7	16	32.4	52.9
Group B	34	3	21	6	4	70.6	88.2
X^2						9.950	10.204
P						0.041	0.037

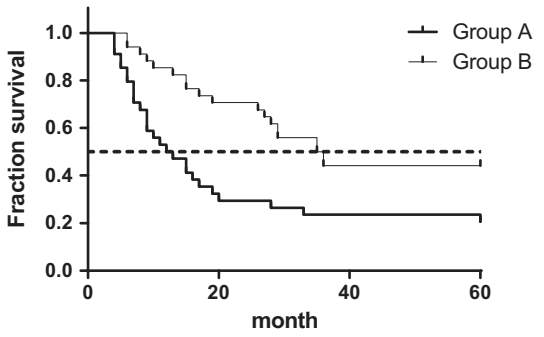

Figure 1 The survival curves of the two groups of patients.

Table 2 Comparison of KPS of patients in two groups before and after treatment.

Groups	n	Before treatment	After treatment	t	P
Group A	34	61.28 ± 4.86	63.79 ± 4.53	2.202	0.034
Group B	34	61.34 ± 4.92	67.11 ± 4.32	5.138	0.000
t		0.055	3.092		
P		0.959	0.004		

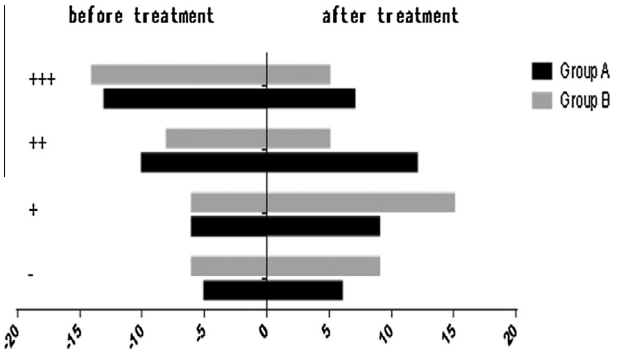

Figure 2 Twist the positive rate of two groups of patients before and after treatment.

3.6. E-cadherin expression in two groups before and after treatment

Before treatment, the positive rate of E-cadherin expression in two groups has no significant difference ($Z = -3.225$, $P = 0.532$). After treatment, however, the positive rate in two groups has statistical significance ($Z = -7.942$, $P = 0.028$) (as shown in Figs. 4 and 5).

4. Discussion

4.1. Current status of neuroglioma treatment

Statistical data show that the annual morbidity of neuroglioma is 10–20/100 thousand people accounting for about 45–66% in primary intracranial tumors. This disease primarily occurs to people in age of 20–50 years old with 5-year survival rate lower than 5% (Killela et al., 2013). However, some researches show that the higher the malignant degree is, the stronger the ability

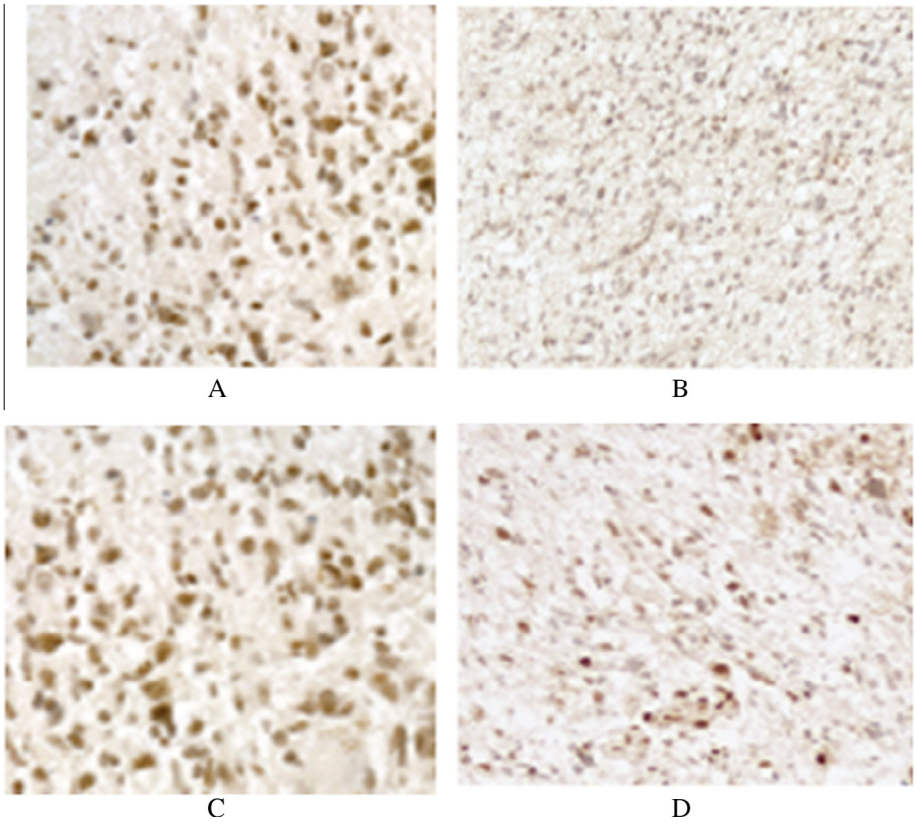

Figure 3 (A, C) is group A Twist protein expression before and after treatment, (B, D) is group B Twist protein expression before and after treatment.

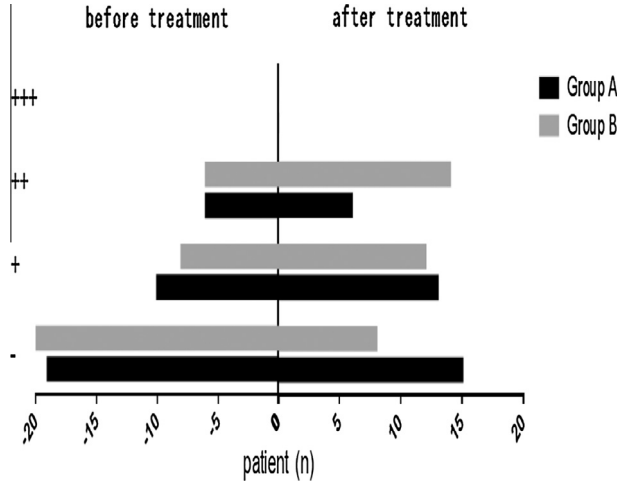

Figure 4 E-cadherin the positive rate of two groups of patients before and after treatment.

of cytotoxin resistance, invasion transfer and proliferation induced by tumor vessel will be because neuroglioma always presents invasive growth and has no obvious demarcation in normal brain tissue (O'Kane et al., 2013). Moreover, the characteristics of "three highs and one low" that is, high morbidity, high fatality rate, high recurrence rate and low cure rate are much more significant. The technology of excising glioma by neuro-navigation-guided or fluorescence-guided operation can overcome problem of incomplete excision or injury to brain tissue to some extent (Nong et al., 2013). However, for patients with high malignant degree or obvious tissue invasion, the curative effects of this technology are not satisfied, while the curative effects of radiotherapy and chemicals treatment are controversial. Therefore, it is significant to deeply understand the mechanism of neuroglioma and make further research on the influence factors on operative effects and postoperative recurrence in view of molecule.

4.2. Application of VM-26 with Me-CCUN in neuroglioma

Traditional chemotherapeutics can be divided into (1) nitrosoureas, including lomustine and carmustine, (2) natulane, and (3) cisplatin. However, most of these drugs have following problems: (1) restriction of drug entrance by blood brain barrier only allows few lipid soluble drug passing; (2) tumor heterogeneity and tolerance; and (3) side effect of drug. References reported that VM-26 with Me-CCUN can not only improve drug effects, but also reduce adverse reaction (He et al., 2015). As a main drug for DNA damage, Me-CCUN can make DNA dead by combing it and interrupting the cell cycle. Also as the drug for DNA damage, VM-26 plays a role in DNA topoisomerase. Therefore, the combination of these two drugs can not only reduce drug dose for each other and decrease the adverse reaction, but also lower the drug resistance. According to the research results in this work, the radiotherapy with medical chemotherapy has obvious advantages compared with single radiotherapy. For example, the effectiveness rate and disease

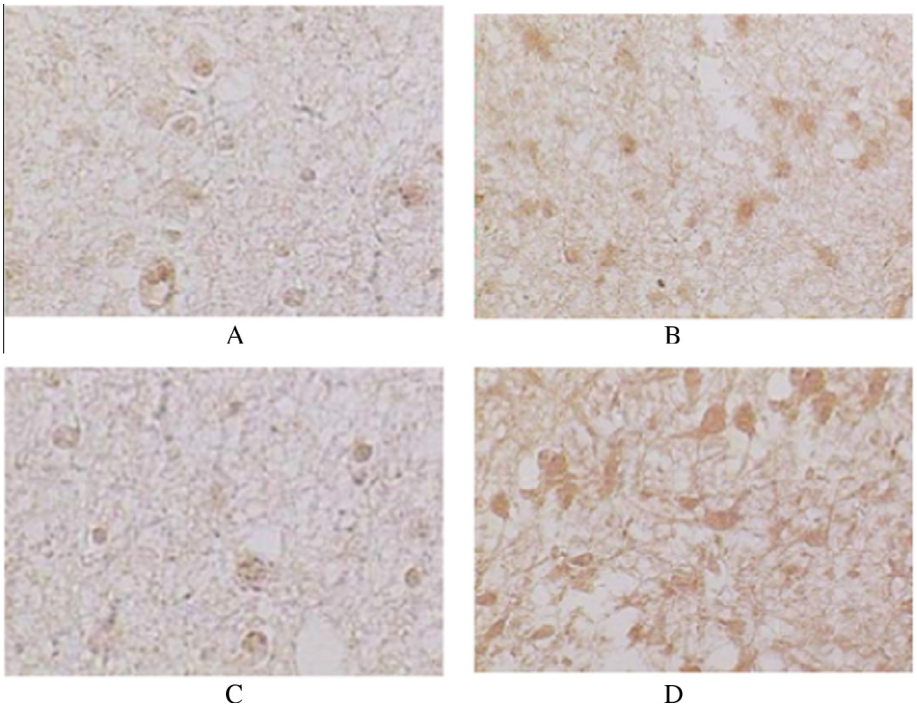

Figure 5 (A, C) is group A E-cadherin protein expression before and after treatment, (B, D) is group B E-cadherin protein expression before and after treatment.

control rate of former are 70.6% and 88.2%, respectively, which are higher than those of later 32.4% and 52.9%. The difference has statistical significance. The result is relatively consistent with that of Smoll et al. (2013), by which the treatment measure for 125 patients with neuroglioma is the same as that in this work. The results in the study by Smoll show that the effective rate is 73% and disease control rate is 90.2%. In addition, for patients taking synchronous radiotherapy, the three-year survival rate is 44.1% and the living quality score is 67.11 ± 4.32. For patients taking single radiotherapy, the three-year survival rate is 23.5% and living quality score is 63.79 ± 4.53. The difference between both is significant. Although the adverse reaction rate for patients taking synchronous radiotherapy is higher than that for patients taking single radiotherapy, the statistical test shows that the difference between both has no statistical significance. With expectant treatment, the adverse symptoms of patients taking synchronous radiotherapy have been relieved to some extent. For example, intravenous injection of 5HT3 receptor antagonist can prevent emesis.

4.3. Twist, E-cadherin and neuroglioma

At present, more and more molecular markers related to tumor are found, including Twist, E-cadherin, PTEN and Ki-67 (Yang and Mao, 2013). Twist is also called as twist protein which has functions of adjusting embryonic development, cell differentiation, organ growth, etc. Some researches show that Twist has high expression in lung cancer, intestinal cancer, breast cancer and tumor tissues. Nordfors et al. (2015) also found in the study on 52 patients with neuroglioma and non-tumor tissue of 30 patients that the Twist expression level of former is significantly higher than that of later ($Z = -8.51$, $p < 0.01$), and the expression level in higher-level glioma tissue is also higher than that in lower-level tissue ($Z = -3.42$, $p < 0.05$). In addition, Khan verified that Twist can not only improve the cell survival and its malignant transformation by inhibiting p53 genes, but also induce epithelial–mesenchymal transition (Khan et al., 2013). One of the specificity marks of later is E-cadherin. The study shows that E-cadherin protein mainly mediates interaction among cells, and it plays an important role in cellular morphological change, signal transduction and structural integrity of cells. E-cadherin expression is abnormal, which can always cause loose cellular adhesion and make tumor cells transfer or invade. For example, Xiong et al. (2012) found in intestinal tumor research that E-cadherin in tumor tissue is always in low expression, and this phenomenon is negatively related to the tumor differentiation degree, lymphatic metastasis and Ducks stages that is, patients with low expression rate always have bad prognosis. Xiong et al. (2014) found that Twist and E-cadherin have specific negative correlation ($r = -0.57$, $p < 0.01$) when studying the relationship between both for patients with neuroglioma. This result helps us to judge the treatment prognosis of patients with neuroglia.

4.4. Influences of chemotherapeutics on Twist and E-cadherin

Former researches show that the present biological marks are mainly applied to the judgment of tumor diagnosis or treatment prognosis. During the treatment, only a few researches focus on the expression of tumor markers. The research on Twist expression before and after radiotherapy or chemotherapy of 54 patients with intestinal cancer shows that Twist expression level is significantly lower than that of patients with radiotherapy. However, Twist expression in both groups before treatment has no difference (Fan et al., 2013).

5. Conclusion

According to the results in this work, the positive Twist rate of patients taking synchronous chemotherapy is lower than that of patients taking radiotherapy. Meanwhile, the positive E-cadherin rate is higher than that in radiotherapy group. At present, however, we cannot effectively explain why chemotherapeutics have effects on protein expression. Even though we can still prove that the combined chemotherapy is beneficial to improve the survival rate and living quality of patients after operation, it is also related to the inhibition or high expression of protein.

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