

Clinical efficacy of gamma knife and surgery treatment of mesial temporal lobe epilepsy and their effects on EF-Tumt and EF-Tsmt expression

X.-Q. WANG, X.-D. ZHANG, Y.-M. HAN, X.-F. SHI, Z.-B. LAN,
X.-X. MEN, Y.-W. PAN

Neurosurgery Department, Lanzhou University No. 2 Hospital, Lanzhou, Gansu, China

Abstract. – **OBJECTIVE:** To study the clinical efficacy of gamma knife and surgery treatment of mesial temporal lobe epilepsy (MTLE) and their effects on EF-Tumt and EF-Tsmt expression.

PATIENTS AND METHODS: The data of 78 cases of MTLE patients treated in our hospital from April 2011 to March 2013 were retrospectively analyzed. The patients were divided into two groups according to the treatment methods: the surgery group (including 41 cases) and the gamma knife group (including 37 cases). The clinical efficacy, the occurrence and recurrence of complications were evaluated, respectively; meanwhile, the expression of the EF-Tumt protein and EF-Tsmt protein in brain tissue were analyzed.

RESULTS: The difference between the efficacy rate of the two groups showed no statistical significance ($\chi^2=0.960$, $p>0.05$). The complication rate of the gamma knife group was significantly lower than that of the control group ($\chi^2=6.430$, $p<0.05$). The recurrence rate of the patients in the gamma knife group was significantly lower than that of the patients in the surgery group ($p>0.05$). Within the two groups, the positive expression granum of EF-Tsmt protein and EF-Tumt protein of the two groups after treatment were significantly lower than that before treatment ($p<0.05$). After treatment, the positive expression granum of EF-Tsmt protein of the patients in the gamma knife group was obviously more than that of the patients in the surgery group ($p<0.05$). The difference between the positive expression granum of EF-Tumt protein of the two groups showed no statistical significance ($p>0.05$). Before and after treatment within the group, the positive cell of EF-Tsmt protein and EF-Tumt protein of the two groups of patients after treatment were significantly lower than that before treatment ($p<0.05$). After treatment, the difference between the EF-Tsmt protein positive cell and the EF-Tumt protein positive cell of the two groups of patients showed no statistical significance ($p>0.05$).

CONCLUSIONS: Both surgery and gamma knife could treat MTLE effectively, and the efficacy may be related to the ability to reduce the expression of EF-Tsmt protein and EF-Tumt protein in brain tissue.

Key Words:

Gamma knife, Surgery treatment, Mesial temporal lobe epilepsy, Clinical efficacy, EF-Tumt, EF-Tsmt expression.

Abbreviations

Mesial temporal lobe epilepsy = MTLE; magnetic resonance imaging = MRI; electroencephalogram = EEG; single photon emission computerized tomography = SPECT; positron emission tomography = PET; vemişon computerized tomography = ECT; phosphate buffer solution; PBS; S-P immunohistochemical method = S-P IHC.

Introduction

Mesial temporal lobe epilepsy (MTLE) is a clinically common epilepsy syndrome, which is suitable to be treated with surgical intervention. The results showed that the effective rate of surgical treatment was 70%^{1,2}. The clinical incidence of this disease is relatively high, with complex pathogenesis, varied attack forms and various clinical symptoms, which will cause great damages to patients' physical and psychological health and greatly increase the burden of the patient's family. Therefore, the research of the clinical treatment of MTLE and the improvement of prognosis have great significance. Gamma knife, as a kind of efficient target radiation damage treatment means, is increasingly used in the clinical

cal treatment of MTLE in recent years; some patients who refused surgery will choose this kind of treatment^{3,4}. Also, some researches show that when epilepsy attacks, the protein translation in mitochondria of patients will increase; the mitochondria protein translation elongation factors EF-Tumt and EF-Tsmt are two kinds of important proteins in the process of mitochondrial translation^{5,6}. At present, there are many clinical studies on gamma knife and surgical treatment of temporal lobe epilepsy, but most of them only analyzed the clinical curative effects. Due to the lack of deep research, it is very difficult to explore the therapeutic mechanism and find new therapeutic targets so as to improve the clinical efficacy. Thus, this study aims at systematically studying the clinical efficacy of gamma knife and surgery treatment of MTLE and their effects on EF-Tumt and EF-Tsmt expression to provide clinically scientific basis for further improvement of the therapeutic effect of this disease.

Patients and Methods

Patients

78 cases of MTLE patients treated in our hospital from April 2011 to March 2013 were retrospectively analyzed. The patients were divided into two groups, the surgery group and the gamma knife group. The surgery group included 41 patients, including 25 males and 16 females, the age were ranged from 15 to 59, the average age was 30.1 ± 2.8 ; the age of patients with the epilepsy history were 2-28, and the average age was 9.3 ± 1.5 ; 18 cases were located in left temporal lobe, 20 cases at right temporal lobe and 3 cases at bilateral temporal lobes. The gamma knife group was 37 patients, including 21 male and 16 female. The age of patients was from 15 to 58, and the average age was 29.4 ± 3.2 . The age of patients with the epilepsy history was 2-27, and the average age was 9.2 ± 1.8 . The focus of 15 cases was located at left temporal lobe, 17 cases at right temporal lobe and 5 cases at bilateral temporal lobes. There was no significant difference between the two groups of patients in age, medical history, gender and other basic data ($p > 0.05$).

Inclusion Criteria and Exclusion Criteria

Inclusion criteria: (1) all patients selected had been diagnosed as MTLE based on the Brain MRI Check, Scalp EEG and Long-term EEG before surgery; (2) all patients selected had not cerebral trauma within three years after treatment; (3) all

patients selected and their families had been informed and agreed to this study and signed informed consent forms. Exclusion criteria: (1) the patients who suffered from serious substantive organ diseases, such as heart, liver, spleen diseases; (2) the patients accompanied with other neurologic diseases.

Treatment method

The treatment method of the gamma knife group

Based on EEG and MRI, the treatment target point was comprehensively considered and determined by combining PET and SPECT. The midpoint of the former combined-posterior combined line was found by using the MRI image under the Leksell type locator, which was considered as the original point. The anatomical location of amygdaloid nucleus was X=21 mm, Y=8 mm, Z=13.5 mm; the location of amygdaloid nucleus was determined and the image characteristics of MRI was combined, and 4 mm or 8 mm collimator treatment was conducted for the patients. The target point at the mesial or lateral temporal lobe combined PET, SPECT, EEG and clinical symptoms was determined, and conducted by 14 mm or 18 mm collimator treatment. The treatment dose was as follows: the dose at the edges of amygdaloid nucleus and hippocampal area: 15-20 Gy (18.5 Gy in average), 45-50% isodose chart coverage: 1.65 cm^3 - 7.20 cm^3 (5.31 cm^3 in average); the dose at the edges of forehead and anterior temporal lobe: 15-25 Gy (19.5 Gy in average), 50-60% isodose chart, 5-8 iso-middle-point. The optic tract radiation dose was lower than 12 Gy. The lateral cleft blood vessels radiation dose was lower than 20 Gy and the pars opercularis radiation dose was lower than 12 Gy.

The treatment method of the surgery group

The conventional cortical or deep EEG monitoring was carried out during surgery to determine the epileptic focus position and the resection scope. If the intraoperative monitoring showed that the abnormal discharge was limited to the temporal lobe, standard anterior temporal lobectomy could be conducted. For the patients whose epileptogenic foci was considered at the mesial temporal lobe before surgery but in fact spread to the temporal lobe during surgery, the temporal lobe or the hippocampal areas could be removed, as well as the amygdaloid nucleus and entorhinal cortex. If the foci had affected the functional area,

intraoperative function test and intraoperative waking technology could be used to determine the functional area, then surgery of epileptogenic foci resection combined with multiple cortical thermocoagulation or multiple soft tissue transection could begin.

Efficacy Evaluation

The clinical efficacy was evaluated according to the Engel Epilepsy Surgical Therapy Results Classification Method recommended by the First International Conference on Epilepsy Surgical Therapy (in 1987)⁷. The specific content was as follows. Satisfactory: the epilepsy was completely under control and antiepileptic drugs were stopped; significant improvement: the attack frequency was reduced by more than 75%, and the dose of antiepileptic drugs was reduced; good: the epilepsy attack frequency was reduced by more than 50%; inefficacy: when compared with the conditions before treatment, the epilepsy attack frequency and the dose of antiepileptic drugs of the patients had no significant changes.

Sample Collection

The cortical brain tissues of the two groups of patients during the operation were collected for preoperative EF-Tumt and EF-Tsmt protein expression analysis; after operation, in the treatment of traumatic brain injury, patients' brain tissues were collected for the evaluation of postoperative EF-Tumt and EF-Tsmt protein expression.

Observed Indicators and Methods

(1) Detection of positive expression granum of EF-Tumt protein and EF-Tsmt protein: the brain tissues were fixed at the temporal lobe cortex by using 4% glutaraldehyde solution. After the processes of dehydration, the tissues were soaked in 1% hydrogen peroxide, and mouse anti-human EF-Tumt antibody or EF-Tsmt antibody produced by Shanghai Bioleaf Biotech Co., Ltd., (Minxing District, Shanghai, China) was added. Phosphate buffered saline (PBS) was used as negative control, and then tissues were placed at indoor temperature for 1 h. After, they were placed at 4°C for 24-36 h. The samples were rinsed with PBS and the incubation of antibodies with colloidal gold was marked. Meanwhile, the samples were dyed with uranyl acetate and uranyl citric acid (Shanghai Yu Sen Biotechnology Co., Ltd., Xuhui District, Shanghai, China) under electron microscope, and five positions were selected for observation: upper left, lower left, upper right,

lower right and center of each section. After the analysis of image analysis software, the counts of the positive expression granum of EF-Tumt protein and EF-Tsmt protein were recorded, and the mean value was calculated. (2) Detection of positive cell of EF-Tumt protein and EF-Tsmt protein: the brain tissue was fixed at the temporal lobe cortex by using 10% formaldehyde solution (Shanghai Bioleaf Biotech Co., Ltd., Minxing District, Shanghai, China); the positive cell of EF-Tumt protein and EF-Tsmt protein was detected by using S-P IHC method. The specific steps were as follows: thermal remediation was conducted on brain tissue section, and mouse anti-human EF-Tumt antibody or EF-Tsmt antibody was added, then the samples were placed at indoor temperature for 10 min and were marked by using second antibody with biotin labeling (streptomycin antibiotic-peroxidase) and diaminobenzidine (DAB) color reagent (Changchun Dingguo Biotechnology Co., Ltd., Chaoyang District, Changchun, China). Then, vitrification management was conducted by xylene (Shanghai Bioleaf Biotech Co., Ltd., Minxing District, Shanghai, China). Finally, the samples were sealed with neutral balsam (Shanghai Bioleaf Biotech Co., Ltd., Minxing District, Shanghai, China), and were observed under 400 x light microscope; after then, the counts of the two kinds of proteins were analyzed by image analysis software, respectively.

Statistical Analysis

SPSS 19 software (SPSS Inc., Chicago, IL, USA) was used for data processing; the measurement data was expressed in mean value $\pm$ standard deviation. *t*-test was used for comparison between the groups while for the comparison before and after the treatment was done by using matched *t*-test. Comparison of the ranked data was done by using rank sum test; count data was expressed in percentage; χ^2 test was used for the comparison between the groups. For all comparisons, if $p < 0.05$, the difference was significant.

Results

Comparison of Clinical Efficacy Between two Groups

The rank sum test showed that the clinical efficacy difference between the two groups had no statistical significance ($u=0.390$, $p>0.05$); the χ^2 test results showed that the efficacy rate differen-

Table I. Comparison of clinical efficacy between two groups.

Groups	Satisfactory	Significant improvement	Good	Inefficacy	Efficacy rate
Surgery group (n=41)	31	4	4	2	39 (95.1)
Gamma knife group (n=37)	27	3	3	4	33 (89.2)
χ^2	0.390	0.960			
p	0.700	0.326			

ce between the two groups had no statistical significance ($\chi^2=0.960$, $p>0.05$) (Table I).

Comparison of Complications and Recurrence between two groups

The difference of the occurrence rate of such complications as mental symptom, visual field defect, extradural hematoma and declination of memory between the two groups was not significant ($p>0.05$). The complication rate of the patients in the gamma knife group was significantly lower than that of the patients in the surgery group ($\chi^2=6.430$, $p<0.05$). The recurrence rate of the patients in the gamma knife group was significantly lower than that of the patients in the surgery group ($p>0.05$) (Table II).

Comparison of the Counts of Positive Expression Granum Between two Groups Before and after treatment

After treatment, the positive expression granum of EF-Tsmt protein and EF-Tumt protein

of the two groups were significantly lower than that before treatment ($p<0.05$). Before treatment, the difference of the positive expression granum of EF-Tsmt protein and EF-Tumt protein between the two groups had no statistical significance ($p>0.05$). After treatment, the positive expression granum of EF-Tsmt protein of the patients in the gamma knife group was more than that of the patients in the surgery group ($p<0.05$). The difference between the positive expression granum of EF-Tumt protein of the two groups had no statistical significance ($p>0.05$) (Table III).

Comparison of Positive cell of Protein Between two Groups Before and After treatment

After treatment, the positive cell of EF-Tsmt protein and EF-Tumt protein of the two groups of patients were significantly lower than that before treatment ($p<0.05$). Before treatment, the difference of the positive cell of EF-Tsmt protein and EF-Tumt protein between the two groups had no

Table II. Comparison of complication rate and recurrence rate between two groups.

Groups	Mental symptom	Visual field defect	Extradural hematoma	Declination of memory	Recurrence rate	Complication rate /n (%)
Surgery group (n=41)	4	4	5	3	1	16 (39.02)
Gamma knife group (n=37)	3	0	1	1	6	5 (13.51)
χ^2	0.060	3.800	2.470	0.850	4.520	6.430
p	0.799	0.051	0.116	0.356	0.034	0.011

Table III. Comparison of positive expression granum of EF-Tsmt protein and EF-Tumt protein between two groups before and after treatment.

Groups	Positive expression granum of EF-Tsmt protein		Positive expression granum of EF-Tumt protein	
	Pre-therapy	Post-treatment	Pre-therapy	Post-treatment
Surgery group (n=41)	109.82±17.08	45.13±8.04	114.72±21.07	38.27±6.17
Gamma knife group (n=37)	108.92±15.17	53.27±7.42	113.09±19.47	42.31±7.41
t	0.245	4.617	0.354	2.626
p	0.807	0.000	0.725	0.010

Note: *indicated that compared with pre-therapy, the difference was significant ($p<0.05$).

statistical significance ($p>0.05$); after treatment, the difference of the EF-Tsmt protein positive cell and the EF-Tumt protein positive cell between the two groups had no statistical significance ($p>0.05$) (Table IV).

Discussion

In recent years, with the maturity of the surgical treatment theory and methods for epilepsy^{8,9}, MTLE has become one of the indications of surgical treatment; meanwhile, surgical treatment can damage the amygdaloid nucleus and hippocampal area of the patients, which cannot only reduce the excitability of the strengthened structure of epileptogenic focus, but also damage the transmission channel of the epilepsy impulse and regulate the dysfunction of limbic systems, so as to control the attack of epilepsy greatly. As a treatment method, it is different from conventional surgery. Gamma knife has been widely used in the clinical treatment of epilepsy and has achieved good clinical efficacy. The principle of treatment is that single high-dose special radiobiological effect is adopted within the fine-focused target volume to damage or functionally suppress cells, and to achieve therapeutic effects, but the therapeutic mechanism of this treatment method is still unclear¹⁰. Also, seizures can cause the imbalance of energy metabolism, increase heat production, and increase the production of enzymes involved in the respiratory chain, that will further make the mitochondrial protein translation extremely active¹¹. EF-Tsmt protein and EF-Tumt protein are important proteins in the process of mitochondrial translation. Some studies have shown that the expression level of the two kinds of proteins is closely related to the epileptic attack. Therefore, this study aims at systematically analyzing the clinical efficacy of gamma knife and surgery treatment of MTLE, and their effects on EF-Tumt and EF-Tsmt expression, so as to provide reference for proving clinical therapeutic

mechanism and exploring new treatment options. In the aspect of clinical efficacy, the results of this work showed that the difference between the efficacy rates of the two groups had no statistical significance. However, the efficacy rate of the gamma knife group was slightly lower, and the recurrence rate of this group was significantly higher than that of the control group. The reasons for the above results included two aspects: on the one hand, in the process of gamma knife treatment, the excess target dose or too large volume will cause radioactive cerebral edema, even cause radiation-induced brain injuries, which results in dysfunction. In general condition, in the process of gamma knife treatment, the dose will be inadequate and it will cause lower efficacy rate. On the other hand, the surgery process will be guided by cortex electrode, so that the focus could be removed sufficiently, and the clinical efficacy will be higher. The complication rate of the gamma knife group was lower than that of the control group, among which, hypomnesia of the patients in the surgery group may be related to the excess excision of hippocampal area during the surgery. Less excision of the hippocampal area will cause poor clinical efficacy and more excision of the hippocampal area will cause poor memory. The analysis of the expression of EF-Tumt protein and EF-Tsmt protein in brain tissue shows that, within the two groups, both the positive expression granum and the positive cells of the EF-Tsmt protein and EF-Tumt protein after treatment were significantly lower than those before treatment. After treatment, the positive expression granum of EF-Tsmt protein of the patients in the gamma knife group was more than that of the patients in the surgery group; the difference between the positive expression granum of EF-Tumt protein in the two groups had no statistical significance. After treatment, the difference of the EF-Tsmt protein positive cell and the EF-Tumt protein positive cell between the two groups of patients had no statistical significance. Combined with animal experiment, it showed that when rats were in expe-

Table IV. Comparison of positive cell of EF-Tsmt protein and EF-Tumt protein between two groups before and after treatment.

Groups	Positive cell of EF-Tsmt protein		Positive cell of EF-Tumt protein	
	Pre-therapy	Post-treatment	Pre-therapy	Post-treatment
Surgery group (n=41)	53.17±7.13	27.13±3.17	54.72±11.08	28.26±4.17
Gamma knife group (n=37)	52.94±6.14	28.27±3.42	53.19±9.47	26.31±5.41
<i>t</i>	0.152	1.528	0.841	1.793
<i>p</i>	0.880	0.131	0.405	0.077

Note: *indicated that compared with pre-therapy, the difference was significant ($p<0.05$).

rimental epileptic state, the expression quantity of EF-Tsmt protein and EF-Tumt protein in brain tissue mitochondria are positively related to the attack time of epilepsy¹². From these results, we could presume that the clinical efficacy of surgery or gamma treatment of MTLE, may be related to the expression quantity of EF-Tsmt protein and EF-Tumt protein after the excision of a part of the brain or damage of a part of the brain cells.

Conclusions

Based on the above analysis, the following conclusions could be drawn: both surgery and gamma knife could treat MTLE effectively, and its effect may play a role in reducing the expression of EF-Tsmt and EF-Tumt protein in brain tissue.

Ethics Committee Approval

The above cases were confirmed by the hospital Ethics Committee Approval and their families signed informed consent.

Conflict of interest

The authors declare no conflicts of interest.

References

- 1) STEINHÄUSER C, GRUNNET M, CARMIGNOTO G. Crucial role of astrocytes in temporal lobe epilepsy. *Neuroscience* 2016; 323: 157-169.
- 2) TELLEZ-ZENTENO JF, LADINO LD. Temporal epilepsy: clinical, diagnostic and therapeutic aspect. *Rev Neurol* 2013; 56: 229-242.
- 3) REGIS J, BARTOLOMEI F, REY M, HAYASHI M, CHAUVEL P, PERAGUT J.C. Gamma knife surgery for mesial temporal lobe epilepsy. *J Neurosurg* 2000; 93 Suppl 3: 141-146.
- 4) REGIS J. Response: gamma knife surgery in mesial temporal lobe epilepsy. *Epilepsia* 2008; 46: 457-459.
- 5) DONATI D, FOTHERINGHAM J, AKHYANI N, FOGDELL-HAHN A, VORTMEYER A, HEISS JD, WILLIAMS E, WEINSTEIN S, BRUCE DA, BONWETSCH R, GAILLARD WD, SATO S, THEODORE WH, JACOBSON S. 68: HHV-6 and mesial temporal lobe epilepsy: detection of viral DNA and antigen in brain tissue primary cultured astrocytes and implication for a viral role in pathogenesis. *J Clin Virol* 2006; 37: S115-S116.
- 6) JEPPESEN MG, NAVRATIL T, SPREMULLI LL, NYBORG J. Crystal structure of the bovine mitochondrial elongation factor tu.ts complex. *J Biol Chem* 2005; 280: 5071-5081.
- 7) TAN QF. *Epilepsy surgery*. Nanjing: Nanjing University Press 1995: 240-241.
- 8) IMATAKA G, ARISUE K. Epilepsy-related automobile accidents in Japan: legal changes about a precedent and penal regulations. *Eur Rev Med Pharmacol Sci* 2016; 20: 491-497.
- 9) HUANG JS, WU Y, HUANG Q, LI SJ, YE JM, WEI X, LIU QD, LIU Y, MA MG. Expression level and distribution of hmgb1 in sombati's cell model and kainic acid-induced epilepsy model. *Eur Rev Med Pharmacol Sci* 2015; 19: 2928-2933.
- 10) GANZ JC, REDA WA. Radionecrosis following Gamma Knife treatment for mesial temporal lobe epilepsy. *Br J Neurosurg* 2011; 25: 649-651.
- 11) SIMEONE KA, MATTHEWS SA, SAMSON KK, SIMEONE TA. Targeting deficiencies in mitochondrial respiratory complex I and functional uncoupling exerts and anti-seizure effects in genetic model of temporal lobe epilepsy and in a model of acute temporal lobe seizures. *Exp Neurol* 2014; 251: 84-90.
- 12) GREENE NP, NILSSON MI, WASHINGTON TA, LEE DE, BROWN LA, PAPINEAU AM, SHIMKUS KL, GREENE ES, CROUSE SF, FLUCKEY JD. Impaired exercise-induced mitochondrial biogenesis in the obese Zucker rat, Respise PGC-1α induction, is due to compromised mitochondrial translation elongation. *Am J Physiol Endocrinol Metab* 2014; 306: E503-E511.