Article

J. Biol. Regul. Homeost. Agents. 2024; 38(7): 5455–5462
https://doi.org/10.23812/j.biol.regul.homeost.agents.20243807.437

Effects of Gamma Knife Irradiation on the Expression of CREB in the Brain of Chronic Epileptic Rats

Yu Yin[1,2], YuanWu Chen[1,2], Yao Wang[1,2], YouWei Zhang[2,3], XuePing Zhao[4], YaQing Su[5], ShaoHua Zhang[6], PeiYuan Lv[2,7], WeiBo Li[8,*]

[1]Department of Rehabilitation, Hebei General Hospital, 050051 Shijiazhuang, Hebei, China
[2]Hebei Provincial Key Laboratory of Cerebral Networks and Cognitive Disorders, 050000 Shijiazhuang, Hebei, China
[3]Department of Rehabilitation, Hebei Medical University, 050011 Shijiazhuang, Hebei, China
[4]Department of Nursing, Hebei General Hospital, 050051 Shijiazhuang, Hebei, China
[5]Department of Rehabilitation, Lixian Hospital, 071400 Baoding, Hebei, China
[6]Department of Medicine, Eighth People's Hospital of Hebei Province, 050000 Shijiazhuang, Hebei, China
[7]Department of Neurology, Hebei General Hospital, 050051 Shijiazhuang, Hebei, China
[8]Department of Gastrointestinal Surgery, The Second Hospital of Hebei Medical University, 050057 Shijiazhuang, Hebei, China
*Correspondence: Liweibo-99@163.com (WeiBo Li)
Submitted: 9 March 2023 Revised: 5 May 2023 Accepted: 12 June 2023 Published: 1 July 2024

Background: Epilepsy surgery has seen numerous technological advances in diagnostic and therapeutic procedures in recent years. However, further understanding of how to integrate traditional and emerging technologies into epilepsy treatment is needed to develop this area. The purpose of this study is to observe the antiepileptic effect of gamma knife irradiation on chronic epileptic rats and the expression of cyclic adenosine monophosphate response element binding protein (CREB) in the brain.
Methods: Sixty Wistar rats were randomly divided into four groups: the control group, the control + gamma knife group, the pentylenetetrazole (PTZ) group and the PTZ + gamma knife group. The rats were injected intraperitoneally with PTZ to establish the epileptic models. Gamma knife irradiation was performed on the bilateral frontal cortex of rats. After 12 weeks of irradiation, the Morris water maze test was used on each group of rats to test their ability to learn and remember, and the expression of CREB in the cortex and hippocampus was detected.
Results: The epileptic seizures of rats in the PTZ + gamma knife group were significantly reduced by the 12th week after low-dose gamma knife irradiation ($p < 0.05$). Compared with the PTZ group, the swimming distance was significantly shorter in the PTZ + gamma knife group ($p < 0.05$). Compared with the PTZ group, the daily escape latency in the Morris water maze of the PTZ + gamma knife group on days 3–5 was significantly shortened ($p < 0.05$). Compared with the PTZ group, the number of times the Morris water maze crossed the platform and the percentage swimming time in the platform quadrant were both significantly higher in the PTZ + gamma knife group ($p < 0.05$).
Conclusion: The cognitive function and the expression of CREB decreased in the brains of epileptic rats, which was increased after low-dose gamma knife irradiation. This may suggest a possible molecular mechanism underlying the effects of gamma knife irradiation on epileptic seizures.

Keywords: cyclic adenosine monophosphate response element binding protein; gamma knife; epilepsy; pentylenetetrazole

Introduction

Epilepsy is one of the most common neurological diseases. It is characterised by recurrent seizures and affects approximately 70 million individuals worldwide [1]. Nowadays, there are a variety of new drugs being marketed for epilepsy treatment, but the seizure rate has not changed. As a result, work is still needed to develop more sophisticated drugs to treat epilepsy. The pathogenesis and progression of epilepsy are related to many aspects, including ion channels, signalling transduction, synaptic transmission, inflammatory reactions and more [2–5]. All these factors are likely to be closely correlated with each other rather than being isolated. In particular, recent studies have revealed that the cyclic adenosine monophosphate response element binding protein (CREB), implicated in multiple signalling pathways, plays an important role in the pathogenesis and progression of epilepsy [6,7]. Meanwhile, CREB can activate or promote the expression of genes related to memory formation, which is closely related to synaptic plasticity, learning and memorising [8]. The treatment of refractory epilepsy using a gamma knife has been reported for a long time, with the advantages of non-invasiveness, safety, accurate positioning and good efficacy. Treatment with a gamma knife can protect the cognitive function of patients [9], although its biological mechanism remains un-

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

clear. From the CREB signalling pathway perspective, this study investigates the antiepileptic and cognitive protective effects of low-dose gamma knife irradiation on rats with epilepsy induced by pentylenetetrazole (PTZ).

Materials and Methods

Animals and the Establishment of Chronic Epilepsy Models

Animals

Sixty healthy adult Wistar rats (Experimental Animal Centre of Hebei Medical University, Shijiazhuang, China) were randomly divided into four groups: ① the control group; ② the gamma knife control group: the rats in this group were only irradiated by a gamma knife; ③ the PTZ group: epilepsy was induced by PTZ in the rats in this group; and ④ the PTZ + gamma knife group: the rats were irradiated with a gamma knife after epileptic seizures were induced by PTZ.

Establishment of Chronic Epilepsy Models

The rats were injected intraperitoneally with the central stimulant PTZ (P6500, Sigma-Adrich Corporation, St. Louis, MO, USA) once a day for 4 weeks to establish chronic epileptic models. After the epileptic seizures gradually appeared in the rats, the severity of the epileptic seizures was evaluated using the Racine scale (RS), which is one of the most commonly used tools to assess the seizure intensity in rodent models of experimental epilepsy. The RS categorises six stages of intensity and is based on the behavioural repertoire of the animals during a seizure, which includes 'represented no response' (stage 0); 'facial movements with saccade of ears and whiskers' (stage I); 'head nodding' (stage II); 'unilateral or bilateral limb clonus' (stage III); 'rearing with bilateral forelimb clonus' (stage IV) and 'seizures characterised by rearing and falling' (stage V) [10]. The success of the model preparation was judged by whether there were three consecutive episodes of stage IV or above. Thereafter, PTZ was injected once a week to maintain epileptic seizures. Experimental epilepsy models were successfully constructed in 24 of the 30 rats, with 12 rats placed in the PTZ group and 12 in the PTZ + gamma knife group. Six rats were excluded from the subsequent experiments. In addition, an electroencephalography (EEG) recording and a water maze were used to evaluate further the changes in the state or neurological function of the epileptic rats. The results of the experiments showed the successful establishment of a chronic epilepsy model in accordance with the RS.

Low-Dose Gamma Knife Irradiation

After the epilepsy models were successfully prepared by low-dose gamma knife irradiation [11], the rats were anaesthetised with 3% sodium pentobarbital (40 mg/kg) (P3761, Sigma-Adrich Corporation, St. Louis, MO, USA) intraperitoneal injections and fixed on the animal frame and the Leksell frame of the gamma knife. The authors used a magnetic resonance instrument (#9197, Cell Signaling Technology, Danvers, MA, USA) to conduct localisation scanning for the rats, and they transmitted the scanning images to the GammaPlan system (version 8.2, Leksell B, Stockholm, Sweden) to make sure that the left and right frontal lobes were the target areas of irradiation. Then, the authors used the gamma knife system (version 8.2, Leksell B, Stockholm, Sweden) to irradiate rats with a marginal dose of 15 Gy and a collimator whose diameter was 4 mm.

Electroencephalographic Recordings

Three rats were randomly selected from each group, and the MS4000U-1 Quantitative Bio-Signal Recording Analyser (MS4000U-1, Longfeida Technology Co., Ltd., Guangzhou, China) was applied at 1, 2, 4, 6, 8, 10 and 12 weeks after gamma knife irradiation to observe the changes in the EEG recordings of the rats. Each recording was made for 40 min, with each group recording for 5 min before and then 35 min after the drug was given intraperitoneally. The latency and frequency of seizure wave emission were recorded separately. The latency period was the time from drug administration to the first appearance of seizure waves, including spike, spine and spike/slow waves. The frequency was the average number of seizure waves per minute, taken for 1 min every 5 min after drug administration.

Morris Water Maze Test

Twelve weeks after gamma knife irradiation, each group of rats underwent the Morris water maze test to test their ability to learn and remember. The water maze experiment was divided into the place navigation test and the spatial probe test.

Place Navigation Test

The experiment lasted for 5 days. On day 1, the rats were allowed to swim freely in the water maze for 60 s to adapt to the water maze environment. On day 2, a transparent platform was placed in the centre of quadrant IV, and the rats were put into the water facing the wall from the central entry point of quadrants I to III. The computer monitored and recorded the trajectory of the rats from the entry point to the platform, the average time required (escape latency), the swimming distance and the strategy for finding the platform. If the rat failed to find the platform within 60 s, the rat was pulled onto the platform and left there for 30 s. The latency period was recorded as 60 s.

Spatial Probe Test

After the rats were trained for 5 days, as described above, the platform was removed. Then, the rats were placed in the water facing the pool wall from the entry point of the I quadrant on the opposite side of the platform. The

number of times the rats swam through the water maze at the place where the platform was originally placed (the number of rings penetrated), the percentage of the rats swimming distance in the original platform quadrant within 60 s and other information were recorded.

Immunohistochemical Detection

After 12 weeks of gamma knife irradiation, the rats in each group were anaesthetised with 3% sodium pentobarbital (40 mg/kg) (Hebei Bio-High Technology Development Co., Ltd., Shijiazhuang, China) and then fixed and routinely disinfected. After opening the chest, the vessels were irrigated with approximately 50 mL of phosphate buffered solution (pH 7.4, in-house) at 37 ℃, followed by approximately 100 mL of room temperature 4% paraformaldehyde (P6148, Sigma, St. Louis, MO, USA). After the rat's neck and limbs had become rigid, the head and neck were disinfected, the head was severed, the skull was opened, and the brain was carefully removed and fixed in 4% paraformaldehyde for 30 min. The brain was routinely dehydrated, transparent, waxed and embedded. The embedded brain tissue was divided into serial coronal sections to a thickness of 5 μm. The sections were routinely dewaxed to water, incubated with 3% H_2O_2 at 37 °C for 10 min, microwave (G80W23MSPP-N5, Galanz, Guangzhou, China) repair antigen at 92 °C~98 °C for 15 min and 10% normal goat serum at 37 °C for 30 min. After embedding and sectioning them, 1:200 Rabbit anti-CREB antibody (#9197, Cell Signaling Technology, Danvers, MA, USA) was added dropwise at 4 °C overnight and incubated with biotin-labeled secondary antibody (1:500) for 20–40 min at room temperature. Then, the authors used Image-Pro Plus 5.0 analysis software (National Institutes of Health, Bethesda, MD, USA) to calculate the number of CREB-positive cells in the frontal cortex and hippocampal CA1 region to measure the average optical density of CREB-positive cells.

Western Blot Detection

After 12 weeks of gamma knife irradiation, the authors decapitated the rats in each group and collected the frontal lobes and hippocampus. The total protein was extracted from these areas and stored at –80 ℃ for later use. Approximately 2~10 μL of the protein was subjected to 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (IPVH00005, Millipore, Burlington, MA, USA). The membranes were blocked with sealing fluid for 15 min. They were then incubated with primary antibodies against Rabbit anti-CREB monoclonal antibody (1:500, #9197, Cell Signaling Technology, Danvers, MA, USA) and goat polyclonal to beta-actin (1:200, Signa 1.5T HDxt, General Electric Company, Boston, MA, USA) overnight at 4 °C. Next, the membranes were incubated with a secondary antibody (1:5000) for 1~2 hours at room temperature. BAND SCAN image 5.0 analysis software (International Business Ma-

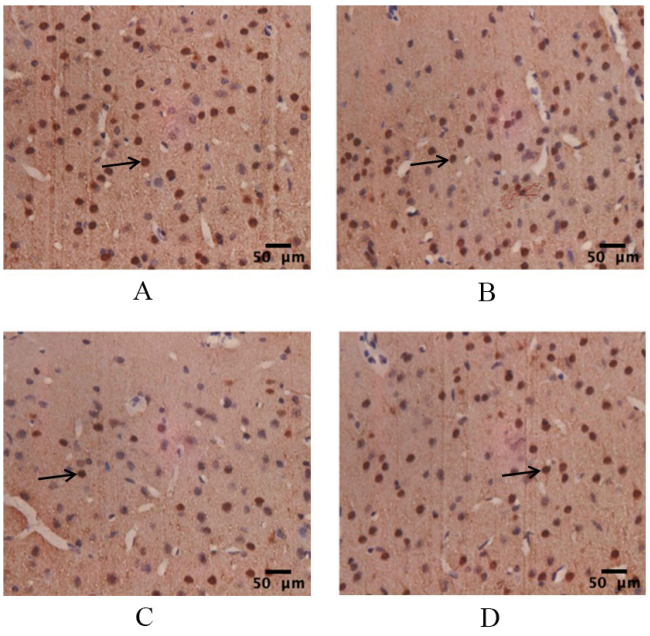

Fig. 1. Expression of CREB in frontal cortex of rats in each group (IHC, ×400). (A) Control group; (B) Control + gamma knife group; (C) PTZ group; (D) PTZ + gamma knife group. Arrows are creb-stained nuclei, and the magnification is ×400, scale bar = 50 μm. IHC, immunohistochemistry.

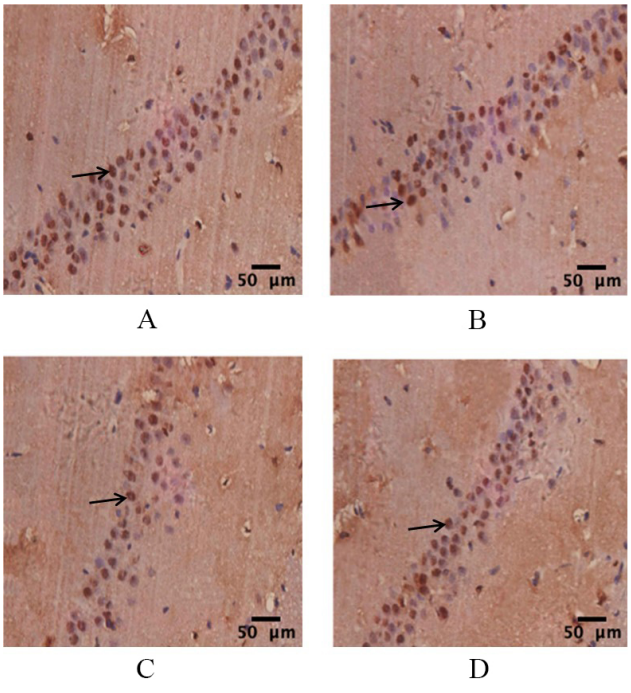

Fig. 2. Expression of CREB in hippocampal CA1 region (IHC, ×400). (A) Control group; (B) Control + gamma knife group; (C) PTZ group; (D) PTZ + gamma knife group. Arrows are creb-stained nuclei, and scale bar = 50 μm.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Table 1. Seizure of rats in the two groups (n).

Groups	Grade	8 w					10 w					12 w				
		I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
PTZ group		1	1	1	5	4	0	1	2	4	5	0	1	1	4	6
PTZ + gamma knife group		1	2	2	4	3	2	2	2	3	3	3	2	2	3	2

Note: Comparison between the two groups at 12 w, Z = –2.27, $p < 0.05$. PTZ, pentylenetetrazole.

Table 2. The comparison of latency and frequency of seizure waves in two groups (means ± SEM).

Weeks	n	PTZ group		PTZ + gamma knife group	
		latency (min)	frequency (n/min)	latency (min)	frequency (n/min)
1 w	12	3.77 ± 0.85	114.56 ± 17.92	3.84 ± 0.87	111.11 ± 13.22
2 w	12	3.55 ± 0.64	110.33 ± 17.71	3.67 ± 0.89	126.44 ± 13.81
4 w	12	3.11 ± 0.71	124.86 ± 20.61	3.65 ± 0.59	103.37 ± 12.79
6 w	12	3.43 ± 0.64	111.82 ± 13.43	4.05 ± 0.85	98.86 ± 19.37
8 w	12	3.25 ± 0.67	123.16 ± 19.34	4.42 ± 0.68[a]	86.13 ± 20.42[a]
10 w	12	2.87 ± 0.63	113.51 ± 20.34	4.77 ± 0.53[a]	83.25 ± 23.48[a]
12 w	12	3.24 ± 0.54	121.58 ± 14.72	5.15 ± 0.85[a]	70.52 ± 20.37[a]

Note: [a] Compared with PTZ group, $p < 0.05$. SEM, standard error of mean.

chines Corporation, Armonk, NY, USA) was used to determine the grey scale values of the protein electrophoresis bands and to compare the grey scale of each target band with the internal reference β-actin band for semi-quantitative analysis.

Statistical Methods

SPSS 21.0 statistical software (International Business Machines Corporation, Armonk, NY, USA) was applied for statistical processing, and the experimental data were expressed as mean ± standard deviation ($\bar{x} \pm s$). When comparing the groups, if the data satisfied the principles of independence, normality and chi-square, the comparison between the means of each group was performed by analysis of variance. If there was a statistical difference between the groups, the SNK-q test was used for further two-by-two comparisons. If the principles of independence, normality and chi-square were not met, the Kruskal-Wallis H rank-sum test was used, and if there was a statistical difference between the four groups, further two-by-two comparisons were made. $p < 0.05$ was considered statistically significant.

Results

Epileptic Seizures in Rats

Compared with the PTZ group, the seizure degree of the PTZ + gamma knife group was gradually reduced, and there was a statistical difference between the two groups after 12 weeks of gamma knife irradiation (Z = –2.27, $p < 0.05$) (Table 1).

EEG Recordings Test

The EEG recordings of the rats in all groups were either α or β waves with a few scattered θ waves before the drug injection. In the control group, there was no significant change in background activity after the injection of saline. In the PTZ group, after the injection of PTZ, the rats began to show a single spike, spine and spike/slow waves at about 2~5 minutes, which then gradually turned into a frequent high-potential spike, spine and spike/slow waves. In the PTZ + gamma knife group, at weeks 8–12 after gamma knife irradiation, compared with the PTZ group, the rats showed significantly longer latency ($p < 0.05$), lower wave amplitude and significantly less frequency ($p < 0.05$) of epileptic waves, and only a few scattered low-amplitude spikes or sharp waves were seen (Table 2).

Morris Water Maze Test

The swimming distance was significantly shorter in the PTZ + gamma knife group than in the PTZ group ($p < 0.05$), and the swimming distance decreased with increasing irradiation time (Table 3). The daily escape latency in the Morris water maze of the PTZ + gamma knife group on days 3–5 was significantly shortened compared with the PTZ group ($p < 0.05$) (Table 4). In addition, the number of times the Morris water maze crossed the platform and the percentage swimming time in the platform quadrant were both significantly higher in the PTZ + gamma knife group than in the PTZ group ($p < 0.05$) (Table 5). These results suggested that gamma knife irradiation ameliorated the cognitive function in the rats exposed to PTZ.

Immunohistochemical Test

Compared with the control group, the number of CREB-positive neurons in the frontal cortex and hippocam-

Table 3. The comparison of swimming distance of every day in Morris water maze in four groups (means ± SEM).

Groups	n	Swimming distance (m)			
		2 d	3 d	4 d	5 d
Control	15	10.99 ± 2.53	7.31 ± 1.96	5.21 ± 1.78	4.72 ± 1.55
Control + gamma knife	15	9.77 ± 2.03	7.24 ± 1.91	6.07 ± 1.98	5.03 ± 1.42
PTZ	12	14.21 ± 3.43[a]	11.03 ± 2.06[a]	10.16 ± 2.59[a]	9.76 ± 1.23[a]
PTZ + gamma knife	12	11.71 ± 2.61[b]	8.22 ± 1.58[b]	7.85 ± 1.65[b]	6.43 ± 1.66[b]

Note: [a] Compared with control group, $p < 0.05$; [b] Compared with PTZ group, $p < 0.05$.

Table 4. The comparison of the escape latency of every day in Morris water maze in four groups (means ± SEM).

Groups	n	Escape latency (s)			
		2 d	3 d	4 d	5 d
Control	15	46.43 ± 12.51	34.17 ± 11.28	22.26 ± 6.62	16.31 ± 4.63
Control + gamma knife	15	47.89 ± 12.05	30.27 ± 10.91	24.47 ± 7.38	15.53 ± 5.41
PTZ	12	54.46 ± 13.25	49.25 ± 12.14[a]	39.18 ± 10.36[a]	34.85 ± 7.24[a]
PTZ + gamma knife	12	47.60 ± 12.65	36.26 ± 11.53[b]	29.53 ± 8.64[b]	23.91 ± 6.36[b]

Note: [a] Compared with control group, $p < 0.05$; [b] Compared with PTZ group, $p < 0.05$.

Table 5. The comparison of the number of platform crossings and the percentage of swimming time in platform quadrant in Morris water maze of four groups (means ± SEM).

Groups	n	Number of platform crossings	Percentage of swimming time (%)
Control	15	3.83 ± 0.94	47.58 ± 10.78
Control + gamma knife	15	3.58 ± 0.84	46.00 ± 8.71
PTZ	12	1.33 ± 0.71[a]	28.00 ± 8.67[a]
PTZ + gamma knife	12	2.67 ± 1.00[b]	35.78 ± 8.23[b]

Note: [a] Compared with control group, $p < 0.05$; [b] Compared with PTZ group, $p < 0.05$.

pal CA1 region of the PTZ group decreased significantly, and the average optical density decreased significantly. Compared with the PTZ group, the number of CREB-positive neurons in the frontal cortex and hippocampal CA1 region of the PTZ + gamma knife group was significantly increased, and the average optical density was significantly increased (number of positive neurons: F frontal = 9.27, F hippocampus = 7.17, $p < 0.01$; F frontal lobe = 5.89, F hippocampus = 4.29, $p < 0.05$). There was no statistical difference between the gamma knife control group and the control group (Figs. 1,2, Table 6).

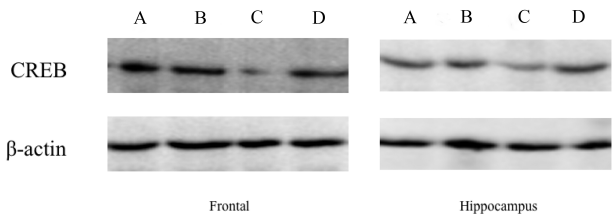

Fig. 3. The expression of CREB in the frontal cortex and hippocampus of each group was detected by Western blot. (A) Control group; (B) Control + gamma knife group; (C) PTZ group; (D) PTZ + gamma knife group.

Western Blot Test

Western blot results showed that the expression of CREB in the frontal cortex and hippocampus of the PTZ group was significantly lower than that of the control group. The expression of CREB in the PTZ + gamma knife group was significantly higher than that in the PTZ group (F frontal lobe = 5.76, F hippocampus = 6.87, $p < 0.01$). There was no statistical difference between the gamma knife control group and the control group (Fig. 3, Table 7).

Discussion

Epilepsy is one of the most frequent and disabling neurodegenerative disorders, with multiple underlying causes. In recent years, studies have shown that the CREB-mediated signalling pathway is closely related to neuronal excitation, which is the target of currently undeveloped antiepileptic drugs [12–14]. Cyclic adenosine monophosphate response element binding protein is a key transcriptional regulator that regulates gene transcription related to neuronal differentiation, synaptic plasticity, learning and memory [15,16]. Study has shown that sustained overexcitation of the hippocampal neural network could inhibit the growth of dendrites and limit the activation of CREB [17]. In addition, a large number of down-regulated genes were found in the hippocampus of epilepsy patients with

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Table 6. Comparison of the number of CREB immunoreactive neurons and the average optical density in each group (means ± SEM).

Groups	n	Number of immunopositive neurons		Average light absorption value	
		Frontal lobe	CA1	Frontal lobe	CA1
Control group	8	17.13 ± 2.80	23.50 ± 3.07	0.28 ± 0.03	0.32 ± 0.04
Control + gamma knife group	8	18.75 ± 2.82	22.38 ± 2.97	0.27 ± 0.03	0.32 ± 0.04
PTZ group	6	11.17 ± 2.79[a]	16.50 ± 3.08[a]	0.21 ± 0.04[a]	0.25 ± 0.03[a]
PTZ + gamma knife group	6	16.83 ± 2.64[b]	20.67 ± 2.58[b]	0.26 ± 0.04[b]	0.31 ± 0.03[b]

Note: [a] Compared with the control group, $p < 0.05$; [b] Compared with PTZ group, $p < 0.05$. CREB, cyclic adenosine monophosphate response element binding protein.

Table 7. Determination of CREB expression in brain tissues of rats by Western blot (means ± SEM).

Groups	n	CREB	
		Frontal lobe	Hippocampus
Control group	7	0.78 ± 0.10	0.86 ± 0.07
Control + gamma knife group	7	0.81 ± 0.07	0.85 ± 0.06
PTZ group	6	0.57 ± 0.11[a]	0.66 ± 0.13[a]
PTZ + gamma knife group	6	0.70 ± 0.16[b]	0.77 ± 0.08[b]

Note: [a] Compared with the control group, $p < 0.05$; [b] Compared with PTZ group, $p < 0.05$.

high seizure frequency and significant inactivation of the CERB signalling pathway [18]. Through its roles in multiple signalling pathways, CREB, as a transcription factor in the nucleus, regulates the transcriptional levels of selective molecular networks and subsequently modulates hippocampal synaptic remodelling and cellular excitability. Moreover, there is an apparent crosstalk between CREB and reactive oxygen species (ROS) production. Mitochondrial ROS production can induce CREB activation [19], whereas CREB directly regulates antioxidant gene expression and protects against oxidative stress-induced neuronal cell death by up-regulating brain-derived neurotrophic factor and heme oxygenase-1 [20,21]. In the present study, the results showed that the expression of CREB in the brain tissues of epileptic rats induced by PTZ was significantly decreased compared with that in the control group, suggesting that the biological mechanism of epileptic rats induced by PTZ may be related to the change in CREB expression in the brain tissues.

Gamma knife has the advantages of accurate positioning, safety and non-invasiveness and has been gradually recognised in the treatment of refractory epilepsy in recent years [22]. Study has found that low-dose gamma knife irradiation of 10~20 Gy can effectively inhibit epileptic seizures while no radiation damage is observed in the ultrastructure [23]. However, the antiepileptic mechanism of the gamma knife remains unclear. In this study, epileptic seizures were significantly reduced ($p < 0.05$), and CREB expression in the cerebral cortex and hippocampus was significantly increased ($p < 0.05$) in the PTZ + gamma knife group of rats after 12 weeks of low-dose gamma knife irradiation. Therefore, the authors speculate that the possible mechanism for epileptic seizure control by low-dose gamma knife irradiation is the increased CREB expression in the cerebral cortex and hippocampus, which causes synaptic structure changes and blocks synaptic transmission [24]. This result is consistent with the findings of the authors' previous study [25]. Moreover, it has been suggested that low-dose gamma knife irradiation can reduce the activity of epileptic neurons without causing necrosis of normal neurons [24]. Epileptogenesis is widely regarded as extremely complicated because many risk factors contribute to its causes, and whether CREB is directly involved in each remains poorly understood. Although several data have been collected in animal models of epilepsy, to the authors' knowledge, few papers have been devoted to studying pCREB expression in human tissues [26,27], particularly in human post-surgical epileptic tissues [28]. Moreover, as more details are revealed regarding CREB-related signalling pathways, CREB can potentially become not only an important biomarker for clinical screening, diagnosis and prognosis of epilepsy but also a novel target for cutting-edge individualised treatments for epilepsy.

Conclusion

The present study revealed the neuroprotective roles of low-dose gamma knife irradiation in the frontal lobe and hippocampus. The activation of CREB in cells can initiate the transcription of downstream genes, trigger the synthesis of other functional proteins, strengthen synaptic connections, form new synaptic connections and promote long-term memory formation. This may represent one of the possible mechanisms underlying the therapeutic effects of gamma knife irradiation on epilepsy. Thus, the authors' findings provide valuable information towards unveiling the mechanisms of human epilepsy and a target for potentially effective clinical therapies in the future. However, it is important to note that this experiment was performed on normal rats, which means that further studies, including animal models and epileptic patients, are needed to elucidate the natural effects of gamma knife irradiation on pathological tissues to achieve more significant clinical outcomes. Extensive pharmacokinetic and pharmacodynamic studies

are necessary to approve the therapeutic success of gamma knife irradiation in the management of epilepsy. Laser interstitial thermocoagulation therapy is now widely used in the United States, where medical care is covered through insurance. Gamma knife may be promising, but long-term results need to be studied. If significant results can be confirmed at a clinical level in the future, it can be expected that gamma knife irradiation will emerge as a novel and effective approach in the comprehensive management of epilepsy and its associated comorbid conditions.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available from the corresponding author, WBL, upon reasonable request.

Author Contributions

YY conceived and designed the research study. WBL procured and prepared research materials and animals. YW, YWZ and XPZ substantial contributed to the conception and design of the work. YWC and YQS collected and integrated data. SHZ and PYL analyzed and interpreted data. All authors participated in manuscript writing. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The experimental protocol was approved by the Animal Experimentation Ethics Committee of The Second Hospital of Hebei Medical University (NO. 2023058) and was carried out according to institutional guidelines for animal care and by the Guide for Care and Use of Laboratory Animals published by the United States National Institutes of Health. Experimental animals underwent all procedures under anesthesia, and every effort was made to minimize their pain, suffering, and death.

Acknowledgment

Not applicable.

Funding

This work was supported by a study on the effect and mechanism of gamma knife irradiation on cognitive function in chronic epilepsy rats (Project No. 20211346, Medical Science Research Program of Hebei Province in 2021).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Thijs RD, Surges R, O'Brien TJ, Sander JW. Epilepsy in adults. Lancet (London, England). 2019; 393: 689–701.
[2] Jiang J, Yu Y. Pharmacologically targeting transient receptor potential channels for seizures and epilepsy: Emerging preclinical evidence of druggability. Pharmacology & Therapeutics. 2023; 244: 108384.
[3] Gribkoff VK, Winquist RJ. Potassium channelopathies associated with epilepsy-related syndromes and directions for therapeutic intervention. Biochemical Pharmacology. 2023; 208: 115413.
[4] Vezzani A, Balosso S, Ravizza T. Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. Nature Reviews. Neurology. 2019; 15: 459–472.
[5] Van Berkel AA, Koopmans F, Gonzalez-Lozano MA, Lammertse HCA, Feringa F, Bryois J, *et al*. Dysregulation of synaptic and developmental transcriptomic/proteomic profiles upon depletion of MUNC18-1. eNeuro. 2022; 9: ENEURO.0186–22.2022.
[6] De Santis D, Rossini L, Tassi L, Didato G, Tringali G, Cossu M, *et al*. pCREB expression in human tissues from epilepsy surgery. Epilepsia. 2020; 61: 1240–1252.
[7] Wang G, Zhu Z, Xu D, Sun L. Advances in Understanding CREB Signaling-Mediated Regulation of the Pathogenesis and Progression of Epilepsy. Clinical Neurology and Neurosurgery. 2020; 196: 106018.
[8] Benito E, Barco A. CREB's control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. Trends in Neurosciences. 2010; 33: 230–240.
[9] Liang CD, Liu N, Cao HX, Wu J, Kang JS, Guo T, *et al*. Effect of gamma knife on cognitive function of patients with drug-refractory epilepsy. Chinese Journal of Minimally Invasive Neurosurgery. 2014; 19: 300–302. (In Chinese)
[10] Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalography and Clinical Neurophysiology. 1972; 32: 281–294.
[11] Li WB, Yin Y, Liang CD, Lv PY, Zhao ZB, Dong CZ. Effect of low-dose gamma knife irradiation on the expression of NMDA receptor subunit in cortex and hippocampus of epileptic rats. Chinese Journal of Nervous and Mental Diseases. 2019; 45: 416–421. (In Chinese)
[12] Han T, Wu Z, Zhu J, Kou Y, Li J, Deng Y. Analysis and Construction of a Molecular Diagnosis Model of Drug-Resistant Epilepsy Based on Bioinformatics. Frontiers in Molecular Biosciences. 2021; 8: 683032.
[13] Martínez-Levy GA, Rocha L, Rodríguez-Pineda F, Alonso-Vanegas MA, Nani A, Buentello-García RM, *et al*. Increased Expression of Brain-Derived Neurotrophic Factor Transcripts I and VI, cAMP Response Element Binding, and Glucocorticoid Receptor in the Cortex of Patients with Temporal Lobe Epilepsy. Molecular Neurobiology. 2018; 55: 3698–3708.
[14] Bergantin LB. The Interplay Among Epilepsy, Parkinson's Disease and Inflammation: Revisiting the Link through Ca^{2+}/cAMP Signalling. Current Neurovascular Research. 2021; 18: 162–168.
[15] Sharma P, Kumar A, Singh D. Dietary Flavonoids Interaction with CREB-BDNF Pathway: An Unconventional Approach for Comprehensive Management of Epilepsy. Current Neuropharmacology. 2019; 17: 1158–1175.
[16] Finkbeiner S. CREB couples neurotrophin signals to survival messages. Neuron. 2000; 25: 11–14.
[17] Nishimura M, Owens J, Swann JW. Effects of chronic network hyperexcitability on the growth of hippocampal dendrites. Neurobiology of Disease. 2008; 29: 267–277.
[18] Hammer MF, Sprissler R, Bina RW, Lau B, Johnstone L, Wal-

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

ter CM, *et al*. Altered expression of signaling pathways regulating neuronal excitability in hippocampal tissue of temporal lobe epilepsy patients with low and high seizure frequency. Epilepsy Research. 2019; 155: 106145.

[19] Barnett M, Hall S, Dixit M, Arany I. Simvastatin attenuates oleic acid-induced oxidative stress through CREB-dependent induction of heme oxygenase-1 in renal proximal tubule cells. Pediatric Research. 2016; 79: 243–250.

[20] Sakamoto K, Karelina K, Obrietan K. CREB: a multifaceted regulator of neuronal plasticity and protection. Journal of Neurochemistry. 2011; 116: 1–9.

[21] Tan YW, Zhang SJ, Hoffmann T, Bading H. Increasing levels of wild-type CREB up-regulates several activity-regulated inhibitor of death (AID) genes and promotes neuronal survival. BMC Neuroscience. 2012; 13: 48.

[22] Bullinger KL, Alwaki A, Gross RE. Surgical Treatment of Drug-Resistant Generalized Epilepsy. Current Neurology and Neuroscience Reports. 2022; 22: 459–465.

[23] Liscák R, Vladyka V, Novotný J, Jr, Brozek G, Naměstkova K, Mares V, *et al*. Leksell gamma knife lesioning of the rat hippocampus: the relationship between radiation dose and functional and structural damage. Journal of Neurosurgery. 2002; 97: 666–673.

[24] Wang JH, Gao DZ, Liu AL, Wan H, Meng FG, Sun YL. Effects of gamma knife irradiation on amino acid transmitter and ultrastructure of hippocampus in epileptic rats. Chinese Journal of Stereotactic and Functional Neurosurgery. 2012; 25: 330–334. (In Chinese)

[25] Yin Y, Zhao WQ, Li WL, Lv PY, Li WB, Liang CD, *et al*. Changes of learning and memory function and expression of calmodulin-dependent protein kinase II in hippocampal neurons of pentylenetetrazol kindled epileptic rats. Basic and Clinical Medicine. 2009; 29: 254–258. (In Chinese)

[26] Moore AN, Waxham MN, Dash PK. Neuronal activity increases the phosphorylation of the transcription factor cAMP response element-binding protein (CREB) in rat hippocampus and cortex. The Journal of Biological Chemistry. 1996; 271: 14214–14220.

[27] Lee B, Dziema H, Lee KH, Choi YS, Obrietan K. CRE-mediated transcription and COX-2 expression in the pilocarpine model of status epilepticus. Neurobiology of Disease. 2007; 25: 80–91.

[28] Rakhade SN, Yao B, Ahmed S, Asano E, Beaumont TL, Shah AK, *et al*. A common pattern of persistent gene activation in human neocortical epileptic foci. Annals of Neurology. 2005; 58: 736–747.