The Effect of Rapamycin on Penicillin-Induced Epileptiform Activity in Rats: An Electrophysiological Study

Rapamisinin Sıçanlarda Penisilinle Oluşturulmuş Epileptiform Aktivite Üzerine Etkisi: Bir Elektrofizyolojik Çalışma

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Abstract

Aim: Approximately fifty million people in the world suffer from epilepsy, and a large part of these patients are resistant to antiepileptic drugs discovered so far. In addition, side effect profiles of these drugs are very wide. Rapamycin that is an inhibitor of mammalian target of rapamycin (mTOR) has antineoplastic, aging-retarding, and anti-inflammatory effects. The studies regarding the effects of mTOR on nervous system have shown that it has neuro-protective effects. Moreover, it has been reported that use of rapamycin reduces epileptic seizures in tuberous sclerosis patients. In this study we aimed to investigate acute effects of the mTOR inhibitor rapamycin on penicillin-induced experimental epilepsy in rats.

Materials and Methods: In this study, a model of forty adult male Wistar rats with penicillin-induced experimental epilepsy was used. The forty rats were divided into five groups, which were saline group, solvent (dimethylsulfoxide) group, and 0.1 mg/kg, 0.4 mg/kg and 0.8 mg/kg rapamycin groups. All substances were administered intraperitoneally. After the administration of 1.25 g/kg urethane for anesthesia, the left part of each rat's skull was opened and electrodes were placed on the brain. Electrocorticography recording was initiated. Penicillin was intracortically administered two hours after the administration of rapamycin. After the administration of penicillin, electrocorticographic data were recorded for another two hours.

Results: In rapamycin-treated rat groups, administration of 0.4 mg/kg and 0.8 mg/kg rapamycin significantly reduced epileptic spike-wave frequency and amplitude of epileptiform activity. However, when compared in terms of latency no significant difference was found between the groups.

Discussion and Conclusion: Acute administration of rapamycin reduced spike-wave frequency and spike-wave amplitude of penicillin-induced epileptiform activity in the rats, and these findings indicate that rapamycin has an antiepileptogenic potential.

Keywords: rapamycin; mTOR; epileptiform activity; electrocorticography; rat

Özet

Amaç: Yeryüzünde yaklaşık elli milyon insan epilepsinin pençesindedir ve bu hastaların büyük bir bölümü şimdiye kadar keşfedilmiş antiepileptik ilaçlara karşı dirençlidir. Bunun yanı sıra, bu ilaçların yan etki profilleri de oldukça geniştir. Memelideki rapamisin hedefi (mTOR) inhibitörü olan rapamisin; antineoplastik, yaşlanmayı geciktirici ve antienflamatuvar etkilere sahiptir. mTOR'un sinir sistemi üzerindeki etkilerine dair çalışmalarda ise nöroprotektif etkisinin olduğu gösterilmiştir. Buna ek olarak, tüberoskleroz hastalarında rapamisin kullanımının epileptik nöbetleri azalttığı bildirilmiştir. Bu çalışmanın amacı mTOR inhibitörü rapamisinin sıçanlarda penisilinle oluşturulmuş deneysel epilepsi üzerindeki akut etkisini araştırmaktır.

Gereç ve Yöntemler: Bu çalışmada penisilinle oluşturulmuş deneysel epilepsili kırk adet erişkin erkek Wistar sıçan içeren bir model kullanılmıştır. Söz konusu kırk sıçan; salin, çözücü (dimetilsülfoksit), ve de 0,1 mg/kg, 0,4 mg/kg ve 0,8 mg/kg rapamisin grupları olmak üzere beş gruba

Geliş Tarihi /*Received* : 11.08.2016
Kabul Tarihi /*Accepted*: 24.08.2016

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ayrıldı. Tüm maddeler intraperitoneal yolla uygulandı. Sıçanlara anestezi için 1,25 g/kg üretan uygulandıktan sonra hayvanların sol kafatası açıldı ve beyin üzerine elektrotlar yerleştirildi. Elektrokortikografi kaydı başlatıldı. Rapamisin uygulamasından iki saat sonra intrakortikal olarak penisilin uygulandı. Penisilin uygulandıktan sonra elektrokortikografi verileri iki saat daha kaydedildi.

Bulgular: Rapamisinle tedavi edilen sıçan gruplarında, 0,4 mg/kg ve 0,8 mg/kg rapamisin uygulamaları epileptiform aktivitenin diken-dalga sıklığını ve genliğini anlamlı olarak azaltmıştır. Fakat latensleri karşılaştırıldığında gruplar arasında anlamlı bir fark bulunmamıştır.

Tartışma ve Sonuç: Akut rapamisin uygulaması sıçanlarda penisilinle oluşturulmuş epileptiform aktivitenin diken-dalga sıklığını ve diken-dalga genliğini azaltmıştır ve bu bulgular rapamisinin antiepileptojenik bir potansiyele sahip olduğunu göstermektedir.

Anahtar Sözcükler: rapamisin; mTOR; epileptiform aktivite; elektrokortikografı; sıçan

INTRODUCTION

Epilepsy is characterized by recurrent seizures and one of the most common neurologic conditions in the world. At the present time, about fifty million people in the world suffer from active epilepsy with continuing seizures and these people need antiepileptic drug treatment. Epilepsy is not a single disease; the term defines the common symptomatic manifestation of numerous brain abnormalities. These abnormalities include genetic syndromes, traumatic brain injuries, central nervous system infections, strokes, or structural brain lesions such as tuberous sclerosis and brain tumors (1). In spite of the increasing variety of antiepileptic drugs developed in recent years, nearly 30% of epilepsy patients are resistant to these drugs (2). In addition, current antiepileptic drugs have many side effects. For these reasons, intensive research continues in order to develop inexpensive and more effective medications with fewer side effects and to explain the mechanism of epilepsy.

mTOR (mammalian target of rapamycin) is a serine/threonine protein kinase, which is a member of the phosphatidylinositol 3-kinase related kinase (PIKK) family. Rapamycin (sirolimus) consists in two multi-protein complexes defined by distinct protein-binding partners with mTOR. The first is the rapamycin-sensitive mTOR, known as mTORC1, and the other is mTORC2, which is largely insensitive to the effects of rapamycin (3). The complex details of mTOR molecule and mTOR pathway have been extensively demonstrated in many studies (4–9). Mammalian TOR provides cellular communication, regulates cellular growth, processes growth factor signals, and so is a molecule that modulates proliferation and survival of cells. It is regulated by different factors such as hormones (insulin), nutrients (amino acids, glucose), cellular energy level and stress. Many studies have shown that the inhibitors of mTOR regulate protein synthesis and other cellular processes.

Rapamycin, which is also a macrolide antibiotic, is used as an immunosuppressive agent in modern medicine. Dysregulation of the mTOR pathway has been implicated in the pathophysiology of a number of neurological diseases. Tuberous sclerosis complex (TSC) is caused by loss-of-function mutations in the mTOR-negative regulators TSC1 or TSC2 resulting in a constellation of neurological phenotypes that can include epilepsy. mTOR hyperactivation among a wide range of cell types can drive epileptogenesis (10,11). In clinical trials, Wong et al. have suggested that rapamycin, an mTOR inhibitor, has anti-seizure effects in tuberous sclerosis and common acquired epilepsies (12). Therefore mTOR inhibitors may be a potential antiepileptogenic medication to treat epilepsy. Studies have shown that mTOR regulates neuronal survival and differentiation, as well as axon growth and migration, dendritic arborization, and synaptogenesis during the developing CNS. In the adult CNS, mTOR is very important for every kind of synaptic plasticity, such as long-term potentiation that plays an important role in the process of learning and memory in hippocampus (13). Moreover, mTOR can affect a variety of cellular and molecular processes, such as neurotransmitter receptor and ion channel expression, synaptic plasticity, neuronal death and apoptosis, and neurogenesis in CNS.

According to the previous studies, the mTOR pathway is abnormally activated by kainate-induced status epilepticus, both acutely during the period of status epilepticus and more chronically for a few weeks

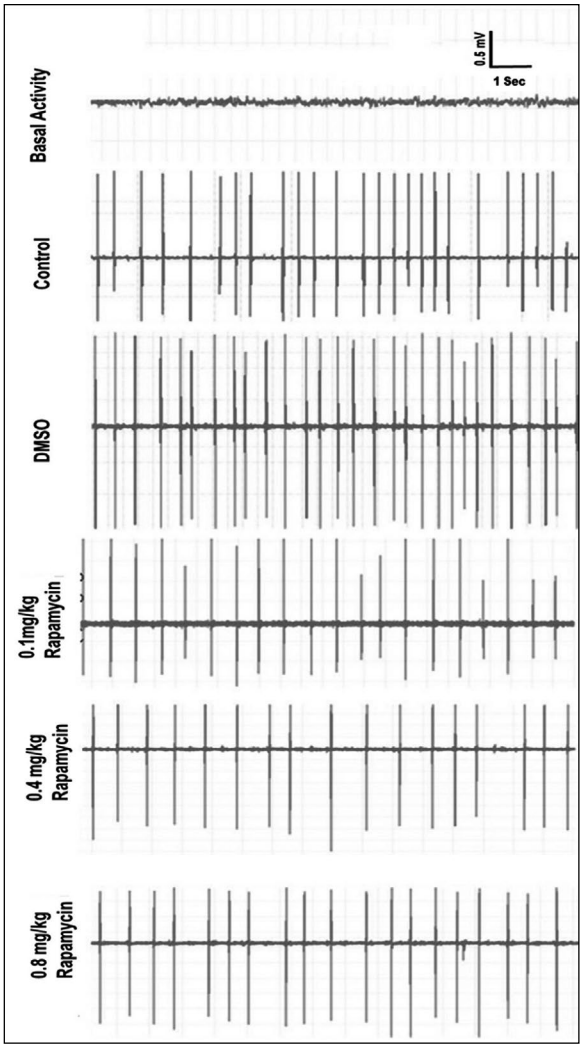


Figure 1. Changes in ECoG activity after administration of penicillin G in the rapamycin-treated and control groups. (A) Baseline ECoG activity (B) Control group (C) DMSO group (D) 0.1 mg/kg rapamycin group (E) 0.4 mg/kg rapamycin (F) 0.8 mg/kg rapamycin group.

during the latent term of epileptogenesis. Rapamycin treatment inhibited this inappropriate mTOR activation and also reduced cellular changes that likely contributed to epileptogenesis in this model, including hippocampal neuronal death, neurogenesis, and axonal sprouting (14,15). Correspondingly, mTOR inhibition with rapamycin also decreased development of spontaneous seizures; thereby it was indicated to have an antiepileptogenic effect. Although some studies showed that rapamycin reduced seizure frequency in some rat models of temporal lobe epilepsy (16,17), low doses used in mice were not sufficient to reduce seizures (18). On the other hand, mTOR inhibitors may decrease somatic growth and interrupt critical mechanisms of brain development and learning, such as long-term potentiation and synaptic plasticity; and high dose rapamycin may have harmful effects on neuronal activity (15).

The diversity of epilepsy syndromes and their causes precludes investigators from using any single animal model system for learning about epilepsy and for testing potential therapies. Animal models for seizures and epilepsy have played a fundamental role in advancing our understanding of the basic mechanisms underlying epileptogenesis, and have been instrumental in the discovery and preclinical development of novel antiepileptic drugs (AEDs). The different doses and different experimental models should be used to determine effectiveness of potential agents before they will become an AED. The penicillin-induced epilepsy is one of the experimental models used for generalized epilepsy, providing electrophysiological evidence.

The aim of this study is to investigate acute effects of rapamycin on penicillin-induced epileptiform activity by using electrocorticography (ECoG) in anesthetized rats.

MATERIALS AND METHODS

Animals and groups

The experimental protocol was approved by the Animal Ethics Committee at University of Duzce (2009-21). Forty male Wistar rats weighing approximately 230–280 g and aged 12 weeks were used in the experiment. Rats were supplied from Medical Research Center of Duzce University. Four animals were housed in each cage and kept under controlled environmental conditions (60±5% humidity; 22±2 °C; 12:12 h reversed light/dark cycle). They were allowed to feed and drink water freely. Animals were randomly assigned to the five experimental groups (control, vehicle, 0.1 mg/kg, 0.4 mg/kg, and 0.8 mg/kg rapamycin groups); each group consisted of eight rats.

Surgical procedure

Animals were anesthetized with 1.25 g/kg i.p. urethane (Sigma, US) and placed in a stereotaxic frame (Harvard Instruments, South Natick, MA, US). The scalps were opened by a rostro-caudal incision and the left part of the skull was carefully removed. Body temperature was maintained at 37°C.

Induction of epileptiform activity

The epileptiform activity was induced by administration of penicillin (500 IU / 2 µl) intracortically (i.c.). The bregma of the skull was used as reference point (coordinates AP=−1 mm, L=1.5 mm) for the intracortical injection. Penicillin was injected into the left sensorimotor cortex by using a Hamilton microinjector (type 701N, Hamilton Co., Reno, NV, US) at 1.2 mm underneath the brain surface.

Electrophysiological recordings

Ag/AgCl electrodes were used for the recording during ECoG. Two top electrodes were placed over the left somatomotor cortex with the common reference electrode being fixed on the right ear of the rats. The coordinates of the recording area were 1 mm anterior to the bregma and 2 mm lateral to the sagittal suture for the first electrode, and 5 mm posterior to the bregma and 2 mm lateral to the sagittal suture for the second electrode. The data acquisition system with multi-channel (PowerLab/8SP, ADInstruments Pty Ltd, Castle Hill, NSW, Australia) was used to record the ECoG signal. The signals from the electrodes were amplified and filtered with 0.1-50 Hz band-pass via the amplifiers (BioAmp, AD Instruments, Australia). It was digitized at a sampling rate of 1024 Hz. ECoG activity was simultaneously monitored and stored using a personal computer. Latency time to onset of first spike wave, spike-wave frequency and amplitude of epileptiform activity were automatically calculated by PowerLab Chart software v.6.0.

Drugs and Applications

Rapamycin (sirolimus) was purchased from LC Labs (Woburn, MA, US) and urethane from Sigma (Saint Louis, MO, US). Rapamycin was dissolved in dimethylsulfoxide (DMSO, Loba Chemie, India) following dilution with saline (99% DMSO; 0.2 ml final solution DMSO/saline 1:4, v/v, respectively). Five minutes after basal activity recording, it was intraperitoneally injected to the rats in rapamycin groups at doses of 0.1 mg/kg, 0.4 mg/kg, and 0.8 mg/kg. We studied the effects of a conventional low dose, a medium dose, and a higher dose of rapamycin. These doses were determined according to the doses used in previous studies (19–22). In equal volume with rapamycin groups

Table 1. The effects of saline (control), DMSO and 0.1 mg/kg, 0.4mg/kg, 0.8mg/kg i.p. rapamycin on frequency of penicillin-induced epileptiform activity

Time (min)	Control		DMSO		0.1 mg		0.4 mg		0.8 mg		P
	Mean ± SEM	Median	Mean ± SEM	Median	Mean ± SEM	Median	Mean ± SEM	Median	Mean ± SEM	Median	
0-5	0±0	0	0±0	0	0±0	0	0±0	0	0±0	0	--
6-10	52,50±8,9	57,5	49,25±18,5	30	82,38±33,9	44	22,50±14,3	8,5	27,25±14,7	6,5	0,268
11-15	144,25±12,0	143,5	97,875±25,0	123	110,75±30,4	93,5	55,625±18,2 *	38,5	54,5±21,2 *	43	**0,032**
16-20	159,63±16,8	148,00	116,88±28,1	122,0	143,00±28,5	143,0	72,63±17,9 *Δ	39,50	62,63±15,4 *Δ	62,50	**0,015**
21-25	148,88±14,6	126,5	136,25±29,5	109	146,88±23,4	139,5	78,25±14,7 *Δ	88	72,00±17,2 *Δ	87	**0,015**
26-30	147,38±13,3	130	122,75±18,9	109	147,88±19,2	140,5	73,13±15,1 *Δ	82	68,00±14,4 *Δ ‡	71,5	**0,002**
31-35	141,50±9,6	157	120,75±16,9	109	143,25±18,4	139	72,25±18,6 *Δ	87,5	78,00±14,5 *Δ	77,5	**0,020**
36-40	129,75±8,9	136,5	122,00±17,3	107	138,63±18,9	143	66,50±19,8	82	96,38±13,6	97,5	0,092
41-45	128,00±10,4	123,5	128,00±22,7	101,5	141,13±20,7	145	59,63±18,9 *Δ ‡	62,5	104,00±17,4	96	**0,050**
46-50	124,00±12,6	107,5	124,50±18,4	102,5	159,75±25,6	160,5	60,13±20,4 *Δ ‡	55	85,88±11,1 Δ	82,5	**0,013**
51-55	132,50±23,1	108	132,88±20,2	138,5	152,38±18,6	161,5	57,50±21,4 *Δ ‡	45,5	80,63±13,9 Δ	69	**0,013**
56-60	114,25±15,3	95,5	126,00±25,1	106,5	156,38±19,3	172,5	58,63±21,8 Δ	42,5	89,25±17,4 Δ	74,5	**0,043**
61-65	108,75±14,8	107,5	129,50±23,2	128	148,75±19,4	151	55,63±20,9 Δ ‡	42,5	92,75±20,4 Δ	74,5	**0,035**
66-70	109,88±10,8	102	122,38±26,8	108,5	143,25±21,7	136,5	55,50±21,7	37	92,63±22,0	68	0,113
71-75	118,25±14,9	114	110,75±24,5	99,5	129,00±22,0	132,5	54,75±21,2	38,5	93,25±23,0	67,5	0,186
76-80	114,00±19,1	112	99,38±25,7	73,5	136,50±24,0	132,5	52,75±21,1	35,5	91,38±23,4	68,5	0,164
81-85	113,38±28,4	92,5	98,38±19,9	92,5	128,13±22,2	130	45,38±20,0	18,5	87,88±21,6	64,5	0,080
86-90	117,75±34,7	110,5	91,88±17,8	83	127,50±22,8	127	40,88±20,5	2,5	87,50±20,6	66	0,087
91-95	114,75±33,0	114,5	93,88±20,1	89,5	130,13±22,8	128,5	35,25±18,9 *Δ ‡	0,5	83,63±20,0	67	**0,037**
96-100	103,75±27,8	107,5	82,63±15,5	73,5	124,63±19,8	124,5	29,63±16,9 *Δ ‡	1	81,63±19,5	68	**0,028**
101-105	91,88±23,5	104	72,50±14,8	68,5	126,00±22,5	117	27,50±15,8 *Δ ‡	0	76,13±18,5	64	**0,019**
106-110	84,38±22,3	91,5	68,63±15,6	66,5	126,50±23,8	129,5	26,00±15,9 *	0	74,38±18,2	63	**0,026**
111-115	85,38±20,4	87,5	68,00±13,2	68	105,63±23,2	75,5	21,88±13,5 *‡Φ	0,5	73,00±17,6	65	**0,025**
116-120	86,58±21,1	90,93	64,68±11,8	62,33	111,39±18,6	94,22	23,27±13,5 *‡Φ	0,5	69,59±16,9	59,64	**0,010**

All values are number/minute. p≤0.05 was considered statistically significant. (*Compared to control group, Δ Compared to 0.1mg/kg group, ‡Compared to DMSO group, Φ Compared to 0.8mg/kg group)

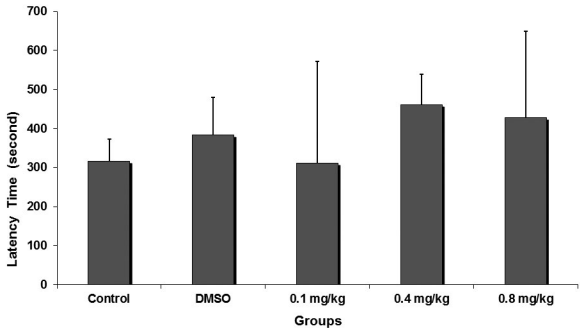


Figure 2. Latency of the first epileptiform activity.

DMSO was injected to vehicle group and saline to control group, intraperitoneally. After administration of substance, ECoG recording was continued for 120 minutes. Then, penicillin G potassium (500 IU/ 2 µl volume, I.E. Ulagay, Turkey) was administered intracortically to produce epileptiform activity and ECoG recordings were continued for 240 minutes.

Statistical analyses

Frequencies and amplitudes of epileptiform activity for each animal were automatically digitized using the software (Chart v.6.0, ADInstruments Pty Ltd, Castle Hill, NSW, Australia). Epileptiform activity was analyzed in every 5-min interval. Descriptive values were computed as mean ± SEM and median. The Kruskal-Wallis test was used to compare the groups in terms of latency spike-wave frequency and wave amplitude in each period. For post-hoc analysis, a method of Dunn's test followed by Kruskal-Wallis test was used. The significance level was $p<0.05$. Statistical analyses were performed using PASW package (version 18).

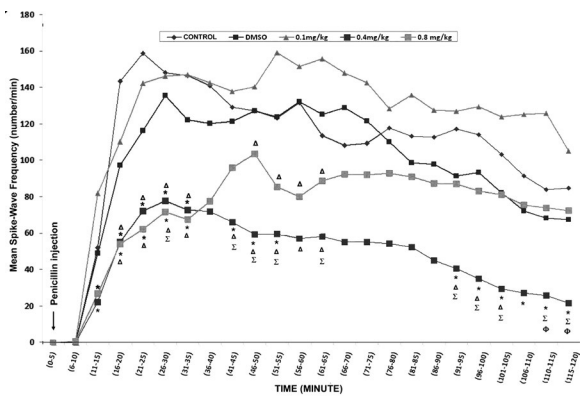


Figure 3. Values of the spike-wave frequency in the control, DMSO and rapamycin-treated groups. *Compared to control group ($p\leq0.05$); Δ Compared to 0.1mg /kg rapamycin group ($p\leq0.05$); ΣCompared to DMSO group ($p\leq0.05$); Φ Compared to 0.8 mg/kg rapamycin group ($p\leq0.05$).

RESULTS

Basal ECoG activity of each rat was recorded before the administration of substances. Spontaneous spike was not detected in any of the animals (Figure 1). The injected substances (rapamycin, DMSO, or saline) before penicillin administration did not cause any epileptiform activity (Figure 1). Epileptiform activities characterized with bilateral spikes began within 5 to10 minutes after penicillin administration and lasted for 3 to 4 h. Frequency and amplitude of spikes reached a constant level about 30 min after penicillin administration.

The effect of rapamycin on latency

When compared to the control and DMSO groups ($P=0.070$), the rapamycin groups showed no significant difference in latency of epileptiform activity (Fig. 2).

The effect on spike-wave frequency of epileptiform activity

After penicillin injection, median spike-wave frequency of epileptiform activity was between 57.50 spike/minute at the 6–10 min interval and 157.00 spike/minute at the 31–35 min interval in the control group. The decrease in the frequency of epileptiform activity continued for 120 minutes (Figure 3, Table 1). Median spike-wave frequency of epileptiform activity of the DMSO group was 109 spike/min at the 21–25 min interval after penicillin injection and there was no statistically significant difference compared to the control group ($p=0.083$) (Figure 3, Table 1).

When compared to the other groups, the 0.1 mg/kg rapamycin group did not show significant difference in spike-wave frequency of epileptiform activity in any of the time periods ($p>0.05$) (Fig 3, Table 1). Rapamycin at 0.4 mg/kg dose reduced the median spike frequency in the first 10 min after the injection, but this decrease was not statistically significant when compared with the other groups (p=0,083). However, the decreasing effects of 0.4 mg/kg dose rapamycin on spike frequencies were statistically significant after the 10th min. The median spike-wave frequency of 0.4 mg/kg dose group was observed to be significantly lower when compared to the control group in most of the periods ($p<0.05$). Moreover, administration of 0.4 mg/kg dose rapamycin decreased spike-wave frequency in the group in

Table 2. The effects of saline (control), DMSO and 0.1 mg/kg, 0.4mg/kg, 0.8mg/kg i.p. rapamycin on amplitude of penicillin-induced epileptiform activity

Time (min)	Control		DMSO		0.1 mg		0.4 mg		0.8 mg		P
	Mean ± SEM	Median	Mean ± SEM	Median	Mean ± SEM	Median	Mean ± SEM	Median	Mean ± SEM	Median	
0-5	0±0	0	0±0	0	0±0	0	0±0	0	0±0	0	--
6-10	1,85±0,1	1,98	2,84±0,5	3,12	2,55±0,6	2,49	1,66±0,3	1,36	1,30±0,44 ‡	1,09	**0,050**
11-15	2,55±0,3	2,60	3,16±0,6	3,42	3,10±0,6	3,37	1,80±0,3	1,61	1,83±0,49	1,44	0,238
16-20	2,96±0,3	2,85	3,48±0,5	3,81	3,30±0,5	3,37	1,97±0,2 ‡	1,88	2,05±0,53 ‡	1,63	**0,049**
21-25	3,10±0,4	2,90	3,67±0,4	3,93	3,43±0,5	3,21	2,05±0,3 Δ ‡	1,75	2,24±0,55 ‡	2,08	**0,050**
26-30	3,27±0,4	3,19	3,82±0,4	3,95	3,43±0,5	3,27	1,82±0,3 * Δ ‡	1,55	2,28±0,4 ‡	2,06	**0.031**
31-35	3,26±0,4	3,28	3,85±0,4	4,00	3,35±0,4	3,49	1,77±0,4 * Δ ‡	1,12	2,27±0,3 ‡	2,25	**0,025**
36-40	3,25±0,4	3,63	3,83±0,4	3,94	3,32±0,4	3,34	1,64±0,4 * Δ ‡	1,12	2,50±0,3	2,14	**0,025**
41-45	3,17±0,5	3,39	3,79±0,4	3,88	3,15±0,3	3,36	1,65±0,4 * Δ ‡	1,14	2,61±0,3	2,36	**0,048**
46-50	3,19±0,5	3,55	3,68±0,4	3,79	3,01±0,3	3,19	1,64±0,5 * Δ ‡	1,12	2,70±0,3	2,62	**0,050**
51-55	3,03±0,4	3,45	3,58±0,4	3,71	3,27±0,4	3,27	1,54±0,4 * Δ ‡	1,36	2,65±0,3	2,58	**0,034**
56-60	2,93±0,4	3,25	3,53±0,4	3,48	3,09±0,3	3,19	1,53±0,4 * Δ ‡	1,27	2,48±0,3	2,23	**0,038**
61-65	2,92±0,4	3,15	3,40±0,4	3,48	3,23±0,5	3,28	1,55±0,5 * Δ ‡	1,10	2,43±0,3	2,20	**0,050**
66-70	2,79±0,4	3,25	3,29±0,4	3,26	3,02±0,3	3,19	1,42±0,4 * Δ ‡	1,04	2,42±0,3	2,20	**0,048**
71-75	2,83±0,4	3,23	3,26±0,5	3,12	3,10±0,5	3,05	1,38±0,4 * Δ ‡	1,17	2,48±0,3	2,34	**0,050**
76-80	2,64±0,4	3,18	3,08±0,4	2,82	2,96±0,4	2,79	1,37±0,4 * Δ ‡	1,02	2,26±0,3	2,31	**0,049**
81-85	2,55±0,4	3,08	2,86±0,4	2,98	3,02±0,5	2,88	1,34±0,4 * Δ ‡	0,81	2,26±0,3	2,35	**0,050**
86-90	2,70±0,5	3,30	2,65±0,4	2,54	2,87±0,5	2,61	1,26±0,4 * Δ ‡	0,65	2,21±0,3	2,31	**0,050**
91-95	2,69±0,5	3,35	2,60±0,4	2,45	2,86±0,5	2,62	1,18±0,4 * Δ ‡	0,69	2,19±0,3	2,27	**0,050**
96-100	0,5	3,28	2,71±0,4	2,66	2,94±0,4	2,54	1,14±0,4 * Δ ‡	0,56	2,12±0,3	2,38	**0,050**
101-105	0,5	3,24	2,60±0,4	2,64	2,77±0,5	2,44	1,08±0,4 * Δ ‡	0,45	1,98±0,2	2,12	**0,049**
106-110	0,4	3,04	2,49±0,4	2,56	2,63±0,4	2,17	1,09±0,4 * Δ ‡	0,50	1,96±0,2	2,13	**0,041**
111-115	,4	2,79	2,38±0,4	2,34	2,73±0,4	2,57	1,00±0,4 * Δ ‡ Φ	0,49	2,12±0,2	2,17	**0,050**
116-120	0,4	3,02	2,52±0,4	2,47	2,70±0,40	2,34	1,04±0,4 * Δ ‡ Φ	0,56	1,91±0,2	2,11	**0,047**

All values are presented as millivolts. p≤0.05 was considered statistically significant. (*Compared to control group, Δ Compared to 0.1mg/kg group, ‡ Compared to DMSO group, Φ Compared to 0.8mg/kg group)

comparison to the DMSO, 0.1 mg/kg rapamycin, and 0.8 mg/kg rapamycin groups (Fig 3, Table 1). Rapamycin at 0.8 mg/kg dose reduced the median spike frequency in the first 10 min interval, but not statistically significantly, as compared to the control group (p=0.058). There was significant difference in median spike-wave frequency of epileptiform activity in the 0.4 mg/kg dose rapamycin group in comparison to the control and 0.1 mg/kg groups during the first 35 min. This reducing effect continued for 65 minutes, except for some periods (p<0.05) (Figure 3, Table 1).

The Effect on Spike-wave Amplitude of Epileptiform Activity

Considering the data obtained from the control group, the median spike-wave amplitude of epileptiform activity reached its maximum value (3.19 mV) at the 26–30 min interval after penicillin administration and gradually reduced for 120 min. (Figure 4, Table 2). In the DMSO group the median spike-wave amplitude of epileptiform activity was between 3.12 mV (6–10 min) and 4 mV (31–35 min) (Figure 3, Table 2). At the same time, effects of DMSO administration on epileptiform activity were investigated. Although it decreased the spike-wave amplitude in comparison to the control group, there was no statistical significance (Figure 4, Table 2).

When compared to the other groups, the 0.1 mg/kg rapamycin group did not show significant difference in spike-wave amplitude of epileptiform activity in any of the time periods (p>0.05) (Fig 4, Table 2). The 0.4 mg/kg rapamycin group showed no significant difference in spike-wave amplitude of epileptiform activity in comparison to the other groups in the first 15 minutes (p>0.05). When results were detailed on data of 0.4 mg / kg rapamycin group, the median spike-wave amplitudes began to appear lower compared to the control group at 26th min, DMSO group at 16th min and 0.1 mg/kg rapamycin at 21st min, significantly. The significant decrease continued throughout the 120 minutes of recording (p≤0.05). Moreover, administration of 0.4 mg/kg rapamycin resulted in lower spike-wave amplitude than administration of 0.8 mg/kg dose rapamycin between the 110th and 120th minutes (p≤0.05) (Fig 4, Table 2). Rapamycin at 0.8 mg/kg dose reduced

the median spike-wave amplitude between the 11th and 40th minutes, except for the 16–20 min interval, as compared to the DMSO groups (p≤0.05) (Figure 4, Table 2).

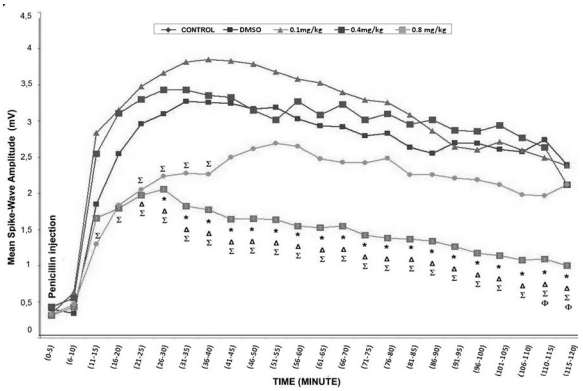


Figure 4. Values of the spike-wave amplitude in the control, DMSO and rapamycin-treated groups. Comparisons: *Compared to control group (p≤0.05); Δ Compared to 0.1mg /kg rapamycin group (p≤0.05); ΣCompared to DMSO group (p≤0.05); Φ Compared to 0.8 mg/kg rapamycin group (p≤0.05).

DISCUSSION

This study is the first investigating the effects of rapamycin on epileptiform activity by using a penicillin-induced epilepsy model of rats. Penicillin-induced epileptiform activity is a good model for focal and generalized epilepsy. It is often used in studies of acute epilepsy research and provides electrophysiological evidence. Intracortical injection of penicillin (500 IU) induces epileptiform activity characterized by bilateral spikes (23). In this study, rapamycin was used at doses of 0.1 mg/kg, 0.4 mg/kg, and 0.8 mg/kg. Administrating rapamycin prior to penicillin did not cause any significant effect on latency of epileptiform activity. However, some of the various doses of rapamycin had a reducing effect on spike-wave frequency and wave amplitude of epileptiform activity, and this effect is not dose-dependent. The reducing effect of administering 0.4 mg/kg rapamycin on spike-wave frequency and wave amplitude of epileptiform activity began at the 10th minute following penicillin administration, and was more prominent than in the other groups. At the dose of 0.8 mg/kg rapamycin also had a reducing effect on spike-wave frequency but the effect was of short duration.

Some studies have suggested that completely blocking mTOR by administering high doses of rapamycin can eliminate the benefits or that the effect of rapamycin may be paradoxical at high and low doses in a non-dose-dependent manner (24,25). More research is needed to further define the mechanism by which low dose rapamycin is more effective. Hartman et al. have suggested that rapamycin is protective against maximally-electroshock-induced (MES) seizures, but not PTZ- and 6 Hz-induced seizures, at doses of 4.5 or 6.0 mg/kg (26). Thus, low doses of rapamycin may be more potent than high doses. On the other hand, we did not determine the tissue levels of rapamycin and its effects on mTOR production levels and these are the most important limitations of this study. In order to assess whether rapamycin indeed inhibited mTOR signaling pathway, researchers examined its effect on p70S6K phosphorylation generally. Many researchers on this issue showed that rapamycin, even at low doses, is enough to inhibit mTOR activation and to distribute in the brain (16).

Animal models demonstrated that mTOR inhibitors could exert both an anticonvulsant action and an antiepileptogenic effect in genetic and acquired epilepsy. The relevance of the mTOR pathway to epileptogenesis and its potential as a therapeutic target in epilepsy treatment by presenting the current results on mTOR inhibitors, in particular, rapamycin in animal models of diverse types of epilepsy. Compared the effects documented in other epilepsy models, effects of rapamycin in this study are remarkable. Penicillin, like bicuculline, is responsible for epileptic discharges by the blockage of the $GABA_A$ receptors and/or excitation-inhibition imbalance with increasing of glutamatergic transmission (23). On the other hand, mTOR hyperactivation in animal models consistently produces epilepsy and the loss of TSC1 in forebrain excitatory neurons causes hyperexcitability and seizures. In this study, epileptic spike-wave discharge was initiated after 5 to 10 minutes from penicillin administration, and it was confirmed by the literature where the pilocarpine model was used (27). Determining the effects of penicillin on mTOR pathway may be a useful way to clarify the similarity of the mechanisms. In contrast, another study reveals that rapamycin does not have antiseizure or antiepileptogenic effects in the pilocarpine model (14). Guo et al. suggest that ra-

pamycin may represent a rational treatment for preventing posttraumatic epilepsy in patients with traumatic brain injury (28).

DMSO is frequently used as a solvent in the studies performed with antiepileptic substances (29). Researchers have reported that DMSO decreases seizure threshold and augments the proconvulsant activity of the substances dissolved in it (30). Intraperitoneal DMSO administration altered absence-like epileptic seizure activities in freely moving WAG-Rij rats (31). DMSO has been reported to have dual effect since it decreases spike-wave frequency at low doses (1.65mg/kg or 1.5 ml/kg) and, contrary to this, increases spike-wave frequency at high doses (1650.6 mg/kg). In our study, DMSO had no effect on latency of the first epileptiform activity, spike-wave frequency, and spike-wave amplitude. Our dose being much lower than those in other studies probably precluded this effect, and DMSO had no effect in the experiment as desired.

Tuberous sclerosis (TS) is a rare genetic disease caused by the mutation of one of the TSC1 and TSC2 genes. TSC1 or TSC2 leads to abnormal disinhibition of the mTOR pathway. This hyperactivation of the mTOR pathway causes epilepsy (32). However, most of the beneficial effect of rapamycin appear to reverse on discontinuation of the drug in both animal models and clinical trials (33–36). The mammalian target of rapamycin (mTOR) regulates protein synthesis related to cell growth and proliferation. Hyperactivation of mTOR pathway causes an increase in neuronal circuits' excitability (37). Probably the abnormal activity of mTOR causes alterations to neurotransmitter receptors and ion channels (16). Previous studies showed that the mTOR pathway could modulate the expression of potassium channels and glutamate receptors (32,38).

Rapamycin treatment in the early stage of seizures prevents the development of epilepsy in mice, and later treatment with rapamycin also decreases seizure-frequency in mice that have already developed epilepsy (16). Despite the lack of controlled clinical trials, it has been reported that mTOR inhibitors reduces epileptic seizures in TS patients (35,36). Results of our study has shown that even a single low dose rapamycin reduces epileptic activity in rats with penicillin-induced epilepsy. Further detailed studies are needed to clarify the mechanism of this action.

Intriguing findings of the frequent hyperactivation of mTOR signaling in epilepsy make it a potential mechanism in the pathogenesis as well as an attractive target for the therapeutic intervention, and have driven the significant ongoing efforts to pharmacologically target this pathway (9). As mTOR can be activated by glutamate receptor stimulation (39,40), it is not surprising that the initial mTOR activation occurs with status epilepticus, which causes massive glutamate release (16,41). Activation of the glutamate release causes calcium to enter the cell, leading to cell death. mTOR increases postsynaptic response of the glutamatergic and GABAergic synapse, and approximately 50% of increased synaptic vesicle release responsible for rising of the postsynaptic response. No study has been conducted yet regarding mTOR pathway in penicillin-induced epilepsy. The presented study has showed that rapamycin reduces epileptiform activity, and it may suggest that penicillin causes epilepsy via mTOR activation pathway.

This study has showed that acute use of rapamycin decreases spike-wave frequency and amplitude and that long-term rapamycin use reduces epileptiform activity. We did not perform molecular and biochemical analyses in this study, but only investigated the effect on epileptiform activity electrophysiologically. Multidisciplinary research including biochemical and histological studies about this issue will help enlighten this matter. Extensive basic clinical research is needed to understand mTOR inhibitors' efficacy and mechanism of action in epilepsy treatment.

ACKNOWLEDGEMENTS

This study was supported by the Committee for Scientific Research of Duzce with the code of 2010.04.01.051.

The study was presented at 38th National Physiology Congress in Trabzon, Turkey, 25–29 September 2012 as poster in Turkish Language and published in abstract book. In addition, it was presented in English language at 11th European Congress on Epileptology in Stockholm, Sweden, 29th June – 3rd July, 2014 and the abstract was published in Epilepsia.

REFERENCES

1. McDaniel SS, Wong M. Therapeutic role of mammalian target of rapamycin (mTOR) inhibition in preventing epileptogenesis, Neurosci Lett. 2011;497(3): 231–9.
2. Ngugi AK, Kariuki SM, Bottomley C, Kleinschmidt I, Sander JW, Newton CR, et al. Incidence of epilepsy: a systematic review and meta-analysis. Neurology. 2011;77(10):1005–12.
3. Sabers CJ, Martin MM, Brunn GJ, Williams JM, Dumont FJ, Wiederrecht G, et al. Isolation of a protein target of the FKBP12–rapamycin complex in mammalian cells. J Biol Chem. 1995;270(2):815–22.
4. Cho CH. Frontier of epilepsy research – mTOR signaling pathway. Exp Mol Med. 2011;43(5):231–74.
5. Weber JD, Gutmann DH. Deconvoluting mTOR biology. Cell Cycle. 2012;11(2);236–48.
6. Hung CM, Garcia-Haro L, Sparks CA, Guertin DA. mTOR-dependent cell survival mechanisms. Cold Spring Harb Perspect Biol. 2012;4(12):a008771.
7. Yang Z, Ming XF. mTOR signalling: the molecular interface connecting metabolic stress, aging and cardiovascular diseases. Obes Rev. 2012;13(Suppl 2):58–68.
8. Jewell JL, Russell RC, Guan KL. Amino acid signalling upstream of mTOR. Nat Rev Mol Cell Biol. 2013;14(3):133–9.
9. Meng XF, Yu JT, Song JH, Chi S, Tan L. Role of the mTOR signaling pathway in epilepsy. J Neurol Sci. 2013;332(1–2):4–15.
10. Bateup HS, Johnson CA, Denefrio CL, Saulnier JL, Kornacker K, Sabatini BL. Excitatory/inhibitory synaptic imbalance leads to hippocampal hyperexcitability in mouse models of Tuberous Sclerosis. Neuron. 2013;78(3):510–22.
11. LaSarge CL, Danzer SC. Mechanisms regulating neuronal excitability and seizure development following mTOR pathway hyperactivation. Front Mol Neurosci. 2014;7:18.
12. Wong M. Mammalian target of rapamycin (mTOR) inhibition as a potential antiepileptogenic therapy: from tuberous sclerosis to common acquired epilepsies. Epilepsia. 2010;51(1):27–36.
13. Cao R, LI A, Cho HY. mTOR signaling in epileptogenesis: too much of a good thing? J Neurosci. 2009;29(40):12372–3.
14. Heng K, Haney MM, Buckmaster PS. High-dose rapamycin blocks mossy fiber sprouting but not seizures in a mouse model of temporal lobe epilepsy. Epilepsia. 2013;54(9):1535–41.
15. Jaworski J, Sheng M. The growing role of mTOR in neuronal development and plasticity. Mol Neurobiol. 2012;34(3):205–19.
16. Zeng LH, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. J Neurosci. 2009;29(21):6964–72.
17. Huang X, Zhang H, Yang J, Wu J. Pharmacological inhibition of the mammalian target of rapamycin pathway suppresses acquired epilepsy. Neurobiol Dis. 2010;40(1):193–9.
18. Sliwa A, Plucinska G, Bednarczyk J, Lukasiuk K. Post-treatment with rapamycin does not prevent epileptogenesis in the amygdala stimulation model of temporal lobe epilepsy. Neurosci Lett. 2012;509(2):105–9.
19. Way WS, Rozas NS, Wu HC, McKenna J, Reith RM, Hashmi S, et al. The differential effects of prenatal and/or postnatal rapamycin on neurodevelopmental defects and cognition in a neuroglial mouse model of tuberous sclerosis complex. Hum Mol Genet. 2012;21(14): 3226–36.
20. Erlich S, Alexandrovich A, Shohami E, Pinkas-Kramarski R. Rapamycin is a neuroprotective treatment for traumatic brain injury. Neurobiol Dis. 2007;26(1):86–93.
21. Kolosova NG, Vitovtov AO, Muraleva NA, Akulov AE, Stefanova NA, Blagosklonny MV, et al. Rapamycin suppresses brain aging in senescence-accelerated OXYS rats. Aging (Albany NY) 2013;5(6):474–84.
22. Zafar I, Ravichandran K, Belibi FA, Doctor RB, Edelstein CL. Sirolimus attenuates disease progression in an orthologous mouse model of human autosomal dominant polycystic kidney disease. Kidney Int. 2010;78(8):754–61.
23. Yildirim M, Marangoz AH, Ayyildiz M, Ankarali S. The interactions of nitric oxide and adenosine on penicillin induced epileptiform activity in rats. Acta Neurobiol Exp. 2011;71(2): 208–19.
24. Chen L, Hu L, Dong JY, Ye Q, Hua N, Wong M, et al. Rapamycin has paradoxical effects on S6 phosphorylation in rats with and without seizures. Epilepsia. 2012;53(11):2026–33.
25. Zhang Z, Wu X, Duan J, Hinrichs D, Wegmann K, Zhang GL, et al. Low dose rapamycin exacerbates autoimmune experimental uveitis. PLoS One. 2012;7(5):e36589.
26. Hartman AL, Santos P, Dolce A, Hardwick JM. The mTOR inhibitor rapamycin has limited acute anticonvulsant effects in mice. PLoS One. 2012;7(9):e45156.
27. Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. Ann Neurol. 2008;63(4):444–53.
28. Guo D, Zeng L, Brody DL, Wong M. Rapamycin attenuates the development of posttraumatic epilepsy in a mouse model of traumatic brain injury. PLoS One. 2013;8(5):e64078.
29. Gebhardt C, Breustedt JM, Nöldner M, Chatterjee SS, Heinemann U. The antiepileptic drug losigamone decreases the persistent Na+ current in rat hippocampal

neurons. Brain Res. 2001;920(1–2):27–31.
30. Wong PT, Tan SF, Lee HS. N-demethylation of methyl and dimethyl derivatives of phenytoin and their anticonvulsant activities in mice. Jpn J Pharmacol. 1988;48(4):473–8.
31. Kovácsa Z, Czurkób A, Kékesib KA, Juhász G. The effect of intraperitoneally administered dimethyl sulfoxide on absence-like epileptic activity of freely moving WAG/Rij rats. J Neurosci Methods. 2011;197(1):133–6.
32. Wang Y, Barbaro MF, Baraban SC. A role for the mTOR pathway in surface expression of AMPA receptors. Neurosci Lett. 2006;401(1–2):35–9.
33. Bissler JJ, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM, et al. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. N Engl J Med. 2008;358(2):140–51.
34. Rauktys A, Lee N, Lee L, Dabora SL Topical rapamycin inhibits tuberous sclerosis tumor growth in a nude mouse model. BMC Dermatol. 2008;8(1):1–9.
35. Muncy J, Butler IJ, Koenig MK. Rapamycin reduces seizure frequency in tuberous sclerosis complex. J Child Neurol. 2009;24(4):477.
36. Krueger DA, Care MM, Holland K, Agricola K. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. N Engl J Med. 2010;363(19):1801–11.
37. Sandsmark DK, Pelletier C, Weber JD, Gutmann DH. Mammalian target of rapamycin: master regulator of cell growth in the nervous system. Histol Histopathol. 2007;22(8):895–903.
38. Graham KF, Haddick PC, Jan YN, Jan LY. Activity- and mTOR-dependent suppression of Kv1.1 channel mRNA translation in dendrites. Science. 2006;314(5796):144–8.
39. Lenz G, Avruch J. Glutamatergic regulation of the p70S6 kinase in primary mouse neurons. J Biol Chem. 2005;280(46):38121–4.
40. Gong R, Park CS, Abbassi NR, Tang SJ. Roles of glutamate receptors and the mammalian target of rapamycin (mTOR) signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons. J Biol Chem. 2006;281(27):18802–15.
41. Brewster AL, Lugo JN, Patil VV, Lee WL, Qian Y, Vanegas F, et al. Rapamycin reverses status epilepticus-induced memory deficits and dendritic damage. PLoS One. 2013;8(3):e57808.