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ANTICONVULSANT ACTIVITY OF RESVERATROL-LOADED LIPOSOMES *IN VIVO*

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Abstract—Resveratrol (3,5,4′-stilbenetriol), a natural polyphenol produced by various plants, has attracted attention over the past decade because of its multiple beneficial properties, including anti-inflammatory, anti-oxidant and chemopreventive, yet, there is limited information about its antiepileptic effects. Moreover, its poor solubility in water and low bioavailability are the challenging issues. In the present study, we aimed to investigate effects of free resveratrol and resveratrol delivered in amphipathic liposomal delivery system, which has a high blood–brain barrier crossing potential, on penicillin-induced epileptic seizure model. For this purpose, adult male Sprague–Dawley rats were divided into four groups as saline (Control), liposome (LIP), free resveratrol (RES) and resveratrol + liposome (RES + LIP). Penicillin-induced epileptic activity was recorded for 120 min by electrocorticography. Glutathione S-transferase (GST), Glutathione (GSH), Superoxide dismutase (SOD) and Malondialdehyde (MDA) assays were performed in brain tissues collected. Our results showed that RES + LIP was the most effective anticonvulsant treatment on penicillin-induced epileptic seizures when compared to control, as RES + LIP immediately decreased the number of spikes per minute. GST and SOD activity, as well as the GSH levels, were significantly increased in the RES + LIP group as compared with the control group. Also, the MDA levels were significantly higher in the RES + LIP compared to RES and control groups. In conclusion, RES + LIP treatment was more effective on the decrease in spike frequency and spike amplitudes than other treatments. Our results suggest that the RES + LIP is more effective than RES on penicillin-induced epileptiform activity. **© 2017 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: resveratrol, epilepsy, penicillin-induced epilepsy model, antioxidant, liposome.

INTRODUCTION

Epileptic seizure is a paroxysmal attack caused by abnormal electrical discharge in the brain. It reveals itself with sudden changes in memory, consciousness, behavior or sensory-motor functions (Fisher et al., 2005). Epileptic seizures are clinical indicators of excessive, hypersynchronous and limited duration activity of neurons. Seizures occur as a consequence of abnormal electrical activity of the brain (Fisher et al., 2005; Silver et al., 1991).

Reactive oxygen species (ROS) are produced as a result of normal aerobic metabolism. Enzymatic (e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and nonenzymatic (e.g., vitamin C, vitamin E, and reduced form of glutathione) antioxidant systems can clear the physiological levels of ROS (Shin et al., 2011; Akkaya et al., 2014). However, excessive ROS generation causes oxidative damage and contributes to neuronal injury during epilepsy (Puttachary et al., 2015). Since the brain tissue uses the highest amount of oxygen compared to other body organs, it is particularly susceptible to oxidative stress. It is also prone to lipid peroxidation due to the presence of very high amounts of polyunsaturated fatty acids in the brain. Oxidative stress may be generated in epilepsy and also in pathogenesis of a number of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (Shin et al., 2011). Flavonoids having a modulating role in the treatment of such neurodegenerative diseases may repair cellular oxidative processes in the central nervous system (Diniz et al., 2015).

Resveratrol (RES; 3,4′,5 trihydroxystilbene) is a phytoalexin that is formed by spermatophytes in plants against biotic and abiotic stresses (Fremont, 2000). It is found in the roots of *Polygonum cuspidatum*, grape, peanuts, plums, berries and red wine. Studies have reported neuroprotective effects of RES in various models of neurological conditions. In cerebral ischemic stroke animal models, RES reduced the total and cortex infarct volume in a dose, time, and sex dependent manner, as well as ROS production (Shin et al., 2010). In Alzheimer's model of rats, 7 days of lateral ventrical injection of 100 μM RES led to decreased amyloid β-induced neuronal damage, improved antioxidant activity and spatial memory

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Abbreviations: ECoG, electrocorticogram; GSH, glutathione; GST, glutathione S-transferase; LIP, liposome; MDA, malondialdehyde; RES, resveratrol; RES + LIP, resveratrol embedded into liposome; SOD, superoxide dismutase.

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(Huang et al., 2011). In mice, 21-day administration of 50 mg/kg RES by gavage reduced neurodegeneration, astroglial activation and inflammatory activity (Lofrumento et al., 2014), and α-synuclein levels, while increasing the midbrain neuronal survival ratio (Wang et al., 2015). In another study on cerebral ischemic animal model, it was indicated that RES exerted antioxidant activity and inhibited the excitatory synaptic transmission, which is important for neuroprotection (Tsai et al., 2007).

The studies investigating possible effects of RES on epilepsy are limited. Pretreatment with RES was found to be protective on pentylenetetrazole (PTZ)-induced seizures (Gupta et al., 2002b). RES pretreatment decreased the seizure scoring and oxidative stress in the PTZ-induced kindling models of rats (Saha and Chakrabarti, 2014). A 10-day treatment of RES was found to be neuroprotective. In rats, RES was found to be protective for the death of hippocampal CA1 and CA3 neurons, but not DG neurons in the kainate-induced temporal lobe epilepsy (Wu et al., 2009) and PTZ-kindling model of epilepsy (Meng et al., 2014). On the other hand, preconditioning with RES did not hamper the kainate-induced seizures in postnatal developing rats, and moderately protected the CA1, but not CA3 neurons (Friedman et al., 2013). Also, RES pretreatment decreased the early inflammatory responses in pilocarpine-induced status epilepticus (Wang et al., 2013). However, the molecular mechanisms underlying the neuroprotective effects of RES await to be elucidated (Shetty, 2011).

In the present study, we have investigated whether the effect of RES on seizure can be improved with liposome drug delivery system. For this purpose, we aimed to elucidate differences between resveratrol and resveratrol embedded in liposome carrier system on penicillin-induced epileptic activity via main aspects involved in the development of this brain disorder.

EXPERIMENTAL PROCEDURES

Animals and treatments

Adult male Sprague–Dawley rats (250–300 g) used in this study were obtained and maintained at Yeditepe University Medical School Experimental Research Center. Experimental procedures were approved by the Yeditepe University local ethics committee. All animals were kept under standard conditions as 12-h light/dark cycle and temperature (22 ± 1 °C). Standard rat diet and water were provided *ad libitum*.

Firstly, effects of three doses of resveratrol (highly purified, Interpharma) on epileptiform activity were examined. In this preliminary study, the animals were randomly divided into four groups as Control (1 ml saline; $n = 3$), 2 mg/kg RES ($n = 3$), 10 mg/kg RES ($n = 3$) and 20 mg/kg RES ($n = 4$). In the second set of experiment, effect of liposome-embedded RES on the epileptiform activity was investigated. For this purpose, animals were randomly divided into five groups subjected to epileptic seizures; Control-untreated (1 ml saline; $n = 7$), Control (+EtOH)-untreated (1 ml saline with 5% Ethanol (EtOH); $n = 5$) LIP-empty liposome ($n = 5$), RES-free RES (20 mg/kg; $n = 8$), RES + LIP-RES loaded in liposomes (20 mg/kg; $n = 6$).

All drugs were injected through a polyethylene cannula (PE 50) inserted into the femoral vein. A syringe pump (Model 400, CMA) was used for the standard flow rate (200 μl/min). Animals in the control group received 1-ml saline (i.v; 0.09% NaCl) and in the control (EtOH) group received 1-ml saline (i.v; 0.09% NaCl + 5% EtOH) 30 min after penicillin administration. Resveratrol was dissolved in 1 ml saline with 5% ethanol and administered at a dose of 20 mg/kg.

Liposome preparation

Liposomes for resveratrol delivery were locally prepared at Yeditepe University, Faculty of Pharmacy. Liposome dispersions were prepared by thin film technique. Briefly, liposome was prepared by dissolving the 100 μM of phospholipids (PL 100 H) in 30 mL chloroform in a round-bottom flask. The chloroform was removed using a rotary evaporator under reduced pressure to form a thin film over the wall of the flask. The dried film was then hydrated over a water bath with 10 mM Tris Buffer pH = 5.5 above phase transition temperature. After multi lamellar vesicle preparation, sonication process and incorporation techniques were applied, respectively. Resveratrol was incorporated into the liposome dispersions so as to be administered at a dose of 20 mg/kg body weight RES loaded liposomes. Final drug concentration was 0.5% (w/v). Then liposomes were sonicated for 3 × 5 min. All dispersions were kept 4 °C until use. Composition of the empty and resveratrol loaded liposomes is indicated in Table 1.

After preparation, size of liposome and liposome with free resveratrol were analyzed with Zeta Sizer (Malvern instruments; Table 2).

Penicillin-induced epileptiform activity and electrocorticography (ECoG) recording

Sprague–Dawley male rats were anesthetized with urethane (1.25 g/kg, i.p., Sigma U2500) and placed in a stereotaxic frame. Rectal temperature was maintained between 36.5 and 37.0 °C using a feedback-controlled heating system (Model 150, CMA). Lidocaine (2%) was used as a local anesthetic to reduce possible pain in the incision area. The skull on the left cerebral cortex was removed (5 mm posterior to bregma and 3 mm lateral to sagittal sutures) (Ayyildiz et al., 2007) with a dental drill. Two Ag—AgCl spherical electrodes were placed over the left somatomotor cortex. Coordinate of the electrodes was arranged as the positive electrode is 2 mm lateral to

Table 1. Composition, ratio and code of the liposome formulations containing trans-resveratrol

Formulation	Composition	Molar Ratio	Code
Liposome	PL 100H:DCP:CHOL	10:1:4	LIPO SHAM
Liposome	PL 100H:DCP:CHOL + 0.5% trans-RES (w/v)	10:1:4	LIPO RES

CHOL, Cholesterol; DCP, dicetylphosphate; PL 100 H, Phospholipon 100H.

Table 2. Characterization of the empty and resveratrol containing liposomes

Formulation	Size (nm)	Zeta potential (mV)
LIPO SHAM	146.18 ± 5.54	−18.6 ± 0.49
LIPO RES	194.55 ± 25.37	−32.6 ± 3.89

sagittal suture and 1 mm anterior to bregma; and negative electrode is 2 mm lateral to sagittal suture and 5 mm posterior to bregma. The reference electrode was clamped on tail. 500 IU of penicillin-G-potassium (I.E. ULAGAY, Turkey) was injected intracortically (AP: −1 mm, L: 1.5 mm, V: 1 mm) (Hamilton microsyringe type 701 N, 10 μl) with a microsyringe pump controller (micro 4, World Precision Instruments) to form epileptic focus. Epileptic seizures were observed with a four-channel recorder (PowerLab, 4/SP, AD Instruments, Castle Hill, Australia).

All ECoG recordings were performed under anesthesia and continuously recorded on a computer's hard drive through a data acquisition software (LabChart 7.3.3, AD Instruments). Basal electrical activity was recorded for 15 min prior to penicillin injection. After penicillin administration, a latent period occurred. Bilateral spikes appeared in the recording line. After 2–4 min, epileptic activity was formed. When the spike frequency and amplitude began to be in the steady state (after 30 min), drugs were administrated according to the groups. When epileptic activity started, drugs were administered accordingly. ECoG recording was proceeded during 1.5 h. Then, the frequency and amplitude of epileptiform activity were analyzed off-line using LabChart 7.3.3 program. At the end of the experiment, the results were analyzed per minute with the macro properties of the software. The number of spikes per minute and average amplitudes were calculated as "peak to peak". This process was followed for each animal's record (Seker et al., 2015).

At the end of experiments, deeply anesthetized animals were decapitated, brains were removed and frozen on dry ice.

Biochemical analysis

Brains were collected and frontoparietal lobe of the left hemisphere was separated on dry ice, then stored at −80 °C for biochemical analysis.

Protein concentrations were measured according to the method reported by Lowry et al. (1951), Akkaya et al. (2014, 2016) and Yilmaz et al. (1996).

GST activity was determined as described by Habig et al. (1974). The reaction was measured by observing the conjugation of 1-chloro, 2,4-dinitrobenzene with reduced GSH. This was done by watching an increase in absorbance at 340 nm.

GSH was determined by a spectrophotometric method based on the use of Ellman's reagent (1961). 5,5′-Dithio-bis (2-Nitrobenzoic Acid) is measured by the enzymatic cycle procedure in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione reductase.

Total SOD activity was determined by the method of Sun et al. (1988) by inhibiting nitroblue tetrazolium reduction with xanthine/xanthine oxidase, which was used as a superoxide generator. One SOD unit was defined as the amount of protein that inhibits the rate of NBT reduction by 50%.

The MDA level in the brain tissue was measured according to the concentration of thiobarbituric acid reactive substances (Placer et al., 1961).

Statistics

As the drugs were applied 30 min post-induction of penicillin-induced epileptic activity, the measurements obtained between the period of 0 and 30 min (7 measurements with 5-min interval) were set to 100% within each treatment group and all the measurements were normalized against the 0–30 min period (normalization period).

For all statistical analysis, GraphPad prism® 6 was used.

For the statistical analyses of electrophysiological measurements, two different approaches were used. First, the differences between groups were compared using a Two-Way ANOVA followed by least significant difference (LSD) test within each row (Time points), comparing the columns (treatments). Secondly, the differences between the normalization period and the measurement points were analyzed using One-way ANOVA followed by LSD. Values were expressed as mean ± standard error of mean (SEM). $P < 0.05$ was considered to be statistically significant.

Outliers in the measurements were identified using the ROUT method by Motulsky and Brown (2006). The Q value was set to two. The statistical analyses for the biochemical analyses of the oxidative stress markers were a One-way ANOVA followed by the LSD test. Values were expressed as mean ± standard deviation (SD). $P < 0.05$ was considered to be statistically significant.

RESULTS

In this study, resveratrol and resveratrol delivered in liposome carrier system were investigated in the rat models of epilepsy induced by penicillin. Brain epileptic activity was measured by ECoG records and enzyme activities were measured to determine the antioxidant activities.

Effects of free resveratrol on penicillin-induced epilepsy

In order to investigate the dose-dependent effect of resveratrol on penicillin-induced epileptiform activity, rats were treated with three different doses of resveratrol (2, 10 and 20 mg/kg) after the induction of epilepsy by intracortical Penicillin G injection (500 IU/2.5 μl). ECoG results showed that neither of the doses did significantly change the spike frequency (Fig. 1A) and spike amplitude (Fig. 1B).

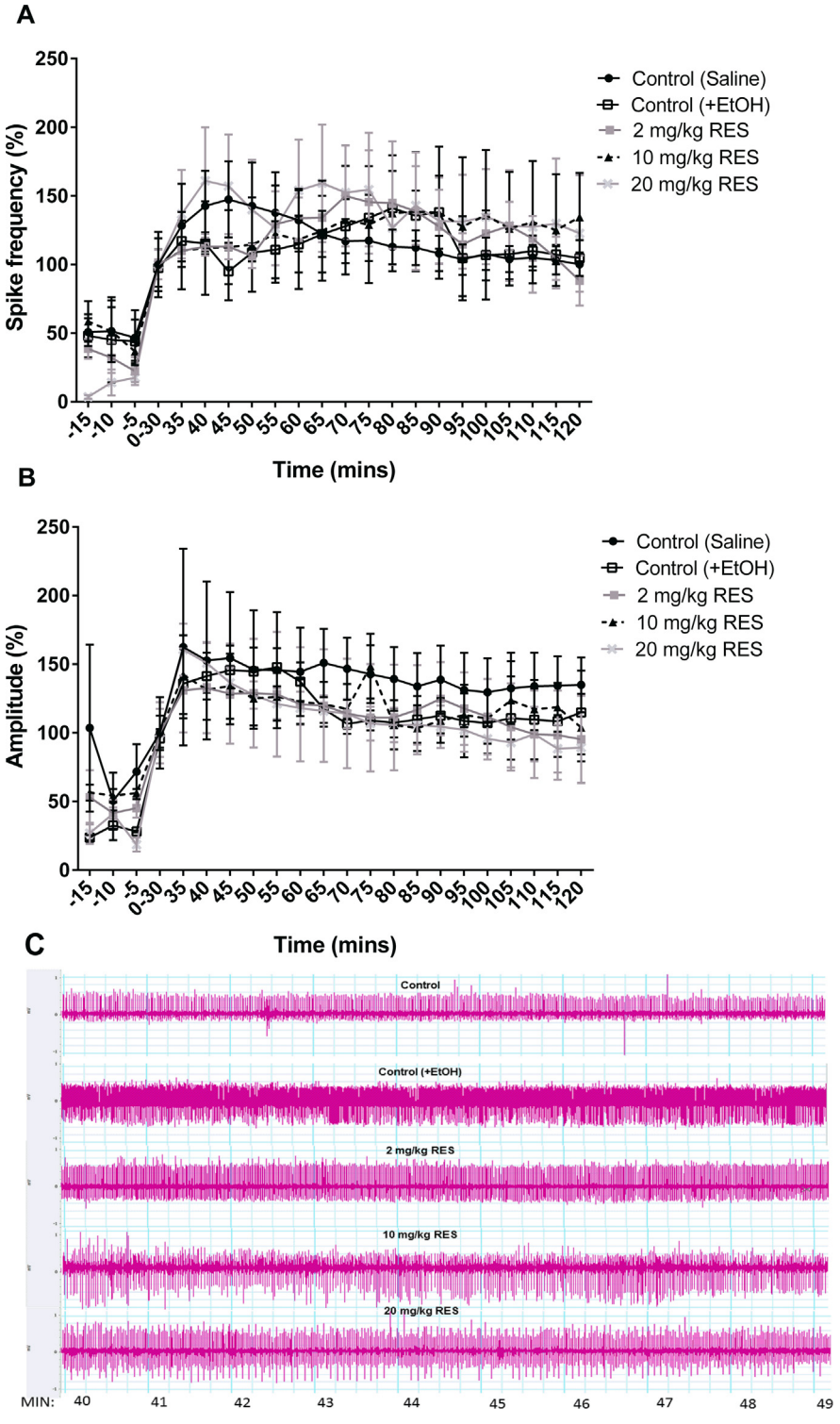

Fig. 1. Free resveratrol did not alter the epileptiform activity in the brain. Dose-dependent effect of resveratrol on (A) spike frequency (%) and (B) spike amplitude (%). (C) Representative traces of the ECoG measurements. Data were expressed as mean ± SEM. (n = 3/group except the 20 mg/kg RES treated group, n = 4). Statistical analyses were done using a Two-way ANOVA followed by LSD.

Characterization of the free and resveratrol-loaded liposomes

The free resveratrol did not have any significant effect on the penicillin-induced epileptiform activity in the brain. We therefore loaded the liposomes with the highest dose of resveratrol (20 mg/kg). Liposomes were characterized by the polarized light microscopy and zeta-sizer. Measurements revealed that the empty liposomes have smaller size (Unpaired t-test; $p = 0.032$) and higher zeta potential (Unpaired t-test; $p = 0.003$) compared to the resveratrol-loaded liposomes (Table 2).

Effects of resveratrol delivered in liposome on penicillin-induced epilepsy

There was no difference between the RES-treated group and Control group, except the beginning of the treatment period, in which higher relative spike frequency was observed in the RES-treated group (min 40 and 45, Fig. 2A). Also, RES did not interfere with the amplitudes (Fig. 2B), also no significant changes were observed throughout the post-treatment period when compared with the normalization period (0–30 min; One-Way ANOVA, $p > 0.05$). We also tested the effects of vehicle solution (5% ethanol dissolved in saline) that caused no significant change in frequency and amplitude of ECoG compared to the control group receiving saline alone, except the measurements at the end of the experiment (min 110 and 115).

RES + LIP group was observed as the most effective on the brain epileptiform activity. Throughout the post-treatment period, relative spike frequency decreased significantly compared to the normalization period (0–30 min; One-Way ANOVA, $P < 0.05$). Also, when compared at different measurement points, the RES-LIP treatment led to significantly lower relative frequency compared to control group, as well as RES group (Fig. 2A). Moreover, RES-LIP-treated rat exhibited significantly lower relative amplitudes when compared to the RES and Control groups between 55 and 95 min (Fig. 2B). On the other hand, we did not observe any difference in relative amplitudes between neither of the groups and Control (+EtOH) group.

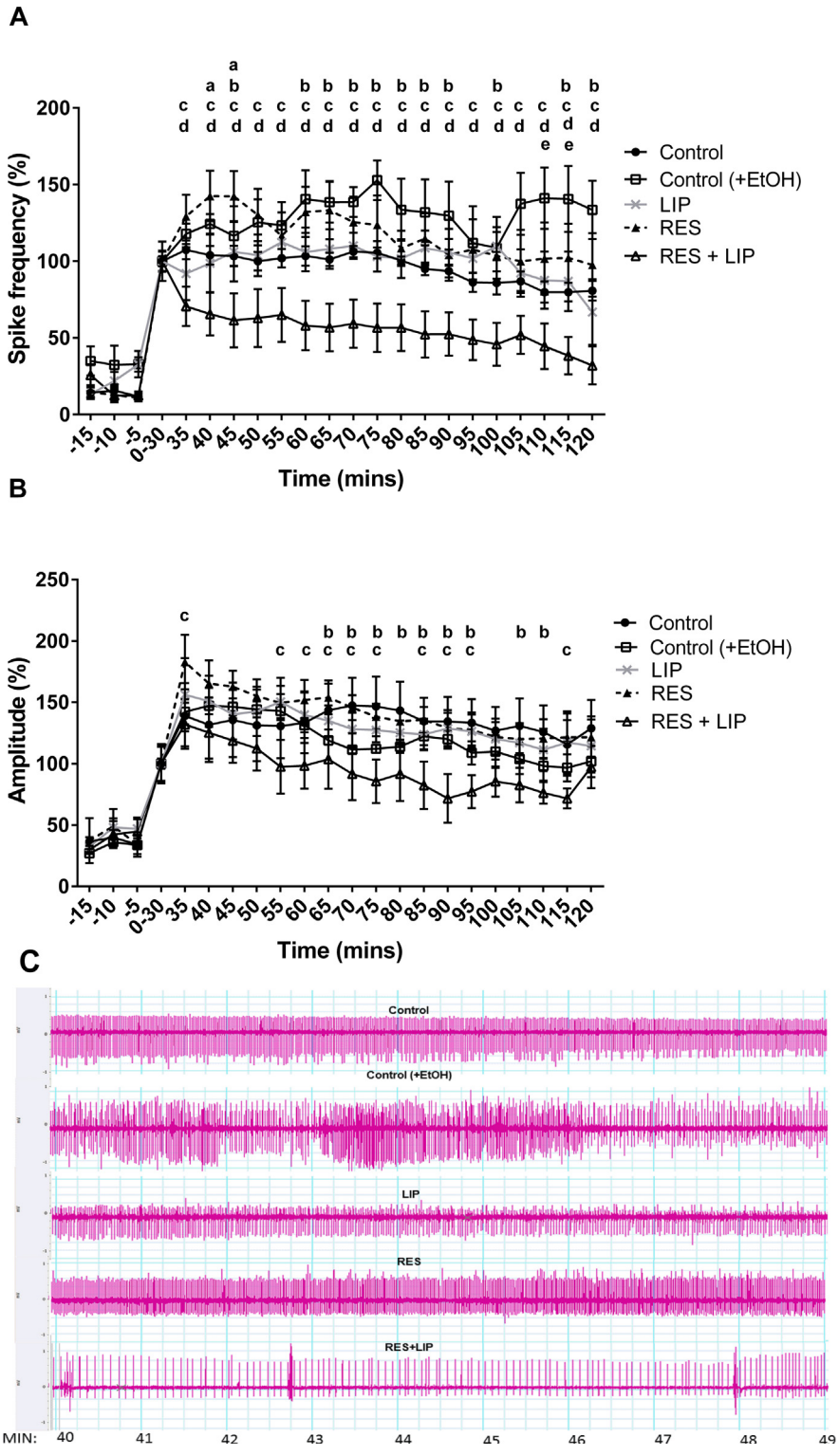

Fig. 2. Resveratrol delivered in liposomes decreased the penicillin-induced epileptiform activity in brain. Effects of free and liposome-entrapped resveratrol on (A) spike frequency (%) and (B) spike amplitude (%). (C) Representative traces of the ECoG measurements. Data were expressed as mean ± SEM (n = 7/group except the Control (+EtOH) group, n = 5). Statistical analysis was done using a Two-way ANOVA followed by LSD. [a]$P < 0.05$ (RES vs. Control (Saline)) [b]$P < 0.05$ (RES-LIP vs. Control (Saline)), [c]$P < 0.05$ (RES vs. RES + LIP) [d]$P < 0.05$ (RES + LIP vs Control (+EtOH)) and [e]$P < 0.05$ (Control (Saline) vs. Control (+EtOH)).

The antioxidant effects of free resveratrol and resveratrol delivered in liposome on penicillin-induced epilepsy

Results of the GST, GSH, SOD and MDA assays are shown in Table 3. GST activity and GSH levels were found significantly higher in RES-LIP group when compared the Control ($P < 0.05$). SOD activity was increased by the RES-LIP treatment significantly, whereas no significant change was observed in RES group. Moreover, RES-LIP treatment significantly reduced the lipid peroxidation, measured as MDA levels, compared to the untreated counterparts and RES group ($P < 0.01$).

DISCUSSUON

The present study shows that liposome carrier system might improve free resveratrol effect on epileptic rat brain with its antioxidant activity.

Acute epileptic seizures may bring about cellular or biochemical alterations, such as permeability of ion channels, neurotransmitter receptor functionality, level of excitability and energy metabolism of neurons (Vaughan and Delanthy, 2002). Penicillin-induced epilepsy begins with focal seizures and may result in grand mal epilepsy depending on its dosage (Akdogan et al., 2008). Penicillin exhibits its proconvulsant epileptic activity via blockage of GABA-gated chloride ion-influx (Tsuda et al., 1994).

RES-mediated neuroprotective effect may include multiple mechanisms, such as inhibition of voltage-activated potassium currents in rat hippocampal neurons, inhibition of electrical activity of CA1 neurons, inhibition of excitatory synaptic transmission in the hippocampus by inhibiting the post-synaptic glutamate receptors and suppression of the activation of astrocytes and microglia (Gao and Hu, 2005; Li et al., 2005). In the literature, there are studies suggesting anticonvulsant properties of resveratrol. RES has been studied for its antioxidant effects in kainic acid model of epilepsy, in which RES prevented the vicious chain by decreasing

Table 3. Brain tissue activity of SOD and GST and level of GSH and MDA

Group	GST (μmol min^{-1} mg^{-1} protein) (N)	GSH (μmol/ml) (N)	SOD (U/mg protein) (N)	MDA (nmol/gr tissue) (N)
Control	15.74 ± 2.04 (5)	8.75 ± 3.42 (5)	1.27 ± 0.09 (4)[x]	2.76 ± 0.53 (5)
LIP	19.29 ± 4.02 (5)	14.04 ± 0.54 (3)	1.35 ± 0.17 (5)	2.11 ± 0.57 (5)
RES	17.42 ± 2.01 (5)	11.84 ± 5.15 (4)	1.17 ± 0.16 (5)	2.43 ± 0.64 (5)
RES + LIP	20.14 ± 4.14[a] (5)	15.49 ± 3.99[a] (5)	1.40 ± 0.25[a] (5)	1.57 ± 0.24[k,l](5)

All data are given as means ± SD.
Statistical analysis was done using a One-Way ANOVA followed by LSD test.
[x] One measurement was determined as an outlier using the ROUT method (Q = 2%; Motulsky and Brown, 2006).
[a] $p < 0.05$ vs. control
[k] $p < 0.01$ vs. control.
[l] $p < 0.01$ vs. RES.

excitotoxicity (Gupta et al., 2002a,b; Friedman et al., 2013). In 2002, researchers aimed to investigate the dose-dependent effect of RES pretreatment on kainic acid model and they found that pretreatment of single dose resveratrol (40 mg/kg) could not be significantly effective on seizures with kainic acid model on rats. They injected multiple doses of trans-resveratrol in two different doses (20 and 40 mg/kg i.p., repeated 2 times after kainic acid). As a result, multiple doses of RES could reduce frequency of convulsions on kainic acid epilepsy model. Brain MDA levels were found to be significantly decreased in the trans-resveratrol-treated groups opposite to treated only kainic acid group (Gupta et al., 2002a).

For evaluating the seizure intensity, number of spikes per minute (frequency) and magnitude (amplitude) were calculated. In our study, we have found that the decrease in frequency started after 30 min RES + LIP administration in comparison to saline and RES group. However, amplitude values started to decrease from 70th min as compared control group. Beside all these results; our results showed that RES + LIP group had less spike frequency than RES group on all post induction period from 35th min till 120th min.

RES has been shown to have anticonvulsant effects in previous studies (Gupta et al., 2002a,b; Friedman et al., 2013). In addition, this anticonvulsant effect was investigated in minutes and significant time intervals were graphically indicated. In preliminary studies, we wanted to investigate that whether different dosages of free resveratrol have an effect on penicillin-induced activity. However, there were no significant results between them. Therefore, we decided to administer the highest dose of free resveratrol for embedding in liposome. According to second part of experiment, we propose that RES + LIP treatment has an action on seizure frequency and seizure propagation. We observed that resveratrol-embedded liposome can be more effective than only resveratrol treatment on acute brain epileptic activity.

To our knowledge, this study was the first using RES treatment on penicillin-induced epilepsy model. Liposomes are carrier systems, which are usually used in cosmetic area or medical drugs (Laura et al., 2006). RES is encapsulated with liposomes and recently used in cancer research (Meng et al., 2016). Encapsulation technique has been reported to be more effective than only RES application in anticarcinoma research (Lu et al., 2012). This method of administration increased its stability and biological activity against oxidative damage and decreased the cytotoxicity at high concentrations (≥100 μM) (Kristl et al., 2009; Caddeo et al., 2008). Liposomes behave like an insert carrier system allowing to sufficient release of resveratrol (Amri et al., 2012). Also, liposomes may act like a cell membrane, they might pass through the blood–brain barrier and release resveratrol. Thus, we hypothesized that RES + LIP might be absorbed in the brain more than RES administered alone. Thus, our findings suggest the notion that liposome improves bioavailibility of resveratrol and its stability.

Under physiological conditions, generation of ROS and antioxidants are balanced with each other. When oxidizable lipids and metals are much more in quantities than antioxidants in the brain, oxidative stress is promoted (Mariani et al., 2005; Uttara et al., 2009). The relationship between oxidative stress and epilepsy was first presented by Armstead et al. (1989). In epilepsy, ROS are produced excessively, over-production of free radicals causes oxidative stress which changes neuronal function dramatically (Uttara et al., 2009).

Animal studies showed that nitrite levels and lipid peroxide formation increase with oxidative stress. Nitric oxide synthases (NOS), SOD, CAT, GP and glutathione reductase, as well as reduced GSH levels can be reduced in hippocampus, striatum, thalamus, cortex or the whole brain in animal epilepsy model (Carmona-Aparicio et al., 2016).

RES has powerful antioxidant potential because it can act both free radical scavenger and antioxidant. Also, it can aid to the activation of antioxidant enzymes. This potent antioxidant feature is likely to influence the epileptic seizures (Alarcón de la Lastra and Villegas, 2007). RES is thought that may suppress seizures through antioxidant mechanism (Lu and Wang, 2015). In the literature, there was no report about antioxidant effects of RES or RES coated by liposome, on penicillin-induced epilepsy models. Therefore, we investigated the possible antioxidant effects of RES and liposome coated RES on penicillin-induced epilepsy model.

The present findings indicate that the animals in RES + LIP group were found to have higher antioxidant activity markers, with statistically significant difference compared to Control group. Individually, the GST and GSH analyzes showed that enzyme activities in the RES + LIP group were significantly higher than in the Control group, suggesting a higher antioxidant activity.

In parallel with this, we observed a significant increase in SOD activity, as well as decreased MDA levels. However, free RES treatment did not cause any change in the oxidative status of the epileptic animals. Our results showed that the resveratrol-coated liposome showed a greater antioxidant property in these experimental groups, the concept of which can be accounted for the biological availability of liposome. Liposome-coated RES may be more efficient in suppression of epileptic seizures as it exhibits a stronger antioxidant activity.

In this study, effects of RES and RES + LIP were analyzed on penicillin-induced epilepsy model. We suggest that when RES is coated with liposome, the antioxidant effect is improved leading to the reduced epileptic seizures. As a result, resveratrol inserted into liposomes showed higher antioxidant and anticonvulsant effects than only resveratrol.

CONCLUSION

Our findings showed that RES + LIP decreases the number of spikes per minute following the drug administration. On the other hand, RES + LIP had no instant effect on amplitudes. Both ECoG results and the biochemical analysis results demonstrated the effectiveness of RES + LIP treatment as an antioxidant.

We suggest that antioxidant and antiepileptic properties of RES are improved by liposome entrapment. Clinical application of resveratrol may be developed using liposomes.

DISCLOSURES

None.

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