The Effect of Ciprofloxacin Injection on Genetically Absence Prone (Wag/Rij) Rat's Electroencephalogram Characteristics

Ali Moghimi[1*], Samaneh Mollazadeh[2], Fatemeh Behnam Rassouli[3], Reza Shiee[4], Mohammad Ali Khalilzade[5]

1. The Applied Research Center for Neurofeedback and Neurobehavioral Sciences (Aren), Ferdowsi University of Mashhad, Iran
2. Biotechnology Research Center, Bu-Ali Research Institute, Mashhad University of Medical Science, Mashhad, Iran.
3. Cell and Molecular Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
4. Department of Signal and Systems, Chalmers University, Sweden.
5. Department of Medical Engineering, Islamic Azad University, Mashhad Branch, Iran.

Article info:
Received: 9 May 2012
First Revision: 27 August 2012
Accepted: 27 October 2012

Key Words:
Ciprofloxacin,
Absence Epilepsy,
Wag/Rij,
EEG.

ABSTRACT

Introduction: Ciprofloxacin which was used in this study is a Fluoroquinolone (FQ). This kind of drug may cause epileptic seizures probably because of the inhibition of GABA binding to its receptors. Wag/Rij rats (an animal model for generalized absence epilepsy), were used as experimental subjects.

Methods: For EEG study, electrodes were inserted into the cortex of animals according to paxinos coordinates. After and before ciprofloxacin injection, EEG was recorded and their SWDs were compared with each others.

Results: Findings showed a significant increase in the mean number of seizures during recording period. But the mean number of SWDs during seizures did not show any significant differences between groups.

Discussion: These results may be due to involvement of GABA antagonistic effects of FQs and/or Mg2+ linked blockade of NMDA receptors. More researches are going to determine physiopathology of SWDs and find new effective substance against this kind of epilepsy.

1. Introduction

Fluoroquinolones (FQs) such as ciprofloxacin and norfloxacin, which are used in the treatment of various infectious diseases, may induce adverse symptoms on the central Nervous System (CNS) including headache, confusion, hallucinations, anxiety, nervousness, and nightmares (Christ, 1990). It also has been reported that FQs may also cause epileptic seizures most frequently in patients with a history of epilepsy (Halliwell, Davey, & Lambert, 1993). Numerous studies conducted in vitro suggested that this central excitatory effect should result from inhibition of γ-aminobutyric acid (GABA) binding to its receptors (Akahane et al., 1989; Tsuji et al., 1988; Tsutomi, Matsubayashi, & Akahane, 1994). Other in vitro experiments suggested that FQs exert their convulsant effects in part by reducing central adenosine-mediated inhibition (Dodd et al., 1989). More recently, it was shown that both central diffusion and affinity for the receptors responsible for the epileptogenic activity vary considerably among FQs, from which it was concluded that these two factors must be considered for prediction of in vivo convulsant activity (Delon et al., 1999).

Ciprofloxacin, a FQ antibiotic, is thought to lower seizure threshold by reducing GABA transmission. There are reports of seizures after internal and intravenous administration of ciprofloxacin (Kushner, Peckman, & Snyder, 2001). Zhang et al. studied the neurotoxicity and toxicokinetics of norfloxacin in freely moving rats.

**** Corresponding Author:***
Ali Moghimi, PhD
Dept. of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Azadi Squ., Mashhad, Iran
Telefax: +98(511)8762227
E-mail: moghimi@um.ac.ir

They observed the epileptiform discharges that were appeared in all norfloxacin injected groups with different latent periods, accompanied with limb twitching and clonic-tonic seizures (Zhang et al., 2003a). In absence epilepsy, the cortico-reticular theory explains the bilateral generalized SWDs. The subcortical pacemaker that is responsible for sleep spindles is also responsible for pathological SWDs (Midzianovskaia et al., 2001). Unseld claimed that FQs act as GABA receptor antagonists (Unseid et al., 1990); which was approved by other researchers This hypothesis was approved by other researchers. Kushner and colleagues showed that ciprofloxacin lowers seizure threshold by reducing GABA transmission (Kushner, Peckman, & Snyder, 2001). Inhibitory effects of Quinolones on the receptor binding of GABAA receptors were investigated (Akahane et al., 1989; Imanishi, Akahane, & Akaike, 1995),

Wag/Rij (Wistar albino Glaxo rat of Rijswijk) rat strain is an animal model for generalized absence epilepsy which shows electrophysiological phenomena, behavioral symptoms and pharmacological responsiveness similar to human non-convulsive epilepsy (Peters et al., 1998). WAG/Rij rats begin to develop Spike Wave Discharges (SWDs) at 3 months and display hundreds of SWDs per day when they are about 6 months (Coenen, & van Luijtelaar, 1987). In, for adult rats the mean number of SWDs per hour is 7.5-10 and their mean duration is 2.5-4/ Sec (Peters et al., 1998).

The objective of this study was to elucidate more about the effects of ciprofloxacin (as a FQ) on electroencephalographic characteristics of absence epileptic seizures in rats. Therefore, the electroencephalogram of WAG/Rij rats was evaluated before and after ciprofloxacin administration.

2. Methods

Six male adult Wag/Rij rats with body weight of 180-220 g were used as experimental animals. They were housed six per cage under artificial 12/12 hours light/dark cycle at a constant room temperature (22±2 ºC) and humidity (60%). All manipulations with animals were according to the locally approved codes of practice for the care and use of experimental animals for scientific purposes.

For EEG recording, animals were anesthetized with intraperitoneal injections of a mixture of Ketamine (60 mg/kg) and Xylazine (6 mg/kg). Standard EEG stainless steel electrodes were inserted according to paxinos coordinates: AP +2.0, L 3.0 for active, AP −6.0, L 4.0 for the indifferent electrode, and the earth electrode was placed on the occipital region of skull. All electrodes were fixed to the skull with dental cement. Then rats were housed individually and allowed to recover for 7 days after electrode implantation.

Before EEG recording, all rats were familiarized with the recording set up and then adapted to experimental cage during 30 min. Then cortical EEGs were recorded for 1800 seconds. Ciprofloxacin was obtained from Samen institute (Mashhad, Iran) and each rat received 6mg/kg ciprofloxacin via i.p injection. Directly after the injection the EEG was recorded for 3600 seconds.

To evaluate the seizure quantitatively, we monitored the EEG in freely moving rats in 3 series of time (indicated as groups): 1st group, 30 min before injection; 2nd group, first 30 min after injection and 3rd group, second 30 min after injection (from 30 min after injection to 60 min). The EEGs were amplified, filtered between 0.01 and 32 Hz, and stored for off-line analyses.

Spike wave extraction has been done based on frequency filtration method. By searching in electrical characteristics of epileptic spike waves, we found the specific frequency range (2-7Hz). Figure 1 shows recorded epileptic spike waves.

The mean number of seizures was counted according to standard criteria. Also, mean frequencies of SWDs in each period of time was counted and analyzed. The results were analyzed using one-way ANOVA and Tukey krammer test. Values are expressed as minimum, average and maximum in figure 2 and 3.

3. Results

SWDs, which are typical criteria of absence epilepsy, can be recognized in Wag/Rij rat's electroencephalogram (fig 1). The number of seizures in each group of Rats before and after ciprofloxacin injection was counted that are the followings: For the 1st group: 21-34. For the 2nd group: 37-131 and for the 3rd group: 17-93. These results are showed in the figure 2 and there is an obvious significant increase in the mean number of seizures during recording period.

To evaluate the probable effects of ciprofloxacin, we also calculate the mean number of SWDs in each period of time (groups). The results are the followings led us result in 4.16-8.42 for the 1st group; : 3.94-7 for the second one and : 4.11-6.57 for the third one. : For the 1st group: 4.16-8.42. For the 2nd groupand for the 3rd

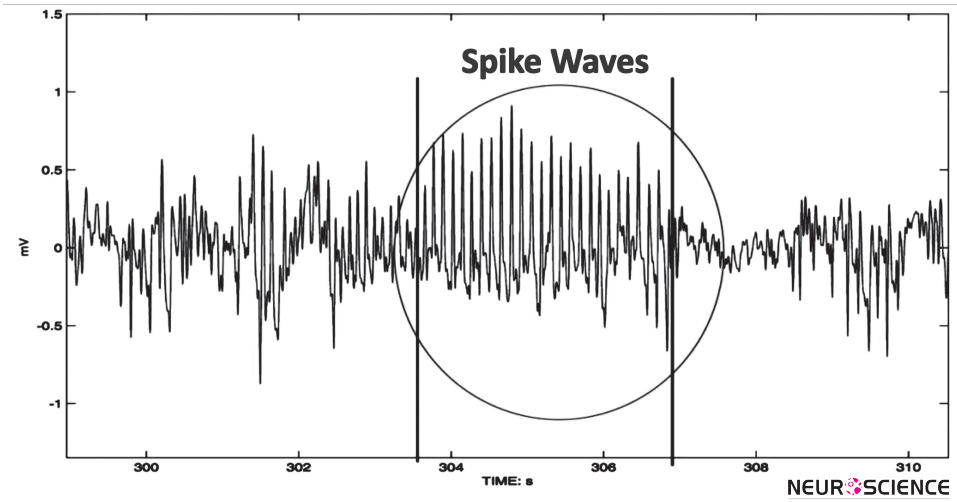

Figure 1. A sample of Spike wave discharges (SWDs) during a seizure.

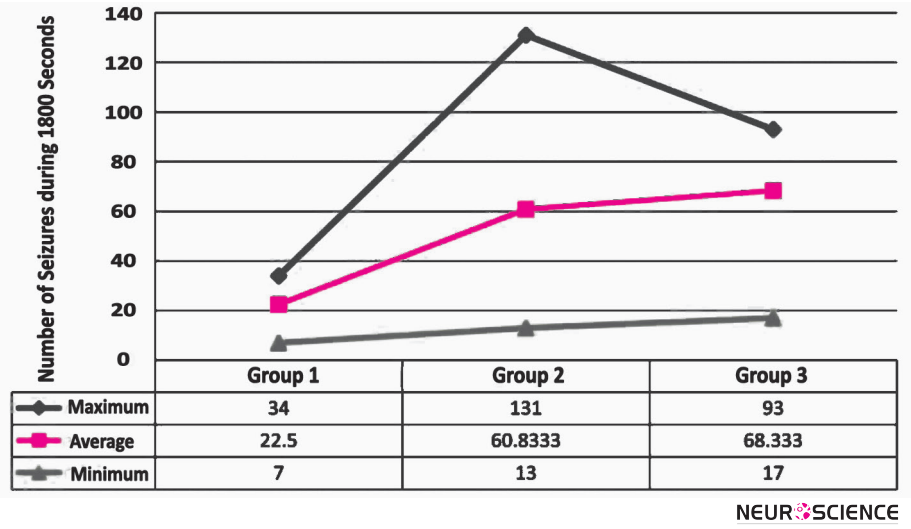

Figure 2. Comparison of minimum, mean and maximum number of seizures between 3 groups.

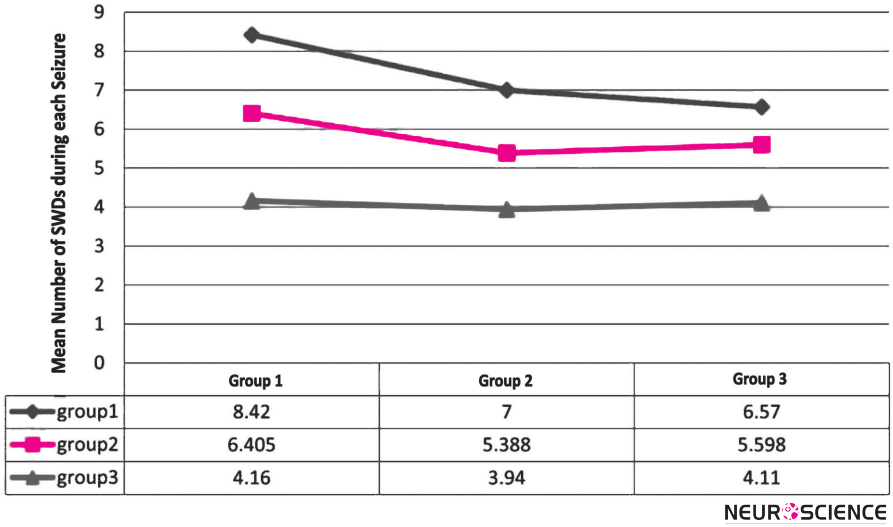

Figure 3. The mean number of SWDs during each seizure.

group. seen in figure 3 there are no significant differences between groups; even though we observed some degrees of decreases in this characteristic recorded from each individual animal, after ciprofloxacin injection the mean number of SWDs during seizures did not show significant differences. As there is obvious in the figure 3 there was not any significant difference between groups.

4. Discussion

The purpose of this study was to investigate the effects of ciprofloxacin on SWDs in Wag/Rij rats. There were some significant changes in seizure incidence (figure 1) after ciprofloxacin injection, yet the exact pathophysiological mechanisms of FQs on the CNS are not completely understood.

Convulsive seizures are reported after quinolones treatment mostly in the elderly or in patients with a history of epilepsy, cerebral trauma, or alcohol abuse. In this report Christ suggested that the proconvulsive activity of fluoroquinolones depends on the chemical structure and might be a critical endpoint of some new representatives of this valuable class of antimicrobials (Christ, 1990). Tsuji et al. have been reported that the dominant mechanism of convulsion by some quinolones is blockading of GABAA receptors in inhibitory neurons and their synergistic enhancement in the presence of Non-steroid anti-inflammatory drugs (Tsuji et al., 1988).

The CNS effects of ciprofloxacin and pefloxacin were attenuated by benzodiazepines (Enginar, & Eroglu, 1991). A clinical case of tonic-clonic convulsion after administration of ciprofloxacin to AIDS patients has been reported by Fan-Harvard et al (Fan-Harvard et al., 1994). The effects of four fluoroquinolones (sparfloxacin, fleroxacin, ofloxacin and levofloxacin) on K+ currents were investigated in pyramidal neurons acutely isolated from rat hippocampus, by Li et al. They found that all four compounds inhibited the delayed rectifier K+ current, but with different potencies (Zhang et al., 2003b). Some investigations revealed that epileptogenic action of quinolones may be related to GABA like structure of ring substitutes (Zhang et al., 2003a). It was suggested that Levofloxacin and ciprofloxacin can induce serious convulsions. They, so they suggested that these quinolones may act their effects through glutamate and GABAB (rather than GABAA) receptors in mice (Akahane, Kato, & Takayama, 1993).

The structural similarities of the FQs to kinurenic acid may suggest an interaction of quinolones with ligand-gated glutamate receptors. Also the field potentials of the CA1 region of the rat hippocampus slices were increased after FQs injections (Schmuck, Schürmann, & Schlüter, 1998). The excitatory effects of FQs were abolished by MK-801(a selective NMDA receptor channel blocker). Also FQs decrease blocking effects of Mg2+ in NMDA receptors (De Sarro et al., 1997). Norfloxacin injections revealed a clear stimulant effect on EEG patterns in normal rats (increase in α and β but decrease in δ rhythms) (Zhang et al., 2003a). By an extensive number of fluoroquinolones tested in an in-vitro model, it was shown that all fluoroquinolones increased the population spike amplitude of the neurons in the CA1 region of the hippocampus. The observation is qualitatively in agreement with the observed convulsant potential of some fluoroquinolones in humans (Hori, & Shimada, 1993).

Some evidences suggested that there is a difference in NMDA receptors discharges in somatosensory cortex of Wag/Rij rats (Luijtelaar, & Sitnikova, 2006). Sen proposed that some newer generations of FQs Such as Levofloxacin causes neurological side effects including convulsion, tremor and chorea-like movements and anxiety (Sen et al., 2007). Finally, as was demonstrated in this research and from some evidences of clinically observations, ciprofloxacin has some excitatory effects that must be under attention when the patients have infections and are epileptic seizures. To find more detailed mechanisms of ciprofloxacin actions on neuronal receptors need to use immunohistochemical methods. Also we suggest studying the effects of ciprofloxacin on other types of experimental epileptic seizures.

Acknowledgements

There is not any conflict of interests. The authors would like to thank the Samen institute for preparing the ciprofloxacin solution. This study was supported by the grant from the Ferdowsi University of Mashhad.

References

Akahane, K., Sekiguchi, M., Une, T., & Osada Y. (1989). Structure-epileptogenicity relationship of quinolones with special reference to their interaction with γ-aminobutyric acid receptor sites. Antimicrobial agents and chemotherapy, 33(10), 1704–1708.

Akahane, K., Kato, M., & Takayama, S. (1993). Involvement of inhibitory and excitatory neurotransmitters in levofloxacin and ciprofloxacin-induced convulsions in mice. Antimicrobial agents and chemotherapy, 37(9), 1764-1770.

Christ, W. (1990). Central nervous system toxicity of quinolones: human and animal findings. The Journal of antimicrobial chemotherapy, 26, 219–225.

Coenen, A. M., & van Luijtelaar, E. L. (1987). The WAG/Rij rat model for absence epilepsy: age and sex factors. Epilepsy research, 1(5), 297–301.

Delon, A., Bouquet, S., Huguet, F., Brunet, V., Courtois, P., & Couet, W. (1999). Pharmacokinetic-pharmacodynamic contributions to the convulsant activity of fluoroquinolones in rats. Antimicrobial agents and chemotherapy, 43(6), 1511–1515.

De Sarro, G. B., Nava, F., Calapai, G., & De Sarro, A. (1997). Effects of some excitatory amino acid antagonists and drugs enhancing gamaaminobutyric acid neurotransmission on pefloxacin – induced seizures in DBA/2 mice. Antimicrobial agents and chemotherapy, 41(2), 427–437.

Dodd, P. R., Davies, L. P., Watson, W. E., Nielsen, B., Dyer, J. A., & Wong, L. S., et al. (1989). Neurochemical studies on quinolone antibiotics: effects on glutamate, GABA and adenosine systems in mammalian CNS. Pharmacology and toxicology, 64(5), 404–411.

Enginar, N., & Eroglu, L. (1991). The effect of ofloxacin and ciprofloxacin on pentylenetetrazol-induced convulsions in mice. Pharmacology, biochemistry, and behavior, 39(3), 587-589.

Fan-Harvard, P. V., Sanchorawala, V., Oh, J., Moser, E. M., & Smith, S. P. (1994). Concurrent use of foscarnet and ciprofloxacin may increase the propensity for seizures. The Annals of pharmacotherapy, 28(7-8), 869–872.

Halliwell, R. F., Davey, P. G., & Lambert, J. J. (1993). Antagonism of GABAA receptors by 4-quinolones. The Journal of antimicrobial chemotherapy, 31(4), 457–462.

Hori, S., & Shimada, J. (1993). Effects of quinolones on the central nervous system. Washington, D.C: American Society for Microbiology. 513–518.

Imanishi, T., Akahane, K., & Akaike, N. (1995). Attenuated inhibition by levofloxacin, l-isomer of ofloxacin, on GABA response in the dissociated rat hippocampal neurons. Neuroscience letters, 193(2), 81-84.

Kushner, J. M., Peckman, H. J., & Snyder, C. R. (2001). Seizures associated with fluoroquinolones. The Annals of pharmacotherapy, 35(10), 1194-1198.

Luijtelaar, G. V., & Sitnikova, E. (2006). Global and focal aspects of absence epilepsy: The contribution of genetic models. Neuroscience and biobehavioral reviews, 30(7), 983–1003.

Midzianovskaia, I. S., Kuznetsova, G. D., Coenen, M. L., Spiridonov, A. M., & van Luijtelaar, G. V. (2001). Electrophysiological and pharmacological characteristics of two types of spike-wave discharges in WAG/Rij rats. Brain research, 911(1), 62-70.

Peters, H. C., Kammer, G., Volz, A., Kaupmann, K., Ziegler, A., & Bettler, B., et al. (1998). Mapping, genomic structure, and polymorphisms of the human GABABR1 receptor gene: evaluation of its involvement in idiopathic generalized epilepsy. Neurogenetics, 2(1), 47-54.

Schmuck, G., Schürmann, A., & Schlüter, G. (1998). Determination of the Excitatory Potencies of Fluoroquinolones in the Central Nervous System by an In Vitro Model. Antimicrobial agents and chemotherapy, 42(7), 1831–1836.

Sen, S., Jaiswal, A. K., Yanpallewar, S., & Acharya, S. B. (2007). Anxiogenic potential of ciprofloxacin and norfloxacin in rats. Singapore medical journal, 48(11), 1028-1032.

Tsuji, A., Sato, H., Kume, Y., Tamai, I., Okezaki, E., & Nagata, O., et al. (1988). Inhibitory effects of quinolone antibacterial agents on _-aminobutyric acid binding to receptor sites in rat brain membranes. Antimicrobial agents and chemotherapy, 32(2), 190–194.

Tsutomi, Y., Matsubayashi, K., & Akahane, K. (1994). Quantitation of GABAA receptor inhibition required for quinolone-induced convulsions in mice. The Journal of antimicrobial chemotherapy, 34(5), 737–746.

Unseid, E., Ziegler, G., Gemeinhardt, A., Janssen, U., & Klotz, U. (1990). Possible interaction of fluoroquinolones with the benzodi-azepine-GABA-A-receptor complex. British journal of clinical pharmacology, 30 (1), 63-70.

Zhang, L. P., Yong-Ming, W., Bin-Yan, C., & Neng, C. (2003a). Neurotoxicity and toxicokinetics of norfloxacin in conscious rats. Acta pharmacologica Sinica, 24(6), 605-609.

Zhang, L. R., Li, M. H., Cheng, N. N., Chen, B. Y., & Wang, Y. M. (2003b). Inhibition by fluoroquinolones of K+ currents in rat dissociated hippocampal neurons. European journal of pharmacology, 462(1-3), 9–13.