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

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A B S T R A C T

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.

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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. 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 group and 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.
As there is obvious in the figure 3 there was not any significant difference between groups.
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