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Original Article

A COMPARATIVE STUDY ON THE EFFECT OF FLUOROQUINOLONES IN EXPERIMENTAL SEIZURES ON WISTAR RATS: AN ACUTE STUDY

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ABSTRACT

Objective: To compare the convulsive profile of three different Fluoroquinolones namely Ciprofloxacin, Levofloxacin and Ofloxacin using Maximal Electro Shock (MES) as an experimental seizure model.

Methods: Wistar rats were divided into 8 groups with 6 animals each. Group I was administered Gum acacia 1 % solution (control), Group II with Standard drug, Sodium Valproate and Group III-VII received 25 mg/kg and 50 mg/kg of Ciprofloxacin, Levofloxacin and Ofloxacin each respectively. After 45 min of administration of drugs, animals were subjected to MES.

Results: Ciprofloxacin prolonged various phases of MES, Ciprofloxacin 50 mg/kg group increased Tonic Hindlimb Extensor (THE) duration by 91% compared to the control group which was statistically significant. Levofloxacin group exhibited pro convulsive activity which was not significant. Ofloxacin 50 mg/kg group exhibited 80% reduction in the duration of tonic hind limb extensor phase and early recovery phase compared to control group (4.2±1.09 s and 169.33±5.3 s) respectively, proven statistically significant. Ofloxacin group exhibited anticonvulsant like activity.

Conclusion: Fluoroquinolones are the popular class of antimicrobials used in a variety of infections. However, conflicting experimental evidence regarding central neurotoxicity especially seizures, complicate their use and required further investigation. Our results suggest that older generation Fluoroquinolones like Ciprofloxacin exhibits significant dose-dependent pro convulsive activity. Hence, their use must be judiciously restricted in patients with predisposing epileptogenic factors. Levofloxacin had no significant pro convulsant activity. Ofloxacin on higher dose appears to be protective exhibiting an anticonvulsant like activity. Hence, if need be, newer generation Fluoroquinolones should be preferred.

Keywords: Fluoroquinolones, Ciprofloxacin, Levofloxacin, Ofloxacin, MES, Anticonvulsant.

INTRODUCTION

A seizure is the physical finding or changes in behavior that occur after an episode of abnormal electrical activity in the brain [1]. Seizures can occur due to many reasons ranging from abnormality in electrolyte levels to serious cerebral infections, injuries or tumors, systemic causes like fever, uremia, hypoglycemia, multi-organ failures, other cause like poisonings, drug withdrawals etc. Seizures are also a serious complication associated with certain drug use. It is estimated that 6.1% of new onset seizures are drug related [2]. It is essential for clinicians to understand drugs causing seizures in order to monitor and to treat the patient promptly. Even though seizures could be a feature of the underlying disease, convulsive episodes are also reported with the use of a number of antimicrobial agents used in therapy (fluoroquinolones, penicillins, monobactams, and carbapenems) [3].

Fluoroquinolones have recently gained much attention recently due to their adversities from different health-care perspectives. Their common adverse effects ranges from mild reactions like headache, nausea, vomiting, diarrhea, to severe ones like photosensitivity, visual disturbances, insomnia, hepatic and renal insufficiency, tendinopathy, prolongation of QT interval, neurotoxicity and neuropathy [4, 5]. Adverse event reporting for antibiotics found that 12.2% of adverse reaction reports concerning fluoroquinolones involved the central nervous system (CNS) versus 3.6% for other antibiotics [6, 7]. CNS adverse effects include headache, dizziness, depression, seizures, hallucination, anxiety, insomnia, dizziness and encephalopathy are well established in preclinical studies and occurs in 1-7% of patients under the treatment with quinolones, complicating their clinical use [8]. Ascertaining the probable clinical course and likelihood for complications related to fluoroquinolone induced seizures can guide clinical decisions, foster cost-effective management, and optimize treatment. Hence, there is a need for continued surveillance of safety and tolerability data is essential. The present study was carried out with the aim to evaluate the same and to compare and rank various generations of fluoroquinolones in the order of their convulsant activity. The drugs studied were Cipro floxacin, Levofloxacin and Ofloxacin using maximal electroshock induced seizures (MES) in Wistar albino rats.

MATERIALS AND METHODS

Adult Wistar albino rats of either sex weighing between 200-250 g were used for this study. Rats were procured from the central animal house of A J Institute of Medical Sciences, Mangalore. The animals were housed in groups of 3 animals per polypropylene cages in temperature-regulated rooms with air cooling and 12 h light and dark cycle, and had free access to food and water ad libitum. They were allowed to acclimatize to the laboratory conditions for a period of one week. The study was approved by the Institutional Animal Ethics Committee, A J Institute of Medical Sciences, Mangalore and all the experiments were performed as per the Committee for the purpose of control and supervision on experiments on animals (CPCSEA) guidelines. The animals were subjected to experimentation between 0900-1600 h in noise-free atmosphere with ambient temperature 23-30 °C.

Chemicals and drugs

The drugs and chemicals used for the experiment were of analytical grade. All the drugs were suspended in vehicle 1% gum acacia solution.

Methodology [9]

Animals were divided into eight groups. For all the procedures, 6 rats were randomly allocated to each of the groups which are given in table 1. Group I served as control and received gum acacia. Group II received the standard drug, (sodium valproate 100 mg/kg). Remaining groups received fluoroquinolones namely ciprofloxacin, levofloxacin and ofloxacin in two doses each (25 and 50 mg/kg orally) respectively for the acute study.

Table 1: Groups & dosages

S. No.	Groups	Drugs*	Dosage
1)	Control	Gum Acasia 1 % solution	10 ml/kg
2)	Standard	Sodium Valproate	100 mg/kg
2)	Test 1	Ciprofloxacin	25 mg/kg
3)	Test 2	Ciprofloxacin	50 mg/kg
4)	Test 3	Levofloxacin	25 mg/kg
5)	Test 4	Levofloxacin	50 mg/kg
6)	Test 5	Ofloxacin	25 mg/kg
7)	Test 6	Ofloxacin	50 mg/kg

*All the drugs were suspended in vehicle-1% gum acacia solution and given once daily, orally

Maximal Electroshock seizures (MES) 10 After 45 min of drug dosing, animals were subjected to Maximal electroshock seizure (MES) by an electrical stimulus of 50 mA (milliampere), for 0.2 s by using electro convulsiometer via ear clip electrodes. The duration in seconds of tonic flexion, tonic extension, clonic convulsion and post ictal depression were noted. The increase in the duration of the tonic extension was considered as the index of epileptic activity and vice versa.

Statistical analysis

The data were represented by mean±Standard error of mean (SEM). Statistical analysis was carried out using IBM SPSS version 22 software. We used one-way ANOVA followed by posthoc Tukey test for analyzing the MES parameters between the groups. A p value less than 0.05 was considered statistically significant.

RESULTS

The results of the effect of Fluoroquinolones on Maximal Electroshock Seizures from our present study is summarized in table 2.

Effect of Fluoroquinolones on onset of tonic hind limb extension (THE) in MES

On application of electroshock, all animals went into different phases of convulsions. The duration of tonic extensor phase was 4.2±1.09 s in the control group (table 2). Ciprofloxacin prolonged various phases of MES in a Dose-dependent pattern. Ciprofloxacin 25 mg/kg and 50 mg/kg significantly increased onset time of tonic hind limb extensor phase at 5.96±0.46 and 5.3±0.03 s respectively from the control groups, effect on onset of THE by Levofloxacin and ofloxacin was neither dose dependent nor statistically significant. The effect on the onset of tonic hind limb extension was not statistically significant in any of the groups.

Effect of Fluoroquinolones on duration of tonic hind limb extension (THE) in MES (fig. 1)

The duration of THE in case of control group was 10.60±1.14 s, whereas ciprofloxacin 25 mg/kg and 50 mg/kg has dose-dependently increased the duration of THE, 15.88±2.04 s and 20.27±3.95 s respectively which was statistically significant for Ciprofloxacin 50 mg/kg group ($p<0.01$). The effect on the duration of THE produced by Levofloxacin was statistically insignificant ($p>0.05$). Ofloxacin reduced the duration of THE with respect to the Control group, in a dose-dependent manner 6.4±2.42 s and 2.08±1.11 s. Ofloxacin 50 mg/kg reduced the duration of THE very significantly ($p<0.001$).

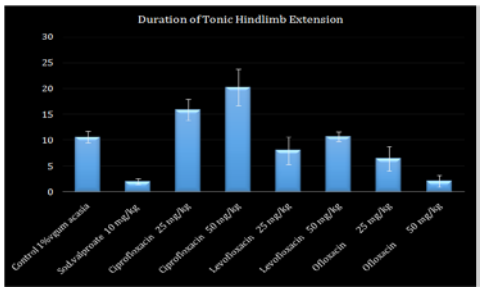
Effect of Fluoroquinolones on recovery time in MES (table 2)

Recovery time signifies the time taken by the animal to recover from the post ictal state after generalized tonic-clonic convulsions. Ciprofloxacin and levo floxacin at both the doses (25 and 50 mg/kg) tested did not alter the recovery time significantly. On the other hand, ofloxacin showed the dose-related reduction in recovery time. But, it was statistically significant only at the highest dose (50 mg/kg) treated group ($p<0.05$). A similar reduction was seen in standard drug, sodium valproate treated group too. None of the animals died, during the observation period and 24 hours after the experiment.

Table 2: Effect of fluoroquinolones on maximal electroshock seizures (MES) in rats n=6)

S. No.	Group mg/kg	Onset of 'THE' (s)	Duration of 'THE' (s)	Duration of recovery (s)
1	Control Gum Acasia 1% soln	4.2±1.09	10.60±1.14	169.33±5.3
2	Standard Sod. valproate 10 mg/kg	3.2±0.54	2.01±0.54**	45.25±1.4**
3	Ciprofloxacin 25 mg/kg	5.96±0.46	15.88±2.04	178±3.12
4	Ciprofloxacin 50 mg/kg	5.3±0.03	20.27±3.95**	188.33±2.4
5	Levofloxacin 25 mg/kg	4.7±1.2	8.05±2.69	140.60±6.15
6	Levofloxacin 50 mg/kg	5.23±0.38	10.69±0.97	180.80±8.22
7	Ofloxacin 25 mg/kg	4.98±1.56	6.44±2.42	153.80±s9.27
8	Ofloxacin 50 mg/kg	3.91±1.2	2.08±1.11**	109.50±7.0*

(Values expressed as mean±SEM). *=P<0.05, ** = p<0.01 compared with control group.)



Values expressed as mean±SEM). *=P<0.05, ** = p<0.01 compared with control group

Fig. 1: Duration of tonic hindlimb extension in maximal electroshock seizures in albino rats

DISCUSSION

Fluoroquinolones are a popular class of antimicrobial agents prescribed in a variety of infections caused by gram negative, gram positive and atypical bacteria. They are mainly classified into four generations based on the year of their discovery. The antimicrobial activity of fluoroquinolones is due to the inhibition of bacterial DNA gyrase [4]. Fluoroquinolones are wonderful antibiotics, however, there is preclinical as well as clinical evidence demonstrating their neurotoxic potential. Neurological toxicity may manifest as mild reactions like headache, dizziness, insomnia, irritability to severe forms like convulsions, psychotic episodes or hallucinations even though they are rarely observed in day to day practice.

However, there is black box warning for fluoroquinolones and they should be avoided in epileptics, and patients presenting with various central nervous system lesions to prevent neurologic complications [11].

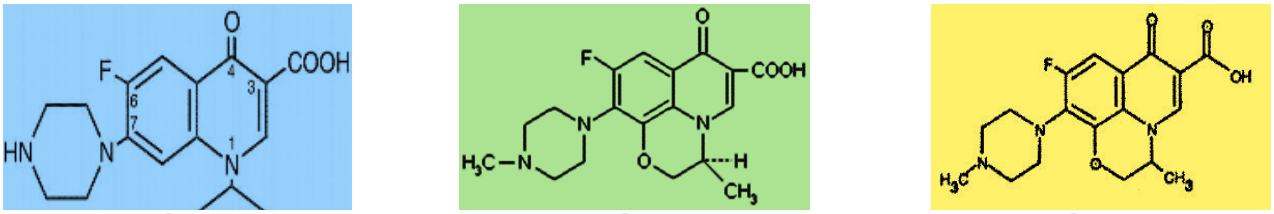
The mechanisms of neurotoxicity have been attributed to their interactions with different receptor complexes such as blockade of the GABA-A receptor complex within the central nervous system, leading to excitotoxic type effects and oxidative stress [12, 13].

In the present study, Ciprofloxacin produced a dose-dependent pro convulsive effect than the other fluoroquinolones studied and the results were similar to studies reported earlier [14]. The pro convulsants activity of fluoroquinolones has a correlation with the chemical structure.

The R7 side chain substituent appears to have maximum influence on the degree of GABA binding inhibition; Larger the side chain substitution (R7) in the chemical structure of fluoroquinolones (fig. 2), lower the binding affinity for GABA receptors [12]. The R7 side chain substituent, particularly unsubstituted piperazinyl, and pyrrolidinyl moieties appear to dictate affinity for the GABA receptor [15]. In agents with an unsubstituted piperazinyl ringlike ciprofloxacin, enoxacin, and norfloxacin,the R7 side chain substituent, demonstrate high-affinity binding to GABA-A and interfere with GABA binding to its receptor. [16] In our study, ofloxacin, which are having heavier side chains at R7 displayed anticonvulsant like activity. The GABA-A antagonistic property of ciprofloxacin was demonstrated previously using rat vagus nerve preparation which further substantiate our results of Ciprofloxacin having high pro convulsant activity [17].

Ofloxacin has higher CNS penetration but the lower affinity to GABA [18] as compared to other fluoroquinolones which could be the reason why it did show protective activity in the present study. Apart from GABA receptor binding theory, other reason attributed for such activity could also be due to by altered pharmacokinetics due to concurrent administration of other drugs [6]. Experimental data suggest that the chondro toxicity of fluoroquinolones could be due to their magnesium chelating property [19]. Similarly, in the central nervous system they might abolish Mg2+in the ion channels associated with activation of excitatory NMDA receptors which may prolong the duration of opening of the channels thus increasing the intracellular Ca2+concentrations and the excitability of the neurons [20]. This magnesium antagonistic property again might be contributive to the excitatory potency and neurotoxicity of fluoroquinolones [21].

From the available clinical literature, it is difficult to put fluoroquinolones in a relative order according to pro convulsive property. Therefore in this preclinical *in vivo* research we tried to compare the relative pro convulsive potential of these drugs so that the results obtained will help the clinicians to make better decisions on choosing the appropriate Fluoroquinolones in patients more prone for convulsive episodes. The same can be considered during drug designing & development of newer fluoroquinolone molecules with better efficacy and negligible neurotoxicity.



Ciprofloxacin **Levofloxacin** **Ofloxacin**

Fig. 2: Chemical structure of Fluoroquinolones

CONCLUSION

The results of the present study suggest that older generation Fluoroquinolones like Ciprofloxacin exhibits significant dose-dependent pro convulsive activity.

Hence, their use must be judiciously restricted in patients with predisposing epileptogenic factors. Levofloxacin had no significant anticonvulsant activity. Ofloxacin higher dose appears to be protective, having anticonvulsant like activity. Hence, if need be, newer generation Fluoroquinolones should be preferred.

CONFLICT OF INTERESTS

Declared None

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