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Research Paper

PROCONVULSIVE PROFILE OF FLUOROQUINOLONES - AN EXPERIMENTAL STUDY WITH CLINICAL CO-RELATIONS

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Background: Fluoroquinolones are a popular class of antibiotics used in variety of infections. However, toxicities are associated with these agents of which seizure provoking action of fluoroquinolones are compared in this study. **Objective**: To compare the proconvulsive profile of various generation of fluoroquinolones namely ciprofloxacin, levofloxacin, sparfloxacin and moxifloxacin in experimental models of convulsions in rat and to correlate the same with the clinical literature. **Material and Methods**: Proconvulsive activity of fluoroquinolones was assessed in rats. The animals were treated intraperitoneally with 12.5 and 25 mg/kg of ciprofloxacin, levofloxacin, sparfloxacin and moxifloxacin. After 30 minutes, animals were subjected to maximal electroshock (MES) & pentylenetetrazole (PTZ) induced convulsions. **Results**: Proconvulsive property of ciprofloxacin was greater compared to other fluoroquinolones in both MES and PTZ method. Levofloxacin produced statistically significant proconvulsive action only in MES, and was insignificant in case of PTZ induced convulsions. Sparfloxacin and moxifloxacin had no significant proconvulsant activity in both the models. **Conclusion**: The results of this study is, if substantiated by further experimental research suggests that fluoroquinolones must be judiciously used in patients with predisposing epileptogenic factors, if mandatory to use these drugs, newer fluoroquinolones of higher generations may be preferred.

Keywords: Ciprofloxacin, Levofloxacin, Moxifloxacin, Pentylenetetrazole, Proconvulsant, Sparfloxacin

INTRODUCTION

Physicians prescribe antibiotics on daily basis, and except for concerns about allergies and selecting the right antibiotic for the right infection, little thought is given to other potential adverse effects. Fluoroquinolones belong to the subset of quinolones, which have a fluorine atom attached to the central ring system, typically at the C-6 position or C-7 position. The antimicrobial spectrum of fluoroquinolones includes gram-negative organisms, gram-positive organisms and some atypical pathogens. Fluoroquinolones are

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broad-spectrum antibiotics, that play an important role in the treatment of serious bacterial infections, especially hospital-acquired infections and others in which resistance to older antibacterial classes is present (Mittmann *et al*., 2002 ; Karageorgopoulos *et al*., 2008).

A study performed by the United States Centers for Disease Control (CDC) projected that adverse events occur at a rate of 9.2/ 10,000 fluoroquinolones prescriptions. This rate is greater than that for cephalosporins 6.1/ 10,000 and macrolides 5.1/ 10,000 (Shehab *et al*., 2008). Fluoroquinolones, overall incidence of CNS adverse reactions, including seizure occurs with an incidence of 1–2 %. Adverse event reporting for antibiotics found that 12.2% of adverse reaction reports concerning fluoroquinolones involved the CNS versus 3.6% for other antibiotics (Owens and Ambrose, 2005). Isolated case reports note the occurrence of seizures with norfloxacin and ciprofloxacin, but underlying neurologic diseases, renal insufficiency, concomitant drug use, or a combination of these factors is known to lower the seizure threshold (Bader, 1992 ; Dembry *et al*., 1999). The association of fluoroquinolones provoking seizures cannot be re-established clinically by a rechallenge due to ethical considerations. Moreover, so far none of the reported studies reveal a comparison of proconvulsive profile of various generations of fluoroquinolones. So, the present study was undertaken to compare the experimental proconvulsive activity of fluoroquinolones by taking one drug from each generation and correlate the same with relative incidences of convulsions occurring clinically. The drugs studied were ciprofloxacin, levofloxacin, sparfloxacin and moxifloxacin on MES and PTZ induced convulsions in rats.

MATERIALS AND METHODS

Animals

Adult Wistar albino rats weighing between 200-250gm were used for this study. Rats were procured from central animal house of Narayana Medical College, Nellore. The animals were housed in 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, Narayana Medical College and all the experiments were performed as per the Committee for the purpose of control and supervision on experiments on animals (CPCSEA) guidelines.

Chemicals and Drugs

Ciprofloxacin, levofloxacin, sparfloxacin, moxifloxacin and pentylenetetrazol were purchased from Sigma Aldrich, Bengaluru. All the drugs were dissolved with distilled water.

Assessment of Proconvulsant Activity

Grouping

Each model consists of nine groups, each group containing 6 animals which were allocated randomly (Tables 1 and 2). Ciprofloxacin was used as reference drug to check proconvulsant activity of I generation fluoroquinolones, levofloxacin for II generation, sparfloxacin for III and moxifloxacin for IV generation (Sharma and Sharma, 2011).

Groups are divided as follows,

Group I	Control group, distilled water 10ml/kg
Group II	Ciprofloxacin 12.5mg/kg.
Group III	Ciprofloxacin 25 mg/kg

Group IV	Levofloxacin 12.5 mg/kg
Group V	Levofloxacin 25 mg/kg
Group VI	Sparfloxacin 12.5 mg/kg
Group VII	Sparfloxacin 25 mg/kg
Group VIII	Moxifloxacin 12.5 mg/kg
Group IX	Moxifloxacin 25 mg/kg

Separate groups of animals were used for different models and all the groups received the drugs intraperitonially half an hour prior to the induction of convulsions.

Maximal Electroshock Seizure (MES)

In this method, electrical stimulation was applied via clipped ear electrodes (moistened with saline solution before each application) which delivered a constant current of 150mA current for 0.2 seconds. Increase in the duration of tonic hind limb extension was taken as an index of proconvulsant activity. Parameters observed were time for onset and duration of tonic hind limb extension (THE) in seconds (Mittal, 2009).

Pentylenetetrazol (PTZ) Induced Seizures

Rats were injected with pentylenetetrazol (40 mg/kg, i.p, subconvulsive dose) half an hour after test drugs and control. The occurrence of the generalized clonus (repeated clonic seizures of the fore and hind limb lasting over 5 sec. with an accompanying loss of righting reflex) or jerky movements were recorded during individual observation of 1 hour. Parameters observed were number of animals showed clonus or jerky movements and twenty four hour mortality was also recorded (Mittal, 2009).

2.3.4. Statistical Analysis

The data was collected in case record forms and then entered into excel spreadsheet 2007. Statistical analysis was performed using Microsoft Excel – 2007 and Sigma Graph pad prism version-5 USA. Data was described as Mean ± Standard deviation. One way ANOVA followed by Tukey - Kramer multiple comparison Test was used for analysis of MES data between the nine group. Data of PTZ method was analysed using Fisher's exact test. For all inferential statistical tests a one tailed $P < 0.05$ was considered significant (as literature and previous studies support proconvulsant activity of fluoroquinolones).

RESULTS

Effect of Fluoroquinolones on Tonic Hind Limb Extension (THE) in MES

All the animals showed various phases of convulsions, on application of maximal electro shock. The duration of THE in case of control group was 10.35±0.74, whereas ciprofloxacin 12.5mg/kg and 25 mg/kg has dose dependently increased the duration of THE, 12.93±0.63 and 15.87±0.47 respectively which was statistically highly significant ($p<0.001$). Levofloxacin 25mg/kg also produced increase in the duration of THE, 13.33±0.73 which was statistically highly significant ($p<0.001$). The increase in the duration of THE produced by Levofloxacin 12.5mg/kg was statistically insignificant. The increase in duration of THE produced by sparfloxacin and moxifloxacin was also not statistically significant (Table 1, Figures 1 and 2).

The percentage increase in duration of THE when compared to control group for ciprofloxacin 12.5 and 25 mg/kg was 24.93% & 53.34% respectively, whereas for levofloxacin 12.5 & 25 mg/kg it was 7.54% & 28.78% respectively. For sparfloxacin and moxifloxacin the percentage increase was less than 10% and not significant. None of the animals died, during the observation period of 24 h after the experiment.

Table 1: Proconvulsant Effect of Fluoroquinolones on Maximal Electro Shock (MES) Induced Convulsions in Rats (n=6)

S. No	Groupsmg/kg	Onset of THE(Sec)	Duration of THE(Sec)	% Increase in Duration of THE
1	Control	2.92±0.30	10.35±0.74	–
2	Ciprofloxacin 12.5	2.3±0.22	12.93±0.63***	24.93%
3	Ciprofloxacin 25	1.92±0.12***	15.87±0.47***	53.34%
4	Levofloxacin 12.5	2.87±0.22	11.13±0.57	07.54%
5	Levofloxacin 25	2.42±0.44	13.33±0.73***	28.78%
6	Sparfloxacin 12.5	3.08±0.22	10.82±0.67	0 4.54%
7	Sparfloxacin 25	2.72±0.40	10.98±0.52	06.08%
8	Moxifloxacin 12.5	2.95±0.36	10.68±0.72	03.19%
9	Moxifloxacin 25	2.86±0.63	10.77±0.61	04.06%

Note: ***p<0.001 as compared to control group, using one way annova followed byTukey - Kramer multiple comparison Test

Figure 1: MES Induced Convulsions – Onset of THE (as per Table 1)

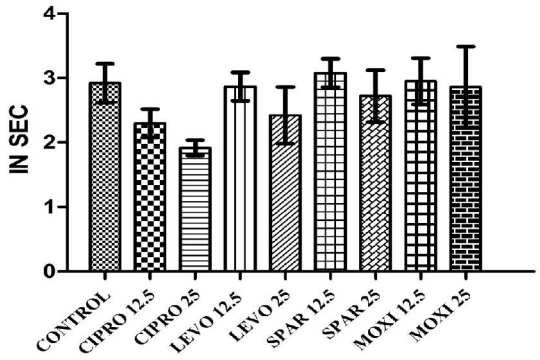
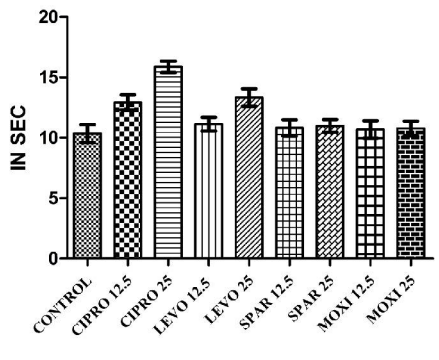


Figure 2: MES Induced Convulsions – Duration of THE (as per Table 1)



Effect of Fluoroquinolones on PTZ Induced Seizures

In control group, PTZ 40mg/kg i.p. has not produced jerky movements or clonus with no incidence of mortality. The percentage of animals showing convulsions for ciprofloxacin 12.5 and 25 mg/kg was 66.67%% and 83.33% respectively, whereas for levofloxacin 12.5 and 25 mg/kg it was 50% and 66.67% respectively. In sparfloxacin and moxifloxacin group, no convulsions were observed. With ciprofloxacin 25 mg/kg the mortality percentage was 66.67%, whereas with ciprofloxacin 12.5 mg/kg the mortality percentage was 16.67%. No mortality was observed for 24 h in case of levofloxacin, sparfloxacin and moxifloxacin groups and the recovery was complete (Table 2 and Figure 3).

DISCUSSION

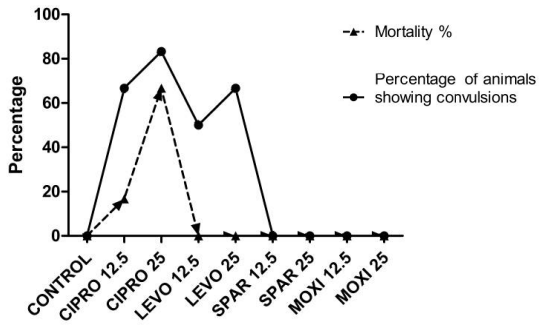
From the animal studies and accumulated clinical experience, attention has been focused on side effects of fluoroquinolones, especially those

Table 2: Proconvulsant Effect of Fluoroquinolones on Pentylenetetrazol (PTZ) Induced Convulsions in Rats (n=6)

S. No.	Groups mg/kg	% of animals showedconvulsions	Mortality%
1	Control	0%	0%
2	Ciprofloxacin 12.5	66.67%%*	16.67%
3	Ciprofloxacin 25	83.33%**	66.67%
4	Levofloxacin 12.5	50%	0%
5	Levofloxacin 25	66.67%*	0%
6	Sparfloxacin 12.5	0%	0%
7	Sparfloxacin 25	0%	0%
8	Moxifloxacin 12.5	0%	0%
9	Moxifloxacin 25	0%	0%

Note: *p<0.05, **p<0.01 as compared to control group, using Fisher's exact test.

Figure 3: PTZ Induced Convulsions - Percentage of Animals Showing Convulsions and Mortality (as per Table 2)



involving central nervous system (CNS). Wide spectrum of neurotoxic effects has been observed with fluoroquinolones. Older non fluorinated quinolones produced neurotoxic symptoms such as dizziness in about 50% of the patients while with fluorinated quinolones the rate is below 10% (Dembry *et al*., 1999; Akahane *et al*; 1989). Mild neurotoxic effects in the form of headache, dizziness, insomnia, restlessness and nightmares have been reported. Severe neurotoxic side effects are seldom observed but they have occurred with most of the fluoroquinolones developed so far. These include convulsions, psychotic reactions and hallucinations. However, all fluoroquinolones are contraindicated in epileptic patients, and close supervision is required when fluoroquinolones are taken by patients with pre-existing CNS lesions, inducing a lower seizure threshold; patients with cerebrocranial injuries or stroke, elderly patients with pronounced arteriosclerosis are likely to suffer from neurologic complications (Sean C Sweetman, 2009; Eric M Scholar, 2003).

With the available literature, it is difficult to categorize proconvulsive profiles of fluoroquinolones. Therefore, the present animal study was undertaken to compare the relative proconvulsive properties of ciprofloxacin, levofloxacin, sparfloxacin and moxifloxacin in two experimental models of convulsions. The results were correlated with the available experimental and clinical reports.

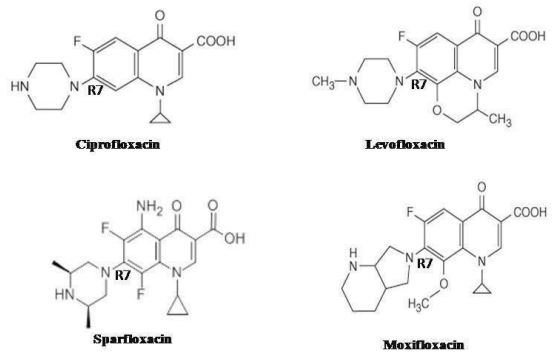
In our study, ciprofloxacin produced a dose dependent proconvulsive effect which was more as compared to other fluoroquinolones in both MES and PTZ (16.67% and 66.67% mortality at

12.5mg/kg and 25mg/kg respectively, Table 2) induced seizure methods. Levofloxacin produced statistically significant proconvulsive action in MES and PTZ induced method only at higher dose of 25 mg/kg. Sparfloxacin and moxifloxacin did not show any significant proconvulsant activity in both the models, (Tables 1 and 2), when compared to ciprofloxacin and levofloxacin which were proconvulsant.

Earlier, it was reported that, ciprofloxacin dose dependently increased the duration of tonic extensor phase in the model of MES and it was the most proconvulsant followed by pefloxacin (Shalini and Prabhu, 1999). Highest proconvulsant activity was observed with pefloxacin, followed by ofloxacin, ciprofloxacin, norfloxacin while nalidixic acid showed least proconvulsant activity (De Sarro *et al*., 1999). Higher incidence of seizures was observed with ciprofloxacin clinically as stated by committee on safety of medicines, which received 26 reports of convulsions associated with ciprofloxacin, 1 with norfloxacin and 1 with ofloxacin (Committee on Safety of Medicines). The $GABA_A$ antagonistic property of ciprofloxacin and sparfloxacin was demonstrated using rat vagus nerve preparation and concluded that sparfloxacin is devoid of $GABA_A$ antagonistic property, at the concentration that reaches human brain (Davey *et al*., 1994). These findings further substantiate our experimental results which portray that proconvulsive profile of ciprofloxacin was much higher than levofloxacin, whereas sparfloxacin and moxifloxacin showed insignificant proconvulsant activity (Tables 1 and 2).

The proconvulsant activity of fluoroquinolones depends on the chemical structure. The R7 side chain substituent appears to have maximum influence on the degree of GABA binding inhibition; larger the side chain lower the binding affinity for $GABA_A$ receptors (Domagala *et al*., 1994). This was substantiated by a clinical study, where it was stated that substitution at R7 with piperazinyl or pyrrolidinyl containing compounds like gatifloxacin and moxifloxacin is associated with reduced seizure provoking potential (Louis and James, 2003). In our study, sparfloxacin and moxifloxacin which are having heavier side chains at R7 showed no proconvulsant activity, supporting the above studies, as shown in Figure 4. So, it may be a decisive endpoint for some new representatives of this valuable class of antimicrobials. It is therefore to be considered during preclinical development.

Figure 4: Comparative Structure of Fluoroquinolones with Substitution at R7 Side Chain (Modified from Betram G Katzung *et al*., 2012)



Other possible mechanism for the proconvulsant activity of quinolones, excitatory potency of quinolones might be based on activation of NMDA receptor by abolishing Mg^{2+} in the ion channel which may prolong the opening time of the channel, thus increasing the intracellular Ca^{2+} concentrations and the excitability of the neurons (Egerbacher *et al*., 2000 ; Stahlmann *et al*., 1997).

PTZ is a better model to evaluate proconvulsive profile of fluoroquinolones (Shalini and Prabhu, 1999). Till date no single animal model for

predicting epileptogenic activity of fluoroquinolones, co-relates with the clinical experience and the mechanism behind this variability needs to be elucidated with further studies.

CONCLUSION

Populations at risk of neurotoxicity associated with various generations of fluoroquinolones include geriatric individuals with critical illness, renal dysfunction, prior neurological disease and drug – drug interaction. Awareness of proconvulsive nature of fluoroquinolones is essential for physicians in order to avoid this avertable complication.

This study may throw some light in selection of an appropriate fluoroquinolones and their judicious use in patients with predisposing epileptogenic factors. If mandatory to use these drugs, newer fluoroquinolones of higher generations may be preferred as they may help in the prevention of neurotoxicity associated with these drugs. Further, research is required to design and develop newer fluoroquinolones with better efficacy and negligible neurotoxicity which will be an attractive therapeutic goal.

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