ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effect of fluoroquinolones for Anticonvulsant activities on PTZ induced seizures in *mice*

Sridharamurthy.N.B[1], *Muralidhar.S.Talkad[2], Juganta das[2], Akanksha[2], Channaveeraswamy.T.H.M[1]

1. Department of Pharmacology, Dayananda sagar College of Pharmacy, Bangalore, India.

2. P.G. Department of Biotechnology, R&D Centre, Dayananda Sagar College of Biological Sciences, Kumaraswamy Layout, Bangalore-560078, India.

Manuscript Info

Manuscript History:

Received: 12 September 2013
Final Accepted: 27 September 2013
Published Online: October 2013

Key words:
Fluoroquinolones Levofloxacin, Norfloxacin, PTZ, theophylline, seizures, Pro-convulsive activity, GABA.

Abstract

The objective of this study was to investigate the Pro-convulsive activity of fluoroquinolones (levofloxacin, norfloxacin) on epilepsy induced methods like PTZ, theophylline at sub convulsive dose. Convulsion is induced by giving sub convulsive dose i.e. 40mg/kg of PTZ intraperitonealy. The pro-convulsive actions of fluoroquinolones levofloxacin, norfloxacin, at two different doses were studied using PTZ methods and co-administered with theophylline and results were compared with the respective control groups and standard groups. Results were showed that, the levofloxacin and norfloxacin demonstrated a significant pro-convulsive profile in PTZ induced seizures. The % decrease in level of GABA treated with LF, NF at 25mg/kg bdw, in mice brain is 7.85, 7.69 respectively as compared to control 16.14% on PTZ induced seizures. Similarly, GABA levels is also showed decreased % to 8.88, 7.44 on treatment with LF, NF at 25mg/kg bdw respectively as compared to control group (16.14%) on co-administration with theophylline.

This indicates that fluoroquinolones may interfere with the synthesis of GABA or may decrease the affinity of GABA towards GABA receptors. The study lend support to the view that role of fluoroquinolones in patho physiology of seizures, the study also provides the protective activity of diazepam against pro-convulsive activity of fluoroquinolones treated animals.

Introduction

Epilepsy is a common chronic neurological disorder characterized by seizures (Leroy O et.al, Presses MED 1989, Blime W et al 1993). These seizures are transient signs and/or symptoms of abnormal, excessive or hyper synchronous neuronal activity in the brain. About 50 million people worldwide have epilepsy, and nearly two out of every three new cases are discovered in developing countries. Epilepsy becomes more common in old ages people age. Onsets of new cases occur most frequently in infants and the elderly. As a consequence of brain surgery, epileptic seizures may occur in recovering patients (Wolters Kluwer et al, 2010.). Epilepsy is usually controlled, but not cured, with medication. However, over 30% of people with epilepsy do not have seizure control, even with the best available medications. Surgery may be considered in difficult cases. Not all epilepsy syndromes are life-long, some forms are confined to particular stages of childhood. Epilepsy should not be understood as a single disorder, but rather as syndrome with vastly divergent symptoms, all involving episodic abnormal electrical activity in the brain and numerous seizures (Surgery for seizures NEJM 1996). However, it has been stressed that accurate differentiation between generalized and partial seizures is especially important in determining the appropriate treatment (Harrison's Principles of Medicine. 15th edition.).

The fluoroquinolones are synthetic broad-spectrum antibiotics. In general, the common side-effects are mild to moderate and self-limiting. On occasion, serious adverse effects can occur. Some of the serious adverse drug

reactions (ADRs) that occur more commonly with fluoroquinolones than with other classes antibiotic drug include central nervous system (CNS) toxicity, phototoxicity, cardio toxicity, arthropathy, and tendon toxicity. Children and the elderly are at greater risk. ADRs may manifest during, as well as sometimes long after fluoroquinolone therapy has been discontinued. Events that may occur in acute overdose are rare and include renal failure and seizure (Jungst, Mohr, 1987). Today, fluoroquinolones are the most commonly-prescribed antimicrobial agents. Ciprofloxacin is considered a benchmark for comparing the efficacy of new fluoroquinolones. The tolerability of these agents is good, with low incidence of adverse effects. Overall rates of adverse reactions are 4.0%–8.0%, and adverse effects have necessitated discontinuation of therapy in 1.0%–2.6% of patients (Christ W, 1990).

Levofloxacin (Kawahara, S. Dec 1998, Nippon Rinsho, 1998) is a broad-spectrum antibiotic that is active against both Gram positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. Norfloxac in (Nelson, JM. Et al, 2007) is a 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1*H*-quinoline-3-carboxylic acid Norfloxacin is a broad-spectrum antibiotic that is active against both Grampositive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division.The concomitant administration of a non-steroidal anti-inflammatory drug (NSAID) with a quinolone, including norfloxacin, may increase the risk of CNS stimulation and convulsive seizures. Therefore, norfloxacin should be used with caution in individuals receiving NSAIDS concomitantly.

The terms fluoroquinolone and quinolone are often used interchangeably, without regard to this distinction (Hakanen, A et al. 2007). With the increasing use of this class of antibiotics, even in sometimes benign indications, an increasing number of patients are exposed to the risk of ADRs (Melhus A (March 2005). Serious adverse reactions can occur in any patient. For example fluoroquinolones induced convulsions can occur in patients with or without a history of convulsions. However, certain patient groups are at increased risk of fluoroquinolone ADRs. A 1998 retrospective survey of the use of the fluoroquinolones in the pediatric population showed that the fluoroquinolones were oftentimes prescribed in children, (although their use is not approved in this age group), and that numerous serious side effects had been recorded (Pariente-Khayat A, et al, 1998). By taking into considerations of review of literature, claims of fluoroquinolones, the present study was planned to assess the pro-convulsive potential of fluoroquinolones in experimental animals.

Material and Methods

Chemicals: Pentylenetetrazole from HI Media Laboratories, Theophylline - Microlab Limited, Bengaluru, Levofloxacin & Norfloxacin -Karnataka Antibiotics Pharmaceuticals Ltd, GABA -S.D. fine Chemicals, other chemicals from SDFCL & Fisher scientific Products

Experimental animals: Albino mice with either sex of average weight (25-35gm) bred in the animal house Dayananda Sagar College of pharmacy, Bangalore were used to induce convulsion by electric shock, Pentylenetetrazole and also by theophylline. The above test animals were divided into three groups, such one group was subjected to electroshock of 150mA intensity for 0.2 seconds, through auricular electrodes, majority of mice showed tonic flexion of fore and hind limbs with tail erection, and stupor followed by post tetanus depression and recovery. Only those mice showing the convulsive responses were used for experiment and divided into 14 groups of six each and further other groups of mice were used for chemo shock (pentylenetetrazole, theophylline) and divided into 14 groups of six each.

All the test animals were allowed for food & water *add libitium* both being withdrawn 24 hrs prior to experimentation (to avoid any possible "kindling" effect). All the preparations were administered intraperitonealy. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (approval no.DSCP/M.Pharmacol/IAEC/82/12-13).

Pentylenetetrazole (PTZ) induced seizures:

Each mouse under the test received a test drug intra-peritoneal 30 minutes before administration of sub convulsive dose of PTZ (40mg/kg body wt) i.p. The animals were kept under observation for the onset of maximal seizures which is evidenced by tonic flexion of forelimbs, tonic extension of hind limbs and clonic convulsions for 15 minutes. (Gabriele schmuck Anja schurmann, Gerhard schlutet Bayer AG, et al, July -1998)

Co-administration with sub convulsive dose of theophylline
Each mouse received the test drug intraperitonealy, 5 minutes before intraperitoneal administration of sub convulsive dose of theophylline (125mg/kg) i.p.

They were observed for the onset of maximal seizures which was evidenced by tonic flexion of fore limbs, tonic extension of hind limbs and clonic convulsions for 2 hours. (Gabriele schmuck Anja schurmann, Gerhard schlutet Bayer AG, et al, July-1998)

Chemoconvulsion Method [Pentylenetetrazole]:
The animals were divided into 14 groups. Group I served as control and received normal saline and group II served as positive control and received diazepam in normal saline. Remaining groups received fluoroquinolones namely levofloxacin and norfloxacin in two doses each (12.5&25mg/kg i.p).

Chemoconvulsion Method [Theophylline]
The animals were divided into 14 groups. Group I served as control and received normal saline and group II served as positive control and received diazepam in normal saline. Remaining groups received fluoroquinolones namely levofloxacin and norfloxacin in two doses each (12.5 & 25mg/kg) i.p. Brain GABA content estimation (Taiwe G.S. et al, 2010.)

Experimental Procedure:
Animals were killed by decapitation at predetermined intervals after the administration of test drug, diazepam and the saline followed by subjecting the animals to Electro convulsion/Chemo convulsions. The brains were rapidly removed, blotted, weighed and taken in ice cold 5 ml trichloro acetic acid (10% w/v), homogenized and centrifuged at 1000rpm for 10 min at 0^0 C. A sample of 0.1 ml of tissue extract was taken in 0.2 ml of 0.14 M ninhydrin solution in 0.5 M carbonate-bicarbonate buffer pH (9.9), was kept in a water bath at 60^0 C for 30 min, then cooled and treated with 5 ml of copper tartarate reagent. After 10 min, the fluorescence reading was taken at 377/451 nm in a spectrofluorimeter.

For GABA standards, different amounts (20, 40, 60, 80,100 µg) mixed with 1.5µM Glutamic acid were dissolved in 0.1 ml of 10% trichloroacetic acid. GABA was determined by the measurement of the formed fluorescent product resulting from the reaction of GABA with ninhydrin in an alkaline medium in the presence of glutamate. The GABA content in brain was expressed in µg g^{-1} of the wet brain tissue.

Results

Table No-1: Effect of fluoroquinolone (LF) on experimental seizures induced by PTZ in mice:

GROUPS	JERKY MOVEMENTS (sec's)	CLONIC CONVULSIONS (sec's)	RECOVERY/DEATH (mint's)
N.SALINE+PTZ	14.5±0.42	15.16±1.13	12±0.36
DZPM+PTZ	5.83±0.54**	12.33±1.22	10.83±0.30
LF(12.5mg)+PTZ	23.83±1.01**	64.83±3.42**	16.83±0.30**
DZPM+ LF (12.5mg) + PTZ	14±0.36	43.83±2.44**	12.97±0.51
LF(25mg)+PTZ	31.5±1.72**	91.5±2.4**	16.66±0.21**
DZPM+ LF(25mg)+PTZ	17±0.96**	49±3.78**	14.66±0.21**

All values are expressed as mean± SEM, n=6, One way analysis of variance (ANOVA) followed by Dun net's Multiple Comparison test. The minimum of $p< 0.0001$ was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05.

Table.No-2: Effect of fluoroquinolone (NF) on experimental seizures induced by PTZ in mice:

GROUPS	JERKY GROUPS MOVEMENTS (sec's)	CLONIC CONVULSIONS (sec's)	RECOVERY/DEATH (mint's)
N.SALINE+PTZ	14.5±0.42	15.16±1.13	12±0.36
DZPM+PTZ	5.83±0.54**	12.33±1.22	10.83±0.30
NF(12.5mg)+PTZ	23.83±1.01**	64.83±3.42**	16.83±0.30**
DZPM+ NF(12.5mg)+PTZ	14±0.36	43.83±2.44**	12.97±0.51
NF(25mg)+PTZ	31.5±1.72**	91.5±2.4**	16.66±0.21**
DZPM+ NF(25mg)+PTZ	17±0.96**	49±3.78**	14.66±0.21**

All values are expressed as mean± SEM, n=6, One way analysis of variance (ANOVA) followed by Dun net's Multiple Comparison test. The minimum of p< 0.0001 was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05.

Levofloxacin (12.5mg) showed 21.83±0.98 sec, 25.83±2.57 sec, jerky movements and clonic convulsions respectively and recovery was increased to 13.66±0.49minutes. Levofloxacin (25mg) showed increased value of 28.66±2.17 sec, 37.0±3.24 sec, jerky movements, clonic convulsion respectively and recovery time was increasedto15±0.36 minutes. Diazepam with levofloxacin (25mg) showed 13.16±0.79 sec jerky movements, 23.5±0.71 sec clonic convulsion and 8.66±0.42 minutes. Norfloxacin (12.5) showed increased value of 23.83±1.01 sec jerky movements, 64.83±3.42 sec of clonic convulsion, and 16.83±0.30 minutes of recovery. Norfloxacin (25mg) showed still increased value of 31.5±1.72 sec and 91.5±2.4 sec Jerky movements, clonic convulsions respectively and recovery of 16.66±0.21 minutes. Diazepam with Norfloxacin (25mg) showed 17.0±0.96 sec, 49.0±3.78 sec, jerky movements, clonic convulsions and recovery respectively and recovery 14.66±0.21 minutes.

Table.No.3- Effect of fluoroquinolone (LF) on experimental seizures induced by theophylline in mice

GROUPS	**TONIC FLEXION OF FORE LIMBS (sec's)**	**TONIC EXTENSION OF HIND LIMBS(sec's)**	**CLONIC CONVULSIONS(sec's)**
N.Saline+TP	37.33±5.18	29.66±2.34	7.8±1.20
Dzpm+TP	24.66±1.74**	23.5±1.40**	4.5±0.56**
LF(12.5mg)+TP	55.16±5.51**	35.87±2.01**	18.33±1.33**
DZPM+	26±4.54**	29.16±2.88	-----
LF(12.5mg)+TP LF(25mg)+TP	240±22.91**	150±8.26**	164.5±5.88***
DZPM+ LF(25mg)+TP	31.16±2.63**	43.33±1.02**	11.16±0.98**

All values are expressed as mean± SEM, n=6, One way analysis of variance (ANOVA) followed by Dun net's Multiple Comparison test. The minimum of p< 0.0001 was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05

Table.No-4:- Effect of fluoroquinolone (NF) on experimental seizures induced by theophylline in mice:

GROUPS	TONIC FLEXION OF FORE LIMBS(sec's)	TONIC EXTENSION OF HIND LIMBS(sec's)	CLONIC CONVULSIONS (sec's)
N.Saline +TP	37.33±5.18	29.66±2.34	7.8±1.20
Dzpm +TP	24.66±1.74**	23.5±1.40**	4.5±0.56**
NF(12.5mg) +TP	62±2.20**	41±1.91**	54.83±2.41**
DZPM+NF(12.5mg)+TP	35.66±4.22	22.33±1.80**	7.5±2.5
NF(25mg) + TP	289.16±10.28**	153.16±11.64**	172.83±19.47**
DZPM+ NF (25mg) +TP	44±5.85**	28±2.38	12.20±1.49**

All values are expressed as mean± SEM, n=6, One way analysis of variance (ANOVA) followed by Dun net's Multiple Comparison test. The minimum of $p< 0.0001$ was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05

Effect of two different concentrations of fluoroquinolones (Levofloxacin and Norfloxacin) on theophylline induced seizures were studied and reported in table no.3. The animals were observed for tonic flexion of fore limbs, tonic extension of hind limbs and clonic convulsions up to two hours. All the drugs are administered through i.p and theophylline was given after 5 minutes of administration of the test drug. Normal saline with theophylline showed 37.33±5.18 sec, 29.66±2.34 sec, 7.8±1.20 tonic flexion of fore limbs, tonic extension of hind limbs and clonic convulsion's respectively. Levofloxacin (12.5mg) showed slight increased value of 55.16±5.51 sec, 35.87±2.01 sec of tonic flexion of fore limbs, tonic extension of hind limbs respectively and no clonic convulsions. Diazepam with Levofloxacin (12.5mg) showed similar observations as that of normal. Levofloxacin (25mg) with theophylline showed increased value of 240±22.91 sec, 150±8.26 sec, 164.5±5.88 sec of tonic flexion of fore limbs, tonic extension of hind limbs and clonic convulsions respectively , as compared to levofloxacin (12.5mg).

Norfloxacin (12.5mg) showed 62±2.20 sec, 41±1.91sec and 54.83±2.41 sec of tonic flexion of fore limbs, tonic extension of hind limbs and clonic convulsions respectively. Norfloxacin (25mg) showed further increased values of 289.16±10.28 sec, 153.16±11.64 sec, 172.83±19.47 sec of tonic flexion of fore limbs, tonic extension of hind limbs and clonic convulsions respectively.

All the values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dun net's Multiple Comparison Test. The minimum of $p< 0.0001$ was considered significant; if the value of q is greater than 2.70 then the p value is less than 0.05.

In normal mice the concentration of GABA was 563.66µg, for other group of mice were subjected to normal saline with electroshock then the concentration of GABA was found 506.421µg. this showed that the electroshock was reduce the GABA level in the mice.

Levofloxacin of two doses (12.5&25mg/kg) was treated with electroshock treatment, the concentration of GABA was slight reduced in low dose of levofloxacin and in high dose it was still reduced. In presence of diazepam the GABA level was increased by treated with both electroshock treatment and test drug levofloxacin.

Table.No.5-Comparison of GABA-Concentration between groups (LF) with PTZ (Sub-convulsive doses) induced Seizures:

SL. NO.	GROUP'S	GABA-CONCENTRATION(µg) MEAN±SEM	% OF GABA
1.	Control	563.06±5.89	14.01%
2.	N.Saline+PTZ	517.55±11.74**	12.88%
3.	Diazepam+PTZ	690.49±12.41**	17.19%
4.	LF12.5mg+PTZ	454.76±11.42**	11.32%
5.	Diazepam+ LF12.5mg+PTZ	735.81±14.81**	18.32%
6.	LF25mg+PTZ	315.39±17.00**	7.85%
7.	Diazepam+LF25mg+PTZ	739.26±17.13**	18.40%

All the values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dun net's Multiple Comparison Test. The minimum of p< 0.0001 was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05

The GABA level at normal saline with PTZ was 517.55µg. it was reduced when compared to normal mice (563.06µg) at both doses 0f levofloxacin, the GABA level was reduced as 454.76 &315.39µg respectively. In the presence of diazepam GABA value was increased as 690.49, 735.81, 739.26µg with PTZ, LF12.5, LF 25mg/kg respectively.

Table.No.6-Comparison of GABA-concentration between groups (NF) with PTZ (subconvulsive dose) induced seizures.

SL. No.	GROUP'S	GABA-CONCENTRATION(µg) MEAN±SEM	% OF GABA
1.	Control	563.06±5.89	15.28%
2.	N.Saline+PTZ	517.55±11.74**	14.04%
3.	Diazepam+PTZ	690.49±12.41**	18.74%
4.	NF12.5mg+PTZ	325.17±17.36**	8.82%
5.	Diazepam+NF12.5mg+PTZ	705.76±19.86**	19.15%
6.	NF25mg+PTZ	280.24±10.68***	7.60%
7.	Diazepam+NF25mg+PTZ	602.24±27.22**	16.34%

All the values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test. The minimum of p< 0.0001was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05.

Similarly, at two doses of norfloxacin, the GABA level was reduced to 325.17 & 280.24µg with low dose and high dose respectively. It was showed very less concentration of GABA when compared to levofloxacin & norfloxacin. In presence of diazepam the GABA concentration was increased but not significantly.

Table.No.7-Comparison of GABA-concentration between groups (LF), Theophylline (sub-convulsive doses) induced Seizures:

SL. NO.	GROUP'S	GABA-CONCENTRATION(µg) MEAN±SEM	% OF GABA
1.	Control	563.06±5.89	16.37%
2.	N.Saline+TP	435.55±10.66**	12.66%
3.	Diazepam+TP	734.44±18.45**	21.35%
4.	LF12.5mg+TP	361.92±9.88**	10.52%
5.	Diazepam+ LF12.5mg+TP	563.55±16.37**	16.38%
6.	LF25mg+TP	305.38±11.76**	8.88%
7.	Diazepam+LF25mg+TP	475.05±12.76**	13.81%

All the values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dun net's Multiple Comparison Test. The minimum of p< 0.0001 was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05

The GABA level at normal saline with theophylline was 435.55µg. it was reduced concentration when compared to normal mice (563.06µg) and also it was less when compared to electroshock and PTZ treated mice, at both doses of levofloxacin 12.5 &25mg/kg, the GABA level was reduced to 361.92 &305.38µg respectively. In presence of diazepam it was increased to 734.44, 563.55, 475.05µg with theophylline, levofloxacin12.5 &25mg/kg respectively, but at 25mg it was less increased in GABA level.

Table.No.8-Comparison of GABA-concentration between groups (NF) with Theophylline (Sub-convulsive dose) induced seizures.

SL. NO.	GROUP'S	GABA-CONCENTRATION(µg) MEAN±SEM	% OF GABA
1.	Control	563.06±5.89	17.09%
2.	N.Saline+TP	435.55±10.66**	13.22%
3.	Diazepam+TP	734.44±18.45**	22.30%
4.	NF12.5mg+TP	284.71±13.51**	8.64%
5.	Diazepam+ NF12.5mg+TP	551.90±12.23**	16.75%
6.	NF25mg+TP	245.29±11.04***	7.44%
7.	Diazepam+NF25mg+TP	478.41±13.48**	14.52%

All the values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dun net's Multiple Comparison Test. The minimum of $p< 0.0001$was considered as significant; if the value of q is greater than 2.70 then the p value is less than 0.05.

Similarly, norfloxacin at both doses, the GABA level was reduced as 284.71 &245.29µg with low dose and high dose respectively. Norfloxacin treated mice were showed a very less GABA concentration when compared to levofloxacin. In presence of diazepam it was increased as 734.44, 551.90, with Theophylline norfloxacin 12.5 & 25mg/kg respectively.

Statistical analysis: The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

Discussion

The fluoroquinolone are one of the most frequently prescribed classes of antimicrobials. Their spectrum of activity includes many gram negative rods, some staphylococci and some atypical gram negative organisms. Besides their inhibition of DNA gyrase, quinolones are also antagonists of inhibitory neurotransmitter gamma amino butyric acid (GABA). CNS adverse effects can be well established both in animal studies and clinical reports. These adverse reactions are uncommon but well described and occur in 1-7% of patients under the treatment with quinolones. CNS side effects include seizures, hallucination, insomnia, dizziness and encephalopathy (Gabriele schmuck Anja schurmann, Gerhard schlutet Bayer AG, et al, July-1998., Ball P, Tillotson G, 1995). Fluoroquinolones have an excellent tissue penetrability and high level of activity against gram positive and gram negative pathogens. On the other hand, through animal studies and accumulated clinical experience, attention has been focused on side effects of these drugs, especially those involving central nervous system. The most common reactions include headache, dizziness and restlessness. Rarely, seizure activity has been associated with quinolone therapy (Gabriele schmuck Anja schurmann, Gerhard schlutet Bayer AG, et al, July-1998, Wolters Kluwer/Lippincott Williams and Wilkins 2010).

Most of the reported data were based on case studies with concomitant administration of other drugs like Theophylline (Turner RA, Robert A, 1965) with the occurrence of seizures. Higher rate of seizures were reported in patients with norofloxacin and moxifloxacin therapy as compared to other fluoroquinolone. In present study, comparison was made with the experimental pro-convulsive activity of levofloxacin, norfloxacin and moxifloxacin and correlated the same in the experimental animals, on electro convulsion and pentylenetetrazole induced convulsion models in mice and co-administered with theophylline. The pentylenetetrazole and theophylline were given as sub convulsive dose (PTZ-40mg/kg, theophylline-125mg/kg) instead of PTZ-80mg/kg, theophylline-250mg/kg. In Pro-convulsive activity of fluoroquinolone on maximal electroshock induced seizures, Levofloxacin were subjected to electroshock model, it was observed that values are increased in all the phases of seizures but in presence of diazepam seizure was controlled but the recovery was delayed.

Norfloxacin showed the pro-convulsive potency at low dose, and tonic extension phase is more compared to control. Norfloxacin at higher dose showed a increased values in all phases of seizures, i.e. tonic extension, clonic convulsion and latency in recovery. Even in presence of diazepam also, it was observed there clonic convulsion and post titanic depression values were not controlled and recovery delayed. By these observations it is clear that

norfloxacin abolishes the activity of GABA on benzodiazepine receptors. In Pro-convulsive activity of fluoroquinolone on pentylenetetrazole induced seizures in Mice, Animals were subjected to a lower dose of Levofloxacin (12.5mg/kg bdw) and sub convulsive doses of pentylenetetrazole (40mg/kg bdw) showed increased values of jerky movements and clonic convulsions as compared to control and the seizure values were further increased as the dose of levofloxacin increased to (25mg/kg bdw) but in presence of diazepam the seizure value's are decreased. Norfloxacin (12.5mg/kg bdw) also showed increased seizures values with PTZ, and the seizure values were further increased as the dose raised to 25mg/ kg bdw but the values were decreased with diazepam 2mg/kg bdw. The seizure values were decreased in presence of diazepam.

Pro-convulsive activity of fluoroquinolone on co-administered with theophylline:

Low dose Levofloxacin (12.5mg/kg bdw) was co-administered with theophylline 125mg/kg bwd, showed increased values of seizures at all the phases like tonic flexion of fore limbs, tonic extension of hind limbs and clonic convulsion, which demonstrate that levofloxacin has pro-convulsant activity when given with theophylline. With diazepam the seizure values were decreased which shows the protective action of diazepam. Sub convulsive dose of theophylline was co-administered with norfloxacin 12.5mg/kg bwd showed increased seizure values and the values were still increased as the dose of norfloxacin increased to 25mg/kg bdw which indicates the pro-convulsive activity of norfloxacin with theophylline. Both the doses of moxifloxacin also showed all the stages of convulsive activity with theophylline which signifies the pro-convulsive
activity of moxifloxacin with theophylline.

The pro-convulsive action of fluoroquinolones; levofloxacin, norfloxacin, at two different doses were studied using PTZ and co-administered with theophylline and the results were compared with the respective control groups and standard groups. Results of the present study show that, norfloxacin and moxifloxacin do have a significant pro-convulsive action with increased severity of convulsions in both PTZ and theophylline treated animals. The levofloxacin and Norfloxacin demonstrated a significant pro-convulsive profile in PTZ induced seizures. The % decrease in level of GABA treated with LF& NF at 25mg/kg bdw, in mice brain is 10.92, 11.50. The % decrease in level of GABA treated with LF & NF at 25mg/kg bdw, in mice brain is 7.85, 8.52, respectively as compared to control 16.14% on PTZ induced seizures. Similarly, GABA levels is also showed decreased % to 8.88, 7.75, on treatment with LF & NF at 25mg/kg bdw respectively as compared to control group (16.14%) on co-administration with theophylline.

In the present study all the three fluoroquinolones produced a dose dependent proconvulsive effect. With the present research findings it is found that the fluoroquinolones (levofloxacin and norfloxacin) have pro-convulsing activity in both *invivo* (PTZ) induced seizures and co-administered with theophylline. The pro-convulsing activity is more with PTZ and with theophylline induced seizures models. It was observed that the norfloxacin was the most potent proconvulsing followed by levofloxacin. Norfloxacin showed prolonged duration of tonic extension phase in PTZ models and also with theophylline (sub convulsive) treated animals. Hence it is concluded that fluoroquinolones especially norfloxacin should be used with caution in patients with predisposing epileptogenic factors.

It was also observed the level of GABA in fluoroquinolone treated animals was low as compared to control and diazepam treated animals. This indicates that fluoroquinolones may interfere with the synthesis of GABA or may decrease the affinity of GABA towards GABA receptors. The study lend support to the view that role of fluoroquinolones in pathophysiology of seizures, the study also provides the protective activity diazepam against pro-convulsive activity of fluoroquinolones treated animals.

Acknowledgement

The authors are extremely grateful to Dr.Premchandra Sagar, Vice Chairman, Dayananda Sagar Institutions and Dr.V.Murugan, Principal, Dayananda Sagar College of Pharmacy. Bangalore-560078, India, for their immense guidance and support for this project.

References

1. An astrocytic basis of epilepsy. Tian G-F, Azmi H, Takano T, Xu Q, Peng W, Lin W, *et al*. An astrocytic basis of epilepsy. *Nature Medicine* 2005; 11(9):973-981.

2. Babb T, Mathem GW, Leite GP et al. Glutamate AMPAreceptors in the fascia dentate of human and kainite rat hippocampal epilepsy. *Epilepsy Res* 1996; 26:193-205.

3. Ball P, Tillotson G. Tolerability of fluoroquinolone antibiotics, past, present and future. Drug safety1995; 13:343-58.

4. Ball P.Adverse reactions and interactions of fluoroquinolones. *Clin Invest Med* 1989; 12:28-34.

5. Barcaults N, De Masure Measure M, Benhamou CL, Guever C. Seizure in the course of prolonged treatment with ofloxacin, *Rev Med* 1989; 18:2050-4.

6. Blime W, Luders H, Mizrahi E, Tassinari C. Commission on Epidemiology and Prognosis, International League against Epilepsy. *Epilepsia* 1993; 34:592-6.

7. Chadwk D. Seizures and other epilepsy disorders.In: Watton J,editor. Brain's diseases of the nervous system. 10th ed. Oxford:oxford medical publications; 1993.

8. Christ W. Central nervous system toxicity of quinolones: human and animal findings. *J Antimicrob Chemother* 1990; 1:219-25.

9. Commission on classification and terminology of ILAE Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia* 1989; 389-91.

10. Commission on classification and terminology of ILAE. Proposal for revisedclinical and EEG classification of epileptic seizures. *Epilepsia* 1981; 22:489-501.

11. Covelli HD, Knodel AR, Heppner BT. Predisposing factors to apparent theophylline induced seizures. *Ann Allergy1985*; 54:411-5.

12. David M. Treiman. The GABAergic mechanisms in epilepsy. Department of Neurology, University of Medicine and Dentistry of New Jersy. *Epilepsia.* 2001; 42:8-12.

13. De Castro FR, Torres A (2003). "Optimizing treatment outcomes in severe community-acquired pneumonia".*Am J Respir Med* 2003; 2 (1):39-54.

14. Delgako-Escueta AV, Wilson WA, Oslen RW, Porter RJ editors. Philadephia:Lippincott Williams and Wilkins;1999:699-708.

15. Dudek FE, Pabryo PR, Wauarin J.P. Mechanisms of neuronal synchronization during epilepiform activity. In: Jasper's basic mechanisms of epilepsies. 3rd ed. Advances in neurology. Vol 49.

16. Duneidde TV. Adenosine and suppression of seizures. In: Jasper's basic mechanisms of epilepsies. 3rd ed. Advances in neurology. Vol 49. Delgako- Escueta AV, Wilson WA, Olsen RW, Porter RJ editors. Philadelphia: Lippincott Williams and Wilkins; 1999:1001-10.

17. During MJ, Spencer DD. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet* 1993; 341:1607-10.

18. During MJ, Ryder KM, Spencer DD. Hippocampal GABA transporter function in temporal-lobe epilepsy. *Nature* 1995; 376:174-7.

19. Ebginar N, Eroglu L. The effect of ofloxacin and ciprofloxacin on Pentylenetetrazole induced convulsions in mice. *Pharmacol Biochem Behay* 1991; 39587-89.

20. Errington AC, Stoh T, Lees G. Voltage Gated ion Channels: Targets for Anticonvlsant Drugs. Current Topics in Medicinal Chemistry 2005; 5:15-30.

21. Esclapez M, Trottier S. Changes in GABA-immuno reactive cell density during motor focal epilepsy induced by cobalt in the rat. *Exp Brain Res* 1989; 76:369-85.

22. Fisher R, Long L, White L Guide to the care of the patient with seizures. In: Buelow J, P Warbel A editors. AANN Reference Series for Clinical Practice *Illinois:* American Association of Neuroscience Nurses. 2004. [Online].'[Cited 2007 Jun 12].

23. Fisher RS.Animal models of the epilepsies. *Brain Res Rev*. 1989; 14(3):245-78.

24. Gabriele schmuck Anja schurmann, Gerhard schlutet Bayer AG, et al. Determination of the Excitatory Potencies of Flouroquinolones in the Central Nervous System by *invitro* Model. Institute of Toxicology, 42096-Wupperial, Germany, Antimicrobial Agents and Chemotherapy. July-1998.

25. Hakanen, A, Kotilainen P, Huovinen P, Helenius H, Sitonen A. "Reduced fluoroquinolone susceptibility in Salmonella enteric serotypes in travelers returning from Southeast Asia". *Emerg Infect Dis* 2007; 7(6):1003.dio:10.3201/eid0706.010613 PMC2631904.PMID11747728.
26. Harrison's Principles of Medicine. 15th edition.

27. Henry TR, Frey KA, Sackellars JC, et al. Invivo cerebral metabolism and central benzodiazepine-receptor binding in temporal lobe epilepsy. *Neurology* 1993; 43:1998-2006.

28. Houser CR, Harris AB, Vaughn JE. Time course of the reduction of GABA terminals in a model of focal epilepsy: a glutamic acid decarboxylase immune cyto chemical study. *Brain Res* 1986; 383:129-45.

29. Houser CR, Swartz BE, Walsh GO, et al. Granule cell disorganization in the dentate gyrus: possible alterations of neuronal migration in human temporal lobe epilepsy. In: Engel J Jr, Wasterlain C, Cavalheiro EA,et al., eds. Molecular neurobiology of epilepsy(Epilepsy Res Suppl . New York :Elsevier Science Publishers, 1992:41-9.

30. Jackson H. On the anatomical, physiological and pathological investigation of epilepsies. West Riding Lunatic Ssylum medical reports1873; 3:315: Reprinted in:Taylor. Selected writngs of John Hugjlings Jackson. London: Hadder and Stoughton;1931: 90-111.

31. Janknegt, Mohr. Side effects of fluoroquinolones in clinical trials and in post marketing surveillance drugs 1989; 34:144-9.

32. Johnson EW, de Lanerolle NC, KIim JH, et al. "Central" and "peripheral" benzodiazepine receptors: opposite changes in human epileptogenic tissue. *Neurology* 1992; 42:811-5.

33. Jungst, Mohr. SIDE effects of fluoroquinolones in clinical trials and in post marketing surveillance drugs 1987; 34:144-9.

34. Karande SC, Kshirsagar NA. "Adverse drug reaction monitoring of ciprofloxacin in pediatric practice" .*Indian Pediatr*1992- 29 (2): 181–8.

35. Kawahara, S. (Dec 1998). "[Chemotherapeutic agents under study]". *Nippon Rinsho* 1998 (12): 3096–9.

36. Leroy O, Beuscart C, Sivery B, Senneville E, Mounton Y. Efficacy and tolerance of intravenous ofloxacin *Presses MED* 1989;18:2050-4.

37. Loyd KG, Bossi L, Morselli PL, et al. Biochemical evidence for dysfunction of GABA neurons in human epilepsy. In:Bartholini G, Bossi L, Loyd KG, et al., eds. Epilepsy and GABA receptor agonists: basic and therapeutic research. New York: *Raven Press* 1985:43-51.

38. McDonald JW, Garofalo EA, Hood T, et al. Altered excitatory and inhibitory amino acid receptor binding in hippocampus of patients with temporal lobe epilepsy. *Ann Neurol* 1991; 29:529-41.

39. McKernan RM, Whiting PJ. Which GABAA receptor subtypes really occur in the brain? *Trends Neuroscience* 1996; 19:139-43.

40. Meldrum BS. Epilepsy and GABA mediated inhibition. *Int Rev Neurobiol* 1975; 17:1-6.

41. Melhus A (March 2005). "Fluoroquinolones and tendon disorders". *Expert Opin Drug Saf* 2005; 4(2):299-309.

42. Merlin LR, Taylor GW, Wong RKS. Role of metabrotropic Glutamate receptor subtypes in the patterning of epileptifirm activities *invitro*. *J Neuroscience* 1995; 74:896-900.

43. Morin F, Beaulieu, Lacaille JC. Alterations of perisomatic GABA synapses on hippocampal CA1 inhibitory intemeutons and pyramidal cells in the kainite model of epilepsy. *Neuroscience* 1999; 93:457.

44. Najm I, Ying Z, Babb T et al. NMDA receptor 2a1B subtype differential expression in human cortical dysplasia: Correlation with insitu epileptogenicity. *Epilepsia 2000*; 41(8):971-976.

45. Najm I, Ying Z, Janigro D. Mechanisms of epileptogenesis. Neurol Clin 2001; 19(2):23750.

46. Nelson, JM.; Chiller, TM. Powers JH, Angulo, FJ. "Fluoroquinolone-resistant Campylobacter species and the withdrawal of fluoroquinolones from use in poultry: a public health success story." *Clin Infect Dis* 2007: 977–80.

47. Niyogi SK. " Shigellosis".*J.Microbial*. 2005;43(2):133-43.

48. Ochoa JG, Richie W. Antiepileptic drugs: an overview. emedicine [online].2007 Apr 5[cited 2007 Jun 15].

49. Olsen RW, Avole M. GABA and epileptogenesis, *Epilepsia* 1997; 38:399-407.

50. Olsen RW, Bureaw M, Houser CR, et al. GABA/benzodiazepine receptors in human focal epilepsy. *Epilepsy Res Suppl* 1992; 8:383-91.

51. Olsen RW. GABA-Benzodiazepine-barbiturate receptor interactions. *J Neurochem* 1981; 37-13.

52. Pariente-Khayat A, Vauzelle-Kervroedan, FdAthis P, BreartG, Aujard Y, Olive G, Pons G. "[Retrospective survey of fluoroquinolone use in children]". *Arch Pediatr* 1998(5):484-8.

53. Pedly TA, Scheur ML, Walizak TS. Epilepsy. In Merritt's Textbook of Neurology. 9th edition edited by Lewis P Rowland; Williams and Wilkins. Baltimore. 1995:845.

54. Prince DA. Neurophysiology of epilepsy. *Ann Rev Neuroscience* 1978; 1:395- 415.

55. R.Schaumann, A.C. Rodloff. "Activities of Quinolones against Obligately Anaerobic Bacteria" (PDF). *Anti-Infective Agents in Medicinal Chemistry* 2007; 6:49-56.

56. Ramzan IM, Levy g. Kinetics of drug action in disease states XIX. Effect of experimental disease on neurotoxicity of theophylline in rats. *J PharmacolExp Ther* 1987; 241:236-8.

57. Ribak CE, Harris AB, Vaughn JE, et al. Inhibitory, GABAergic nerve terminals decrease at sites of focal epilepsy. *Neuroscience* 1979; 205:211-4.

58. Sarkisian MR. Overview of the current animal models for human seizure and epileptic disorders. Epilepsy and Behavior. 2001; 201-16.

59. Savic I, Persson A, Roland P, et al. In-vivo demonstration of reduced benzodiazepine receptor binding in human epileptic foci. *Lancet* 1988; 2:863-6.

60. Schwartz RD. The GABAA receptor-gated ion channel: biochemical and pharmacological studies of structure and function. *Biochem Pharmacol* 1988; 37:3369-75.

61. Shalini Rewari, S Prbhu. Comparative experimental study of pro-convulsive potential of fluoroquinolones. *Indian Journal of Pharmacology*1999; 31:29-32.

62. Steintein OK, Mulley JC, Propping P, *et al.* A missense mutation in neuronal nicotinic acetylcholine receptor alpha- 4-subunit is associated with autosomal dominant noctumal frontal lobe epilepsy. *Nat Genet* 1995; 11:201-10.

63. Surgery for seizures *NEJM* 1996; 647-652.

64. Taiwe G.S. et al., Department of animal physiology. International *J.of Pharmacology*2010.

65. Taylor GW, Merlin LR, Wong RKS. Synchronized oscillations on hippocampal CA3 neurons induced by metabotropic Glutamate receptor activation. *J Neuroscience* 1995; 15:8039-52.

66. Tillotson G. Tolerability of fluoroquinolone antibiotics, past, present and future. Drug safety 1995; 13:343-58.

67. Toman JEP, Guy M. Anticonvulsants In: Laurence DR, Bacharach AL. Evaluation of drug activities Pharmacometrics Vol.1London and New York, *Academics Press*1964:290.

68. Turner RA,Robert A. Screening methods in Pharmacology, New York and London, *Academic Press* 1965:164-5.

69. Waterlain CG, FarberDB, and Fairchild D. Cholinergic kindling: what has it taught us about epilepsy? *J Biol Sci* 1985; 63(2)119-32.

70. Wiljnands WIA, Vree TB, Vanherwaarden. The influence of quinolones derivatives on theophylline clearance.*BJ Clin Pharmacol* 1986; 22:677-83.

71. Williams PD, Helton DR. The pro-convulsive activity of quinolone antibiotics in animal model. *Toxicol Lett* 1991; 58:23-8..

72. Wolfson JS. Overview of fluoroquinolone safety. *Am J Med* 1991; 91 (Suppl 6A):153-61.

73. Wyllie's treatment of epilepsy: principles and practice. (5th ed). Philadelphia: Wolters Kluwer/Lippincott Williams and Wilkins2010.

74. Wyllie's treatment of epilepsy: principles and practice. (5thed.). Philadelphia*:* Wolters Kluwer/Lippincott Williams and Wilkins 2010:653-61.

75. Ysada H, Yoshida A, Masuda Y.Levofloxacin-induced neurological adverse effects such as convulsion, visual hallucination in two elderly patients. *Nippon Ronen I gakka I Zasshi*1999; 36:213-7.