Anxiogenic potential of ciprofloxacin and norfloxacin in rats

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ABSTRACT

Introduction: The possible anxiogenic effects of fluoroquinolones, namely ciprofloxacin and norfloxacin, were investigated in adult Charles Foster albino rats of either sex, weighing 150–200 g.

Methods: The drugs were given orally, in doses of 50 mg/kg for five consecutive days and the experiments were performed on the fifth day. The tests included open-field exploratory behaviour, elevated plus maze and elevated zero maze, social interaction and novelty-suppressed feeding latency behaviour.

Results: The results indicate that ciprofloxacin- and norfloxacin-treated rats showed anxious behaviour in comparison to control rats in all the parameters studied. However, ciprofloxacin- and norfloxacin-treated rats did not differ significantly from each other in various behavioural parameters.

Conclusion: The present experimental findings substantiate the clinically observed anxiogenic potential of ciprofloxacin and norfloxacin.

Keywords: anxiogenic effects, ciprofloxacin, fluoroquinolone, norfloxacin, rat

Singapore Med J 2007; 48(11):1028–1032

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INTRODUCTION

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.[1] Patterns of organ-system involvement and of signs and symptoms are quite similar, with gastrointestinal effects predominating (nausea, vomiting, diarrhoea or abdominal pain in 1.0%–5.0% of the patients). This is followed by effects on the central nervous system (CNS) (i.e. dizziness, headache, insomnia, anxiety, euphoria, seizures, depression and/or tremor in 0.1%–3.0% of the patients) and skin (0.5%–2.2% of the patients). These adverse effects are reversible after drug withdrawal and are generally not dose dependent.[2]

Levofloxacin, representative of the newer generation of fluoroquinolones, causes neurological adverse effects, such as convulsion, tremor, chorea-like movements and visual hallucination in two elderly patients.[3] In clinical settings, psychopathological and neurological adverse effects have been repeatedly reported during treatment with gyrase inhibitors (fluoroquinolones). Fluoroquinolones have been reported to produce an anxiogenic-like action in the elevated plus maze test,[4,5] shorten the pentobarbitone-induced sleeping time,[5] depress locomotor activity[6] and are known to have analgesic activity in acetic acid writhing and hot plate tests in rodents.[5] The effect of fluoroquinolones in the elevated plus maze test in rats and clinical reports both suggest that fluoroquinolones may cause anxiety. Therefore, the present study was planned to elucidate the behavioural effects of two commonly-used fluoroquinolones, ciprofloxacin and norfloxacin, on anxiety patterns in rats by employing various models of anxiety, viz., elevated plus maze, elevated zero maze, open-field behaviour, social interaction and feeding latency tests.

METHODS

Male adult Charles Foster rats (150–200 g) were used for this study. Animals were housed in groups of 5–6 in standard polypropylene laboratory cages at an ambient temperature of 25 ± 2°C and 45%–55% relative humidity with reversed 12:12 hour light/dark cycle. They had free access to rodent chow (Brook-Bond, Lipton, India) and tap water *ad libitum*. The experiments were performed after approval from the Institutional Ethics Committee, and principles of laboratory animal care (NIH publication No. 86–23, revised 1985) guidelines were followed throughout.

Ciprofloxacin (Ranbaxy Laboratories, New Delhi, India) and norfloxacin (Albert David, Calcutta, India) were freshly dissolved in distilled water and administered orally in the morning for five consecutive days, keeping the volume constant at 0.5 ml / 100 g of the body weight. A dose of 50 mg/kg/day for both the drugs was chosen,

for the present study, on the basis of an earlier study.[4,5] The control animals were given the same volume of distilled water orally for five consecutive days. Separate groups of rats were used for each behavioural test. Open-field exploratory behaviour, elevated plus maze behaviour, elevated zero maze behaviour, social interaction and feeding latency tests were employed in the study. Experiments were performed at 09.00 hr on the fifth day 45 min after the drug administration.

An open field apparatus similar to that of Bronstein[7] was used to study the open field exploratory behaviour of rats. It was made of plywood and consisted of a cube (61 cm × 61 cm × 61 cm). The entire apparatus was painted black, except for 6 mm white lines that divided the floor into 16 squares. The open field was lighted by a 100 W bulb focusing onto the field from a height of about 100 cm from the floor. The entire room, except the open field, was kept dark during the experiment. Each animal was centrally placed in the test apparatus for 5 min and the following behavioural aspects of anxiety were recorded:

i) Ambulation: measured by the number of squares crossed by the animal;
ii) Rearings: measured by the number of times the animal stood on its hind limbs;
iii) Self-groomings: measured by the number of times the animal made these responses; viz., grooming the face, licking/washing, and scratching the various parts of the body;
iv) Faecal pellets: measured by the number of faecal pellets excreted during the period;
v) Activity in centre: measured by the number of central squares crossed by the animal.

The elevated plus maze consisted of two opposite arms (50 cm × 10 cm) crossed with two opposite enclosed arms of the same dimension with 40 cm high walls. The arms were connected with a central square (10 cm × 10 cm) to give the apparatus a plus sign appearance. The maze was kept elevated 50 cm above the floor in a dimly-lit room. The rats were individually placed on the central square of the plus maze facing an enclosed arm. The time spent and number of entries made by the rat, during the next 5 min, on open and enclosed arms were recorded. An arm entry was defined when all the four limbs were on the arm.[8]

The elevated zero maze comprised an annular black perplex platform (105 cm diameter, 10 cm width), elevated 65 cm above the ground level and divided equally into four quadrants. The two opposite quadrants were enclosed by black perplex walls (27 cm high) on both the inner and outer edges of the platform, while the remaining two opposite open quadrants were surrounded only by a perplex "lip" (1 cm high), which served as a tactile guide to animals on these open arms. The apparatus was illuminated by indirect dim white light (10 lux) arranged in such a manner to provide similar lux levels in open and closed quadrants. Rats were placed in one of the closed quadrants for a 5-min test period. During the 5-min test period, time spent on open quadrants, number of 'head dips' over the edge of the platform, and number of 'stretched attend postures' (when the rat stretches its body from enclosed arms to open quadrants of the maze) were recorded. Animals were scored as being in the open area when all the four paws were in the open quadrants, and in the enclosed area only when all four paws had passed the open-closed divide.[9]

Rats were housed singly for 5 days prior to the social interaction test. The social interaction arena was a dimly-lit wooden box (60 cm × 60 cm × 35 cm) with a solid floor. The rats received two 7.5 min familiarisation sessions individually, at an interval of 1 hr, 24 hr before final testing. The next day, rats of the same gender and similar weight were paired, and placed on the test arena for 7.5 min. The time spent by the rat pair in active social interaction, characterised by sniffing, following, grooming, kicking, boxing or crawling over or under the partner was scored.[10]

The test apparatus for the feeding latency test was an iron box (60 cm × 60 cm × 35 cm), placed in a dimly-lit room. The box floor was covered with a 2.5 cm layer of wooden chips, on which 15 laboratory chow pellets were placed. A similar arrangement was made in the home cage of the rats. Food was removed from the home cage 48 hr prior to testing, but water was provided *ad libitum*. Naïve rats were placed individually in the test chamber and the latency to begin eating (defined as chewing of the pellet, and not merely sniffing or playing with it) was recorded. If the rat had not eaten within 300 s, the test was terminated and a latency score of 300 s was assigned. The results were compared with that of another group of rats, where latency to feed was recorded in the home cage under identical conditions.[11]

All the apparatuses were cleaned with 5% ethanol/water solution and dried thoroughly between the sessions. A neutral blind observer, unaware of the nature of treatment given to the animals, made the observations. The data are expressed as mean ± standard error of the mean (SEM), and were subjected to one-way ANOVA followed by multiple group comparisons using Newman-Keuls test.[12]

RESULTS

The effect of ciprofloxacin and norfloxacin treatments on the behaviour of rats in the open-field, elevated plus maze, elevated zero maze, feeding latency

Table I. Effects of ciprofloxacin and norfloxacin treatments on open-field exploratory behaviour in rats.

Groups (each n = 7)	Ambulation	Number of rearings	Number of groomings	Number of faecal pellets	Immobility (s)	Activity in centre (s)
Control	67.57 ± 1.53	22.57 ± 3.26	2.71 ± 1.11	3.00 ± 1.15	14.86 ± 5.12	3.00 ± 0.82
Ciprofloxacin	55.71 ± 1.39**	14.00 ± 3.46**	9.14 ± 4.06**	1.71 ± 1.11	26.04 ± 8.42*	2.00 ± 0.82*
Norfloxacin	50.29 ± 1.84**	15.71 ± 4.42**	8.43 ± 3.51**	2.86 ± 1.57	34.79 ± 9.65**	1.57 ± 0.53**

Values are expressed as mean ± SD.

* indicates statistical significance in comparison to the control treatment; * and ** denote $p < 0.05$ and $p < 0.01$, respectively.

Table II. Effects of ciprofloxacin and norfloxacin treatments on elevated plus maze behaviour in rats.

Groups	Time spent (s)			No. of entries		
(each n = 7)	Enclosed arms	Open arms	Ratio	Enclosed arms	Open arms	Ratios
Control	174.83 ± 16.36	76.01 ± 5.45	0.44 ± 0.04	5.14 ± 1.77	4.29 ± 1.11	0.67 ± 0.17
Ciprofloxacin	249.99 ± 27.14**	46.17 ± 4.28**	0.19 ± 0.02**	7.14 ± 1.35	2.00 ± 0.82**	0.29 ± 0.11**
Norfloxacin	231.29 ± 9.99**	40.67 ± 6.41**	0.18 ± 0.03 **	7.00 ± 1.41	2.14 ± 0.90**	0.31 ± 0.13**

Values are expressed as mean ± SD.

* indicates statistical significance in comparison to the control; ** denotes $p < 0.01$.

Table III. Effects of ciprofloxacin and norfloxacin treatments on elevated zero maze behaviour in rats.

Groups	No. of stretched attend postures	No. of head dips	Open arms	
(each n = 7)			No. of entries	Time spent
Control	5.57 ± 1.72	8.43 ± 1.51	5.57 ± 1.51	22.51 ± 3.71
Ciprofloxacin	3.29 ± 1.38*	3.14 ± 1.68**	2.14 ± 1.07**	9.44 ± 2.32**
Norfloxacin	2.86 ± 1.35**	3.00 ± 1.41**	2.43 ± 1.13**	12.11 ± 3.26**

Values are expressed as mean ± SD.

* indicates statistical significance in comparison to the control; * and ** denote $p < 0.05$ and $p < 0.01$, respectively.

Table IV. Effects of ciprofloxacin and norfloxacin treatments on feeding latency and social interaction in rats.

Groups	Feeding latency (s)		Time in social interaction (s)
(each n = 7)	Home cage	Novel cage	
Control	45.71 ± 5.14	84.89 ± 11.71	190.91 ± 31.07
Ciprofloxacin	47.03 ± 5.58	110.27 ± 13.71**	121.16 ± 17.88
Norfloxacin	50.79 ± 4.03	140.80 ± 21.82**, ##	141.07 ± 9.02

Values are expressed as mean ± SD.

* and # indicate statistical significance in comparison to the control and ciprofloxacin treatments, respectively; ** and ## denote $p < 0.01$.

and social interaction tests are respectively shown in Tables I–IV. Ciprofloxacin and norfloxacin treatments caused significant reduction in ambulation and activity in the centre squares, and increase in self-grooming and immobility in the open field, in comparison to the control treatment. In the elevated plus maze test, ciprofloxacin- and norfloxacin-treated rats spent significantly more time, and made more number of entries to the enclosed arms, with concomitant less time and fewer number of entries to the open arms, as compared to control rats. The results of the ratio between open arm and enclosed arm time and entries also indicated that both ciprofloxacin and norfloxacin caused significant anxiogenic behaviour in rats. Similarly, in the elevated zero maze test, ciprofloxacin-treated rats made significantly fewer entries, spent less

time on the open arms and showed a significant decrease in the number of head dips and stretched attend postures in comparison to control rats. The drug treatments also had significant effect on novelty-induced suppressed feeding (Table IV). Ciprofloxacin and norfloxacin treatments caused significantly enhanced feeding latencies in comparison to control treatment in the novel environment. They also reduced the social interaction time in paired rats in comparison to the control group, but the results are not statistically significant (Table IV).

DISCUSSION

The question of reliability and validity is of prime importance in establishing experimental paradigms of practical predictable value. These assume further importance when animal models of human behaviour, and its perturbations, are being used. The paradigms used in the present study have been subjected to thorough critical appraisal and validated as animal models of anxiety.[13-16] Thus in the open-field and similar tests, when the animals are taken out from their home cage, and placed in a novel environment, they express their anxiety and fear by a decrease in ambulation, rearings, and other exploratory behaviours. Likewise, the elevated plus and zero maze tests are based on the principle that exposure to the open part of the maze leads to an approach conflict which is considerably stronger than evoked by exposure to the enclosed part of the maze.[17] In the social interaction test, when naïve rats are placed in pairs in a novel test arena, they show a decline in the time spent in active social interaction. Anxiolytic drugs prevent this decline. This test is one of the few animal tests of anxiety that has been validated behaviourally and physiologically as well as pharmacologically.[18] Similarly, in the feeding latency test, when hungry rats are placed in a novel cage, there is a delay in eating of the food pellets.[19] All these behaviours are increased by anxiogenic agents and attenuated by anxiolytics under identical experimental conditions.

The findings of the present study indicate that ciprofloxacin and norfloxacin treatments induced anxiogenic behaviour patterns in rats in open-field exploratory behaviour, elevated plus maze behaviour, elevated zero maze behaviour and feeding latency tests. The data of the open-field test also suggests that reduction in various responses may be due to a non-selective behavioural inhibition (e.g. sedation) with both compounds. This non-selective behavioural inhibition may also reduce the open-arm time and entries in the elevated plus and zero maze tests. However, this possibility is reduced because of the fact that the closed arm entries are not affected in the drug-treated rats. This suggests that both ciprofloxacin and norfloxacin have potential anxiogenic effects. Earlier studies also support the present observations wherein investigators have reported. Earlier, fluroquinolones have been reported to reduce locomotor activity[6] and induce anxiogenic behaviour in elevated plus maze in rodent experiments.[4] These findings support the observations of the present study.

It is well known that serum concentration of norfloxacin is far less than other fluoroquinolones, limiting its clinical use in urinary tract infections. However, the data of the present study reveals CNS adverse effect potential of norfloxacin is comparable to ciprofloxacin. The pathophysiological mechanisms involved in the development of adverse CNS effects are not completely understood.[20] The role of γ-aminobutyric acid (GABA) in anxiety is well documented.[21] Some studies indicate that fluoroquinolones function as GABA receptor antagonists,[22] and the epileptogenic action of quinolones has been proposed to be related to the GABA-like structure of ring substitutes. The CNS effects of pefloxacin in clinical settings have been explained by some reported biochemical studies. Quinolones have an inhibitory effect on the receptor binding of $GABA_A$, and may thus exert an inhibitory CNS stimulant action.[23,24] Benzodiazepine agonists have been reported to attenuate the central stimulating effects of ciprofloxacin and pefloxacin.[22] Likewise, they potentiate chemically-induced convulsions, which could be antagonised by benzodiazepines.[25] The adenosine or $GABA_A$ receptor has therefore been proposed as a possible target for quinolones, particularly with older agents like norfloxacin and ciprofloxacin, and less so with pefloxacin.[26] The structural similarities of the fluoroquinolones to kynurenic acid and other similar compounds, which are endogenous ligands of the glutamate receptor, might suggest an interaction of quinolones with ligand-gated glutamate receptors as well.[27]

The excitatory potentials of fluoroquinolones have been reported to be increased in a dose dependent manner in the electrophysiological studies of the field potentials in the CA 1 region of the rat hippocampus slice.[27] N-methyl-D-aspartate (NMDA) receptors present in the hippocampus may also be responsible for the anxiogenic property exhibited as side effects of fluoroquinolones. The increased excitability caused by fluoroquinolones may be due to activation of NMDA receptors. It has been shown that MK-801, a selective channel blocker of the NMDA receptor, abolishes the excitatory effects of fluoroquinolones. This strongly suggests that fluoroquinolones may exert their excitatory response in the hippocampus through NMDA-gated ion channel.[28-30] Fluoroquinolones did not bind to the

glutamate or glycin-binding site of the NMDA receptor. It has been shown that fluoroquinolones decrease blocking effects of Mg^{2+} and MK-801 binding to the NMDA receptor. Magnesium chelating properties of fluoroquinolones have been postulated as mechanisms of fluoroquinolone-induced atrophy, and the excitatory potency of fluoroquinolones might also be based on activation of the NMDA receptor by abolishing the Mg^{2+} block in the ion channel. This would prolong the opening time of the channel, thus increasing intracellular Ca^{2+} concentration in the neurons.[27]

The present findings of anxiogenic potentiality of ciprofloxacin and norfloxacin on different experimental models substantiate the CNS adverse effects observed in a clinical scenario. However, it is not out of place to mention that the anxiogenic property of the commonly-used fluoroquinolones, ciprofloxacin and norfloxacin, may add to the pre-existing anxiety in a patient suffering from serious infections.

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