EFFECT OF CIPROFLOXACIN ON ISOLATED SMOOTH AND CARDIAC MUSCLES AND NEUROMUSCULAR JUNCTIONS

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A B S T R A C T

The pharmacodynamic effects of ciprofloxacin on smooth muscles were investigated in isolated organs. The effect of graded increased concentrations of ciprofloxacin on isolated rat's uterine muscles was examined during various stages of sex cycles. Ciprofloxacin in the tested concentrations produced a dose-dependent negative inotropic effect on isolated rabbit's heart and guinea pigs auricles. Ciprofloxacin in all tested concentrations did not induce any effects on the isolated guinea pig's tracheal chain and rabbit's aortic strips. Neuromuscular blockade effect was investigated on isolated frog's gastrocnemius muscles and rectus abdominis muscle preparations. It was concluded that ciprofloxacin directly stimulates smooth muscles of gastrointestinal tract and depresses those of uterus in various stages of sex cycle as well as cardiac muscles. Ciprofloxacin might act directly to induce neuromuscular blockade. It was concluded that ciprofloxacin scarcely possess any pharmacological properties which might be leading to sever adverse reaction in clinical use.

Key words: cardiac muscles, ciprofloxacin, smooth muscles, neuromuscular junctions

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1. INTRODUCTION

Ciprofloxacin is an important member of the fluoroquinolone group of antibiotics. It is a broad spectrum antibiotic used to combat various infectious diseases in animals and humans. Information regarding bio disposition of ciprofloxacin shows that it has not been studied in local ruminant species [1]. Several studies have shown that the pharmacokinetic characteristics, optimal dosage, renal clearance and urinary excretion of the investigated drugs were different under indigenous conditions when compared with the values given in the literature or in the product inserts supplied by the manufacture [2]. The purpose of this study was to investigate the pharmacodynamic effects of ciprofloxacin on smooth, cardiac and skeletal muscles.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1 Drug:

Ciprofloxacin (INN) is a synthetic antibiotic of the fluoroquinolone drug class [3] and [4]. It is a member of second-generation of fluoroquinolone antibacterial. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of DNA and protein.

2.1.2. Laboratory animals:

Guinea pigs of both sexes and different weights (300:450 gm) were used for investigating the effect of ciprofloxacin on the isolated ileum, auricles and tracheal chain smooth muscle. Rabbits of both sexes and different weights (1500-4000 gm) were used for studying the effect of ciprofloxacin on isolated duodenum, heart, aortic strip.

Rats of both sexes and different weight (150-220 gm) were used for studying the effects of ciprofloxacin on isolated colon, fundic strip, uterine muscle in different stages of sex cycle and phrenic nerve hemidiaphragm. Egyptian toads were used for studying the effect of ciprofloxacin on isolated rectus abdominus muscle and sciatic nerve gastrocnemius muscle preparations.

2.2. Methods:

The method described by [5] was used for studying the effect of ciprofloxacin on the isolated ileum of guinea pigs. The method described by [6] were used for studying the effect of ciprofloxacin on isolated rabbit duodenum, rat's colon and uterine muscle of the rats in various stages of sex cycles. The method described by [7] was used for studying the effect of ciprofloxacin on rat's fundic strip. The method described by [8] was used for studying the effect of ciprofloxacin on isolated guinea pig's tracheal smooth muscle using the glass jar bath apparatus. The glass jar bath was used as described by [9] for studying the effect of ciprofloxacin on isolated guinea pig's auricles. The method explained by [10] using Gunn's apparatus (heart infusion assembly) was used for studying the effect of ciprofloxacin on rabbit's heart. The method explained by [11] was used for studying the effect of ciprofloxacin on rabbit's aortic strip. The method described by [12] was used for investigating the effect of ciprofloxacin on the frog's gastrocnemius muscle- sciatic nerve preparation. The effect of ciprofloxacin on the isolated frog's rectus abdominis muscle was investigated by using the method described by [6].

3. RESULTS

The effect of graded increased concentrations of ciprofloxacin on the contractility of guinea pig's ileum, rabbit s duodenum, rat's colon and rat's fundic strip and guinae pig s tracheal chain are recorded in table (1). The effect of ciprofloxacin on the uterine motility of rats at various stages of sex cycle was presented in table (2). Trials were performed to locate the site of action of ciprofloxacin on the gastrointestinal motility and the results showed that ciprofloxacin had a direct intestinal smooth muscle stimulant effect and an antihistaminic like effect on rat's fundic strip. Ciprofloxacin exerts its stimulatory effect on uterine muscles in non estrous, estrous, while its depressant effect in early and late pregnancy stages of sex cycle which revealed to a direct effect of ciprofloxacin on uterine motility as shown in (figure1). Ciprofloxacin depressed the isolated guinea pig auricles, rabbit's heart (figure 2) and this negative inotropic effect of ciprofloxacin was not referred to β_1 adrenergic blocking effect as adrenalin (1µg/ml bath). Ciprofloxacin (1000µg/ml bath) was able to produce its cardiac stimulatory effect in presence of adrenalin (1µg/ml bath). Ciprofloxacin (1000µg/ml bath) was able to produce its inhibitory effect in the presence of atropine sulphate (figure2). The effect of graded increased concentrations of ciprofloxacin on isolated frog's rectus abdominis muscle had a neuromuscular blockade in presence of acetyl choline.

4. DISCUSSION

The present investigation showed that, ciprofloxacin *in-vitro* stimulated the contractility of guinea pig's ileum, rat's colon and rabbit's duodenum. The stimulatory effect of ciprofloxacin was proportional to the graded tested concentrations. Presence of atropine sulphate as muscarinic cholinergic receptor blocker and large dose of nicotine sulphate as ganglionic (Nicotinic receptor) blocker did not inhibit the stimulatory effect of ciprofloxacin. In addition, the adrenaline as adrenoceptor agonist produced its inhibitory effect in presence of ciprofloxacin. These results proved that, the

ciprofloxacin might directly stimulate the intestinal smooth muscles of rabbit's duodenum, guinea pig's ileum and rat's colon. These obtained results were similar to those obtained by [13] who studied the effects of quinolone carboxylic acid derivatives on GABA receptor — mediated stimulation of gastric acid secretion and intestinal motility. The study investigated the effects of some quinolone carboxylic acid derivatives on GABA receptor - mediated excitatory responses in gastrointestinal preparations in *vitro* and in *vivo*. Also, these results were similar to those obtained by [14] who proved the fluoquinolone - induced motor changes in the guinea - pig isolated ileum. The effects of norfloxacin and enoxacin were examined on spontaneous motor activity in guinea - pig isolated ileum and lead to excitatory effects in the guinea pig isolated ileum.

Ciprofloxacin stimulated the uterine motility during non pregnant stages (estrous and non estrous) and inhibited the uterine motility during early and late pregnant stage. The effect was dose dependant. These effects might be attributed to the direct action of the ciprofloxacin on the isolated uterus. During non pregnant stages (estrous and non estrous), in presence of atropine sulphate (0.25 µg/ml bath), ciprofloxacin induced its stimulant effect and the adrenaline (0.5 µg/ml bath) relaxed the uterus after its stimulation with 500 µg/ml bath ciprofloxacin. During the early and late pregnant stages, the addition of acetylcholine in a small concentration (0.25 µg/ml bath) produced its stimulatory effect in the presence of ciprofloxacin (500 µg/ml bath) and the ciprofloxacin in the same concentration, relaxed the uterus after its stimulation with 1µg propranolol /ml bath. The obtained results were consistent with those recorded by [15] who summarized that the general pharmacology of levofoxacin, were examined DR-3355 at 200/600 mg/kg orally should depressant activity on the C.N.S, as was indicated by the depressant syndrome in mice, decreased spontaneous motor activity and hypothermia in mice and rabbit. And inhibition of nicotine-induced contraction in ileum and spontaneous or oxytocin induced motility in pregnant uterus this result was consistence with other antibacterial**,** [16] who concluded that erythromycin produced a decrease in the pregnant rat myometrial activity *in-vitro*, independent of the stimulant. Also, [17] reported that clarithromycin inhibited myometrial contractions in isolated human myometrium independent of stimulus.

The guinea pig's tracheal smooth muscles seemed to be insensitive to the tested concentrations of ciprofloxacin. In presence of ciprofloxacin, histamine was not able to produce its stimulatory effect, thus ciprofloxacin blocked the action of histamine on the tracheal smooth muscles. The obtained results in this study were similar with those obtained by other antibacterial [18] and [19] who recorded that cefprozil and cefamandole respectively in different graded concentrations had no effect on the tracheal smooth muscles. The two antibiotics blocked the stimulatory effect of histamine on the guinea pig's tracheal muscles in a dose dependant manner.

The obtained results in this study on the cardiovascular muscles proved that ciprofloxacin had a negative inotropic effect on the isolated guinea pig's auricles and rabbit's heart. Ciprofloxacin produced a direct and dose dependant depression of the myocardial contractility. This negative inotropic effect of ciprofloxacin was not referred to either ß1 adrenergic blocking effect or cholinergic stimulant effect, as adrenaline (2 µg/ml canula) was able to produce its cardiac stimulatory effect in presence of ciprofloxacin (500 µg/ml canula) and after addition of atropine sulphate (25 µg/ml canula), ciprofloxacin (500 µg/ml canula) was able to produce its inhibitory effect. Contraction of the cardiac cells is believed to be dependent upon the intracellular concentration of available calcium ions in the vicinity of the

contractile apparatus [20]**.** The direct myocardial depressant effect of ciprofloxacin in the present work might be attributed to a modification of calcium function. The negative inotropic effect of ciprofloxacin on guinea pig's auricles and rabbit's heart in the present work was similar to that result obtained by [15] who summarized the general pharmacology of levofloxacin were examined DR-3355 produced a hypotensive and bradycardia effect after epinephrin. [21] recorded that the calmodulin antagonist W-7 prevents sparfloxacin-induced early after depolarizations in isolated rabbit purkinje fibers. It had been concluded that present findings support the hypothesis that CaM kinase may be a proarrhythmic signaling molecule and demonstrated that CaM kinase may be involved in the generation of early after depolarizations in drug-induced long QT and enhanced beat-to-beat instability of repolarization is essential for the genesis of early after depolarizations in rabbit in *vitro.*

Regarding the effect of ciprofloxacin on skeletal muscle preparations (frog's gastrocnemius muscle sciatic nerve, frog's rectus abdominis muscle and rat's phrenic nerve hemidiaphragm), ciprofloxacin elicited a marked neuromuscular blocking activity in response to indirect muscle twitches; also ciprofloxacin exhibited a local anaesthetic activity on frog's gastrocnemius sciatic nerve preparation. Trials were performed to detect the site of action of ciprofloxacin on the skeletal muscle preparations. The results showed that, ciprofloxacin did not impair the stimulatory effect of neostigmine and acetylcholine on rat's phrenic nerve hemidiaphragm preparation. Therefore, the neuromuscular blocking effect of ciprofloxacin seemed to be attributed to two mechanisms; the first might be due to local anesthetic effect of ciprofloxacin which is responsible for blocking of conduction through sciatic and phrenic nerve. The second mechanism might be attributed to calcium ions antagonistic effect of the ciprofloxacin. Calcium ions influx is necessary for acetylcholine release as well as other neurotranmittors and hormones [22]. The neuromuscular blocking activity of ciprofloxacin on skeletal muscle preparations in the present work was similar to those obtained by [23] who determined the inhibitory effects of quinolone antibacterial agents on gamma-amino butyric acid binding to receptor sites in rat brain membranes. The specific binding of 3H - labeled gamma- amino butyric acid ([3H] GABA) to synaptic plasma membranes from rat brains was inhibited by various quinolone carboxylic acid derivatives (quinolones), and these inhibitions were concentration dependant. The binding of [3H] muscimol to GABA sites were also inhibited. These inhibitory potencies differed widely among the quinolones examined. The Dixon plots showed that a newly developed diflourinated quinolone, NY - 198 [1- ethyl-6,8-diflouro-l,4-dihydro-7-(3-methyl-l-piperazinly)-4-oxo-3-quinolone carboxylic acid hydrochloride], competitively inhibited the receptor bindings of [3H] GABA and [H] muscimol in conclusion. These findings suggested that the inhibition of GABA binding to receptors (including uptake sites) in the brain may be involved in the induction of epileptogenic neurtoxicitles by qiunolones. [20] examined.DR-3355 at 200- 600 mg/kg orally showed depressant activity on the central nervous system, as was indicated by the depressant syndrome in mice, decreased spontaneous motor activity and hypothermia in mice and rabbit.

Table (1) Effect of ciprofloxacin on isolated guinea pig's ileum, rabbit's duodenum, rat's colon, rat's fundic strip and guinea pig's tracheal chain.

Concentration (µg/ml bath)	Guinea pig's ileum	Rabbit's duodenum	Rat's colon	Rat's fundic strip	Guinea pig's tracheal chain
0.025	No effect	No effect	No effect	No effect	No effect
5	No effect	No effect	Slight stimulation in the force	Slight inhibition in the force	No effect
10	Slight stimulation in the force	Slight stimulation in the force	Marked stimulation in the force	Moderate stimulation in the force	No effect
25	Slight stimulation in the force and rate of contraction	Slight stimulation in the force	Marked stimulation in the force and rate of contraction	Marked inhibition in the force	No effect
50	Marked stimulation in the force and rate of contractions	Marked stimulation in the force	Marked stimulation in the force and contraction	Marked inhibition in the force	No effect
100	Marked stimulation	Marked stimulatin in the force of contraction	Maximum stimulation	Maximum inhibition in the force and rate of contraction	No effect
250	Maximum stimulation	Marked stimulation			No effect
500		Maximum stimulation			No effect

Table (2): Effect of ciprofloxacin on uterine motility of rats at various stages of sex cycle

Concentrations (µg/ml bath)	Non estrous	Estrous	Early pregnant	Late pregnant
0.025 -10	No effect	No effect	No effect	No effect
25 - 50	Slight increase in frequency	Slight increase in frequency	Slight decrease in force and frequency	Slight decrease in force and
100	Marked increase in force and frequency	Slight increase	Moderate decrease	Moderate decrease
250		Marked increase	Marked decrease	Marked decrease
500	Maximum stimulation	Maximum stimulation	Complete relaxation	Complete relaxation

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Figure (1A): Site of aciton of ciprofloxacin (Cip.) on isolated rat's uterus during late pregnant stage.

* 1 μg/ml bath propranolol (Prop.) followed by 500 μg/ml bath ciprofloxacin (Cip.).

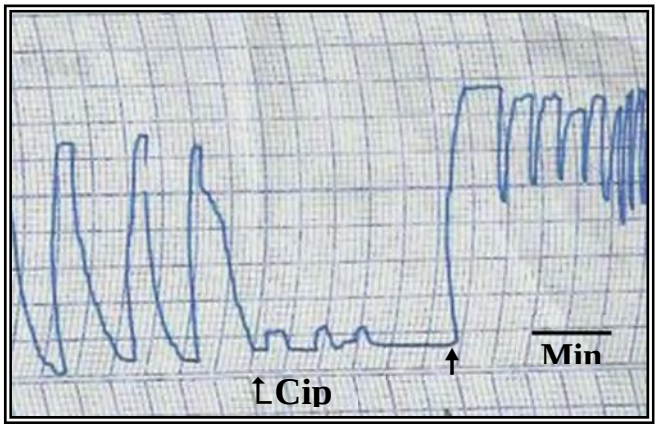

Figure (1B): Site of aciton of ciprofloxacin (Cip.) on isolated rat's uterus during late pregnant stage.

*500 μg/ml bath ciprofloxacin (Cip.) followed by 0.25 μg/ml bath acetylcholine (Ach.).

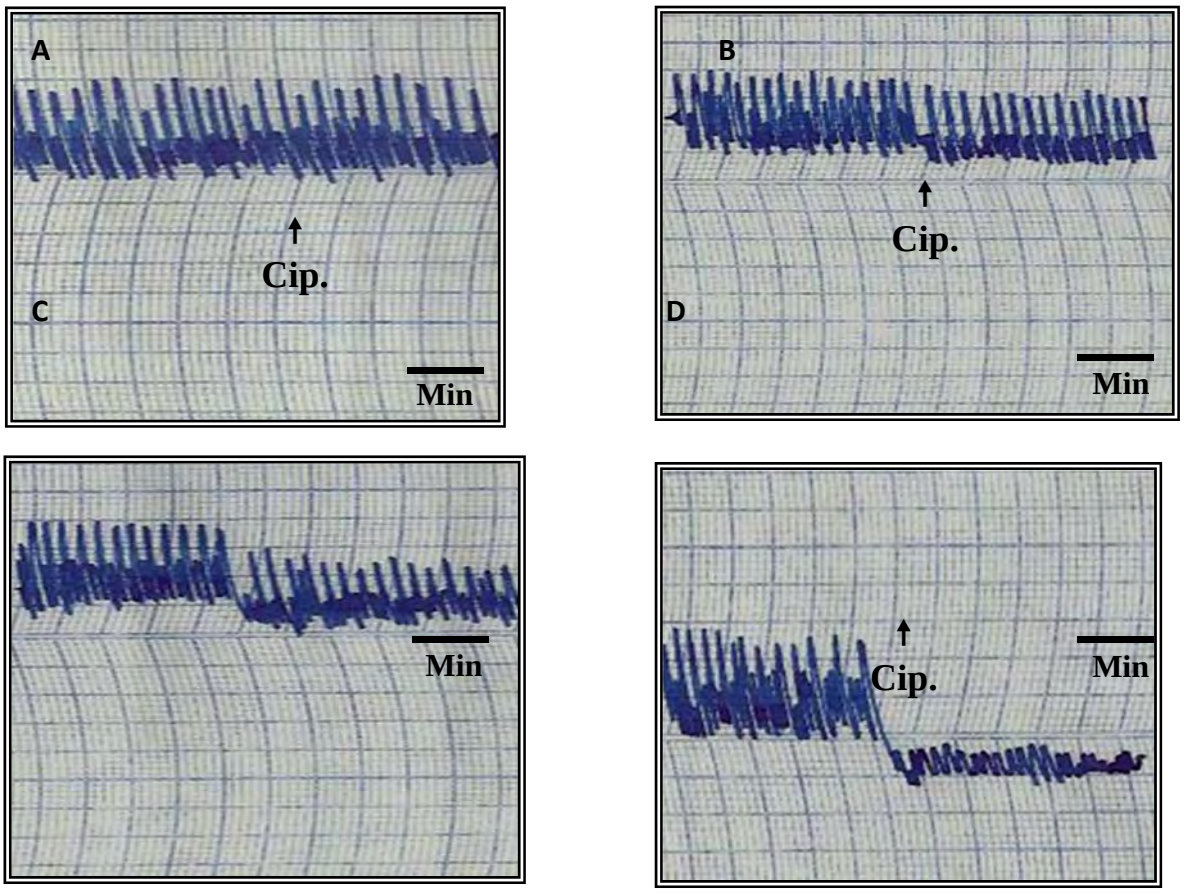

Figure (2): Effect of ciprofloxacin (Cip.) on isolated guinea pig's auricles.

(A) 5 μg/ml bath ciprofloxacin (Cip.)
(B) 100 μg/ml bath ciprofloxacin (Cip.)
(C) 250 μg/ml bath ciprofloxacin (Cip.)
(D) 1000 μg/ml bath ciprofloxacin (Cip.)u

CONCLUSION

From the present study, it could be concluded that ciprofloxacin directly stimulates the smooth muscles of gastrointestinal tract and uterine muscles in non-estrous and estrous and depresses those of uterus in pregnancy as well as cardiac muscles. Ciprofloxacin in all tested concentrations didn't induce any effect on the resting tonus of guinea pig's tracheal chain and rabbit's aortic strip. Ciprofloxacin had a neuromuscular blocking activity on the skeletal muscle preparations.

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تأثير السيبروفلوكساسين على العضلات الملساء والعضلات القلبية وتأثيره على التوصيل العضلي العصبي

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الملخص العربي

تمت دراسة التأإثير الفارماكوديناميكى لعقار السيبروفلوكساسين على حركة العضلات الملساء موضحا على العضلات المعزولة حيث تمت دراسة التأثير التزايدى التدريجي لعقار السيبروفلوكساسين فى عضلة الرحم فى الجرذان فى المراحل المختلفة من الدورة الجنسية حيث يعطى عقار السيبروفلوكساسين تأثيرا إيجابيا يعتمد على الجرعة فى العينات المعزولة من قلب الأرانب واذينين الأرانب الغينية. ووجد أن عقار السيبروفلوكساسين ليس له أي تأثير على العينات المعزولة من عضلة القصبة الهوائية للأرانب الغينية وشريان الأورطى فى الأرانب، بينما يظهر تأثيره على التوصيل العضلي العصبي للعينات المعزولة من عضلة الساق البطنية بالعصب الوركي وعضلة البطن المستقيمة المنزوعتين من الضفادع المصرية. وخلص البحث الى أن عقار السيبروفلوكساسين له تأثير تنشيطي مباشر على العضلات الملساء للقناة المعد معوية وتأثير تنشيطي لعضلة الرحم فى مراحل الشبق وما قبل الشبق وتأثير تثبيطي لعضلة الرحم فى مراحل الحمل والعضلات القلبية وهذه النتائج تدل على أن عقار السيبروفلوكساسين له خصائص فارماكولوجية من الممكن أن تؤدى الى تأثيرات مضادة فى الاستخدام الإكلينيكي.

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