Possible Cardiac Adverse Effects Induced by Therapeutic Doses of Ciprofloxacin in Juvenile Rats

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Abstract

Ciprofloxacin is widely used in treating adults infected with Gram-negative bacteria. It is contraindicated in children, growing adolescents and during pregnancy due to joint toxicity. Its toxicity concerning other organs needs to be clarified. Thus, this study was designed to study the possible cardiac damage induced by two selected doses of ciprofloxacin in juvenile rats.Eighteenth healthy juvenile rats (4 weeks old and their weight 30 ± 2 gm) were utilized in this study and divided into three groups. Group-I control; group II and group III, respectively injected IP with 25 mg/kg and 50 mg/kg ciprofloxacin every 12 hours for one week. Serum enzymes activities alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatin kinase -muscle brain isoform (CK-MB), and lactate dehydrogenase (LDH) were assessed. Histological examination of heart tissues was also performed. The results of this study showed that, ALT, AST and CK-MB enzymes were significantly elevated only in group III compared to control. LDH enzyme was elevated in both group II and III. Concerning histological examination of the heart's gross sections, the results obtained from this work demonstrated the degeneration and necrosis in the hearts of group II and III juvenile rats compared to control animals. In conclusion, our results showed that the selected therapeutic doses of ciprofloxacin utilized in this study caused cardiac damage in juvenile rats.

Key words: Fluoroquinolones, cardiac adverse effects, juvenile rat .

التأثيرات الجانبية المحتملة في القلب والمستحدثة بواسطة الجرع المعالجة للسيبروفلوكساسين في الجرذان اليافعة

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الخلاصة

يعد عقار السبروفلوكساسين من الادوية الشائعة الاستعمال في المرضى البالغين لعلاج الالتهابات التي تسببها بكتريا الصبغة كرام السالبة (Gr-ve). ان العقار أعلاه لا يمكن اعطاءه للاطفال واليافعين وللنساء خلال فترة الحمل بسبب سميته للمفاصل. ان سميته في اعضاء الجسم الاخرى تحتاج الى ان توضح لذلك صمم هذا البحث لدراسة احتمالية تلف القلب الذي يمكن ان تسببه جرعتان من عقار السبروفلوكساسين والتي تعد جرع علاجية في الجرذان اليافعة. تم استخدام ثمانية عشر جرذ يافع أعمارهم ٤ أسابيع واوزانهم ٣٠ ± ٢ غراما وقسمت الى ثلاث مجموعات: المجموعة الاولى/ مجموعة سيطرة، المجموعة الثانية / ٢٥ ملغم لكل كيلوغرام وزن الجسم اعطيت مرتان يوميا داخل الصفاق، المجموعة الثالثة/ ٥٠ ملغم لكل كيلوغرام وزن الجسم اعطيت مرتان يوميا داخل الصفاق. تم قياس فعالية الانزيمات (ALT, AST, CKMB, and LDH) في مصل الدم كما تم عمل دراسة نسيجية لنسيج القلب.أظهرت الدراسة بأن هناك زيادة معنوية في فعالية الانزيمات المذكورة اعلاه في مصل الدم في المجموعة الثالثة مقارنة بمجموعة السيطرة. ان فعالية الانزيم (LDH) في مصل الدم قد زاد بصورة معنوية في المجموعتين الثانية والثالثة. كما اظهرت الدراسة النسيجية ان هناك تلف في نسيج القلب في مجموعتي الجرذان اليافعة (المجموعة الثانية والثالثة) التي اعطيت عقار السبروفلوكساسين مقارنة بمجموعة السيطرة.من خلال النتائج التي تم الحصول عليها يمكن الاستنتاج بأن الجرع المستخدمة في هذه الدراسة من عقار السبروفلوكساسين سببت تلف في نسيج القلب لدى الجرذان اليافعة.

الكلمات المفتاحية :فلوروكونيولونرز، التأثيرات الجانبية في القلب ، الجرذان اليافعة .

Introduction

Fluoroquinolones (FQs) are widely used antibacterial agents in adults because of their strong antibacterial effect against Gram-negative bacteria [(1)]. They target bacterial DNA gyrase and topoisomerase IV [(2)]. In pediatric patients, growing adolescents and during

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therapeutic indications, where they are active against *P. aeruginosa*–induced respiratory infections in children with cystic fibrosis, chronic suppurative otitis media or malignant otitis externa; acute or chronic osteomyelitis or osteochondritis; multidrug-resistant Gram-negative bacterial infections especially in immunocompromised hosts , bacterial septicemia or meningitis cases for which the causative organism is resistant to other approved agents; exposure to aerosolized *Bacillus anthracis*; serious infections in the children with life-threatening allergy to alternative antibacterial agents and multidrug-resistant mycobacterial infections [(4)].The underlying mechanism of FQs-induced joint toxicity is not well-known' but possible involvement of oxidative stress and nitric oxide ($NO^{\cdot}$) in the tendinopathic effects of the drugs have been demonstrated *in vivo* and *in vitro* [(5)]. Their toxicity concerning other organs needs to be clarified. Thus, this work was designed to study the possible cardiac damage induced by two therapeutic doses of ciprofloxacin in juvenile rats.

Materials and Methods

Animals

Eighteenth, (4-week) healthy albino rats of both sexes weighing 30 ± 2 g were utilized in the study. They were obtained from the Animal Laboratory House of the College of Pharmacy, University of Baghdad. The animals were fed standard diet *ad libitum* and were free access to tap water. They were divided into three groups of 6 animal each as follows: group-I Juvenile rats were received 25 IU I.P saline solution two times daily at twelve-hour intervals for one week, this group served as control; group II- Juvenile rats were injected intraperitoneally (I.P.) with 25 mg/kg ciprofloxacin two times daily at twelve-hour intervals for one week and group III- Juvenile rats injected I.P. with50 mg/kg ciprofloxacin two times daily at twelve-hour intervals for one week. At the end of the treatment period, blood samples were collected via cardiac puncture after euthanizing the animals by anesthetic ether. Blood samples were centrifuged for 10 minutes at 4,000 rpm, to obtain serum, which was utilized for the assessment of cardiac enzymes activities {(alanine aminotransferase (ALT) [(6)], aspartate aminotransferase (AST) [(6)], creatin kinase-muscle brain isoform (CK-MB) [(7)], and lactate dehydrogenase (LDH) [(8)]}. The hearts were dissected and stored in formaldehyde 10% until they were utilized for histological examination under light microscope [(9)] that was performed in the College of Veterinary/ Al-Mousel University. Analysis of data was performed using Microsoft Excel version 2007 two - ways to analyze student t-test. The data were expressed as mean ± standard deviation. *P* value of less than 0.05 was considered significant.

Results

Table 1 showed that serum ALT activity in the juvenile rats was significantly ($P<0.05$) elevated in group of animals treated with 50mg/kg ciprofloxacin (group III) compared to groups I and II. Additionally, no significant differences ($P>0.05$) concerning serum ALT were observed in juvenile rats treated with 25mg/kg ciprofloxacin (group II) compared to control juvenile rats (group I).Although there was an elevation in the serum activity of AST in group of juvenile rats treated with 50 mg/kg ciprofloxacin, but the results of table 1 showed no significant differences ($P>0.05$) in the activity of serum AST in all groups of juvenile rats (I, II and III) used in this study. Concerning serum enzyme activity of CK-MB, table 1 showed that there were significant ($P<0.05$) elevation in the serum activity of CK-MB in groups of juvenile rats treated with either 25mg/kg- (group II) or 50mg/kg-(group III) ciprofloxacin compared to control animals (group I). Furthermore, a significant elevation ($P<0.05$) in the serum activity of CK-MB was observed in group III of juvenile rats compared to the corresponding level of group II animals.The results of table 1 also showed a significant elevation ($P<0.05$) in the serum activity of LDH in group of juvenile rats treated with either 25mg/kg- (group II) or 50mg/kg-(group III) ciprofloxacin compared to control animals (group I). Furthermore, a significant elevation ($P<0.05$) in the serum activity of LDH in group III of juvenile rats compared to group II animals. Concerning the examination of histological sections of juvenile hearts' rats received either 25mg/kg- or 50mg/kg ciprofloxacin, respectively [figures (2 and 3)], the results showed the presence of vaculations, degenerations in addition to absence of striation of cardiac muscle were seen compared to control juvenile heart sections (figure 1).

Table 1:Serum Enzymes activities(ALT, AST, CK-MB and LDH)in cardiac tissues of the juvenile rats treated with either 25mg/kg- or 50mg/kg-ciprofloxacin compared to control group.

	Serum ALT IU	Serum AST IU	Serum CK-MB IU	Serum LDH IU
Group I Normal Saline 25IU (Control) N=6	47.8± 23.82^{A}	163.8±12.25^{A}	90.6 ± 61.65^{A}	1749± 191.9^{A}
Group II Ciprofloxacine 25 mg/kg-treated group N=6	47.33±22.83^{A}	157.66±11.21^{A}	267.83±263.6^{*B}	2580±334.21^{*B}
Group III Ciprofloxacine 50 mg/kg- treated group N=6	64.16±16 ^{*B}	216.16±34.36^{A}	425.66±234.16*^{C}	4030.33±228^{*C}

Values are expressed as mean± SD.
*: P<0.05 significant difference compared to control group.
Values with non-identical superscripts (A, B and C) are considered significantly different (P<0.05) among groups.
N= number of animals.

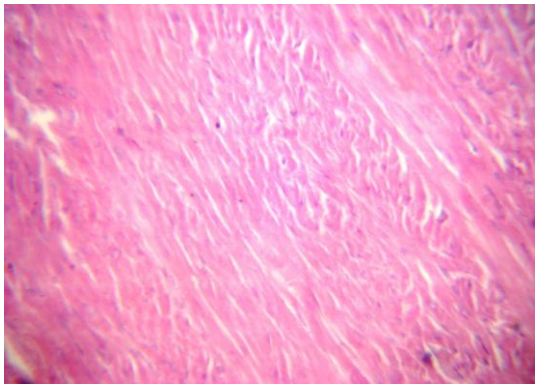

Figure 1: Section showing normal heart cell of juvenile rats.
Staining: Haematoxylline & Eosin;
amplification: 450 X.

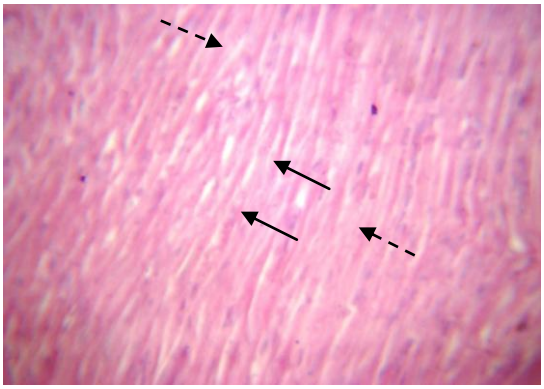

Figure 2: rat's heart aged 37 days, administered 25mg/kg ciprofloxacin I.P. for 7 days, demonstrates vaculation ( ) and degeneration ( ). Staining: Haematoxylline & Eosin; amplification: 450 X

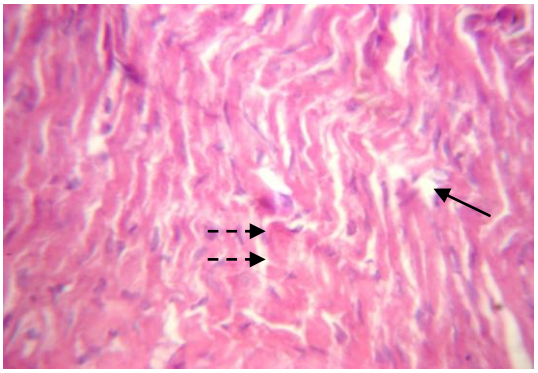

Figure 3: rat's heart aged 37 days, administered 50mg/kg ciprofloxacin I.P. for 7 days, demonstrates degeneration ( ) and vaculation ( ) with necrosis more clearly from that of 25mg/kg ciprofloxacin receiving animals .
Staining: Haematoxylline & Eosin;
amplification: 650 X.

Discussion

Although there were no clinical data concerning the cardiac adverse effects induced by FQs, it has been reported that therapeutic doses of a drug belongs to FQs groups, ciprofloxacin, may induce adverse effects in healthy rats following sub-acute treatment [4]. Furthermore, FQs have been associated with risk of QT prolongation and arrhythmias [2, 3].Increased ALT, AST, CK-MB and LDH activities that were observed in this study (Table 1), demonstrated myocardiocyte tissue damage due to ciprofloxacin a dministration

compared to control juvenile rats. The obtained biochemical results were consistent with that reported by others [(10)] and the biochemical changes were further confirmed by histological findings. Figures 2 and 3.Different myocardial markers were utilized to determine cardiac function; first, CK-MB resides in the cytosol and facilitates high energy phosphates into and out of mitochondria. It is distributed in a number of tissues including heart and skeletal muscle. In animal experiments, it was demonstrated that an increased in CK-MB activity in blood has been associated with evidence of irreversible cardiac injury (cell disruption) [(11)].Second, lactate dehydrogenase (LDH) catalyses the conversion of pyruvate to lactate. LDH-1 isozyme is normally found in the heart muscle and LDH-2 is found predominately in blood serum. A high LDH-1 level to LDH-2 suggests MI [(12)]. Third, aspartate transaminase (AST), an enzyme was the first used. It is not specific for heart damage, and it is also one of the liver function tests [(12)]. The mechanism of heart damage induced by ciprofloxacin has been demonstrated to implicate oxidative stress and many studies had been demonstrated that the antioxidant enzyme levels are relatively lower in the myocyte making this organ susceptible to reactive oxygen species [(4), (13-14)]. The results of this study provide an evidence, for the first time, to our knowledge on the effect of treatment of healthy juvenile rats with ciprofloxacin on cardiac markers in vivo. Therefore, we did not have the chance to compare the results of this study with other reports.

Conclusion

According to results obtained from this study, one can conclude that cardiac adverse effects manifested by the selected doses of ciprofloxacin used in this study (25 and 50mg/kg) injected IP to juvenile rats caused myocardial damage and may be another limitation for the use of this drug in the pediatric patients. Thus further studies are required to support this finding.

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