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Ciprofloxacin Chondrotoxicity, A Biochemical Analysis for Serum Magnesium, Calcium, Zinc, and Vitamin E in Wistar Albino Rats

Ameera Kamal Khalee[1], Ramizu Bin Shaari[1], Mohamad Arif Awang Nawi[1], and Ali Mihsen Hussein Al-Yassiri[2]

*[*1]Health Campus, Universiti Sains Malaysia, Kubang Kerian, 16150 Kota Bharu, Kelantan, Malaysia*

[2]Head of Dentistry Department, Hilla University College, Babylon, Iraq

Abstract

Ciprofloxacin is the most potent fluoroquinolone and was seen active against a broad range of bacteria. Several researchers revealed that ciprofloxacin can caused a chondrotoxic effects and supplementation with magnesium, calcium, zinc or vitamin E can diminished the induced chondrotoxicity. The aim of present study was to assess the rat's serum magnesium, calcium, zinc, and vitamin E, after the subcutaneous injections of ciprofloxacin to male Wistar rats. Ten rats were used in the study and divided in to two equal groups. On day 32 of age, all the animals in the control group were subcutaneously injected by physiological saline, while the animals in study group were subcutaneously injected by 600 mg/kg b.w. of ciprofloxacin eight hours apart. On day 34 of age, rats were anaesthetized and blood collection by cardiac puncture was taken. The present study revealed that mean serum levels for rat's magnesium, calcium, and zinc in the control group were 2.258±0.199 mg/dl, 9.416±0.369 mg/dl, and 95.51±1.81 µg/dl respectively, and the mean serum levels for rat's magnesium, calcium, and zinc in the study group were 2.138±0.257mg/dl, 9.254±0.292 mg/dl, and 94.19±2.929µg/dl respectively; The mean serum levels for vitamin E in the control and study group were 23.772±1.029 and 21.97±1.419 ng/ml respectively. Statistical analysis showed that ciprofloxacin hydrochloride caused a non-significant decrease in rat's serum magnesium, calcium, zinc, and vitamin E levels ($p>0.05$). The research concluded that ciprofloxacin chondrotoxicity was not caused by the decrease in rat's serum magnesium, calcium, zinc, and vitamin E levels, and other researchers are recommended to measure their levels in the chondroid tissue after ciprofloxacin therapy.

Key words: Fluoroquinolone, Arthropathy, Chondrotoxicity, Biochemistry

Introduction

Nalidixic acid (NA) is considering as one of the oldest quinolone antibiotic that was used in treating the urinary tract infection, it is considered as a first-generation quinolone. NA is an organic acid, but is also a base due to its amine functional groups, it is effective basically against gram-negative bacteria, but also has a minor effect on gram-positive. In lesser concentrations, it acts as a bacteriostatic manner; that is, it inhibits growth and reproduction, but in high concentrations, it kills bacteria. Historically, NA was used for treating urinary tract infections "as it was explaind by Emmerson and Jones 2003; Thakuria and Sarma 2017 [1, 2]". Later in the 1980, the fluorinated derivatives were synthesized, which possess a broad antibacterial spectrum that includes the gram negative and the gram positive aerobic with the anaerobic species "Sharma et al 2010 [3]". Ciprofloxacin is an antibiotic that considered as fluoroquinolone compound, and it is also used to treat different types of bacterial infections, it is considered as one of the most widely used and successful compounds of the fluoroquinolone, is ciprofloxacin, which was first patented by Bayer AG in 1983 and then approved by United States FDA (Food and Drug Administration) for use in the year of 1987. It is considered as the second generation of the quinolones and considered as a broad-spectrum antibiotic and the most potent fluoroquinolone "as explained by Tamma et al 2012 [4]".

Ciprofloxacin and other newer quinolone antimicrobial agents such as NA, exhibit increased potency and decreased frequency of spontaneous bacterial resistance in comparison with older analogues such as nalidixic acid "Hooper et al., 1987 [5]". Ciprofloxacin, a yellowish crystalline substance, is a monohydrochloride monohydrate salt of 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-1-

*Corresponding author e-mail: *ramizu@usm.my*

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piperazinyl-3-quinolinecarboxylic acid. Its formula is C17H18FN3O3 with a molecular weight is 331.4. A number of ciprofloxacin derivatives are also present which showed improved potency "Sharma et al 2010 [3]". Inside bacterial cells, ciprofloxacin has target to the DNA gyrase enzyme (type II topoisomerase), and a negative super helical twist into bacterial DNA was introduced. By this inhibition to DNA gyrase enzyme, ciprofloxacin can inhibit the DNA synthesis. The second target for the ciprofloxacin is topoisomerase IV which carries out the relaxation of DNA, and thus helps in the segregation of the replicating chromosomes. Inhibition of topoisomerase IV is important factor which contributing to the bactericidal activity of the ciprofloxacin. It can interrupt the DNA replication and preventing the cell division in bacterial cells "as discussed by Fief et al., 2019; Brar et al., 2020 [6, 7]".

The reasons for ciprofloxacin widely use are susceptibility of the multi-resistant pathogens only to ciprofloxacin treatment, higher plasma concentrations, greater bioavailability, and increased tissue penetration "Sharma et al 2010 [3]". Oral ciprofloxacin or intravenous injections of ciprofloxacin are used for treatment of bone infections "[8]", anthrax "[9]", gastrointestinal infections "[10]", Lower respiratory tract infections "[11]", urinary tract infection "[12]", and complicated pyelonephritis "[13]".

Various fluoroquinolone derivatives have been screened for their antifungal activities, and was seen exhibited potency against fungi "[14]", but they exhibit a low antiviral activity against SARS-CoV-2 and MERS-CoV "[15]".

Ciprofloxacin is readily absorbed following oral administration, but the absolute bioavailability is within a range of 70–80% for the oral dose. The intravenous infusion of 400 mg given twice daily has shown to produce a serum concentration the same like that which is produced by oral dose of 500 mg given twice daily. The glomerulus filtration and tubular secretion are important route for elimination, and the liver is the secondary route of excretion "as discussed by Sharma et al 2010 [3]".

The use of fluoroquinolones during the first months of pregnancy was not seen associated with the risk of premature births or birth defects, but other studies are needed to evaluate the side effects of ciprofloxacin in the developing fetuses. Ciprofloxacin was considered as a first-line for treatment of dysentery in the WHO Pocket Book of Hospital Care for Children "[16]", but they recommended that its use in children is only when the benefits outweigh the risk of arthropathy "as discussed by [17]".

Toxicity of ciprofloxacin is mild at therapeutic dose, in general, vomiting, nausea, hepatotoxicity, diarrhea "[18]", nephrotoxicity "[19]", exacerbation of myasthenia gravis "[20]", and hypersensitivity to ciprofloxacin "[21]" were reported. Previous studies found that ciprofloxacin can induce chondrotoxicity and tendinopathy. It must not be used as first line agents in children due to its risk of the injury to the joints "as discussed by Halawa 2010 [22]". It was found that it caused necrosis of the chondrocytes, extracellular matrix degeneration and cleft formation in the center of articular cartilage which may be detached and lead to the formation of erosions "as discussed by Sendzik et al., 2005 [23]". Previous studies explained that the supplementation with magnesium "as described bu Kat 2008 [24]", calcium "[25]", zinc "[26]", or vitamin E "[27]", can diminish articular cartilage chondrotoxicity.

The objectives of the present research were to study and review the effect of ciprofloxacin on serum levels of magnesium, calcium, zinc, and vitamin E in Wister albino rats.

Materials and methods

The total number of the animals used in the study was 10 male Wistar Albino rats. They were aged 32 days, weighing 50-70 g. The source of the animals was from the Laboratory Animal Research Center, College of Pharmacy, University of Karbala, Iraq. All rats were kept in a standard room condition, and fed with a standard rat chow, and allowed to drink water ad libitum. The research project was approved by the USM Institutional Animal Care and Use Committee (USM IACUC) and Alhilla University College, Hilla/ Iraq.

Experimental design

Ciprofloxacin hydrochloride (Bactiflox, Switzerland, 750 mg, Figure 1a) was used as two subcutaneous injections of 600 mg/kg of body weight, eight hours apart "as discussed by Pfister et al 2007 [28]". To prepare the injection solution, ciprofloxacin tablets were dissolved in 0.9% NaCl (7500 mg of ciprofloxacin in 100 ml of 0.9% NaCl yield a final ciprofloxacin strength of 600 mg/ 8 ml, which is corresponding to an injection volume of 8 ml/kg). The skin overlying ventral body wall was penetrated by a needle which entered at about 25 ° angles into a pocket which was formed beneath the tented skin of the rat. Table 1 shows the experimental design of the study.

Rats anesthesia, blood collection and euthanization

On day 34 of age, rats were first anaesthetized with intra muscular injection of 5 mg xylazine and 100 mg ketamine "as explained by Parasuraman et al., 2010 [29]". For each animal, a new needle and syringe was used. Check for anesthesia was done by lack of spontaneous movement and response to different stimuli. The blood collection by cardiac puncture was taken from the ventricle of the heart, and very slowly to avoid the collapsing of the heart.

Table 1: Experimental design of the study

Group	Description
Control Group	On day 32 of age, all the animals in this group were subcutaneously injected by two doses of physiological saline (0.9% NaCl), eight hours apart, in the same manner like ciprofloxacin (8 ml/kg).
Study Group	On day 32 of age, all the animals in this group were subcutaneously injected by two doses of ciprofloxacin hydrochloride (600 mg/kg), eight hours apart, prepared as 8 ml/kg).

The rat was placed on its back. The heart is present nearly one centimetre above the level of the lowest rib. The syringe was hold and the needle was then inserted between the two ribs and watched carefully for the drop of blood to ascertain that it was inside the heart (Figure 1b). The rats were immediately euthanized post blood collection. Bilateral thoracotomy with scissor was done to insure death. The whole blood was collected in a covered test tube and allowed for clotting. Then all the samples were centrifuged in a refrigerated centrifuge at 4℃ for 10 minutes at 3000 RCF. The serum was placed on ice for detection, but when the serum was not analyzed on the same day of blood collection, the serum was stored at –80°C "Abdelhalim et al., 2020 [30]".

The protocols of Magnesium Assay Kit and Calcium Assay Kit were followed by using a fully automatic Abbott Arcchitect *c*4000 clinical chemistry analyzer, Colorado, USA (Figure 2a). Serum Zn was quantified by using Dirui Auto-Chemistry Analyzer (Figure 2b), and following the protocol of Zinc Colorimetric Assay Kit. Rat vitamin E ELISA Kit was used for determination of rat's serum vitamin E using Elisys Uno, fully automated ELISA analyzer (Figure 2c). All parameters recommended by the manufacturers were followed and "as explained by [31]".

Figure 1: Photographs shows: (A). Ciprofloxacin tablets, Bactiflox; (B) Blood collection by cardiac puncture

Figure 2: Photographs shows: (a)Abbott Arcchitect *c*4000 clinical chemistry analyzer. (b) Fully automatic DIRUI, Auto-Chemistry Analyzer. (c) Elisys Uno, fully automated ELISA analyzer

Data analysis

Results were given as mean ± standard deviation. Statistical calculations were done using SPSS computer program (Statistical Package for the Social Science; SPSS Inc., version 24). The non-parametric statistical Kruskal-Wallis test was used to analyze the significant differences on biochemical data. *P* -Value less than or equal to 0.05 was considered statistically significant.

Results and Discussion

Serum magnesium, calcium, and zinc levels

The present study revealed that mean serum levels for rat's magnesium, calcium, and zinc in the control group were 2.258±0.199 mg/dl, 9.416±0.369 mg/dl, and 95.51±1.81 µg/dl respectively, and the mean serum levels for rat's magnesium, calcium, and zinc in the study group were 2.138±0.257mg/dl, 9.254±0.292 mg/dl, and 94.19±2.929µg/dl respectively. Statistical analysis showed that the subcutaneous injection by ciprofloxacin hydrochloride as two subcutaneous injections of 600 mg/kg of body weight, eight hours apart caused a non-significant decrease in rat's serum magnesium, calcium, and zinc levels ($p>0.05$) as seen in Table 2.

Previous studies found that the normal serum concentration of magnesium and calcium in male rats was 2.26 ± 0.12 mg/dl "[32]" and 9.9±0.2mg/dl "[33]" respectively. "Ahmed [34]". found that the normal serum level of zinc in rats was 115.08 ±2.04 ug/dl. "Djurdjevic *et al.* [35]" study found non-significant decrease in plasma zinc after taking ciprofloxacin.

Serum vitamin E level

The serum levels for vitamin E in the control and study group were 23.772±1.029 and 21.97±1.419 ng/ml respectively Statistical analysis showed that the subcutaneous injection by ciprofloxacin hydrochloride as two subcutaneous injections of 600 mg/kg of body weight, eight hours apart caused a non-significant decrease in rat's serum vitamin E levels ($p>0.05$) as seen in Table 2.

Vitamin E is the generic descriptor for all tocopherol and tocotrienol derivatives exhibiting the biological activity of α-tocopherol. It functions as a lipid-soluble biological antioxidant and protects against lipid peroxidation through the scavenging of free radicals. It may also confer anti-inflammatory effects and function in enzyme regulation and gene expression "as discussed by [36]". Previous study found that the normal serum levels of vitamin E in albino rats were 0.83±0.08mg/dl "[37]". "Pfister *et al.* [28]" found that the plasma concentration of vitamin E after the subcutaneous administration of two doses of 600 mg of ciprofloxacin/kg of b.w to rats was 93.6 ± 21.1 nmol/l. The differences may be due to different methodology used.

It was found that ciprofloxacin chondrotoxicity was caused by a deficiency of functionally available magnesium in the condylar tissue "[38]". Ciprofloxacin also can decrease the initial rates of calcium entry to the cells and affects the cellular energy metabolism and calcium homeostasis "[39]". Vitamin E level may be also decreased in this tissue, but not in the serum. "Sriram *et al*. [40]" found that a significant decrease in the lung tissue level of vitamin E was evident after taking bleomycin antibiotic when compared to control rats. Other causes of condylar chondrotoxicity was also found, like the DNA oxidative damage of the chondrocyte's cells"[41]" inhibition of mitochondrial dehydrogenase"[42]" and altered metabolism of DNA"[43]".

Table 2: Biochemical analysis for rat's serum in the control and study groups

Groups	Mean± SD with level of significance							
	Magnesium mg/dl	P-value	Calcium mg/dl	P-value	Zinc ug/dl	P-value	vit E ng/ml	P-value
Control group	2.258±0.199	0.403	9.416±0.369	0.601	95.51±1.81	0.464	23.772±1.029	0.2656
Study group	2.138±0.257		9.254±0.292		94.19±2.929		21.97±1.419	

Conclusion

- ✓ Previous studies explained that the supplementation with magnesium, calcium, zinc, or vitamin E, can diminished the chondrotoxicity induced by ciprofloxacin in juvenile animals.
- ✓ But the present study found that the ciprofloxacin can causes a non- significant decrease in serum magnesium, calcium, zinc, and vitamin E in a rat model.
- ✓ Other studies are recommended to measure their levels in the condylar tissue of Wistar albino rats after taking ciprofloxacin.

Conflict of interests

The authors declare no conflict of interest.

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