ORIGINAL ARTICLE

The Possible Protective Role of Magnesium on Ciprofloxacin Induced Chondrotoxicity on the Epiphyseal Plate Growth of Juvenile Albino Rat: A Histological and Morphometric Study.

Joseph Aziz[1], *Michel Morgan*[1]

[1]*Professor, Department of anatomy, Faculty of Medicine, Cairo University, Egypt*

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ABSTRACT

Background: Ciprofloxacin is one of the most effective antibiotics used in the treatment of many infectious diseases in adults, but it has undesirable toxic effects on the growing cartilage. The present study was designed to detect the toxic effect of ciprofloxacin on growing cartilage in the immature albino rats and the possible protective role of magnesium in prevention of such toxicity. **Methods:** Seventy rats were used in the current study. They were divided into five groups Group I: control group, group II: ciprofloxacin treated group, group III: magnesium given before ciprofloxacin, group IV: ciprofloxacin with magnesium and group V: magnesium given after ciprofloxacin). The region of the knee joint was prepared and stained with hematoxylin and eosin stain for light microscopic examination. Morphometric studies were also done using the image analyzer. **Results:** ciprofloxacin treated group revealed different changes in the tibial epiphyseal growth plate of juvenile rats in the form of decreased thickness, loss of chondrocytes, karryorrhexis, pyknotic nuclei, interlacunar hemorrhage, irregularity in the columns and myxomatous degeneration. Partial improvement was observed in the group treated with magnesium before ciprofloxacin and in the group treated with magnesium concurrently with ciprofloxacin, meanwhile no improvement was detected in the group treated with magnesium given after ciprofloxacin. **Conclusion:** The present study concluded that the use of ciprofloxacin in juvenile rats caused epiphyseal plate growth retardation; accordingly the use of this drug in pediatrics should be restricted to carefully selected indications. Owing to its protective role in reducing such chondrotoxic effects, the use of magnesium before (in chronic cases like cystic fibrosis of the lung) and concomitantly with ciprofloxacin is highly recommended.

Keywords: *Ciprofloxacin, Epiphyseal plate, Histology, Magnesium, Morphometry.*

INTRODUCTION

Ciprofloxacin is one of the quinolone group of antibiotics widely used in clinical practice. It is generally well tolerated by adults; however, its use in children is controversial. It is indispensable in the treatment of children suffering diseases as pseudomonas aeruginosa infection in patients with cystic fibrosis[1] and anthrax.[2]

The adverse effect of ciprofloxacin on growing cartilage has been studied by many authors.

Name & Address of Corresponding Author
Dr Joseph Aziz
Professor, Department of anatomy,
Faculty of Medicine,
Cairo University, Egypt.
E mail: jo_anatomy@yahoo.co.uk

Li et al.[3] said that the thickness of epiphyseal cartilages in juvenile rats receiving ciprofloxacin was markedly reduced compared to the control group, also

it shows histological changes in the form of matrix swelling and marked loss of chondrocytes. Channaet al.[4] reported that administration of ciprofloxacin to albino rat litters induced retardation in the growth of their epiphyseal plates and reduced the mean length of their humora and femora.

Other authors reported that Magnesium exerted a protective role on the growing cartilages exposed to ciprofloxacin. [5,6]

The aim of the present work is to study the possible effect of ciprofloxacin on the growth and architecture of the epiphyseal plate of juvenile albino rat and evaluate the possible protective role of magnesium.

MATERIALS AND METHODS

The present study was carried out on 70 female, immature albino rats weighing 75 – 85 gm. Only female rats were used to exclude possible sex differences. The rats were housed in cages, 5 rats / cage under standard environmental conditions with food and water ad libitum. The rats were divided into five groups. In each group the animals were sacrificed at the age of 39 days.

Group I: control group consisted of 10 rats. They were sacrificed at the age of 39 days for comparison with other groups. **Group II:** consisted of 15 rats. Each received a single subcutaneous injection of ciprofloxacin 20 mg / kg of body weight / day, for five days, starting at the age of 34 days. **Group III:** consisted of 15 rats. They were given magnesium 0.5% via naso-gastric tube for 10 days, starting at the age of 24 days, before receiving the above mentioned dose of ciprofloxacin by the same route and for the same period. **Group IV:** consisted of 15 rats. They were given a single subcutaneous injection of ciprofloxacin 20 mg / kg of body weight / day, for five days, starting at the age of 34 days and magnesium 0.5% via naso-gastric tube for the same period. **Group V:** consisted of 15 rats. They were given a single subcutaneous injection of ciprofloxacin 20 mg / kg of body weight / day, for five days, starting at the age of 24 days, followed by magnesium 0.5% by gastric gavage for 10 days.

All 70 rats were sacrificed by inhalation of high dose of ether. The hind limbs were disarticulated and the upper part of the femur and the lower part of tibia together with the soft tissues were removed, leaving the region of the knee joint. The specimens were fixed in 10 % formol saline, trimmed, washed and decalcified using nitric oxide 10% then processed for paraffin sections. 4 μm thick longitudinal sections of long bones were cut with a rotary microtome. The tibial and femoral epiphyseal plates were excised and processed for light microscopic examination using hematoxylin and eosin stain.[7]

Chemicals: Ciprofloxacin was supplied as ciprofloxacin vials 200 mg/200 ml (a product of the Al Ameria pharmaceutical company). It was given as a single subcutaneous dose of 20 mg/kg body weight/day, for 5 days. Magnesium was supplied as magnesium oxide powder (a product of the El gomhoria company) in a concentration of 0.5 %.[4] It was given orally by a special blunt-tipped syringe in the Animal house, ophthalmology institute, Giza, once per day.

- For 10 days before ciprofloxacin in group III.
- For 5 days in group IV simultaneously with ciprofloxacin.
- For 10 days in group V after the stoppage of ciprofloxacin.

The specimens were cut out immediately after death and the tissues were fixed in paraffin and Hematoxylin and eosin staining was done.

Morphometric study

Hematoxylin and eosin stained sections were examined by the use of the image analyzer computer system to measure the thickness of the tibial epiphyseal plates and to count the number of chondrocytes per field.

The data were obtained using Leica Qwin 500 image analyzer computer system (England). The image analyzer consisted of a coloured video camera, coloured monitor and hard disc of IBM personal computer connected to the microscope and controlled by Leica Qwin 500 software. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units.

Using the interactive measure menu the thickness of the tibial epiphyseal plate was measured using an objective lens of magnification 4 i.e of total magnification 40. Ten readings were obtained for each specimen and the mean value was obtained for each group [Figure 1].

Using the interactive measure menu the number of chondrocytes was counted in 10 HPF (x 200) in each specimen and the mean value was obtained for each group [Figure 2].

The data obtained were tabulated and subjected to statistical analysis using ANOVA (Analysis of Variants).

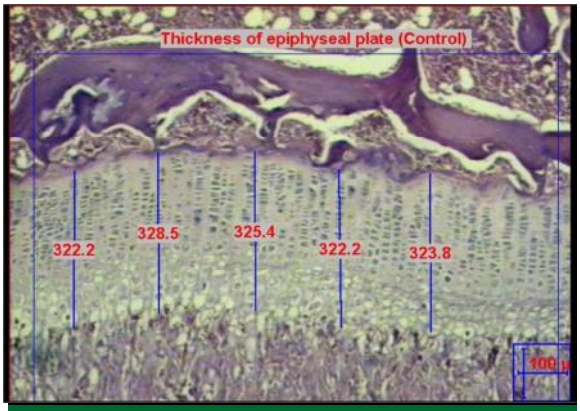

Figure 1: A copy of a display seen in the monitor's screen of the image analyzer of a longitudinal section of rat tibial epiphyseal plate specimen of control group showing the average thickness of the plate in the standard frame. (H & E x 40).

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Figure2: A copy of a display seen on the monitor's screen of the image analyzer of a longitudinal section of rat tibial epiphyseal plate specimen of the control group showing the average number of chondrocytes inside the standard frame. (H &E X 200).

RESULTS

Group I (control group)
Histological examination of the sections of the control group showed the normal architecture of the tibial epiphyseal plate. The plate revealed five different zones; the resting zone showing small non grouped cartilage cells, the proliferative zone containing flat chondrocytes arranged in regular columns, the hypertrophic zone containing hypertrophied chondrocytes, the calcification zone showing degenerated hypertrophied cells and resorption zone displaying absence of chondrocytes. The average thickness of the epiphyseal plate of cartilage and the homogenous matrix was also observed.

Group II (ciprofloxacin treated group)
Examination of sections of this group revealed a marked decrease in epiphyseal plate thickness secondary to the diminution of the size of all zones with short distorted columns focal areas of karryorehxis in the proliferative zone, karyolysis in the hypertrophy zone and pyknosis in the hypertrophy zone. The proliferative zone presented spots of interlacunar hemorrhage and areas of myxomatous degeneration of the matrix. Note that the cells are arranged in clusters in the resting zone and the hypertrophy zone showed focal areas of mucoid degeneration.

Group III (group treated with magnesium for 10 days before ciprofloxacin)
Examination of sections of this group revealed preserved epiphyseal plate thickness. The columns of chondrocytes in the proliferative zone were regularly arranged in some specimens and distorted in others with minimal myxomatous degeneration of the matrix. Focal areas of karryorehxis of nuclei were also observed.

Group IV (group treated with Ciprofloxacin and magnesium at the same time)
Examination of sections of this group revealed preserved thickness of the epiphyseal plate, regular columns of chondrocytes in the proliferative zone with homogenous matrix. Karyorrhexis (fragmentation of nucleus), karyolysis (disappearance of nucleus), pyknosis (shrinkage of the nucleus), short columns of chondrocytes with faint cell boundaries and minimal myxomatous degeneration were also found in most of the sections.

Group V (group treated with magnesium for 10 days after Ciprofloxacin)
Examination of this group revealed regular columns of chondrocytes in some specimens. Mild myxomatous degeneration of the matrix and decreased epiphyseal plate thickness. Focal areas of pyknosis and karyorrhexis were encountered.
Severe interlacunar hemorrhage, disorganization of chondrocytes in the proliferative zone, marked decrease in the epiphyseal plate thickness and homogenous matrix were noticed.
Focal areas of karyolysis, preserved epiphyseal plate thickness and marked myxomatous degeneration of the matrix were also found.

Morphometric measurements: [Histogram 1 and 2; Tables 1 and 2]
In group I (the control group), the mean value of the epiphyseal plate thickness was 325.37 ± 31.13 while the mean value of the number of chondrocytes was

11.44 ± 3.54.

In group II, the mean value of the epiphyseal plate thickness was 248.13 ± 36.37, which was highly significantly decreased as compared with the control group. Regarding the number of chondrocytes, the mean value was 8.4 ± 2.64, which was significantly decreased as compared with the control group.

In group III, magnesium given before ciprofloxacin minimized the decrease in epiphyseal growth plate thickness and the number of chondrocytes. The mean value of the epiphyseal growth plate thickness was 298.85 ± 31.97, which was statistically insignificantly decreased as compared with the control group. Regarding the number of chondrocytes, the mean value was 10.2 ± 3.33, which was insignificantly decreased as compared with the control group.

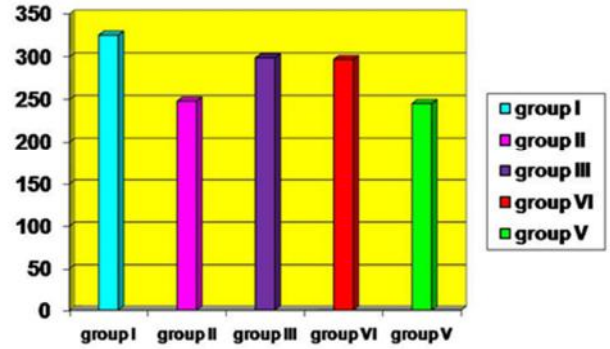
In group IV, magnesium given simultaneously with ciprofloxacin also minimized the decrease in the epiphyseal growth plate and the number of chondrocytes as the mean value of the epiphyseal plate thickness was 296.9 ± 38.7 which was statistically insignificantly decreased as compared with the control group. Regarding the number of chondrocytes, the mean value was 10.7 ± 4.25 which was insignificantly decreased as compared with the control group.

Table 1: Showing the mean and SD of the thickness of the epiphyseal plate of the experimental groups.

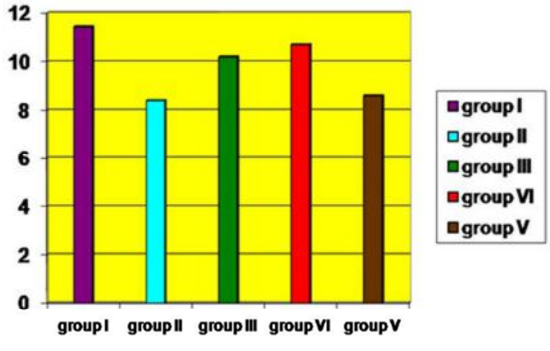
	Group I	Group II	Group III	Group IV	Group V
Mean	325.37	248.13	298.85	296.9	245.3
SD ±	31.13	36.37	31.97	38.7	28
P- value	----------	0.001	0.122	0.121	0.001

Table 2: Showing the mean and SD of the number of chondrocytes in the experimental groups.

	Group I	Group II	Group III	Group IV	Group V
Mean	11.4	8.4	10.2	10.7	8.6
SD ±	3.54	2.64	3.33	4.25	5.23
P- value	----------	0.014	0.124	0.124	0.015



Histogram (1) showing comparison of the thickness of the epiphyseal plate between the experimental groups



Histogram (2) showing comparison of the number of the chondrocytes between the experimental groups

DISCUSSION

Ciprofloxacin is a second generation fluoroquinolone. It is one of the most effective antibiotics, owing to its wide spectrum antibacterial activity and high degree of bioavailability.[8] It is well tolerated in adults, but its use in children and adolescents is controversial due to its potential chondrotoxic effect in immature animals.[9] It acts by inhibiting DNA gyrase and topoisomerase IV enzymes, in the bacterial cell; both of these targets are essential for bacterial DNA replication. Fluoroquinolones inhibit these enzymes by stabilizing either the DNA–DNA gyrase complex or the DNA–topoisomerase IV complex.[10]

In the present study, there was marked decrease in thickness of the tibial epiphyseal growth plate in ciprofloxacin treated group which was highly significantly decreased (248.13 ± 36.37) as compared with the control group (325.37 ± 31.13). This concluded that the hind limbs of the experimental animals were affected by the adverse effects of ciprofloxacin. These findings might be attributed to chondrocyte proliferation depression as shown by diminution of the proliferative zone of the epiphyseal growth plate.

kato et al[9] reported that the use of ciprofloxacin in children and adolescents is controversial due to its potential chondrotoxic effect. Chevalier et al.[11] recorded a considerable number of case reports showing symptoms of arthropathy in juvenile and even adult patients. On the other hand, Schaad and Wedgwood[12] suggested that most juvenile patients have shown no signs of arthropathy after treatment with quinolones.

Our observations were also in consistence with the finding of Arora[13], who reported that cartilage damage that may influence linear growth retardation remains a possibility due to fluoride accumulation with repeated quinolones administration and with Stahlman[14], who found that the epiphyseal growth plate could be damaged in immature animals during early post-natal developmental phase by quinolones and that these effects were associated with irreversible bone damage and growth inhibition. However, he neither specified the type of growth plate damage, nor clarified the form of the irreversible bone damage. Stahlmann and Lode[15] stated that the chondrotoxicity of quinolones as observed in immature animals can affect the particular cartilage and/or the epiphyseal growth plate, depending on the developmental stage as juveniles are especially sensitive. The reduction of the magnesium concentration in cartilage via the chelating properties of quinolones was discussed as the underlying mechanism.

One of the most characteristic findings in the present study, in ciprofloxacin treated group was the presence of myxomatous degeneration and matrix damage.

Morphological changes included the loss of the orderly columnar arrangement in the growth plate and shortened chondrocyte columns were observed in the current study. Also ,The present study revealed a decreased number of chondrocytes in group II (8.4 ± 2.64) as compared with the control group (11.4 ± 3.54) and this was in agreement with the findings obtained by many researchers[16-20] who noticed a decrease in the number of chondrocytes.

Gruber et al.[20] in 1994attributed the reduction of the number of chondrocytes in the cartilage columns after ciprofloxacin treatment to magnesium deficiency that generally enhances cytotoxicity by increasing membrane permeability, particularly for calcium. While Gruber et al.[9] in 2004 attributed it to the direct toxic effect and free radical formation secondary to ciprofloxacin treatment.

Nuclear changes were noticed in the current study in group II in the form of karyolysis, karryorehxis and pyknosis. Similar findings were encountered by Shakibaei et al.[21] who mentioned that integrins activate the mitogen-activated protein kinase (MAPK) signal transduction pathway which was important for differentiation and survival of chondrocytes and Inhibition of this pathway in chondrocytes resulted in apoptotic cell death.

Owing to their chelating properties, quinolones form complexes with magnesium ions, decreasing its availability for the physiological reactions it catalyses in cartilage. Magnesium had been used in the current study to confirm the possibility of its protective role in ciprofloxacin induced chondrotoxicity.

With the use magnesium 10 days before ciprofloxacin in group III and in conjunction with ciprofloxacin in group IV, partial improvement was observed in the present study as compared with the control group. Accordingly, decreased magnesium leads to interruption of the normal function of the signal receptors of the β1 integrin family which crucially depends on magnesium. These receptors have an important role in chondrocyte-matrix interaction and thus seem to contribute to cartilage stability and integrity.[22]

Regarding the thickness of the epiphyseal plate, no significant decrease was observed in using magnesium with ciprofloxacin either before in-group III (298.85 ± 31.97) or concomitant in group IV (296.9 ± 38.7) as compared with the control group (325.37 ± 31.13). These findings were in agreement with Hickory et al.[23] who stated that magnesium helps in excess formation of collagen, which increase osteoblastic activity and rate of longitudinal growth and bone remodelling in experimental rats. Moreover, Prassad[24] added that magnesium directly stimulates

DNA synthesis by enzyme stimulation.

The current study observed a protective effect of magnesium with no significant decrease in the number of chondrocytes in using magnesium with ciprofloxacin either before in group III (10.2 ± 3.33) or concomitant in group IV (10.7 ± 4.25) as compared with the control group (11.4± 3.54). Similar findings were detected by different studies.[7,11,12,24]

The protective effect of magnesium was detected in the present work as minimal to moderate myxomatous degeneration of the matrix was encountered in groups III and IV. This is matching the results obtained by Sendziket al.[25] who mentioned that the partial protective effect of Magnesium on ciprofloxacin induced toxicity was attributed to its responsibility for the normal function of the signal receptors of the β_1 integrin family, which is essential for cell–cell and cell-matrix interactions involved in cartilage growth.

In the present work group III (magnesium was given 10 days before ciprofloxacin) and group IV (magnesium was given concomitantly with ciprofloxacin) revealed partial improvement in the epiphyseal plate thickness, number of chondrocytes and matrix homogeneity. On the contrary, no improvement was detected in the previous parameters regarding group V (magnesium was given for 10 days after ciprofloxacin). The requirement of magnesium for both the desired antibacterial action and the toxic effect on cartilage could explain chondrotoxicity of all known quinolones and the decreased extracellular magnesium induced by ciprofloxacin was compensated by the administration of magnesium either before ciprofloxacin in group III or concomitantly with it in group IV. On the other hand, the present study revealed no protective role of magnesium when administered after ciprofloxacin treatment in group V, and this could be explained by the inability of magnesium to reverse the chondrotoxicity when given after the end of ciprofloxacin treatment

Similar findings were detected by Grzesiak et al.[26] who attributed the partial improvement encountered in immature rats treated with ciprofloxacin and magnesium to the later as the integrin-mediated fibroblast migration on type I collagen, observed only in the presence of magnesium ions, is influenced by minor changes in the concentration of divalent cations.

CONCLUSION

The present study concluded that the use of ciprofloxacin in juvenile rats caused epiphyseal plate growth retardation, accordingly the use of this drug in pediatrics should be restricted to carefully selected indications. Owing to its protective role in reducing such chondrotoxic effects, the use of magnesium before (in chronic cases like cystic fibrosis of the lung) and concomitantly with ciprofloxacin is highly recommended.

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