EFFECT OF CIPROFOXACIN ON GROWING CARTILAGE IN ALBINO RAT PUPS

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Background: Administration of quinolone therapy is controversial during growing age as stated by earlier worker. The flroquinolones are currently not indicated for young children, because of arthropathy and adverse effect on growing cartilage shown by studies. However the effects of ciprofloxacin on epiphyseal growth plate has remained undocumented. This study is therefore, undertaken to determine the risk of ciprofloxacin administration an growing cartilage by prospective experimental animal study model using Wistar albino rat pups. **Methods:** Ciprofloxacin was administered to newly born Wistar albino rat pups with a doze of 20mg/kg body weight intraperitonealy twice a day from day-1 to day-14 after birth. The animals were sacrificed by deep ether anesthesia. The limbs were disarticulated from axial skeleton, soft tissue was removed. The intact bone mean length in millimeter of right and left humerus and femur was measured with the help of electronic vernier caliper and bones were fixed in 10% buffered farmalin. Decalcification was done in 10% nitric acid and 10% formic acid changes. After paraplast embeding, 4 µm thick longitudinal sections of the proximal long bones were cut by a rotary microtome. Routine staining with haemotoxylin and eosin was performed. Histomorphometry was done measuring the thickness of epiphyseal cartilage and was compared with similar value of control animals. The results were statistically analysed to find out the significance. **Results:** The ciprofloxacin induces a mordanting effect as abviated by increased basophilia. Our study reveales that cirprofloxacin administration in the newly born pups decreased the width of epiphyseal growth plate cartilage by 10.43% in humerus and 4.72% in femur as compared to the growth of control cartilage. The decrease in the width was brought about mainly by the reduced count of the proliferative cells in the proliferative zone and the diminuation in the average size of the hypertrophic condryocytes in the hypertrophic zone. The reserve zone has become markedly reduced in thickness. **Conclusion:** The ciprofloxacin post-natal administration effected growth plate retardation by inhibiting the mitosis in the proliferative zone and also effected the mean length of humora & femora leading to reduction in limb length of rat pups.

Key words: Ciprofloxacin – Cartilage Toxicity- Cartilage growth – Bone Ossification – Growth rate – Epiphyseal plate – Chondrocyte.

INTRODUCTION

The newer quinolones, the fluroquinolones are represented by ciprofloxacin, norloxacin and temofloxacin, these drugs represent an improvement over their non-fluroquinolone counterpart in many ways, including a wider spectrum of antimicrobial activity, improved pharmacokinetic properties and clinical efficacy against wide range of organisms [1]. Ciprofloxacin is one of the more active drugs in this class that possesses an extended spectrum of the antimicrobial activity[2], has better bioavailability 80-95% serum level and is distributed widely in body fluids and tissues[3]. It has sufficiently long serum half life to suit twice daily dosing[4].

The ciprofloxacin is one of the most commonly used antibiotics now a days for different kinds of infection. Concomitant with its wide range of activity and common usage, it inherits many side effects, i.e. hepetotoxicity[5], nephrotoxicity[6] and damages the growing cartilage in experimental animals [7].

In the rat epiphyseal growth plate cartilage, chondrocytes are organized in discrete layers. The progenitor cells are assembled with in the frame of reserved cell zone (RZ). Thus adjacent to the epiphyseal trabecular bone are found single cells, and/or small cluster of two or four chondrocytes. Adjacent to RZ cells are small flat cells, the proliferative cells which to form columns of cells, thus forming the proliferative cell zone (PZ) with subsequent cellular maturation, the PZ cells loose their ability to multiply and undergo hypertrophy, thus, giving rise to hypertrophic zone (HZ) which under goes mineralization and is partially resorbed by chondroclasts. The remainder of mineralized calcified cartilage constitutes the scaffold for the opposition of metaphysical trabecular bone[8].

Ciprofloxacin is an inhibitor of gyrase enzyme which is important for metabloic activity of

bacteria[9]. Although the ciprofloxacin is contraindicated in pregnancy and children[10] but quicks and doctors are liberally using the drug unchecked, which may be prohibited by the government under rules.

Arora reported that subtle bone and cartilage damage that may influence linear growth retardation remains a possibility particularly due to floride accumulation with repeated fluroquinolone administration[11].

This study is undertaken to determine the effect of ciprofloxacin administration on epiphyseal growth plate of long bone in limbs of juvenile laboratory Wistar albino rat pups to confirm the possibility of Arora's speculation and find out its magnitude.

MATERIAL AND METHODS

Twenty spontaneously ovulating female and 10 fertile male Wistar albino rats of 10-12 weeks age were taken from the animal house of Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi. The female rats were mated with fertile males of same strain according to the method described by Rough[12]. Thus one male rat was mated with two female rats in a separate cage. On next morning the female rats were examined for signs of mating such as blood stained vagina or a vaginal plug of mucoid greenish white material. Presence of both or any of these signs was considered as day-1 of pregnancy[13] . The mean gestation period of the albino rat is 21 to 23 days[14]. Hundred delivered pups were randomly selected irrespective of the sex to make two groups i.e. A and B, each group comprised of 50 animals.

GROUP "A" : Experimental pups were given injection ciprofloxacin developed in Bayer Research Laboratories., AG, Germany, as single dose of 20mg/kg body weight[15]. Thus 0.12mg ciprofloxacin in 0.1 ml of daily for 14 days from day-1 of birth.

GROUP "B" : Control pups were injected normal saline in equal volume (0.1ml)[16] intraperitoneally twice daily for 14 days from day-1 of birth.

All pups of both groups were sacrificed on 15th post-natal day by giving deep ether anesthesia. Fore and hind limbs were disarticulated from axial skeleton and at the joints, soft tissue were removed. The intact bone mean length in millimeters of right and left humerus & femur was measured with the help of electronic digital vernier caliper and fixed in 10% buffered formaline, decalcified in 10% nitric acid and 10% formic acid changes. Embeded in paraplast and 4 µm thick longitudinal sections of long bones were cut by a rotary microtome. These sections were stained with Haemotoxylin and eosin (H &E)[17] Histomorpho metery was done and the data was subjected to statistical analysis. Students 't' test was employed to determine the statistical significance of the results as described in introduction of medical statistics[18].

RESULTS

In present study we have observed the effects of ciprofloxacin on neonatal skeletal growth. At the end, the experimental humerus and femur from fore and hind limb respectively was chosen for Histomorphometry of epiphyseal growth plate (EGP) to determine the thickness and morphological changes in the growth plate.

EGP THICKNESS IN CIPROFLOXACIN TREATED AND CONTROL RAT PUPS.

HUMERUS: The mean EGP thickness Proximal & distal ends in control animals was 131.65 + 0.63 µm, while in ciprofloxacin treated animals it was reduced to 117.60 + 1.05 µm. The difference noted was 13.7 + 0.42 µm as shown in the table-1. The decrease in thickness of EGP in ciprofloxacin treated animals was 10.43% which is highly significant ($P<0.001$).

FEMUR: The mean EGP thickness proximal distal ends in control animals was 139.65+0.39 µm while the ciprofloxacin treated animals it was reduced to 133.05 + 1.6 µm. The difference noted was 6.6+1.21 µm as shown in the table-1. The decrease in thickness of EGP in ciprofloxacin treated animals was 4.72% and was highly significant ($P<0.001$).

The width of EGP cartilage in this group decreased by 10.43% in humerus and 4.72% in femur as compared to control cartilage as shown in the table[-10]. It is noted Fig-1, that decrease in width of epiphyseal growth plate cartilage was brought about mainly by reduction in proliferation of chondrocytes in proliferative zone (humerus from 131.65 + 0.63 µm) in controls to 117.6 + 1.05 µm in experimental pups and femur from 139.65 + 0.39 µm in controls to 133.05 + 1.6 µm in experimental pups) and in diminution of the size of the individual zones and virtual absence of hypertrophic zone. Most of the reserve cells were organized in cluster and some times formed columns that reached the middle of the growth plate fig.-2. The epiphyseal growth plate cartilage was demarcated from the underlying bone marrow by a thin demineralized layer of bone that was devoid of vascular elements.

This observation was constant in all ciprofloxacin treated pups along the whole thickness of cartilage-marrow interface. As a result of absence of osteoclastic activity at this bone marrow-growth plate junction, decreased metaphysical trabecular bone was formed. There were no chondronecrosis or stromal cells observed at the zone of hypertrophy.

INTACT BONE LENGTH (mm) IN CIPROFLOXACIN TREATED AND CONTROL RAT PUPS

The mean value of intact bone length as determined by measuring the length of long bones of right and left fore limbs humerus and hind limbs femur respectiely with the help of electronic digital vernier caliper of groups A and B are presented in table-2.

1. Humerus: The mean length of humerus in control animals was 13.67+0.93 mm. While in ciprofloxacin treated animals, it was reduced to 9.90+0.17 mm. The mean difference and standard error of mean was noted 3.77+0.76 mm. Which is statistically highly significant (P<0.001), decrease in length is 27.50% as shown in Table-2.

2. Femur: The mean length of femur in control animals was 14.88 + 0.04 mm. While in ciprofloxacin treated animals it was reduced to 11.49+0.11 mm. The mean difference and stand standard error of mean was noted 3.39 + 0.07. Which is statistically highly significant (P<0.001) decrease in length is 22.78% as shown in table-2.

DISCUSSION

The fluroquinolnes are the direct inhibitors of DNA synthesis, by binding to the enzyme DNA complex. They stabilize the DNA strand breaks created by DNA gyrase and Topoisomerase IV. Ternary complexes of drug, the enzyme and DNA block the progress of the replication fork.[19,20,21,22]

The present study was therefore aimed to determine the extent of chondrotoxicity of ciprofloxacin administered in newly born albino rat pups by Histomorphometry of epiophyseal growth plate (EGP) as a parameter.

Our observation revealed that there was decrease in thickness of epiphyseal growth plate in ciprofloxacin treated animals by 10.67% in humerus and 4.72% in femur as compared to control cartilage. The decrease in thickness of epiphyseal growth plate of humerus was noted from 131.65 + 0.63 µm in controls to 117.6 + 1.05 µm in experimental rat pups and femur from 139.65 + 0.39 µm in controls to 133.05 + 1.6 µm in experimental rat pups. These findings are attributed mainly to reduction of thickness in proliferative zone and virtual absence of the hypertrophic zone.

Our observations are in consistence with stahlmann who reported that the chondrotoxicity of quinolones as observed in immature animals, can effect articular cartilage and / or the epiphyseal growth plate, depending on the development stage.[23] Stahlmann noted that juveniles are especially sensitive and in animal at an early developmental phase the epiphyseal growth is also damaged by the quinolones and these effects are associated with reversible bone damage and growth inhibition.[24]

Table-1: Comparison of epiphyseal growth plate thickness (mm) of Humerus and Femur in albino rat pups between experimental Group A and control Group B.

Bones		**Group A Experimental (n = 50)**	**Group B Control (n = 50)**	**Reduction of epiphyseal thickness**
Humerus	Upper End	117.6 ± 1.05 µm*	131.3 ± 0.63 µm	13.7±0.42µm (10.43%)
	Lower End	117.6±1.05µm*	131.3±0.63µm	13.7±-.42 µm (10.43%)
Femur	Upper End	133.05 ± 1.6 µm*	139.65 ± 0.39 µm	6.6 ± 1.21 µm 4.72%
	Lower End	133.05±1.6µm*	139.65±0.39µm	6.6±1.2 µm (4.72%)

Values are given as mean ± standard error of mean (SEM).

Table-2: Comparison of intact bones length (mm) of Albino rat pups between postnatal treated and control pups. Values are given as mean ± standard error of mean (SEM).

Bones		**Group A Experimental (n = 50)**	**Group B Control (n = 50)**	**Reduction of bone length**
Humerus	Right	9.90 ± 0.17 mm*	13.67 ± 0.93 mm	3.77±0.76mm (27.57%)
	Left	9.90±0.17mm*	13.67±0.93mm	3.77±0.76mm (27.57%)
Femur	Right	11.49 ± 0.11mm*	14.88 ± 0.04 mm	3.39 ± 0.07mm (22.78%)
	Left	11.49±0.11mm	14.88±0.04mm	3.39±0.07mm (22.78%)

µm = micrometer.
* = P<0.001 (Highly Significant decreased).
n = Total numbers of animals used in group.
mm = Millimeter.

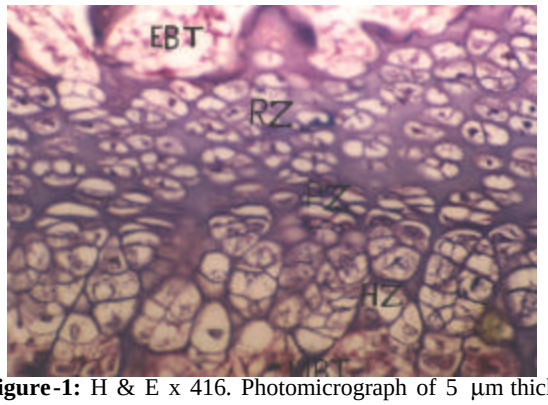

Figure-1: H & E x 416. Photomicrograph of 5 µm thick longitudinal section of proximal end of Humerus in control animals showing thickness of epiphyseal growth plate EBT, RZ, PZ, HZ and MBT represents epiphyseal bone trabeculae, reserve cartilage zone, proliferative zone, hypertrophic zone and metaphyseal bone trabeculae respectively.

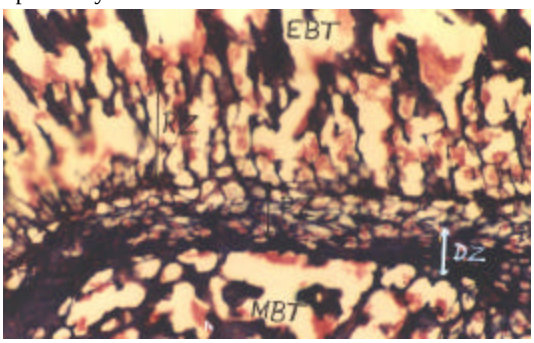

Figure-2: H & E x 486. Photomicrograph of 5µm thick longitudinal section of proximal end of Humerus showing epiphyseal growth plate in ciprofloxacin treated albino rat pups, with narrowness of reserve cell zone RZ, and proliferate zone PZ and virtual absence of HZ and widening of cartilage degeneration DZ followed by capillary invasion.

Arora reported that subtle bone and cartilage damage that may influence linear growth (linear growth retardation) remains a possibility particularly due to fluoride accumulation with repeated fluroquinolone administration.[11] Our study thus not only confirms the Arora's speculation but specifies the extent of linear growth retardation.

The increased staining intensity of the cartilage matrix of ciprofloxacin treated cartilage reflects the increased concentration of acidic, sulphated proteoglycans which is greatest around the fully differentiated cells and least in the perichondrium[25]. Thus the increased basophilia in ciprofloxacin treated cartilage indicates the increased concentrations of the acid sulphated proteoglycans as a feature of metabolic alteration.

We recommend the follow up of ciprofloxacin treated pups to assess the duration and extent of reversibility of changes with & without chondrotrophic agent because this would indicate the prognosis of the affected children.

No difference was noted in the gait and posture of control and treated animals. It would be interesting to perform an electron microscopic study backup to find out the changes at the ultra structural level in the organelles of the retarded chandrocytes. Determining the extent of reversibility of such ultra structural changes would be of more clinical acumen in determining the therapeutic appropriation.

CONCLUSION

Our results conclude that ciprofloxacin causes epiphyseal growing plate retardation by inhibiting the mitosis in chondrocytes of proliferative zone in newly borne rat pups, when administered postnataly. As juveniles are especially sensitive, use of these drugs in human neonates should be restricted to carefully selected indications. And the increase risk of prolonged therapy should not be under estimated.

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