PREVENTIVE ROLE OF ZINC CHLORIDE AGAINST TOXICITY OF CIPROFLOXACIN ON THE GROWING CARTILAGE OF WISTAR ALBINO RAT LITTER

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Objective: To assess the preventive role of zinc chloride on toxicity of ciprofloxacin administration in wistar albino rat litter. It was a Prospective experimental study. The study was carried out in the department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, Pakistan during March 2002 to February 2003 one year study. **Method:** Ciprofloxacin and zinc chloride were administered to newly born albino rat litters separately and simultaneously at a dose of 20 mg/kg body weight and 1200 μg/Kg body weight respectively, intraperitonealy twice daily from 1–14 day after birth. The animals were sacrificed by deep ether anaesthesia. The fore and hind limbs were dis-articulated from the axial skeleton, soft tissue was removed and bones were fixed in 10% buffered formalin. Decalcification was done in 10% nitric acid and 10% formic acid changes. After paraplast embedding, 4μm thick longitudinal sections of proximal & distal ends of long bones were cut by a rotary microtome. Routine staining with haemotoxylin and eosin was performed. Histomorphometery was done to measure the thickness of epiphyseal cartilage and was compared with similar values of the control animals. The results were statistically analyzed to evaluate the significance. **Result:** Our study revealed that ciprofloxacin administration in new born albino rat litter decreased the width of epiphyseal growth plate cartilage by 13.7±0.42 μm, 10.43% in humerus and 6.6±1.2 μm 4.72% in femur as compared to control, whereas, simultaneous zinc chloride administration restricted the decrease to 1.27 μm±SD in humerus and 2.05 μm±SD in femur. **Conclusion:** Simultanous zinc chloride administration minimized the epiphseal cartilage damage induced by ciprofloxacin in Wistar albino rat litter.

Keywords: Ciprofloxacin, Cartilage toxicity, Cartilage growth, Zinc chloride, Epiphyseal growth plate, Growth rate, Chondrocyte.

INTRODUCTION

A new drug application for intravenous ciprofloxacin was submitted in 1980's to United States Food and Drug Administration.[1] Ciprofloxacin is fluorinated derivative of quinolone. It is a bactricidal substance with wide bacterial spectrum of activity and is entirely synthetic.[2] The ciprofloxacin is among the most commonly used antibiotics for different kind of infections caused by susceptible bacteria in lower respiratory tract, skin, bone, joint, urinary tract and infectious diarrhoea.[3] Ciprofloxacin is contra-indicated in pregnancy and children.[4] because it has many adverse effects such as hepatotoxicity[5] nephrotoxicity[6] and causes the damage to the growing cartilage in young animals[7]. But quicks and doctors are librally using the drug unchecked, which may be prohibited by the government under rules.[8]

Ciprofloxacin acts as a gyrase inhibitor which is important for metabolic activity of bacteria.[9]

Epiphyseal grwoth plate shows a typical hyaline cartilage with chondrocytes arranged singly or in small clusters surrounded by a large amount of moderately basophilic stained matrix. This is known as zone of reserve cartilage (Rz). Adjacent to the Rz, the chondroytes undergo successive mitotic divisions to form the columns of chondrocytes separted by strongly basophilic stained matrix rich in acid proteoglycans. This is known as proliferative cell zone (Pz). The cells of Pz undergo hypertrophy & loose their ability to divide and form the hypertrophic zone (Hz). In Hz chondrocytes are greatly enlarged, vacuolated and the matrix becomes calcified. The mineralized Hz adjacent to the metaphysis gets partially resorbed by the chondroclasts. This remainder mineralized calcified cartilage constitutes the scaffold for the deposition of metaphyseal trabecular bone.[10] However the study involved the magnetic resonance images. Neither the images are of real dimension nor their sharpness is comparable to the resolution of the light Microscopy. The reliability of the direct histomorphometery is comparatively great. Therefore the potential to retard the linear growth by criprofloxacin that has been regarded a distinct possibility by Narendra K. Arora in his/her annotation, needs to be scientifically confirmed.[11]

Zinc is one of the essential trace elements and is necessary for the synthesis of DNA & RNA proteins and functions as a catalyst for several enzymes. Zinc stabilizes the structure of nucleic acid protein and thereby preserves the integrity of intracellular organelles

such as mitochondrion.[12] Zinc participates in the regulation of cell proliferation in several ways, it is essential to enzyme systems that influence cell division and proliferation.[13] Zinc is an essential nutrient that is required in humans and animals for many physiological functions, including immunological and antioxidant functions including normal growth and reproduction.[14] However despite the long-term study of the zinc metabolism, the limiting role of zinc in cell proliferation remains undefined.[15]

This study is performed to find out the preventive role of zinc chloride against the toxicity of ciprofloxacin on the growing cartilage of Wistar albino rat litter.

MATERIAL AND METHODS

In this study 30 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 Center, Karachi. The female rats were mated with fertile males, according to the method described by Rough.[16] One male rat mated with two female rats in one cage. On next morning the female rats were examined for sign of mating in the form of blood stained vagina or a viginal plug consisting of a mucoid greenish white material. Presence of any one of these signs was considered as day-1 of pregnancy.[17] The gestation period of albino rat averages between 21 to 23 days.[18]

Thirty pregnant rats delivered their litters. Randomly selected 150 rat litters were divided into three groups i.e. A, B, and C. Each group comprised of 50 animals regardless of sex.

Group "A": Experimental rat litters were given injection ciprofloxacin (developed in Bayer Research Laboratories, A.G, German) at a dose of 20 mg / kg body weight[19] i.e. 0.12 mg of drug dissolved into 0.1ml of the solvent.

Group "B": Experimental rat litters were injected zinc chloride salt (developed as laboratory chemical, in West Germany) at a dose of 1200μgm/kg weight[20] i.e. 7.4μgm salt dissolved into 0.1 ml of the solvent. The salt was injected intraperitoneally 30 minutes before the administration of ciprofloxacin twice daily for 14 days from day one after birth.

Group "C": Control rat-litters were injected normal saline in equal volume[21] i.e. 0.1 ml intra-peritoneally twice daily for 14 days from day-1 after birth.

The rat-litters of all three groups were scarified on 15th post-natal day by giving deep ether anesthesia. The fore and hind limbs were dis-articulated from the axial skeleton at the proximal joints. The soft tissue was removed and bones were fixed in 10% buffered formalin. The decalcification was done in 10% Nitric acid and 10% Formic acid changes.[22] Embedding was done in paraplast and 4 μm thick longitudinal sections of long bones were cut by the rotary microtome. The sections were stained with Haemotoxylin and Eosin (H&E).[23] Histomorphometery was done and the data was statistically analyzed. Student's 't' test was employed to determine the statistical significance of results.[24]

RESULTS

We observed the effects of ciprofloxacin and zinc chloride, on neonatal skeletal growth plate separately and simultaneously. The experimental humerus and femur were chosen for histo-morphometry of epiphyseal growth plate (EGP) and morphological changes were also noted. Results were compared with the control.

Epiphyseal Growth Plate (EGP) Thickness.

Humerus:

Mean normal humerus EPG thickness at proximal and distal ends of control animals was measured 131.65±0.63 μm. While in ciprofloxacin treated humera it was reduced to 117.6±1.05 μm. However, simultaneous treatment of zinc chloride restricted the thickness to 130.3±0.38 μm. Percentage decrease in EPG in ciprofloxacin treated animal was 10.43 μm which is statistically highly significant (p<0.001). Simultaneous zinc chloride minimized the decrease of EPG thickness to 1.02% which is statistically insignificant (p<0.001) as compared to the control.

Femur:

Mean EGP thickness at proximal & distal ends of control was 139.65±0.29 μm which was reduced in ciprofloxacin treated animals to 133.05±1.6 μm. However, simultaneous treatment with zinc chloride restricted the decrease of thickness of EGP to 137.6±0.4 μm.

Table-1: Comparison of epiphyseal growth plate thickness (μm) of Humerus and Femur in albino rat-litter between experimental Group A, B and C

Bones		Group A Experimental (n=50)	Group B Experimental (n=50)	Group C Control (n=50)	Reduction of epiphyseal thickness in group A.
Humerus	Upper End	117.6±1.05 μm*	130.3±0.38 μm**	131.65±0.63 μm	13.7±0.42μm (10.43%)
	Lower End	117.6±1.05μm*	130.3±0.38 μm**	131.65±-0.63 μm	13.7±0.42μm (10.43%)
Femur	Upper End	133.05 ± 1.6 μm*	137.6 ± 0.4 μm**	139.65±0.39 μm	6.6± 1.21μm (4.72%)
	Lower End	133.05±1.6μm*	137.6±0.4 μm**	139.65±0.39 μm	6.6±1.2μm (4.72%)

Values are given as mean ± standard deviation (SD). *=P<0.001 (Highly Significant decrease). ** =P>0.05 (insignificant change)

Thus the EGP decrease is statistically highly significant (p<0.001) due to chondrotoxicity of ciprofloxacin as shown in Figure-1. This was very effectively prevented by simultaneous zinc chloride treatment as proved by statistical analysis (p>0.05) (Figure-2). Which is only 1.46% reduction in EGP thickness as compared to the control.

Epiphyseal Growth Plate Cartilage of Ciprofloxacin Treated Rat-Litters:

The width of epiphyseal growth plate cartilage in this group decreased by 10.43% in humerus and 4.72% in femur relatively to that of control cartilage as shown in Table-1. It is noted in Figure-1 that decrease in width of epiphyseal growth plate cartilage was brought about mainly by reduction in proliferation of chondrocytes in proliferative zone Pz (Humerus from 131.65±0.63 μm in controls to 117.6±1.05 μm in experimental rat-litters and femur from 139.65±0.39 μm in controls to 133.05±1.6 μm in experimental rat-litters) and in diminuation the size of the individual zones and virtual absence of hypertropic zone HZ. Most of reserve cells were organized in cluster and some times formed columns that reached the middle of growth plate Figure-2. The epiphyseal growth plate cartilage was demarcated from the underlying bone marrow by a mineralized layer of bone that was devoid of vascular elements. This observation was consistent in all ciprofloxacin treated rat-litters along the whole thickness of cartilage/marrow interface. As result of total absence of osteoclastic activity at this bone marrow/growth plate junction, decreased metaphyseal trebecullar bone was formed. There was no chondronecrosis and stromal cells were absent at the Hz.

Epiphyseal growth plate in ciprofloxacin and zinc chloride treated rat-litters:

Simultaneous therapy with zinc chloride resulted in recovery of the width of epiphyseal growth plate cartilage and even surpassed that of control as shown in Figure-2 and Table-1. This was brought about by an increase in the number of proliferative cells per columns in the Pz (Humerus from 131.65±0.63 μm in controls to 130.3±0.4 μm in experimental rat-litters and femur from 139.65±0.39 μm in controls to 137.6±0.4 μm in experimental rat-litters) an increase in size of individual hypertrophic cells in the Hz also fully recovered. The cells of Rz were organized mainly as single cells at the heads of the Pz columns and adjacent to the epiphyseal trabecular bone. The neat columnar organization of epiphyseal growth plate cartilage was fully restored.

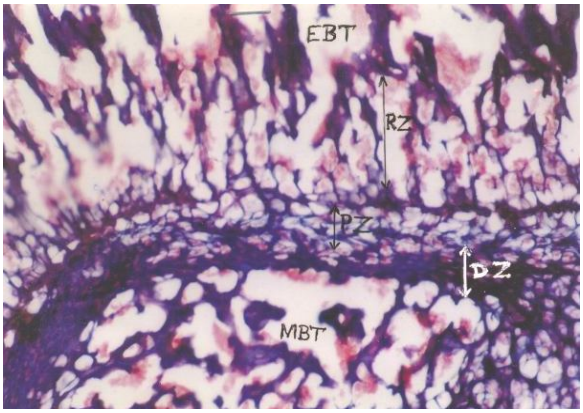

Figure-1: H & E ×486

Photomicrograph of 4μm thick longitudinal section of proximal end of Humerus showing epiphyseal growth plate in ciprofloxacin treated albino rat-litters with narrowness of reserve cell zone Rz, proliferate zone Pz, absence of Hz and widening of cartilage degeneration zone Dz followed by capillary invasion.

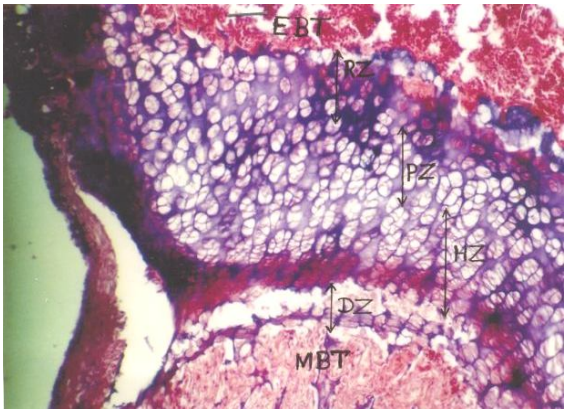

Figure-2: H & E ×486

Photomicrograph of 4 μm thick longitudinal section of proximal end of Humerus showing thick epiphyseal growth plate in ciprofloxacin + zinc chloride treated albino rat-litters with EBT, epiphyseal bone trabiculae, Rz reserve cell zone, Pz proliferative zone Hz hypertrophic zone and MBT metaphyseal bone trabeculae.

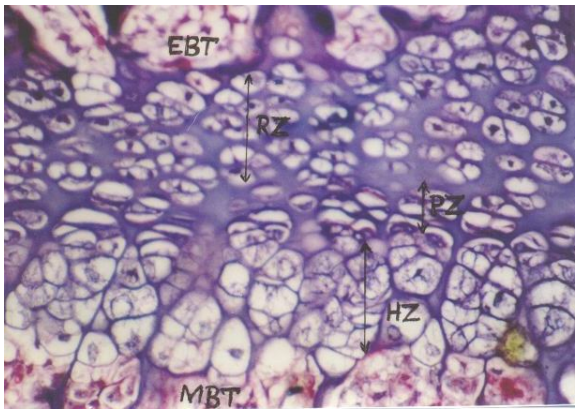

Figure-3: H & E ×486

Photomicrograph of 4μm thick longitudinal section of proximal end of Humerus in control animals showing well formed epiphyseal growth plate with EBT epiphyseal bone trabaculae, Rz reserve cell zone, Pz proliferative zone, Hz hypertrophic zone and MBT metaphyseal bone trabeculae.

DISCUSSION

The fluroquinolones are the direct inhibitors of DNA synthesis, by binding to the enzyme DNA complex. They stabilize DNA strand breaks created by DNA gyrase and Topoisomerase IV. The increased antibacterial potency of cirprofloxcin corelates with its increased potency inhibiting the DNA supercoiling activity of the purified enzyme.[9]

Nuclear magnetic resonance (NMR) scamming although a sensitive indicator of early cartilage damage picking up minimal synovial effusion.[11,25] NMR was not found helpful by our preliminary model because of relatively miniature structure of rat litters growing cartilages and joints.

The present study was therefore aimed to determine the effects of ciprofloxacin and preventive effects of Zinc chloride when administered separately and simultaneously in newly born albino rat litters by histomorphometry of epiphyseal growth plate cartilage as a parameter.

Our observation revealed that there was decrease in thickness of epiphyseal growth plate in ciprofloxacin treated animals was found to be 10.43% in humerus and 4.72% in femur relatively to that of control cartilage. The decrease in thickness of epiphyseal growth plate of humerus was noted from 131.65±0.63 μm in control to 117.6±1.05 μm in experimental rat-litters and femur from 139.65±0.39 μm in controls to 133.05±1.6 μm in experimental rat-litters. This concluded that fore and hind limb bones were affected by adverse effect of ciprofloxacin. These findings are attributed mainly to reduction of thickness in proliferative zone Pz and virtual absence of hypertrophic zone in Hz. Our observations are in consistance with the finding of stahlmann[26], who found in animals during early post-natal developmental phase the epiphyseal growth plate can be damaged by quinolones and that these effects are 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. Irreversible bone damage could be either in the form of retarted formation or abnormal formation. Our study suggests that the growth plate damage is due to chondroycte proliferation depression as shown by diminuation of proliferative zone of the epiphyseal growth plate. Stahlmann R stated that the chondrotoxicity of quinolones as observed in immature animals, can affect particular cartilage and/or the epiphyseal growth plate, depending on the developmental stage as juveniles are especially sensitive.[27]

The non significant change we obtained in thickness of epiphyseal growth plate simultaneously given zinc chloride animals in group B was found to be in humerus 1.02% (over corrected) and in femur 1.46% (over corrected) when compared with age matched controls, humerus from 131.65 ± 0.63 μm in controls to 130.3±0.38 μm in experimental rat-litter and femur from 139.65±0.39μm in controls to 137.6 ±0.4 μm in experimental rat-litters. These findings are attributed to protective role of zinc chloride. Our observations are in agreement with those by Hickory W, who found that zinc helps in excess formation of collagen increase osteoblastic activity and increase rate of longitudinal growth and bone remodelling in experimental rats.[28] Prassad AS, stated that zinc directly stimulates DNA synthesis either by enzyme stimulation or by altering the binding of F_1 and F_3 histones to DNA, so as to effect RNA synthesis.[29]

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.[30] Thus the increased basophilia in ciprofloxacin treated cartilage indicates the increased concentrations of the acid sulphated proteoglycans as a feature of metabolic alteration.

Our study proved that there is a definite chondrotoxic effect of the ciprofloxacin over the skeletal growth in rat litters.

Our study also proved that the simultaneous administration of the zinc chloride effectively prevents the ciprofloxacin condrotoxic effects.

We suggest the follow-up of ciprofloxacin rat litters after different defined periods to find out the maximum period lag after which the toxic effects are irreversible or insignificantly reversible.

CONCLUSION

These result strongly suggested that ciprofloxacin causes epiphyseal growth plate retardation in newly born rat-litters. However, the ciprofloxacin chondrotoxicity could be partially prevented by simultaneously administration of Zinc chloride in albino rat-litters post-natally.

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