ORIGINAL PROF-760

TOXICITY OF CIPROFLOXACIN;
PREVENTIVE ROLE OF ZINC CHLORIDE ON APPEARANCE OF SECONDARY OSSIFICATION CENTERS IN WISTAR ALBINO RAT LITTERS

DR. MUHAMMAD ASLAM CHANNA.
M. Phil (Anat)
Department of Anatomy
Basic Medical Science Institute,
Jinnah Postgraduate Medical Center, Karachi.

DR. MUHAMMAD ASHFAQUE
M.Phil (Anat)
Department of Anatomy
Basic Medical Science Institute,
Jinnah Postgraduate Medical Center, Karachi.

DR. SIBGHATULLAH SANGHI
M.Phil (Pharma)
Department of Anatomy
Basic Medical Science Institute,
Jinnah Postgraduate Medical Center, Karachi.

Dr. Muhammad Baqir Soomro
M.Phil (Anat)
Department of Anatomy
Basic Medical Science Institute,
Jinnah Postgraduate Medical Center, Karachi.

Correspondence:
Dr. Muhammad Aslam Channa
Department of Anatomy
Basic Medical Science Institute,
Jinnah Postgraduate Medical Center, Karachi.

ABSTRACT ... draslamchanna2000@yahoo.com **Objectives:** To evaluate the preventive role of Zinc Chloride on Toxicity of Ciprofloxacin administration on secondary ossification centers in wistar albino rat litters. **Design of Study:** Prospective and comparative animal study carried out on experimental Wistar Albino Rats. **Setting:** The study was carried out in the department of Anatomy Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center (JPMC) Karachi from 2001 to 2002. **Methods**: Ciprofloxacin and Zinc Chloride were administered to newly born albino rat pups separately and simultaneously at a dose of 20 mg/kg body weight and 120 ug/100g body weight respectively intraperitoneally twice daily from day 1 to 14 after birth. These animals were killed by deep either anaesthesia and fixed in 80% alcohol. They were then bulk stained with alizarin Red S and alcian blue. Finally they were cleared in 4% KOH and stored in Glycerin. The fore and hind limbs were disarticulated from the axial skeleton and observed under stereo-microscope for evidence of skeletal differentiation in the form of presence of secondary ossification centers in ling bones. The time of appearance of these centers were noted and compared statistically with those in the control animals. **Results:** This study revealed that the secondary ossification centers in long bones were delayed in experimental animals and simultaneous treatment with Zinc Chloride partially prevented toxicity of ciprofloxacin as compared with controls. **Conclusion:** these results strongly suggest that ciprofloxacin causes a risk to skeletal differentiation

and therefore to its growth. However the cirpofloxacin toxicity could ne partially prevented by simultaneous administration of Zinc Chloride in wistar albino rat litters.
Key Words: Ciprofloxacin, Zinc Chloride, Secondary ossification centers, bone differentiation.

INTRODUCTION

A new drug application for intravenous ciprofloxacin in 1980's was submitted to United States Food and Drug Administration[1]. New quinolones carboxylic acid compounds are currently being investigated for therapeutic use in many infections, cirpofloxacin is one of the most active in this class that posses an extended spectrum of activity as good bioavailability and sufficient long serum half life to allow twice daily dosing[2-8]. Use of fluoroquinolones is restricted in pediatric age group because of possible adverse effect on the growing cartilage and bones[9]. After initiation of ciprofloxacin in clinical trials in the early 1980s it has been administrated to children despite restrictions. The first report from a child treated with ciprofloxacin was in May 1983[10]. Since that time ciprofloxacin has been continuously used in children and adolescent when conventional therapy failed or was not available.

Ciprofloxacine is described as gyrase inhibitor because of its mode of action gyrase enzyme is important for metabolic activity of bacteria[11].

Zinc is one of the trace elements and is known to be essential for synthesis of DNA, RNA, proteins and physiological functions of several enzymes. Zinc stabilizes the structure of proteins and nucleic acids and preserves the integrity of sub cellular organelles such as mintochandria[12]. Zinc modulates insulin sensitivity and insulin is involved in insulin like growth factor-1 (IGF-1) insulin receptors may be involved in the zinc link[13]. This study was done to evaluate the effects of ciprofloxacin and Zinc Chloride administration during infancy separately and simultaneously of differentiation of long bones in extremities of postnatal Juvenile Laboratory Wistar albino rats.

MATERIALS & METHODS

Two hundred and ten pups were used in this study. They were obtained from 40 pregnant female, wistar albino rats, were 12-16 weeks of age and spontaneously ovulating, when taken from the animal house of Basic medical Sciences Institute, JPMC, Karachi for this study they were mated with fertile males of same strain, allowing one male rat with two female rats in one cage[14] on next morning the female rats were examined for signs of mating in the form of blood stained vagina or a vaginal plug (a mucoid greenish white material) presence of any one of these signs was considered as day-1 of pregnancy [15]. Pregnancy lasted for 21 days[16].

Forty pregnant albino rats were allowed to deliver their pups. Randomly selected 210 pups were divided into three groups i.e A, B and C, each comprising 70 animals, sex of these offsprings was omitted.

Group-A (experimental n=70). Pups were given injection ciprofloxacin (developed in Bayer Research Laboratories, AG, Germany) at a dose of 20 mg/kg body weight[17]. (0.12mg in 0.1ml) intra-peritoneally twice daily for 14 days from day-1 to day-14 after birth.

Group-B (protected n=70). Pups were given simultaneously Zinc Chloride (developed in laboratory chemical, West Germany) at a dose of 120ug/100gm body weight[18] (7.4 ug in 0.1ml) intraperitoneally 30 minutes before administration of ciprofloxacin twice daily for 14-days)from day-1 to day-14 after birth).

Group-C (Control n=70). Pups were given normal saline in equal volume (0.1 ml)[19] intraperitoneally twice daily for 14 days (from day-1 to day-14 after birth).

Five specimen were then randomly selected for the study from each group for each day mentioned (Total

70 pups from group experimental which received ciprofloxacin and total 70 pups from preventive group, which received simultaneously zinc chloride 30 minutes before administration of ciprofloxacin and total 70 pups from control group which received normal saline) pups were then killed by deep Ether anaesthesia and fixed in 80% alcohol after removing their skin and visceras. They were then bulk stained in Alizarin Red S and Alcian blue, cleared in 4% KOH (to reveal ossification centers) and stored in glycerin[20].

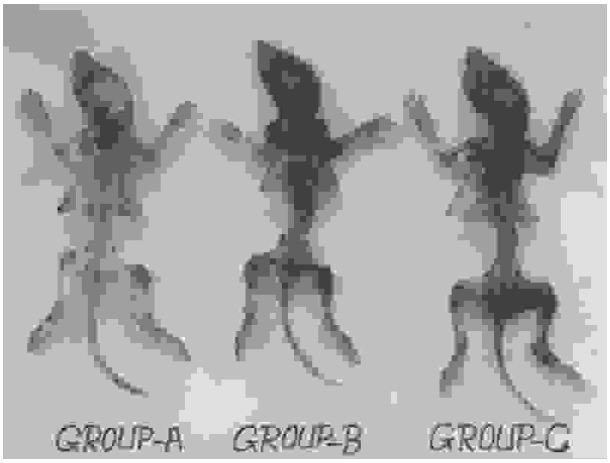

Fig-1. Photograph og postnatal treated pups whole skeleton with double staining technique, i.e. Alizarin red stained bone and Alincian blue stained cartilage showing comparison of appearance of secondary ossification centers at proximal and distal ends of long bones between experimental (Group-A left), (Group-B Central) and control (Group-C right) animals used in this study.

Left fore and hind limbs were separated from axial skeleton at their joints and viewed under stereo-microscope. The presence or otherwise of secondary ossification centers in Humerus, Radius Ulna of Fore limbs and Femur, Tibia and Fibula of hind limbs at their proximal and distal ends were observed and recorded. The said bones were observed throughout the experimental period (day-1 ot day-14 postnatally). Even after the detection of secondary ossification centers to see appearance of any additional ossification center in shaft or end of these long bones. The mean value of the time of first appearance of secondary centers in experimental and control bones were given as mean ± SEM. Student "t" test was employed to see the significance of result[21].

RESULTS

The mean time of appearance of secondary ossification centers in major skeletal components of both extremities in experimental and control animals are given in table and shown in figure-1,2 and 3.

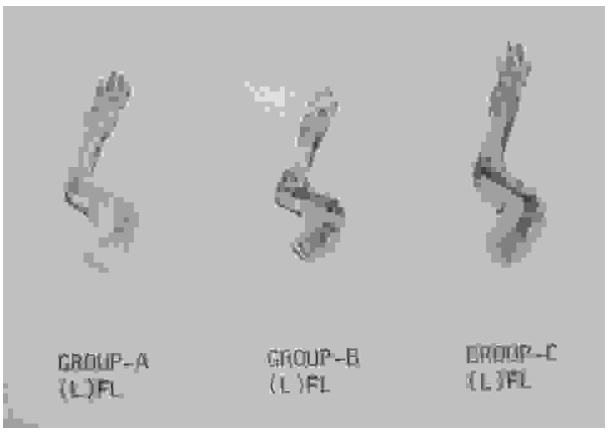

Fig-2. Photograph of left fore limb (Humeus, Radius & Ulna bones) with double staining technique, i.e. Alizarin red and Alician blue stained cartilage showing comparison in presence of secondary ossification centers at proximal and distal ends between experimental (Group-A left), (Group-B Central) and Control (Group-C Right) animals used in this study.
Key: L=left, FL = Fore Limb.

Left Humerus, Ulna and Tibia:
In control animals (group C n=70) the secondary ossification centers at proximal and distal ends, were present on 7th postnatal day in all specimens observed (5 specimens of each bone).

No additional ossification center was observed in any bones during the rest of study period. In experimental animal (group A, n=70 and B, n=70) the secondary ossification centers at proximal and

distal ends were seen to be present on 9th and 7th postnatal day in 3 specimens and 10th, 8th, postnatal day in 2 specimens respectively (average 9.4 ± 0.2 days and 7.3 ± 7.01 days with delay of 2.4 ± 0.2 and 0.3 ± 0.1 days respectively).

Left Radiu, Femur and Fibula:
In control animals (group C, n=70) the secondary ossification centers were present at proximal end on 14th day after birth in all specimen observed (5 specimens of each bone) while at distal end were present on 7th post natal day in all specimen (5 specimens of each bone). Any additional postnatal ossification center was not observed in any of these bones during rest of study.

In experimental animals (group A, n=70 and group B, n=70) the secondary ossification centers at proximal end was seen to be present on 16th and 14th postnatal day in 3 specimens of each group and on 17th, 15th postnatal day in 2 specimens of each group respectively (average 16.4 ± 0.2, 14.4 ± 0.2 days with delay of 2.4 ± 0.2 and 0.4 ± 0.2 days respectively) while at the distal end the secondary ossification centers were present on 9th, & 7th postnatal days in 4 specimens of each group and 10th, 8th postnatal day in one specimen of each group respectively (Average 9.2 ± with delay of 2.2 ± 0.2 and 0.3 ± 0.2 days respectively).

Table-I. Comparison of time (days) first appearance of secondary ossification centers in post natal experimental Group-A, B and control Group-C, at their proximal and distal ends of left side long bones in juvinicle Albino rat litters.

Bones		Experimental animals		Control Animals	Time Days Delayed
		Group-A (n=70)	Group-B (n=70)	Group-C (n=70)	
Humerus	PE	9.4 ± 0.2*	7.3 ± 0.1**	7.0 ± 00	2.4 ± 0.2
	DE	9.4 ± 0.2*	7.3 ± 0.1**	7.0 ± 00	2.4 ± 0.2
Radius	PE	16.4 ± 0.2*	14.4 ± 0.1**	14.0 ± 00	2.4 ± 0.1
	DE	9.6 ± 0.2*	7.4 ± 0.1**	7.0 ± 00	2.4 ± 0.2
Ulna	PE	9.4 ± 0.2*	7.2 ± 0.2**	7.0 ± 00	2.4 ± 0.1
	DE	9.4 ± 0.1*	7.3 ± 0.2**	7.0 ± 00	2.4 ± 0.2
Femur	PE	16.4 ± 0.2*	14.2 ± 0.1**	14.0 ± 00	2.4 ± 0.2
	DE	9.2 ± 0.2*	7.3 ± 0.2**	7.0 ± 00	2.2 ± 0.2
Tibia	PE	9.2 ± 0.3*	7.3 ± 0.2**	7.0 ± 00	2.2 ± 0.2
	DE	9.2 ± 0.2*	7.4 ± 0.2**	7.0 ± 00	2.2 ± 0.1
Fibula	PE	16.0 ± 0.2*	14.2 ± 0.0	14.0 ± 00	2.2 ± 0.0
	DE	9.2 ± 0.2*	7.2 ± 0.1	7.0 ± 00	2.2 ± 0.2

*P.E = Proximal end, D.E =Distal end, n = Total number of animals, * = P < 0.001 significantly delayed, ** = P> 0.05 non-significantly delayed.*

DISCUSSION

The appearance of ossification centers is the first indication of skeletal differentiation. The effects of ciprofloxacin treatment and by simultaneous administration of Zinc chloride treatment on skeletal

differentiating were therefore studied by determining the time of first appearance of representative ossification centers in postnatal fore and hind limb long bones in experimental and control animals.

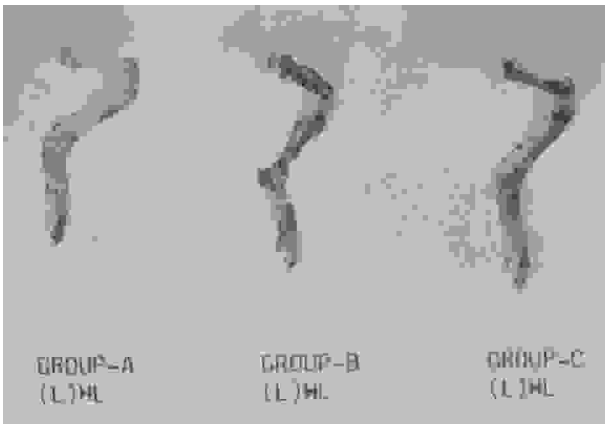

Fig-3.Photograph of left and hind limb (Femur, tibia and Fibula bones) with double staining technique, i.e. Alizarin red stained bone and Alician blue stained cartilage showing in presence of secondary ossification centers at proximal and distal ends between experimental (Group-A left), (Group-B Central) and (Group-C Right) animals used in this study.
Key L = leftHL = Hind Limb

In control animals the secondary centers of ossification in humerus, ulna and tibia bones at proximal and distal ends were present at 7th day postnatally in all specimens and in Radius, Femur and fibula bones were present at proximal ends on 14-day after birth in all specimens while at distal ends were present on 7th day after birth in all specimens observed, our observations are in agreement with the findings of patton and kaufmann[20], who state that the pattern of ossification in fore limb the secondary ossification are evident in the head, greater tubrosity, capitulum and trochlea by 7th day after birth. The radius has secondary centers appeared in distal part of cartilage primordium by 7th day after birth and proximally by 14th after birth, the secondary center visible in the distal regions of tibia and fibula and proximal part of tibia by 7 day after birth.

Ciprofloxacin delayed the appearance of secondary ossification centers in long bones at their proximal and distal ends average by 2.2 ± 0.2 day as compared to those in control animals this delay was highly significant ($P > 0.01$). However simultaneous administration of Zinc chloride delayed the appearance of secondary ossification centers by 0.3 ± 0.1 day i.e. non significant ($P > 0.05$) when compared with those in control long bone. This indicates that fore limb and hind limb bone were subjected more to the adverse effects of ciprofloxacin and effect was partially prevented by simultaneous use of Zinc chloride.

In this study the delay in appearance of ossification centers by ciprofloxacin may be attributed to tissue accumulation of floride which may inhibit the action of calcification process. The mechanism by which ciprofloxacin act is still unknown. Our observations are in agreement with the findings of stahlmann [22] and Arora [23] who states that subtle bone and cartilage damage that may influence linear growth (linear growth retardation) remains a possibility particularly due to fluoride accumulation with repeated fluoroquinolone administration, while the partial prevention made by simultaneous administration of Zinc Chloride. This result may be attributed that zinc directly stimulates DNA synthesis either by enzyme stimulation or by altering the binding of F_1, F_3 histones to DNA so as to effect RNA synthesis Prasad[24]. Our results are in agreement with findings of WuFy[25], who states that several enzymes required for nucleic acid synthesis and is well known now that zinc is needed for DNA polymerase-1, RNA poly merase and reverse transcriptase.

CONCLUSION

These results strongly suggest that ciprofloxacin causes a risk to skeletal differentiation and therefore to its growth. However the ciprofloxacin toxicity could be partially prevented by simultaneous administration of Zinc chloride in wistar albino rat litters.

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