ORIGINAL ARTICLE

EFFECTS OF CIPROFLOXACIN AND ZINC CHLORIDE IN ADULT ALBINO RAT AND PRE-NATAL CONCEPTUS

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Background: Administration of quinolone therapy is controversial during growing age as stated by earlier authors. The flouroquinolones are currently not indicated for young children because of arthropathy and adverse effect as new born shown by studies. However the effects of ciprofloxacin and $ZnCl_2$ on prenatal conceptus have remained undocumented. The present study was designed to compare the effects on conceptus after maternal ingestion of ciprofloxacin and $ZnCl_2$ using Wastar albino rats. **Methods:** Ciprofloxacin and $ZnCl_2$ was administrated to pregnant female albino rats. Ciprofloxacin with a dose of 20 mg/Kg bodyweight and $ZnCl_2$ 120 µg/100 gm bodyweight two times therapeutic dose for 10 days (from day 8–18 of pregnancy). Each animal was weighted on day 1, day 8 and day 18 of pregnancy. Abortion resulted on day 18^{th} of pregnancy. Each group of pregnant animals were sacrificed on day 18 of gestation by over dose of either anaesthesia, abdomen opened, uterus and both cornua containing conceptus identified, removed, there weight recorded, crown rump length was measured 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 obviated by increased basophilia. Our study reveals that ciprofloxacin administered in maternal, decreased maternal body weight to 38.4±0.9 gm. However simultaneous $ZnCl_2$ maintained the body weight to 41.4±0.7 gm, while $ZnCl_2$ increased the body weight to 46.5±2.25 gm. The body weight and Crown Rump length (CR Length) in conceptus decreased by 4.52±0.10 gm and 3.06±0.09 Cm respectively. That ciprofloxacin and $ZnCl_2$ administration maintained the body weight and CR length by 5.46±0.09 gm and 3.79±0.13 Cm respectively. That $ZnCl_2$ administration increased the body weight and CR length by 6.71±0.05 gm and 4.15±0.08 Cm respectively. **Conclusion:** Prenatal administration of Ciprofloxacin caused reduction in growth rate and CR length, and $ZnCl_2$ maintained body weight and CR length and growth of the rat conceptus.

Keywords: Ciprofloxacin, $ZnCl_2$, body weight, crown rump length, conceptus

INTRODUCTION

Fluoroquinlones are the fluorinated derivatives of quinolones. These are Ciprofloxacin, Sparfloxacin, Travofloxacine, Ofloxacine and Norfloxacine.[1] Ciprofloxacin is a synthetic antibiotic of quinolone group.[2] Ciprofloxacin is one of more active drug having an extended spectrum of antimicrobial activity.[3] It has best bioavailability 80–95% serum level and is remained widely in body fluids and tissues.[4] It has better long serum half life to suit twice daily dosing.[5]

The ciprofloxacin is one of the most commonly used antibiotics now a day for different kinds of infections. Concomitant with it's wide range of activity common usage, it inherits many toxic effects on liver,[6] kidneys[7] and damage the epiphyseal growth plate in experimental animals.[8] Ciprofloxacin is a gyrase enzyme inhibitor, which is important for metabolic activity of bacteria.[9] Although quinolones are contra-indicated for children and adolescents and during pregnancy and nursing due to potential for joints cartilage lesion found in immature animals.[10] But quick doctors and physicians are liberally using the drug unchecked, which may be prohibited by government under rules. Arora reported that subtle bone and cartilage damage that may cause gradual retardation of growth remains a possibility particular due to fluoride accumulation with repeated use of quinolones.[11]

Zinc is one of the essential trace minerals and is necessary for synthesis of DNA and RNA proteins and functions as catalyst for several enzymes. Zinc stabilises the structure of nucleic acid protein and thereby preserves the integrity of intracellular organelles such as mitochondrion. Zinc plays many significant roles in metabolism as component of many metalloenzymes and transcription factors.[12]

The study was undertaken to determine the effect of ciprofloxacin and $ZnCl_2$ on the crown rump (CR) length and body weight of juvenile laboratory Wistar albino rat off-springs to confirm the possibility of Arora's speculation and find out its magnitude.

MATERIAL AND METHODS

Forty spontaneously ovulating female and 20 fertile male Wistar albino rats of 16–18 weeks age were obtained from animal house of basic medical science institute, Jinnah Postgraduate Medical Centre Karachi. The female rats mated with same strain.[13] Thus one male rat was mated with two female rats in a separate cage. On next day one female rat was examined for signs of mating such as blood strained vagina or vaginal plug of mucoid greenish white material. Presence of both or any of these signs were considered a day zero of pregnancy, All pregnant rats were weighed on day one of pregnancy. Forty prenatal-natal conceptus were randomly selected irrespective of the sex to make four groups each comprising of 10 animals.

Group-A consisted of control prenatal conceptus, their mothers were injected normal saline in equal volume[14], i.e., 0.1 ml intra-peritonially twice daily for 18 days from day 1 of pregnancy. Group-B included prenatal conceptus, their mothers were injected ciprofloxacin at a dose of 20 mg/Kg body weight[15], i.e., 0.12 mg of drug dissolved in 0.1 ml of the solvent, intra-peritoneally twice daily for 18 days from day 1 of pregnancy. Group-C had prenatal conceptus, their mothers were injected ciprofloxacin at a dose of 20 mg/Kg body weight, i.e., 0.12 mg of the drug dissolved into 0.1 ml of the solvent and $ZnCl_2$ at a dose of 120 µg/Kg bodyweight, i.e., 7.4 µg salt dissolved in 0.1 ml of the solvent. Intra-peritoneally 30 minutes before the administration of ciprofloxacin twice daily for 18 days from day 1 of pregnancy. Group-D consisted of prenatal conceptus, their mothers were injected $ZnCl_2$ at a dose of 120 µg/Kg body weight, i.e., 7.4 µg salt dissolved in 0.1 ml of the solvent, Intra-peritoneally twice daily for 18 days from day 1 of pregnancy. Each animal was weighed on day 1; day 8 and day 18 of pregnancy and recorded in Performa.

The animals of each group were sacrificed on day 18 of gestation by an overdose of ether anaesthesia, abdomen opened, uterus and both cornua containing offspring were identified and removed, harvested, washed in normal saline. Each conceptus were weighed on sartorious balance and their body weight were recorded. The CR lengths were also measured.

Morphometery was done and data was statistically analysed using Student's *t*-test.[16]

RESULTS

The mean body weight and CR length of experimental and control animals are given in Table-1, 2, 3 and Figure-1, 2. Ten mothers of Group B aborted on 18th day of gestation, and these mothers showed abrupt average weight reduction of 50 gms.

Table-1: Body weight gain/loss during gestation in control and treated groups

S. No.	A (Control)				B (Ciprofloxacin)				C (Ciprofloxacin+$ZnCl_2$)				D (Zinc Chloride)			
	GD1 (I)	GD8	GD18 (F)	Wt. G/L	GD1 (I)	GD8	GD18 (F)	Wt. G/L	GD1 (I)	GD8	GD18 (F)	Wt. G/L	GD1 (I)	GD8	GD18 (F)	Wt. G/L
1	153	170	196	42	148	170	184	36	150	170	190	40	149	160	185	36
2	155	174	198	43	149	175	189	40	148	160	185	37	155	165	190	35
3	150	168	180	30	150	185	190	40	150	170	192	42	154	170	205	51
4	151	170	198	47	152	175	185	38	155	175	195	40	150	165	200	50
5	150	172	190	40	150	188	190	40	154	174	196	42	158	170	209	50
6	150	170	190	40	150	180	192	42	150	175	195	45	150	160	188	38
7	150	172	185	35	152	175	188	36	150	170	190	40	150	170	200	50
8	150	175	192	42	149	180	190	41	154	175	195	41	155	172	205	50
9	150	170	194	44	150	185	192	42	148	170	190	42	155	180	208	53
10	150	170	192	42	150	180	189	39	150	175	195	45	150	170	202	52
Mean	150.9	171.1	191.5	40.5±1.52	150	179.3	188.9	38.4±0.9	150.9	171.4	192.3	41.4±0.7	152.6	168.2	199.2	46.5±2.25
SD	1.728	2.131	5.681	4.813	1.247	5.618	2.643	2.96	2.514	4.671	3.529	2.41	3.134	5.97	8.573	7.121
SEM	0.54	0.67	1.79	1.52	0.394	1.776	0.835	0.936	0.795	1.477	1.11	0.76	0.99	1.89	2.711	2.25

Key: Wt. G/L=Weight Gain/Loss, I=Initial, F=Final, GD=gestational day

The body weight of Group-B was reduced compared to controls which was statistically significant ($p<0.001$). However, simultaneous treatment with $ZnCl_2$ prevented the body weight loss in Group-C which is statistically non-significant ($p>0.064$) while in $ZnCl_2$ treated animals Group-D increased the body weight which is statistically highly significant ($p<0.001$).

The mean normal bodyweight in control animals at day 1, day 8 and day 18 was measured 40.5±1.52 gm. While ciprofloxacin treated animals' body weight was reduced to 38.4±0.9 gm ($p<0.001$). However simultaneous treatment with $ZnCl_2$ prevented body weight loss which is statistically non-significant ($p>0.064$). In animals treated with $ZnCl_2$ only (Group-D) the body weight was 46.5±2.25 gm which is statistically highly significant ($p<0.001$).

The mean normal baby weight of control conceptus was measured 5.44±0.12 gm, while in ciprofloxacin treated conceptus the body weight reduced to 4.52±0.10 gm which is statistically highly significant ($p<0.001$). However simultaneous $ZnCl_2$ treated animals maintained the weight, which is statistically non-significant ($p>0.89$). In $ZnCl_2$ treated conceptus increased weight to 6.71±0.05 gm, which is statistically highly significant ($p<0.001$).

The mean normal CR length in control conceptus was measured 3.97±0.07 Cm. In ciprofloxacin treated conceptus CR length reduced to

3.06±0.09 Cm (p<0.001). However simultaneous $ZnCl_2$ maintained the CR length to 3.79±0.13 Cm, which is statistically non-significant (p>0.24). $ZnCl_2$ treatment increased the CR length of the conceptus to 4.15±0.08 Cm (p<0.001).

Table-2: Body Weight of albino rat conceptus in control and treated groups

S#	A (Control) GD18	B (Ciprofloxacin) GD18	C (Ciprofloxacin+$ZnCl_2$) GD18	D ($ZnCl_2$) GD18
1	5.17	4.51	5.65	6.70
2	5.39	4.61	5.30	6.80
3	5.00	4.17	5.45	6.85
4	5.00	4.70	5.95	6.78
5	5.02	4.90	5.00	6.70
6	5.96	4.13	5.10	6.75
7	5.90	4.99	5.90	6.30
8	5.70	4.80	5.35	6.70
9	5.75	4.31	5.60	6.65
10	5.50	4.13	5.30	6.85
n	n=10	n=10	n=10	n=10
Mean	5.44±0.12	4.52±0.10	5.46±0.09	6.71±0.05
SD	0.378	0.325	0.315	0.1583
SEM	0.119	0.10300	0.0996	0.0500

GD=Gestational Day

In Group-B ciprofloxacin treated conceptus reduced body weight which is statistically highly significant (p<0.001). However in Group-C simultaneous $ZnCl_2$ treated animals maintained the weight which is statistically non-significant (p>0.89) while in Group-D treated conceptus increased weight which is statistically highly significant (p<0.001).

Table-3: CR length of albino rat conceptus in control and treated groups

S#	A (Control) GD18	B (Ciprofloxacin) GD18	C (Ciprofloxacin+$ZnCl_2$) GD18	D ($ZnCl_2$) GD18
1	3.8	2.9	4.0	3.8
2	3.9	3.0	4.0	3.9
3	4.0	3.0	4.5	4.0
4	3.8	3.8	3.9	3.8
5	3.8	2.9	3.8	3.8
6	4.5	3.0	4.0	4.5
7	4.2	2.8	3.0	4.2
8	4.0	3.0	3.7	4.0
9	3.9	3.2	3.3	3.9
10	3.8	3.0	3.7	3.8
n	n=10	n=10	n=10	n=10
Mean	3.97±0.07	3.06±0.09	3.79±0.13	4.15±0.08
SD	0.226	0.279	0.4121	0.271
SEM	0.07	0.09	0.13	0.08

In Group-B ciprofloxacin treated conceptus crown rump length reduced which is statistically highly significant (p<0.001). However in Group-C simultaneous $ZnCl_2$ maintained the CR length which is statistically non-significant (p>0.24). In Group-D $ZnCl_2$ treated conceptus crown rump length increased which is statistically highly significant (p<0.001).

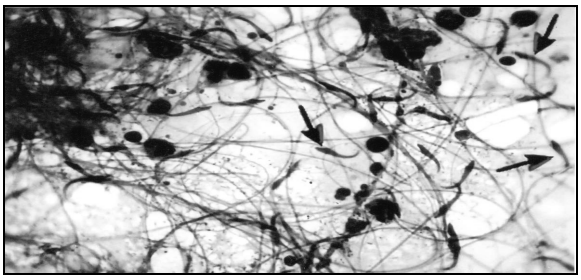

Figure-1: Photomicrograph of staining vaginal smear with H and E which contained spermatozoa confirming mating

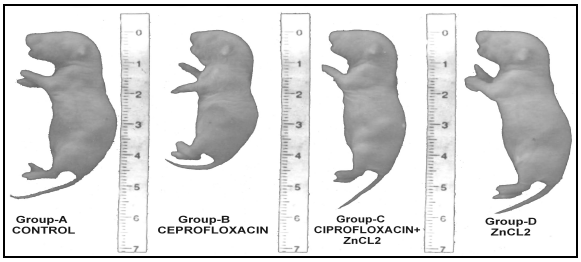

Figure-2: Albino rat conceptus on 18 pre-natal day showing comparison of CR length between control group A and experimental Groups B, C and D used in this study

DISCUSSION

Ciprofloxacin is a broad-spectrum antibiotic active against both gram-positive and gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase IV enzyme necessary to separate bactreial DNA, thereby inhibiting cell division.[17] This mechanism also affects mammalian cell replication. In particular, some cogners of this drug family, for example those that contain the C-8 fluorine[18] display high activity not only against bacterial topoisomerases but also against eukaryotic topoisomerases and are toxic to the cultured mammalian cells and *in vivo* models.[19]

Present study is therefore aimed to determine the effects of ciprofloxacin and $ZnCl_2$ when administered separately and simultaneously during gestation of albino rat the effects on the mother and the conceptus during day 18 are assessed as the measurement of the body weight and CR length, as the parameter.

A reduction in body weight in Group-B mothers, and weight and CR length in conceptus was noted when compared to Group-A mothers and conceptus. The results confirmed that reduction of the body weight and CR length were affected by the adverse effects of ciprofloxacin. These findings are attributed mainly to reduction of body weight and CR length due to less intake of food. Our observations are in consistence with findings of Dowd.[20] Retarded conceptus growth can result in humans to limb length

discrepancy by Ciprofloxacin. Similarly Mehlman,[21] found that complete or partial growth retardation may result in limb length discrepancies.

The statistically insignificant differences in body weight and CR length of the mother and the conceptus was noted when simultaneous administration of $ZnCl_2$ in animals in Group-C when compared with age matched controls of Group-A. These findings confirmed the protective role of $ZnCl_2$. Our observations support those of WHO contributors.[22] Who found that zinc supplements help prevent disease and reduce mortality specially among children with low birth weight or stunted growth.

The highly significant differences are seen in body weight and CR length of mother and conceptus in Group-D when given $ZnCl_2$ only to and compared with age matched control Group-A. These findings reflect the role of the $ZnCl_2$ on the growth of mature and developing living being. WHO contributors,[22] study indicated that $ZnCl_2$ enhanced the children growth prenatally in partially retarding or stuntedly growing children. But our study indicated the body weight and CR length of the conceptus very significantly improved the values of even normal growing pups.

Three mothers aborted on 18th day of gestation. However the abortus was not available and was believed to have been eaten by the rat herself, indicated by telltale blood stains in the cage and grossly reduced dietary intake. Histerotomy confirmed the abortion.

CONCLUSION

Ciprofloxacin retards the conceptus growth and mother body weight. $ZnCl_2$ has definite preventive role in the ciprofloxacin retarding effect on the conceptus. $ZnCl_2$ has a role on the exaggerated growth in length and weight in the conceptus. The effect of the $ZnCl_2$ on the appetite and satiety centres in the hypothalamus need to be further explored.

REFRENCES

1. Gangopadhyay Nibaren, Daniell Mark, Weigh LO Anna, Taylor Heigh R. Fluoroquinlone and fortified antibiotics for treating bacterial corneal ulcers. Br J Ophthalmol 2000;84(4):378–84.
2. Nelson JM, Chiller TM, Powers Jh, Angulo FJ. Fluoroquinolone-resistant compilobacter species and the withdrawal of fluoroquinlones from use in poultry: a public health success story Clin Infect Dis 2007;44(7);977–80.
3. Peterson LR, Lissack LM, Canter K, Fasching CE, Clabots C, Gerding DN. Therapy of lower extremity infections with ciprofloxacin in patient with diabetes mellitus, peripheral vascular disease or both. Am J Med 1987;86:801–8.
4. Katzang BG. fluoroquinlones in: Basic & Clinical Pharmacology 11th Ed. New york: Me Graw Hill 2007;p. 797–801.
5. Gillman GA, Hardman JG, Limbird LE. The quinolones. In: Goodman & Gillman: The pharmacological basis of therapeutics. 10th ed. New York. McGraw Hill 2007;11:1179–83.
6. Grassmick BK, Lehr VJ, Sundareson AS. Fulminant hepatic failure possibly related to ciprofloxacin. Ann Pharmacother 1992;26:636–9.
7. Simpson J, Wasten AR, Mellersh A, Nelson CS, Dodd K. Typhoid fever ciprofloxacin and renal failure. Arch Disc Child 1991;66:1083–4.
8. Greenberg RN, Kennedy DJ, Reilly PM, Luppen KL. Treatment of bones joint and soft tissue infections with oral ciprofloxacin Anti Microb Agent Chemother 1987;31:151–5.
9. Hooper DC, Wolfsons Js, Swartz MN. Mechanism of action and resistance to ciprofloxacin. Am J Med 1987;82(54):12–20.
10. Pfister K, Manzur D, Vormann J, Stahlmann R. Diminshed ciprofloxacine-induced chondrotoxicity by suplimentation with Magnecium and vitamin E in immature rats. Anti Microb Agent and Chemother 2007;51(3)1022–7.
11. Arora NK. Are fluroquinolones safe in children? Indian J Pediatr: 1994;61(6):601–3.
12. Williams SB, Southern LL, Bidner TD. Effects of supplemental dietary phytase and pharmacological concentrations of Zinc on growth performance and tissue Zinc concentration of weanling pigs. J Anim Sci 2005;83:386–92.
13. Rough R. Reproductive system in the mouse. 2nd ed, Minneapolis; Burgess Pub Co. 1968; p. 269–99.
14. Lori EK, Slick KK. Experimental foetal alcohol syndrome proposed pathogenic basis for a variety of associated facial and brain anomalies. Am J Gen Med 1992;44:168–76.
15. Martindale W. The complete drug reference 33rd ed. Sweetman SC London, Chicago Pharmaceutical Press 2002;182–5.
16. Bland M. Introductioin of medical statistics 1st ed. Oxford, Oxford University Press 1987;p.165–87.
17. Drlica K, Zhao XK. DNA gyase, topoisomerase IV and 4-quinolones. Microbiol Mol Biol Rev 1997;61(3):377–92.
18. Robinson MJ, Mortin BA, Gootz TD, McGuirk PR, Osheroff N. Effects of Novel fluoroquinolones on the catalytic activities of eukaryotic topoisomerase II: Influnce of the C-8 fluorine group. Anti Microb Agent Chemother 1992;36(4):751–6.
19. Sissi C, Palumbo MC. The quinolone family: from antibacterial to anticancer agents. Curr Med Chem Anticancer Agents 2003;3(6):439–50.
20. Dowd RO, Kent JC, Moseley JM, Wlodek ME. Effects of uteroplacental insufficiency and reducing litter size on maternal mammary function and post natal offspring growth. Am J Physiol 2007;39:101–21.
21. Mehlman CT, Methew E, Koeppling DO. Growth plate (physeal) fractures: Treatment 2010. Available at: http://www.emedicine.medscape.com
22. WHO contributors. The impact of zinc supplemention on childhood mortality and severe morbidity. World Health Organization 2007. Available at: http:/www.who.int/child-adolesecent-health/zinc-mortality.html.

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