Original article

Evaluation of the teratogenic potentials of ciprofloxacin in albino rat

Siddiqui, MA.[1]* and Naqvi, SNH.[2]

[1]Hamdard College of Medicine and Dentistry, Hamdard University, Karachi, Pakistan
[2]Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan
*E-mail: draslam72@yahoo.com

Abstract

The safe use of medicines, both modern as well as traditional, during gestation is becoming an increasingly contentious issue because many therapeutic agents have proved to be menace as they produce congenital malformations in offspring when used during pregnancy. Objective: The present study was undertaken to investigate the effects of ciprofloxacin administration during gestation on young ones of albino rat. Material and Methods: Three hundred off springs of albino rat were used in this study. They were obtained from 60 pregnant rats at term or at 20th day of gestation. The pregnant rats were divided in four groups. Group A received ciprofloxacin in a dose of 15 mg.kg^{-1}/day orally from 6th to 12th days of gestation. Groups B and C received 30 and 60 mg.kg^{-1}/day orally for same period. Group D behaved as control and received 0.5 ml of distilled water orally for same period. The pregnant rats were observed for duration of pregnancy, weight gain, abortions and number of pups given birth. The obtained pups were examined for any structural malformations. Results: The weight gain of ciprofloxacin treated pregnant rats showed a significant decrease of 12.10, 17.91 and 24.87% as compared to control group animals. The incidence of abortion in treated animals was 7.1 and 9.1% as compared to none in controls. Mean number of pups delivered by treated animals (showed a highly significant decrease of 14.4, 21.6 and 27.7% as compared to controls. Mean weight of pups from treated animals showed a highly significant decrease of 16.12, 24.75 and 35.68% as compared to controls. Mean CRL of pups from treated animals showed a highly significant decrease of 17.42, 24.63 and 28.5% as compared to controls There were no resorptions in control group. Treated group A, B and C had incidences of 8.7, 30.6 and 36.4% resorptions respectively. No gross structural malformation was found in control or any of the treated groups. Conclusion: Ciprofloxacin was found to be embryo/feto toxic in terms of causing abortions, reduced no: of litter, retarded growth of young ones, fetal death and fetal resorptions in all doses tested.

Keywords: ciprofloxacin, pregnancy, congenital malformation.

1 Introduction

Almost all therapeutic agents cross placental barrier (SCHLEGEL, CHANG and MARSHALL, 1991) and enter fetal circulation. Every agent given during pregnancy therefore has a tendency to produce some sort of structural abnormality in the neonate at birth until proved otherwise.

The safe use of medicines, both modern as well as traditional, during gestation therefore, is becoming an increasingly contentious issue. On one hand regulatory authorities appear to be adopting the policy that if there is no clear evidence of safety from experimental and clinical trials then a drug or a herb should not be recommended during pregnancy. On the other hand medical practitioners in our society are using drugs and herbs as frequently during pregnancy as without it.

A birth defect or a congenital malformation is a structural abnormality of any type present at birth. It may be macroscopic or microscopic, on the surface or within the body (MOORE, 1988).

Major structural anomalies occur in 2-3% of live born infants, and an additional 2-3% is recognized in children by age 5 years making a total of 5-6% (SADLER, 2000).

The most vulnerable period for malformation to take place is the period of organogenesis. Agents given during this period are more likely to cause birth defects. This critical time of fetal development in rats and mice is from 6-12 days of their gestation (SOMER, 1962; FARRIS, 1967).

Ciprofloxacin is the prototype broad spectrum flouroquinolone antibacterial agent. Flouroquinolones are the fluorinated derivatives of quinolones, which belong to a chemical class of 4- aminoquinolones that all contain a carboxylic moiety in third position of their basic ring structure. The original fluroquinolone is norfloxacin; others in the group include ciprofloxacin, ofloxacin, clinofloxacin, ternofloxacin, levofloxacin lomefloxacin and sparfloxacin. All are entirely synthetic (SCHULD, SMALL and HARRIS, 2001).

1.1 Purpose of study

Flouroquinolones are extensively used in our society for treatment of various infections. There is every likelihood that these may be used during pregnancy intentionally or unintentionally. There is, therefore, an urgent need for evaluation of its safety when used during pregnancy.

Many reproduction studies have been performed in rats, mice and rabbits using oral doses of up to 100 mg.kg^{-1} of ciprofloxacin with conflicting results. There is no clear evidence that its use during gestation is safe.

1.2 The present study was therefore designed

To study and compare the effects of ciprofloxacin administration during gestation on offspring of albino rats in terms of induced abortion, reduction in litter size, reduction in weights and/or length of fetuses and pups, resorptions and any gross malformation.

2 Material and methods

About 360 off-springs (fetuses and pups) of albino rat were used in the present study. To obtain these off-springs of albino rat, 60 normal healthy female albino rats 12 weeks of age were taken in phases from the animal house of HEJ Institute of Chemistry Karachi University. They were mated with normal healthy male albino rats allowing one male for two female rats in one cage (MACINTYRE, CHANG and KAUFMAN, 1995). Presence of a vaginal plug the following morning was taken as evidence of mating and was counted as day 1 of pregnancy. Normal delivery in albino rat takes place on day 21-23 (ROUGH, 1968).

The pregnant rats were divided in four groups:

- Group A contained 10 pregnant rats. They received ciprofloxacin orally in a dose of 15 mg.kg^{-1}/day from 6th to 12th days of gestation.
- Group B contained 10 pregnant rats. They received ciprofloxacin orally in a dose of 30 mg.kg^{-1}/day from 6th to 12th days of gestation.
- Group C contained 10 pregnant rats. They received ciprofloxacin orally in a dose of 60 mg.kg^{-1}/day from 6th to 12th days of gestation.
- Group D contained 10 pregnant rats, behaved as control group and received 0.5 mL of distilled water orally for the same period.

The pregnant rats were observed for duration of pregnancy, weight gain, abortions and number of pups given birth. The obtained pups were weighed and their crown rump length noted. They were then examined under dissecting microscope for any structural malformations. Subsequently they were processed in the lab: for measurements of bones.

2.1 Statistical analysis

In the present study the data was subjected to students 't' test. By this test the statistical significance of the difference between two means of various parameters between control and experimental groups was evaluated.

The P value was found by means of 't' distribution table and was read against the degree of freedom i.e n1 + n2 – 2 . A p value less than 0.05 was considered significant.

3 Results (Tables 1-8)

The weight gain of ciprofloxacin treated pregnant rats showed a significant decrease of 12.10, 17.91 and 24.87% as compared to control group animals. About 80.0% of ciprofloxacin treated pregnant rats reached full term

Table 1. Comparison of mean maternal body weight (gm) gain/loss between gestational day 1 and 20 in different group of rats.

S. N°.	Groups	Maternal body weights		
		Gestational day-1	Gestational day-20	Weight gain/loss
01	A	175.4 ± 3.961	228.4 ± 5.044	53.0 ± 1.173
02	B	171.6 ± 7.841	220.5 ± 8.567	49.5 ± 1.147
03	C	189.0 ± 4.197	234.3 ± 3.614	45.3 ± 0.843
10	Control "D"	183.5 ± 5.155	243.8 ± 4.627	60.3 ± 1.044

* Mean + standard error

Table 2. Fate of pregnancy in rats.

Groups		Animals				
		Total used	Non pregnant		Pregnant	
			Pseudopregnent (%)	Infertile (%)	Abortion (%)	Full term (%)
D Control	-	22	2 (9.1)	0	0	20 (90.9)
A Ciprofloxacin	Dose I	10	1 (10)	1 (10)	0	8 (80)
B Ciprofloxacin	Dose II	11	2 (18.1)	0	1 (9.1)	8 (72.8)
C Ciprofloxacin	Dose III	14	1 (7.1)	2 (14.2)	1 (7.1)	10 (71.4)

Table 3. Average number of fetuses and pups obtained at birth.

Groups	Dose	Average number	Gain/loss as compared to control
A Ciprofloxacin	I	7.1 ± 0.54	14.40%
B Ciprofloxacin	II	6.50 ± 0.26	21.60%
C Ciprofloxacin	III	6.0 ± 0.21	27.70%
D Control	-	8.3 ± 0.36	-

* Mean + standard error.

Table 4. Comparison between average weight (gm) of pups in different experimental groups at birth.

Groups	Dose	Average weight	Gain/loss as compared to control
A Ciprofloxacin	I	4.97 ± 0.124	16.12%
B Ciprofloxacin	II	4.46 ± 0.112	24.75%
C Ciprofloxacin	III	3.81 ± 0.086	35.68%
D Control	-	5.93 ± 0.067	-

* Mean + standard error.

Table 5. Comparison between average crown rump length (mm) of pups in different experimental groups at birth.

Groups	Dose	Average crl	Gain/loss as compared to control
A Ciprofloxacin	I	37.4 ± 0.100	17.42%
B Ciprofloxacin	II	34.1 ± 0.092	24.63%
C Ciprofloxacin	III	32.4 ± 0.115	28.50%
D Control	-	45.3 ± 0.076	-

***** Mean + standard error.

Table 6. Detail of foetuses and pups of rats

Group	Animals	Foetuses				Resorption	Total	Foetal
		Alive (%)	Dead (%)	Premature (%)	Total	(%)	implantation	mortality (%)
Control Group D	30	250 (100)	0	0	250	0	250	0
Ciprofloxacin A I	10	71 (97.2)	0	2 (2.7)	73	2 (2.3)	84	3.5
Ciprofloxacin B II	10	65 (95.5)	1 (1.4)	2 (2.9)	68	5 (5.7)	87	8.0
Ciprofloxacin C III	10	60 (88.2)	3 (4.4)	5 (7.3)	68	15 (15.3)	98	18.3

Table 7. Comparison between mean weight (gm) of placenta and liver of pups in different experimental groups.

Groups	Dose	Average weight of placenta	Average weight of liver
A Ciprofloxacin	I	0.52 ± 0.016	0.49 ± 0.013
B Ciprofloxacin	II	0.50 ± 0.018	0.47 ± 0.013
C Ciprofloxacin	III	0.42 ± 0.012	0.40 ± 0.016
D Control	-	0.61 ± 0.007	0.58 ± 0.014

***** Mean + standard error.

Table 8. Comparison of mean intact bone length (mm) in control and treated albino rat pups.

Groups	Dose	Humerus	Ulna	Femur	Tibia
A Ciprofloxacin	I	4.90 ± 0.076	4.93 ± 0.047	4.75 ± 0.039	4.80 ± 0.055
B Ciprofloxacin	II	4.57 ± 0.037	4.53 ± 0.044	4.51 ± 0.046	4.55 ± 0.042
C Ciprofloxacin	III	4.13 ± 0.056	4.11 ± 0.062	4.05 ± 0.059	4.09 ± 0.067
D Control	-	6.54 ± 0.076	6.60 ± 0.074	6.19 ± 0.079	6.30 ± 0.087

***** Mean + standard error.

pregnancy as compared to 90% in controls. The incidence of abortion in treated animals was 7.1 and 9.1% as compared to none in controls. Mean number of pups delivered by treated animals (7.1 ± 0.27, 6.50 ± 0.26, 6.0 ± 0.21) showed a highly significant decrease of 14.4, 21.6 and 27.7% as compared to controls (8.3 ± 0.36). Mean weight of pups from treated animals (4.97 ± 0.124, 4.46 ± 0.112, 3.81 ± 0.086 gm) showed a highly significant decrease of 16.12, 24.75 and 35.68% as compared to controls (5.93 ± 0.067 gm). Mean CRL of pups from treated animals (37.4 ± 0.100, 34.1 ± 0.092, 32.4 ± 0.115 mm) showed a highly significant decrease of 17.42, 24.63 and 28.5% as compared to controls (45.93 ± 0.076 mm). There were no resorptions in control group. Treated group A, B and C had incidences of 8.7, 30.6 and 36.4% resorptions respectively. No gross structural malformation was found in control or any of the treated groups.

4 Discussion

Approximately 3% of live born infants have defects at birth (DUDEK, 2001). Additional anomalies can be detected after birth so the incidence reaches about 6% in 2 year olds and 8% in 5 year olds (MOORE and PERSAUD, 2003).

It has been estimated that 25% of congenital malformations are due to genetic and chromosomal abnormalities, 10% due to environmental causes including drugs and 65% of unknown etiology (RUBIN, 1995).

About 2-3% of all birth defects result from use of drugs (Merck and Company, 2003).

Ciprofloxacin was used in our study in doses of 15, 30 and 60 $mg.kg^{-1}$ bodyweight in rats. Numerous reproduction studies have been carried out to see the teratogenic effects of ciprofloxacin. There is no direct evidence of this drug having a tertogenic effect on human embryos. Data on ciprofloxacin use during pregnancy from the Teratogen Information System indicate that therapeutic doses during pregnancy are unlikely to pose a substantial teratogenic risk, but data are insufficient to determine that there is no risk (FRIEDMAN and POLIFKA, 2000).

One publication described six pregnant women exposed to longer durations of ciprofloxacin therapy (3 weeks to 3 months) who delivered normal babies (BOMFORD, LEDGER, O´KEEFFE et al., 1993).

Another prospective follow-up study reported on 549 pregnancies with fluoroquinolone exposure (93% first trimester exposures). There were 70 ciprofloxacin exposures,

all within the first trimester and showed that the rate of congenital malformations in live-born children exposed during the first trimester was 4.7%. No specific patterns of congenital abnormalities were found. The study did not reveal any clear adverse reactions due to in utero exposure to ciprofloxacin (SCHAEFER, AMOURA-ELEFANT, VIAL et al., 1996).

In a company-sponsored prospective registry of 116 human pregnancies, 54% were exposed during the 1st trimester and resulted in live births. Of these, six were malformed (SCHAEFER, AMOURA-ELEFANT, VIAL et al., 1996).

Nahum, Uhl, Kennedy et al. (2006) put ciprofloxacin in category C as far the relative risk of human teratogenesis by drugs used in pregnancy is concerned. FDA Pregnancy Category C means that there isn't sufficient information or there are concerns from animal studies but no confirmation of birth defects in humans (GREENFIELD, 2004).

The safety and effectiveness of ciprofloxacin in pregnant and lactating women have not been established (NET DOCTOR, 2007). Flouroquinolones are not recommended for use in pregnancy because of their effects on growing cartilage (KATZUNG and TREVOR, 1998).

In our study 80% of the animals receiving 15 $mg.kg^{-1}$ ciprofloxacin went to full term pregnancy while those receiving 30 and 60 $mg.kg^{-1}$ doses had an incidence of full term pregnancy reduced to 72.8 and 71.4% respectively. The incidence in control group was 90.9%.

The incidence of abortion in group A-1 (cipro dose I) and control group D was 0. Where as the same in group A-2 and A-3 was 9.1 and 7.1%. Similarly the incidence of resorption in group A-1 and control group D was 0 while in groups A-2 and A-3 it was 30 and 36%.

The last two doses (dose II and III) seem to be more toxic to embryos in terms of abortion and resorption. In this regard our findings are in contradiction to Loebstein, Addis, Ho et al. (1998) who reported no difference in rate of spontaneous abortions in women exposed to ciprofloxacin. But their study refers to human exposure while we report our findings in rat.

The incidence of prematurity and dead fetuses with 30 and 60 mg dose was 2.7 and 2.9% and 0 and 1.4% respectively in our study which again is unlike the report of Loebstein, Addis, Ho et al. (1998) who mentioned no difference in the rate of prematurity or intrauterine death in humans.

Thus ciprofloxacin proved to be toxic to embryos/fetuses by its effect of reducing the mean number and average weight of the young ones at birth. The reduction in number may be due to prematurity and number of resorptions while decrease in weight due to stunted growth. These findings in our study are in contradiction to the findings of Bomford, Ledger, O'Keeffe et al. (1993), Koul, Wani, Wahid (1994), Schaefer, Amoura-Elefant, Vial et al. (1996), Wilton, Pearce and Mann (1996), Ludlam, Wreghitt, Thornton et al. (1997), Loebstein, Addis, Ho et al. (1998), Friedman and Polifka (2000), Greenfield (2004), Nahum, Uhl, Kennedy et al. (2006) and Net Doctor (2007), who all have reported against the observation of any adverse teratogenic effects in women exposed to ciprofloxacin during pregnancy. Majority of the exposures reported were during first trimester of pregnancy. Some of them e.g. Schaefer, Amoura-Elefant, Vial et al. (1996) did mention a 4.7% incidence of congenital malformations in live-born children exposed to ciprofloxacin during the first trimester. However no specific patterns of congenital abnormalities were found. In another study Shaefer reports birth of 6 malformed children out of 116 human pregnancies, 54% of which were exposed during the 1st trimester and resulted in live births.

Even more pronounced embryo/feto toxic effect of ciprofloxacin was observed on skeletal growth as evidenced by decrease in crown rump length (CRL) and decrease of intact bone length in long bones of extremities.

There were no observable external or internal (visceral) structural malformations seen with all doses of ciprofloxacin. However the weight of liver and placenta of pups exposed to ciprofloxacin during gestation was significantly lower than those of pups obtained from control group D animals.

5 Conclusion

In conclusion we report that ciprofloxacin proved to be fetotoxic/ embryotoxic when administered during gestation to female albino rats.

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Received January 5, 2010
Accepted June 15, 2010