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A Survey of Ofloxacin Histopathological Effect on Fetus Rat Heart

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Abstract

Objective: Ofloxacin is an antibiotic of the fluoroquinolone group consisting of broad-spectrum antibiotics widely used in various infectious diseases. Nearly 600 teratogenic factors are known that cause congenital disease in laboratory animals. One of these factors is drugs. The aim of this study was to determine the effect of ofloxacin on the development of fetus rat heart.

Materials and Methods: In this study, 4-month-old Wistar rats with 300 gram weight were used and were housed in an environmentally controlled room. A group of 3 females were caged with a single male of proven fertility overnight. Finding of vaginal plug on the following morning was regarded as a gestational day 0. Pregnant rats were divided into 2 groups (control and experimental). The first were fed with rodent food and the second with rodent food plus 50 mg/kg ofloxacin every day. After collection of tissue specimen from rat newborns the heart was dissected and prepared for light microscopy.

Results: The results showed that in the group receiving ofloxacin, in comparison with the control group, myocardial cells were smaller and contain highly dense nuclei.

Conclusion: In conclusion, the results show that the above mentioned drug could be transferred through placenta and affect the normal development of myocardial cells. These changes could have negative effects on the function of the heart after birth.

Keywords: Histopathologic, Ofloxacin, Teratogen

Introduction

Experimental teratology was performed for the first time in the nineteenth century on non-mammalian species. It is clear that many environmental factors (temperature, microbial toxins, and drugs) cause growth and evolution disorders in birds, fish, and amphibians. Almost 10% of birth defects are related to genetic mutation, 5% to chromosomal changes, and less than 3% are related to teratogenicity. To this date, about 600 teratogenic factors have been identified which have caused birth defects in laboratory animals, of which drugs could be mentioned (1-3). Ofloxacin is one of these drugs. This antibiotic medicine is of the fluoroquinolone group. This group of drugs consists of broad-spectrum antibiotics that are prescribed to treat bacterial infections. These drugs are used to treat certain infections of the respiratory tract, genitourinary tract, gastrointestinal tract, skin, soft tissues, and etcetera. Most current researches have mentioned the convenience of taking antibiotics during pregnancy. However, the use of quinolones should be avoided during this period. The mechanism of action of this class of drugs is through the inhibition of DNA gyraze bacterial enzyme. This enzyme is necessary for bacterial DNA replication and other aspects of transcription, replacement, and repair (4,5). Takayama et al. concluded in their study that female rats that received 810 mg/kg of ofloxacin showed an increase in salivation, decreased body weight, and increased mortality and bone problems at higher doses (6). Another report indicated that oral administration of norfloxacin can cause bone problems in young animals (7).

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Materials and Methods

In this study, 4-month-old Wistar rats weighing 300 g were used. The animals were kept in 23 to 25 °C, 50 % humidity, and light cycle of 12 hours light and 12 hours dark. For mating, 3 female rats and 1 male rat were kept for one night in a cage. Viewing vaginal plug the following morning was considered as gestational day zero. Then, 20 pregnant female rats were placed in the control group and were only given rodents' food (typical compact food purchased from the Pars Lame Inc.) during pregnancy until birth. The 20 other female pregnant rats were placed in the experimental group. During pregnancy until birth, in addition to rodent's food, they were administered ofloxacin with a dosage of 50 mg/kg which was added to their food in powder form.

Dose of the drug was determined according to information obtained from a previous study on laboratory rats (6). The drug used was manufactured by Iran's chemical drug company. After completion of pregnancy, the neonates were immediately weighted, and were studied under loop for anatomical abnormalities. Then, from their hearts 4 mm samples were taken and placed in containers with 10% buffered formalin. After fixation, tissue processing was performed on the samples, which included the following steps: A- Dewatering; B- Transparency; C- Imbued with paraffin; and D- Molding.

The tissue blocks were sectioned using a rotary microtome with a thickness of 5 microns. Then, the sections were floated gently in a water bath of 45 °C until the sections were smoothed. The sections were placed on slides that had been previously defatted and smeared with albumin glue. Then, the tissues were stained with hematoxylin and eosin (H & E). For imaging, Olympus microscope and Konica 400 ASA film were used. For statistical analysis of quantitative data, t-test was used. The significance level was considered 0.05.

Results

Anatomic study of neonates did not show any anomalies in various organs. The mean birth weight of the control group was 6.3 + 0.37 g and the mean birth weight of neonates of the experimental group was 6.06 + 0.48 g. Statistical analysis showed that the weight difference between experimental and control groups was significant ($P < 0.03$). Results showed that myocardial cell nuclei of rat neonates of the experimental group were dense and dark, and smaller in size than the control group. Due to the increased density of nuclei, nucleoli were not visible and the overall reduction in size of the cells resulted in the empty spaces in the intercellular spaces.

Figure 1 shows the heart muscle of a neonatal rat in the control group, in which the muscle cells in longitudinal (L) and transverse (C) sections can be seen. Figure 2 shows the heart muscle of a neonatal rat in the control group with larger magnification. As can be seen in the picture, the nucleus is large and clear and has one or two nucleoli. Figure 3 shows the heart muscle of a neonatal rat in the experimental group. As can be seen, the nucleus is dense and dark and smaller than the control group.

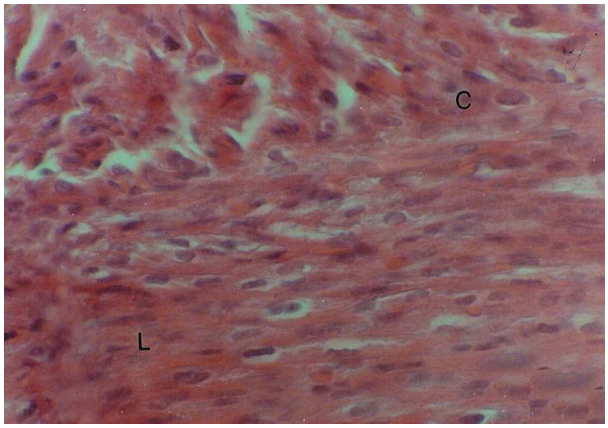

Figure 1. Sectional photomicrography of fetal rat heart in the control group, pay attention to the muscle fibers in the longitudinal (L) and transverse (C) form (H & E staining, magnification 260 times)

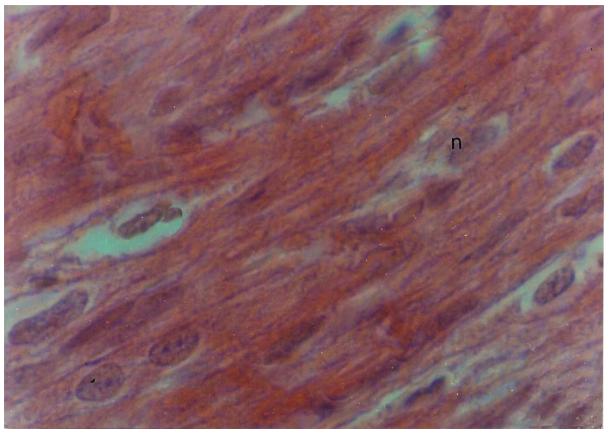

Figure 2. Sectional photomicrography of fetal rat heart in the control group, pay attention to the shape of nuclear (N) and nucleolus within it and the cellular arrangement (H & E staining, magnification 650 times)

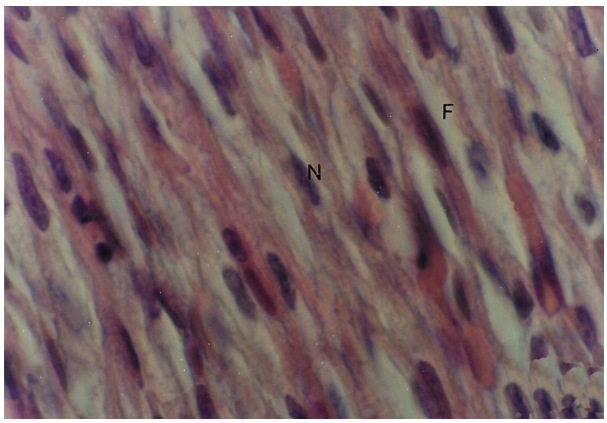

Figure 3. Sectional photomicrography of fetal rat heart in the experiment group, pay attention to the shape of nuclear (N) and the large intercellular spaces (F) (H & E staining, magnification 650 times)

Ofloxacin is an antibiotic medicine of the fluoroquinolone group. The mechanism of action of this

drug is through the inhibition of DNA gyraze bacterial enzyme. This enzyme is necessary for the bacterial DNA replication, transcription, and repair. This mechanism could be a reason for the highly teratogenic property of the mentioned drug. Another report indicated that oral administration of ofloxacin can cause bone problems in young animals (7). Loebstein et al. in their investigation in 1998 concluded that children who were exposed to fluoroquinolones in the womb did not have a clinically significant musculoskeletal disorder (8). It was also shown in this study that the risk of miscarriage during pregnancy in women exposed to quinolones had increased (8). The ability of the drug to cross the placenta was also shown in previous studies. These findings are in agreement with that of the present study. In a study it was shown that in rats that had received levofloxacin at doses higher than DR-3355 increased salivation, decreased number of neutrophils, cecum expansion, and increase in goblet cells present in the mucosa cecum were observed (9).

Takayama et al. concluded 810 mg/kg of ofloxacin tend to an increase in salivation, decrease in body weight, and increase in mortality and bone problems at higher doses (6). Weight measurement of rat neonates in the experimental and control groups showed that the weight of neonates who were exposed to ofloxacin during the study had significantly reduced compared to the weight of the control group ($P < 0.03$).

Conclusion

These findings indicated intrauterine growth retardation due to the mentioned drug and showed that overall embryonic growth and activity of cells in all organs were reduced and ultimately lead to weight loss at birth and pathological changes. Based on the present study, norfloxacin reduced nuclear volume and myocardial cells. Therefore, it can be concluded that the use of this drug during pregnancy due to the disorders it can cause should be used with absolute caution, with a doctor's prescription, and only when necessary.

Ethical issues

We have no ethical issues to declare.

Conflict of interests

We declare that we have no conflict of interests.

Acknowledgments

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