

Reproductive and Developmental Effects of Moxifloxacin on Female Mice and Embryos

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Abstract

Moxifloxacin (Avelox®) is a fluoroquinolone antibiotic with a broad spectrum of activity and bactericidal action. Moxifloxacin has *in vitro* activity against a wide range of Gram-positive and Gram-negative organisms. The safe use of moxifloxacin in human pregnancy has not been established. In order to evaluate the genotoxic and embryo toxic effects of (Avelox)® during pregnancy, Avelox was administered orally to female mice with doses (8.7, 17 and 26 mg/kg/day) from 1 to 17 days of pregnancy. Caesarean sections were completed on gestation day 18 and complete fetal examinations and cytogenetic analysis were conducted. Decreases in the fetal body weights and increases in the external visceral and skeletal anomalies were found in all doses of (8.7, 17 and 26 mg) Avelox compared to the controls. Cytogenetic analysis in mothers and embryos revealed that all the tests doses produced chromosomal aberrations and micronuclei (MN) formations in a dose dependent manner compared to the controls. These results indicate that Avelox has a maternal and embryotoxic effects on the female mice and their embryos when administered with a recommended and above the recommended dose during pregnancy.

Key words: Moxifloxacin - chromosomal aberrations – micronuclei – mice – embryos

Introduction

Antibiotics are medicines designed to treat bacterial infections. A new fluoroquinolone antibacterial for the treatment of respiratory tract infections is known as Avelox® (Moxifloxacin). Moxifloxacin works Reliably against all relevant respiratory pathogens and is characterized by its rapid efficacy, excellent tolerability and high cure rates. Patients begin to feel much better soon after starting therapy. Moreover, clinical trials involving more than 8.000 patients to date indicate that Avelox has a low propensity for the development of bacterial resistance (Minenko *et al.*, 2004). Moxifloxacin inhibit the functionally related mammalian to topoisomerase II (Tomas *et al.*, 2004). As can sequence of these effects Fluoroquinolones have shown genotoxic effects in pro-and eukaryotic systems. However, it has to be taken into account that several orders of magnitude higher concentrations as for gyrase are normally needed for topoisomerase-11-inhibition (Drago *et al.*, 2004).

Based on their specific mode of action, enzyme, inhibition, and in contrast to chemical mutagens which directly interact with DNA, no-effect levels can be defined for Fluoroquinolones which act by enzyme inhibition if no enzyme inhibition take place no genotoxic effect can be induced (Mah, 2004).

The safe use of Moxifloxacin in human pregnancy has not been established. Therefore, we study the effect of Avelox® (Moxifloxacin) on the pregnant females and embryos (*in vivo*) if given orally to mice during the pregnancy.

Materials and Methods

Test drug:

Moxifloxacin (Avelox) was provided from Bayer Laboratories (Moxifloxacin hydrochloride) is a synthetic broad spectrum antibacterial agent) and is available as Avelox Tablets for oral administration and as Avelox I.V. for Intravenous administration.

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Moxifloxacin, a fluoroquinolone, is available as the monohydrochloride salt of 1-cyclopropyl-7-[s,s]-2,8-diazabicyclo[3.3.0]non-8-yl]-6-fluoro- slightly yellow to yellow crystalline substance with a molecular weight of 437.9. Its empirical formula is $C_{21}H_{24}FN_3O_4$.

Animals and Treatments:

Young adult male and virgin female mice of about 8-12 weeks of age were used and housed five or six per cage in a 12h light: 12h dark cycle with food and drinking water available ad libitum.

The recommended dose for Moxifloxacin is 400mg once daily. 250 ml solution contain 336.8 mg Moxifloxacin hydrochloride, equivalent to 400 mg Moxifloxacin. The adjustment of dosage is required for mice. Three groups of pregnant females were treated orally with 0.5 ml of Avelox, equivalent to 8.7, 17 and 26 mg/kg/day Moxifloxacin. Females were mated with males 3:1 per cage the day on which the vaginal plug appears positive was considered 1 day of pregnancy. Immediately on 1 day of pregnancy females were treated with three doses of Moxifloxacin the lowest dose 8.7 mg/kg used correspond to the recommended dose of human and the two other doses 17 and 26 mg/kg correspond to 2 and 3 times the recommended dose respectively. Control females were treated with 0.5 ml/kg of saline. The treatments of Moxifloxacin with doses of (8.7, 17 and 26 mg) once daily from days 1 to 17 of gestation. The general behaviour of females was observed every day. All females were sacrificed on day 18 of gestation. The uterus was opened and the number of live fetuses and dead fetuses were recorded. Live fetuses were examined for external malformations. Part of fetuses from each female were preserved in 95% ethanol for subsequent skeletal malformations and developmental toxicity. The remaining fetuses were used for cytogenetic analysis (micronucleus assay and chromosomal aberrations).

Micronucleus assay: (in bone marrow cells):

The animals were sacrificed by cervical dislocation. For each treatment 3-4

animals were used in different groups. Bone marrow smears and staining were done following the method of (Schmid 1976) with slight modifications. Briefly, both the femora were removed and the bone marrow was flushed out into a centrifuge tube with 1% sodium citrate solution from syringe. The bone marrow cells were dispersed by gentle pipetting and collected by centrifugation at 1000 rpm for 5min at 4°C. The cell pellet was resuspended in a small volume of 5% fetal calf serum. Drop of this suspension was smeared on a clean slide, air-dried, fixed in absolute methanol for 15 min and stained the following day with Giemsa stain. One thousand of polychromatic erythrocytes (PCEs immature erythrocytes) normochromatic erythrocytes (NCEs, mature erythrocytes) and immature white cells (reddish blue) were analysed for the presence of micronuclei (MN).

Micronucleus assay: (in embryonic cells):

On day 18 of gestation embryos were taken and a small volume of blood from the embryo tail was resuspended in a small volume of 5% fetal calf serum. A drop of this suspension was smeared on a clean slide, air dried, fixed in absolute methanol for 15 min and stained with Giemsa stain. The frequency of micronuclei in embryos were analyzed according to the method of (Schmid 1976).

Chromosomal preparation (in bone marrow):

Animals were injected i.p. with 0.2 ml colchicine 90 min before sacrifice, which occurred 24h after treatment. Bone marrow preparations for the chromosome aberrations in metaphase cells were obtained by the technique of Yosida *et al* (1971) only well-spread metaphases were analyzed in order to determine the frequencies of chromosomal aberrations. 50-spread metaphases per animal were analyzed.

Chromosomal preparation (in embryos):

On day 18 of gestation embryos were taken and minced in 5ml T.C.M. media and centrifuged at 1000 r.p.m. for 5

min. 2mM of colchicines was added to each tube. After 90 min tubes were taken and centrifuged for 5min. Embryonic cell preparations for the chromosome aberrations in metaphase cells were obtained by the technique of (Romagnano *et al* 1986). Spread metaphases were analyzed in order to determine the types of chromosomal aberrations. 50-spread metaphases per embryo were analyzed.

Statistical Analysis:

The incidences of resorption, skeletal variation, delayed ossification of fetuses between experimental and control values were calculated non-parametrically using wilcoxon's rank sum test (Siegal, 1956).

The data of chromosomal aberrations in the females and embryos were subjected to analysis of variance (ANOVA) according to snedecor and Cochran (1990). Least significant differences were used compare between means according to Waller and Duncan (1969) at probability 5%. The data

of micronucleus tests were expressed as percentage.

Results

Reproductive and foetal effects:

There were treatment-related effects on the number of implantations, the number of the live fetuses and the number of dead fetuses (Table 1). There was a significant dose- dependent decreases in the average fetal body weights compared to controls. There was an increase in the incidence of fetuses with external malformations between treated groups compared with the control (Table 2). Among the malformations increased in the treated groups agnothia, omphalocele and palatoschisis. The skeletal anomalies are shown in (Table 2). There was a significant dose-dependant increase in the skeletal anomalies between treated groups (8.7, 17 and 26) respectively and control. The major skeletal anomalies observed is a verterbral anomalies and none of the fetuses showed any major skeletal malformations. However, visceral

Table (1): Reproductive and foetal effects.

| Parameters | Daily dose of Avelox (mg/kg/b.w.) | | | |
|-------------------------|-----------------------------------|-----------|----------|----------|
| | Control | 8.7 mg/kg | 17 mg/kg | 26 mg/kg |
| No. of females | 20 | 20 | 20 | 20 |
| No. of pregnant females | 20 | 20 | 20 | 19 |
| No. of implantations | 220 | 215 | 212 | 198 |
| No. of live fetuses | 215 | 208 | 203 | 188 |
| % | 97.7% | 96.7% | 95.8% | 94.9% |
| No. of dead fetuses | 5 | 7 | 9 | 10 |
| % | 2.2% | 3.2% | 4.2% | 5% |
| Mean fetal weight (g) | 3.9 | 3.8 | 3.7 | 3.5 |

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Table (2): Incidence of fetal malformations and Anomalies.

| Parameters | Daily dose of Avelox (mg/kg/b.w.) | | | |
|------------------------|-----------------------------------|-----------|----------|----------|
| | Control | 8.7 mg/kg | 17 mg/kg | 26 mg/kg |
| External malformation | 1/107 | 3/104 | 4/102 | 5/94 |
| Affected fetuses | | | | |
| Agnothia | 0 | 1 | 2 | 2 |
| Omphalocele | - | - | 1 | 2 |
| Palatoschisis | 1 | 2 | 1 | 1 |
| Skeletal anomalies | 5/107 | 11/104 | 14/102 | 13/94 |
| Affected fetuses | | | | |
| Extra ribs | 1 | 4 | 3 | 3 |
| Wavy ribs | 1 | 3 | 3 | 0 |
| Vertebral-anomalies | 3 | 4 | 8 | 10 |
| Skeletal malformations | 0/107 | 0/104 | 2/102 | 3/94 |
| Affected fetuses | | | | |
| Ribs fused | 0 | 0 | 1 | 1 |
| Ribs missing | 0 | 1 | 1 | 2 |
| Visceral anomalies | 2/107 | 4/104 | 6/102 | 8/94 |
| Affected fetuses | | | | |
| Abnormal ovary | 0 | 0 | 3 | 4 |
| Abnormal tests | 0 | 0 | 1 | 4 |
| Enlarged ureter | 2 | 4 | 2 | 0 |

anomalies are shown in Table (2). There was an increase in the visceral anomalies between treated groups especially Enlarged Urter. All these malformation were considered to be treatment related.

Micronucleus test:

The total number of micronuclei and the distribution of NCE in treated females and their embryos are given in table (3). Generally, in treated females and embryos with (8.7, 17 and 26 mg) Avelox, an increase in the total number of micronuclei in the cells compared to the control was observed and these increases were higher in the treated female groups than in the treated embryo groups and theses increases were dose-dependent.

Chromosomal aberrations: (females and their embryos)

The results of total number of chromosomal aberrations and their types in treated females and their embryos and in a control group are given in Table (4) and (5). The total number of chromosomal aberrations (structural and numerical) was increased significantly in treated groups (females and embryos) compared with the control. These increases were higher in the groups of treated females than treated embryos. The most frequent structural aberrations among the treated groups were chromatid gaps, breaks, deletions, fragments, centric fusions and endometosis, and the most frequent numerical aberrations were aneuploidy and polyploidy.

Table (3): Results of micronucleus tests in mothers and embryos after mothers maternal oral administration with (Avelox)

| Test Substance | Dose (mg/kg/day) | Number of assessed PCE | NCE per 1000 PCE | Frequency of micronucleated cells% |
|----------------|------------------|------------------------|------------------|------------------------------------|
| Mothers | | | | |
| Control | 0 | 10000 | 450 | 45% |
| Avelox | 8.7 | 10000 | 500 | 50% |
| | 17 | 10000 | 720 | 72% |
| | 26 | 10000 | 800 | 80% |
| Embryos | | | | |
| Control | 0 | 10000 | 400 | 40% |
| Avelox | 8.7 | 10000 | 430 | 43% |
| | 17 | 10000 | 550 | 55% |
| | 26 | 10000 | 620 | 62% |

Table (4): Effect of oral administration of Avelox on pregnant females.

| Dose mg/kg/day | Structural aberrations | | | | | | | T.S. A | Numerical aberrations | | |
|----------------|------------------------|------------------|--------------------|-----------|-----------|--------------------|---------------|--------------------|-----------------------|---------------------|---------------------|
| | Chromatid gaps | Chromosomal gaps | Chromatid breaks | Deletions | Fragments | Centric fusions | Endo. mitosis | | Aneu-ploidy | Poly-ploidy | T.N. A |
| Control | 5.667 | 1.333 | 2.667 ^B | 4.333 | 4.333 | 0.000 ^B | 2.667 | 21.00 ^C | 6.333 ^D | 3.333 ^B | 9.667 ^D |
| Avelox | | | | | | | | | | | |
| 8.7 mg/kg/day | 7.000 | 2.333 | 3.667 ^B | 4.667 | 2.667 | 1.333 ^B | 3.667 | 25.33 ^B | 7.000 ^C | 4.333 ^{AB} | 11.333 ^C |
| 17 mg/kg/day | 6.000 | 0.000 | 8.667 ^A | 6.000 | 2.667 | 3.667 ^A | 1.333 | 28.33 ^A | 9.667 ^A | 4.000 ^B | 13.667 ^B |
| 26 mg/kg/day | 7.667 | 1.000 | 9.667 ^A | 5.333 | 1.667 | 4.333 ^A | 0.000 | 29.67 ^D | 10.323 ^B | 5.333 ^A | 15.667 ^A |

Means of different letters (A, B, C, D) in the same column are significantly different. The column without letters is not significant. 50 metaphase cells were examined from each animal.

Table (5): Effect of (Avelox) treatment on embryos at the 18th day of gestations.

| Dose mg/kg/day | Structural aberrations | | | | | | | T.S. A | Numerical aberrations | | |
|----------------|------------------------|------------------|------------------|-----------|-----------|---------------------|---------------------|--------------------|-----------------------|--------------------|--------------------|
| | Chromatid gaps | Chromosomal gaps | Chromatid breaks | Deletions | Fragments | Centric fusion | Endo. mitosis | | Aneu-ploidy | Poly-ploidy | T.N. A |
| Control | 4.667 ^B | 1.667 | 4.667 | 2.000 | 3.333 | 0.000 ^C | 2.000 ^{AB} | 18.33 ^D | 6.667 ^B | 2.333 ^B | 9.000 ^B |
| Avelox | | | | | | | | | | | |
| 8.7 mg/kg/day | 5.000 ^B | 2.333 | 5.333 | 4.333 | 2.667 | 1.333 ^{BC} | 0.333 ^C | 21.33 ^C | 8.667 ^{AB} | 4.000 ^A | 12.67 ^A |
| 17 mg/kg/day | 7.333 ^A | 1.000 | 5.333 | 3.333 | 1.333 | 3.000 ^B | 3.000 ^A | 24.33 ^B | 10.670 ^A | 2.667 ^B | 13.33 ^A |
| 26 mg/kg/day | 7.000 ^A | 1.333 | 5.333 | 4.000 | 3.000 | 5.667 ^A | 1.000 ^{BC} | 27.33 ^A | 9.667 ^A | 4.333 ^A | 14.00 ^C |

Means of different letters (A, B, C, D) in the same column are significantly different. The column without letters is not significant. 50 metaphase cells were examined from each animal.

Discussion

Moxifloxacin (Avelox) is a fluoroquinolone antibacterial which is approved for the treatment of acute exacerbations of chronic bronchitis (AECB) and acute bacterial sinusitis. Moxifloxacin has a broad spectrum of antibacterial activity, including activity against penicillin resistant *Streptococcus-Pneumonia*. The safe use of moxifloxacin during pregnancy for the mothers and embryos has not been established so in this study we try to evaluate the cytogenetic and developmental effects of (Avelox) on pregnant women and future embryos.

In the present study we found that treatment with moxifloxacin from 1 day to 17 day of gestations with a dose of 8.7 mg/kg/day which corresponds to the therapeutic parenteral dose of 400 mg per day caused a slight increase in chromosomal and micronuclei in maternal bone marrow cells and in embryonic cells and also caused development toxicity in embryos. However, the treatment with a dose of (17 mg/kg/day) which corresponds to 2x of therapeutic dose caused a highly significant increase in the chromosomal aberrations and micronuclei in the maternal bone marrow cells and also highly increases in the chromosomal aberrations and micronuclei in the embryonic cells with skeletal malformation were observed in embryos at 18 day of gestations while, the treatment with a dose of 25 mg/kg/day which corresponds to 3x of the therapeutic dose of (Avelox) caused a very significant increase in the frequencies of both chromosomal aberrations and micronuclei in the maternal bone marrow cells and in the embryonic cells but these increases were more frequent in the maternal cells than embryonic cells. Skeletal malformation were highly increased in mice treated with (26 mg/kg/day).

These results were in agreement with many reports by (Kowalski *et al.*, 2004) who observed that in rabbits that had been treated with an intravenous dose of 20 mg/kg skeletal malformations were observed.

Also (Mukherjee *et al.*, 1993) observed that there was an increase in the

incidence of abortions in monkeys and rabbits at human therapeutic concentrations. Recently (Ferguson, *et al.* 2004) published the results of an in vivo bone marrow chromosomal assay. Mice were intraperitoneally treated with 0.6, 6 and 20 mg/kg ciprofloxacin which is similar to moxifloxacin. This chromosomal aberration assay showed positive results for 6 and 20 mg/kg.

However, negative results were observed in another study by (Herbold *et al.*, 2001) who found that ciprofloxacin had no genotoxic effects in mice and rats.

Also, negative results observed by (Ball *et al.*, 2004) found that in rats and monkeys treated with Moxifloxacin did not show evidence of genotoxicity. Considering the above mentioned results it was shown that when moxifloxacin (Avelox) is administered during the pregnancy with a dose equal to the recommended dose of human (Avelox) caused a slight increase in the frequencies of chromosomal aberrations and micronuclei in maternal cells and embryonic cells. Also the administration of a dose above the recommended dose of human 2x and 3x respectively, has a mutagenic effects on both bone marrow cells and fetal cells. Also it has teratogenic effects and skeletal malformations to the fetuses. These increases were dose related. The significant increase in the chromosomal aberrations and in skeletal malformations can be taken as a clear indication that drugs can cross the placenta and interact with fetal DNA increasing the genetic load in future generations causing chromosomal abnormalities.

Also, from the above result we found that Moxifloxacin affected maternal cells more than fetal cells. This may be as a result of the directional injection of the Moxifloxacin to the pregnant female during the pregnancy which reached directly to the mothers bone marrow cells and indirectly through the placenta to the fetal cells therefore, moxifloxacin is considered not to be safe for therapeutic dose and above therapeutic dose it has a mutagenic and developmental effects. Consequently, the

use of moxifloxacin during pregnancy is contraindicated.

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التأثير التكاثرى والنموى للأيموكسفلاكسين
فى إناث الفئران الحوامل والأجنة

هناء محمود رشدى

قسم بيولوجيا الخلية المركز القومى للبحوث – الدقى - القاهرة

أفيلوكس (أيموكسفلاكسين) مضاد حيوى جديد واسع المجال يعتبر من المضادات الحيوية الفعالة والأمنة فى معالجة البكتيريا (الموجبة والسالبة) التى تصيب الجهاز التنفسى ولكن الاستخدام الآمن خلال فترة الحمل لم يتم دراسته الدراسة الكافية ولم يعرف حتى الآن تأثير هذا الدواء على الأمهات والأجنة أثناء الحمل.

لذلك هذه الدراسة تبحث فى تأثير (الأفيلوكس) الوراثة على الأمهات والأجنة ودراسة التشوهات فى الأجنة التى يمكن أن يسببها استخدام هذا الدواء.

تم فى هذا البحث أخذ فئران أنثى مايس صغيرة حوامل وأعطائها جرعات مختلفة من (الأفيلوكس) حوالى (8.7، 17، 26 مجم/كجم/ فى اليوم) وهذه الجرعات تعتبر الجرعة المسموح بها دولياً ومضعفاتها، وتم أخذ هذه الجرعات خلال فترة الحمل من اليوم الأول للإخصاب إلى اليوم السابع عشر للحمل وتم تشريح الأمهات فى اليوم الثامن عشر للحمل وأخذ عينات من النخاع الشوكى للأمهات وأخذ عدد من الأجنة لمعرفة التأثير الوراثة والمورفولوجى للدواء.

أوضحت النتائج أن دواء (أفيلوكس) يسبب تشوهات مورفولوجية للأجنة ونقص عدد الأجنة الحية وأزدياد عدد الأجنة الميتة عند مقارنتها بالكنترول وأن هذا التأثير للدواء يتزايد بإزدياد الجرعة المأخوذة من الدواء أما من حيث التشوهات الكروموسومية وعدد الأنوية فى خلايا الأمهات والأجنة تجد أن (أفيلوكس) يسبب تشوهات كروموسومية (عددية وتركيبية) فى الأمهات والأجنة ويسبب أيضاً زيادة فى عدد الأنوية الموجودة فى خلايا الأمهات والأجنة ولذلك تنصح الدراسة بعدم استخدام (الأفيلوكس) خلال فترة الحمل لما يسببه من أضرار على الأم والجنين حتى فى الجرعة المسموح بها دولياً.