RISKS OF EXPOSURE TO VETERINARY MARBOFLOXACIN RESIDUES THROUGH MILK

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ABSTRACT

The veterinary antibacterial Marbofloxacin (Marbo) is used in food-producing animals, including dairy cows, so the residues of this drug will be excreted through milk. This study was aimed to investigate a risk of developmental and neurobehavioral toxicity effects in newborn consumers exposed to the residues of this drug. To achieve this goal, suckling pups of rats were selected as a model. Four groups of female lactating rats (5 dams for each group – 8 pups per dams) were divided into (A,B,C,D) treated with Marbo (0 (control) , 3, 6 ,9 mg/kg, i.m.) given once daily for 10 consecutive days (first nursing period) respectively.The exposure of suckling pups to the drug through milk in group (D) led to neurobehavioral toxicity effects represented in the occurrence of a significantly prolongation in both the time required to terminate the surface righting reflex on postnatal day (PND) 5 and the time of induction and duration of anesthesia by (ketamine and xylazine) on the (PND) 21 . the newborns in group (C) recorded a significantly prolongation of both in the period of appearance of pinna detachment and in induction and duration of anesthesia accompanied by a significantly decrease in numbers of squares crossed in open field activity test on the (PND) 21 .It could be conclude from our current study that there are risks of causing developmental and neurobehavioral toxic effects in newborn consumers exposed to Marbo residue in animal products such as milk.

Key words: Marbofloxacin; neurobehavioral toxicity; suckling pups of rats; veterinary drugs residues.

مخاطر التعرض لبقايا الماربوفلوكساسين البيطري عن طريق الحليب

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المستخلص

يستعمل المضاد البكتيري البيطري الماربوفلوكساسين (ماربو) الحيوانات المنتجة للغذاء ، بما في ذلك الأبقار الحلوب ، لذلك فان بقايا هذا الدواء سوف تطرح عن طريق الحليب .السؤال الذي يطرح نفسه فيما إذا كان هناك خطورة من حدوث تأثيرات سمية تطورية وسلوكية عصبية في المستهلكين الصغار المعرضين لبقايا هذا الدواء. لتحقيق هذا الهدف تم اختيار صغار الجرذان الرضع كنموذج.اربعة مجاميع من اناث الجرذان المرضعات (خمسة أمهات في كل مجموعة وبواقع 8 صغار لكل ام) قسمت الى (أ , ب ,جـ , د) عوملت بالماربو (0 (السيطرة) ,3 ,6 ,9 ملغم /كغم ,بالعضل) لمرة واحدة باليوم ولمدة 10 أيام متتالية (خلال فترة الرضاعة الاولى) وعلى الترتيب . أدى تعرض الصغار الرضع للدواء عن طريق الحليب في المجموعة (د) الى حدوث تأثيرات سمية سلوكية عصبية تمثلت في حدوث إطالة معنوية في كل من الوقت اللازم لأنهاء اختبار منعكس تصحيح الجسم في اليوم الخامس بعد الولادة ووقت احداث ومدة التخدير (الكتامين والزيلازين) في اليوم 21 بعد الولادة . ومن جهة أخرى فقد سجل الصغار من المجموعة (جـ) إطالة معنوية في كل من مدة انفصال صيوان الاذان وفي وقت احداث ومدة التخدير رافقها حدوث انخفاض معنوي في عدد المربعات المقطوعة في اختبار نشاط الميدان المفتوح في اليوم 21 بعد الولادة .نستنتج من هذه الدراسة وجود مخاطر من حدوث تأثيرات سمية تطورية وسلوكية عصبية في المستهلكين الصغار المعرضين لبقايا الماربو في المنتجات الحيوانية مثل الحليب.

الكلمات المفتاحية: ماربوفلوكساسين. سمية عصبية سلوكية الجراء الرضع من الفئران؛ مخلفات العقاقير البيطرية

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INTRODUCTION

Veterinary medicines are mostly used in the management of dairy cows to treat and prevent diseases, and these medicines may leave residues in milk, human exposure to medication residues and unintended utilization of drug residues can result in adverse consequences posing a serious threat to consumer health (25) . Especially if consumers of animal products (milk) that contain the residues of veterinary drugs are newborns ,because the ability to expel drugs is often impaired in this newborns compared with adults, indicating that even exposure to seemingly insignificant doses could be cause adverse consequences on the central nervous system due to the incomplete development of the blood-brain barrier in these newborns (2, 24) The most prominent adverse effects resulting from exposure to veterinary drug residues are allergies, which are frequently linked to β -lactam antibiotics; genotoxic and carcinogenic responses, which are frequently linked to chloramphenicol, sulfamethazine, and nitrofurans (18 ,9). In the field of research studies on this subject, (23, 26) have indicated that there are developmental toxicological effects in breast feeding offspring of rats exposed to diclofenac via milk. Marbofloxacin is a third generation fluorinated quinolone (fluoroquinolone), It is used exclusively in the field of veterinary medicine as broad spectrum antibacterial (6). It is clinically used to treat of bovine respiratory disease (BRD) (11) and mastitis (17, 16, 14) Marbo is excreted through milk(21,22,15) where mention the penetration of the Marbo into the milk of lactating buffaloes after single intravenous (IV) and single intramuscular (IM) injections at a dose of 2 mg/kg .body.weight was rapid and extensive with Marbofloxacin concentration exceeding those of serum(8). The most prominent adverse effects caused by fluoroquinolone compounds, to which Marbofloxacin belongs, is their neurotoxicity (19) and arthropathy (27). Since, Marbo is excreted through milk (21,22,15,8), hence its danger in causing the above-mentioned adverse effects in newborns when they consume milk containing Marbo residues. The occurrence of these adverse effects confirm the problem of exposure to veterinary drug residues, which has become a global problem , Therefore, researchers were keen to find ways to detect the presence of veterinary drug residues in animal products such as milk in order to dispose of these products and not consume them because of their risks to consumer health (7) .Based on what was mentioned above and because of the lack of research studies on the risk of exposure of newborns to the residues of veterinary medicines, so the target of our research was to reveal the developmental, neurobehavioral, toxicity in newborns of rats exposed to Marbofloxacin via milk.

MATERIAIS AND METHODS

In this study, female white Albino rats were used, which were raised in the laboratory animal house of the College of Veterinary Medicine , University of Mosul, and their weights were between (166-294 g) . Bearing in mind that the weights of female rats are close in each experiment. After a 1-week adaptation of lighting (10 h-light/14 h-dark) and the temperature of the laboratory in which the breeding was carried out was (22±2°C), they were pair off with males. A positive -sperm vaginal smear was taken to show the first day of pregnancy. They were placed in special plastic cages prepared for this purpose and provided with water and feed in abundant quantities and continuously. The birth day was reflected as day 0 of lactation, in order to equally expose lactating pups to the drug through milk, eight neonates per dams were determined (20) In this experiment, each breastfeeding mother and her pups (8 pups per mother) were placed in a cage separately for the purpose of monitoring and following up this newborns from day zero (the day of birth) until the age of weaning, in order to record any changes that occur in the body weight , developmental index ,appearance of developmental landmarks and neurobehavioral toxicity changes that may appear on suckling pups as a result of exposure to Marbofloxacin (Marbosav 2%-solution for injection – manufactured by Saudi pharmaceutical industries) through milk. All search were done in accordance with the advice for the care and use of experimental laboratory animals (3) The twenty (28) healthy lactating female rats

were distributed into four groups (5 dams per group) **:**

Group A (control group): included 40 suckling pups of rats from five mothers (8 pups per mother), each of the lactating female rats in this group was treated with normal saline (2 ml/kg. intramuscularly (i.m.)) once daily for10 consecutive days during the initial lactation period.

Group B : included 40 suckling pups of rats from five mothers (8 pups per mother),each of the lactating female rats in this group was treated with Marbo (3 mg/kg.B.Wt i.m) once every 24 hours for ten sequential days during the initial lactation period.

Group C : included 40 suckling pups of rats from five mothers (8 pups per mother), each of the lactating female rats in this group was treated with Marbo (6 mg/kg B.Wt i.m) once every 24 hours for ten sequential days during the initial lactation period.

Group D: included 40 suckling pups of rats from five mothers (8 pups per mother), each of the lactating female rats in this group was treated with Marbofloxacin (9 mg/kg B.Wt. i.m) once every 24 hours for ten sequential days during the initial lactation period. In this study, the doses of Marbo (3, 6 and 9 mg/kg, i.m.) were selected based on preliminary experiments. For the purpose of detecting the developmental and neurobehavioral toxicity in newborns rats exposed to Marbo through milk, the following criteria were studied.

1-Study of changes in the rate of body weight in the suckling pups of rats in postnatal days (PND) (0, 5, 10, 15,20) as a result of exposure this pups to the drug through milk

2-Study of changes in the rate of developmental index in the suckling pups of rats , by measuring the length of body from the top of the head to the top of the tail in PND (0, 5, 10, 15,20) as a result of exposure to the drug through the milk (20).

3- Study of changes in the time of the appearance of developmental lanmarks (in days), such as (pinna detachment, eye opening, tooth eruption and hair development) in newborns as a result of exposure to the drug (20).

4-Study of changes in the neurabehavioral response (choosing the surface righting reflex test) as this test shows the functions of the nervous system where the newborn is placed on his back and then according to the time required for the newborn to be able to corrected its position and the test was performed on newborns for all groups in PND (2,3,4 and5),and to detect any changes in the activity levels, gross locomotors activity, and exploration habits of CNS this was done by using the open field activity test (neurobehavioral test) in PND 21

5-The pharmacological challenge is one of the methods used to detect the latent effects of some chemicals on the function of the nervous system This challenge was carried out using both (xylazine 5mg/kg B.Wt.i.p and ketamine 50 mg/kg B.Wt. i.p) on the young pups that survived until the age of weaning . The induction and duration of anesthesia were recorded on the animals on which the pharmacological challenge was performed.

RESUITS AND DISCUSSION

Suckling pups of rats from mothers treated with Marbo at a dose of (6 mg/kg B.Wt.) showed a significant rise in the average weight and index of development on the PND (10, 15 and 20) (Table 1,2) accompanied by a significant prolongation of both in the period of appearance of signs of developmental landmarks (Pinna detachment) (Table 3)and in the time of induction and duration of anesthesia by (ketamine and xylazine) in compared with control group(Table 5) . The newborns of this group also recorded a significant decline in motor activity in open field test (neurobehavioral test) compared to the control group(Table 6). The exposure of young rats to Marbo through milk in group (D) (lactation from mothers treated with high doses of Marbo 9 mg/kg) led to neurobehavioral toxicity effects represented by the appearance of a significantly prolongation, firstly in the time required to terminate the surface righting reflex on 5 (PND) (Table 4) and secondly in time of induction and duration of anesthesia by (ketamine and xylazine) on the 21 (PND) (Table 5) as well as a significant increase in body weight on 20 (PND) in compared with control group (Table 1) With regard to suckling pups from injected mothers with Marbo at a dose (3mg/kg)B,Wt.),both revealed a significant rise in the body weight and index of development on the PND (10, 15

and 20) (Table 1,2) accompanied by a significant prolongation of time of induction of anesthesia by (ketamine and xylazine) (table 5).

Table 1. Show the change in the body weight rate of breastfeeding pups of rats from dams injected with Marbofloxacin (0,3,6,9 mg/kgB.Wt..i.m.) given once every 24 hours for ten sequental days (first breastfeeding period)

Days (PND)	Body Weight (gram) Marbofloxacin (mg/kg .i.m.) O(control)	3	6	9
0	6.1393±0.12983	5.8485±0.09284	5.7681±0.08648	5.9880±0.06210
5	10.7230±0.16463	11.1217±0.13326^{b}	10.3507±0.27499^{ac}	10.9867±0.18582^{b}
10	16.2445±0.22960	18.8300±0.29847^{*bc}	17.3592±0.43906^{*a}	17.7220±0.30736^{*a}
15	22.2770±0.29031	26.3935±0.79727^{*bc}	24.7155±0.42561^{*a}	23.4642±0.47115^{a}
20	25.3268±0.53304	29.3533±0.75405^{*b}	30.0402±0.75288^{*ac}	27.1220±0.85504^{*ab}

The values represent the mean ± standard error of forty pups/group

*The value differs significantly comparison to the values of the group 0(control) at the level of significance A < 0.05

a The value differed significantly compared with the values of the group treated with a dose of (3 mg/kg B.Wt) at a significant level A <0.05

b The value differed significantly compared to the values of the group treated with a dose of (6 mg/kg B.Wt) at a significant level A <0.05

c The value differed significantly compared with the values of the group treated with a dose of (9 mg/kg B.Wt.) at a significant level A <0.05

Table 2. Show the change in the index of development rate of breastfeeding pups of rats from dams treated with Marbofloxacin (0,3,6,9 mg/kg.i.m.) given once every 24 hours for ten sequential days (first breastfeeding period)

Days	Developmental index (mm) Marbofloxacin (mg/kg, i.m.) 0(control)	3	6	9
0	39.03±0.256	37.68±0.543	38.19±0.428	39.59±135
5	47.15±.0719^{ac}	51.80±0.448^{*bc}	48.19±0.722^{a}	49.04±0.611^{*a}
10	55.63±1.395^{abc}	62.79±0.674^{*b}	60.16±0.627^{*ac}	63.2320±0.405^{*b}
15	67.02±0.969^{abc}	70.90±0.611*	72.86±686*	70.09±1739*
20	79.13±1.440^{ab}	85.39±0.792^{*c}	86.26±0950^{*c}	79.58±1.333^{ab}

The values represent the mean ± standard error of forty pups/group

*The value differs significantly comparison to the values of the group 0(control) at the level of significance A < 0.05

a The value differed significantly compared with the values of the group treated with a dose of (3 mg/kg B.Wt) at a significant level A <0.05

b The value differed significantly compared to the values of the group treated with a dose of (6 mg/kg B.Wt) at a significant level A <0.05

c The value differed significantly compared with the values of the group treated with a dose of (9 mg/kg B.Wt.) at a significant level A <0.05

Table 3. Shows the appearance of developmental landmarks like (pinna detachment, eye opening, tooth eruption and hair development) (in days) in breastfeeding pups of rats from dams treated with Marbofloxacin (0 ,3 ,6, 9 mg/kg. B.Wt. i.m.) given once every 24 hours for ten sequential days (first breastfeeding period)

Marbofloxacin (mg/Kg)	Appearance of developmental landmarks (In days) Pinna detachment	Hair development	Tooth eruption	Eye opening
control	2.35±0.08^{bc}	6.550±.10712	9.40±0.202	15.15±.11065
3	2.15±0.05^{b}	6.625±.08539	9.15±0.150	14.35±.07638^{c}
6	2.77±0.10*^{ac}	6.725±.07150	9.35±0.177	14.85±.13180^{c}
9	2.1±0.05*^{b}	6.675±.07500	9.05±0.123	15.1250±.13487^{ab}

The values represent the mean ± standard error of forty pups/group

*The value differs significantly comparison to the values of the group 0(control) at the level of significance A < 0.05

a The value differed significantly compared with the values of the group treated with a dose of (3 mg/kg B.Wt) at a significant level A <0.05

b The value differed significantly compared to the values of the group treated with a dose of (6 mg/kg B.Wt) at a significant level A <0.05

c The value differed significantly compared with the values of the group treated with a dose of (9 mg/kg B.Wt.) at a significant level A <0.05

Table 4. Shows the changes in the neurobehavioral responses (Surface Righting Reflex test) in the PND (2,3,4,5) for breastfeeding pups of rats from dams treated with Marbofloxacin (0,3,6,9 mg/kg.i.m.) given once every 24 hours for ten sequential days (first breastfeeding period)

Marbofloxacin dosage (mg/Kg)	Time of surface righting reflex in seconds			
	PND 2	PND 3	PND 4	PND 5
Control	5.4770±.65186	4.6778±.55346	2.8685±.36133	2.3862±.23731[ac]
3	4.7682±.58627[b]	4.1955±.75842	3.8547±1.45057	3.4318±.42537[*b]
6	7.9072±1.60179[a]	3.4248±.36940	2.8322±.39384	1.9998±.16174[ac]
9	6.1315±.95670	5.0945±.88884	2.6940±.25349	3.8466±.40837[*b]

The values represent the mean ± standard error of forty pups/group

*The value differs significantly comparison to the values of the group 0(control) at the level of significance A < 0.05

a The value differed significantly compared with the values of the group treated with a dose of (3 mg/kg B.Wt) at a significant level A <0.05

b The value differed significantly compared to the values of the group treated with a dose of (6 mg/kg B.Wt) at a significant level A <0.05

c The value differed significantly compared with the values of the group treated with a dose of (9 mg/kg B.Wt.) at a significant level A <0.05

Table 5. shows the time of induction and the duration of anesthesia caused by pharmacological challenge (ketamine and xylazine) in the surviving young pups of rats to the age of weaning (PND 21) from dams treated with Marbofloxacin (0,3,6,9 mg/kg.i.m.) given once every 24 hours for ten sequential days (first breast feeding period)

Marbofloxacin (mg/Kg)	Time of induction (minute)	Time of duration (minute)
control	1.4000±.11239[abc]	94.503±.43779[bc]
3	3.7222±.44871[*bc]	99.50±3.21959[c]
6	2.6000±.37975[*a]	111.40±7.07494[*c]
9	2.8000±.30435[*a]	129.95±7.07459[*ab]

The values represent the mean ± standard error of twenty pups/group

*The value differs significantly comparison to the values of the group 0(control) at the level of significance A < 0.05

a The value differed significantly compared with the values of the group treated with a dose of (3 mg/kg B.Wt) at a significant level A <0.05

b The value differed significantly compared to the values of the group treated with a dose of (6 mg/kg B.Wt) at a significant level A <0.05

c The value differed significantly compared with the values of the group treated with a dose of (9 mg/kg B.Wt.) at a significant level A <0.05

Table 6. shows the changes in the neurobehavioral responses (open – field activity test) in the surviving young pups of rats to the age of weaning (PND 21) from dams treated with Marbofloxacin (0,3,6,9 mg/kg B.Wt .i.m.) given once every 24 hours for ten sequential days (first breastfeeding period)

Marbofloxacin (mg/Kg)	Open field activity test During 3 mintes		
	Numbers of Squares crossed	Numbers of rearing behavior	Numbers of fecal bolus
control	33.4±4.58395[b]	4.50±0 .63037	0.40±0 .22243[b]
3	27.65±6.08894	3.45±0.72720	0.65±0.19568
6	18.95 ±3.55148*	4.60± 0.64236	1.15±0.33462[*]
9	25.95± 4.69573	4.950±1.00910	1.05±0.28539

The values represent the mean ± standard error of twenty pups/group

*The value differs significantly comparison to the values of the group 0(control) at the level of significance A < 0.05

a The value differed significantly compared with the values of the group treated with a dose of (3 mg/kg B.Wt) at a significant level A <0.05

b The value differed significantly compared to the values of the group treated with a dose of (6 mg/kg B.Wt) at a significant level A <0.05

c The value differed significantly compared with the values of the group treated with a dose of (9 mg/kg B.Wt.) at a significant level A <0.05

The problem of exposure to veterinary drug residues found in animal products like (milk and meat) has become a global problem (25, 7), There is a lack of research studies on the risks of exposure to veterinary drug residues, especially newborns, these risks come because the behavior of pharmacokinetic in these newborns is different from that of adults, so their nervous system will be the target organ for these residues due to the incomplete growth and development of the blood-brain barrier (24), In this study, the antibacterial Marbo was chosen because of its wide use in the field of veterinary medicine to treat food-producing animals such as (cows), The Marbo has many adverse effects like neurotoxicity (19) and arthropathy (27) and it is excreted through milk. Is there a risk of adverse effects in newborns exposed to the drug through milk? To answer this question, this study conducted to detect the developmental and neurobehavioral toxicity in newborns of rats exposed to Marbo via milk The breastfeeding pups of rats from the group of mothers treated with Marbo at a dose (3 and 6 mg) showed a significant rise in both body weight and index of development in compared to the control group. This increases could be explained by the fact that Marbo has a stimulating effect on growth, which confirms this explanation is the use of Marbo as feed additives for food-producing livestock and in sub-therapeutic doses as a growth promotor (10, 28) This study was showed the neurobehavioral toxicity effects in newborns of rats exposed to Marbo by milk, which were detected using both the surface righting reflex test on (PND) 2, 3, 4 and 5 and the pharmacological challenge test (ketamine and xylazine) was done on the young pups surviving to weaning (21 days of age), the neurobehavioral toxicity effect appeared clearly in the group of suckling pups from mothers injected with high dose of Marbo (9 mg/kg B.Wt.) which was in the form of a delay in the response time to surface righting reflex in (5 PND) and a significantly prolongation of the anesthesia time . The reason for this neurotoxicity effects in this pups could be due to the fact that when lactating female rats were injcted with a high dose of Marbo (9 mg/kg), this led to the transmission of high concentrations of the drug through the milk to the suckling pups, and then the young will be exposed to high levels of the drug (5), and as it is known that there are changes in the pharmacokinetic behavior in these suckling pups in compared adults , because the process of metabolism in the liver and excretion in the kidney is not fully developed and functional. It also has a very high volume of distribution of pharmaceutical compounds due to the presence of a high percentage of water in the body of these young children. In addition, the growth of the blood-brain barrier is not complete, which facilitates the passage of the drug to the central nervous system (4,1,13) ,and all these reasons combined will lead to an increase in the concentration of the drug and its accumulation in the different body tissues of young pups of rats and the appearance of these effects on the central nervous system . Another reason for the prolonged time needed to complete the surfacr righting reflex test is likely to be due to the adverse effects of the Marbo on the musculoskeletal system , where the exposure of sucking pups to Marbo in high concentrations through milk (because they were breastfed by mothers treated with the high dose of Marbo (9 mg/kg) led to the appearance of the adverse effects of the drug on the musculoskeletal system, which led to a delay in the time required for the newborn to be able to corrected its position. In addition, the young pups from the group of mothers treated with Marbo at a dose of (6 mg/kg B.Wt.) showed significantly decreases in numbers of squares crossed in open-field activity . The reason for this hypoactivation of the young pups in this test could be attributed to the fact that, as it is known, the all internal organs, their growth is completed during pregnancy, except for the nervous system, immune systems, and the reproductive system, which did not fully developed during pregnancy, but these organs continue to grow until after birth (12) .Therefore, it is possible that when suckling pups of rats are exposed to Marbo from breastfeeding, it will affect the growth and development processes of the central nervous system, leading to incomplete brain functions which was shown as a significant decreases in motor activity in the neurobehavioral tests Thus, the results of this

study agree with Spigset O, and S. Hägg. 1998 point about the ability for drug elimination is often decreased in newborns comparison to adults, showing that even exposure to apparently insignificant doses could be cause adverse effects on the central nervous system due to the incomplete development of the blood-brain barrier in these newborns

Conclusions

We deduce from the results of this study that the exposure of newborns of rats to Marbo residues in milk led to toxic effects at the level of the central nervous system. This result confirms the risks of exposure of the consumer, especially the newborns, to the residues of veterinary drugs that are found in animal products such as milk because they have a pharmacokinetic behavior that differs from adults.

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