Effects of Meloxicam on Implantation and Parturition of Rat

Sahar M. Jaffal,[1] Abdulazim S. Salhab, [2] Ahmad M. Disi [1] and Farouq Al-Qaadan[3]*

Abstract

Meloxicam, a selective cyclooxygenase-2 preferential inhibitor, was studied for its anti-implantation and parturition effects on pregnant rats. Regarding the effect of meloxicam on implantation, rats were dosed orally by 7.5 and 10 mg/kg/day from day 1 through 3 or from day 3 through 5 of gestation, respectively. While for the parturition effect, rats were dosed orally by the above doses from day 20 through 22 of gestation. The results of implantation experiments showed that the number of implantation sites was significantly decreased in all treated groups in a dose- and time-dependent manner. Whereas the number of resorption sites were significantly increased in all meloxicam treated groups. On the other hand, the results of parturition experiments indicated that meloxicam significantly prolonged the duration time of delivery in a dose-dependent manner. Further, significantly less viable fetuses and pups were delivered per female treated with meloxicam. In conclusion, the results indicate that meloxicam exhibited potential effect on implantation and parturition processes of pregnant rats.

Keywords: Meloxicam; Anti-implantation; Parturition; Oxytocin; Resorption.

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Introduction

Nowadays, the Non- Steroidal Anti-Inflammatory Drugs (NSAIDs) are considered as one of the most widely prescribed drugs. These drugs are effective in the treatment of acute and chronic painful inflammatory musculoskeletal conditions. Specifically, the NSAIDs are considered as prophylactic agents against cardiovascular disease and to relief the discomfort associated with minor injuries, such as headaches, and to alleviate the severe pain caused by a variety of inflammatory and degenerative joint diseases.

Further, NSAIDs are recently used to treat colorectal cancer and brain tumors.[1- 5]

Historically, the mechanism of NSAIDs action was proposed by John Vane et al. [6] Vane et al. proposed that the ability of most NSAIDs to suppress inflammation resides primarily on their capability to inhibit the enzymes cyclooxygenases (COXs) which are required for the conversion of arachidonic acid to prostaglandin G_2. [6]

1- Department of Biological Sciences, Faculty of Science, University of Jordan, Amman, Jordan.
2- Department of Pharmacology, Faculty of Medicine, University of Jordan.
3- Department of Biological Sciences, Hashemite University, Zarqa, Jordan.
*** Correspondence should be addressed to:**
Professor Abdulazim S. Salhab,
Department of Pharmacology, University of Jordan, Amman-Jordan.
E-mail: Assalhab@ju.edu.jo

Lately, two cyclooxygenase isoforms have been identified and referred to as COX-1 and COX-2. [7, 8] The COX-1 enzyme is produced constitutively in most tissues and facilitates the production of prostaglandins involved in physiological functions such as platelet aggregation, the vasodilation of the kidney veins and to induce stomach cytoprotection.[7,8] In contrast, COX-2 is an inducible enzyme at sites of inflammation and its expression is elevated in chronic inflammatory diseases and in colon tumors, in the ovaries and in fetal membranes. [9, 5, 10]

Concerning the reproduction processes, both COX-1 and COX-2 are involved in almost all stages of reproduction such as ovulation, fertilization, implantation, and decidualization. Further, prostaglandins are reported to act on the ovary and uterus to help in mediating the delivery process. [11, 12]

Meloxicam is a relatively new non-steroidal anti-inflammatory agent that appears to have a greater selective inhibitory activity against the inducible COX-2 isoform, than against the constitutive isoform COX-1 and was promoted for its safer profile with less side effects. Recently, meloxicam was reported to posses a potential inhibitory effect on rabbit ovulation. The extent of inhibition was dose-and time-dependent.[13, 14] Since a scant information is available in the literature on the effect of meloxicam on the implantation and parturition processes and because prostaglandins are playing vital roles in both processes, the major objective of this study was to assess the effects of meloxicam on implantation and parturition using the pregnant rat as a model.

Materials and Methods

Meloxicam: Authentic meloxicam, one gram powder (Batch no. 8250381, sent on 12/98, kept refrigerated most of the time and was used only as reference for UV and HPLC) was donated by Boehringer Ingelheim Pharma KG (Biberach an der Riss, Germany). Additional meloxicam powder (Batch no.1105376001, manufacturing date: 11/01 and expiry date: 11/04) used for experiments was obtained from Advanced Pharmaceutical Industries Co, Ltd. (Amman, Jordan). The purity of meloxicam was checked by HPLC and UV spectrum against the authentic sample. The different dilutions of meloxicam were freshly prepared in warm 0.15 M NaOH solution (pH 8.5) as recommended by Boehringer Ingelheim researchers (personal communications).

Animals: Adult male and female albino rats (*Rattus norvegicus* UJ-1) weighing 200-300g body weight were housed individually in plastic cages with a 12-hr light/dark cycle. Rats were kept for 2 weeks for acclimatization at laboratory conditions before they were used in experiments. Rats were provided with pelleted diet and tap water *ad libitum*.

Implantation Experiments: Female rats were introduced individually for mating with proven fertile males and kept overnight in stainless steel mating cages. The detection of vaginal plug was considered as day one of gestation. In order to study the effect of meloxicam on early implantation stage, meloxicam dilutions of 7.5 mg/kg (n=7), 10 mg/kg (n=8) or vehicle (n=9) were administered orally to vaginally positive plug rats on days 1, 2 and 3 of gestation. In order to study the effect of meloxicam on late implantation stage, meloxicam doses 7.5 mg/kg (n=9), 10 mg/kg (n=8) and vehicle (n=8) were administered to vaginally plug positive rats on days 3, 4 and 5 of gestation.

All treated rats were laparotomised on day 10 of gestation under ether anesthesia. The uteri were examined visually and implants were counted and reported as resorptions, dead or live fetuses. Meanwhile, fresh uterus tissues were kept in 10% formalin for histopathological examination after macrophotography using 35-mm camera.

Parturition Experiments: Pregnant rats were received orally meloxicam dilutions of 7.5 mg/kg (n=8) and 10 mg/kg (n=8) or vehicle (n=10) on days 20, 21 and 22 of gestation. After treatment, one group was laporatomised on the day 23 (morning) to assess the effect of meloxicam on the outcome of late pregnancy, the other group of rats went through natural delivery to assess the effect of meloxicam on the parturition onset. In both cases, the number of live and dead pups were recorded. The body weights of pups were recorded and the pups were macrophotographed. From rats which were sacrificed on day 23 of gestation, heart blood samples (1 ml) were collected for oxytocin determination and the uteri were opened to count the number of live, dead or resorbed fetuses.

Oxytocin Determination: Upon sacrificing the animals, heart blood samples were collected in heparinized tubes and then centrifuged at 4000 rpm for 10 min. Plasma aliquots were collected and stored at –20C till hormonal analysis. Plasma samples were assayed in duplicate for oxytocin using immunoassay kits (Assay Designs, Inc. Ann Arbor, USA).

Statistics: The difference between the means within the groups were analyzed using one-way ANOVA followed by Students t-test. Results are given as mean±SD considering a p-value of <0.05 as statistically significant.

Results

The Effect of Meloxicam on Implantation

Table (1) summarizes the effect of meloxicam on the implantation outcome of pregnant rats. The results show meloxicam treatment resulted in a significant decrease in the number of viable implanted fetuses in 7.5 and 10 mg/kg groups compared to vehicle group. Further, the decrease in the number of viable fetuses was higher among rats of the late implantation period (3 through 5 days of gestation) compared to early implantation group. Moreover, the resorption rate was significantly more among rats treated with meloxicam compared to placebo group, being higher in the late implantation group which received 10 mg/kg/day compared to early implantation group. Thus, one can conclude that meloxicam resulted in a significant inhibition of implantation in rats by dose-and time-dependent fashion.

Photography is a convenient way to record permanent reproductive effects. Figure 1 presents the uterine horns of placebo treated rat containing 10 tiny viable fetuses. While, Figure 2 shows meloxicam treated-rats (10 mg/kg) with several adsorbed sites (arrows). Whereas Figure 3 shows unspaced clumped fetuses (arrows).

The Effect of Meloxicam on Parturition

Table (2) summarizes the results of laparotomised rats on the early morning of day 23 of gestation. The results indicate a significant decrease in the number of viable fetuses of treated rats compared to placebo group. Further, a significant decrease in the number of viable fetuses was observed. Meanwhile, no effect on fetuses, body weight was found. Moreover, it was observed that there was a significant increase in the number of adsorbed sites of treated groups compared to the placebo group (Table 2). Besides, a six fold increase in the oxytocin plasma level of treated rats (10 mg/kg) was observed compared to placebo.

Table (3) summarizes the outcome of spontaneously delivered pregnant rats. The results indicate that 10 out of 11 (91%) spontaneously delivered their pumps in early afternoon of the day 23 of gestation. Whereas as long as 37 hr and 51 hr delay in parturition onset were recorded for rats treated with 7.5 and 10 mg/kg, respectively. Concerning the alive pups body weight, it was comparable to pups of placebo group. Apparently, normal healthy pups were borne to placebo pregnant rats (Figure 4). Meanwhile, dead pups were born to meloxicam treated group (10 mg/kg/day) with shrinking bluish skin (Figure 5).

Table 1: Effect of meloxicam treatment on rat implantation.

Parameters	***Early implantation (1-3 days)***			***Late implantation (3-5 days)***		
	Vehicle	***7.5mg/kg***	***10mg/kg***	***Vehicle***	***7.5mg/kg***	***10mg/kg***
Number of pregnant rats	*9*	*8*	*8*	*8*	*9*	*8*
Viable implanted sites (M ± SD)	*9.78 ± 1.99*	*7.4 ± 5.0*	*0.13 ± 0.35**	*7.63 ± 0.5*	*5.89 ± 6.11*	*0.0 (all dead)*
Adsorption sites (M ± SD)	*0.56 ± 1.67*	*5.4 ± 5.6**	*7.6 ± 2.1**	*0.5 ± 1.4*	*6.56 ± 5.7**	*8.0 ± 1.85**
Number of pregnant rats with viable implantation sites	*9*	*6.5*	*1*	*7*	*5*	*0.0 (all dead)*
(%):	*100*	*75.0*	*12.5*	*87.5*	*55.6*	*0.0 (all dead)*

**** P < 0.05 as compared to vehicle values.***

Table 2: Effect of meloxicam treatment on rat late pregnancy outcome.

		Treatment	
Parameter	***Vehicle***	***7.5 (mg / kg)***	***10.0 (mg / kg)***
Number of pregnant rats	*10*	*8*	*8*
Viable fetuses (M ± SD)	*8.18 ± 1.6*	*6.37 ± 3.85**	*6.38 ± 4.1**
(%)	*(93.7)*	*(79.9)*	*(79.9)*
Fetus body weight (g)	*5.62 ± 1.0*	*5.53 ± 0.57*	*4.76 ± 0.82*
Dead fetuses (M ± SD)	*0.55 ± 0.82*	*1.6 ± 3.03**	*2.75 ± 4.53**
Maternal plasma oxytocin (ng/ml)	*104 ± 83*	*116 ± 67*	*739 ± 311**

**** P < 0.05 as compared to vehicle values.***

Table 3: Effect of meloxicam on rat parturition.

		Treatment	
Parameter	***Vehicle***	***7.5 (mg / kg)***	***10.0 (mg / kg)***
Number of pregnant delivered rats	*11*	*11*	*11*
Viable pups (M ± SD)	*6.2 ± 2.64*	*2.0 ± 2.9**	*0.57 ± 1.51**
(%)	*(80)*	*(25.9)*	*(7.0)*
Pup body weight (g)	*5.5 ± 0.76*	*5.3 ± 0.67*	*5.6 ± 0.42*
Dead pups (M ± SD)	*1.5 ± 2.5*	*5.73 ± 3.43**	*7.56 ± 1.24**
Parturition day (PD) (%)			
22:	*1 (9.1)*	*1 (9.1)*	*0.0*
23:	*9 (81.2)*	*1 (9.1)*	*0.0*
24:	*1 (9.1)*	*2 (18.2)*	*2.0 (18.2)*
25:	*0.0*	*5 (45.5)*	*5.0 (45.5)*
26:	*0.0*	*2 (18.2)*	*4.0 (36.4)*
Parturition day (M ± SD):	*23.0 ± 0.63*	*24.55 ± 1.2**	*25.14 ± 1**

**** P < 0.05 as compared to vehicle values.***

Figure 1: Normal uterus with intact ovaries of control rats. Rats were sacrificed on day 10 of gestation. Ten normal fetuses appear with no absorbing sites. Bar represents 10 mm.

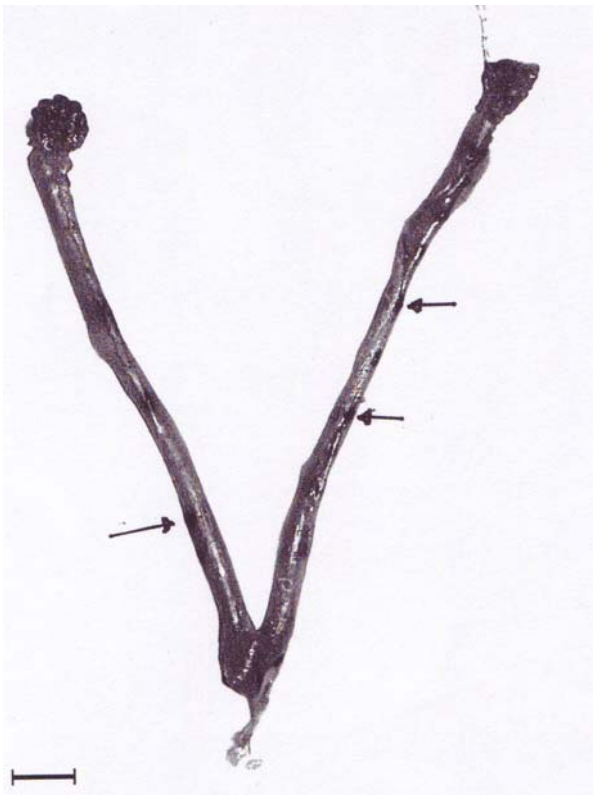

Figure 2: The uterus of meloxicam-treated rats (10 mg/kg) on days 3,4 and 5 of gestation (Late implantation). Notice the resorping sits (arrows) and the absence of fetuses. Bar represents 10 mm.

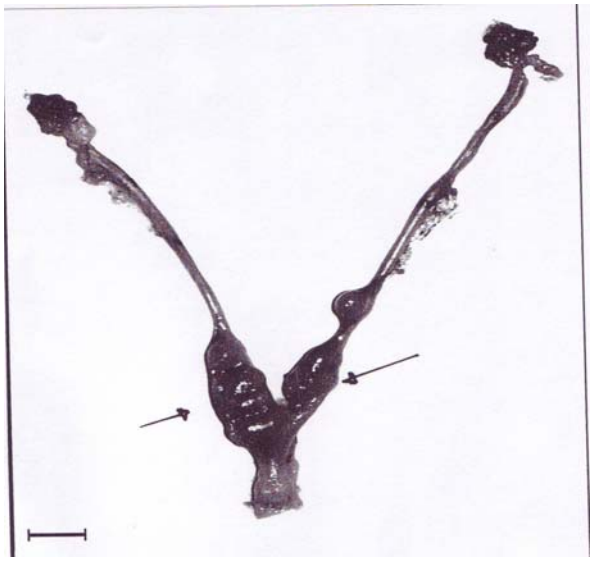

Figure 3: The uterus of meloxicam-treated (7.5 mg/kg) on days 3,4 and 5 of gestation. Notice the clumping of fetuses (arrows). Bar represents 10 mm.

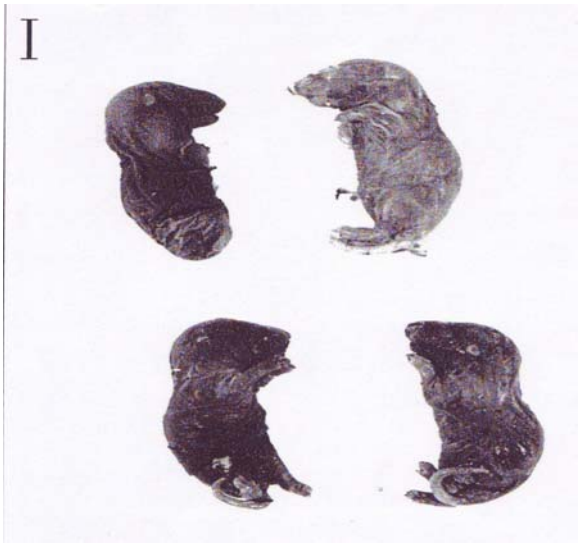

Figure 4: Pups normally delivered by control rat on day 23 of gestation. The appearance of pups were normal with pinkish colored skin. Bar represents 10 mm.

Figure 5: Dead pups delivered on day 26 of gestation by meloxicam treated rat (10 mg/kg) on days 20, 21 and 22 of gestation. The appearance of pups were blue in color with shrieked skin. Bar represents 10 mm.

Discussion

In this investigation, we studied the effect of meloxicam on two important reproductive processes namely, implantation and parturition.

Implantation involves a direct interaction between embryonic trophoplast and maternal uterine tissue. In rat, blastocyte transplantation depends mainly upon hormonal conditioning of the uterus. The uterus is in receptive state for about 12 hours during the late fourth to fifth day of pregnancy. To be receptive, the uterine endometrium must be exposed to progesterone for a minumum of 48 hrs, and estrogen must be present at the end of this period. Not only the maternal hormonal state is important to the blastocyte but other biological factors produced by the blastocyte such as steroid hormones, histamine, uterine fluid proteinases and surface charges of the blastocytes have been also implicated in the implantation process. Moreover, prostaglandins play vital roles in blastocytes implantation process.

It is reported that prostaglandins and their receptors were detected in the uterus and traphoblasts prior to blastocytes implantation and remained detectable for 5-6 days after blastocytes attachments.[15, 16] Therefore, it is expected that meloxicam might affect implantation due to its action on prostaglandins biosynthesis. The results of this investigation showed that meloxicam inhibited implantation in rats at a dose- and time-dependent manner. Similar findings were also observed by Matsuo et al (1997).[17, 18]

Parturition, the physiological process by which the fetus is born at term, because of the continuous rhythmic contraction of muscle uterus. This process is affected mainly by hormonal and mechanical factors. Uterine contraction is influenced by oxytocin (either maternal or fetal origin), released at higher concentration level by fetal membranes at time of labor. Generally, prostaglandins induce labor by exerting a contractile effect on the myometrium.[19] Dong et al (1996) reported that the rat uteri during labor, at term, demonstrated a 217% increase in prostaglandin E2 compared with day 18 of pregnancy. [20] Therefore, inhibition of prostaglandins by meloxicam is expected to induce uterine relaxation and a delay in parturition. Further, it is known that oxytocin is responsible for the induction of labor at the time of parturition. However, the effect of this hormone is correlated with prostaglandins that stimulate the induction of oxytocin receptors. [20, 21] Thus, meloxicam delays delivery by decreasing the myometrial sensitivity to oxytocin rather than decreasing oxytocin release itself. [24] This explains the delay in the onset of delivery in rats treated with meloxicam although oxytocin release is not inhibited.

The clinical implications of meloxicam effects on implantation and parturition in human await further investigations. For example, meloxicam could be clinically employed if given in proper dose and proper time to prevent early implantation after unwanted pregnancy.

On the other hand, meloxicam could be used in proper doses for the treatment of preterm labor without serious maternal or fetal side effects.[25]
In conclusion, the results of this investigation clearly demonstrate that meloxicam possesses the potential to inhibit rat implantation and parturition in time and dose dependent manner.

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