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Evaluation of the contraceptive effects of carprofen, flunixin meglumine and meloxicam in rats

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ABSTRACT: The objective of this study was to determine the suitability of carprofen, flunixin meglumine and meloxicam for use in emergency contraception. Forty-eight pregnant Sprague-Dawley rats were used as material. Five groups were subjected to treatments while one group served as a control. The numbers of animals in each group were equal (n = 8). Treatment groups were administered carprofen (10 mg/kg, single or double dose, *s.c.*), flunixin meglumine (5 mg/kg, single or double dose, *i.m.*) and meloxicam (2 mg/kg, a single dose, *s.c.*) on the third day after mating. The control group received saline. The rats were sacrificed on Day 7 of gestation. Luteal spots and implantation sites were recorded. Pre-implantation loss was calculated by subtracting the number of luteal spots from the number of implantation sites. Compared with the control, the administration of flunixin meglumine (double dose), carprofen (double dose) and meloxicam highly significantly decreased the implantation rate ($P < 0.001$). Single dose administration of flunixin meglumine and carprofen led to significant decreases ($P < 0.01$). In conclusion, this study indicates that carprofen, flunixin meglumine and meloxicam treatment cause a decline in implantation rate in rats.

Keywords: cox inhibitors; embryo implantation; NSAIDs; luteal spots; pregnancy

Prevention of unwanted pregnancy is very important in pet animals and contraception is a commonly used method for this purpose. Cyclooxygenase enzyme (COX) inhibitors are non-steroid anti-inflammatory drugs (NSAIDs); the contraceptive effects of COX inhibitors have recently been discovered (Eilts 2002; Jewgenow et al. 2006; Wiebe and Howard 2009; Goericke-Pesch 2010). The inhibition of the COX enzymes prevents the formation of prostaglandins that cause pain and fever. The COX enzyme is not a single molecule; there are different isoforms that perform different tasks. COX-1 is found in the stomach, intestine, kidneys and platelets while COX-2, considered as the inducible form, is produced by macrophages, synoviocytes, chondrocytes, osteoblasts, and endothelial cells (Crofford 1997; Vane and Botting 2003). While classical NSAIDs inhibit both enzymes, COX-2 inhibitors inhibit only the inducible COX-2 and exhibit anti-inflammatory effects in the gastrointestinal tract and other tissues (Isakson 2003; Botting 2006; Rao and Knaus 2008). Chandrasekharan et al. (2002) identified COX-3, an enzyme sensitive to inhibition by acetaminophen, phenacetin, antipyrine and dipyrone. The enzyme was identified in the canine cerebral cortex and its inhibition may represent a primary central mechanism of analgesic and antipyretic drugs.

Studies have been conducted to determine the impact of COX inhibitors on reproduction. The effects of these enzymes include inhibition of ovulation, prevention of implantation as well as adhesion after surgery and the inhibition of premature labour (Muzii et al. 1998; Salhab et al. 2001; Slattery et al. 2001; Shafiq et al. 2004; Botting 2006; Gaytan et al. 2006).

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Numerous studies have been carried out to determine the importance of the COX enzyme in implantation. During implantation in mice it was determined that COX-2 was produced by the luminal epithelium and stroma of the uterus surrounding the blastocyst. This suggests that COX-2 plays an important role in implantation (Chakraborty et al. 1996; Reese et al. 1999). Reese et al. (1999) determined that the fertility and number of offspring of female mice with COX-1 enzyme deficiency are normal. The deficiency in COX-1 enzyme is compensated for by COX-2. However, COX-2-deficient female mice are infertile due to the fact that ovulation, fertilisation, implantation and decidualization processes are impaired (Lim et al. 1997).

No clinical studies are available regarding the preventive effects of flunixin meglumine and carprofen on implantation. We hypothesized that flunixin meglumine, carprofen and meloxicam may reduce the implantation rate in rats. Therefore, the objective of this study was to assess the contraceptive effect of flunixin meglumine and carprofen in a rat model. The contraceptive efficacy of meloxicam has been proven in previous studies (Jaffal et al. 2006) and it was used as a positive control here.

MATERIAL AND METHODS

Animal material. Forty-eight female Sprague-Dawley rats, approximately three months of age and weighing 150–200 g, were used as material for the study. Permission was obtained from the Animal Experimentation Ethics Board of Ataturk University (Decision number: 113).

This study was carried out at the Ataturk University Medical Experimental Research and Application Center (ATADEM). The rats were caged one week before the experiment for acclimatisation and were housed in transparent cages manufactured from polycarbonate material. Feed and water were given *ad libitum*. Sawdust was used as litter. The animals were kept at ambient room temperature (22 ± 2 °C) with light for 12 h and 12 h of darkness.

Experimental design. The female rats were placed with male rats of proven fertility (one male and two females) in the same cage for two days. After the female rats had mated, they were removed and placed into separate cages. The female rats were separated into six groups (n = 8). The following was administered on the third day after the mating: Group I – Carprofen (Rimadyl, Pfizer, UK, 50 mg/ml) (10 mg/kg, single dose) was given *s.c.* Group II – Carprofen (10 mg/kg, double dose at 12 h intervals) was injected *s.c.* Group III – Flunixin meglumine (Flumeglin, Teknovet, Turkey, 50 mg/ml) (5 mg/kg, single dose) was given *i.m.* Group IV – Flunixin meglumine (5 mg/kg, double dose at 12 h intervals) was injected *i.m.* Group V – Meloxicam (Ekomel, Ekomed, Turkey, 5 mg/ml) (2 mg/kg, single dose) was given *s.c.* Group VI – This group received saline on the third day and served as control.

The rats were euthanized on the seventh day post mating and the luteal spots in the ovary and the implantation sites in the uterus were counted. The number of possible implantation sites was deducted from the number of luteal spots to determine the pre-implantation losses.

Statistical analysis. The statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, USA). The number of luteal spots and implantation sites in the groups were compared using the Kruskal-Wallis test, and group comparisons were performed using the Mann-Whitney *U*-test. Pregnancy rates were compared using Fisher's exact test because data distribution was not suitable for the chi-squared test. *P*-values of less than 0.05 were considered significant.

RESULTS

There were no significant differences in the number of luteal spots between the groups (Table 1). While a decrease in the average number of implantations was observed in all the treatment groups after drug administration compared to control, significant losses were observed only in the rats which had been administered double doses of flunixin meglumine and carprofen as well as rats which had been administered a single dose of meloxicam (Table 1). It was determined that seven of the rats administered a single dose of carpofen became pregnant while only three of those administered with double doses became pregnant. Five rats from the group administered a single dose of flunixin meglumine became pregnant while only three rats became pregnant after being administered a double dose. Only three animals out of the group injected with meloxicam became pregnant. All animals in the control group became pregnant (Table 2).

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Table 1. Effects of carprofen, flunixin meglumine and meloxicam in rats before implantation (mean ± SEM)

Groups	*n*	Luteal spots	Implantation sites
Group I	8	12.25 ± 0.88	7.75 ± 1.56[ab]
Group II	8	13.50 ± 0.82	1.87 ± 1.12[b]
Group III	8	12.00 ± 0.70	7.12 ± 2.12[ab]
Group IV	8	12.25 ± 1.03	1.00 ± 0.50[b]
Group V	8	12.63 ± 0.49	3.00 ± 1.51[b]
Group VI	8	11.38 ± 0.86	11.38 ± 0.86[a]
P		ns	< 0.05

ns = not significant

[a,b]Means in the same columns with different letters are significantly different

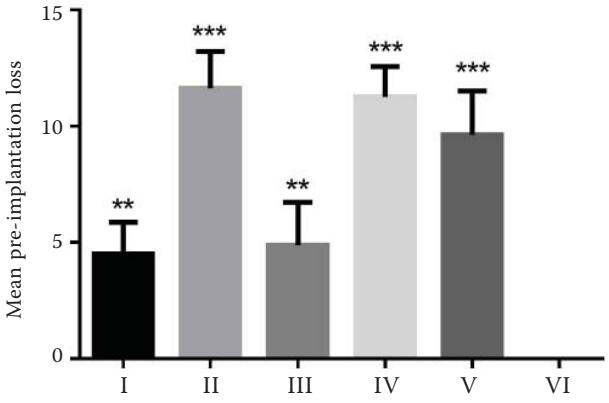

Figure 1. Comparison of pre-implantation losses in the Groups I–VI (results are expressed as mean ± SEM)
$^{**}P < 0.01$, $^{***}P < 0.001$

The implantation losses of the groups were found to be statistically significant using the Kruskal-Wallis test ($P < 0.001$). When these data were analysed in the Mann-Whitney *U*-test, it was determined that the losses, particularly in the groups administered double doses of flunixin meglumine, carprofen and a single dose of meloxicam, were highly significant ($P < 0.001$) when compared with the control group. In comparison with the control group, significant losses were also observed in groups administered single doses of carprofen and flunixin meglumine ($P < 0.01$; Figure 1).

DISCUSSION

The most commonly used drugs for contraceptive purposes in animals are anti-progestins, prolactin inhibitors, progestagens, prostaglandins, progesterone synthase inhibitors, GnRH agonists, androgens and melatonin implants. Some of these drugs have not been licensed in all countries (Deslorelin, GnRH agonist, Suprelorin®), while others are available in pharmacies as human medicine and are very expensive (Leuprolide, GnRH agonist, Lupron®). Cox inhibitors are emerging as a new option since these drugs are both inexpensive as well as widely available (Gobello 2006; Wiebe and Howard 2009; Goericke-Pesch 2010). We, therefore, set out to investigate the contraceptive effects of flunixin meglumine, carprofen and meloxicam.

Table 2. The pregnancy rates of the groups on Day 7

Groups	*n*	Pregnancy (%)
Group I	8	87.5[a]
Group II	8	37.5[b]
Group III	8	62.5[a]
Group IV	8	37.5[b]
Group V	8	37.5[b]
Group VI	8	100[a]
P		< 0.05

[a,b]Pregnancy rates with different superscripts in the same column are significantly different

In a study carried out by Sookvanichsilp and Pulbutr (2002) the effectiveness of indomethacin and celecoxib in preventing implantation in rats was investigated. The researchers administered indomethacin (2.5 or 5 mg/kg daily) and celecoxib (40, 80 or 160 mg/kg daily) to the rats 3–5 days after mating. The rats were killed on the eighth day of the presumed pregnancy and the implantation sites were counted. The researchers reported that no rats administered with 160 mg/kg of celecoxib developed implantations but implantation developed in all the rats in the control group as well as those administered with 40 mg/kg of celecoxib. Indomethacin and celecoxib caused implantation losses in the other groups in a dose-dependent manner. Our findings were similar to the work of Sookvanichsilp and Pulbutr (2002) in terms of implantation rates; we observed maximum losses in groups administered with double doses of carprofen and flunixin meglumine.

Pre-implantation losses in rats administered with indomethacin, nimesulide and celecoxib were studied by Shafiq et al. (2004). During Days 1–7 of pregnancy rats were repetitively administered a daily dose of 2.5 and 10 mg/kg of indomethacin, 10 and 40 mg/kg of nimesulide and 10 and

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40 mg/kg doses of celecoxib in the form of gavage. In comparison with the control group, significant pre-implantation losses were observed in the groups administered with 10 mg/kg of indomethacin, 40 mg/kg of nimesulide and 40 mg/kg of celecoxib. When comparing the results obtained by Shafiq et al. (2004) and Sookvanichsilp and Pulbutr (2002), difference are evident in implantation loss in response to 40 mg/kg celecoxib. Treatment duration was three days in Sookvanichsilp and Pulbutr (2002), and seven in Shafiq et al. (2004). Thus, the reason for the difference in implantation loss might be the longer duration of treatment in Shafiq et al. (2004). Our findings are in agreement with that study and indicate that pre-implantation losses in groups administered high doses of carprofen and flunixin meglumine were high.

In the study of Salhab et al. (2001) rabbits were administered a single intraperitoneal dose of meloxicam at four different concentrations (20, 10, 5, or 2.5 mg/kg meloxicam) 2, 5, 8 or 24 h after mating. Their results indicated that the pregnancy rates had decreased at all times and with all dosages. Furthermore, pregnancy was completely prevented in all animals after administration of meloxicam (20 mg/kg). Another study by Salhab et al. (2003) reported that the oral administration of 20 mg/kg meloxicam to rabbits 5 h after mating achieved a 100% contraceptive effect. The researchers reported that a 62.5% contraceptive effect was achieved with vaginal administration of meloxicam.

In conclusion, it was observed that treatment with carprofen, flunixin meglumine and meloxicam may be effective in preventing implantation a few days after mating. These effects were dependent on the dosage, i.e., the contraceptive effect was higher in groups that were administered larger amounts of drugs. We conclude that further research into the impact of these drugs on implantation in both domestic and laboratory animals would be beneficial.

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