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Rat ovulation, implantation and decidualization are severely compromised by COX-2 inhibitors

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1. ABSTRACT

Although Cyclooxygenase-2 (COX-2) is essential for mouse ovulation, fertilization, implantation and decidualization, the regulation and function of COX-2 in rat reproduction are still unknown. This study was designed to examine the action of COX-2 in rat ovulation, implantation and decidualization by using two specific inhibitors of COX-2 (nimesulide and niflumic acid). Compared to control, either nimesulide or niflumic acid significantly inhibited the ovulation in the superovulated rats. Although nimesulide had no obvious effects on the number of implantation sites and the vascular permeability, the expression of PPARdelta, HB-EGF and vimentin proteins was down-regulated in the nimesulide-treated groups. COX-1 protein was upregulated by nimesulide treatment. Nimesulide also had an inhibitory effect on decidualization during early pregnancy and under artificial decidualization. Moreover, nimesulide caused the increase of the gestation period and the reduction of litter size and birth weight compared to controls. Based on our data, rat implantation and decidualization were delayed by nimesulide treatment, resulting in the reduction of litter size and birth weight and the prolongation of gestational length, suggesting that COX-2 plays an important role in implantation and decidualization.

2. INTRODUCTION

Prostaglandins (PGs) are considered to be proinflammatory and play important roles during female reproduction (1,2). Cyclooxygenase (COX) is a rate-limiting enzyme that produces PGs from arachidonic acid and includes two isoforms, COX-1 and COX-2. COX function in mice has been demonstrated with gene knockout experiments. COX-1-deficient females are fertile with specific parturition defects, whereas COX-2-deficient females are infertile with abnormalities in ovulation, fertilization, implantation and decidualization (2-4). In another study, COX-2-deficient mice did not show any abnormality in embryo implantation except for the reduction in the number of ovulated and fertilized eggs, and a delay in decidual growth (5). Recently, Wang et al (6) reported that the reproductive defects of COX-2-mutant mice mainly depend upon genetic background.

COX-2 immunostaining was also strongly detected in the subluminal stroma when the attachment reaction began in rat uterus. At the implantation site on day 6, COX-2 immunostaining was detected in the stromal cells, but not in the primary decidual zone at the anti-mesometrial side (7). To date, COX-2-deficient rats are still not available for this kind of study. Although COX-2(-/-)

mouse is very powerful in understanding the physiological functions, the early lethality often caused a large problem for further study on late development. Thus, the use of a COX-2 inhibitor at specific times during pregnancy should have less severe effects than the total absence of activity throughout the life span as occurs in COX-2 null mice (8).

Although there are several reports on the effects of COX inhibitors in rats, the results remain controversial. In Wistar rats, indomethacin, nimesulide or celecoxib caused the increase of preimplantation and postimplantation loss, and gestation length (9). Sookvanichsilp and Pulbutr (10) reported that indomethacin at a dose of 5 mg/kg/day as well as celecoxib at doses of 80 and 160 mg/kg/day significantly reduced the proportion of pregnant rats. However, Kennedy (11) showed that indomethacin had no significant effect on the mean number of implantation sites when indomethacin was given s.c. at a dose of 1 mg/rat twice on day 5 of pregnancy. Poyser (12) reported that NS-398 (a COX-2 inhibitor) at a s.c. dose of 12 mg/kg/day on days 3-4 of pregnancy failed to inhibit implantation in rats. It is possible that the dose of NS-398 in that study was still too low to inhibit COX-2 activity adequately in the rat uterus.

Both nimesulide and niflumic acid are selective inhibitors for COX-2 (13,14). The aim of this study was to examine whether COX-2 is important for ovulation, implantation and decidualization in the rat. Specific inhibitors of COX-2 (nimesulide and niflumic acid) were used in this study to inhibit COX-2 action.

3. MATERIALS AND METHODS

3.1 Animals

Mature rats (Sprague-Dawley strain) were caged in a controlled environment (14 hr light: 10 hr dark). All animal procedures were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University.

3.2 Superovulation

Because of irregularities in rat estrous cycles, a standard protocol of superovulation was used to compare ovulation potential of the drug-treated rats with that of the vehicle treated controls as described in the mouse (8). Female rats at 24-26-days old were superovulated with 40 IU eCG, followed by 40 IU hCG 48 h later. These rats were injected i.p. with different dosages of nimesulide or niflumic acid (Cayman Chemical, Ann Arbor, MI, USA) in a volume of 0.2 ml 30 min before hCG injection. The oocytes were collected from the oviducts 24 h after hCG injection and counted. Because immature rats show ovulatory defects (15), sexual mature rats were also used for superovulation.

3.3. Early pregnancy and pseudopregnancy

Female rats were mated with fertile males of the same strain to induce pregnancy. Pregnancy was confirmed by examining the spermatozoa in vaginal smear (day 1 = day of vaginal sperm positive). The implantation sites on days 6-7 of pregnancy were identified by intravenous injection of 1 ml of 1% (w/v) Chicago blue solution in 0.85% (w/v) NaCl. Pseudopregnancy was induced by co-caging female rats with vasectomized males and confirmed by checking vaginal plug (either in the vagina or on the cage floor) next morning.

3.4. Artificial decidualization

Artificial decidualization was induced by intraluminally infusing 100 μl of sesame oil into one uterine horn on day 5 of pseudopregnancy, whereas the contralateral, uninjected horn served as a control. The uteri were collected 96 h after the injection of sesame oil (i.e., on day 9 of pseudopregnancy).

3.5. Measurement of vascular permeability of rat implantation sites

Vascular permeability was assayed as described by Satchi-Fainaro et al. (16). Briefly, the implantation sites on day 6 were identified by intravenous injection of 1 ml of 1% (w/v) Chicago blue solution in 0.85% (w/v) NaCl. The same weight of implantation sites were collected in 500 μl formamide. The optical density at 620 nm was measured after 120 h extraction.

3.6. Treatments with COX-2 inhibitors

COX-2 inhibitor, either nimesulide or niflumic acid (Cayman Chemical, Ann Arbor, MI, USA) was dissolved in DMSO and diluted to proper concentrations in 0.2 ml of DMSO for each injection/rat. The same amount of DMSO was injected *i.p.* for controls. The dosages of nimesulide and niflumic acid used in this study were based on previously described (9,17,18).

3.7. Immunohistochemistry

Rat uteri were immediately fixed in Bouin's solution for 24 h, dehydrated and embedded in paraffin wax. Immunostaining was performed as described (19). Briefly, nonspecific binding was blocked with 10% (v/v) normal horse serum in PBS at 37 °C for 1 h. The sections were incubated with rabbit anti-human vimentin, goat anti-human PPARδ, goat anti-human HB-EGF or goat anti-human COX-1 (Santa Cruz Biotechnology, Inc., CA) in horse serum at 4 °C for 16 h. The sections were then incubated with biotinylated goat anti-rabbit IgG or rabbit anti-goat IgG followed by a streptavidin-alkaline phosphatase complex and Vector Red according to the manufacturer's protocol (Vectastain ABC-AP kit, Vector Laboratories, Burlingame, CA). Vector Red was visualized as a red color. Endogenous alkaline phosphatase activity was inhibited by supplementing 1 mM levamisole (Sigma) into Vector Red substrate solution.

3.8. Statistical Analysis

At least 3 rats were used in each stage or treatment in this study. Quantitative data were expressed as mean ± SEM and analyzed by SPSS software. Statistical significance was calculated using Student's two-tailed, unpaired t-test.

4. RESULTS

4.1. Effects of COX-2 inhibitors on rat ovulation

In order to examine the effects of COX-2 inhibitors on rat ovulation, superovulated rats at 24-26-days

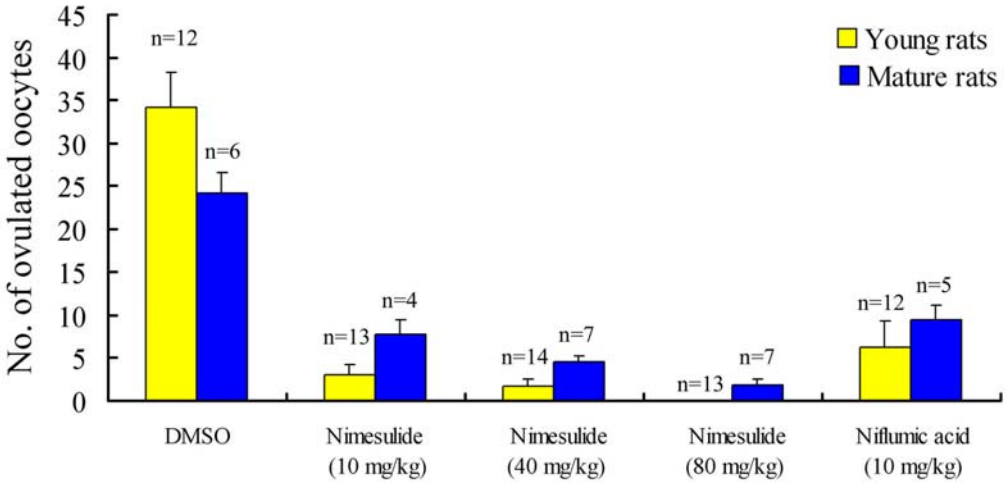

Figure 1. Female rats were superovulated with 40 IU eCG, followed by 40 IU hCG 48 h later. These rats were injected i.p. with nimesulide or niflumic acid 30 min before hCG injection. The oocytes were collected from the oviducts 24 h after hCG injection and counted. Sexual mature rats (designated as mature rats) and 24-26 days old rats (designated as young rats) were used in this study, respectively. Ovulation was significantly inhibited by either nimesulide or niflumic acid compared to controls. The number of rats used in each treatment was shown in each bar. Values are presented as the mean ± SEM.

old were treated i.p. with different doses of nimesulide or niflumic acid 30 min before hCG injection. The oocytes were collected from the oviducts 24 h after hCG injection, counted and shown in Figure 1.

In control group, the number of ovulated oocytes was 34.25±4.01 per rat. When the superovulated rats were treated with three doses of nimesulide, there were 3.07±1.13 (10 mg/kg), 1.71±0.87 (40 mg/kg), and 0 (80 mg/kg) ovulated oocytes, respectively. In order to further verify the specificity of nimesulide on ovulation, the superovulated rats were treated with niflumic acid (10 mg/kg), another COX-2 specific inhibitor, in which the number of ovulated oocytes was 6.25±3.02.

Because immature rats were showed to have ovulatory detects (15), sexual mature rats were also used for superovulation. Similarly, the ovulation in sexual mature rats were also significantly inhibited by treating with nimesulide or niflumic acid (Figure 1). We found that ovulation was significantly inhibited by either nimesulide or niflumic acid compared to controls.

4.2. Effects of nimesulide on rat embryo implantation

Since attachment reaction already occurred in the morning on day 5 of pregnancy in the rat, the pregnant rats in the day 4 evening (20:00) were used to examine whether COX-2 inhibitors have any effects on attachment reaction. Compared to controls, nimesulide had no obvious effects on the number of implantation sites (Figure 2A).

In order to check the effects of nimesulide on implantation, pregnant rats were treated with nimesulide (40 mg/kg, i.p.) daily from day 5 of pregnancy. After these treated rats were sacrificed 24 h after last injection on days 6, 7, 8, and 9 of pregnancy, respectively, the number of implantation sites was evaluated (Figure 2B). Compared to control groups, there were no significant differences for implantation sites in the nimesulide-treated groups on days 6, 7 and 9, respectively. Nevertheless, the number of implantation sites in the nimesulide-treated group was 11.40±0.68 on day 8 of pregnancy, which was significantly less than control group (13.14±0.67).

4.3. Effects of nimesulide on vascular permeability

Endometrial vascular permeability has been considered as a marker for embryo implantation (20). Because prostaglandins play a key role in endometrial vascular permeability (21-23), vascular permeability was measured as described by Satchi-Fainaro et al (16). Compared to the vascular permeability of the implantation sites on day 6 of normal pregnancy or DMSO treatment, there were no significant changes in vascular permeability after pregnant rats were treated with twice on day 4 (20:00) and Day 5 (08:00), or once on day 5 (08:00) (Figure 3).

4.4. Effects of nimesulide on the expression of implantation-related proteins

Vimentin is a molecular marker for stromal cells and decidualization (24). On day 6 of pregnancy, vimentin immunostaining was strongly seen in the subluminal stroma at implantation site in the control (Figure 4A), but not detected in the uterus of the nimesulide-treated group (Figure 4B).

PPARdelta has been shown to play a key role in mouse implantation (25). After pregnant rats were treated with nimesulide once on day 5, uterine expression of PPARdelta protein was examined on day 6. On day 6 of pregnancy, PPARdelta immunostaining was strongly seen in a larger area of subluminal stroma surrounding the implanting blastocyst in the control (Figure 4C), but was only seen in a smaller area of subluminal stroma

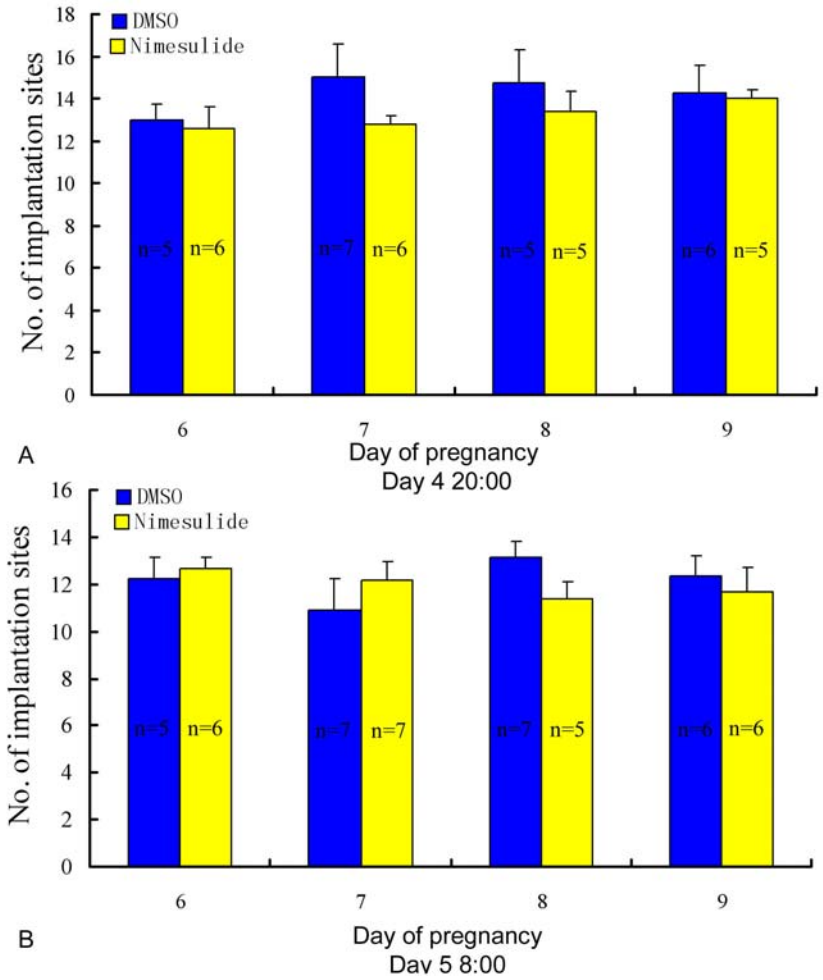

Figure 2. Effects of nimesulide on rat implantation. (A) Pregnant rats were first treated with nimesulide (40 mg/kg, i.p.) in the evening (20:00) of day 4 and once daily (08:00) from day 5 of pregnancy. (B) Pregnant rats were first treated with nimesulide (40 mg/kg, i.p.) once daily (08:00) from day 5 of pregnancy. After these treated rats were sacrificed 24 h after last injection on days 6, 7, 8, and 9 of pregnancy, respectively, the number of implantation sites was evaluated. Compared to control groups, there were no significant differences for implantation sites in the nimesulide-treated groups on days 6, 7 and 9, respectively. Nevertheless, the number of implantation sites in the nimesulide-treated group on day 8 of pregnancy was significantly less than control group. The number of rats used in each treatment was shown in each bar. Values are presented as the mean ± SEM.

surrounding the implanting blastocyst in the nimesulide-treated group (Figure 4D).

Because HB-EGF is specifically expressed in the luminal epithelium during the apposition phase in mouse uterus (26), uterine expression of HB-EGF protein was examined following nimesulide treatment. On day 6 of pregnancy, HB-EGF immunostaining was strongly detected in the luminal epithelium of implantation site in the control (Figure 4E), but weakly seen in the nimesulide-treated group (Figure 4F).

Because there was an up-regulation of COX-1 expression in COX-2 deficient mice of CD-1 background (6), COX-1 immunostaining was examined to see whether COX-1 is up-regulated following nimesulide treatment. Indeed, COX-1 immunostaining was only seen in the luminal epithelium of implantation site in the control. After pregnant rats on day 5 were treated with nimesulide, COX-1 immunostaining was detected in both luminal epithelium and the subluminal stroma surrounding the implanting blastocyst (Figure 5).

4.5. Effects of nimesulide on rat decidualization

In order to examine whether nimesulide treatment before attachment reaction has any effects on following decidualization, pregnant rats were first injected i.p. with nimesulide (40 mg/kg) in the evening (20:00) of day 4 and then injectd daily from day 5 of pregnancy. Compared to controls, the weights of implantation sites on days 7, 8 and 9 of pregnancy were also significantly reduced by nimesulide treatments (Figure 6A).

In order to see the effects of nimesulide on decidualization, pregnant rats were injected i.p. with nimesulide (40 mg/kg) daily from day 5 of pregnancy. The

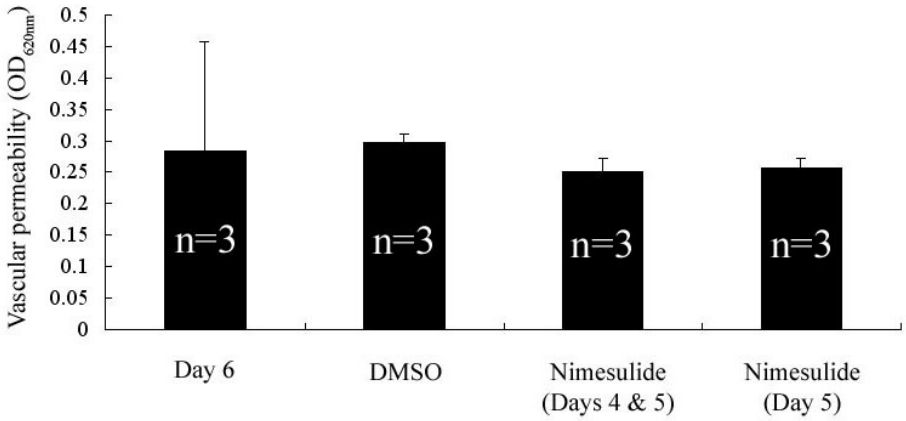

Figure 3. Vascular permeability of implantation sites. The optical density at 620 nm was shown as mean ± SEM. The value on day 6 of normal pregnancy was measured as a reference. The vascular mermeability was measured on day 6 of pregnancy after pregnant rats were treated with DMSO for control, nimesulide on days 4 and 5, and nimesulide on day 5, respectively. The number of rats used in each treatment was shown in each bar. Values are presented as the mean ± SEM.

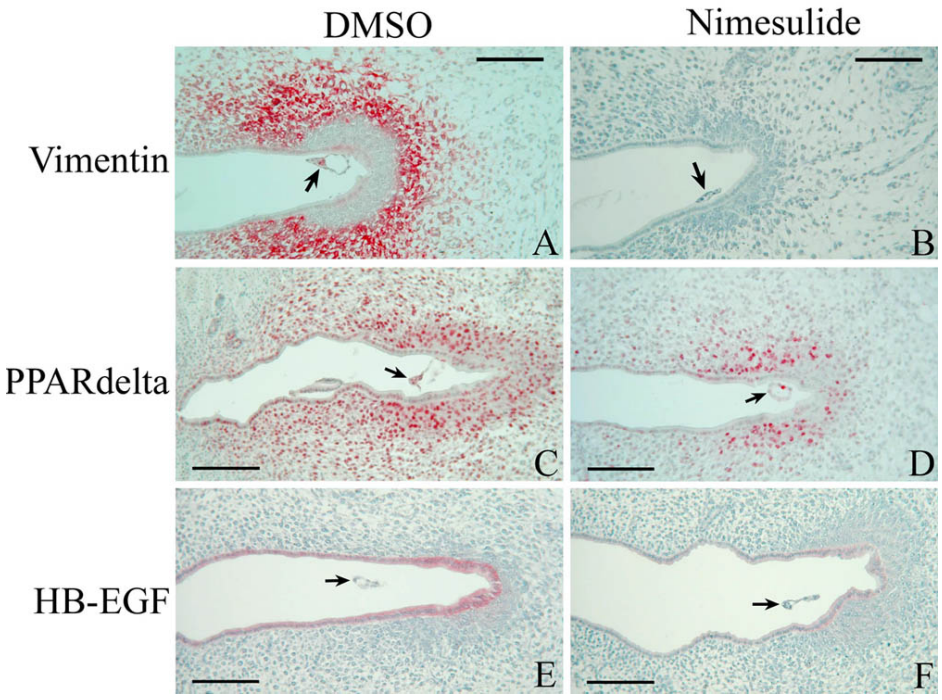

Figure 4. Vimentin, PPARdelta and HB-EGF immunostaining on day 6 of pregnancy after pregnant rats were treated with either vehicle (DMSO) or nimesulide (40 mg/kg, i.p.) on day 5 of pregnancy for 24 h. Arrow: Embryo; Bar=80 μm.

weight of implantation sites was examined on days 7, 8 and 9 of pregnancy, respectively (Figure 6B). After pregnant rats were treated with nimesulide, the weights of implantation sites were significantly reduced on days 7, 8 and 9 of pregnancy compared to control groups. On day 9 of pregnancy, the weight of implantation site in thenimesulide-treated group (21.74±0.57) was only similar to that on day 7 of pregnancy in the control (19.14±0.26). The sizes of the implantation site on days 8 and 9 of pregnancy were much smaller than that in the controls, respectively (Figure 6C).

4.6. Effects of COX-2 inhibitors on artificial decidualization

In order to check the effects of nimesulide or niflumic acid on artificial decidualization, pseudopregnant rats were treated with nimesulide or niflumic acid (40 mg/kg) daily for 4 days, beginning from day 5 of pseudopregnancy 30 min before the intrauterine injection of oil. The weights of the uterine horns with oil injection were examined on day 9 of pseudopregnancy. We found that either nimesulide or niflumic acid could significantly reduce the weights of each uterine horn compared to

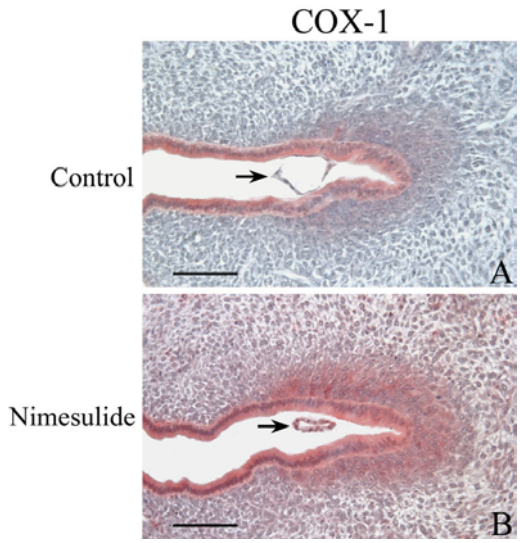

Figure 5. COX-1 immunostaining on day 6 of pregnancy after pregnant rats were treated once with vehicle (DMSO) or nimesulide (40 mg/kg, i.p.) on day 5 for 24 h. Arrow: Embryo; Bar=60 μm.

controls. In the control group, the weight of each uterine horn was 1216.6±71.1 mg. When these rats were treated with nimesulide and niflumic acid, the weights of the uterine horn with oil injection significantly decreased to 296.66±35.5 and 466.9±64.4 mg, respectively (Figure 7A). From uterine morphology, the size of uterine horn under artificial decidualization was much bigger than that in the control (Figure 7B).

4.7. Effects of COX-2 inhibitors on rat gestational length and birth weight

Since nimesulide was shown to have significantly inhibitory effects on rat decidualization in this study, the effects of nimesulide on litter size, birth weight and gestational length were further examined after pregnant rats were treated once with nimesulide (40 mg/kg, i.p.) on day 5 or twice on both days 5 and 6 of pregnancy. Compared to control, the litter size was not significantly different in the nimesulide-treated group on day 5, but was significantly less in the nimesulide-treated group on both days 5 and 6 (Figure 8A). The birth weight was significantly less in both nimesulide-treated groups compared to control (Figure 8B). The gestational length was 22.44±0.18 days in the control. After pregnant rats were treated with nimesulide once or twice, the gestational lengths were 23.11±0.11 and 23.71±0.29 days, respectively, which were significantly longer than control (Figure 8C).

5. DISCUSSION

5.1. COX-2 action in rat ovulation

Because of irregularity in rat estrous cycles, superovulation was used to examine the effects of nimesulide on rat ovulation in our study. In both 24-26 days old and sexual mature rats, the number of ovulated oocytes was significantly reduced by either nimesulide or niflumic acid compared to control. The role of prostaglandins in ovulation was first suggested by Orczyk and Behrman (27). NS-398, another COX-2 inhibitor, also caused a reduction of ovulation and prostaglandin production (28). When mice were treated with celecoxib (600 mg/kg/dose) for 4 days before the induction of superovulation, a smaller number of recovered eggs and fewer fertilized eggs were obtained compared to the vehicle-treated females (8). The action of COX-2 in ovulation was also confirmed in COX-2-deficient mice, in which ovulation and fertilization were severely compromised (2-4). Gonadotropin stimulation and simultaneous treatment with PGE2 or interleukin-1beta resulted in ovulation of COX-2(-/-) mice comparable to that in COX-2(+/+) (29). mPGES-1, the key enzyme for PGE2 synthesis, was highly expressed in granulose cells of mouse ovary and induced by hCG treatment (30). The induction of COX-2 in rats was very rapid, 2–4 h after hCG treatment, and preceded ovulation by 10 h (31). COX-2 was considered as a potential regulator of the mammalian ovulatory clock (32,33). Our data showed that COX-2 is essential for rat ovulation as in the mouse. Recently, it was suggested that nonsteroidal anti-inflammatory drugs (NSAIDs) and selective cyclooxygenase-2 inhibitors may interfere with ovulation and the rupture of the follicle, causing reversible infertility (34,35). Meloxicam, a COX-2 inhibitor, resulted in a reversible delay of ovulation, an increase in follicular diameter, and a decrease in plasma progesterone level (36). These data suggest that COX-2 inhibition may have a potential for contraceptive use.

5.2. COX-2 action on rat implantation

In this study, nimesulide had no significant effects on the number of implanted embryos and vascular permeability when nimesulide was initially treated in the evening (20:00) of day 4 (before attachment reaction) or in the morning (08:00) of day 5 (after attachment reaction). In the mouse uterus on day 5 of pregnancy, PPARdelta and HB-EGF are specifically expressed at implantation sites and essential for implantation (25,26,37). PPARdelta was also highly expressed at implantation sites in rat uterus (38). In our study, the intensities of PPARdelta and HB-EGF immunostaining were severely declined on day 6 of pregnancy following nimesulide treatment. mPGES-1 is also highly expressed at implantation site in the mouse and rat (7,19). In our previous study, mPGES-1 protein expression was completely inhibited when pregnant rats on day 5 were treated with nimesulide for 24 h (7). This suggests that molecular changes already happened before the effects on the number of implantation sites and vascular permeability became noticeable. In COX-2(-/-) female mice, there are severe deficiencies in mouse implantation and decidualization at a C57BL/6J/129 genetic background (2). However, COX-2-knockouted mice in another genetic background only show an approximately 24 h delay in the initial rate of decidual growth after implantation compared to that in heterozygous or wild-type recipients (5). Recently, Wang et al (6) indicated that the deficiency in COX-2 null mice at CD-1 background could be rescued by COX-1 overexpression, but not at C57BL/6J/129 background. In this study, COX-1 expression was indeed up-regulated after nimesulide treatment at a background of Sprague-Dawley strain. It has been shown that COX-2 inhibition in COX-1-deficient mice will lead to complete

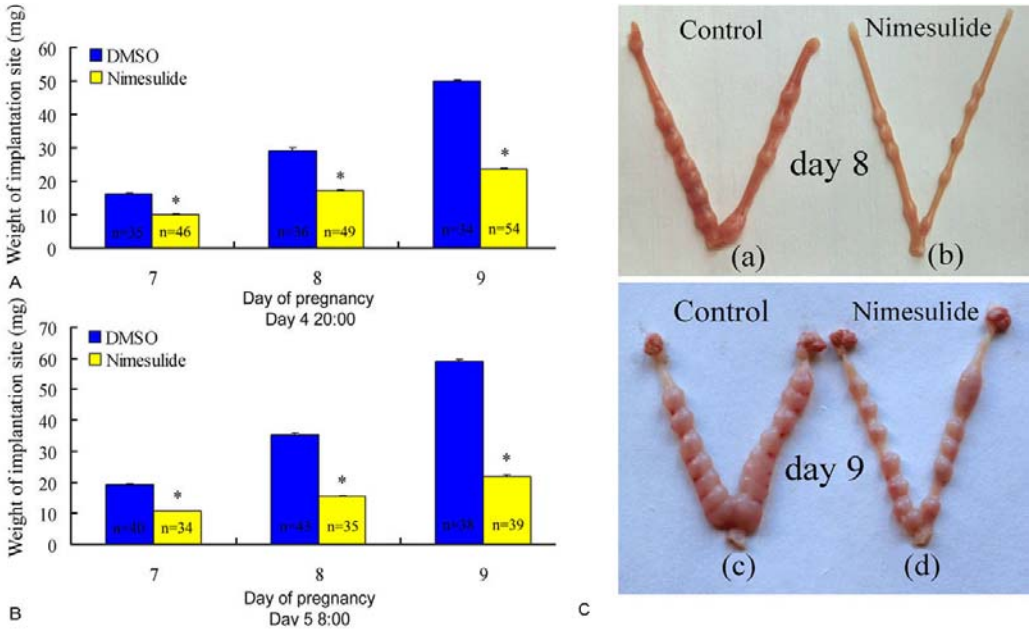

Figure 6. Effects of nimesulide on rat decidualization. (A) The weights of implantation sites on days 7, 8 and 9 of pregnancy after pregnant rats were first treated with nimesulide (40 mg/kg, i.p.) in the evening (20:00) of day 4 and once daily (08:00) from day 5 of pregnancy, respectively. (B) The weights of implantation sites on days 7, 8 and 9 of pregnancy after pregnant rats were injected i.p. with nimesulide (40 mg/kg) once daily from day 5 of pregnancy, respectively. (C) Uterine morphology on days 8 and 9 of pregnancy in the controls and nimesulide-treated groups from day 5 of pregnancy. The weights of implantation sites were significantly reduced on days 7, 8 and 9 of pregnancy in the nimesulide-treated groups compared to control groups. The number of implantation sites weighed in each treatment was shown in each bar. Values are presented as the mean ± SEM.

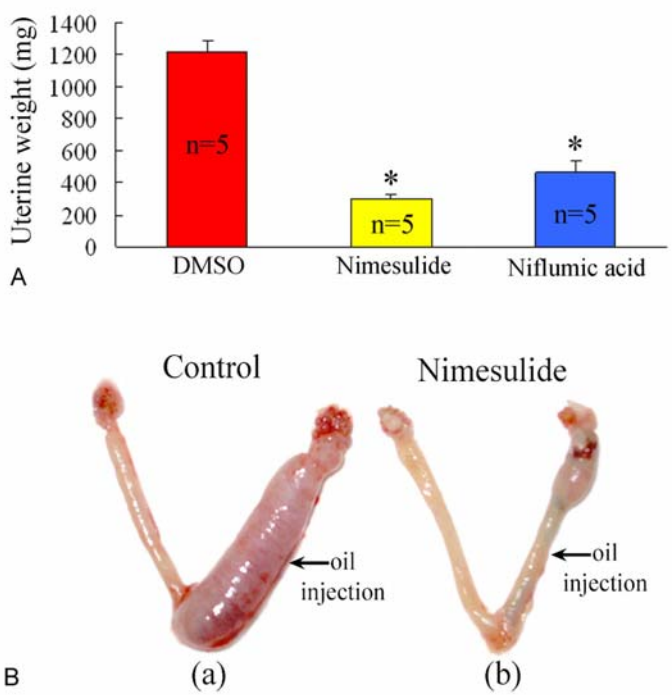

Figure 7. Effects of COX-2 inhibitors on artificial decidualization. Pseudopregnant rats were treated with nimesulide or niflumic acid (40 mg/kg) daily for 4 days, beginning from day 5 of pseudopregnancy 30 min before the intrauterine injection of oil. (A) The weights of the uterine horns with oil injection on day 9 of pseudopregnancy. (B) Uterine morphology on day 9 of pseudopregnancy. Compared to controls, uterine weights were significantly reduced by nimesulide or niflumic acid. The number of rats used in each treatment was shown in each bar. Values are presented as the mean ± SEM.

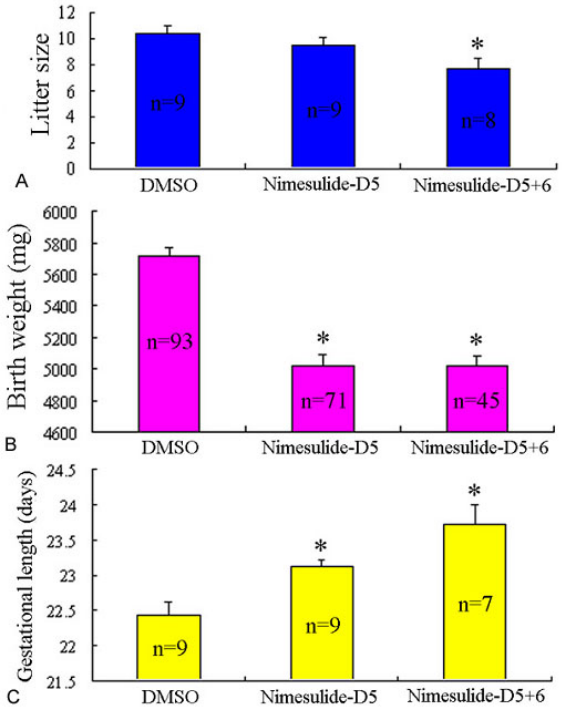

Figure 8. Effects of COX-2 inhibitors on litter size, birth weight and gestational length. After pregnant rats were treated once with nimesulide (40 mg/kg, i.p.) on day 5 or twice on both days 5 and 6 of pregnancy, the litter size, birth weight and gestational length were recorded at birth. The number of rats used in each treatment was shown in each bar. Values are presented as the mean ± SEM.

reproductive failure, suggesting a lack of alternative sources of prostaglandin synthesis (8). It should be checked whether rat implantation is blocked after both COX-1 and COX-2 are inhibited.

5.3. COX-2 action on rat decidualization

In our study, the size and weight of implantation sites were not only reduced by nimesulide treatment, but artificial decidualization was also severely inhibited by nimesulide treatment. Vimentin is a marker for stromal cell decidualization (24). On day 6 of pregnancy, vimentin was highly expressed in the subluminal stroma surrounding the implanting blastocyst in the control, but not detected in the nimesulide-treated rat uterus. COX-2-knockouted mice show a delay in the initial rate of decidual growth after implantation compared to that in heterozygous or wild-type recipients (5,6). In the mouse, lower doses of celecoxib showed little effects on the number of implantation sites, and there was a reduction only in the weight of implantation sites by day 6 (8). Celecoxib, a specific COX-2 inhibitor, could cause the failure of decidual reaction in the mouse (8). In Wistar rats, preimplantation, postimplantation loss and gestation length were all increased by the gavage administration of indomethacin, nimesulide or celecoxib (9). Sookvanichsilp and Pulbutr (10) demonstrated that indomethacin at a dose of 5 mg/kg/day as well as celecoxib at doses of 80 and 160 mg/kg/day could significantly reduce the proportion of pregnant rats. At the anti-implantation dosages, they exhibited no significant effect on proportion of rats with blue dye sites in the endometrial vascular permeability study, but they could significantly reduce the uterine decidualization.

5.4. The secondary effects of nimesulide treatment on rat reproduction

Although the number of implantation sites and vascular permeability were not affected by nimesulide treatment, the expression of implantation-related genes, the weight of decidual tissues, litter size, birth weight and gestational length were all severely affected by nimesulide treatment. Interestingly, COX-2-derived PGs contribute to the onset of parturition after the decrease in serum progesterone level (39). COX-2 inhibitor has been reported to possibly prevent human preterm delivery (40). However, it is impossible for COX-2 to act on late pregnancy directly since the estimated mean terminal half-life for nimesulide varied from 1.96 to 4.73 hours (41). In this study, the last injection of nimesulide was done on day 8 of pregnancy. All of the abnormalities in litter size, birth weight and gestational length should result from the effects of nimesulide on implantation and decidualization during early pregnancy. In our study, except for the expression implantation-related genes, the size and weight of implantation sites were severely compromised by nimesulide treatment. However, implantation and decidualization were not completely blocked by nimesulide treatment, and gestational length was only prolonged, suggesting a delay in implantation and decidualization following nimesulide treatment. Our data on immunostaining and decidualization also suggest a delay in implantation and decidualization. Similar results have been observed with cPLA2a-, COX-2- and LPA3-deficient mice (2,42,43). Deferred implantation in these mice leads to defective postimplantation development, including retarded fetal development, embryo crowding, sharing of placentas by multiple embryos and reduced litter size. In most successful human pregnancies, the conceptus implants 8 to 10 days after ovulation. The risk of early pregnancy loss increases with later implantation (44).

In conclusion, ovulation was significantly inhibited by nimesulide treatment. The expression of PPARdelta, HB-EGF and vimentin proteins was down-regulated in the nimesulide-treated groups. Our data suggested that rat implantation and decidualization were delayed by nimesulide treatment, resulting in the reduction of litter size and birth weight and the prolongation of gestational length. Our data also indicated that proper implantation and decidualization are very important for following embryonic development.

6. ACKNOWLEDGMENT

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Abbreviations: COX: Cyclooxygenase, COX-2: cyclooxygenase-2, cPLA2alpha: cytosolic phospholipase A2alpha, eCG□equine chorionic gonadotropin, HB-EGF: heparin-binding epidermal growth factor, hCG: human chorionic gonadotropin, LPA3: lysophosphatidic acid receptor A3, mPGES-1: microsomal prostaglandin E synthase, PGE2: prostaglandin E2, PPARdelta: peroxisome proliferator-activated receptor delta

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