Effect of a Selective Nonsteroidal Anti-inflammatory Drug, Celecoxib, on the Reproductive Function of Female Mice

Peng-Hui Wang[1,2]*, Huann-Cheng Horng[1,2], Yi-Jen Chen[1,2], Shie-Liang Hsieh[3,4,5], Hsiang-Tai Chao[1,2], Chiou-Chung Yuan[1,2]

[1]Department of Obstetrics and Gynecology and [4]Immunology Research Center, Taipei Veterans General Hospital, [2]Institute of Clinical Medicine and [3]Department of Microbiology and Immunology, National Yang-Ming University School of Medicine, and [5]Genomics Research Center, Academia Sinica, Taipei, Taiwan, R.O.C.

Background: The aim of the present study was to determine if long-term use of a cyclooxygenase-2 (COX-2) inhibitor affects fertility or ovulation in female mice.

Methods: Twenty-four female mice, 25 days of age, were given a selective COX-2 inhibitor: 3 mg/kg celecoxib ($n=8$), 5 mg/kg celecoxib ($n=8$), or placebo ($n=8$) in a random fashion. Eight female mice, 10–11 weeks old, given 3 mg/kg celecoxib ($n=4$) or placebo ($n=4$) were subjected to continuous mating studies.

Results: Results from the 24 mice ($n=8$ for each group) showed that oocyte number was not significantly different between female mice treated with either 3 mg/kg or 5 mg/kg celecoxib and placebo (21.4 ± 2.5, 21.5 ± 3.3, 23.3 ± 3.8, respectively). From the continuous mating study, the litter size of female mice treated with celecoxib was not significantly different (8.2 ± 1.3 pups/litter) compared to those treated with placebo (8.3 ± 1.2 pups/litter). In addition, female mice treated with celecoxib had an average of 2.8 ± 0.5 litters in a 12-week period, which was similar to female mice treated with placebo (3.0 ± 0.8 litters/female).

Conclusion: This study suggests that use of low-dose (≤ 5 mg/kg) selective COX-2 inhibitor in a mouse model does not significantly impair the female reproductive function. [*J Chin Med Assoc* 2007;70(6):245–248]

Key Words: cyclooxygenase-2 (COX-2) inhibitor, female, reproductive function, selective nonsteroidal anti-inflammatory drug

Introduction

Ovarian follicular development lies with the interaction between pituitary gonadotropins, follicle stimulating hormone and luteinizing hormone (and glucocorticoid hormone and prolactin in some species), and intraovarian factors such as steroid, cytokines and other growth factors.[1] Among these, prostaglandins (PGs) possess vasoactive, mitogenic, and differentiating properties and are implicated in various female reproductive functions.[2] In particular, PGs have been shown to play key roles in ovarian physiology, the periovulatory period, and female reproduction.[3] Several studies have suggested a role for PGs in the maintenance and function of the cumulus–oocyte complex (COC).[4] PGE_2 was one of the earliest substances shown to induce cumulus expansion *in vitro*.[5] Additionally, COCs obtained from rats and mice after superovulation synthesize PGE_1, PGE_2 and $PGF_{2\alpha}$.[5,6] Therefore, any drug that blocks synthesis of PGs would affect female reproductive function. In fact, it is reported that high doses of indomethacin (an inhibitor of both cyclooxygenase-1 [COX-1] and -2 [COX-2], one of the nonsteroidal anti-inflammatory drugs [NSAIDs]) or a "specific" COX-2 inhibitor (NS-398) can block ovulation in rats.[3,7] In addition, treatment of COCs with indomethacin greatly reduces the *in vitro* fertilization rate of oocytes, and this effect is reversed if PGE_1 and PGE_2 are added to

*Correspondence to: Dr Peng-Hui Wang, Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan, R.O.C.
E-mail: phwang@vghtpe.gov.tw • Received: June 14, 2006 • Accepted: May 16, 2007

the media in the presence of indomethacin.[6] These data suggest that PGs may be critical for maintaining an optimal microenvironment for oocyte survival and fertilization.

However, although use of low-dose indomethacin can abolish ovarian PGE_2 synthesis, low-dose indomethacin failed to affect ovulation.[8] In contrast, high-dose indomethacin resulted in significant inhibition of ovulation.[8] It was also noted that absence of COX-2 in mice led to defects in ovulation, fertilization, and implantation,[9] suggesting that COX-2 enzyme may play an important role in female reproduction. Selective COX-2 inhibitors are commonly used in clinical practice, including for the management of dysmenorrhea, menorrhagia or other rheumatism, all of which often attack women in reproductive age. In this study, a low dose of the selective NSAID COX-2 inhibitor, celecoxib, was used to evaluate its effects on the reproductive function of female mice.

Methods

All mouse experiments (BALB/C strain; weight, 20–30 g) were approved by the Institutional Animal Care and Use Committee of Taipei Veterans General Hospital. Animals were housed in an air-conditioned room with free access to a commercial pellet diet and tap water. In order to maintain stable biological rhythms, 12 hours of artificial light and 12 hours of darkness were provided.

Superovulation with exogenous gonadotropins

To assess the ovarian function of female mice treated with low-dose celecoxib, we induced superovulation with exogenous gonadotropins in immature female mice (weight, 20–25 g) at 25 days of age to determine the oocyte production per female. Reasons for using immature female mice for the superovulation test included: (1) to avoid the possible effect of endogenous hormone inducing follicle growth or ovulation in the mice; and (2) to ignore the cyclic change of female mice after sexual maturation. This approach can be found in the literature.[10–13] Superovulation assays[11,12] were carried out on female mice ($n=24$) feeding with COX-2 inhibitor ($n=16$) or not ($n=8$) at 25 days of age. The COX-2 inhibitor, celecoxib (Celebrex®; Pfizer Inc., New York, NY, USA), at doses of 3 mg/kg ($n=8$) and 5 mg/kg ($n=8$) was administered by oral intubation once daily to each group initially from 3 days before entering ovulation induction to the day of ovulation induction for a total of 4 days. The superovulation protocol consisted of a single intraperitoneal (IP) injection with 5 IU pregnant mare's serum gonadotropin (PMSG) (Sigma, St Louis, MO, USA) followed 48 hours later with IP 7.5 IU human chorionic gonadotropin (hCG) (Sigma) injection. The animals were sacrificed 20 hours after hCG injection, and the ovaries, oviduct, uterus and vagina removed to 1X phosphate-buffered saline (PBS) (pH 7.4) for washing. The oviduct–ovary complexes were excised and placed into 2 mL Dulbecco's modified Eagle's medium (DMEM) containing 25 U/mL penicillin, 25 U/mL streptomycin and 20% fetal calf serum (FCS). After separating the ovary (preparing for ovarian histology and follicle counting) from the oviduct–ovary complex, the oviducts were removed to IVF dishes (Corning Inc., Corning, NY, USA) with DMEM containing 10% FCS, supplemented with 0.3% hyaluronidase (Sigma). The oocyte/cumulus mass was surgically extracted from the oviduct.

Fertility of female mice

Eight 10–11-week-old female mice (weight, 25–30 g) were subjected to continuous mating studies. Celecoxib 3 mg/kg ($n=4$) was administered by oral intubation once daily from 25 days of age until the end of the study. Two female mice were housed with 1 fertile male mouse aged 4–6 weeks, and the male mice were rotated weekly. Cages were monitored daily, and the numbers of pups and litters were recorded.

Statistical analysis

Quantitative data were expressed as mean ± standard error of the mean and analyzed by Student's 2-tailed unpaired t test using Microsoft Excel 2003. Fisher's exact test was used for the analysis of qualitative data. Statistical significance was set at $p < 0.05$.

Results

No effect on ovulation capacity

Results from the 24 mice ($n=8$ for each group) indicated that oocyte number was not significantly different between female mice treated with either 3 mg/kg or 5 mg/kg celecoxib, and placebo (21.4 ± 2.5, 21.5 ± 3.3, 23.3 ± 3.8, respectively), indicating that the ovulation function of the female mice were not affected by doses of 3 mg/kg and 5 mg/kg Celebrex®.

No effect on female fertility

We employed a continuous mating study using sexually mature female mice ($n=4$ for each group) at 10–11 weeks of age and known fertile male mice. After 12 weeks of mating, the litter size of the female mice treated

with celecoxib was not significantly different (8.2 ± 1.3 pups/litter) from that of female mice treated with placebo (8.3 ± 1.2 pups/litter). In addition, female mice treated with celecoxib had an average of 2.8 ± 0.5 litters in a 12-week period, which was similar to that of female mice treated with placebo (3.0 ± 0.8 litters/female).

Discussion

The COX enzyme complex, a key enzyme in the synthesis of PGs from arachidonic acid, consists of at least 2 enzymes existing as distinctly regulated forms expressed by separate genes and differing in distribution, regulation, and expression.[14,15] COX-1 is constitutively expressed in all tissues and regulates the action of PGs, whereas COX-2 is induced in immune cells and other tissues following diverse stimuli, such as mitogens, proinflammatory cytokines, growth factors, and tumor promoters.[16–20] NSAIDs, acting largely through inhibition of COX, are widely used as anti-inflammatory agents and as analgesia for various disorders.[21] Due to nonspecific inhibition of the COX family, NSAIDs possess many side effects since they block the constitutive function of COX-1. Because COX-2 is primarily responsible for increased PG production during inflammation, this isoform is the target for development of selective anti-inflammatory drugs.[2] Development of specific antagonists to COX-2 has permitted the use of potent anti-PG agents without many of the side effects commonly attributed to the nonspecific NSAIDs.[22]

The processes of ovulation and implantation are considered analogous to proinflammatory responses; thus, participation of PGs in these events has been speculated.[23] For example, PGs are considered to participate in follicular rupture during ovulation.[2] This is consistent with gonadotropin-mediated induction of COX-2 in ovarian follicles preceding ovulation.[24] There is evidence to indicate that COX inhibitors have major effects on ovulation and other reproductive processes, including fertilization, decidualization, implantation and parturition, and that they are a link to reversible female infertility.[21] However, COX-2 also regulates PGs, which are important in many reproductive processes.[14] Selective blockage of COX-2 using pharmacologic agents reduces PG production and ovulation both *in vivo* and *in vitro* in the rat.[21] However, in this study of a low-dose COX-2 inhibitor in female mice, we failed to demonstrate any impairment in ovulation since oocyte number was not statistically significantly different between female mice treated with 3 mg/kg or 5 mg/kg celecoxib and placebo (21.4 ± 2.5, 21.5 ± 3.3, 23.3 ± 3.8, respectively).

In clinical practice, Pall et al studied the effects of the selective COX-2 inhibitor, rofecoxib, on the ovarian function of women.[25] The average maximum preovulatory follicle diameter in women taking rofecoxib was larger than that in the placebo group (30 mm *vs.* 21 mm). In addition, 4 out of 6 women taking rofecoxib showed delayed follicle rupture (> 48 hours after the LH peak), which is characteristic of luteinized unruptured follicle syndrome (LUFS), and the phenomenon disappeared when the medication was discontinued,[25] suggesting that the clinical implications of COX-2 inhibition in human fertility are important. Thus, it is clear that the critical role of PGs in reproductive processes, especially ovulation, can be regulated by COX-2. The implications of these findings for women who are attempting to become pregnant and who are taking COX-2 inhibitors are important because there is increasing evidence of the potential danger of these drugs. These drugs may prevent or delay ovulation, with no change in endocrine markers of ovulation, such as progesterone levels, with the resulting luteinized unruptured follicle discovered only by ultrasonography and LH evaluation. In contrast, the low-dose effect may be different from the above finding because the recent report from Moon et al shows the benefit of using a low dose of NSAID when performing assisted reproductive technique, because treatment with piroxicam before embryo transfer increases the pregnancy rate after *in vitro* fertilization and embryo transfer.[26] Our study agreed with the latter finding, suggesting that low-dose NSAID use may be safe, although we did not apply this strategy in a human study.

COX-2 is also expressed in the luminal epithelium and underlying stromal cells solely at the site of blastocyst attachment.[2] Using the delayed implantation model, this study also established that the expression of COX-2 in the receptive uterus requires the presence of active blastocysts, suggesting that COX-2 expression during the attachment reaction is critical to implantation.[27] Indeed, gene targeting experiments have demonstrated that COX-2-derived PGs are essential for implantation and decidualization.[9] However, from this study, COX-2 inhibitor may be safe if the dose is low because we clearly demonstrated that low-dose COX-2 inhibitor of ≤ 5 mg/kg celecoxib per day did not seem to affect the normal fertility of female mice, suggesting that implantation may not be impaired when using a low dose of COX-2 inhibitor.

Since differences exist between primates and other species in the sites and timing of COX-2 expression in the ovary, COX-2 inhibitors may produce different results in these species. In addition, emerging evidence shows that COX-2 inhibitors may impair fertilization,

implantation, and maintenance of pregnancy. From this study, we found that low-dose COX-2 inhibitor did not affect fertilization, implantation and maintenance of pregnancy in a mouse model. Overall, the therapeutic window between pain relief and fertility impairment in using COX-2 inhibitors in clinical practice is worthy of further investigation.

Acknowledgments

This work was supported by grant number 93VGH-111 from Taipei Veterans General Hospital.

References

1. Findlay JK, Drummond AE, Dyson ML, Baillie AJ, Robertson DM, Ethier JF. Recruitment and development of the follicle; the roles of the transforming growth factor-beta superfamily. *Mol Cell Endocrinol* 2002;191:35–43.
2. Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, Wang H. Molecular cues to implantation. *Endocr Rev* 2004; 25:341–73.
3. Elvin JA, Yan C, Matzuk MM. Growth differentiation factor-9 stimulates progesterone synthesis in granulosa cells via a prostaglandin E2/EP2 receptor pathway. *Proc Natl Acad Sci USA* 2000;97:10288–93.
4. Eppig JJ. Maintenance of meiotic arrest and the induction of oocyte maturation in mouse oocyte–granulosa cell complexes developed *in vitro* from preantral follicles. *Biol Reprod* 1991;45:824–30.
5. Schuetz AW, Dubin NH. Progesterone and prostaglandin secretion by ovulated rat cumulus cell–oocyte complexes. *Endocrinology* 1981;108:457–63.
6. Viggiano JM, Herrero MB, Cebral E, Boquet MG, de Gimeno MF. Prostaglandin synthesis by cumulus–oocyte complexes: effects on *in vitro* fertilization in mice. *Prostaglandins Leukot Essent Fatty Acids* 1995;53:261–5.
7. Mikuni M, Pall M, Peterson CM, Peterson CA, Hellberg P, Brannstrom M, Richards JS, et al. The selective prostaglandin endoperoxide synthase-2 inhibitor, NS-398, reduces prostaglandin production and ovulation *in vivo* and *in vitro* in the rat. *Biol Reprod* 1998;59:1077–83.
8. Tanaka N, Espey LL, Kawano T, Okamura H. Comparison of inhibitory actions of indomethacin and epostane on ovulation in rats. *Am J Physiol* 1991;260:170–4.
9. Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, Dey SK. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 1997;91:197–208.
10. Wandji SA, Wood TL, Crawford J, Levison SW, Hammond JM. Expression of mouse ovarian insulin growth factor system components during follicular development and atresia. *Endocrinology* 1998;139:5205–14.
11. Durlinger ALL, Kramer P, Karels B, De Jong FH, Uilenbroek JTJ, Anton Grootegoed J, Themmen APN. Control of primordial follicle recruitment by anti-mullerian hormone in the mouse ovary. *Endocrinology* 1999;140:5789–96.
12. Hu YC, Wang PH, Yeh S, Wang RS, Xie C, Xu Q, Zhou X, et al. Subfertility and defective folliculogenesis in female mice lacking androgen receptor. *Proc Natl Acad Sci USA* 2004; 101:11209–14.
13. Burdette JE, Kurley SJ, Kilen SM, Mayo KE, Woodruff TK. Gonadotropin-induced superovulation drives ovarian surface epithelia proliferation in CD1 mice. *Endocrinology* 2006;147: 2338–45.
14. Norman RJ, Wu R. The potential danger of COX-2 inhibitors. *Fertil Steril* 2004;81:493–4.
15. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol* 1998;38:97–120.
16. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem* 2000; 69:145–82.
17. Chen WS, Wei SJ, Liu JM, Hsiao M, Kou-Lin J, Yang WK. Tumor invasiveness and liver metastasis of colon cancer cells correlated with cyclooxygenase-2 (COX-2) expression and inhibited by a COX-2-selective inhibitor, etodolac. *Int J Cancer* 2001;91:894–9.
18. Chen WS, Liu JH, Wei SJ, Liu JM, Hong CY, Yang WK. Colon cancer cells with high invasive potential are susceptible to induction of apoptosis by a selective COX-2 inhibitor. *Cancer Sci* 2003;94:253–8.
19. Chou YC, Chen YJ, Lai CR, Wang PH, Hsin-Chan, Yuan CC. Cyclooxygenase-2 expression is higher in ovarian cancer tissue adjacent to endometriosis than in ovarian cancer without comorbid endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2006;124: 101–5.
20. Chen YJ, Wang LS, Wang PH, Lai CR, Yen MS, Ng HT, Yuan CC. High cyclooxygenase-2 expression in cervical adenocarcinomas. *Gynecol Oncol* 2003;88:379–85.
21. Norman RJ. Reproductive consequences of COX-2 inhibition. *Lancet* 2001;358:1287–8.
22. De Witt DL. COX-2-selective inhibitors: the new super aspirins. *Mol Pharmacol* 1999;55:625–31.
23. Espey LL. Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. *Biol Reprod* 1994;50:233–8.
24. Sirois J. Induction of prostaglandin endoperoxide synthase-2 by human chorionic gonadotropin in bovine preovulatory follicles *in vivo*. *Endocrinology* 1994;135:841–8.
25. Pall M, Friden BE, Brannstrom M. Induction of delayed follicular rupture in the human by the selective COX-2 inhibitor rofecoxib: a randomized double-blind study. *Hum Reprod* 2001; 16:1323–8.
26. Moon HS, Park SH, Lee JO, Kim KS, Joo BS. Treatment with piroxicam before embryo transfer increases the pregnancy rate after *in vitro* fertilization and embryo transfer. *Fertil Steril* 2004;82:816–20.
27. Chakraborty I, Das SK, Wang J, Dey SK. Developmental expression of the cyclo-oxygenase-1 and cyclo-oxygenase-2 genes in the peri-implantation mouse uterus and their differential regulation by the blastocyst and ovarian steroids. *J Mol Endocrinol* 1996;16:107–22.