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Effect of Indomethacin on Progesterone Profile in Rabbit

Jun-hong Park* and Jong-kuk Kwun
College of Veterinary Medicine, Seoul National University
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Indomethacin이 가토의 Progesterone 수준에 미치는 영향

박 전 홍·권 종 국
서울대학교 수의과대학
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초록 : 배란 및 착상시기에 indomethacin 투여가 혈액의 progesterone 수준과 착상율에 미치는 영향을 조사하였다.

Progesterone수준은 HCG 주사후 4시간이내에 9ng/ml로 증가 되었다가 배란시에는 3ng/ml로 감소하였다. indomethacin투여구에서는 배란시 8ng/ml를 나타내기 까지 계속증가하였다.

Indomethacin투여는 착상을 억제하였다. 대조구의 착상율은 83%이나, indomethacin을 HCG 주사후 1일부터 4일까지 투여하면 착상율은 63%, HCG주사후 5일 부터 8일까지 투여하면 착상율은 27%로 감소하였다.

Progesterone 수준은 HCG 주사후 12ng/ml의 높은 수준을 나타내다가 HCG 주사후 3일까지는 3ng/ml이하의 낮은 수준을 나타내었으며 4일 부터는 10ng/ml의 높은 수준을 나타냈다.

Indomethacin을 HCG 주사후 5일부터 8일까지 투여하면 HCG 주사후 3일까지는 대조구와 비슷하지만 4일 부터는 5ng/ml이하로 대조구 보다 낮은 수준을 나타냈다.

Introduction

Indomethacin delays the appearance of uterine dye sites and inhibits decidualization in rats.[37] Indomethacin and such a nonsteroid antiinflammatory drugs inhibit prostaglandin synthetase in numerous species.[10,33,35,40]

The prostaglandins have been shown to be nearly ubiquitous in the effects on the various aspects of reproductive process.[17,25,41] Early findings of the effects of prostaglandins on ovulation included that $PGF_2\alpha$ caused luteal degeneration and ovulation in the pregnant hamster.[24,30] Prostaglandin level was elevated approximately two-fold in uterine areas where the permeability was increased relative to nondye sites.[21] Rbbit's blastocysts contain PGE and PGF which might therefore diffuse from the blastocyst to increase vascular permeability, or play a role in blastocyst steroidogenesis.[5,6]

Apart from the mention of the role of prostaglandin, no detailed description were given of the mode of action during early pregnancy. In the present experiment the effect of the indomethacin on ovulation and implantation was recorded.

Materials and Methods

The animals used in the experiment were sexually matured mixed breeds weighing from 3.0 to 3.5kg,

* a Present address: Animal Feed Resources Laboratory, KAIST, P.O. Box 131, Dong Dae Mun, Seoul 131, Kore

which was bred at the Animal Breeding House in the Seoul National University. They were fed on a cubed diet, crude protein 19% and given tap water ad libitum. Ovulation was induced with 50 i.u. HCG injection intravenously. Diluted semen was introduced to dose as previously described.[13,22,42] The day of artificial insemination was designated as day 0. Indomethacin (10mg/kg body weight) was given intramuscularly at 9 : 00 and 17 : 00 on day 1 to day 4 or on day 5 to day 8 after insemination. The number of implant and of corpora lutea were recorded on day 10.

Progesterone was obtained from the Sigma Chemical Company, USA. Labeled progesterone(specific activity 53 Ci/mmole) was obtained from the New England Nuclear, USA. Progesterone antiserum(P-3-CMO-BSA) was obtained from Deigog Jogi Company, Japan. Plasma Progesterone was assayed by radioimmunoassay using a liquid scintilation counter (Beckman LS-100).[23]

The data was statistically analized by Student test and least square deviation method for independent samples. The following statistic was employed to calculate implantation percentage: 100 x No. of implant/No. of corpus luteum on day 10.

Results

Plasma progesterone was assayed after HCG administration(Fig. 1). It was increased stepwise up to 8ng/ml during the presumed ovulation period in the indomethacin treated rabbit. While the preovulatory surge of progesterone reached a peak level, 9ng/ml, in 6 hours after HCG injection and declined rapidly before ovulation.

Indomethacin administration showed a period difference in the implantation inhibition(Table 1). Implantation percentage was much decreased in the late indomethacin treatment during day 5 to day 8. Control rabbit showed 83% of implantation, but that of indomethacin treatment was only 27%. However, the number of copora lutea was not different with both treatments.

Plasma progesterone was decreased below 3ng/ml in short period after ovulation, but it was increased there after and showed plateau 12ng/ml in control rabbit(Fig. 2). It remained above 10ng/ml before implantation period, 4 days after HCG injection. This profile was similar to that of indomethacin treatment during periovulatory period, but this showed lower level, 5ng/ml, in the indomethacin treated rabbit.

Table 1. Effect of Indomethacin on Implantation in the Rabbit

Parameter	Control	Indomethacin	
		Day 1-4	Day 5-8
No. of animal	6	6	6
No. of pregnant	6	5	3
No. of corpora lutea	62	59	52
No. of implant	52	37	14
Implantation %	83.49 ±6.50	63.26 ±7.35	26.92 ±7.20*

*$p<0.01$

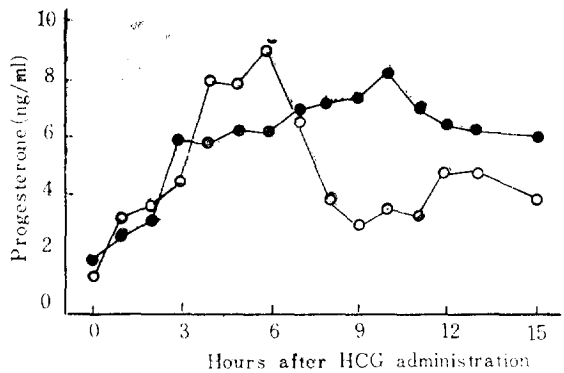

Fig. 1. Effect of indomethacin on the peripheral plasma progesterone concentration during ovulatory process in rabbbits. Indomethacin(10mg/kg bw) was administered 30 minutes prior to HCG. ○—○ : Control (n=6), ●—● : Indomethacin (n=12)

Discussion

The experiment confirmed the earlier finding that the action of indomethacin was through a steroidogenesis mediated mechanism.[1,4,15] The preovulatory surge of progesterone was observed and it was related to some other findings.[34] Indomethacin increased the biosynthesis of progesterone *in vitro*.[8] However,

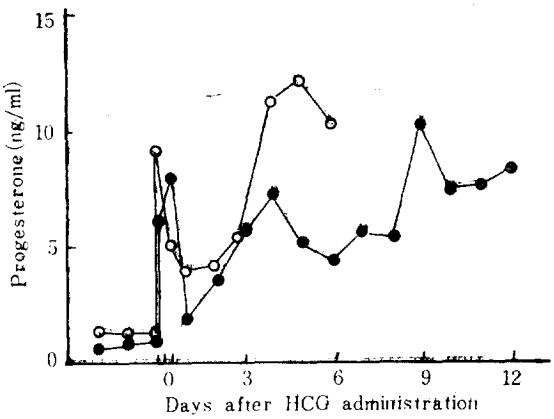

Fig. 2. Effect of indomethacin on the peripheral plasma progesterone concentration during implantation in rabbits. Indomethacin (10mg/kg bw) was administered from day 5 to day 8. ○—○ : Control (n=6), ●—● : Indomethacin(n=12)

we observed the decreased progesterone level *in vivo* (Fig. 1). The concentration of progesterone was not affected dy indomethacin.[14,36] This difference may be due to the different use of indomethacin. Indomethacin suppressed LH-stimulated ornithine decarboxylase activity at higher concentration but no effect at lower concentration.[29]

The present experiment showed that indomethacin delayed the preovulatory surge of progesterone(Fig. 1). While the changes in peripheral progesterone was not significantly affected by indomethacin.[12]

This difference was due to the timing of indomethacin administration. A single injection of indomethacin(20mg/kg) given simultaneously with HCG did not block ovulation.[27] However, exogenous gonadotropin induced ovulation was blocked with a single injection of indomethacin if given 30 minutes after an ovulatory dose of human LH. From this result, the timing of indomethacin administration was critical for blokage of ovulation and the specific effect was not related to toxicity.[27] Harper *et. al.*[15] reported that the action of indomethacin was through a prostaglandin mediated mechanism or a direct toxic effect on uterine tissue.

In the HCG induced ovulated rabbit, the dose of 10mg/kg of indomethacin produced an inhibition of ovulation that was propotional to the dose, which was confirmed by previous report.[30,32] It has been reported that prostaglandins play a role in regulating the release of LH necessary for ovulation.[5,26] The concentration of follicular PGE and PGF increased markedly and reached maximum values at about the expected time of ovulation.[26,39] The granulosa cell component had 4 fold the capacity of the residual wall and the follicular fluid synthesized no prostaglandins.[28] So it seemed that the ovary is the main source of increased plasma $PGF_{2\alpha}$ during the ovulatory process induced with the PMS-HCG treatment.[20]

The different schedule of indomethacin administration affected the implantation inhibition(Table 1). It was suggested that prostaglandin had a role in implantation.[38] The uterine prostaglandin content was elevated at implantation sites as compared with non dye sites in the rat and hamster. And indomethacin reduced uterine prostaglandin concentration.[7,21] Vane found that aspirin-like drugs inhibited prostaglandin biosynthesis.[40] Aspirin acetylated the cyclo-oxygenase protein at the active sites and nonsteroid antiinflammatory drug blocked prostaglandin synthetase in numerous species.[10,33]

The peripheral progesterone level during implantation was similar to those previously reported(Fig. 2).[16,34] Indomethacin injection during day 5 to day 8 inhibited implantation in the rabbit(Table 1) and the level of progesterone was slightly increased prior to implantation in the indomethacin treated rabbit (Fig. 2). This result was contradictory to the early findings. Indomethacin alone did not prevent implantation but it reduced blastocyst size.[16,19] Indomethacin did not influenced viability, but the possibility of a direct toxic to blastocyst could not be ruled out.[18,21] This discrepancy may be due to the different use of indomethacin. The period of indomethacin administration was critical.[27] We confirmed that early injection of indomethacin did not significantly influenced the viability of embryo, but the reverse was true in the case of late injection of indomethacin.

From the above result we inferred that prostaglandins had a obligatory role in ovulation and in implantation which might be mediated via ovarian ster-

oidogenesis in the rabbit. However, the possibility of a direct hypothalamo-hypophysis axis control could not be ruled out. The effect of $PGF_{2\alpha}$ upon decidualization was mediated by an alteration in ovarian steroidogenesis, but that of PGE_2 acted independently on the ovary.[2)]

Conclusion

Preovulatory progesterone surge was monitored in control rabbit, but it was delayed 4 hours in the indomethacin treated rabbit which was administered 30 minutes before HCG injection.

Indomethacin inhibited implantation, which was dependent on the administration schedule. Implantation ratio in control rabbit was 83%, but it was decreased 63% and 27% according to the injection schedule. Later injection from day 5 to day 8 after insemination showed lower implantation ratio, 27%.

Plasma progesterone was increased during implantation in control rabbit, but it was decreased in the indomethacin treated rabbit which was administrated from day 5 to day 8 after insemination.

It was considered that the effect of indomethacin on ovulation and implantation was progesterone med iated mechanism which was disturbed by prostaglandin synthesis inhibition.

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