ANTI-FERTILITY EFFECT OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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Abstract—The anti-fertility activity of prostaglandin synthesis inhibitors namely the non-steroidal anti-inflammatory drugs: acetyl salicylic acid, indomethacin and oxyphenbutazone were investigated in male and female albino rats and female albino rabbits. Oxyphenbutazone and indomethacin affected the reproductive process in male rats. Indomethacin 3 mg/kg. and acetyl salicylic acid 300 mg/kg produced significant anti-ovulatory activity in the rabbit. In female rats, all three drugs given in high doses over a period of two oestrous cycles reduced mating significantly, while only indomethacin given in a low dose of 0.8 mg/kg over a period of six oestrous cycles could reduce mating significantly. Anti-implantation activity was seen with indomethacin 4 mg/kg. alone, and though post-implantation activity was seen with all three drugs, it was associated with maternal deaths. It appears that non-steroidal anti-inflammatory drugs affect reproduction in female animals.

High concentrations of prostaglandins in semen, menstrual and amniotic fluid suggest a physiological role for prostaglandins in reproduction and they have been implicated at practically every step in the sequence of reproduction from conception to parturition although in certain aspects, the role remains obscure. Semen from persons with low fertility rates has a lower concentration of prostaglandins than that from those with normal fertility (1), while in the female, the increased sensitivity of the myometrium at ovulation (2, 3) may be related to a physiological role for prostaglandins at coitus (4).

Flower et al. (5) have shown prostaglandin synthetase inhibition with a number of nonsteroidal anti-inflammatory drugs, and a number of clinical actions of these drugs could be explained by inhibition of prostaglandin production (6, 7).

Looking to the probable physiological roles for prostaglandins in conception it was thought worthwhile to study the effects of prostaglandin synthesis inhibitors on reproduction. Moreover, at least one anti-inflammatory drug— aspirin has been shown to induce congenital malformations in foetal mice (8) and cause a large number of foetal resorptions and foetal deaths in rats (9). With this in mind, the present work of evaluating the antifertility activity of anti-inflammatory drugs was undertaken.

MATERIAL AND METHODS

Anti-fertility effects were tested in the rat-Haffkine strain, male and female, and the female rabbit-Haffkine strain. For every test, 5 groups of animals were used: the control group which was given saline, and the control group which was given 4% gum acacia—the suspending agent used for the preparation of drug suspensions. Drug treated groups were
the indomethacin treated group, the oxyphenbutazone treated group and the aspirin treated group.

For short term experiments in rats, the drugs were used in their anti-inflammatory doses of 4 mg/kg, 100 mg/kg, and 400 mg/kg for indomethacin, oxyphenbutazone and aspirin respectively, (10) and for long term experiments in rats, in one-fifth of the above mentioned doses. In rabbits, the doses were calculated from the anti-inflammatory doses according to surface area ratios (11). The anti-inflammatory doses were about one-tenth of the LD50 doses and they did not produce mortality. Body weights were recorded daily throughout the duration of the experiments. Symptoms of toxicity such as loss in body weight, listlessness and decreased food consumption were looked for. The presence of gastric ulceration with the doses mentioned above was observed in a separate group of animals.

The tests that were carried out were as follows:

1) Effect of treatment in males:

Thirty male albino rats of proved fertility weighing between 175-225 g were used with six rats in each group. Drugs were given orally through a rubber catheter daily for a period of twenty-eight days (12, 13) in the doses of 0.8 mg/kg, 20 mg/kg and 80 mg/kg, for indomethacin, oxyphenbutazone and aspirin, respectively.

Males were mated with female rats of proved fertility in a ratio of 1 male to 2 females for a period of twelve weeks starting from the first day of feeding. Females were replaced every week of the experiment (12, 14). Daily vaginal smears were examined and those showing the presence of spermatozoa were labelled as fertile matings. In this way, the number of males which had the capacity to mate successfully with females every week during and after treatment could be estimated. The pregnant females were surgically observed on the tenth day of pregnancy (day 1 of pregnancy being the day the sperms were detected in the vaginal smear), and all pregnant females were allowed to deliver normally.

2) Effect of treatment in females:

(1) Effect on oestrous cycle and mating: Female albino rats of proved fertility weighing 150-200 g were screened beforehand for the presence of at least two normal oestrous cycles. Each oestrous cycle was counted from the day of pro-oestrous and the cycle was generally 5 days in duration.

The study was carried out in two parts:

(a) Effect of short term treatment with high doses-fifty rats in groups of ten were used. The anti-inflammatory doses of the test drugs were administered daily, orally, starting from the day of prooestrus over a period of two oestrous cycles. The rats were mated singly with fertile males in the pro-oestrous phase of the third oestrous cycle.

(b) Effects of long term treatment with low doses-Groups of six rats each were assessed. The test drugs indomethacin 0.8 mg/kg, oxyphenbutazone 20 mg/kg and aspirin 80 mg/kg were given orally from the pro-oestrous stage for a period of six cycles (15). Mating was allowed in the pro-oestrous stage of the seventh cycle. From this study, therefore, any
change in duration or types of the oestrous cycles before and after treatment could be gauged. Reduction in the number of animals mating successfully in the treated groups could also denote an anti-fertility effect.

(2) Anti-implantation effect: Fifty rats of proved fertility in the weight range of 150-200 g were separated into five groups of ten each. The females were mated in pro-oestrous singly with fertile males and oral treatment was given from day 1 to day 7 of pregnancy (16). Anti-implantation activity was studied by the method described by Khanna et al. (17, 18). The animals were allowed to deliver normally and the litter size was recorded in every group.

(3) Post-implantation effect: Fifty rats of proved fertility in the weight range of 150-200 g were taken. On the tenth day of pregnancy, the rats were surgically observed and the number of implantation sites counted. Oral treatment was given from days 10 to 16 and the size of the litter born at term was recorded (19). Vaginal bleeding was looked for during the course of pregnancy as an indication of abortifacient activity of the drug (17).

In all the above mentioned tests, the pups born of treated males or females were observed for one month from the date of birth, for the presence of gross abnormalities (18).

(4) Anti-Ovulatory effect: This effect was assessed in adult female rabbits weighing between 1-2 kg. Ovulation was induced by injecting copper acetate 4 mg/kg. in the marginal ear vein. Forty-eight hours later, laparotomy was performed and the presence of fresh bleeding points on the ovaries was indicative of ovulation (20). The drugs were given as oral pretreatment for two days before the injection of copper acetate (21) in the following doses—indomethacin 3 mg/kg, oxyphenbutazone 75 mg/kg and aspirin 300 mg/kg.

RESULTS

I. Effect of treatment in males (Table 1): The results were analysed by the chi square test. The total number of females inseminated by the treated males over a period of 12 weeks was less than that of the control, but the difference was not significant. Week-wise analysis of the number of females inseminated by the males also did not reveal any significant differences. The total number of pregnancies was less in the females mated with Indomethacin and oxyphenbutazone treated groups. The number of pregnant females in the aspirin treated group was also less than that of the control but this difference was not significant.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Gumaceia</th>
<th>Oxyphenbutazone</th>
<th>Indomethacin</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of inseminated females</td>
<td>73</td>
<td>73</td>
<td>58*</td>
<td>57*</td>
<td>56*</td>
</tr>
<tr>
<td>Total no. of pregnant females</td>
<td>37</td>
<td>24</td>
<td>20*</td>
<td>22*</td>
<td>25*</td>
</tr>
</tbody>
</table>

* Significant difference from control P<0.05

† No significant difference from control
statistically. The females kept for mating with indomethacin treated males during the 2nd and 6th week of the experiment were inseminated, but no pregnancies occurred. Similarly, the females mated with oxyphenbutazone treated males during the 3rd, 6th and 10th week of the experiment did not become pregnant.

II. Effect of treatment on oestrous cycles and mating in females: The results of the oestrous cycle duration were analysed by variance analysis while the data on mating in females was analysed by the four-fold table test.

1(a) Effect of low doses on the oestrous cycle: There was no significant change in the duration of oestrous cycles after treatment.

1(b) Effect of low doses on mating: With low dose administration of the test drugs over a period of six oestrous cycles, only the indomethacin treated group showed a significant decrease in the number of mated females.

2(a) Effect of high doses on oestrous cycle: There was no significant change in the duration of oestrous cycles after treatment.

2(b) Effect of high doses on mating (Table 2): All three drug treatments resulted in a significant decrease in mating. There was no significant variation among the drugs themselves.

3. Anti-implantation activity (Table 3): This was analysed by variance analysis and Duncan's multiple range test. The aspirin and oxyphenbutazone treated groups showed absence of implantation sites in four out of ten animals each. With indomethacin, seven animals did not show implantation sites, the difference being statistically significant. The average litter size of all the drug treated groups was significantly smaller than that of the control showing thereby that foetal resorption had taken place in the drug treated group.

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**Table 2.** Effect of high doses on mating of female rats. Drugs given over a period of two oestrous cycles in the following doses: Oxyphenbutazone: 10 mg/kg Indomethacin: 4 mg/kg Aspirin: 400 mg/kg Analysis by four fold table test.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Gumacacia</th>
<th>Oxyphenbutazone</th>
<th>Indomethacin</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of females inseminated</td>
<td>10</td>
<td>9</td>
<td>4*</td>
<td>6*</td>
<td>5*</td>
</tr>
<tr>
<td>Total no. of females</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

* Significant difference from control  P<0.05

**Table 3.** Anti-implantation activity in female rats (Mean ± S.E.). Treatment given from day 1 to day 7 of pregnancy in the following doses: Oxyphenbutazone: 100 mg/kg. Indomethacin: 4 mg/kg. Aspirin: 400 mg/kg. Analysis by variance analysis and Duncan's multiple range test.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Gumacacia</th>
<th>Oxyphenbutazone</th>
<th>Indomethacin</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of implants</td>
<td>6.8±0.8</td>
<td>7.2±0.9</td>
<td>4.3±1.5</td>
<td>1.4±0.8*</td>
<td>3.9±1.3</td>
</tr>
<tr>
<td>Litter size</td>
<td>6.1±0.7</td>
<td>6.4±0.8</td>
<td>2.6±1.2*</td>
<td>0.5±0.4*</td>
<td>3.4±1.2*</td>
</tr>
</tbody>
</table>

*: Significant difference from control  P<0.05
In the oxyphenbutazone group, one animal delivered a pup with an absent tail and lower limb. This pup did not survive.

4. Post-implantation effect (Table 4): Variance analysis and Duncan's multiple range tests were carried out. All the animals in the control group delivered at term, while in the gumacacia group, one animal failed to deliver. With oxyphenbutazone two rats died during treatment and only one out of the remaining eight delivered. In the indomethacin group, five rats died during the treatment period and the remaining five did not deliver at term. Of these five, one had vaginal bleeding suggestive of abortion on the sixteenth day of pregnancy. In the aspirin treated group, one rat died during treatment. Of the two rats which delivered, at term, one gave birth to three pups which had blue spots on their backs. The blue spot on dissection was found to be local haemorrhage. All the drug treatments though showing a significant decrease in the number of deliveries as compared to control, did not show significant variation among themselves.

5. Anti-ovulatory activity (Table 5): This was analysed by variance analysis and Duncan's multiple range test. All the rabbits in the control and gum acacia groups showed bleeding points after injection of copper acetate. In the oxyphenbutazone group, two out of six animals did not ovulate. However, the mean number of bleeding points did not differ significantly from the control group. Both the aspirin and indomethacin treated groups showed a significant decrease in the number of bleeding points.

The body weights and food consumption were more or less the same in both the control and treated animals in all groups except those where the anti-implantation activity was tested. In the group where anti-implantation activity was tested, the treated pregnant females did not show as much of an increase in weight, with the advancement of pregnancy as the corresponding control females. In the group where post-implantation activity was tested,

Table 4. Post-implantation activity in female rats (Mean ± S.E.). Treatment given from day 10 to day 16 of pregnancy in the following doses: Oxyphenbutazone: 100 mg/kg. Indomethacin: 4 mg/kg Aspirin: 400 mg/kg. Analysis by variance analysis and Duncan's multiple range test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of pups delivered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.3 ± 0.3</td>
</tr>
<tr>
<td>Gumacacia</td>
<td>5 ± 0.6</td>
</tr>
<tr>
<td>Oxyphenbutazone</td>
<td>0.87 ± 0.7*</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>0.89 ± 0.7*</td>
</tr>
</tbody>
</table>

*: Significant difference from control  P<0.05

Table 5. Anti-ovulatory activity in female rabbits (Mean ± S.E.). Pretreatment given for two days before the injection of copper acetate, in the following doses: Oxyphenbutazone: 75 mg/kg. Indomethacin: 3 mg/kg and Aspirin: 300 mg/kg. Analysis by variance analysis and Duncan's multiple range test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of bleeding points on ovaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6 ± 0.8</td>
</tr>
<tr>
<td>Gumacacia</td>
<td>6.8 ± 1</td>
</tr>
<tr>
<td>Oxyphenbutazone</td>
<td>3.1 ± 1.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>2.4 ± 0.8*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>2 ± 0.8*</td>
</tr>
</tbody>
</table>

*: Significant difference from control  P<0.05
the treated females showed loss of weight. Food consumption was also less in this group. Mortality also occurred though this was not related to loss of weight. The doses of the anti-inflammatory drugs used in the present work, failed to produce gastric ulcerations though hyperaemia and congestion were evident.

DISCUSSION

**Effect of treatment in males:** It has been reported that a relationship exists between the prostaglandin content of seminal plasma and male fertility (22). The number of fertile matings or pregnancies was significantly less in the groups mated with indomethacin and oxyphenbutazone treated males. Sperm transport and capacitation could have been adversely affected by prostaglandin inhibition, but this can be ruled out since spermatozoa are ejaculated directly into the uterine cavity in the rat (23). Hawkins (24) has demonstrated a relationship between low concentrations of prostaglandins in the semen and a high incidence of abnormal spermatozoa in human ejaculates. This could explain the decreased fertility in the treated males. The complete absence of fertile matings in females put for mating with indomethacin and oxyphenbutazone treated males during certain isolated periods is difficult to correlate with inhibition of any particular stage of spermatogenesis.

**Effect of treatment in females:**

**Effect on oestrous cycle and mating:** The lengthening of the oestrous cycle seen with short term and long term administration of the anti-inflammatory drugs was not significant statistically. This was probably because the doses used could not attain the high intrauterine concentrations required to inhibit corpus luteum regression or that corpus luteum regression does not govern the duration of the oestrous cycle.

Aspirin and indomethacin (25) inhibit ovulation in rats. This process involves the intervention of prostaglandins which are directly inhibited by these two compounds or are rendered ineffective by the luteinizing hormones whose functions they regulate. This anti-ovulatory effect could explain the reduction in pregnancies in the indomethacin group in the long term experiment and with indomethacin, oxyphenbutazone and aspirin in the short term experiment. However, since the number of females inseminated was also less, a decrease in the libido of the treated female rats cannot be ruled out. An anti-ovulatory activity was also demonstrated in rabbits in the present study.

**Anti-implantation activity:** All three anti-inflammatory drugs produced a decrease in the number of implantation sites though the decrease was significant only with indomethacin. The anti-inflammatory drugs could act as anti-implantation agents by inhibiting prostaglandins and thereby hastening the tubal passage of ova (26, 27). This would allow the ova to enter the uterus at a time when proper cleavage of the ova has not taken place and when the uterus has not been prepared for implantation. Thus a large number of ova would fail to nidate. A direct blasto-cystotoxic effect cannot be ruled out in the present work, because single day drug administrations were not carried out. Further work in this direction is under progress. Small groups of pregnant rats were given indomethacin in a dose of 4 mg/kg on day 1, 2, 3, 4, 5 or 6 of pregnancy and preliminary studies revealed that indomethacin
when given on day 2 alone, can markedly inhibit implantation and PGE₂ given in a dose of 10 µg orally can reverse this inhibitory effect by 50%.

In the present study, all the anti-inflammatory drugs produced a significant decrease in full term litter size. In addition to those rats having no implantation sites, one rat each in the three drug treated groups failed to deliver, which means that foetal resorption had taken place. The foetal resorption seen when the drugs are administered during the implantation period could be due to implantation of ova at the wrong sites.

Post implantation activity:

All the three drug treated groups showed a significant decrease in the full term litter size. The decrease in litter size could be due to abortions or foetal resorptions. Except for one rat in the indomethacin group, which aborted on the 16th day of pregnancy as seen from vaginal bleeding, all the other rats in the drug treated groups showed a decrease in the litter size because of foetal resorption. Resorption could be due to the embryotoxicity of the drugs (28–33). The drugs in the doses used, also produced maternal mortality which can be explained on the basis of lowering of LD₅₀ values with pregnancy (34, 35). However no teratogenic effects were seen unlike the reports of Trasler (8) who showed the occurrence of malformation such as cleft lip, cleft palate with aspirin. In the present work, since high doses of the drugs were administered over a period of 7 days from the 10th to the 16th day of pregnancy, the embryotoxic effects could have masked the teratogenic effects. The occurrence of blue spots born to one of the aspirin treated group is not indicative of any specific teratogenic effect.

The present study clearly shows that the nonsteroidal anti-inflammatory drugs such as indomethacin, oxyphenbutazone and aspirin have an antifertility activity in the form of anti-ovulatory, anti-implantation and foetal resorptive properties. As we have explained some of the properties on the basis of prostaglandin synthesis inhibition, further work is in progress to determine if administration of PGE₂ and PGF₂α could reverse the anti-fertility affects of the anti-inflammatory drugs.

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