Effect of non-steroidal anti-inflammatory drugs on fertility of male rats

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Summary. In an attempt to study the influence of seminal prostaglandin reduction on male fertility, the effect of prolonged treatment with 4 non-steroidal anti-inflammatory drugs (acetylsalicylic acid, indomethacin, naproxen and phenylbutazone) on fertility was determined in male rats. Before the fertility experiments, the pharmacokinetics of the drugs were determined to find dosage regimens by which drug concentrations known as active from human anti-inflammatory therapy could be reached and maintained in the animals. Except for phenylbutazone, all drugs decreased prostaglandin E-2 level in seminal fluid by 80–90%, but only indomethacin reduced fertility significantly. The results suggest that reduction of prostaglandin synthesis in male rats does not affect fertility, which might be related to the very low seminal prostaglandin levels in rats compared to those in animals of other species.

Introduction

Human semen is the richest known source of prostaglandins (PGs) in vertebrates. However, in contrast to the enormous amount of research which has been carried out on the pharmacological effects and physiological roles of PGs in the female, research into the function of PGs in semen has remained at a comparatively low level despite the absence of any convincing explanation for the presence of these compounds in semen at such large concentrations (for review see Cenedella, 1975; Kelly, 1978). Non-steroidal anti-inflammatory drugs, such as acetylsalicylic acid, which inhibit prostaglandin synthetase in various tissues including those of the male genital tract (see Flower, 1974), decrease human seminal PG concentrations (Collier & Flower, 1971; Horton, Jones & Marr, 1973; Freixa et al., 1984). However, the consequences of reduction of seminal PGs by acetylsalicylic acid and related drugs for male fertility are unknown, although there is some evidence that continuous daily intake of large doses of such drugs may impair fertility in male patients (Boyd, 1970). The few animal studies available on this subject are controversial. Marley & Smith (1974) reported that treatment of male mice for 7 days with high doses of indomethacin reduced the fertility of the animals, but it was not clear whether this antifertility effect was related to the measured decrease in seminal PGs or to unspecific effects of the treatment on sexual drive. Cenedella & Crouthamel (1973) found that prolonged administration of acetylsalicylic acid to male mice did not change fertility in most animals but seemed to be associated with an increased level of fertility in males initially judged as sub-fertile. However, effects on PG levels were not measured, and salicylate concentrations determined in the animals were far below those known to inhibit PG synthesis (Flower, 1974; Matsuda, Ohnishi, Misaka & Yamazaki, 1983). Hallesy, Shott & Hill (1973) reported that chronic treatment of male rats with naproxen did not affect fertility, but again PG levels were not analysed. Chronic treatment of male rats with very high, toxic doses of non-steroidal anti-inflammatory drugs, such as paracetamol and phenacetin, is known to cause testicular atrophy, inhibition of spermatogenesis and sterility (e.g. Boyd, 1970; Jacqueson et al., 1984), but this is apparently an
unspecific part of a toxicity syndrome known from many drugs and chemicals which exert no
effects on PG synthesis (Neumann, 1984).

In the present paper, the effect of prolonged administration of various non-steroidal anti-
inflammatory drugs on male fertility was studied in rats. To reach and maintain drug concentrations
similar to those occurring during anti-inflammatory therapy in humans, the pharmacokinetics of
the drugs in male rats were determined before the fertility experiments and were taken into account
for dose regimens. The efficacy of the treatments with respect to inhibition of PG synthesis was
determined by analysis of concentrations of PGE-2 in seminal fluid.

Materials and Methods

Animals. Male and female rats of the Wistar strain (Winkelmann Versuchstierzucht, Borchern,
F.R.G.) were used. Body weight of the male rats ranged between 340 and 480 g, that of the females
between 180 and 220 g. Male rats were kept alone and female rats in groups of 3 in polypropylene
cages at an ambient temperature of 24–26°C and controlled humidity (50%) with a 12-h light cycle
from 07:00 to 19:00 h, and were supplied with food (Altromin® 1324, Altromin, Lage, F.R.G.) and
water ad libitum.

Drugs. Acetylsalicylic acid was purchased from Caelo (Hilden, F.R.G.). Naproxen was kindly
provided by Syntex Research (Lovain-la-Neuve, Belgium) and indomethacin by Sharp & Dohme
GmbH (Munich, F.R.G.). Phenylbutazone was used as commercial 20% solution (Butazolidin,
Ciba-Geigy GmbH, Wehr, F.R.G.) All drugs except phenylbutazone were freshly dissolved in
water before each injection by means of NaHCO₃ (acetylsalicylic acid), Na₂CO₃ (indomethacin) or
NaOH (naproxen) and were buffered to pH 7.5 with dilute HCl. Drugs were injected intraperi-
toneally; injection volumes were 100 μl/kg (phenylbutazone), 1 ml/kg (indomethacin, naproxen) or
3 ml/kg (acetylsalicylic acid). Control rats were injected with the same volume of saline (9 g
NaCl/l).

Pharmacokinetic studies. The pharmacokinetics of the drugs were studied after their i.p.
injection in male rats. The following doses (per kg body wt) were administered to groups of 6–11
animals: 50 or 150 mg acetylsalicylic acid, 2 mg indomethacin, 10 mg naproxen and 20 mg
phenylbutazone. Rats were killed by decapitation at different times after administration and blood
was collected for drug analysis in plasma (see below).

Fertility studies. Before the drug experiments, all male rats were mated with females to prove
their fertility. Only male rats of proven fertility were then used for the drug studies. For these
studies, groups of 5 male rats were treated i.p. twice daily at 07:00 h and 18:00 h for 7 consecutive
days with the following doses (per kg body wt): 150 mg acetylsalicylic acid, 2 or 4 mg indomethacin,
10 or 20 mg naproxen and 20 mg phenylbutazone. With each drug-treated group, one group of 5
age-matched control rats was injected twice daily with saline for 7 days. From the 3rd day of treat-
ment, each male was housed with 3 females from 19:00 to 22:00 h. Successful insemination was con-
firmed by the presence of vaginal spermatozoa or a copulatory plug. Females without such proof of
insemination were again placed with the males on the next evening, and, if necessary, this was
repeated each evening for a maximum of 4 mating sessions. At 2 h after the morning administration
on Day 7 of treatment, the males were killed and blood was sampled for drug determinations. The
gastrointestinal tract of the animals was examined for the presence of lesions or bleedings. The
seminal vesicles were excised and seminal fluid was collected for analysis of PGE-2. However, in
many animals (treated and controls), the seminal vesicles were almost empty after the 4 mating
periods and so PG analysis was not possible. The effect of drug treatment on seminal fluid PGE-2
was therefore determined in separate experiments (see below). The female rats were killed 9 days
after insemination and their uteri were examined for the presence of implantation sites.

Effect of drug treatment on concentrations of PGE-2. The effect of the drugs on seminal fluid
Seminal prostaglandins and male rat fertility

PGE-2 concentration was studied in groups of 6–9 male rats after 7 days of treatment. Drugs were administered i.p. twice daily (150 mg acetylsalicylic acid/kg, 2 mg indomethacin/kg, 20 mg phenylbutazone/kg, 20 mg naproxen/kg) and the animals were killed 1 h after the morning dose on Day 7. Blood was sampled for drug determination. Seminal fluid (about 500 mg) was collected as rapidly as possible from the excised seminal vesicles by squeezing it into a polypropylene tube, was weighed and immediately homogenized by an Ultra-Turrax in 5 ml ice-cold distilled water containing 10 µg indomethacin/ml, and was then deep-frozen in isopropanol/solid CO₂ until analysis (maximum time until analysis was 2 h). A group of 13 rats that were injected i.p. twice daily with saline (9 g NaCl/l) served as control.

Analysis of PGE-2. Concentrations of PGE-2 in seminal fluid were determined by means of a sensitive commercially available radioimmunoassay (New England Nuclear, Boston, MA, U.S.A.). To extract the very low amounts of PGE-2 in rat seminal fluid, different extraction procedures were compared and the method described by Olson, Bowen, Behrendt, Olson & Nett (1984) for PGE-2 in plasma gave the highest extraction recoveries and the most reproducible results. Briefly, each seminal fluid sample was thawed, acidified to pH 4.5 with 1 M-formic acid and then extracted twice with ethyl ether. The extracts were evaporated to dryness under a stream of nitrogen and the residue was dissolved in assay buffer (500 µl buffer per 100 mg seminal fluid). Then 100 µl of the reconstituted sample extract were used for the radioimmunoassay. All samples were analysed in duplicate. Sensitivity of the assay was about 0.2 pg PGE-2 per tube which corresponded to a lower limit of the assay of about 10 pg/g seminal fluid. Recovery of PGE-2 was determined by adding known amounts of the PG to seminal fluid before extraction: the extraction yield thus determined was 93–3%.

Drug analysis in plasma. Acetylsalicylic acid and its main metabolite salicylic acid were determined in plasma fluorometrically as described by Frey & El-Sayed (1977). Phenylbutazone was assayed spectrophotometrically as described by Kaergaard-Nielsen, Østergaard & Frey (1969). Indomethacin and naproxen were measured by spectrofluorometry by the methods of Lindquist, Møller Jensen, Johansson & Hansen (1974) and Mortensen, Jensen, Petersen, Husted & Andreasen (1979), respectively.

Results

Pharmacokinetics

As shown in Fig. 1, with all drugs, plasma concentrations within or above the anti-inflammatory concentration range known from man (50–150 µg phenylbutazone/ml, 100–250 µg salicylate/ml, 20–70 µg naproxen/ml, and 0.5–3 µg indomethacin/ml; Bochner, Carruthers, Kampmann & Steiner, 1978) were reached at the dosages administered. For naproxen and indomethacin, a monophasic exponential decline of plasma concentrations was found, while phenylbutazone exhibited a biexponential decay. After administration of 50 mg acetylsalicylic acid/kg, only its metabolite salicylic acid could be quantified in plasma, which may be explained by a very marked first-pass metabolism of the parent drug. Salicylate levels declined monoexponentially up to 10 h after administration, but a considerable acceleration of elimination was observed thereafter, which may point to saturation of salicylate metabolism at high concentrations. After injection of 150 mg acetylsalicylic acid/kg, the parent drug could be quantified during the first hours, but concentrations remained much below those of salicylic acid, again indicating a pronounced first-pass metabolism of acetylsalicylic acid in the liver. A monoexponential decline of salicylate levels was found for the first 12 h after administration, but again elimination was accelerated thereafter.
Phenylbutazone, 20 mg/kg i.p.  
Acetylsalicylic acid, 50 mg/kg i.p.  
Acetylsalicylic acid, 150 mg/kg i.p.

Fig. 1. Plasma concentrations of non-steroidal anti-inflammatory drugs after i.p. injection in male rats. Each symbol refers to the plasma concentration determined in one rat. The straight lines through the experimental values were constructed by means of log-linear regression analysis of data and were used for calculation of the elimination rate constants and half-lives shown in Table I. ASA, acetylsalicylic acid; SA, salicylic acid.

Pharmacokinetic parameters calculated from the respective concentration/time curves are shown in Table 1. Half-lives of 4.1, 5.2 and 5.5 h were calculated for indomethacin, naproxen and phenylbutazone, respectively, which correspond to previous studies in rats (Hucker, Zacchel, Cox, Brodie & Cantwell, 1966; Kampmann & Frey, 1966; Runkel et al., 1973). Acetylsalicylic acid exhibited a very short half-life of 1.2 h, whereas the elimination half-life of salicylic acid was dose-dependent: 6.4 h and 13 h were calculated after injection of acetylsalicylic acid at doses of 50 and 150 mg/kg, respectively. From these data, it was concluded that two daily injections of the different drugs would be sufficient to maintain effective concentrations in the fertility studies. The higher dose of acetylsalicylic acid (150 mg/kg) was chosen for these experiments.
Table 1. Pharmacokinetics of non-steroidal anti-inflammatory drugs after intraperitoneal injection in male rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg i.p.)</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$t_{\text{max}}$ (h)</th>
<th>$k_{\text{el}}$ (h$^{-1}$)</th>
<th>$t_{0.5}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>2</td>
<td>19</td>
<td>2</td>
<td>0.171</td>
<td>4.1</td>
</tr>
<tr>
<td>Naproxen</td>
<td>10</td>
<td>66</td>
<td>1</td>
<td>0.133</td>
<td>5.2</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>50</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>as metabolite</td>
<td>190</td>
<td>0.66</td>
<td>0.109*</td>
<td>6.4*</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>150</td>
<td>144</td>
<td>0.66</td>
<td>0.585</td>
<td>1.2</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>as metabolite</td>
<td>280</td>
<td>0.66</td>
<td>0.053†</td>
<td>13†</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>20</td>
<td>110</td>
<td>0.5</td>
<td>0.126</td>
<td>5.5</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$, maximal drug concentration in plasma; $t_{\text{max}}$, time at which $C_{\text{max}}$ was determined; $k_{\text{el}}$, elimination rate constant; $t_{0.5}$, elimination half-life; n.d., not detectable.

* For the first 10 h, then rapid decline (see Fig. 1).
† For the first 12 h, then rapid decline (see Fig. 1).

Table 2. Effect of subacute treatment (twice per day) with non-steroidal anti-inflammatory drugs on fertility in male rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of males</th>
<th>Drug level in plasma (µg/ml)†</th>
<th>No. of females</th>
<th>No. of females with vaginal spermatozoa or plug</th>
<th>No. of pregnancies</th>
<th>No. of implantation sites per pregnant rat†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>30</td>
<td>—</td>
<td>90</td>
<td>66 (73%)</td>
<td>57 (86%)</td>
<td>10±2.5</td>
</tr>
<tr>
<td>Indomethacin (2 mg/kg)</td>
<td>10</td>
<td>15.9± 4.9</td>
<td>30</td>
<td>24 (80%)</td>
<td>15 (63%)*</td>
<td>9±5.2</td>
</tr>
<tr>
<td>Naproxen (10 mg/kg)</td>
<td>5</td>
<td>38.0± 15.9</td>
<td>15</td>
<td>15 (100%)</td>
<td>12 (80%)</td>
<td>10±9.2</td>
</tr>
<tr>
<td>Naproxen (20 mg/kg)</td>
<td>5</td>
<td>96.4± 8.3</td>
<td>15</td>
<td>14 (93%)</td>
<td>13 (93%)</td>
<td>10±7.2</td>
</tr>
<tr>
<td>Acetylsalicylic acid (150 mg/kg)</td>
<td>5</td>
<td>23.0± 8.4 (salicylic acid 304±19.4)</td>
<td>15</td>
<td>12 (80%)</td>
<td>9 (75%)</td>
<td>11±0.7</td>
</tr>
<tr>
<td>Phenylbutazone (20 mg/kg)</td>
<td>5</td>
<td>84.5± 7.1</td>
<td>15</td>
<td>11 (73%)</td>
<td>7 (64%)</td>
<td>10±1.6</td>
</tr>
</tbody>
</table>

† Arithmetic mean ± s.d.

* Significantly different from concurrent controls; $P < 0.05$ ($\chi^2$ test).

Effects on male fertility

As shown in Table 2, the different drugs did not impair the sexual behaviour of the animals as indicated by the number of females with vaginal spermatozoa or a copulatory plug. The number of pregnancies in inseminated females was only decreased significantly by indomethacin. This anti-fertility effect of indomethacin was reproduced in a second group of 5 rats. Fertility was also reduced with phenylbutazone and acetylsalicylic acid, but these reductions were not significant when results for the treated rats were compared with those of the concurrent control groups. Naproxen caused no decrease in fertility at either dose level studied. None of the treatments affected the number of implantation sites in pregnant rats. Except for indomethacin (see below), higher doses of the drugs were not studied because of toxic side-effects.

At the dosage levels shown in Table 2, none of the drugs affected body weight in the rats during the period of treatment. The general behaviour of the rats was only altered in the animals treated with indomethacin: 1 of these rats became apathetic (but still showed sexual drive) and died after 5
days of treatment. Severe gastrointestinal lesions and haemorrhages were found in this animal. A fertility experiment in which 5 male rats received a higher dose of indomethacin (4 mg/kg twice daily) was interrupted because the animals lost weight, became apathetic and 1 rat died after 4 days of treatment. Plasma concentrations of indomethacin in these rats were about 30 μg/ml at 1 h after injection. In all of these rats, gastrointestinal haemorrhage was found. Similar but less marked gastrointestinal bleeding was also observed in some rats after treatment with acetylsalicylic acid (150 mg/kg twice per day), but not in the naproxen- and phenylbutazone-treated animals.

**Effect on PGE-2 concentrations in seminal fluid**

At the dose levels used for the fertility studies, all the drugs except phenylbutazone decreased significantly PGE-2 concentrations in seminal vesicle fluid by 80–90% (Table 3). Phenylbutazone reduced PG levels by only 25%, which was not significant.

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of rats</th>
<th>Drug conc. in plasma (µg/ml)†</th>
<th>PGE-2 conc. in seminal fluid (pg/g)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>12</td>
<td>—</td>
<td>201 (94–428)</td>
</tr>
<tr>
<td>Indomethacin (2 mg/kg)</td>
<td>9</td>
<td>14:6 ± 3:5</td>
<td>43 (21–89)*</td>
</tr>
<tr>
<td>Naproxen (20 mg/kg)</td>
<td>6</td>
<td>65:1 ± 5:4</td>
<td>17 (14–21)*</td>
</tr>
<tr>
<td>Acetylsalicylic acid (150 mg/kg)</td>
<td>6</td>
<td>9:2 ± 7:1</td>
<td>14 (10–21)*</td>
</tr>
<tr>
<td>Phenylbutazone (20 mg/kg)</td>
<td>6</td>
<td>92:6 ± 13:5</td>
<td>150 (61–373)</td>
</tr>
</tbody>
</table>

† Arithmetic mean ± s.d.
‡ Geometric mean with range for 1 s.d.
* Significantly different from controls; \( P < 0.001 \) (Student's \( t \) test with log transformation of data).

**Discussion**

Recent evidence suggests that PGs can enhance or decrease male fertility, depending upon the site of action. There are several reports of a correlation in men between low levels of seminal PGs, particularly the PGEs, and otherwise unexplained infertility (Bygdeman, Fredricsson, Svanborg & Samuelson, 1970; Sturde & Glowania, 1974; Gstöttner, Seifert, Beissert & Gstöttner, 1975; Svanborg, Bendvold, Bygdeman & Eneroth, 1983). Accordingly, a beneficial effect of PGE upon idiopathic male infertility has been suggested (Gstöttner et al., 1975; Aitken & Kelly, 1985). Inactivation of prostaglandins in semen from humans or rabbits by incubation with prostaglandin 15-hydroxydehydrogenase has been reported to result in a dramatic decrease in sperm motility, which in rabbits was associated with a marked reduction of fertilization rate after insemination (Schlegel, Rotermund, Färber & Nieschlag, 1981; Schlegel, Fischer, Beier & Schneider, 1983). The fertilizing ability of rabbit spermatozoa was also inhibited by incubation of semen with antisera to PGF-2α or PGE-2 (Schlegel et al., 1983). On the other hand, marked decreases of seminal PG
concentrations by a 4-day treatment of volunteers with the non-steroidal anti-inflammatory drugs flurbiprofen and lysine salicylate had no effect on sperm motility and other sperm parameters (Freixa et al., 1984). Addition of PGs to intravaginally inseminated semen has been shown to increase the fertilization rate in rabbits (Chang, Hunt & Polge, 1973; Spilman, Finn & Norland, 1973) and sheep (Dimov & Georgiev, 1977), most probably by increasing sperm transport in the female reproductive tract through effects on the smooth musculature (Mandl, 1972; Chang et al., 1973). More recent studies on human sperm function have shown that addition of PGE-2 to human semen samples increased the motility and ovum-penetrating ability of the spermatozoa (Aitken & Kelly, 1985). Besides effects of seminal PGs on sperm motility and transport, there are studies which indicate that PGs may contribute to the ejaculatory process by effects on the smooth muscles of the male reproductive tract (see Cenedella, 1975). While most of the above-cited data indicate that seminal PGs enhance male fertility, some animal studies have shown that intrascrotal or intratesticular deposition of high doses of PGEs or PGF-2α induce temporary sterility in rats and rabbits, probably by impairment of spermatogenesis (Saksena, Lau & Chang, 1978; Saksena & Lau, 1979; Rej & Chatterjee, 1980). From these results it was suggested that inhibition of testicular PG synthesis could improve male fertility (Cenedella, 1975), but there is no clear evidence for this suggestion. The available information on the role of PGs for male fertility suggests that infertility can be induced both by reduction of endogenous PG concentrations in semen or by marked increase of PG concentrations in the testis. Theoretically, drugs which decrease seminal PGs could be interesting agents for male fertility control.

In the present study, we found that inhibition of PG synthesis by non-steroidal anti-inflammatory drugs in rats did not affect male fertility. Corresponding to previous experiments in mice (Marley & Smith, 1974), indomethacin decreased fertility significantly in rats. However, this effect was apparently not due to the decrease in PG levels induced by indomethacin, because both naproxen and acetylsalicylic acid reduced PGE-2 levels in seminal fluid to an extent similar to that of indomethacin without any significant effect on fertility. Reduction of fertility by indomethacin may therefore be secondary due to toxic side-effects, which are more pronounced in the rat than with any of the other non-steroidal anti-inflammatory drugs studied. Phenylbutazone, which displayed a certain inhibitory effect on fertility, exerted no significant effect on PGE-2 levels, most probably because the dose used was not high enough for complete inhibition of PG synthesis in rats (Matsuda et al., 1983). However, the effects of phenylbutazone and the other drugs on PGE-2 were studied by determination of basal concentrations, as rats that were not mated with females were used. Thus, the effects of the drugs on PGE-2 concentrations during mating may have been different. Furthermore, PGE-2 concentrations measured in the seminal vesicle fluid may not reflect PGE-2 concentrations in the semen. Nevertheless, the present finding that almost complete inhibition of PG synthesis as accomplished with naproxen or acetylsalicylic acid had no significant effect on fertility would seem to indicate that PGs do not participate in the control of reproductive physiology in male rats. The PG content of rat semen is about 1·5 ng/ml (Ventura & Freund, 1973) compared to about 50 ng/ml in rabbit semen (Schlegel et al., 1983), about 40 µg/ml in ram semen (Bydgeom & Holmberg, 1966) and about 400 µg/ml in human semen (Gerozissis & Dray, 1981). The major PG present in accessory sex tissues of male rats is PGE-2 (Gerozissis & Dray, 1977) which is the only PG detectable in rat seminal fluid (Gerozissis & Dray, 1981). Because of the low endogenous PG concentrations in semen of rats and rabbits, a physiological role in sperm transport has been questioned (Mandl, 1972; Ventura & Freund, 1973). However, Schlegel et al. (1983) have shown that reduction or inactivation of PGs in rabbit semen significantly reduces the fertilizing ability of the semen. In view of the present data, this indicates that seminal PGs play different roles in the fertility of male rabbits and rats, and we therefore plan to test these same drugs in rabbits.

In conclusion the present study has shown that reduction of seminal prostaglandins in rats by different non-steroidal anti-inflammatory drugs does not affect fertility. This does not exclude that such drugs may affect fertility in other species, especially those with high seminal prostaglandin levels, such as primates and sheep.
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References


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