Pharmacokinetics of non-steroidal anti-inflammatory drugs in male rabbits after acute and chronic administration and effect of chronic treatment on seminal prostaglandins, sperm quality and fertility

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Summary. The pharmacokinetics of various non-steroidal anti-inflammatory 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 rabbits during continued administration. Based on the pharmacokinetics and side-effects of the different drugs, phenylbutazone was selected for the fertility experiments. Treatment of male rabbits with phenylbutazone for 9 consecutive days significantly reduced seminal concentrations of PGE-2 and PGF-2 α and tended to increase ejaculate volumes, sperm motility, and fertility. These results indicate that, at least in rabbits, inhibition of PG synthesis by prolonged treatment with non-steroidal anti-inflammatory drugs does not impair male fertility. Instead, chronic treatment with the drugs at non-toxic doses may improve sperm quality and fertility.

Keywords: prostaglandins; anti-inflammatory drugs; rabbits; male fertility

Introduction

Prostaglandins (PGs) were named for the prostate gland because they were discovered in semen and assumed to originate from this gland (von Euler, 1934). Nevertheless, the role of PGs in male reproduction has not received as much attention as their role in female reproduction. A variety of findings show that PGs may play some role in male fertility and thus may warrant further attention (see Cenedella, 1975; Kelly, 1978; Poyser, 1981). Non-steroidal anti-inflammatory drugs, such as acetylsalicylic acid, lysine salicylate, naproxen, and flurbiprofen, have been shown to lower the PG content of human semen (Collier & Flower, 1971; Horton *et al.*, 1973; Freixa *et al.*, 1984; Bendvold *et al.*, 1985) but the consequent effects of such drugs on fertility have not been fully examined. Such drugs are widely used for subacute and chronic treatment of rheumatoid diseases. There is some evidence that continuous daily intake of large doses of the drugs may impair fertility in male patients (Boyd, 1970). However, chronic treatment of oligospermic men with indomethacin or ketoprofen increased fertility (Berkay *et al.*, 1984). Animal studies on this subject are also controversial. Cenedella & Crouthamel (1973) reported that prolonged administration of acetylsalicylic acid (50 mg/kg twice daily for 12 days) 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. Hallesy *et al.* (1973) found that chronic treatment of male rats with naproxen (up to 30 mg/kg/day for 60 days) did not affect fertility. Marley & Smith (1974) reported that treatment of male mice for 7 days with high doses of indomethacin (about 5 mg/kg/day), which decreased seminal PG levels by 95%, reduced the fertility of the animals, whereas treatment with lower doses (about 3 mg/kg/day), which caused a similar reduction of seminal PGs, had no effect on fertility. Saksena *et al.* (1975) found

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that treatment of male rats with indomethacin (1–2.5 mg/kg/day for 30 days) did not alter fertility. However, in none of these studies, except the report of Marley & Smith (1974), was the effect of the drugs on seminal PGs measured. Differences between the results of these studies could be related to the extent of PG reduction, the duration of treatment, the dose of drug administered, the pharmacokinetics of the drug in the respective species, and the experimental approach to evaluate fertility. Rodents eliminate most non-steroidal anti-inflammatory drugs much more rapidly than do humans (see Löscher & Blazaki, 1986), which may result in misleading data if dose regimens used in human anti-inflammatory therapy are also used in animal experiments. For instance, salicylate concentrations reached in mice by the daily dosage of acetylsalicylic acid used in the study of Cenedella & Crouthamel (1973) were far below those known to inhibit PG synthesis (Flower, 1974; Matsuda *et al.*, 1983). By use of dosage regimens by which drug concentrations known as active from human anti-inflammatory therapy could be reached and maintained, we found in male rats a significant reduction of fertility after prolonged administration of indomethacin (2 mg/kg twice daily for 7 days) but not of other drugs, such as acetylsalicylic acid (150 mg/kg twice daily) and naproxen (10 or 20 mg/kg twice daily), although these drugs induced a decrease in seminal PGs similar to that of indomethacin (Löscher & Blazaki, 1986). The results suggested that reduction of PG synthesis in male rats does not affect fertility. However, in view of the marked species differences in seminal PG content and function (Cenedella, 1975; Kelly, 1978; Poyser, 1981), these data for rats do not exclude that reduction of seminal PG levels by non-steroidal anti-inflammatory drugs may affect fertility in other species. In rabbits the fertility of ejaculates can be significantly reduced by inactivation of PGs (Schlegel *et al.*, 1983). We have therefore determined the pharmacokinetics of several non-steroidal anti-inflammatory drugs in rabbits to find dosage regimens by which active drug concentrations could be reached and maintained during chronic treatment. The effects of drug treatment on fertility were then examined.

Materials and Methods

Animals. Sexually mature male and female rabbits of the Chinchilla-strain (Versuchstierzucht Dr Karl Thomae GmbH, Biberach, F.R.G.) were used. Body weight of the bucks ranged between 3000 and 4000 g, that of the does between 3000 and 4300 g. Animals were kept singly in cages at the Central Animal Laboratories in Berlin at a room temperature of $22 \pm 2^\circ\text{C}$ and a humidity of 55–65% with a 12-h light cycle from 07:00 to 19:00 h, and were supplied with food (Altromin 2010 standard diet, Altromin, Lage, F.R.G.) and water *ad libitum*. To prevent induction of pseudopregnancy, does were not handled or otherwise disturbed for at least 3 weeks before the fertility studies.

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 MSD 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 NaHCO_3 (acetylsalicylic acid), Na_2CO_3 (indomethacin) or NaOH (naproxen) and were buffered to pH 7.5 with dilute HCl. Drugs were injected subcutaneously; injection volumes were 375–500 $\mu\text{l/kg}$ (phenylbutazone), 1 ml/kg (indomethacin, naproxen) or 3 ml/kg (acetylsalicylic acid). In the control trial, bucks were injected with the same volume of saline (9 g NaCl/l).

Pharmacokinetic studies. The pharmacokinetics of the drugs were studied after their s.c. injection in male rabbits. The following doses were administered to groups of 5 animals: 150 mg acetylsalicylic acid per kg body weight, 2 or 20 mg indomethacin/kg, 20 mg naproxen/kg, and 100 mg phenylbutazone/kg. The respective doses were chosen on the basis of previous experiments with these drugs in rats (Löscher & Blazaki, 1986). Blood was sampled from the ear vein at different times after administration for drug analysis in plasma (see below). With indomethacin and phenylbutazone, drug concentrations in plasma were also determined during prolonged treatment with different doses (see 'Results') in order to find dosage regimens suited for the fertility studies.

Fertility studies. Ten bucks with normal sperm quality were used. In a control trial, the animals were injected twice daily at a 12-h interval with saline for 9 consecutive days. On each day, body weight of the animals was measured. Blood was sampled at each day before the morning injection and 80 min thereafter. At 1 h after the morning injection on Days 3, 8 and 9 of the trial, semen was collected with an artificial vagina (Büsing, 1973). Ejaculate volume was determined after removing the seminal coagulum. Aliquants of semen collected on Days 3 and 9 were immediately deep-frozen in liquid nitrogen and stored at -80°C until PG analysis. Aliquants of semen samples collected on Days 8 and 9 were examined immediately after collection for different measures of sperm quality, i.e. sperm density, total sperm count, percentage of spermatozoa with forward locomotion, quality of sperm motility (scored from 0, immotile, to 5, vigorously progressive), and sperm viability. The methods used in these determinations have been described

in detail elsewhere (Busch *et al.*, 1982). At 1 h after the morning injection on Days 5, 6 and 7 of the trial, each buck was housed with one doe so that a total of 30 does were mated during the 3 days. The animals were observed until mating was finished, and the does were then housed singly again, killed 14 days later and their uteri were examined for the presence of implantation or resorption sites. The numbers of corpora lutea were also counted in each doe. At 5 weeks after the control trial, the 10 bucks were treated with phenylbutazone at an initial dose of 100 mg/kg followed by maintenance doses of 75 mg/kg administered twice daily for 9 days. Seminal PG levels, sperm quality and fertility were studied as described for the control trial. Blood samples collected before the morning dose and 80 min thereafter were used for determinations of plasma concentrations of phenylbutazone. Once weekly evaluation of sperm quality in the 5 weeks between control and drug trial showed that the different semen parameters did not change during this period (data not illustrated).

PG analysis. Concentrations of PGE-2 and PGF-2 α were determined in the ejaculates by radioimmunoassay as previously described (Schlegel *et al.*, 1977, 1983). In brief, semen aliquants were acidified with 1 N-HCl and extracted with ethyl acetate. After extraction, PGE-2 and PGF-2 were purified by silicic acid column chromatography using columns of silicic acid BIO-Sil A, 100–200 mesh (Bio-Rad, Richmond, CA, U.S.A.) as described by Jaffe & Behrmann (1974). PGE-2 and PGF-2 α were then analysed by using antisera raised in rabbits (see Schlegel *et al.*, 1983). The overall recovery of added PGE-2 and PGF-2 α was 70–75%. PGE-2 determination of some rabbit ejaculates by a commercially available radioimmunoassay (New England Nuclear, Boston, MA, U.S.A.) yielded considerably lower levels than those determined with the antiserum used for the present study. The difference between the two assays might be related to the rapid decomposition of PGE-2 to PGA-2 which is known to occur during storage and extraction of ejaculates (Kelly, 1978), since our PGE-2 antiserum cross-reacts with PGA-2 (70%), whereas the commercial PGE-2 assay shows no cross-reactivity with PGA-2. Also, cross-reactivity with PGE-1 might be involved which was 50% for our antiserum but only 3.7% for the commercial assay (cross-reactivity data are expressed as the ratio of the PG concentration to the cross-reacting substance concentration at 50% inhibition of maximum binding). In any event, differences between the PGE-2 assays were not due to in-vitro synthesis of PGE-2 during collection and storage of ejaculates, since rabbit ejaculates do not contain PG synthetase (W. Schlegel, unpublished experiments). The antibody to PGF-2 α used in the present study was highly specific with cross-reactivities below 0.1% for all available PGs (Schlegel *et al.*, 1983).

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 *et al.* (1969). Indomethacin and naproxen were measured by spectrofluorometry with the methods of Lindquist *et al.* (1974) and Mortensen *et al.* (1979), respectively.

Statistics. Log-linear regression analysis was used for determination of elimination kinetics in the pharmacokinetic studies as well as for calculation of correlations between semen measures and PG concentrations in the fertility studies. Significance of differences in PG levels and sperm quality between the control trial and phenylbutazone treatment was calculated by the Wilcoxon signed-rank test for paired replicates. For statistical evaluation of fertility data, the χ^2 test was used.

Results

Pharmacokinetics

Mean plasma concentrations of naproxen, indomethacin, acetylsalicylic acid and phenylbutazone after s.c. administration of single doses in groups of 5 male rabbits per drug are shown in Fig. 1. With all drugs, plasma concentrations within or above the anti-inflammatory concentration range known from man were reached at the dosages administered (see Fig. 1). For acetylsalicylic acid, only the active metabolite salicylic acid could be measured in plasma, because levels of the parent drug were too low for reliable determination, indicating a very rapid metabolism after the injection. In fact, all drugs were rapidly eliminated from the plasma after maximum concentrations had been reached. Consequently, with naproxen, acetylsalicylic acid and the lower dose of indomethacin, active concentrations were only maintained for 2–4 h after the injection. When indomethacin was injected at a higher dose (20 mg/kg), the drug was eliminated more slowly and so active plasma concentrations were attained for up to 14 h after s.c. administration. This was similar with phenylbutazone at the dose administered (100 mg/kg). None of the drugs caused side-effects after acute single-dose administration.

Pharmacokinetic parameters calculated from the respective plasma concentration/time curves are shown in Table 1. Half-lives of the different drugs varied between 1 and 3 h. Based on the concentration/time curves, indomethacin and phenylbutazone were chosen for experiments with prolonged administration, whereas elimination of the other drugs was considered too rapid to

Fig. 1. Plasma concentrations (mean $\pm$ s.d.) of drugs after s.c. injection in male rabbits (5 animals per drug). For acetylsalicylic acid, symbols refer to plasma levels of the metabolite salicylic acid. The straight lines through the experimental values were constructed by means of log-linear regression analysis. The horizontal broken lines indicate the anti-inflammatory plasma concentration range of the respective drugs known from man (Bochner *et al.*, 1978).

allow maintenance of active drug concentrations during chronic experiments. Plasma drug concentrations in male rabbits during prolonged treatment with indomethacin and phenylbutazone are shown in Figs 2 and 3. Indomethacin was administered to 2 bucks twice daily at a dose of 10 mg/kg s.c. for 7 days. Plasma concentrations before each injection were mostly below the therapeutic concentration range (Fig. 2a). On Days 3, 4 and 6 of this experiment, plasma concentrations 1–2 h after the morning dose ranged between 7 and 12 µg/ml (data not illustrated). Both animals lost weight (120 and 240 g) during the period of treatment with indomethacin and appeared depressed. Phenylbutazone was administered in two different dosage regimens. In the experiment illustrated in Fig. 2(b), the drug was injected into 2 bucks twice daily at 100 mg/kg s.c. Plasma concentrations

Table 1. Pharmacokinetics of non-steroidal anti-inflammatory drugs in male rabbits

Drug	Dose (mg/kg s.c.)	C _{max} (µg/ml)	t _{max} (h)	k _{el} (h ⁻¹)	t _{0.5} (h)
Indomethacin	2	1.7 (1.3–2.2)	1	0.718 (0.481–0.942)	1.0 (0.7–1.4)
	20	58 (47–70)	1	0.366 (0.263–0.455)	2.0 (1.5–2.6)
Naproxen	20	62 (59–65)	1	0.511 (0.439–0.613)	1.4 (1.1–1.6)
Acetylsalicylic acid	150	213 (197–230)	1.2 (1–2)	0.482 (0.327–0.676)	1.6 (1.0–2.1)
Phenylbutazone	100	263 (234–319)	2.4 (2–3)	0.231 (0.176–0.279)	3.1 (2.5–4.0)

Drugs were administered subcutaneously to groups of 5 bucks. Data are given as means and range. For acetylsalicylic acid, the results refer to the metabolite salicylic acid (levels of acetylsalicylic acid were too low for reliable quantification).

C_{max}, maximal drug concentration in plasma; t_{max}, time at which C_{max} was determined; k_{el}, elimination rate constant; t_{0.5}, elimination half-life.

were within the therapeutic range throughout the course of the experiment, but the animals lost weight (240 and 400 g), appeared depressed, and died 3–4 h after the morning dose on Day 6. Severe haemorrhages and gastrointestinal lesions were found in the animals. In the second experiment with phenylbutazone, the daily dosage was decreased in that the drug was injected at an initial dose of 100 mg/kg followed by maintenance doses of 75 mg/kg injected twice daily (except the first day, at which this dose was injected 12 h after administration of the initial dose). During the course of the 7-day experiment with this dosage regimen in 4 bucks (Fig. 2c), no weight reduction and no behavioural alterations were noted. Furthermore, sexual drive was not impaired as indicated by collection of semen with an artificial vagina. This dosage regimen of phenylbutazone was therefore chosen for the fertility studies. Plasma concentrations of phenylbutazone in the 10 bucks selected for the fertility studies are shown in Fig. 3. Active concentrations of phenylbutazone were attained at the time of semen collection and mating, respectively (see below).

Effect of phenylbutazone on seminal PG concentrations, sperm quality and fertility

PG concentrations and measures used for evaluation of sperm quality during the 9-day control trial and the trial with phenylbutazone are shown in Table 2. During the control trial, there was no significant correlation between PG values and any of the different semen measures. Treatment with phenylbutazone significantly reduced seminal PGF-2α concentrations by 87 and 77% on Days 3 and 9 of the trial, respectively. A comparable reduction was found for PGE-2 on Day 9, whereas on Day 3, PGE-2 concentrations were only reduced by 26%. There was a moderate but significant increase in ejaculate volume during treatment with phenylbutazone which may, in part, have been associated with an increased sperm output, since on Day 8 sperm density was not altered and total sperm output appeared to be increased, although the difference from control was not significant. However, on Day 9, sperm density was significantly reduced. The percentage of motile spermatozoa in the ejaculates and viability of the spermatozoa were not altered by treatment with phenylbutazone, but in several ejaculates collected during the phenylbutazone trial the spermatozoa exhibited a more progressive motility compared to the control trial, which is illustrated by the trend for higher motility scores (see Table 2).

Sexual drive of the animals was not altered by treatment with phenylbutazone, but erection was slightly impaired. Body weight of the animals did not change during the phenylbutazone trial.

Fig. 2. Plasma concentrations of drugs during subacute treatment of male rabbits with (a) twice daily s.c. injection of 10 mg indomethacin/kg for 7 days, (b) twice daily s.c. injection of 100 mg phenylbutazone/kg for 6 days (the rabbits died (†) 2–3 h after the morning dose of Day 6), and (c) s.c. initial injection of 100 mg phenylbutazone/kg followed by twice daily injection of 75 mg/kg. The symbols indicate the plasma levels determined in the individual rabbits before each injection. The horizontal broken lines indicate the lower level of the anti-inflammatory plasma concentration range of the drugs known from man (Bochner *et al.*, 1978).

Results of the fertility studies are shown in Table 3. All bucks were fertile in the control and phenylbutazone trial, but fertility appeared to be increased during treatment with phenylbutazone as indicated by the significant increase in the number of implantation (and resorption) sites in relation to the number of corpora lutea in the mated does.

Fig. 3. Plasma concentrations of phenylbutazone in male rabbits during the fertility experiments. Phenylbutazone was administered at an initial dose of 100 mg/kg s.c. followed by twice daily injection of 75 mg/kg. The symbols indicate the mean ($\pm$ s.d.) plasma concentrations determined in 10 bucks before each morning dose and 80 min thereafter. The horizontal broken lines indicate the anti-inflammatory plasma concentration range of phenylbutazone known from man (Bochner *et al.*, 1978).

Discussion

The present results demonstrate that prolonged treatment of male rabbits with phenylbutazone at doses which significantly decrease seminal PGs does not impair sperm quality and fertility. Instead, there was a trend to increased semen volume, sperm output, sperm motility and fertility in the phenylbutazone-treated bucks. These results seem to be in contrast to previous data in rabbits which showed that inactivation of PGE-2 or PGF-2 α in semen by incubation with selective antisera or reduction of seminal PG levels by incubation with PG-15-hydroxydehydrogenase (which was inseminated with the ejaculate) reduced the fertilizing ability of the ejaculates (Schlegel *et al.*, 1983). However, these data were derived from in-vitro treatment of rabbit ejaculates with selective antisera or enzymes, whereas the present results obtained with systemic administration of an inhibitor of PG synthesis have to be related to in-vivo alterations of the arachidonic acid pathway in different parts of the male reproductive tract. Recent evidence suggests that PGs can enhance or reduce male fertility, depending upon the site of action (see Cenedella, 1975; Kelly, 1978; Poyser, 1981). Testicular function is impaired by chronic s.c. administration of high doses of PGE-2 and PGF-2 α to rats and mice as indicated by a decrease in testosterone production and spermatogenesis (Bartke *et al.*, 1973; Saksena *et al.*, 1973; Abbatiello *et al.*, 1975; Chinoy *et al.*, 1980), which may suggest that male fertility is 'down-regulated' by endogenous prostaglandins present in the testis (Cenedella, 1975; Poyser, 1981). In humans a high sperm density is associated with a low concentration of PGs, especially PGE (Kelly *et al.*, 1979; Bendvold *et al.*, 1984). In mice, spermatogenesis is increased by prolonged treatment with acetylsalicylic acid (100 or 200 mg/kg twice daily for 15 days) or indomethacin (1 mg/kg/day for 15 days) (Abbatiello *et al.*, 1975). In oligospermic patients, chronic treatment with indomethacin or ketoprofen increased seminal sperm concentrations (Padrón & Nodarse, 1979; Barkay *et al.*, 1984), whereas in normospermic volunteers, non-steroidal anti-inflammatory drugs had no effect on sperm density or total sperm output (Freixa *et al.*, 1984;

Table 2. Effect of subacute treatment with phenylbutazone on various semen parameters of male rabbits

Measure	Day of the trial	Control trial	Phenylbutazone trial
PGE-2 (ng/ml)	3	9.1 (2.6–15)	6.7 (0–23)
	9	11 (2.5–37)	3.3 (1.8–5.3)***
PGF-2 α (ng/ml)	3	5.3 (2.0–8.1)	0.7 (0.3–1.6)***
	9	6.6 (2.4–15.3)	1.5 (0.6–3.3)***
Ejaculate volume (ml)	3	0.39 (0.1–0.7)	0.5 (0.3–0.7)**
	8	0.36 (0.2–0.55)	0.48 (0.3–0.7)*
	9	0.42 (0.2–0.95)	0.55 (0.35–1.0)
Sperm density ($\times 10^{-6}/\text{mm}^3$)	8	0.18 (0.02–0.48)	0.24 (0.05–0.76)
	9	0.23 (0.06–0.45)	0.10 (0.03–0.17)*
Total sperm count ($\times 10^{-6}/\text{ejaculate}$)	8	66 (9–233)	108 (19–227)
	9	83 (19–180)	56 (9–93)
Sperm motility			
Motile spermatozoa (%)	8	80 (65–90)	80 (50–90)
	9	71 (55–90)	71 (60–90)
Quality of motility (score)	8	2.8 (0–5)	3.9 (2–5)
	9	2.0 (0–5)	2.6 (0–5)
Sperm viability (%)	8	72 (36–93)	73 (54–90)
	9	Not determined	74 (48–89)

Phenylbutazone was administered twice daily (initial dose 100 mg/kg, maintenance dose 75 mg/kg s.c.) to 10 bucks for 9 days. A control trial with twice daily injection of saline was carried out in the same bucks 5 weeks before the experiment with phenylbutazone. At the days of the experiments indicated, semen was collected with an artificial vagina and examined immediately after collection, except for PG concentrations for which aliquants of ejaculates were immediately deep-frozen in liquid nitrogen until analysis.

Data are given as means and range. Significance of differences compared to control data was calculated by the Wilcoxon signed rank test for paired replicates and is indicated by asterisks: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$.

Bendvold *et al.*, 1985). In contrast, chronic treatment of male rats with very high, toxic doses of drugs, such as indomethacin, phenacetin and paracetamol, is known to cause testicular atrophy, inhibition of spermatogenesis and sterility (Boyd, 1970; Jacqueson *et al.*, 1984; Neumann, 1984), but this is apparently a non-specific part of a toxicity syndrome known from many drugs and chemicals which exert no effects on PG synthesis (Neumann, 1984).

Testicular prostaglandins seem to reduce spermatogenesis also in rabbits as indicated by data showing that intrascrotal deposition of PGF-2 α caused a significant reduction in sperm number per ejaculate and induced temporary sterility (Saksena & Lau, 1979). However, chronic subcutaneous administration of PGF-2 α or PGE-2 did not reduce fertility of male rabbits (Hunt & Nicholson, 1972; Hafs *et al.*, 1974) but did increase sperm output (Hafs *et al.*, 1974) which may be explained by direct and indirect effects of the PGs on the contractility of the testicular capsule, seminiferous tubules, epididymis and vas deferens leading to enhanced sperm transport through the male reproductive tract (see Poyser, 1981). In addition to effects on testicular function, PGs administered to mice and rats tend to reduce the weight of the accessory sexual glands (Poyser, 1981). This may be due to the reduction in circulating testosterone concentrations. Castrated rats receiving testosterone and indomethacin had significantly enlarged seminal vesicles and ventral prostates compared to animals treated with testosterone alone. Treatment with testosterone and PGF-2 α reduced the growth promoting effect of testosterone on these glands, indicating that PGF-2 α has a direct inhibitory effect on the growth of these glands (Joseph & Siwela, 1976). The present finding that ejaculate volumes increased during treatment with phenylbutazone could thus be related to the significant reduction of PGF-2 α .

Table 3. Effect of subacute treatment with phenylbutazone on fertility in male rabbits

	Control trial	Phenylbutazone trial
No. of does with corpora lutea	27/27	29/30
Total no. of corpora lutea	340	353
No. of does with implantations	18/27	25/30
Total no. of implantations	141	245
No. of does with resorptions	5/27	1/30
Total no. of resorptions	10	6
No. of corpora lutea per pregnant doe	12.7 $\pm$ 1.6	12.0 $\pm$ 1.7
No. of implantations or resorptions per pregnant doe	7.9 $\pm$ 3.8	9.7 $\pm$ 2.8
Fertilization (%)		
No. of pregnant does/total no. of mated does	70	87
No. of implantations and resorptions/no. of corpora lutea	44	70*
No. of implantations and resorptions per pregnant doe/no. of corpora lutea per pregnant doe	58	80

Phenylbutazone was administered twice daily (initial dose 100 mg/kg, maintenance dose 75 mg/kg s.c.) to 10 bucks for 9 days. After 4 days of treatment, each buck was mated with 3 does on three subsequent days. Does were killed 14 days after insemination for evaluation of corpora lutea as well as implantation and/or resorption sites in the uterus. A control trial with twice daily injection of saline was carried out in the same bucks 5 weeks before the experiment with phenylbutazone. Three does of the control trial were not evaluated because of endometritis.

Data for corpora lutea per pregnant doe and for implantation and resorption sites per pregnant doe are means $\pm$ s.d. of 19 (control trial) or 26 (phenylbutazone trial) does, respectively. Significance of differences in fertilization compared to controls was calculated by the χ^2 test: $P < 0.01$.

Data on PGs and sperm motility are controversial which, at least in part, may be related to the fact that in most studies on sperm motility unphysiologically high levels of PGs were added to semen samples and changes in sperm motility were evaluated by subjective ranking systems (Cenedella, 1975). By use of time-exposure photomicrography, Aitken & Kelly (1985) found that addition of physiological concentrations of PGE-1, 19-hydroxy PGE-1, PGE-2 or PGF-2 α to human semen samples increased sperm velocity and the frequency of sperm head rotation. Furthermore, these authors reported increases in the ovum-penetrating ability of human spermatozoa after exposure to PGs, particularly PGE-1 and PGE-2. These results therefore seemed to substantiate reports of a correlation in men between low levels of PGs, particularly the PGEs, and otherwise unexplained infertility (Bygdeman *et al.*, 1970; Sturde & Glowania, 1974; Gstöttner *et al.*, 1975; Svanborg *et al.*, 1983), although, because of the wide ranges of PGE concentrations in normal fertile men and the instability of the PGEs, such findings of lower PGE values in men with idiopathic infertility need to be interpreted with caution (Kelly, 1978; Schlegel *et al.*, 1981). In line with the data of Aitken & Kelly (1985) on the increase of sperm motility by addition of PGs to human semen, we have shown that inactivation of PGs in rabbit or human semen by incubation with PG-15-hydroxydehydrogenase resulted in a dramatic fall in sperm motility (Schlegel *et al.*, 1981, 1983) which in rabbits was associated with a reduction of fertilization rate. When individual PGs were selectively inactivated by incubation with antisera, inactivation of PGE-2 reduced sperm motility only slightly, whereas inactivation of PGF-2 α seemed to improve motility (Schlegel *et al.*, 1983), which might indicate that the effect of incubation with PG-15-hydroxydehydrogenase on sperm motility was due to reduction of other PGs. In the present study, however, marked reduction of seminal PG levels by systemic inhibition of PG synthesis did not reduce sperm motility. These data strongly suggest that effects resulting from in-vivo alterations of endogenous PG levels in males differ from effects which are obtained by selective in-vitro changes in seminal PGs. Indeed, Freixa *et al.* (1984) reported that marked decreases of seminal PGE-1, PGE-2 and 19-hydroxy PGE-2 levels by a 4-day treatment of volunteers with the non-steroidal anti-inflammatory drugs flubiprofen

and lysine salicylate had no effect on sperm motility. Administration of naproxen to volunteers for 2 weeks also did not significantly influence sperm motility, although the concentration of seminal PGs was reduced by more than 70% (Bendvold *et al.*, 1985). In oligospermic patients, chronic treatment with indomethacin or ketoprofen even increased sperm motility (Padrón & Nodarse, 1979; Barkay *et al.*, 1984). In fact, as in the present experiments with phenylbutazone, treatment with indomethacin and ketoprofen seemed to increase male fertility (Barkay *et al.*, 1984) and it was suggested that non-steroidal anti-inflammatory drugs may have a beneficial effect in the therapy of male infertility (Padrón & Nodarse, 1979; Barkay *et al.*, 1984). The antifertility effect of high doses of indomethacin determined previously in mice (Marley & Smith, 1974) and rats (Löscher & Blazaki, 1986) may be unrelated to PG content and synthesis, since it is known that, at high concentrations, this drug inhibits various other enzymic processes (Flower, 1974).

Besides effects of PGs on the male reproductive systems, PGs are known to exert pronounced effects upon the female reproductive tract (Cenedella, 1975; Poyser, 1981). Semen instilled into the human vagina usually, but not invariably, causes the uterus to contract. In addition, when the uterus contracts, the cervix usually relaxes. Consequently, these two actions could be looked on as a suction pump transporting the spermatozoa into the uterus, and may be due to the PGs present in human semen (Poyser, 1981). In fact, addition of PGs to intravaginally inseminated ejaculates has been shown to increase the fertilization rate in rabbits (Chang *et al.*, 1973; Spilman *et al.*, 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). However, the present data obtained by in-vivo alterations of PG levels in rabbits show that reduction of seminal PGs does not decrease the fertilization rate.

Like other non-steroidal anti-inflammatory drugs, phenylbutazone inhibits the fatty acid cyclooxygenase (PG synthetase) which is responsible for the first step of the synthesis of PGs from arachidonic acid (Flower, 1974). An unexpected finding in the present study was therefore that, after 3 days of treatment with phenylbutazone, reduction of PGF-2 α levels was much more marked than reduction of PGE-2. This finding may be explained by the fact that in various tissues, including those of the male reproductive tract, PGE-2 is converted to PGF-2 α by the enzyme PGE-2 9-ketoreductase (Thuy & Carpenter, 1978). This enzyme is inhibited by non-steroidal anti-inflammatory drugs, such as indomethacin (Krüger & Schlegel, 1986), which would explain the present finding in that concomitant inhibition of PG synthetase and PGE-2 9-ketoreductase will lead to a more marked reduction of PGF-2 α than of PGE-2 as long as inhibition of PG synthetase is not complete. As indicated by the dose regimen studies, highly toxic doses of phenylbutazone would have been required to block PG synthesis completely.

The present pharmacokinetic data in rabbits also deserve comment. We have previously shown that rats eliminate non-steroidal anti-inflammatory drugs, at least in part, more rapidly than do humans (Löscher & Blazaki, 1986). The present determinations in rabbits show that the drugs are eliminated in this species even more rapidly than in rats and high doses had to be administered to obtain plasma concentrations which correspond to those associated with anti-inflammatory action in humans (Bochner *et al.*, 1978) and inhibition of PG synthesis in animals (Flower, 1974; Matsuda *et al.*, 1983). With respect to chronic studies with the drugs in rabbits, the present experiments with indomethacin and phenylbutazone showed that, due to the rapid elimination, maintenance of active drug concentrations during prolonged treatment was only possible by twice daily administration of high doses which led to toxic side-effects. An alternative to administration of high doses would have been a more frequent administration of the drugs which, however, could have biased the fertility studies. The dosage regimen of phenylbutazone used for the fertility studies was therefore a compromise, since active drug concentrations were not maintained throughout the interval between the two daily injections, but significant inhibition of PG synthesis could be reached without concomitant development of toxic side-effects. These results emphasize that the pharmacokinetics of a given drug have to be taken into consideration to find dosage regimens which lead to pharmacologically relevant concentrations during chronic treatment.

In conclusion, the present study has shown that reduction of seminal PGs by drugs, such as phenylbutazone, which inhibit PG synthesis does not impair male fertility. The data seem to indicate that, at least in rabbits, male fertility does not critically depend on the formation and content of PGs within the reproductive tract. The possibility that chronic administration of non-steroidal anti-inflammatory drugs improves sperm quality and might thus be suited for therapy of infertile men with oligospermia deserves further study.

We thank Professor D. Rohloff (Department of Reproductive Physiology, School of Veterinary Medicine, Berlin) and Professor K. F. Weitze and Dr D. Rath (Department of Andrology and Insemination, School of Veterinary Medicine, Hannover) for helpful discussion and advice; Mr F. Müller for the assistance with the drug level determinations; and Mr R. Pund for the assistance in the fertility studies. The results are part of the doctoral thesis of H.L.

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Received 8 July 1987