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Sex-related Differences in Disposition and Response to Phenprocoumon in Rats

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Abstract—The pharmacokinetics and the pharmacological response to phenprocoumon have been studied in female and male inbred Lewis-Wistar rats. A significantly lower clearance was found in female than in male rats ($7{\cdot}9 \pm 1{\cdot}4$ vs $24{\cdot}5 \pm 2{\cdot}5$ mL h^{-1} kg^{-1}, respectively; $t = 15{\cdot}09$, $P < 0{\cdot}001$) as well as a lower apparent volume of distribution (288 ± 46 vs 617 ± 105 mL kg^{-1}; $t = 7{\cdot}58$, $P < 0{\cdot}001$) and a longer half-life ($25{\cdot}5 \pm 3{\cdot}4$ vs $17{\cdot}5 \pm 1{\cdot}8$ h; $t = 5{\cdot}16$, $P < 0{\cdot}001$). The binding of phenprocoumon was higher in female than in male rats (f_u: $0{\cdot}0096 \pm 0{\cdot}0008$ vs $0{\cdot}0124 \pm 0{\cdot}0007$, respectively; $t = 6{\cdot}66$, $P < 0{\cdot}001$). The total (C) as well as the unbound concentration (C_u) needed to elicit a 50% decrease in the prothrombin complex synthesis rate was substantially higher in female rats: C50 was 377 ± 98 ng mL^{-1} in female and 155 ± 29 ng mL^{-1} in male rats ($t = 5{\cdot}32$, $P < 0{\cdot}001$), whereas C_u50 was $3{\cdot}6 \pm 0{\cdot}7$ ng mL^{-1} in female and $1{\cdot}9 \pm 0{\cdot}3$ ng mL^{-1} in male rats ($t = 5{\cdot}50$, $P < 0{\cdot}001$). However, because of the lower clearance and volume of distribution and the longer half-life in female rats, the female rats experienced a higher cumulative effect than male rats to $0{\cdot}34$ mg kg^{-1} i.v. doses.

Hagan & Radomski (1953) reported a substantially higher LD50 of warfarin in male rats than in female rats. Pyörälä (1968) found qualitatively similar differences in rats for warfarin and also determined the kinetics of warfarin and found a half-life of 18 h in males and 28 h in females. As the apparent volume of distribution was marginally smaller in female than in male rats (114 vs 132 mL kg^{-1}), it is therefore likely that the higher LD50 in male rats might be due to the drug's pharmacokinetics. With a longer half-life in female rats, the inhibition of the synthesis of clotting factors is expected to be longer-lasting in females, resulting in a more severe suppression of the clotting factors. It is not clear if phenprocoumon, an anticoagulant similar to warfarin, shows a similar effect in rats.

This study was undertaken to investigate sex differences in the anticoagulant activity of phenprocoumon and to determine if sex differences in the disposition of phenprocoumon exist. In addition, the potential influence of sex differences in pharmacokinetics on sex differences in response to anticoagulants was investigated.

Materials and Methods

Phenprocoumon

Racemic unlabelled and ^{3}H-labelled phenprocoumon (803 μCi mg^{-1}, purity >99%) were kindly provided by Hoffmann-La Roche (Basel, Switzerland).

Animals

Inbred Lewis-Wistar rats (240–303 g) were used (Zentralinstitut für Versuchstiere, Hannover, FRG). The rats were age-matched. Inbred rats were used to decrease the inter-individual variability in metabolism of coumarins (Pyörälä & Nevanlinna 1968) and therefore to characterize any sex-differences better.

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Animal experiments

Seven female (240–260 g) and six male rats (280–303 g) were injected with $0{\cdot}34$ mg kg^{-1} [^{3}H]phenprocoumon in the tail vein under light ether anaesthesia. Eight 450 μL blood samples were obtained from the tail artery under light ether anaesthesia (Wingard & Levy 1973) at 12, 18, 24, 30, 36, 42, 48 and 54 h post phenprocoumon administration. The samples were mixed with 50 μL of a solution containing 1·34% sodium oxalate and 0·7% sodium chloride. The samples were subsequently centrifuged (12 000 rev min^{-1}, Eppendorf 3200 centrifuge), the plasma harvested and stored frozen (−20 °C) until assayed.

After termination of the study (54 h), the animals were placed under ether anaesthesia, the abdomen opened and the animals exsanguinated via the inferior vena cava. The blood was allowed to clot, the samples were subsequently centrifuged (4500 rev min^{-1}) for 15 min, the serum collected and immediately used for protein binding determination and for total phenprocoumon assay. After exsanguination the liver was removed, the remaining blood removed and the tissue was then coarsely minced with scissors, mixed with 3 parts 0·9% NaCl (saline) and homogenized, using an Ultra-Turrax homogenizer (Janke & Kunkel KG, Staufen, FRG). The homogenized samples were immediately assayed for phenprocoumon.

Assay

Phenprocoumon was measured, using the method described by Schmidt & Jähnchen (1979).

Protein binding. Serum protein binding was determined in the rat serum obtained at the end of the study, using an equilibrium dialysis technique described earlier (Schmidt & Jähnchen 1979).

Pharmacokinetic analysis. The phenprocoumon data was fitted with a one-compartment model. The half-life ($t_{\frac{1}{2}}$) and elimination rate constant (k) were obtained from the fitted

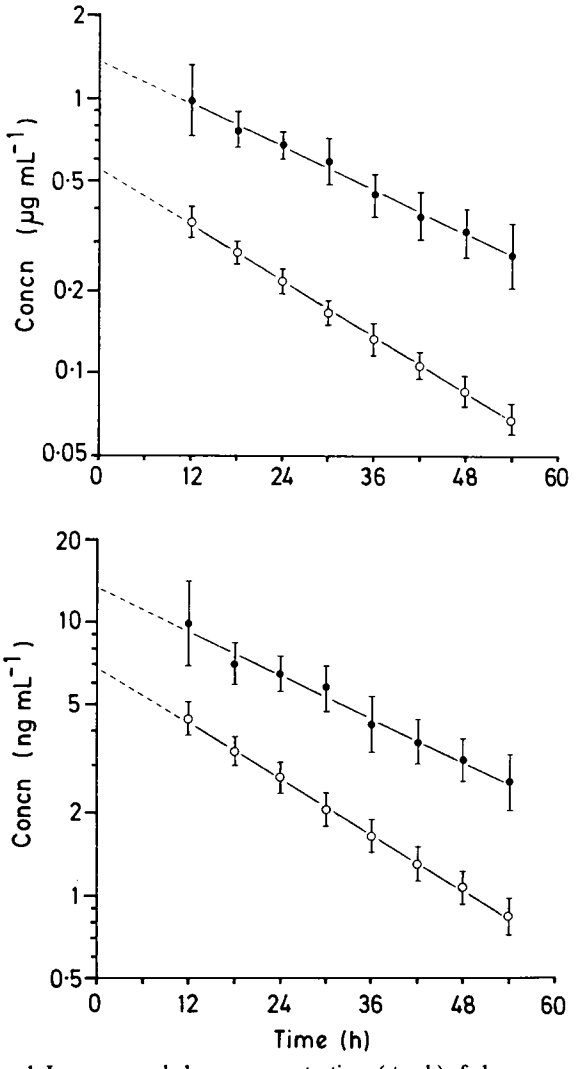

FIG. 1. Log-averaged plasma concentrations (±s.d.) of phenprocoumon after 0·34 mg kg^{-1} i.v. doses to 7 female (●) and 6 male rats (○). Upper panel: total plasma concentration. Lower panel: unbound plasma concentration.

line, the apparent volume of distribution (V) from the back-extrapolated zero time concentration and the clearance (CL) from the i.v. dose given and the fitted area under the plasma concentration-time curve.

The intrinsic clearance (CL_i) was determined using the ratio CL/f_u, whereby f_u is the unbound fraction of phenprocoumon determined. Since phenprocoumon is a low extraction ratio drug, the use of CL/f_u for CL_i is a reasonable approximation (Wilkinson & Shand 1975). The unbound apparent volume of distribution (V_u) was determined by the ratio V/f_u.

Prothrombin complex activity. The prothrombin complex activity was measured at each time as described by Schmidt & Jähnchen (1977).

The synthesis rate of the prothrombin complex activity was carried out as described by Nagashima et al (1969) using an apparent elimination rate constant of the prothrombin complex of 0·133 h^{-1} (Martini & Jähnchen 1977). The synthesis rate was subsequently plotted versus the log concentrations, log unbound concentrations or log amounts (apparent volume of distribution × concentration). A linear regression was made of the points in the straight line portion and the value for 50% inhibition of synthesis rate determined.

The area under the effect curve was obtained from a normalized prothrombin complex activity-time curve by using the trapezoidal rule. The curves were normalized by setting the prestudy value to 100% and adjusting all values by the factor (measured prestudy value of the prothrombin complex activity in % divided by 100%).

Statistics. Comparison of mean values was done using the non-paired *t*-test. Statistical sigrificance was set at the 5% level.

Results

The log-averaged plasma concentrations (±s.d.) of total and unbound phenprocoumon for male and female rats are given in Fig. 1. The clearance of phenprocoumon was found to be 3·1-fold lower in female than in male rats ($t=15·09$, $P<0·001$; Table 1) and the unbound clearance 2·4-fold lower ($t=10·72$, $P<0·001$; Table 2). The apparent volume of distribution of both total and unbound phenprocoumon was found to be smaller in female than in male rats (2·1 and 1·7-fold respectively, $t=7·58$, $P<0·001$ and $t=4·79$, $P<0·001$; Tables 1, 2). Because the ratio of clearances was higher than the ratio of the volumes between males and females, the half-life was significantly shorter in males than in females ($t=5·16$, $P<0·001$).

The binding of phenprocoumon in serum was significantly stronger in female rats with a 23% lower unbound fraction

Table 1. Pharmacokinetic parameters of total phenprocoumon in female and male rats after 0·34 mg kg^{-1} intravenous doses (mean±s.d.).

	Female rats	Male rats
n	7	6
Weight, g	249±6	295±7
CL, mL h^{-1} kg^{-1}	7·9±1·4	24·5±2·5*
V, mL kg^{-1}	288±46	619±105*
$t_{\frac{1}{2}}$, h	25·5±3·4	17·5±1·8*
Liver/plasma concn ratio	5·2±1·0	5·9±0·6*

* Statistically significantly different from female rats, $P<0·001$.

Table 2. Pharmacokinetic parameters of unbound phenprocoumon in female and male rats after 0·34 mg kg^{-1} i.v. intravenous doses (mean±s.d.).

	Female rats	Male rats
n	7	6
CL_i, mL h^{-1} kg^{-1}	824±130	1988±252*
V_u, L kg^{-1}	30·1±5·9	50·3±9·2*
f_u × 100	0·96±0·08	1·24±0·07*
f_u liver × 100	0·190±0·026	0·210±0·026*

* Statistically significantly different from female rats, $P<0·001$.

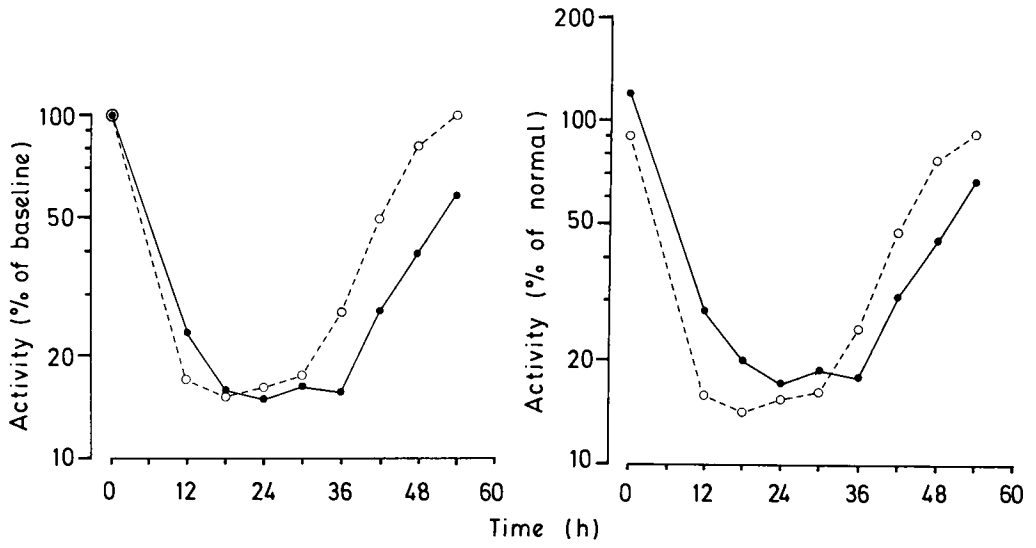

FIG. 2. Average prothrombin complex activity in 7 female (●) and 6 male rats (○) after 0·34 mg kg^{-1} i.v. phenprocoumon doses. Right panel: values determined using reference serum = 100%. Left panel: normalizing the values by setting the initial activity = 100%.

Table 3. Pharmacodynamic parameters* of phenprocoumon in female and male rats (mean ± s.d.).

	Female rats	Male rats
n	7	6
C50, ng mL^{-1}	377 ± 98	155 ± 29**
C_u50, ng mL^{-1}	3·6 ± 0·7	1·9 ± 0·3**
Ab50, ng kg^{-1}	110 ± 38	95 ± 16
AUEC, Δ% h	3636 ± 568	3081 ± 260***

* C50 = total plasma concentration needed to reduce the synthesis rate by 50%.
C_u50 = unbound plasma concentration needed to reduce the synthesis rate by 50%.
Ab50 = amount of phenprocoumon in the body needed to suppress the synthesis rate by 50%.
AUEC = area under effect curve.
** Statistically significantly different from female rats, $P < 0·001$.
*** In comparison with female rats: $t = 2·202$, $P = 0·05$.

($t = 6·66$, $P < 0·001$; Table 2), but no differences in the apparent liver binding or in the liver plasma concentration ratio were found (Tables 1, 2).

The average measured prothrombin complex activities and the normalized average prothrombin activities for male and female rats are given in Fig. 2. The area under the normalized effect-curve is only marginally larger in female rats than in male rats for the duration of the study (54 h) ($t = 2·20$, $P < 0·05$; Table 3). However, because the effect did not reach baseline values in the female rats at the time the study was terminated, in contrast to the male rats, one would expect that the differences in the areas under the effect curves would be larger if followed for a longer time (Fig. 2 lower panel). From the non-normalized prothrombin complex activity the female rats also had a higher activity (119 ± 12%) than male rats (91 ± 7%) that was statistically significant ($t = 5·01$, $P < 0·001$). Similar results have previously been reported for warfarin by Pyörälä (1965).

Because the effect-time curves were not substantially different between male and female, while the disposition showed dramatic differences, the total as well as the unbound concentration needed to achieve a 50% suppression of the normal synthesis rate of the prothrombin complex was much lower in male than in female rats ($t = 5·32$, $P < 0·001$ and $t = 5·50$, $P = 0·001$, respectively; Table 3). However, because the apparent volume of distribution is smaller in female than in male rats, when the amount in the body needed to suppress the synthesis rate 50% is calculated, the difference between female and male rats is negligible ($t = 0·90$, $P > 0·30$; Table 3).

Table 4. Simulated response to various phenprocoumon doses in female and male rats.

Dose mg kg^{-1}	Total area under the effect curve (0–∞) % h		Effect < 80% of normal h		Effect < 50% of normal h		Effect < 10% of normal h		Effect < 1% of normal h	
	F	M	F	M	F	M	F	M	F	M
0·1	410	510	1	12	0	0	0	0	0	0
0·3	3910	3090	57	46	42	33	5	0	0	0
1·0	8360	6140	101	77	86	63	56	38	22	5
3·0	12370	8930	142	105	126	91	97	66	63	38

Discussion

The slower metabolism found for phenprocoumon in female rats is consistent with a slower metabolism found for a number of drugs metablized by the P450 system in the rat (Kato et al 1961; Kato 1974; Chaplin et al 1981; Vodicnik et al 1981; Billings 1983). This difference appears to be sex hormone-dependent, as the difference in metabolism can be abolished by castration and the addition of androgen hormones to castrated females give metabolic activity equal to those of males (Kato et al 1961; Pyörälä 1968; Kato 1974). Kamataki et al (1982) have also isolated different P450-moieties from male and female rats and Kato & Kamataki (1982) have identified two P450-enzymes that are sex hormone-dependent.

The apparent volume of distribution was more than 2-fold higher in males than in females. Some of this higher apparent volume in males can be explained by the lower unbound fraction of phenprocoumon in the serum of males. However, using the model of Øie & Tozer (1979) for the relationship between apparent volume of distribution and plasma protein binding, it is only possible to account for 20% of the higher volume by the higher unbound fraction. The main difference lies rather in the fact that a significant higher fraction of phenprocoumon is located outside the albumin space and the liver in male rats. With the model of Øie & Tozer (1979) 65% of phenprocoumen in females and 84% in males is found to be located outside the albumin space. The reason for this higher tissue affinity for phenprocoumon in male rats is not clear. Data from the literature about the volume of distribution for other drugs in male and female rats are limited but suggest that no or only small differences may exist (Hagan & Radomski 1953; Kato et al 1961). Similarly, data from humans suggest that differences between males and females are usually small when corrected for plasma protein binding (Wilson 1984).

The small but significantly lower unbound fraction of phenprocoumon in serum in female vs male rats appears to be different from usual findings from the genders in man (for overview cf Wilson 1984). In man the binding is usually lower in females (higher unbound fraction). As a multitude of factors appears to be involved in differences in binding (Routledge et al 1981; Wilson 1984), it is difficult to give a mechanistic explanation for the observed differences.

The sensitivity of the synthesis rate of the prothrombin complex activity to phenprocoumon was substantially higher in male than in female rats, when either the unbound or total concentrations needed to inhibit the synthesis by 50% were used. On the other hand, female rats had a higher normal prothrombin complex activity than male rats (see Results). Because the decrease in the clotting factors occurred at the same fractional rate in male and female rats after doses sufficient to block the synthesis rate of the prothrombin complex activity temporarily (Fig. 2), it can be assumed that the synthesis rate of clotting factors may be higher in female than in male rats. It is has also been reported that male rats are more sensitive to vitamin K deprivation than female rats; this appears to be linked to the presence of oestrogens (Matschiner & Willingham 1974). It is therefore possible that, due to their oestrogen, female rats conserve vitamin K better than males by having a lower elimination of this vitamin and/or a higher ability to recycle vitamin K_1-epoxide. However, in preliminary studies in our laboratory (unpublished) we found no differences in the vitamin K_1-epoxide concentration or of the vitamin K_1-epoxide/vitamin K_1 ratio at identical unbound concentrations in male and female rats, suggesting that inhibition of the epoxide reductase by phenprocoumon is not sex-dependent. At this point we therefore can speculate that female rats in the presence of oestrogen have a higher utilization of the available vitamin K in the synthesis of clotting factors.

Although female rats are less sensitive to phenprocoumon than male rats at identical unbound or total concentrations, the differences in kinetics are sufficiently large that, overall, female rats do not show less response to phenprocoumon at equivalent doses (see values for Ab50 in Table 3). Because female rats have a lower apparent volume of distribution, approximately equipotent concentrations are achieved in both genders when identical doses are administered. However, the shorter half-life of phenprocoumon in male rats causes the concentrations to decrease more rapidly in males. Thus, recovery from anticoagulation is usually faster in males. Higher doses may therefore give rise to a prolonged and deeper anticoagulation in female rats due to a slower return to non-inhibiting concentrations of phenprocoumon. Simulations of the time course of prothrombin complex activity in male and female rats based upon the data obtained in this study* are illustrated in Table 4. As the values are normalized (initial values set to 100%), it appears that female rats only show a lower response to phenprocoumon than male rats at low doses when the blockage of the prothrombin complex synthesis is incomplete. At high doses the effect is significantly prolonged in female rats.

In summary, the disposition of phenprocoumon differs between male and female rats, with a substantially lower apparent volume of distribution and clearance in female rats. Although female rats had a lower sensitivity to phenprocoumon (measured as C50), the differences in kinetics caused an apparent equal response to the same doses (measured as Ab50) and a longer duration of the effect.

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* The average effects and concentrations time curve were fitted to the E_{max} equation:

$$E = \frac{E_{max}\ C^n}{E50^n + C^n}$$

where E is the fractional reduction in the synthesis rate, C is total plasma concentration and n a parameter factor. E_{max} is the maximum ability to block the prothrombin complex synthesis and was set to 1 here; E50 is the concentratioin required to reach a 50% effect.

In the simulation the fitted parameters were used (n = 5·0, E50 (females) = 0·360 μg mL^{-1}, E50 (males) = 0·141 μg mL^{-1}.

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