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Gender and oral contraceptive steroids as determinants of drug glucuronidation: effects on clofibric acid elimination

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The disposition of clofibric acid, a drug metabolised largely by glucuronidation, was studied in eight males, eight females and eight females receiving oral contraceptive steroids (OCS). Clofibric acid plasma clearance was not significantly different in males compared to the control female group but was 48% greater ($P < 0.01$) in women receiving OCS compared to non-pill using females. This difference was due to an alteration in clofibric acid metabolic clearance as there were no differences between the groups in clofibric acid free fraction. Along with previous data the results suggest that induction of drug glucuronidation by OCS may be of clinical importance, although any sex-related differences are unlikely to be of clinical significance.

Keywords clofibric acid drug metabolism glucuronidation contraceptive steroids sex differences

Introduction

A number of reports have shown that paracetamol clearance is greater in males compared to females (Wojcicki *et al.*, 1979; Mucklow *et al.*, 1980; Divoll *et al.*, 1982; Miners *et al.*, 1983). More recently, it was demonstrated that this difference was entirely due to increased activity of the glucuronidation pathway in males (Miners *et al.*, 1983). In addition, paracetamol clearance was markedly induced in females using oral contraceptive steroids (OCS) (Abernethy *et al.*, 1982; Miners *et al.*, 1983; Mitchell *et al.*, 1983), largely due to enhanced glucuronic acid conjugation (Miners *et al.*, 1983; Mitchell *et al.*, 1983). The present study was initiated to determine the effects of gender and OCS on clofibric acid disposition to characterise further the influence of these factors on the elimination of drugs metabolised by UDP-glucuronyl transferase.

Methods

Subjects

The subjects were 24 volunteers; eight males (age 19–34 years, weight 63–82 kg), eight females not taking OCS (age 19–29 years, weight 54–74 kg) and eight females using OCS (age 19–30 years, weight 51–69 kg). All subjects were healthy as determined by medical history, physical examination and standard haematological and biochemical parameters. In addition, all subjects were non-smokers and no medications, other than those required for the study, were taken for 1 week before and during the study. The OCS used included; Biphasil (ethinyloestradiol 0.05 mg, levonorgestrel 0.05/0.125 mg), Microgynon and Nordette (ethinyloestradiol 0.03 mg, levonorgestrel 0.15 mg) and Norinyl (mestranol 0.05 mg, norethisterone 1 mg). The control female group was studied between days

10 and 23 of the menstrual cycle and the OCS users between days 13 and 24 of the pill cycle. The study was approved by the Clinical Investigation Committee of Flinders Medical Centre and written informed consent was obtained from each subject.

Protocol

On the study day an indwelling cannula was inserted into a forearm vein of each subject. Venous blood samples (10 ml) were collected prior to and at 1, 2, 3, 4, 5, 6, 8 and 10 h after a single oral 1 g dose of clofibrate (Aterioflexin). The clofibrate was administered following an overnight fast. Additional blood samples were collected by venepuncture at 24, 28, 32, 48, 52 and 56 h post dose. Plasma was separated and stored at –20°C until analysis.

Analytical procedures

Plasma concentrations of clofibric acid were determined using a specific high performance liquid chromatographic method (Veenendaal & Meffin, 1981). Plasma protein binding of clofibric acid was measured by equilibrium dialysis (Veenendaal *et al.*, 1981).

Analysis of results

Area under the clofibric acid plasma concentration-time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity. Elimination half-life ($t_{1/2}$) was determined from the slope of terminal portion of the plasma concentration-time curve by linear least squares regression. Assuming complete bioavailability of clofibric acid (Mannisto *et al.*, 1975; Sedaghat & Ahrens, 1975), plasma clearance was calculated as,

$$CL = D/(AUC \times B.W.)$$

where B.W. is the body weight in kg. Volume of distribution at steady state (V_{ss}) was calculated by the model-independent procedure of Benet & Galeazzi (1979).

Results are expressed as mean ± s.e. mean. Differences among the group means for each pharmacokinetic parameter were examined by analysis of variance, with the Newman-Keuls test being used to detect differences between the individual study groups. Values of $P < 0.05$ were regarded as significant.

Results

Clofibric acid pharmacokinetic parameters in each of the three study groups are summarised in Table 1. Plasma clearance (CL) in the OCS-users (0.133 ± 0.008 ml $min^{-1}kg^{-1}$) was significantly higher than for both males (0.103 ± 0.004 ml $min^{-1}kg^{-1}$; $P < 0.01$) and the control female group (0.090 ± 0.006 ml $min^{-1}kg^{-1}$; $P < 0.01$). The difference in CL between males and the control female group was not statistically significant. Volume of distribution at steady state was 26–30% larger ($P < 0.05$) in both female groups (control group, 0.184 ± 0.016 l kg^{-1}; OCS-users, 0.190 ± 0.009 l kg^{-1}) compared to males (0.146 ± 0.011 l kg^{-1}). Half-life was approximately 65% longer in the control females compared to both males and the OCS-users ($P < 0.01$). The fraction of plasma protein bound clofibric acid was determined in the 4, 10,

Table 1 Clofibric acid pharmacokinetic parameters in males, females and OCS-using females.

Parameter	*Males*	*Females*	*OCS-users*
CL (ml $min^{-1}kg^{-1}$)	0.103 ±0.004	0.090 ±0.006	0.133** ±0.008
$t_{1/2}$ (h)	13.54 ±0.58	22.78** ±2.44	13.97† ±0.79
V_{ss} (l kg^{-1})	0.146 ±0.011	0.184* ±0.016	0.190* ±0.009
Free fraction (%)	2.49 ±0.19	2.56 ±0.11	2.78 ±0.12

Compared to males * $P < 0.05$, ** $P < 0.01$
Compared to females: † $P < 0.01$

24 and 48 h plasma samples from each subject. There was no difference in free fraction, either with respect to time or between groups. The mean time-averaged free fraction for each group is shown in Table 1.

Discussion

This study has investigated whether the effects of sex and OCS previously demonstrated with paracetamol glucuronidation were apparent for another glucuronidated drug, clofibric acid. While clofibric acid clearance was 14% higher in males compared to females, this difference was not significant. However, clofibric acid clearance was 48% greater in OCS-users compared to the control female group. Indeed, the magnitude of induction due to OCS was such that the clearance in pill users was 29% higher than in males. Data presented here also demonstrate that clofibric acid volume of distribution is greater in females compared to males.

The primary route of clofibric acid metabolism in man is glucuronide conjugation (Sedaghat & Ahrens, 1975; Caldwell *et al.*, 1979) and alterations in clofibric acid clearance therefore are likely to reflect changes in this pathway. Unlike paracetamol, which forms an ether glucuronide, clofibric acid forms an ester glucuronide. At least three different forms of UDP glucuronyl transferase have now been identified (Bock *et al.*, 1983) but at this stage it is not generally known whether different forms of the enzyme are involved in the formation of ether and ester conjugates. Apart from the data for paracetamol and clofibric acid, little other information is available concerning the effects of gender or OCS on xenobiotic glucuronidation. Patwardhan *et al.* (1981) reported that elimination of the glucuronidated benzodiazepines, oxazepam and lorazepam, was markedly induced in women receiving OCS, but this result was not confirmed in a later study (Abernethy *et al.*, 1983). Thus at this time it cannot be predicted for which groups of compounds induction of glucuronidation will occur as a result of OCS treatment. However, where enhanced glucuronidation due to OCS has been reported the increases observed in plasma drug clearance are large (approximately 50% or greater) (Patwardhan *et al.*, 1981; Miners *et al.*, 1983) and may have clinical consequences in terms of dosing schedules and duration of action. In contrast, reported sex-related differences in drug glucuronidation are small and unlikely to be of clinical importance.

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References

Abernethy, D. R., Divoll, M., Ochs, H. R., Ameer, B. & Greenblatt, D. J. (1982). Increased metabolic clearance of acetaminophen with oral contraceptive use. *Obstetrics and Gynecology*, **60**, 338–341.

Abernethy, D. R., Greenblatt, D. J., Ochs, H. R., Weyers, D., Divoll, M., Harmatz, J. S. & Shader, R. I. (1983). Lorazepam and oxazepam kinetics in women on low-dose oral contraceptives. *Clin. Pharmac. Ther.*, **33**, 628–632.

Benet, L. Z. & Galeazzi, R. L. (1979). Noncompartmental determination of the steady-state volume of distribution. *J. pharm. Sci.*, **68**, 1071–1074.

Bock, K. W., Burchell, B., Dutton, G. J., Hanninen, O., Mulder, G. J., Owens, I. S., Siest, G. & Tephly, T. R. (1983). UDP-Glucuronosyltransferase activities: guidelines for consistent interim terminology and assay conditions. *Biochem. Pharmac.*, **32**, 953–955.

Caldwell, J., Emudianughe, T. S. & Smith, R. L. (1979). Species variation in the taurine conjugation of clofibric acid. *Biochem. Soc. Trans.*, **7**, 421P–422P.

Divoll, M., Abernethy, D. R., Ameer, B. & Greenblatt, D. J. (1982). Acetaminophen kinetics in the elderly. *Clin. Pharmac. Ther.*, **31**, 151–156.

Mannisto, P. T., Tuomisto, J., Jounela, A. & Penttila, O. (1975). Pharmacokinetics of clofibrate and chlorophenoxy isobutyric acid. I. Cross-over studies in human volunteers. *Acta Pharmac. Tox.*, **36**, 353–365.

Miners, J. O., Attwood, J. & Birkett, D. J. (1983). Influence of sex and oral contraceptive steroids on paracetamol metabolism. *Br. J. clin. Pharmac.*, **16**, 503–510.

Mitchell, M. C., Hanew, T., Meredith, C. G. & Schenker, S. (1983). Effects of oral contraceptive steriods on acetaminophen metabolism and elimination. *Clin. Pharmac. Ther.*, **34**, 48–53.

Mucklow, J. C., Fraser, H. S., Bulpitt, C. J., Kahn, C., Mould, G. & Dollery, C. T. (1980). Environmental factors affecting paracetamol metabolism in London factory and office workers. *Br. J. clin. Pharmac.*, **10**, 67–74.

Patwardhan, R., Mitchell, M., Johnson, R. & Schenker, S. (1981). Induction of glucuronidation by oral contraceptive steroids. *Clin. Res.*, **29**, 861A.

Sedaghat, A. & Ahrens, E. H. (1975). Lack of effect of cholestyramine on the pharmacokinetics of clofibrate in man. *Eur. J. clin. Invest.*, **5**, 177–185.

Veenendaal, J. R. & Meffin, P. J. (1981). The

simultaneous analysis of clofibric acid and probenecid and the direct analysis of clofibric acid glucuronide by high performance liquid chromatography. *J. Chromatogr.*, **223**, 147–154.

Veenendaal, J. R., Brooks, P. M. & Meffin, P. J. (1981). Probenecid-clofibrate interaction. *Clin. Pharmac. Ther.*, **29**, 351–358.

Wojcicki, J., Gawronska-Szklarz, B., Kazimierczyk, J., Baskiewicz, Z. & Raczynski, A. (1979). Comparative pharmacokinetics of paracetamol in men and women considering follicular and luteal phases. *Arzneim-Forsch.*, **29**, 350–354.

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