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Influence of gender and oral contraceptive steroids on the metabolism of salicylic acid and acetylsalicylic acid

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1 Salicylic acid and acetylsalicylic acid (aspirin) disposition after an oral dose of aspirin, 900 mg (equivalent to 689.7 mg of salicylic acid) was studied in eight males, eight females and eight females receiving oral contraceptive steroids (OCS).

2 Salicylic acid clearance was 61% higher in males compared to the control female group, an effect due largely to enhanced activity of the glycine conjugation pathway (salicyluric acid formation) in males.

3 Salicylic acid clearance was 41% higher in OCS-users compared to the control female group due to increases in both the glycine and glucuronic acid conjugation pathways in the pill users. There was no difference in any salicylic acid disposition parameter between males and OCS-users.

4 Area under the plasma concentration–time curve (AUC) and elimination half-life of aspirin was significantly greater and aspirin plasma hydrolysis rate was significantly lower in both female groups compared to males. There was no difference between OCS-users and the control female group in any of these parameters. Aspirin AUC and elimination half-life were significantly correlated with aspirin plasma hydrolysis rate.

5 These data confirm the importance of hormonal factors in the regulation of drug conjugation reactions in humans and suggest that sex-related differences in salicylic acid and aspirin disposition may be of clinical importance.

Keywords salicylic acid contraceptive steroids aspirin sex differences drug metabolism

Introduction

Oral contraceptive steroids (OCS) are known to enhance the elimination of a number of drugs metabolized by UDP-glucuronyltransferase. It has been demonstrated that paracetamol glucuronidation (Miners *et al.*, 1983; Mitchell *et al.*, 1983) and the clearances of clofibric acid (Miners *et al.*, 1984) and temazepam (Stoehr *et al.*, 1984) are increased in women receiving OCS. Patwardhan *et al.* (1981) reported that the elimination of oxazepam and lorazepam was also induced in OCS-users, but this result was not confirmed in a later study (Abernethy *et al.*, 1983). In contrast to the inductive effect of OCS on the glucuronidation of paracetamol and other drugs, the sulphate conjugation of paracetamol is unaffected by OCS (Miners *et al.*, 1983; Mitchell *et al.*, 1983).

Recent studies have also demonstrated sex-related differences in the elimination of a number of drugs dependent on glucuronide conjugation for their elimination. Paracetamol glucuronidation is greater in males than in females (Miners *et al.*, 1983) and the clearances of the glucuronidated benzodiazepines oxazepam and temazepam

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are similarly higher in males compared to females (Greenblatt *et al.*, 1980; Divoll *et al.*, 1981). However, there appears to be no significant difference between sexes in the clearances of clofibric acid (Miners *et al.*, 1984) and lorazepam (Greenblatt *et al.*, 1979). The sulphate conjugation of paracetamol is unaffected by gender (Miners *et al.*, 1983).

The major metabolic product of salicylic acid in humans is the glycine conjugate, salicyluric acid. Other metabolites formed are the glucuronic acid conjugates salicyl phenolic glucuronide and salicyl acyl glucuronide, and the oxidation product gentisic acid (Levy, 1979). To characterize further the influence of gender and OCS on drugs metabolised by conjugation reactions, we have studied salicylic acid metabolism in males, females and females using OCS. In addition, differences in acetylsalicylic acid (aspirin) disposition were studied in the same groups of subjects.

Methods

Twenty four subjects participated in the study; eight males (mean weight 76.5 ± 11.3 kg; mean age 23.9 ± 6.4 years), eight females not taking OCS (mean weight 69.1 ± 3.6 kg; mean age 22.4 ± 3.6 years) and eight females receiving OCS (mean weight 64.3 ± 8.0 kg; mean age 22.4 ± 2.9 years). All subjects were non-smokers and no other medications, apart from those required for the study, were taken for 1 week before and during the study. The OCS used included Triqualar (three subjects: levonorgestrel/ethinyloestradiol; 50 30 μg, 75 40 μg, 125 30 μg), Triphasal (three subjects: levonorgestrel/ethinyloestradiol; 50 30 μg, 75 40 μg, 125 30 μg), Norinyl (one subject: norethisterone, 1 mg; mestranol, 50 μg) and Nordette (one subject: levonorgestrel, 150 μg; ethinyloestradiol, 30 μg). Of the control group females four were studied in the follicular phase and four in the luteal phase, while all OCS-users were studied between days 8 and 23 of the pill cycle. All subjects were healthy as determined by medical history, physical examination and standard haematological and biochemical parameters. The study was approved by the Clinical Investigation Committee of Flinders Medical Centre and written informed consent was obtained from each subject.

Protocol

Subjects reported to the laboratory at 07.00 h on the study day after an overnight fast. An indwelling cannula was inserted into a forearm vein of each subject and blood samples (1.5 ml) were collected at 5 min intervals to 1 h, 10 min intervals from 1 to 2 h, 15 min intervals from 2 to 4 h and then at 4.5, 5, 5.5, 6, 7, 8, 9, 10 and 12 h after administration of a 900 mg oral dose of aspirin (Aspro, Clear, Nicholas-Kiwi; equivalent to 689.7 mg of salicylic acid). A 20 ml blood sample was collected prior to the aspirin dose for the determination of salicylic acid protein binding and aspirin esterase activity. Upon collection the post-dose blood samples were immediately treated with azide/fluoride to prevent aspirin hydrolysis (see Analytical). Urine was collected for 24 h following the aspirin dose and assayed within 12 h of collection.

Analytical

Plasma aspirin and salicylic acid concentrations were measured using a modification of previously published high performance liquid chromatographic (h.p.l.c.) procedures (Lo & Bye, 1980; Rumble *et al.*, 1981). Post-dose blood samples were immediately transferred to 1.5 ml Eppendorf tubes containing sodium azide (16 mg) and sodium fluoride (13 mg) and thoroughly mixed. After centrifugation at 9,000 *g* for 1 min duplicate 0.1 ml aliquots of plasma were transferred to Eppendorf tubes containing 0.2 ml of the internal standard solution (*p*-toluic acid, 5 mg l^{-1} in methanol) and 0.03 ml of 10% perchloric acid. Tubes were then frozen on dry ice and later transferred to a −20° C freezer. Samples treated in this manner were stable for at least 35 days. The plasma sample tubes were recentrifuged (9,000 *g* for 2 min) prior to analysis by h.p.l.c. A 0.02 ml aliquot of the plasma supernatant was injected onto a Waters Associates μ-Bondapak reversed phase column (30 cm × 3.9 mm i.d.), using a mobile phase of 1:1 methanol-phosphoric acid (pH 2.5). Aspirin, salicylic acid and internal standard eluted at 3.25, 5.1 and 6.8 min respectively. With ultraviolet detection at 238 nm, the response was linear over the concentration range 1–200 mg l^{-1}. The method is reproducible, with both the intra-assay and inter-assay coefficients of variation for aspirin and salicylic acid being less than 5%.

Urinary concentrations of salicylic acid, salicyluric acid and gentisic acid were determined by the h.p.l.c. procedure on Cham *et al.* (1980). The amount of salicyl glucuronides in urine was determined by difference after acid hydrolysis of all conjugates (Dromgoole *et al.*, 1983) and estimation of total salicylic acid by the h.p.l.c. method described above.

Salicylic acid plasma protein binding was measured after dialysis of blank plasma from each subject for 3 h at 37° C against isotonic

phosphate buffer, pH 7.4 containing trace amounts of [^{14}C]-salicylic acid and added concentrations of 10, 25, 50 and 100 mg l^{-1} unlabelled salicylic acid. Aspirin esterase activity was also determined in the blank plasma of each subject using a standard procedure (Gupta & Gupta, 1977). Plasma (0.9 ml) was incubated for 1 h at 37° C with 0.1 ml of an aqueous solution of aspirin giving a final concentration of 50 mg l^{-1}. Samples were treated with azide/fluoride (see above) and salicylic acid concentrations were determined by h.p.l.c.

Analysis of results

Areas under the plasma concentration–time curves for both acetylsalicylic acid and salicylic acid were calculated by the trapezoidal rule with extrapolation to infinite time. Elimination half-lives ($t_{½,z}$) of acetylsalicylic acid and salicylic acid were determined from the slope of the terminal portions of the log plasma concentration–time curves for each of these compounds by linear least squares regression. Assuming complete absorption of the dose (as aspirin plus salicylic acid) (see Results) and quantitative biotransformation of aspirin to salicylic acid (Rowland & Riegelman, 1968), salicylic acid plasma clearance was determined 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). Salicylic acid is known to exhibit non-linear kinetics and Levy (1965) has estimated that the threshold dose for zero-order elimination is 276 mg salicylate. In the present study linear semilogarithmic plasma salicylate concentration–time plots were observed in all subjects following administration of the 589.7 mg dose of salicylic acid. Miaskiewicz *et al.* (1982) have reported apparent first order kinetics following administration of similar doses of salicylic acid (9 mg kg^{-1}) to males and females. Nevertheless, the 'apparent' kinetic parameters calculated in this study may represent nett first- and zero-order processes. Although each subject in the present study received the same dose of aspirin, differences between males and the control female group and between the control female group and OCS-users in the dose normalized for body weight were not significant. The body weight normalized aspirin dose was, however, 18% greater in the OCS-users compared to males.

Partial metabolic and renal clearances of salicylic acid were calculated as,

$$CL_m = f_m \times CL$$

where CL_m is the metabolic clearance to salicyluric acid, salicyl glucuronides or gentisic acid or the renal clearance of unchanged salicylic acid and f_m is the fractional urinary recovery of each metabolite or unchanged drug.

Results are expressed as mean ± s.d. Differences among the group means for each parameter were examined by analysis of variance, with the Newman-Keuls test being used to detect differences between the individual study groups. Correlations between aspirin disposition parameters were determined by linear regression analysis. The null hypothesis was rejected when $P < 0.05$. The factors required for the conversion of plasma concentrations of salicylic acid and aspirin from mg l^{-1} to S.I. units (μmol l^{-1}) are 7.24 and 5.55, respectively.

Results

Salicylic acid kinetic parameters in each of the three study groups are summarized in Figure 1. Total plasma clearance of salicylic acid (CL) was significantly higher ($P < 0.01$) in both males (0.563 ± 0.102 ml min^{-1} kg^{-1}) and OCS-users (0.493 ± 0.110 ml min^{-1} kg^{-1}) compared to the control female group (0.350 ± 0.063 ml min^{-1} kg^{-1}). There was a corresponding prolongation of elimination half-life ($t_{½,z}$) in the control females (4.53 ± 1.19 h) compared to males (3.03 ± 0.74 h; $P < 0.01$) and OCS-users (3.36 ± 0.85 h; $P < 0.05$). Neither CL nor $t_{½,z}$ were significantly different in males compared to OCS-users. The volume of distribution at steady-state of salicylic acid was similar in the three study groups. There was no difference between study groups in salicylic acid protein binding for added concentrations of 10, 25, 50 and 100 mg l^{-1} (Table 1). However, salicylic acid free fraction was significantly higher ($P < 0.01$) at 100 mg l^{-1} than at 50, 25 and 10 mg l^{-1} in all groups.

The mean renal and metabolic clearances of salicylic acid in each study group are shown in Figure 2. Clearance to salicyluric acid was greater ($P < 0.01$) in both males (0.390 ± 0.056 ml min^{-1} kg^{-1}) and OCS-users (0.334 ± 0.073 ml min^{-1} kg^{-1}) compared to the control females (0.241 ± 0.044 ml min^{-1} kg^{-1}). Salicyluric acid formation was not significantly different between males and the OCS-users. The difference between males (0.101 ± 0.038 ml min^{-1} kg^{-1}) and females (0.064 ± 0.022 ml min^{-1} kg^{-1}) in clearance to salicyl glucuronides was not statistically significant but clearance to the glucuronides was significantly enhanced ($P < 0.05$) in OCS-users (0.110

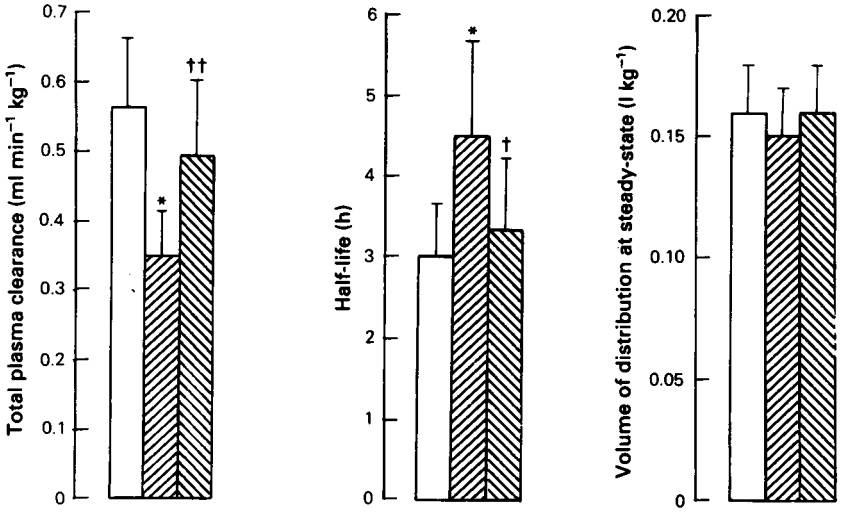

Figure 1 Mean pharmacokinetic parameters of salicylic acid in males (□), females (▨) and OCS-using females (▩). Compared with males, $^{*}P < 0.01$; compared to females, $\dagger P < 0.05$, $\dagger\dagger P < 0.01$.

Table 1 Salicylic acid plasma protein binding in males, females and OCS-users

Salicylic acid concentration (mg l^{-1})	*Salicylic acid free fraction × 100*		
	Males	*Females*	*OCS-users*
100	8.21 ± 1.89	8.38 ± 1.81	9.35 ± 2.35
50	5.91* ± 1.35	6.21* ± 0.98	6.40* ± 1.07
25	5.51* ± 0.77	5.66* ± 0.98	6.17* ± 0.82
10	5.09* ± 0.93	4.96* ± 0.81	5.47* ± 1.04

*Compared to free fraction for 100 mg l^{-1}, $P < 0.05$.

± 0.040) compared to the control females. There were no significant differences between any of the groups in renal clearance of unchanged drug and in clearance to gentisic acid. Mean urinary recovery of salicylic acid-derived products was essentially quantitative in all groups; 99.7 ± 6.0% for males, 101.5 ± 5.7% for the control female group, and 104.6 ± 4.9% for the OCS-users.

Aspirin AUC was significantly greater ($P < 0.05$) in both female groups (control, 882 ± 145 mg l^{-1} min; OCS-users 910 ± 158 mg l^{-1} min) compared to males (665 ± 111 mg l^{-1} min) (Table 2). Differences in AUC remained significant after normalization for body weight. Aspirin $t_{½,z}$ was significantly shorter ($P < 0.05$) in the males (10.6 ± 1.9 min) than in either of the female groups (control females, 15.5 ± 3.2 min; OCS-users, 16.0 ± 3.7 min). The aspirin plasma hydrolysis rate was significantly higher ($P < 0.01$) in the males (363 ± 14 mg h^{-1} l^{-1}) compared to both the OCS-users (262 ± 25 mg h^{-1} l^{-1}) and the control females (277 ± 17 mg h^{-1} l^{-1}). There was no difference between the non-pill using females and the OCS-users in AUC, normalized AUC, $t_{½,z}$ and aspirin plasma hydrolysis rate. When the data from all groups were combined, there were significant correlations ($P < 0.01$) between AUC/$t_{½,z}$ ($r = 0.79$), AUC/plasma hydrolysis rate ($r = -0.69$), normalized

AUC/$t_{½,z}$ (r = 0.75), normalized AUC/plasma hydrolysis rate (r = −0.76) and $t_{½,z}$/plasma hydrolysis rate (r = −0.69).

Discussion

This study has demonstrated that gender and OCS have a major effect on the disposition of salicylic acid. Salicylic acid clearance was greater in both males and OCS-using females compared to non-pill using females. The higher clearance of salicylic acid in males compared to females was due to greater activity of the glycine conjugation pathway whereas both glucuronic acid and glycine conjugation were induced in OCS-users compared to the control females. Although there was no difference in aspirin disposition parameters between OCS-users and the control female group, aspirin AUC and elimination half-life were shown to be greater in both female groups than in males. The slower elimination of aspirin in females would appear to be due to an intrinsically lower activity of aspirin esterase in that sex.

Our results are consistent with previous studies which have demonstrated higher salicylic acid clearance in males than in females (Graham *et al.*, 1977; Ho *et al.*, 1985) or higher plasma salicylate concentrations in males compared to females (Coppe *et al.*, 1981; Kelton *et al.*, 1981) after administration of aspirin. Interestingly, gender was found not to influence salicylic acid clearance in a study where subjects were administered sodium salicylate (Miaskiewicz *et al.*, 1982). However only one of the above studies (Ho *et al.*, 1985) investigated the influence of gender on individual salicylic acid metabolic pathways and none of the studies compared salicylic acid disposition in males, females and

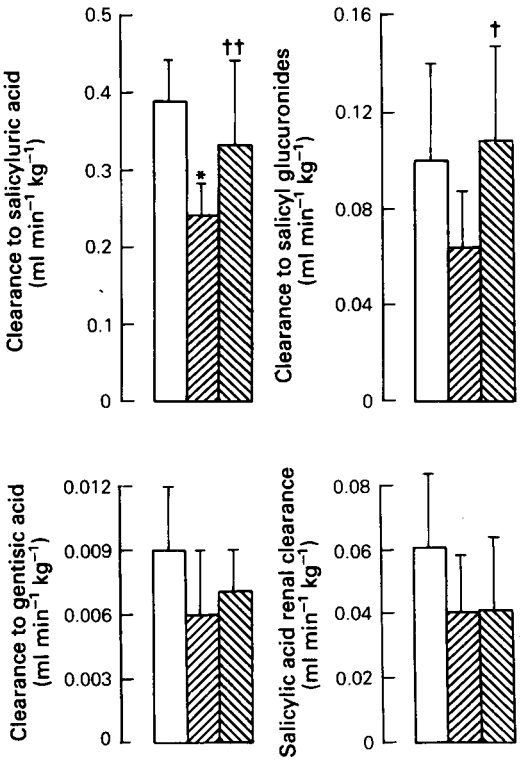

Figure 2 Mean renal and metabolic clearances of salicylic acid in males (□), females (▨) and OCS-using females (▩). Compared with males, $P < 0.01$; compared to females, †$P < 0.05$, ††$P < 0.01$.

OCS-users. It has been reported (Gupta *et al.*, 1982) that salicylic acid AUC was reduced by 32% in a group of women who received a low oestrogen combination oral contraceptive for 2 months and the magnitude of this effect is similar to the enhancement (41%) in salicylic acid clearance in OCS-users compared to control females

Table 2 Acetylsalicylic acid disposition and metabolic parameters in males, females and OCS-users.

Disposition/metabolic parameter	*Study group*		
	Males	*Females*	*OCS-users*
AUC (mg l^{-1} min)	665 ± 111	882* ± 145	910* ± 158
Normalized AUC (mg l^{-1} min kg^{-1})	8.86 ± 2.57	13.05* ± 3.03	14.67** ± 3.36
$t_{½,z}$ (min)	10.6 ± 1.9	15.5* ± 3.2	16.0* ± 3.7
Plasma hydrolysis rate (mg l^{-1} h^{-1})	363 ± 14	277** ± 17	262** ± 25

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

observed in the present study. The earlier study did not determine the effects of OCS on individual salicylic acid metabolic pathways. No sex or OCS-related differences in salicylic acid volume of distribution or plasma protein binding were apparent in our subjects and these observations are consistent with the results of previous studies which have investigated sex-related differences in salicylic acid kinetics and protein binding (Miaskiewicz *et al.*, 1982; Ho *et al.*, 1985). The concentration-dependent protein binding of salicylic acid observed in the present study is also in agreement with previous reports (Ekstrand *et al.*, 1979; Lesko *et al.*, 1985).

The sex-related difference in salicylic acid plasma clearance was due to increased activity of the glycine conjugation pathway in males. Ho *et al.* (1985) estimated the maximum velocity (V_{max}) for salicyluric acid formation is 79% higher in males compared to females. Our data also demonstrates that the glycine conjugation process, like glucuronidation, is inducible by OCS. Salicylic acid plasma clearance is known to be increased in patients co-administered corticosteroids (Klinenberg & Miller, 1965; Graham *et al.*, 1977) but neither of these studies investigated effects on individual metabolic pathways. It should be noted that salicylic acid is converted to salicyluric acid in a two-step reaction sequence; the first or activating step involves formation of a coenzyme A ester which is then acylated by glycine *N*-acyltransferase (Tishler & Goldman, 1970; Forman *et al.*, 1971). The activating step to form the salicyl-coenzyme A ester is known to be rate-limiting in salicylurate biosynthesis (Forman *et al.*, 1971; Levy, 1979). Thus, it would appear that the effects of gender and OCS on salicylurate formation occur due to changes in the enzyme(s) responsible for salicyl-coenzyme A synthesis rather than changes in glycine *N*-acyltransferase activity.

Salicyl glucuronide formation was markedly induced in OCS-users; clearance to the salicyl glucuronides was 72% higher in OCS-users compared to non-pill using women. Since salicyl acyl- and phenolic-gluruconides were not separately assayed in the present study it was not possible to determine whether these pathways were differentially affected by OCS. However, the clearance of drugs forming either ether glucuronides (paracetamol, temazepam, oxazepam and lorazepam) or ester glucuronides (clofibric acid) have previously been shown to be increased in OCS-users (Patwardhan *et al.*, 1981; Miners *et al.*, 1983, 84; Mitchell *et al.*, 1983; Stoehr *et al.*, 1984). Although the clearance to the salicyl glucuronides was 57% higher in the male subjects studied here compared with the control females, the difference was not statistically significant. Again, separate sex-related effects on the formation of the acyl- and phenolic-glucuronides were not determined. Sex-related differences have been shown to occur for the glucuronidation of the phenolic drugs oxazepam, temazepam and paracetamol (Greenblatt *et al.*, 1980; Divoll *et al.*, 1981; Miners *et al.*, 1983) whereas clofibric acid-acylglucuronidation was not significantly different between males and females (Miners *et al.*, 1984).

This report has also demonstrated that there is no difference in aspirin disposition between OCS-users and non-pill using women. Aspirin AUC and elimination half-life were, however, greater in the female groups compared to males. In addition, there were significant correlations between aspirin AUC and aspirin plasma hydrolysis rate and elimination half-life. These data are suggestive of intrinsically lower aspirin esterase metabolic activity in females, although it is acknowledged that the regulation of aspirin esterases in plasma may be different to that for the esterase(s) present in other tissues. Similar sex-related differences in aspirin AUC have also been reported by Ho *et al.* (1985), but other studies have found no difference in aspirin AUC between males and females (Husted *et al.*, 1983) or a larger aspirin AUC in males compared to females (Buchanan *et al.*, 1983). In the present study more plasma samples were collected in the 2 h following aspirin administration than in the previous studies and this should provide a more accurate estimate of aspirin AUC and half-life. Additional steps were also adopted in the plasma collection procedure followed in the present study to avoid *in vitro* aspirin hydrolysis. As in our study, other investigations (Windorfer *et al.*, 1974; Gupta & Gupta, 1977) have noted higher aspirin plasma esterase activity in males than in females. A close correlation ($r = 0.90$) between aspirin plasma esterase activity and aspirin AUC has been reported (Seymour *et al.*, 1984) in patients with post-operative dental pain but the possible sex-dependency of this association was not determined. Interestingly, these authors also demonstrated a close correlation ($r = 0.96$) between aspirin AUC and analgesic effect suggesting that pain relief in post-operative dental pain is determined by aspirin hydrolysis rate.

In summary, this study has confirmed the importance of hormonal factors in the regulation of drug glucuronidation in humans and demonstrated that these factors may also affect other phase II reactions such as glycine conjugation. The magnitude of the sex-related differences in salicylic aid and aspirin disposition is likely to be of clinical importance and is consistent with the

observation (Samter & Beers, 1963; Duggan & Chapman, 1970; Ali Abrishami & Thomas, 1977) that salicylate toxicity appears to be more pronounced in females.

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