Inhibition of antipyrine metabolism by low-dose contraceptives with gestodene and desogestrel

To examine the effects of the two newest monophasic oral contraceptives on liver microsomal drug metabolism, plasma kinetics and urinary metabolite excretion of antipyrine, a model substrate for liver microsomes, were evaluated. Plasma lipid and lipoprotein levels, and in particular the high-density lipoprotein subfractions, were also monitored in view of their apparent regulation by a P450-dependent system. Ten healthy volunteers were treated with each contraceptive for a period of 3 months in a crossover trial. Both contraceptives significantly reduced antipyrine clearance by 34.6% (gestodene) and 43.3% (desogestrel) by impairing the oxidative metabolism, particularly to the 4-hydroxy and 3-hydroxymethyl metabolites, with little difference between the two associations. In addition, with both a comparable highly significant rise of plasma triglyceride levels, apolipoproteins A-I and A-II and the high-density lipoprotein-3 subfraction was observed. Treatment with these new monophasic contraceptives may reduce the metabolism of concomitantly given drugs undergoing oxidative transformations. (CLIN PHARMACOL THER 1991;49:278-84.)

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Oral contraceptive treatment is now used by more than 50 million women in the western world. Several new molecules, particularly progestins, have been developed in recent years to reduce daily doses and maximize safety.[1] These newer, very potent compounds have been suspected to interact with the liver microsomal enzyme system, in some way affecting the metabolism and activity of concomitantly given drugs.[2,3] Unfortunately, most clinical studies on the impact of oral contraceptives on liver microsomal drug metabolism have been hampered by the evaluation of subjects treated with different oral contraceptives, varying in doses and types of estrogens and progestins.[4,5]

Progestogens contained in the last generation of monophasic oral contraceptives, gestodene and desogestrel,[1] now allow the administration of minimal doses of both components of the pill. However, gestodene and desogestrel undergo extensive liver microsomal handling: in the case of gestodene there is a progressive rise of plasma drug levels and the elimination half-life ($t_{1/2}$) during each cycle of treatment[6]; desogestrel is converted to an active metabolite (3-ketodesogestrel) whose levels also tend to grow, albeit to a lesser extent, during each cycle.[7] There is therefore suggestive evidence for both compounds that they may to some extent interfere with microsomal drug metabolism. In addition, recent data indicate that the ethinyl estradiol component itself may interfere with microsomal drug oxidation by suicide inhibition effected by the ethinyl group.[8]

In view of interest in predicting possible drug interactions occurring after administration of these two major oral contraceptives, the metabolism of antipyrine (a model compound, allowing assessment of the major oxidative and nonoxidative pathways of microsomal drug metabolism in humans)[9] was evaluated in healthy young women during treatment with the two oral contraceptives. In addition, in the same subjects, plasma lipid and lipoprotein changes, in particular high-density lipoprotein (HDL) levels and subfraction distribution, were also examined. The induction of mi-

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crosomal drug metabolism is in fact generally associated with increased HDL levels,[10] but contradictory findings have been reported in previous studies investigating HDL levels and microsomal metabolism after oral contraceptives.[11]

SUBJECTS AND METHODS

Ten female volunteers of reproductive age (27.4 ± 1.1 years) were selected for the study. All were fully informed on the modalities and end points of the study, and all signed consent forms. None was a cigarette smoker, and medical illnesses were excluded on the basis of history, physical, and laboratory examinations. In the case of subjects already receiving oral contraceptive treatments, these were discontinued 3 months before the beginning of the study. The study was approved by the hospital internal review board.

Protocol. The volunteers were randomly allocated to two periods of contraceptive treatment, starting with either 20 μg ethinyl estradiol plus 150 μg desogestrel (Mercilon) or 30 μg ethinyl estradiol plus 75 μg gestodene (Minulet). Each oral contraceptive treatment was taken for three menstrual cycles, followed by the other treatment without interruption. During the 6 months of oral contraceptive treatment, the subjects were constantly monitored for the usual possible side effects of oral contraceptives.

At the beginning of the study and the end of every 28-day cycle, body weight and blood pressure were determined, in addition to standard plasma lipid and lipoprotein measurements: total cholesterol,[12] triglyceride,[13] HDL cholesterol,[14] and apolipoprotein A-I, A-II, and B[15] levels. At the beginning of the study and at the end of each 3-month cycle the HDL cholesterol subfractions (HDL_2 and HDL_3) were separated by zonal ultracentrifugation[16] and their cholesterol and protein content was determined. Serum γ-glutamyl transpeptidase levels were also monitored as a possible marker of microsomal induction.[17]

The plasma kinetics of antipyrine were examined at the beginning of the study and at the end of each 3-month cycle of treatment. Antipyrine was given orally: the usual dose (18 mg/kg, rounded to 1 gm) was dissolved in flavored orange juice; the time interval between the intake of the test drug and the last dose of oral contraceptive was approximately 10 hours. Blood samples were collected by separate venipunctures 0, 3, 6, 8, 10, 12, and 24 hours after antipyrine administration. Urine samples were collected after 4, 8, 12, and 24 hours. This urine collection protocol follows the indications of Scavone et al.,[18] according to whom pharmacokinetic parameters can be estimated with adequate precision by using an abbreviated monitoring period of 24 hours.

Analysis of antipyrine and metabolites in plasma and urine. Antipyrine and 4-hydroxyantipyrine were purchased from Aldrich-Chemie (Steinheim/Albuch, Germany) norantipyrine and phenacetin were from Merck (Darmstadt, West Germany). 3-Hydroxymethylantipyrine was furnished by D. Breimer, Department of Pharmacology, University of Leiden, The Netherlands. The plasma concentrations of unchanged antipyrine were measured according to the analytic procedure described by Danhof et al.,[19] with minor modifications. One-milliliter plasma samples were spiked with 10 μg phenacetin as the internal standard. After alkalinization with 0.1 ml 2N NaOH, antipyrine and phenacetin were extracted with 3 ml dichloromethane by vortexing for 1 minute. After centrifugation, the organic phase was dried under N_2. The residue was dissolved in 0.2 ml methanol and an aliquot of the solution was analyzed by reverse-phase HPLC. A Varian 5000 HPLC (Varian Associates, Inc., Palo Alto, Calif.) equipped with a Spherisorb ODS2 S5 column (250/4.6 mm inside diameter; Phase Separation Inc., Norwalk, Conn.) with a precolumn RCSS Guard-Pak C_{18} (Waters Associates, Milford, Mass.) was used. The mobile phase was methanol, 0.05 mol/L, phosphate buffer pH 6.5 (28/72 vol/vol), with a flow rate of 2 ml/min. The eluted compounds were monitored at 254 nm. Retention times were 3.6 and 5.9 minutes for antipyrine and phenacetin, respectively. The detection limit of the assay was 250 ng/ml and the mean coefficient of variation calculated in the linear range was 3.2%.

Urine samples, 0.5 ml in screw-cap tubes, were added to 40 mg Na metabisulfite (Merck), 10 mg limpet acetone powder Patella vulgata (Sigma Chemical Co., St. Louis, Mo.), 100 μg phenacetin as internal standard, and 0.5 ml 0.2 mol/L acetate buffer, pH 4.5. After vortexing, samples were incubated at 37° C for 3 hours, saturated with NaCl, and extracted with 5 ml chloroform ethanol (9:1 vol/vol). The organic phase, recovered after centrifugation, was dried under N_2. The residue was dissolved in 0.2 ml methanol and an aliquot was used for HPLC analysis. A Lichrosorb RP-2 column (250/4.6 mm; Unimetrics Corp., Anaheim, Calif.) with a precolumn RCSS Guard-Pak C_{18} (Waters Associates) was used. The eluting solvent was methanol 0.5 mol/L phosphate buffer, pH 6.5 (26/74 vol/vol), added to 0.2 ml/L triethylamine (Merck) at a flow rate of 1.5 ml/min. The eluted compounds were monitored at 254 nm. Retention times were as follows: 3-hydroxymethylantipyrine, 6.0 min-

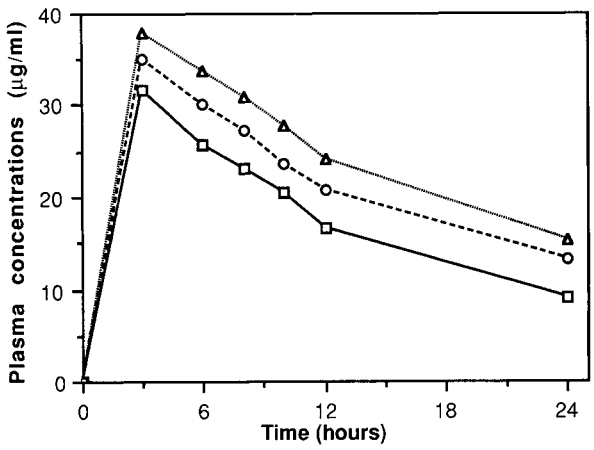

Fig. 1. Mean plasma concentrations of antipyrine in the 10 volunteers, at baseline (□) and after 3 months of treatment with, respectively, desogestrel (△) and gestodene (○) oral contraceptives. See Table I for the analysis of the kinetic data.

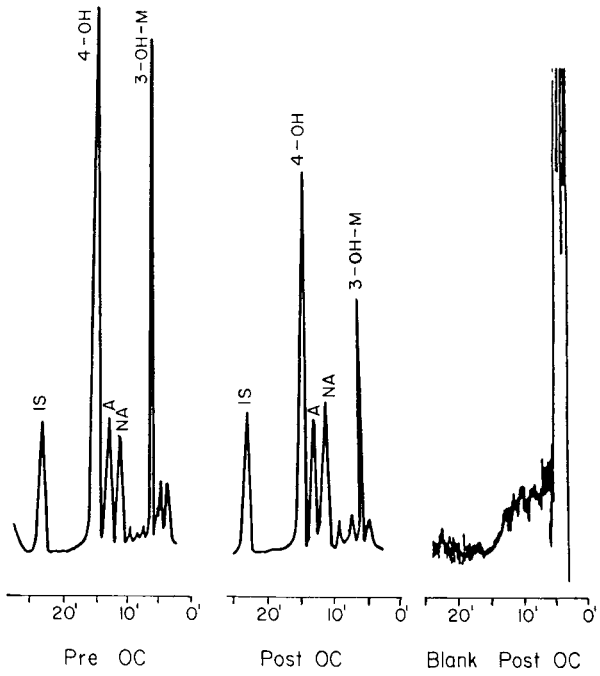

Fig. 2. Separation of antipyrine (A) and its major metabolites, 4-hydroxyantipyrine (4-OH), norantipyrine (NA), and 3-hydroxymethyl antipyrine (3-OH-M), before (Pre OC) and after (Post OC) treatment with gestodene. A marked reduction in the urinary excretion of the 4-OH and 3-OH-M metabolites can be observed. A blank urine sample after the end of the study is also shown. IS, Internal standard.

utes; norantipyrine, 11.5 minutes; antipyrine, 13.7 minutes; 4-hydroxyantipyrine, 16.0 minutes; and phenacetin, 23.3 minutes. Linearity, accuracy, and intraday and interday reproducibility were demonstrated by multiple analyses of calibration curves prepared by adding known amounts of internal standard, pure antipyrine, or metabolites to plasma and urine samples of control subjects. The detection limit for all of the examined compounds was 2 μg/ml and the coefficients of variation for the assay, calculated in the linear ranges, were 2.2 (antipyrine), 2.6 (hydroxyantipyrine), 3.5 (hydroxymethylantipyrine), and 2.6 (norantipyrine), respectively.

Analysis of the kinetic data. Plasma antipiryne kinetic parameters were analyzed with the SIPHAR program (SIMED, Creteil, France), assuming complete systemic bioavailability of antipyrine[19] and one-compartment (single exponential) approximation, thus obtaining the total area under the concentration-time curve (AUC), $t_{1/2}$, total plasma clearance (CL), and volume of distribution (V_{area}). Clearance of each metabolite was calculated from the equation: CL_M = M/AUC, where M is the amount of the metabolite excreted during the 24-hour interval and AUC is the area under the plasma concentration curve for antipyrine deering 24 hours. This formula is based on the amount of each metabolite excreted during the collection period and does not require corrections, not being based on percentages over total plasma CL. The length of the collection period is adequate, because the antipyrine kinetics are linear and urinary clearance is not modified significantly over time.[20]

Statistical evaluation of the differences was carried out for all obtained data by analysis of variance followed by Duncan's *t* test. In the case of plasma triglyceride values, data were log transformed before the statistical evaluation. Statistical analyses were carried out with the aid of the statistical package SPSS/PC+ (SPSS Inc., Chicago, Ill.).

RESULTS

Antipyrine kinetics and metabolism. Both oral contraceptives significantly reduced antipyrine metabolism by increasing elimination $t_{1/2}$ and plasma AUC (Fig. 1), as well as reducing CL. Antipyrine CL (Table I) went down 34.6% with gestodene and 43.3% with desogestrel (both $p < 0.01$ versus baseline), but no significant difference between the two oral contraceptive treatments could be ascertained.

When examining the fractional clearances of urinary antipyrine metabolites, only the major oxidative metabolites were found to be significantly affected (Fig. 2). The clearance of 3-hydroxymethylantipyrine decreased by 51.3% (Table I) with gestodene and

Table I. Plasma kinetic data for antipyrine and urinary metabolite elimination patterns before and after the two oral contraceptives (n = 10)

	Base	*Gestodene + EE*	*Desogestrel + EE*
Plasma			
$t_{1/2}$ (hr)	11.654 ± 1.030	15.559 ± 1.522*	17.285 ± 2.773*
CL (ml/hr/kg)	43.641 ± 4.219	27.933 ± 2.639*	24.355 ± 2.433*
AUC (μg/ml/hr)	585.673 ± 62.290	834.410 ± 67.507	1009.147 ± 150.954†
V_{area} (ml/kg)	680.272 ± 23.235	579.294 ± 26.967†	542.156 ± 34.677*
CL (ml/hr/kg)			
Antipyrine	1.032 ± 0.134	0.953 ± 0.188	0.899 ± 0.092
3-OH-M	4.826 ± 0.634	2.823 ± 0.264*	3.198 ± 0.252*
4-OH-A	8.794 ± 1.321	6.201 ± 0.459†	6.449 ± 0.372†
Norantipyrine	5.762 ± 0.818	4.837 ± 0.468	4.720 ± 0.603

Data are mean values ± SEM.
EE, Ethinyl estradiol; $t_{1/2}$, half-life; CL, clearance; AUC, area under the curve; V_{area}, volume of distribution; A, antipyrine; 3-OH-M, 3-hydroxymethylantipyrine; 4-OH-A, 4-hydroxyantipyrine.
*$p < 0.01$ versus baseline.
†$p < 0.05$.

58.8% with desogestrel (both $p < 0.01$ versus baseline). Clearance of 4-hydroxyantipyrine was reduced to a lesser extent (i.e., by 39.4% with gestodene and 38.7% with desogestrel) (both $p < 0.05$ versus baseline); again no statistical significance could be demonstrated between the two oral contraceptives. In contrast, the elimination of unmodified antipyrine and norantipyrine were not affected significantly by the oral contraceptive treatments.

Changes in plasma lipid and lipoprotein levels. A comparative evaluation of the two compounds on the major plasma lipid and lipoprotein parameters did not show major differences (Table II). Both oral contraceptives, when given first, led to the expected progressive rise of triglyceridemia and HDL cholesterol levels, with no significant change of total cholesterolemia.[21] When examining the pooled mean data at baseline and the end of each oral contraceptive treatment, very similar changes were noted with the two drugs. Triglyceride levels rose by about 50% with both compounds; apoprotein A-I levels rose by 12.4% with gestodene and 14.6% with desogestrel. More marked rises occurred in the case of apolipoprotein A-II (i.e., by 24.5% with gestodene and 20.7% with desogestrel).

HDL cholesterol levels rose somewhat with both treatments (i.e., by 5.1% with gestodene and 9.6% with desogestrel), neither statistically significant. When examining the two HDL subfractions, separated by rate zonal ultracentrifugation, only the HDL_3 mass and cholesterol level were significantly elevated, with an HDL_3 cholesterol rise of 26.8% with gestodene and 25.3% with desogestrel HDL_2 cholesterol levels decreased, nonsignificantly, with both oral contraceptives. No significant changes were noted in the γ-glutamyl transpeptidase activity (data not shown).

DISCUSSION

Interactions with the most commonly used drugs are of practical interest for all physicians. In the case of oral contraceptives, up to now the major emphasis has been placed on the possibility that the metabolism of these compounds may be adversely affected (generally resulting in loss of activity) by enzyme inducers (e.g., rifampin and barbiturates).[22] The possible impact of oral contraceptives on the metabolism of other concomitantly given drugs has received less attention. Early studies indicated that subjects treated with oral contraceptives could show either induction or inhibition of microsomal drug metabolism.[5] More recently, in at least three studies on better-defined oral contraceptive treatments, inhibition of metabolism was described, respectively, versus aminopyrine[2] and antipyrine.[11,23] In these latter studies, carried out with an *l*-norgestrel-containing triphasic oral contraceptive, the plasma $t_{1/2}$ and metabolism of antipyrine were evaluated; in spite of a clear inhibition of microsomal metabolism, plasma HDL cholesterol levels were raised,[11] similar to what could be expected from microsomal inducers.[10,24]

Antipyrine is the best known and more readily available marker of microsomal metabolism in humans.[9] Although the liver microsomal system(s) responsible for the oxidative steps has not been identified clearly, it may belong to the P450IIB or P450IIC subfamilies in mammals, responsible for the oxidative metabolism of numerous commonly used drugs including theophylline, barbiturates, and tolbutamide.[25]

Table II. Major lipid and lipoprotein changes after the two oral contraceptive treatments (n = 10)

	Base (mg/dl)	*Desogestrel + EE (mg/dl)*	*Gestodene + EE (mg/dl)*
Total cholesterol	205.9 ± 10.32	209.2 ± 9.18	209.8 ± 7.75
Triglycerides	61.7 ± 4.99	99.5 ± 5.89*	98.4 ± 8.38*
HDL cholesterol	66.6 ± 4.23	70.0 ± 4.29	73.0 ± 3.41
HDL_2 cholesterol	33.8 ± 3.94	28.4 ± 3.24	31.9 ± 3.03
HDL_3 cholesterol	32.8 ± 1.81	41.6 ± 2.18*	41.1 ± 1.36*
Apolipoprotein A-I	165.9 ± 5.08	186.5 ± 7.96†	190.1 ± 5.16†
Apolipoprotein A-II	36.3 ± 1.15	45.2 ± 2.26*	43.8 ± 1.59*
Apolipoprotein B	98.3 ± 4.92	101.5 ± 7.72	104.3 ± 6.28

Data are mean values ± SEM.
EE, Ethinyl estradiol; HDL, high-density lipoprotein.
Conversion factors for triglycerides to mmol/L = 0.0112272; for cholesterol = 0.0258545.
*$p < 0.01$ versus baseline.
†$p < 0.05$.

Other potential candidates are P450IA2, responsible for the metabolism of phenacetin,[26] and P450IIIA4, oxidizing midazolam and triazolam.[25] It should be emphasized, however, that direct correlations between the in vitro and in vivo approaches to drug metabolism are not found consistently[27]; a clear lack of correlation between the two has been shown (e.g., tolbutamide and mephenytoin).[28]

When examining the formation of oxidative metabolites of antipyrine in oral contraceptive–treated volunteers, it was apparent that both oral contraceptives reduced the clearance of the two major metabolites, 3-hydroxymethylantipyrine and 4-hydroxyantipyrine, with a minor activity on the normetabolite. Because both oral contraceptives contain, in addition to the progestogen, a low dose of ethinyl estradiol, this latter might also be responsible for the inhibited metabolism. In vitro, ethinyl estradiol is a powerful inhibitor of P450III, responsible for the metabolism of the calcium antagonist nifedipine (nifedipine oxidase), demethylation of amphetamine, and particularly 4-hydroxylation of estradiol.[29] Interestingly, ethinyl estradiol metabolism itself can be reduced during treatment with desogestrel- or gestodene-containing oral contraceptives,[30] resulting in progressively increased ethinyl estradiol levels during each cycle of treatment. In addition to the above-reported reservations,[27] against the hypothesis of direct inhibitory role of ethinyl estradiol on antipyrine oxidation, is the finding that nifedipine oxidation and related enzyme activities do not seem to be linked to antipyrine metabolism.[31] Recently a parallel evaluation of different microsomal substrates in volunteers[32], showed no linkage between antipyrine oxidation and the activity of P450III, with the possible exception of norantipyrine formation, minimally affected, however, by the presently evaluated oral contraceptive treatments. The reported findings show therefore that oral contraceptives containing both desogestrel and gestodene can impair microsomal enzyme function, particularly as relates to oxidative changes. The metabolism and elimination of concomitantly administered drugs undergoing similar metabolic transformations may be similarly delayed.

Another finding of interest from this study relates to the somewhat unexpected lipoprotein changes. Both oral contraceptives affected lipid and lipoprotein metabolism in an essentially identical way (i.e., by raising triglyceridemia and HDL cholesterol levels, mainly in the HDL_3 subfraction). Rises in triglyceride levels are attributable to the estrogen component of the pills[21]; the HDL changes may also be hormone related,[33] but a possible link with the microsomal system should not be excluded. Enzyme inducers reportedly increase both HDL_2 and HDL_3 levels (somewhat more in the former subfraction), but the study was not carried out with ultracentrifugal separation.[34] Ethanol, an enzyme inducer on long-term administration, clearly raises HDL_3 levels.[35] Among the scant data pertaining to the effect of microsomal inhibitors on lipoprotein metabolism, there is indication that treatment with cimetidine, a powerful H_2-antagonist, as well as an inhibitor of oxidative metabolism,[35] can lead to significant rises in HDL levels, again in the HDL_3 subfraction.[37] A preliminary report had indicated that cimetidine might delay the turnover of a P-450 form, possibly responsible for HDL catabolism.[38] That alterations in the P450 system should be deemed at least partially responsible for the changes in HDL levels rather than direct hormonal effects, is suggested by the similarity in the results with these oral contraceptives, containing weakly estrogenic progestogens, and the triphasic pill, containing the androgenic *l*-norgestrel.[11]

In conclusion, the administration of oral contraceptives containing both desogestrel and gestodene was associated with impaired oxidative transformation of antipyrine, possibly of significant interest for a variety of commonly used medications. Apparently the inhibited microsomal metabolism is related to a combined effect of both the progestogen and estrogen components of these pills. In addition, and partly in contrast to what was expected, both oral contraceptives modified lipid metabolism in an essentially identical way, with a mild triglyceride-raising effect and more marked increases of HDL cholesterol levels, particularly in the HDL_3 subfraction. The association of these lipoprotein changes to microsomal induction or inhibition requires further clarification.

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