

EFFECTS OF TOBACCO SMOKING AND ORAL CONTRACEPTIVE USE ON THEOPHYLLINE DISPOSITION

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- 1 The independent as well as interactive effects of chronic (> 6 months) oral contraceptive (OC) use and cigarette smoking on single-dose (4 mg/kg) theophylline disposition were assessed in 49 young, healthy women.
- 2 Significant elevations (40%) in theophylline plasma clearance were found in women who smoked. OC use resulted in decreases in clearance of a similar magnitude (28%). These factors do not appear to interact with respect to theophylline disposition. The combination of main effects tended to cancel one another (clearance of $49.1 \text{ ml h}^{-1} \text{ kg}^{-1}$ ideal body weight for OC non-user, non-smoker, vs $49.7 \text{ ml h}^{-1} \text{ kg}^{-1}$ for OC user-smoker).
- 3 Single dose exposure to OC in non-users did not significantly alter theophylline pharmacokinetics for the group as a whole. However, in the subgroup of smoking subjects, significant decreases in clearance were evident ($P < 0.05$). Analogous results were found for half-life. Volume of distribution was slightly diminished in smokers, but was unaffected in OC users.
- 4 Areas under the serum concentration-time (AUC) profiles of norgestrel and ethinyloestradiol were examined in 27 women as indices of OC exposure. The smallest values of theophylline clearance were found in the subjects with largest AUC of both OC steroids.
- 5 Appropriate statistical analyses of data which are influenced by multiple factors are discussed. Special concern is needed when the factor partitioning process yields subgroups of unequal sizes.

Keywords smoking theophylline oral contraceptives

Introduction

Oral contraceptives (OC) are likely to alter drug disposition and clinical effects because of their frequent and protracted use and known capability to affect biotransformation rates. Many oxidative pathways such as for antipyrine (Carter *et al.*, 1974; Teunissen *et al.*, 1982), and theophylline (Tornatore *et al.*, 1982) and reductive processes such as for nitrazepam (Jochemsen *et al.*, 1982) exhibit decreased metabolism rates. On the other hand, the conjugation of lorazepam and oxazepam is increased in OC users (Patwardhan *et al.*, 1981).

Another ubiquitous source of altered drug elimination is tobacco smoking which seems to cause selective enzyme induction and increased biotransformation rates of some drugs (Jusko, 1978). Marked increases in theophylline clearances are exhibited in some smokers (Jusko *et al.*, 1978).

The dual effects of smoking and oral contraceptive use are of special interest. The combined use of tobacco and OC is now well recognized as contributory to increased risk of cardiovascular disease and mortality (Beral & Kay, 1977; Stadel, 1981). The nature of any combined effects of these factors on hepatic function and drug metabolism has not been elucidated.

Theophylline has a low therapeutic index and is subject to extensive hepatic metabolism (Cornish & Christman, 1957), the rate of which is sensitive to numerous drug/disease/physiologic/environmental factors (Jusko *et al.*, 1979). With OC and tobacco use causing opposite effects on theophylline clearances in man, it is of clinical and pharmacological interest to examine the net effects of these factors. Another purpose of this report is to consider the appropriate

statistical methods used in assessing the influences of both independent and interactive factors on drug disposition.

Methods

Subjects

The data collected from 49 young, healthy women who had been studied at the Millard Fillmore Hospital of Buffalo were employed in this analysis. A portion of these data (22 subjects) was extracted from a data base constructed between 1975–1979 whereas the remainder was collected subsequently through 1981. These were essentially part of continuous studies of factors affecting theophylline disposition in man and identical drug, clinical, record, assay, and pharmacokinetic procedures were involved. Protocols were approved by university and hospital Human Investigation Committees and all subjects provided informed consent in participating.

The ages of the subjects ranged from 19 to 30 years. None of the women examined were considered to be obese (i.e., total body weight did not exceed estimated ideal body weight (Diem & Lentner, 1970) by more than 15%), abusers of alcohol, excessively habituated to caffeine, or suffering from any underlying hepatic/renal disease. Sixteen of the subjects admitted to smoking marijuana in the months prior to participation. In such instances, however, the amount of the substance smoked did not exceed that normally associated with social use (i.e., less than one joint per week). Twenty-two subjects used OC on a chronic basis (i.e., longer than 6 months). Of these, 14 were using Ovral (Wyeth Laboratories: 0.05 mg of ethinylloestradiol/0.50 mg norgestrel) at the time of study, while two were using Ovulen (Searle Co.: 0.10 mg mestranol/1.0 mg ethynodiol diacetate). Six subjects in the former group had been taking this product routinely, whereas eight were using various other commercially available combinations and switched to Ovral only for the monthly cycle prior to study. Information concerning the OC type used by the remaining six subjects was not available.

Twenty-seven subjects used tobacco daily. The average daily use exceeded 20 cigarettes per day, while the duration of use spanned a period in excess of 7 years.

Table 1 presents the demographic data for the subjects considered. The group has been partitioned into four disjoint subgroups based upon tobacco and OC use.

Posology and assays

Subjects were requested to fast overnight prior to participation in the investigation. In addition, all

xanthine containing foods/beverages were deleted from their normal diets both during the study day and for the 24 h period preceding examination. In the morning of the study day, theophylline was administered orally as an aqueous solution of its ethylenediamine salt, aminophylline. A weight adjusted dose (4 mg/kg) of this salt was placed in 200 ml of orange juice, which was then consumed by the subject. Venous blood samples (7 ml) were serially collected from an indwelling catheter at times: 0 (just before administration of the drug), 0.5, 1, 2, 3, 5, 7, 11 and 23 h after the dose was given. The patency of this catheter was maintained using a 20 unit/ml solution of heparin in normal saline. When slight deviations from this sampling protocol occurred, such sampling times provided for adequate estimations of both area under the serum concentration versus time profile and the slope of the terminal disposition phase. The samples were allowed to clot and then were centrifuged. Serum was harvested and frozen pending analysis via high performance liquid chromatography (Jusko & Poliszczuk, 1976). For 27 subjects, a single Ovral tablet was ingested 60 min before the test dose of aminophylline. For 11 of these women who were using OC as a method of birth control, this tablet was their routine daily intake of contraceptive steroids. For the remaining 16, such treatment represented single dose exposure. In these cases, two additional blood samples were collected, corresponding to the zero time with respect to OC administration and 0.5 h post-administration. For these 27 subjects, all serum samples were assayed for both ethinylloestradiol and norgestrel (Cook *et al.*, 1974; Stanczyk *et al.*, 1975). Appropriate time corrections were implemented where necessary. Contraceptive steroid serum concentrations were not measured for the remaining 22 subjects.

Pharmacokinetics

Area under the serum concentration vs time curve (AUC) was estimated either by numerical integration using the trapezoidal rule or via mathematical integration of fitted interpolating polynomials (Yeh & Kwan, 1978) in combination with log-trapezoidal approximation. Both approaches provided for inclusion of the terminal area extrapolated to infinite time. Plasma clearance was obtained from the relationship, $CL = \text{Dose}/\text{AUC}$. Since it has been shown that theophylline in solution is completely absorbed from the gastrointestinal tract following oral administration (Hendeles *et al.*, 1977), bioavailability was assumed to be unity. Clearance was normalized for both total body weight (TBW) and ideal body weight (IBW), the latter estimated from body frame size and height (Diem & Lentner, 1970). The slope of the post-absorptive decline of serum concentrations (λ) was obtained via log-linear least-squares regression analy-

Table 1 Characteristics of subjects

Characteristics	Non-smokers		Smokers	
	Group: I Non-users	II OC users	III Non-users	IV OC users
Number of subjects	12	10	15	12
Age range (years)	22–28	19–28	19–30	21–29
Mean age (years)	24.4	24.2	24.1	24.7
(s.d.) ^a	(2.3)	(2.6)	(3.3)	(2.5)
Total body weight (kg)	54.5	59.3	59.9	55.7
(s.d.)	(4.7)	(8.2)	(7.9)	(6.9)
Duration of OC use (years)	0	4.5	0	6.8
(s.d.;n) ^b		(2.5;8)		(2.8;3)
Tobacco use (pk/day)	0	0	1.2	1.1
(s.d.)			(0.4)	(0.4)
Duration of tobacco use (years)	0	0	7.5	5.9
(s.d.;n)			(3.6;15)	(3.2;10)
Marihuana use ^c	0.17	0.20	0.47	0.50
Alcohol use ^c	1.0	1.0	0.93	0.92
Caffeine use ^d	2.0	2.1	4.8	2.0
(s.d.)	(1.8)	(1.3)	(2.4)	(0.1)
Bilirubin (0.1–1.1 mg%) ^e	0.71	0.42	0.49	0.45
(s.d.;n)	(0.20;12)	(0.23;10)	(0.19;15)	(0.18;11)
SGPT (0–43 u/l) ^e	17.8	19.0	14.1	11.9
(s.d.;n)	(7.3;12)	(9.3;9)	(6.0;14)	(9.4;8)
SGOT (11–46 u/l) ^e	18.3	21.6	20.7	23.3
(s.d.;n)	(5.6;12)	(12.9;10)	(7.5;15)	(9.8;11)
Alkaline phosphatase (34–133 u/l) ^e	53.7	47.7	58.7	50.3
(s.d.;n)	(16.7;12)	(15.0;10)	(15.6;15)	(12.2;11)

^a Standard deviation^b Standard deviation; number of subjects^c Coded as 0 = none, 1 = social, 2 = daily use^d Number of cups of coffee or tea per day^e Normal range in parentheses

sis. The apparent volume of distribution (V_D) was then generated as $V_D = CL/\lambda$ and subsequently normalized for TBW.

Statistics

The disparate backgrounds of the subjects with respect to factors known to affect theophylline disposition such as caffeine (Mitoma *et al.*, 1969) and marihuana (Jusko *et al.*, 1978) precluded a direct assessment of OC and smoking effects on theophylline disposition via a two-way analysis of variance. An additional factor lacking adequate control was related to the fact that 16 of the subjects examined were not chronic users of OC, yet were exposed to a single OC dose shortly before aminophylline administration. Consequently, a four-way analysis of covariance was deemed the most appropriate statistical approach to pursue. The main effects were defined as cigarette smoking, OC use, marihuana use, and acute ex-

posure to contraceptive steroids. Daily caffeine intake served as the covariate. This method of analysis was chosen over a two factor approach (cigarettes and OC) with a more stringent subject selection process, since the technique of subject matching is not always optimal and often results in a dramatic reduction in the number of data available for consideration (McKinlay, 1977). In addition, confirmation of various factor effects on theophylline disposition tends to enhance the credibility of the particular analysis conducted.

Since the four-factor partitioning process resulted in subgroups of unequal sizes, the main effects were rendered dependent upon one another. As a consequence, additivity of individually determined sums of squares did not hold. This necessitated pursuing a hierarchical approach, whereby all main effects were assigned status values relative to one another. With this form of analysis, sums of squares were adjusted only for factors possessing higher priorities, those

with lower statuses having no effect (Overall & Spiegel, 1969). Cigarette smoking was given the highest priority due to its well documented effect on theophylline disposition (Hunt *et al.*, 1976; Grygiel & Birkett, 1981). This was followed by daily caffeine intake, OC use, use of cannabis, and acute exposure to contraceptive steroids. This ordering of main effects reflects our suspicions with regard to the abilities of these factors to alter theophylline disposition. Although the net effect of acute contraceptive steroid exposure was unknown, its effect, if any, was not anticipated to be as dramatic as those induced by the other factors, and consequently was assigned the lowest priority. The covariate, caffeine use, was introduced into the analysis at the second stage. This provided for the removal of extraneous caffeine related influences prior to making assessments regarding the contributions of OC use, marijuana smoking, and acute exposure to OC. Such an ordering did permit caffeine effects to confound the analysis of tobacco related influences. It was felt, however, that tobacco effects would be considerably stronger than those of caffeine and hence any contamination of this nature would be minimal. Generally, it is desirable to feather out any covariate influences prior to assessment of all main effects. However, interchanging the positions of tobacco use and caffeine intake in the analysis could result in an exaggerated estimate of caffeine effect, since it would be entered into the analysis first and was shown to be significantly correlated with the magnitude of the subject's tobacco habit.

All two-way interactions were evaluated. Each respective sum of squares was corrected for the influences of all main effects and all other two-way interactions. The question regarding higher order interactions was not addressed.

Since a pre-written digital computer program capable of performing the type of analysis desired was unavailable, appropriate sums of squares were generated following the fitting of coded data to various linear regression models (Kim & Kohout, 1975).

This approach to data analysis was employed in order to assess the effects of these factors on theophylline clearance, volume of distribution, and half-life ($t_{1/2}$).

Least-squares fitting of serum concentration-time data was conducted employing conventional log-linear regression theory (Yeh & Kwan, 1978). All other bivariate regression analyses were performed recognizing the fact that both variables involved were subject to random error (Riggs *et al.*, 1978).

Results

Serum concentration *vs* time profiles for individual subjects representing each of the four groups con-

sidered are presented in Figure 1. In each instance, absorption is rapid, peak concentrations being attained within 2 h following administration of the dose. Post-absorptive declines of serum concentration reflected apparent first order processes.

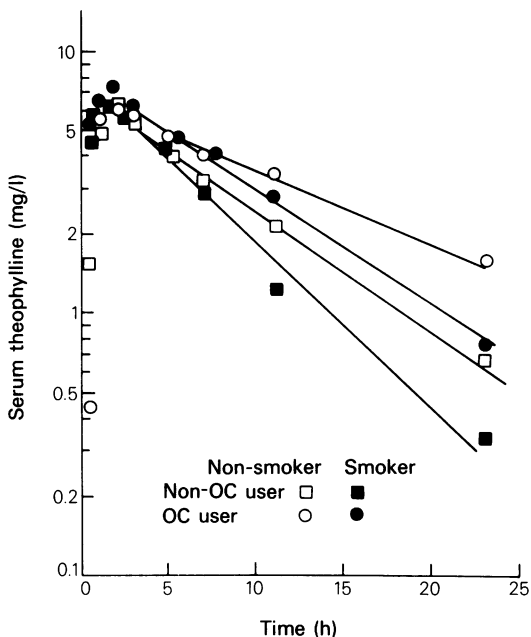


Figure 1 Representative serum concentration *vs* time profiles of theophylline for subjects in each of the four groups examined. The symbols are defined in the key. Solid lines are the results of least-squares fittings of data in terminal phases.

The results of the statistical analysis, conducted with theophylline plasma clearance normalized for IBW as the dependent variable, are exhibited in Table 3. The same type of analysis, performed with clearance values adjusted for TBW, resulted in qualitatively similar conclusions (i.e., the rank order of significant main effects was maintained but the levels of significance were found to be somewhat lower). As indicated in Table 3, cigarette smoking, chronic OC ingestion, and daily caffeine intake all significantly alter theophylline clearing processes in the subjects examined. On the other hand, the social use of cannabis and acute exposure to contraceptive steroids both failed to induce remarkable changes in this parameter. The latter finding suggests that the clearance data presented for smoking and nonsmoking groups of non-OC users (groups 1 and 3) have not been rendered misrepresentative as a consequence of the data blending process.

Figure 2 illustrates the effects that cigarette smoking and OC use have upon theophylline clear-

Table 2 Summary of theophylline pharmacokinetics in smoking and non-smoking female subjects who were users/non-users of oral contraceptives

Parameter (s.d.)	Group:	Non-smokers		Smokers	
		I	II	III	IV
		Non-users	OC users	Non-users	OC users
$t_{1/2}$ (h)		7.76 (2.39)	9.57 (1.34)	5.13 (1.09)	6.22 (1.53)
V_D (l/kg TBW ^a)		0.55 (0.09)	0.55 (0.13)	0.51 (0.06)	0.46 (0.10)
CL (ml h ⁻¹ kg ⁻¹ IBW ^b)		49.1 (14.3)	39.4 (10.1)	74.3 (17.6)	49.7 (22.3)
CL (ml h ⁻¹ kg ⁻¹ TBW ^a)		51.0 (12.7)	37.2 (11.8)	68.7 (17.8)	54.0 (21.8)

^a Normalized for total body weight

^b Normalized for ideal body weight

ance in the entire group of subjects. These data have been normalized for both TBW and IBW and are slightly contaminated by the disparate use of caffeine. Regardless of the normalization approach, the data suggest that OC use exerts a significant inhibitory effect on theophylline clearance, whereas cigarette smoke exposure markedly enhances theophylline's hepatic metabolism. The effect of tobacco use appears to be more pronounced than that of OC exposure when clearance values are normalized for IBW vs TBW. As indicated previously, these obser-

vations were confirmed statistically. In addition, visual inspection of the data in this figure suggests that perhaps a slight interactive effect occurs between cigarette smoking and OC use. The average clearance of group 4 (smokers, OC users) approaches control values (49.7 ml/h/kg IBW vs 49.1 ml/h/kg IBW) in spite of the apparent differences in the magnitudes of the individual main effects. However, when the underlying effects of caffeine and other factors were removed, this interaction was shown not to attain statistical significance ($P > 0.3$). This suggestion of

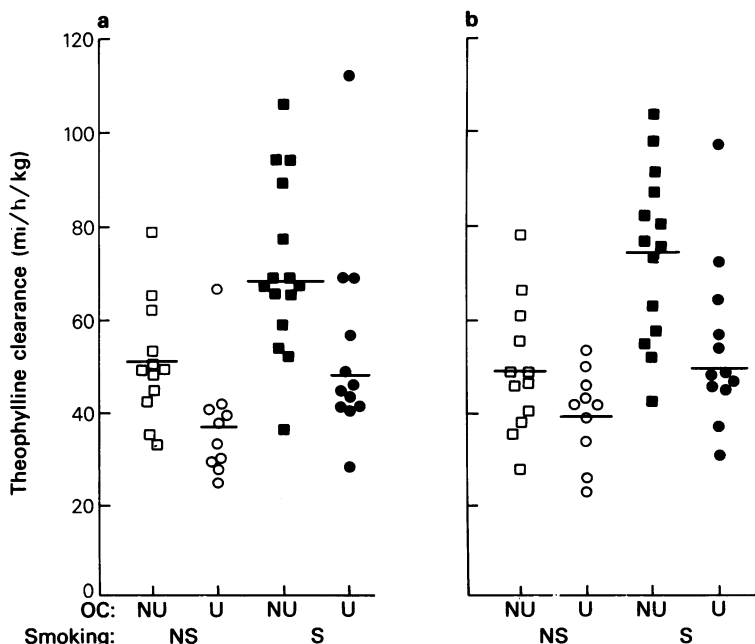


Figure 2 Plasma clearances of theophylline in the four designated subject groups. Clearances were normalized for a) total body weight and b) ideal body weight. Horizontal lines denote mean values for each group. U user, NU non-user, S smoker and NS non-smoker.

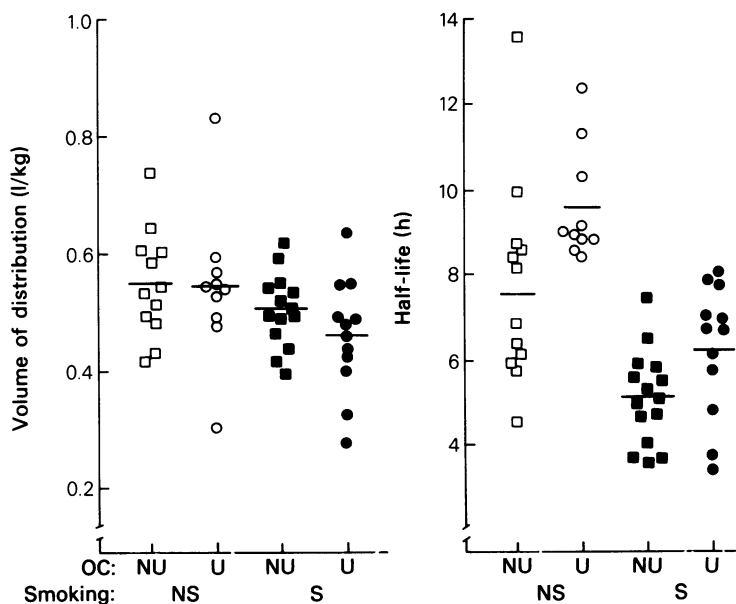


Figure 3 Volume of distribution and half-life of theophylline in the four subject groups. Horizontal lines denote mean values. U user, NU non-user, S smoker and NS non-smoker.

potential interaction is not as evident when normalization is conducted using TBW. In this case, the main effects of OC and tobacco use are significant but appear to be simply additive.

The apparent volume of distribution of theophylline was unaffected by chronic OC exposure (Figure 3). However, a small but significant reduction in this parameter was detected in the cigarette smoking group ($P < 0.05$). This was the only factor considered in the model which exerted such an effect on the distributional space. Figure 3 also presents the effect of these two main factors on the theophylline half-life. As with plasma clearance, both cigarette smoking (shorter $t_{1/2}$) and OC (longer $t_{1/2}$) effects were determined to be significant. The effect induced by cigarette use on $t_{1/2}$ is the combined result of both increased clearance and reduction of distribution volume, whereas the OC effect is primarily mediated via inhibition of theophylline elimination. In parallel with the results of the clearance data analysis, both caffeine intake and the interaction between cigarettes and acute exposure to OC were shown to have significant effects. A summary of the pharmacokinetic parameters for each group is contained in Table 2.

The effects of OC components on theophylline clearance is displayed in an exposure-response format in Figure 4. Areas under the serum concentration-time profiles were employed as indices of OC exposure. The smallest values of theophylline clearance were noted in the subjects with largest AUC of both norgestrel and ethinylestradiol.

Discussion

The present investigation assessed the independent effects of daily OC ingestion and cigarette smoking as well as the potential interactive effect of these and other factors on theophylline disposition in young women. Both cigarette smoke exposure and daily OC intake significantly alter theophylline disposition. In particular, tobacco use enhances the metabolism of theophylline, while chronic OC exposure inhibits the clearance of this bronchodilator. Together, the two factors offset each other.

Primary smoking and OC effects

The marked enhancement of theophylline clearance associated with cigarette use confirms the original findings of Jenne *et al.* (1975) and Hunt and co-workers (1976). Although this increase in metabolic activity is evident regardless of the normalization approach taken (IBW vs TBW), Gal and associates (1978) found the use of the subject's IBW more appropriate. Examination of the clearance data revealed that an increase of roughly 40% was characteristic of the cigarette smoking group. These values are in reasonable agreement with those previously reported from this laboratory (Jusko *et al.*, 1978), although we now have more selective inclusion of subjects as the original report did not control OC and caffeine use. Increases in CL are likely due to the inductive effects of cigarette smoke components such

Table 3 Summary of statistical analysis conducted to assess the effects of various factors on theophylline plasma clearance normalized for IBW

Source of variation	Sum of squares	d.f.	Mean square	F	Significance of F ^a
<i>Covariates</i>					
Caffeine (Caf)	1408.62	1	1408.62	6.80	0.013
<i>Main effects</i>					
Cigarettes (65.2;44.7) ^b	7303.88	4	1825.97	8.82	< 0.001
OC use (47.3;63.1)	5129.13	1	5129.13	24.78	< 0.001
Marijuana use (MJ)	1909.96	1	1909.96	9.23	< 0.005
Acute exposure (AE)	186.02	1	186.02	0.90	NS ^c
	78.77	1	78.77	0.38	NS
<i>Two-way interactions</i>					
	3569.47	9	396.61	1.92	0.082
Cig-Caf	0.26	1	0.26	0.01	NS
Cig-OC	152.28	1	152.28	0.74	NS
Cig-MJ	20.30	1	20.30	0.10	NS
Cig-AE (59.8;53.4)	2305.26	1	2305.26	11.14	0.002
Caf-OC	10.36	1	10.36	0.05	NS
Caf-MJ	51.77	1	51.77	0.25	NS
Caf-AE	389.72	1	389.72	1.88	NS
OC-MJ	199.40	1	199.40	0.96	NS
MJ-AE	67.23	1	67.23	0.33	NS
Explained	12281.96	14	877.28	4.24	< 0.001
Residual	7038.09	34	207.00	—	—
Total	19320.05	—	—	—	—

^a Given as *P*-value for *F*-distribution with appropriate degrees of freedom

^b (Average CL for group indicated; average CL for complementary group)

^c *P* > 0.05

as the polycyclic aromatic hydrocarbons on distinct forms of cytochrome P-450 (Jusko, 1978).

Daily OC exposure was shown to markedly inhibit the clearing processes responsible for theophylline removal from plasma, although the magnitude of this effect was not as striking as that elicited by the use of tobacco. Subjects employing this form of birth control were shown to have clearance values which averaged approximately 28% below those exhibited by non-OC users. This difference is similar to that reported previously in a subgroup of non-smoking normal volunteers (Tornatore *et al.*, 1982). Patwardhan and associates (1980) presented results which indicate that similar metabolic inhibition occurs following the single-dose administration of caffeine which is generally sensitive to the same factors affecting theophylline disposition. Daily exposure to OC for periods longer than 6 months in nonsmoking subjects resulted in a 40% reduction in caffeine plasma clearance. Also, examination of blood concentration data obtained from women using OC have led others to note concomitant elevations in plasma concentrations of caffeine and ethinylestradiol (Ahluwalia *et al.*, 1977; Kaul *et al.*, 1981). Since theophylline is almost exclusively eliminated via hepatic biotransformation pathways, it is reasonable to conclude that such inhibitory behavior reflects either specific competition for metabolic sites or

generalized depression of hepatic function secondary to contraceptive steroid exposure. The primary results of the present analysis should be considered qualitative in nature, and the numerical values presented should serve only as an approximation of the magnitude of any metabolic perturbations which might occur during chronic dosing of patients with theophylline.

Crawford and co-workers (1981) reported that cigarette smoking had no effect on plasma concentrations of either ethinylestradiol or norgestrel when compared to nonsmoking subjects. Similar observations have been made in our laboratory with respect to the plasma clearances of these compounds (to be published). The results of the present investigation as well as those of others have shown that the primary metabolic pathways responsible for theophylline degradation are very sensitive to such exposure, thus suggesting minimal overlap of metabolic mechanisms or pathways. The observation that acute exposure to OC agents apparently has no significant effect on theophylline clearance may partly support this contention. However, the possibility does exist that a competitive effect occurs, but that single dose exposure to these agents does not produce sufficient concentrations in the milieu of the metabolic sites to elicit a discernible effect.

The most likely explanation for this inhibitory

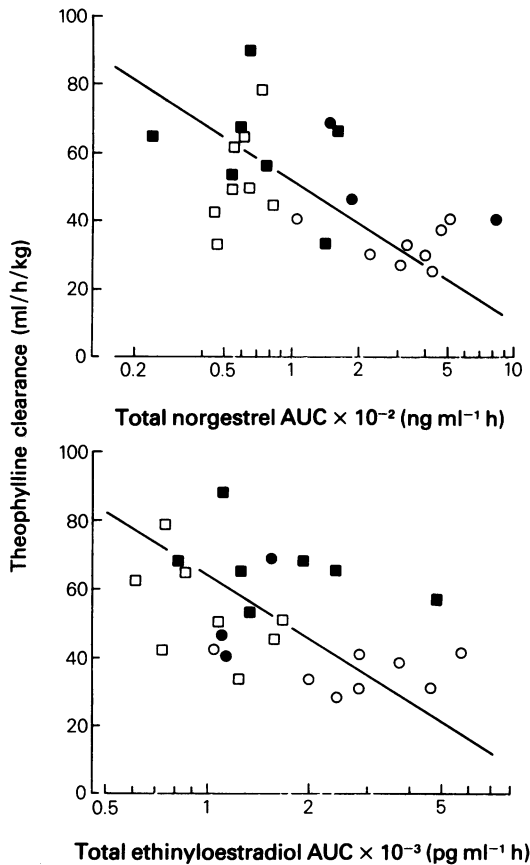


Figure 4 Relationships between theophylline clearance and the areas under the curves (AUC) for norgestrel and ethinyloestradiol for 26 women who received single or multiple doses of Ovral. A log transformation of the AUC data was performed in adherence to classical dose-response theory. Symbols are defined in Figure 1. Norgestrel results from one subject were excluded as grossly misrepresentative. Regression lines were generated employing an orthogonal least-squares approach. Significant negative correlations were found for both norgestrel ($r = -0.53$, $P < 0.01$) and ethinyloestradiol ($r = -0.46$, $P < 0.02$) exposure.

phenomenon is that chronic exposure to these steroidal agents results in a reduction of the non-conjugative activities of the liver. This is strongly suggested by the numerous observations that contraceptive steroids are capable of markedly inhibiting oxidative pathways that are mediated through distinct cytochromic apoproteins (i.e., antipyrine via cytochrome P-450 and theophylline via P-448). More direct evidence in this regard has also been reported (MacKinnon and co-workers, 1977), as rats subjected to a 5 day exposure to ethinyloestradiol ($5 \text{ mg kg}^{-1} \text{ day}^{-1}$), exhibit a significant reduction in the activity of

hepatic ethylmorphine *N*-demethylase and of cytochrome P-450, cytochrome b_5 , and NADPH cytochrome-c-reductase. The strong correlation between theophylline CL and AUC of the OC steroids supports this hypothesis.

Secondary factors

The social or occasional use of marihuana was shown not to significantly alter theophylline disposition. Jusko and co-workers (1978) had concluded that regular marihuana use results in an enhancement of theophylline metabolism. In this earlier instance, the marihuana users were subjects whose use of cannabis greatly exceeded that of current subjects. A more rigorous analysis of this phenomenon showed that occasional use of marihuana was not a primary factor influencing theophylline CL (Jusko *et al.*, 1979). In fact, the analysis suggested that the social use of marihuana results in a reduction of metabolic activity. This finding, however, appeared as a low priority possibility in the statistical cascade of influential factors. Minimal use of tobacco has also been reported not to affect theophylline clearance (Piafsky *et al.*, 1977; Grygiel *et al.*, 1979).

The use of caffeine has been implicated in markedly altering theophylline clearing processes. Both enhancement (Mitoma *et al.*, 1969) and inhibition of theophylline metabolism (Monks *et al.*, 1979) have been reported. In the subjects examined in the present investigation, it appears that a significant, but rather small enhancement of clearance occurred in caffeine users.

The apparent volume of distribution of theophylline was unaffected by OC use in the subjects studied. Similar findings have been reported for caffeine (Patwardhan *et al.*, 1980). However, a modest reduction in V_D was evident in the cigarette smoking group. This phenomenon was noted earlier (Hunt *et al.*, 1976) but cannot be explained.

A significant interaction between cigarette smoking and acute exposure to OC was noted. As previously discussed, it is possible that a portion of the inhibitory effect exhibited by these agents may be ascribable to competitive binding to metabolic sites, and hence could manifest itself acutely. Although such acute exposure was deemed insignificant when considered alone, a notable effect was present when superimposed upon smoking. This finding is consistent with those of Conrad & Nyman (1980) who reported that the inhibitory effects of both propranolol and metoprolol are most pronounced in subjects whose theophylline plasma clearance values were initially elevated (i.e. in smokers). Qualitatively similar findings were presented by LaForce and co-workers (1981) who reported that subjects with the highest initial clearance rates of theophylline, tended to have the largest reductions in clearance subsequent to

treatment with erythromycin ethylsuccinate. Carter and co-workers (1975) reported that only subjects with the shortest antipyrine half-lives exhibited significant increases in this parameter following a 3 month exposure to 2 mg norethindrone/0.1 mg mestranol. In addition, it has been apparent that the enzyme inductive effects of smoking on disposition of several drugs does not occur in patients with cirrhosis (Farrell *et al.*, 1978) or in the elderly whose metabolic capabilities are intrinsically low (Vestal *et al.*, 1980). It thus appears that a higher degree of metabolic activity may be associated with greater sensitivity to metabolic inhibition.

These findings suggest that perturbations in theophylline elimination may occur during pregnancy, a period when circulating concentrations of endogenously produced gonadotropins are relatively high. Preliminary data do not appear to support this (Sutton *et al.*, 1978). Presently we are engaged in a more comprehensive examination of the effects of pregnancy on theophylline disposition. To date our results are in agreement with those of Sutton and co-workers (i.e., there is a trend towards a reduction in theophylline clearance during pregnancy albeit statistically insignificant).

Statistical complexities

Evaluation of data upon which multiple factors have exerted diverse effects is difficult. Such difficulty is compounded when partitioning of the study group by these factors results in disjoint subgroups of unequal sizes. Attempts are generally made to avoid such a situation by carefully matching experimental units with regard to extraneous factors known to influence the results. This approach is not always feasible. In the present study the occurrences of factors known to affect theophylline disposition (e.g. marijuana use and caffeine intake) were unevenly distributed

among the four subgroups partitioned by OC and tobacco use. Exclusion of data in an attempt to match subjects often results in prohibitive reductions in the number of data available for analysis as well as loss of potentially valuable information associated with outlying values which are often difficult to adequately match. The approach pursued in this study provided for inclusion of all available data, while allowing for assessments of specific effects to be made. *A priori* knowledge of the system being investigated facilitates rational definition of the sums of squares associated with factors and interactions of interest. Since many pre-written computer programs are available for analysis of non-orthogonal designs, an effort should be made to fully understand the procedure by which such sums of squares are generated. The fact that various pre-written computer programs produce identical results when analysing data containing subgroups of equal sizes does not guarantee identical performances when unequal sized groups are involved.

Alternative approaches to factor definition exist as well. Just as sum of squares definition can influence the final results of an analysis, so too can the factorial/coding scheme adopted. In the present analysis, a three level OC factor (never exposed, acutely exposed, chronically exposed) could have effectively been used in lieu of the individual factor design we adopted. Although hierarchical adjustment rendered identical results for these two schemes, other approaches may produce markedly different ones.

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