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# 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 ml $h^{-1}$ $kg^{-1}$ ideal body weight for OC non-user, non-smoker, *vs* 49.7 ml $h^{-1}$ $kg^{-1}$ 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 contributary 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 marihuana 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 ethinyloestradiol/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 ethinyloestradiol 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 = Dose/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 ($\lambda$) was obtained via log-linear least-squares regression analy-

**Table 1** Characteristics of subjects

| | Group: | *Non-smokers* | | *Smokers* | |
|---|---|---|---|---|---|
| *Characteristics* | | *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) (s.d.)[a] | | 24.4 (2.3) | 24.2 (2.6) | 24.1 (3.3) | 24.7 (2.5) |
| Total body weight (kg) (s.d) | | 54.5 (4.7) | 59.3 (8.2) | 59.9 (7.9) | 55.7 (6.9) |
| Duration of OC use (years) (s.d.;*n*)[b] | | 0 | 4.5 (2.5;8) | 0 | 6.8 (2.8;3) |
| Tobacco use (pk/day) (s.d.) | | 0 | 0 | 1.2 (0.4) | 1.1 (0.4) |
| Duration of tobacco use (years) (s.d.;*n*) | | 0 | 0 | 7.5 (3.6;15) | 5.9 (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] (s.d.) | | 2.0 (1.8) | 2.1 (1.3) | 4.8 (2.4) | 2.0 (0.1) |
| Bilirubin (0.1–1.1 mg%)[e] (s.d.;*n*) | | 0.71 (0.20;12) | 0.42 (0.23;10) | 0.49 (0.19;15) | 0.45 (0.18;11) |
| SGPT (0–43 u/l)[e] (s.d.;*n*) | | 17.8 (7.3;12) | 19.0 (9.3;9) | 14.1 (6.0;14) | 11.9 (9.4;8) |
| SGOT (11–46 u/l)[e] (s.d.;*n*) | | 18.3 (5.6;12) | 21.6 (12.9;10) | 20.7 (7.5;15) | 23.3 (9.8;11) |
| Alkaline phosphatase (34–133 u/l)[e] (s.d.;*n*) | | 53.7 (16.7;12) | 47.7 (15.0;10) | 58.7 (15.6;15) | 50.3 (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 exposure 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 hierarchial 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, marihuana 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 ($t_{½}$).

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 considered 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.

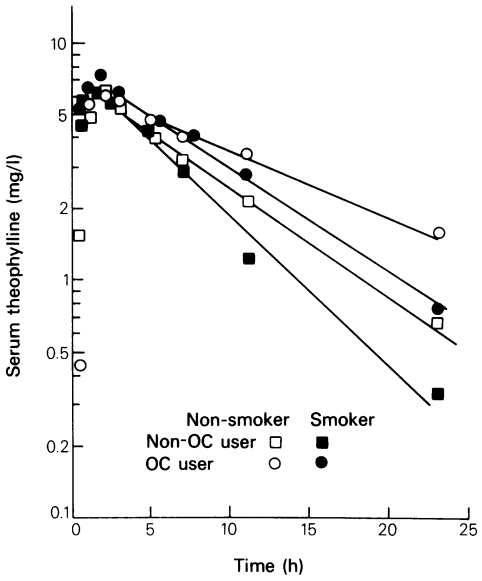


**Figure 1** Representative serum concentration *vs* time profiles of theophylline for subjects in each of the four groups examined. The symbols are defined in the key. Solid lines are the results of least-squares fittings of data in terminal phases.

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* *I* *Non-users* | *Non-smokers* *II* *OC users* | *Smokers* *III* *Non-users* | *Smokers* *IV* *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 observations 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

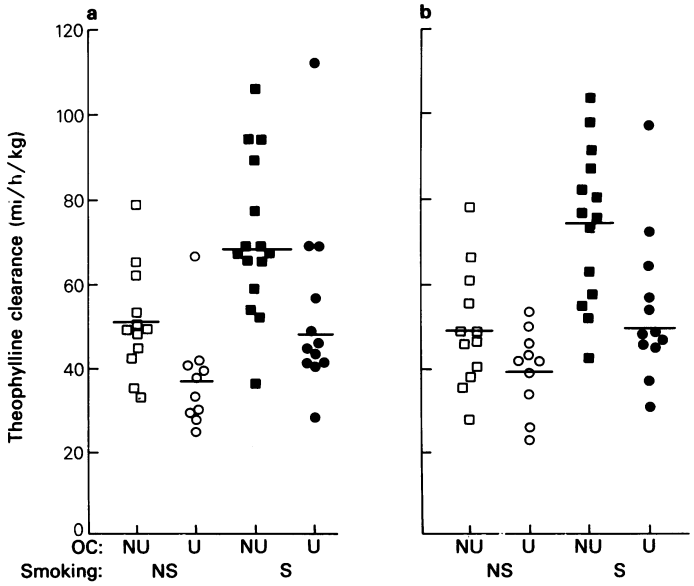


**Figure 2** Plasma clearances of theophylline in the four designated subject groups. Clearances were normalized for a) total body weight and b) ideal body weight. Horizontal lines denote mean values for each group. U user, NU non-user, S smoker and NS non-smoker.

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