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Influence of menstrual cycle phase on serum concentrations of α_1-acid glycoprotein

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Serum concentrations of α_1-acid glycoprotein (AAG) were studied in nine healthy women at four times in their menstrual cycles. AAG concentrations were significantly higher on day 4 than on days 12, 20, and 28 (with the first day of menstrual flow considered to be day 1). The mean AAG concentration (mg dl^{-1}) on day 4 was 78.55 ± 5.03 (mean ± s.e. mean), 70.19 ± 4.80 on day 12, 70.63 ± 6.67 on day 20, and 70.40 ± 5.97 on day 28. Although these results should be considered preliminary because of the small sample size, we conclude that physiologic changes over the course of the menstrual cycle may affect serum AAG concentrations. Since AAG is a major binding protein for several important drugs, the potential exists for altered drug binding and drug effects, and further study of individual drugs is justified.

Keywords α_1-acid glycoprotein menstrual cycle

Introduction

α_1-acid glycoprotein (AAG) is a sialated glycoprotein that has recently gained attention as an important low-concentration, high-affinity plasma binding protein for basic drugs (Müller & Stillbauer, 1983). Binding in excess of 90% has been reported for verapamil (McGowan *et al.*, 1983), disopyramide (Maughey *et al.*, 1985), tricyclic antidepressants (Freilich *et al.*, 1984; Javaid *et al.*, 1983), and triazolam (Kroboth *et al.*, 1984). Variation in AAG concentration has been shown to account for approximately 75% of the variation in lignocaine plasma protein binding (Routledge *et al.*, 1981), and elevated AAG concentrations following myocardial infarction have been associated with elevated lignocaine concentrations (Routledge *et al.*, 1980). The physiologic function of AAG is poorly understood, but it appears to be involved in immunoregulation; AAG has been reported to alter leucocyte chemotaxis (Costello *et al.*, 1984) and natural killer cell target recognition (Okumura *et al.*, 1985). In addition, AAG appears to have antiplatelet (Snyder & Coodley, 1976) and antiheparin activity (Andersen *et al.*, 1981).

AAG is considered to be an acute phase reactant, based on findings that its concentration in serum and plasma is increased following myocardial infarction (Giardina *et al.*, 1985), cancer (Abramson *et al.*, 1982), infection (Kenny *et al.*, 1984), and rheumatoid arthritis (Killingsworth *et al.*, 1975). Concentrations of AAG are increased by amitriptyline (Baumann *et al.*, 1982) and cigarette smoking (Chao *et al.*, 1982), but not snuff or chewing tobacco (Parish *et al.*, 1989).

The effects of pregnancy and hormonal changes upon AAG concentration have also been investigated. Pregnancy has been reported to be associated with lowered AAG concentrations (Adams & Wacher, 1968; Ganrot, 1972). It is unclear whether the state of pseudopregnancy induced by oral contraceptives affects AAG concentrations; one study in 24 healthy women taking oral contraceptives did not demonstrate an effect (Blain *et al.*, 1985), but other studies have noted lowered AAG concentration (Routledge *et al.*, 1981; Song *et al.*, 1970). These studies suggest that variations in plasma oestrogen levels associated with the time course of the menstrual cycle may affect AAG levels. It is possible that such variations may be sufficiently large to alter to a clinically important degree the free fraction of highly bound drugs, with variations in therapeutic response or toxicity as a result. The present study was therefore undertaken to assess the variability of AAG concentrations over the course of the menstrual cycle.

Methods

The procedures were approved by the University Institutional Review Board. All potential subjects were informed of the risks of venepuncture and gave written informed consent before being screened for inclusion.

Non-smoking women between the ages of 21 and 41 years were considered for inclusion. The health of each

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potential subject was assessed by means of a medical and drug history and appropriate laboratory studies, including erythrocyte sedimentation rate and a serum chorionic gonadotropin pregnancy test. Subjects reporting a history of infectious disease, tricyclic antidepressant use, or oral contraceptive use within 60 days of the interview or a history, however remote, of arthritis, hepatitis, neoplastic disease, or other inflammatory disease, were excluded.

Blood was obtained by antecubital venepuncture from each subject on days 4, 12, 20, and 28 of the menstrual cycle, counting the first day of menses as day 1. The subjects entered the study on the first sample day occurring after the completion of screening and continued until samples had been obtained on all four sample days. All subjects underwent sampling on contiguous sampling days. All blood samples were obtained between 12.00 and 17.00 h, since circadian variation in AAG concentration has been reported (Yost & DeVane, 1985). The serum was separated immediately from the clotted blood by centrifugation at 800 *g* and was promptly frozen and stored at −15° C for subsequent analysis.

The AAG content of each serum sample was measured in duplicate by radial immunodiffusion assay (Nor-Partigen RID Plates, Behring Diagnostics). Appropriate calibration and quality control measures were undertaken, with a serum control placed on each RID plate. The coefficient of variation of this assay in our laboratory during the period of the study was 7.35% at an AAG concentration of 85.7 mg dl^{-1} (that of the manufacturer's serum control).

Observed differences in AAG concentrations were evaluated by analysis of variance (ANOVA) with day and subject as main effects, followed by the Ryan-Einot-Gabriel-Welsch multiple *F*-test (SAS Institute, 1988) for comparisons of means among days 4, 12, 20, and 28. The statistical procedures were performed using the General Linear Models procedure in the SAS-PC statistical software, release 6.03 (SAS Institute, Cary, North Carolina, USA).

Results

Nine subjects met the inclusion criteria and were admitted to the study. All subjects had regular menstrual cycles of approximately 28 days duration. Raynaud's phenomenon was observed in one subject during a sampling visit; questioning revealed a history of similar manifestations extending over several years, and this subject was dropped from the study. One subject was unavailable for sampling on days 12 and 20; all other samples contributed information.

Table 1 shows the individual observations for each subject. The ANOVA and multiple comparison procedures indicated that both intersubject variability and cycle day contributed significantly to observed differences in AAG concentration ($P = 0.0001$ for subject, 0.0149 for day, 0.0001 overall). Mean AAG concentrations on days 12, 20, and 28 were significantly different from those on day 4 but not from one another at the 0.05 significance level. If the serum concentration of each subject on day 4 is considered her baseline for purposes of comparison with concentrations on other days, the mean percentages of baseline are 89.2% on day 12, 88.7% on day 20, and 88.9 on day 28. Individual subjects' AAG concentrations were 78–100% of baseline on day 12, 77–103% on day 20, and 73–103% on day 28. Two subjects (5 and 7) had AAG concentrations equal to or greater than baseline concentrations on two of days 12, 20, and 28, when mean concentrations for the group were lower than baseline. One subject (8) had a lower concentration on day 12.

Discussion

Some caution is necessary in extending our findings to a larger population. First, the sample size was small and considerable intra- and inter-subject variation in serum AAG concentration has been demonstrated in various populations (Blain *et al.*, 1985; Parish *et al.*, 1989; Yost & DeVane, 1985). Second, we observed the subjects over the course of only one menstrual cycle, and all observations were made during two calendar months. Third, we made no attempt to control environmental factors. Blain *et al.* (1985) concluded that environmental factors may be as important as endogenous factors in determining serum AAG concentration. We did not measure serum concentrations of hormones or undertake other assessment of endocrine function. Finally, we measured serum AAG concentrations only on selected days during the menstrual cycle, and it is likely that the actual minima and maxima were missed.

We observed statistically significant changes in the mean serum AAG concentration in a population of women. Our findings are consistent with the variable nature of serum AAG concentration previously reported; Killingsworth (1980) reports a coefficient of variation of approximately 12% for intrasubject variation in AAG concentration. Our mean variations are of approximately the same magnitude and may be entirely due to random intrasubject variation. However, the fact that 18 of 22 observations on days 12, 20, and 28 were lower than the individuals'

Table 1 Changes in AAG concentration during the menstrual cycle

Subject	*Day 4 (mg dl^{-1})*	*Day 12 (mg dl^{-1})*	*Day 20 (mg dl^{-1})*	*Day 28 (mg dl^{-1})*
1	72.9	62.3	64.2	62.5
2	71.5	62.8	55.0	66.2
3	73.8			67.0
4	96.0	75.0	87.9	78.6
5	52.7	52.7	46.8	38.2
6	88.2	83.2	70.3	74.7
7	79.0	66.7	72.9	79.0
8	94.3	88.6	97.3	97.2
Mean	78.55**	70.19*	70.63*	70.40*
s.e. mean	5.03	4.80	6.67	5.97

Means: (*) different from (**) at 0.05 significance level (ANOVA followed by Ryan-Einot-Gabriel-Welsch multiple *F*-test)

baseline concentration on day 4 argues in favour of a systematic variation. We therefore believe our observations suggest that in populations similar to ours, AAG concentrations are higher during the first week of the menstrual cycle than at other times.

The alteration of the binding of specific drugs as a result of these changes has not been studied. It would be expected that a decrease in available binding protein would reduce the extent of drug-protein binding, with important increases in free drug fraction only for highly bound drugs. Alterations in protein binding affect steady-state unbound drug concentration, volume of distribution, and drug half-life in a complex, non-linear fashion that depends on hepatic extraction ratio and baseline pharmacokinetic parameters (Wilkinson & Shand, 1975). Specifically, highly bound drugs with high extraction ratios (e.g., verapamil, tricyclic antidepressants) would be expected to have increased volume of distribution, increased free fraction and increased biologic half-life (particularly for drugs with large volumes of distribution). Drugs with low extraction ratios would be expected to display decreased half-life if the drug has high tissue distribution but little change if tissue distribution is high. Although our findings should be considered preliminary because of the small sample size and limited duration of observation, we believe that we have shown the potential for variability in drug protein binding for drugs extensively bound to AAG and further study of individual drugs is warranted.

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