Endotoxin depresses hepatic cytochrome P450-mediated drug metabolism in women

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Aims In men, the inflammatory response to intravenous endotoxin depresses apparent oral clearances of antipyrine, hexobarbitone, and theophylline. The aim of this study was to investigate whether there might be gender differences in the regulation of hepatic cytochromes P450.

Methods Experiments were carried out in seven healthy women volunteers (ages 19–51, median 22 years). Each woman received a cocktail of the three drugs on two occassions, once after a saline injection and again after endotoxin.

Results Endotoxin injections, but not saline, caused the expected physiologic responses of inflammation including fever and increases in circulating tumor necrosis factor-α, interleukin-6, and C-reactive protein. When compared with the saline control studies, endotoxin significantly decreased clearances of all probes: antipyrine, 31% (95%CI 21%–41%); hexobarbitone, 20% (95%CI 10–31%); and theophylline, 20% (95%CI 10%–30%). The decreases were comparable with those found in the men previously studied (35%, 27%, and 22%, respectively).

Conclusions These data show that endotoxin-induced inflammation decreases hepatic cytochrome P450-mediated metabolism of selected probe drugs in women as it does in men.

Keywords: antipyrine, cytochrome P450, drug metabolism, endotoxin, gender, hexobarbitone, inflammation, theophylline

Introduction

TGram-negative bacterial sepsis is still a leading cause of death in the intensive care setting [1, 2] and both men and women are susceptible to the sequelae of sepsis. A preliminary study [3] of drugs given to these patients revealed that a mean (±s.d.) of 12.1±3.9 systemically active xenobiotic drugs are administered during the period of sepsis. Animal models of sepsis using endotoxin (lipopolysaccharide or LPS) to elicit the inflammatory response [4–8] and administration of inflammatory cytokines such as interleukin-1, tumor necrosis factor, and interleukin-6 [7, 9–14] have demonstrated significant decreases in hepatic cytochrome P450-mediated drug metabolism. Therefore, it is likely that septic patients who have high levels of inflammatory cytokine production have depressed hepatic P450 activities which could be clinically significant. Our group recently addressed the question of whether inflammation significantly depresses hepatic P450-mediated drug metabolism in humans by studying affects of LPS administration in men [15]. The clearances of the probe drugs antipyrine (AP), hexobarbitone (HB), and theophylline (TH), ingested as a 'cocktail' [16, 17], were decreased by 35%, 27%, and 22%, respectively, after the male volunteers received two consecutive doses of LPS.

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Gender differences in the metabolism of a number of drugs have been reported [(18–21)]. Differences for the most part were small, most medications appeared to be metabolized similarly in men and women, and a recent report [22] saw no gender differences in amounts of specific P450 proteins in samples of normal liver. However, whether there are important gender differences in the way inflammation affects regulation of P450s has not yet been investigated. To address this question, the current study administered AP, HB, and TH drug probe cocktails to female volunteers and assessed the effects of two consecutive LPS injections on expression of the acute phase response and on clearances of the drug probes.

Methods

Nine female volunteers aged 21–51 years participated in this study. Two were excluded as explained below. The study was approved both by the University of Kentucky Institutional Review Board and the General Clinical Research Center. The protocol was identical to the previously reported study using male volunteers [15], except that each clearance study was performed within the first 10 days (follicular phase) of each woman's menstrual cycle and immediately prior to each study a plasma β-HCG pregnancy test was negative. At enrolment, women were deemed to be healthy by medical history, physical examination, blood chemistry, urinalysis and ECG, and written consent was obtained. All were non-smokers and ingested no caffeine,

theophylline products, non-steroidal anti-inflammatory drugs or other medications for at least 3 days prior to the study.

Each woman underwent two separate clearance studies: one with saline as a control and the other with LPS treatment. Subjects were not randomized formally to have the saline or LPS clearance study first, but chose which would be their first study based on their schedules. For each clearance study, AP (250 mg), HB (500 mg), and TH (150 mg) were given as a 'cocktail' [16, 17] after an overnight fast. For the LPS drug clearance study, each woman received two i.v. doses of U.S. FDA Bureau of Biologics endotoxin (LPS) (Lot EC-5, 20 Endotoxin units kg^{-1}) on consecutive mornings. Saline and LPS injections were given at 08.00 h and the drug cocktail at 08.30 h after the saline or the second dose of LPS. Blood was collected at 0, 10, 20, 30, 45 min and 1, 2, 3, 4, 6, 9, 12, 30, and 48 h after the drug cocktail for plasma drug concentrations and at various time points for plasma TNFα, IL-6, and serum C-reactive protein determinations. Blood samples for complete blood count and clinical chemistry screens were collected 48 h after the second dose of LPS for comparison with baseline samples collected at enrolment.

Plasma concentrations of the cytokines tumor necrosis factor-α (TNF), and interleukin-6 (IL-6) were determined by commercial kits (Quantikine, R&D Systems, Minneapolis, MN.) and the acute phase reactant C-reactive protein (C-RP) was determined in serum using nephelometry [23]. The TNF, IL-6, and C-reactive protein data were published recently in another report [24] combined with the results from the men volunteers [15]. Plasma AP, HB, and TH were analyzed as described previously [25].

AP, HB, and TH plasma concentration *vs* time data for each clearance study were analyzed by a non-linear least squares regression analysis program (R-STRIP®, Micromath Scientific Software, Salt Lake City, Utah). Area under the concentration-time curve (AUC) was calculated by the trapezoidal rule from 0 to the last time point and then extrapolated to infinity using the following formula: C_{last}/λ_z where C_{last} is the last measured concentration time point and λ_z is the terminal elimination rate constant obtained from R-STRIP. Apparent oral clearance (CL_o) of drug was defined as: $CL_o = ORAL\ DOSE/AUC$ and calculated as such. In all cases, the extrapolated AUC (C_{last}/z) represented less than 20% of the total AUC utilized in the calculations of CL_o.

Statistical analysis

It was assumed that a 30% change in drug clearance would be clinically significant and a power analysis was performed using the known intraindividual coefficient of variation (6.8–8.2%) from previous clearance studies for antipyrine [26] and determined that an $n=6$ would be sufficient to detect this change. Although six women were initially enrolled, one subject refused the second dose of LPS and was excluded from analysis. Another subject had an unexpected 55% increase in TH clearance and a 164% increase in HB clearance. (The inclusion of these subjects in a preliminary report [27] led to the suggestion that women might have 'less inhibition than men of P450-mediated drug metabolism' with inflammation.) However, after studying three more women, the subject with the increases was the only one noted to have any increases in clearances after LPS injections. She would not consent to being studied further. Using Rosner's Extreme Studentized Deviate Test [28], her results for the effects of LPS on TH and HB clearances were calculated to be outliers. Therefore, all of her data were excluded from the final analysis. The data presented in this report are from the seven remaining women (median age 22, range 19 to 51 years), four of whom had the LPS clearance study first. Analysis for possible crossover effects found no evidence of an inequality for any of the endpoints.

A statistical package [Pharmaceutical Calculation System, Springer-Verlag, NY] was used for data calculations. Apparent oral clearance data between groups (saline control and LPS) were analyzed by two-tailed paired *t*-tests using standard techniques [29]. Confidence intervals for the changes in clearances were determined at the 95% level. Changes in peak serum cytokines, peak serum acute phase proteins, bilirubin, albumin, white blood cell count, liver enzymes, temperature, and heart rate, were analyzed by paired Student's *t*-test. The relationship between alterations in clearances caused by LPS with changes in plasma TNF, plasma IL-6 and serum C-reactive proteins were correlated to assess a statistical association using regression analysis and *F*-test. Statistical significance was defined as $P<0.05$ for all tests.

Results

LPS administration to the women subjects caused the expected physical signs consistent with inflammation. Each woman reported onset of chills, headache, malaise, and very mild nausea beginning after approximately 1 h and lasting approximately 2 h. Statistically significant ($P<0.05$) peak elevations in temperature from 97.8 ± 0.1 to 100.4 ± 0.2°F and pulse rates from 62 ± 3 to 85 ± 6 beats min^{-1} were seen 2.5 h after the first LPS injection with similar responses after the second LPS dose. No such symptoms or physical signs were seen after the saline injection. The LPS did not lead to changes in platelet counts (314 ± 53 to 280 ± 40 X$10^3/\mu l$), liver alanine aminotransferase (18 ± 2 to 15 ± 2 iu l^{-1}), alkaline phosphatase (58 ± 4 to 52 ± 3 iu l^{-1}), or total bilirubin (0.7 ± 0.2 to 0.4 ± 0.1 mg 100 μl^{-1}) when these parameters were assessed 48 h after the second LPS dose and compared with baseline values obtained at recruitment. However there were minor but significant ($P<0.05$) changes in white blood cell count (5.8 ± 0.5 to 5.2 ± 0.1 $\times 10^3$ μl^{-1}), haematocrit (39.6 ± 1.1 to 34.9 ± 0.6), and albumin values (4.6 ± 0.1 to 4.1 ± 0.1 g 100 ml^{-1}), probably related to the inflammatory process and/or the amount of blood removed for the protocol.

The expected changes in the plasma cytokines IL-6 and TNF and the serum acute phase C-reactive protein were seen after each dose of LPS. A plasma TNF peak of 67.9 ± 17.9 units ml^{-1} (mean $\pm$ s.e. mean) occurred at 1.5 h after the first LPS dose and promptly returned to undetectable baseline concentrations by 4 h. A smaller peak of 28.3 ± 9.3 occurred 1.5 h after the second dose of LPS. A plasma IL-6 peak of 74.2 ± 34.7 units ml^{-1} occurred at 1.5 h after the

first LPS dose and remained elevated (67.1 ± 11.4) at 4.5 h before returning to a baseline of 6–10 by 6.5 h. After the second LPS dose, a smaller IL-6 peak of 59.4 ± 23.7 was seen at 1.5 h, remaining elevated (39.0 ± 10.2) at 4.5 h, and returning to baseline by 6.5 h. Marked increases in C-reactive protein were seen from a baseline concentration of 0.14 ± 0.04 mg 100 ml^{-1} to 2.80 ± 0.63 at 24 h and 3.37 ± 0.51 at 48 h.

Figure 1 shows the changes in clearances for each of the three drug probes for each subject. In all except one subject, for each probe there was a decrease in clearance found after LPS. In one subject, there was no decrease in TH clearance (2.166 to 2.163 l h^{-1}). The mean decrease in clearance for AP was 31% (95% confidence interval 21%-41%), for HB 20% (95% confidence interval 10%–31%), and for TH 20% (95% confidence interval 10%–30%). These decreases in clearances were all statistically significant. When changes in AP clearances for individual women were correlated with peak TNF and IL-6 plasma responses after the first LPS dose as an indication of the 'intensity' of the inflammatory response, there were only weak, non-statistically significant correlations found with $r = 0.71$ for IL-6 and $r = 0.54$ for TNF (Figure 2).

Discussion

The above findings show that women volunteers have a similar response to administered endotoxin (LPS) as do men volunteers [15] with respect to decreases in activities of the hepatic cytochromes P450 responsible for the clearances of AP, HB, and TH. The 20%-30% changes seen probably underestimate what occurs in acutely ill septic patients who have a much more intense inflammatory response and multisystem organ failure. Because these studies were not prospectively designed to rule out gender differences in hepatic P450 responses to inflammation, it is possible that subtle differences may exist and might be more pronounced with clinically severe inflammation. However, since the findings in women are so much like those in men, it is likely that female septic patients are just as vulnerable to inhibition of hepatic P450-mediated drug metabolism as male patients.

A limitation of the current study is that the clearances of the three drug probes reflect mainly activities of only some hepatic P450s; namely, CYP1A2 (AP and TH), CYP2C isoforms (AP and HB), and to a very limited extent CYP3A (AP) [30–32]. *In vivo* inflammatory effects on other important human P450s have not yet been studied. Data from whole animal studies [12, 13] and from cultured human hepatocytes [33–35] have analyzed effects of inflammatory cytokines on individual P450 isoforms. All these studies suggest that although inflammatory mediators generally depress P450 isoforms, a great deal of variability exists. As an example, Nadin *et al.* [13] showed that TNF given to male rats depressed CYPs 2C11 and 3A2 mRNA and proteins, but that CYPs 2A1 and 2C6 were 'refractory'. Because of the potential clinical importance of these changes for septic patients who are likely to receive medications requiring metabolism by various P450 isoforms [3], *in vivo* human studies should be performed assessing inflammatory effects on activities of all the important P450s.

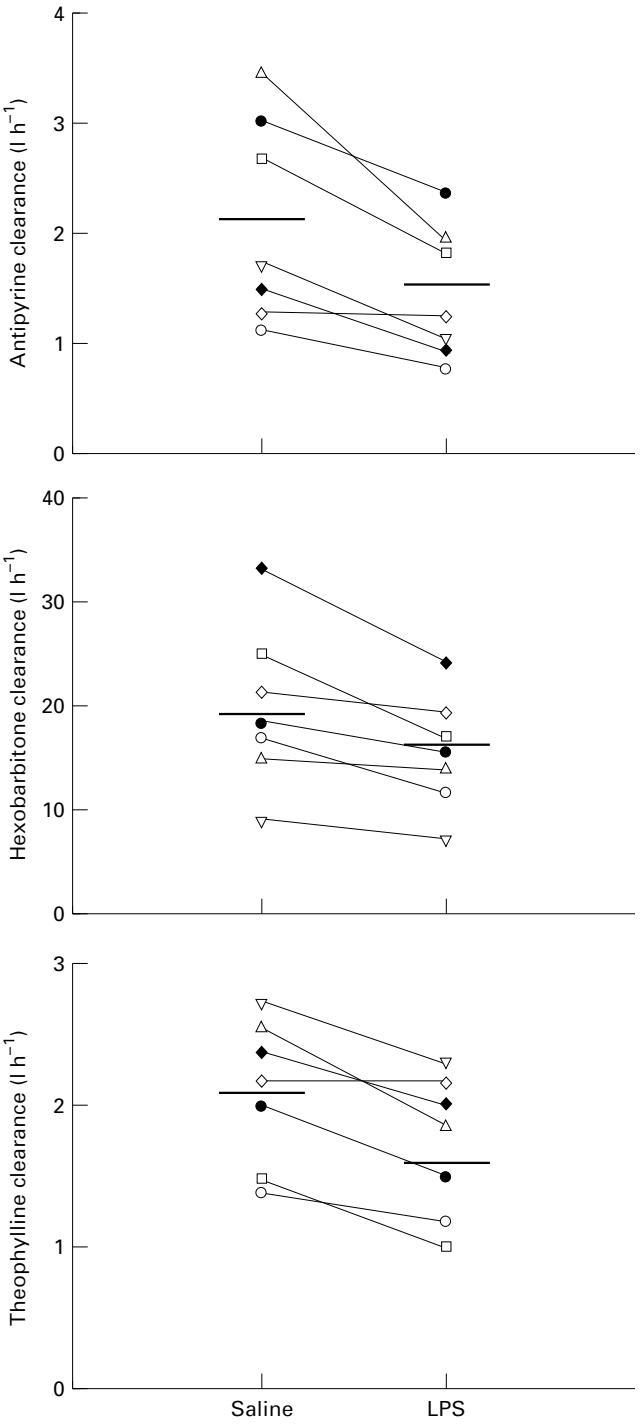

Figure 1 Oral drug clearances of antipyrine (250 mg), hexobarbitone (500 mg), and theophylline (150 mg) administered as a 'cocktail' in seven female subjects given on one occasion saline i.v. 0.5 h prior to the drugs and on another occasion LPS (20 endotoxin units i.v.) 24.5 h and 0.5 h prior to the drugs. The symbols identify data for individual subjects for each drug clearance comparison and are consistent between graphs. The bar is the arithmetic mean for clearance data. Means $\pm$s.e. mean of baseline and post-LPS clearances were for antipyrine 2.13 ± 0.34 and 1.44 ± 0.22 l h^{-1} respectively, for hexobarbitone 19.8 ± 2.9 and 15.6 ± 2.1 l h^{-1}, and for theophylline 2.12 ± 0.19 and 1.71 ± 0.19 l h^{-1}.

Several recent reviews of the effects of gender on drug metabolism [18–21] have concluded that there may be gender differences in humans, but that these differences

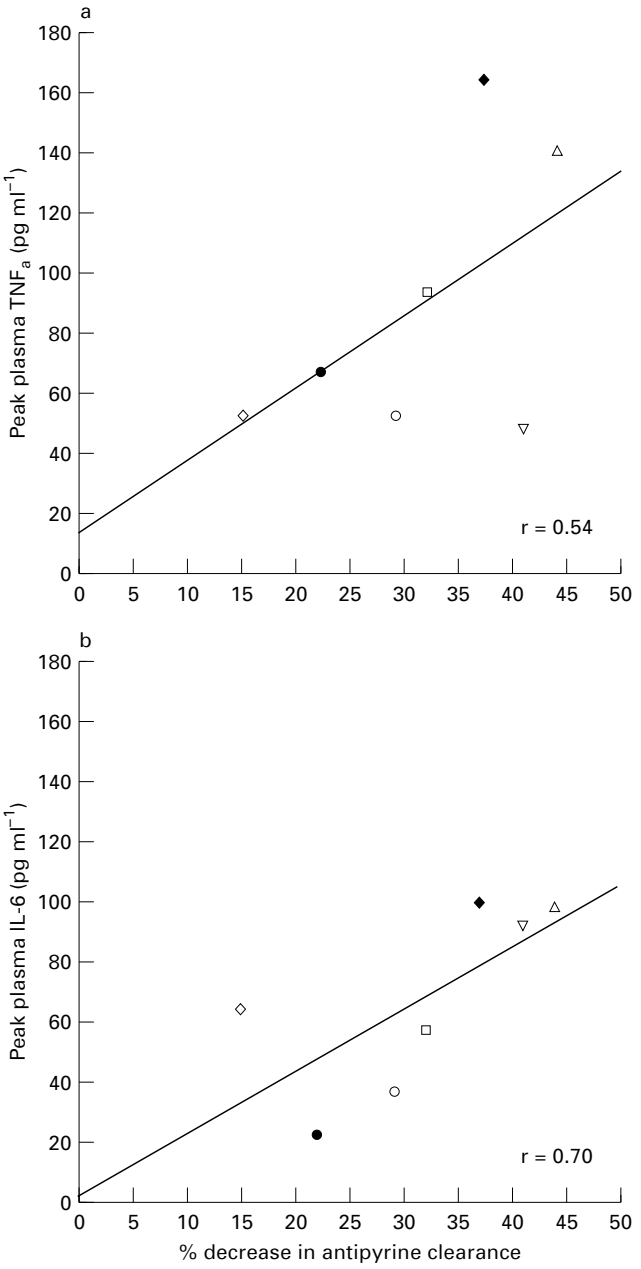

Figure 2 Regression analyses comparing the changes (%decreases after LPS) in antipyrine clearances and a) interleukin-6 (IL-6) plasma concentrations (pg ml^{-1}) or b) the peak tumor necrosis factor-α (TNF) after the first dose of LPS in each subject. For IL-6, $r=0.705$ and for TNF $r=0.538$, neither of which were statistically significant.

must be subtle. George *et al.* [22] did not find gender-related differences in concentrations of CYPs 1A2, 2C, 2E1, and 3A proteins in human livers. The effects of gender on clearances of drug probes similar to those used in the current study also have been reported [36–42]. No gender effects on clearance were seen for AP [36], although oral contraceptive use depressed clearance. Studies using caffeine as a probe for 1A2 reported both decreased activity in nonsmoking women [37] and no gender difference [38]. Studies of TH metabolism, also reflecting primarily 1A2 activity [29], showed increased clearances in nonsmoking women [39] or no gender differences [40]. An explanation for these conflicting findings might be that timing within the menstrual cycle was only considered in one study [40] and TH clearance decreases by about 30% from the beginning of the cycle (follicular phase) to the end of the cycle (luteal phase) [41]. The current study controlled for this variation by assessing women within the first 10 days of their cycle and none of the women were taking oral contraceptives.

With regard to HB, a report [42] that young women cleared R-mephobarbitone much slower than young men (3.5-fold slower!) suggested significant gender differences in the activity of CYP2C19. However, 4/8 of the women were on oral contraceptives which might have depressed clearances, and 2/8 of the men smoked, which might have increased their clearances. Data from the current study suggest that control HB clearances in the women of 19.7 ± 2.9 l h^{-1} (mean ± s.e. mean) were not different from the HB clearances of the men (15.8 ± 4.4 l h^{-1}) in the earlier study [15].

The well known gender-related differences in P450 expression due to growth hormone were recently reviewed [21]. Certainly in rodents, expression of particular P450 isoforms such as the rat CYP2C11 (male) and 2C12 (female) are related to concentration profiles of plasma growth hormone. Whether any human P450 isoforms are so regulated is not known. Morgan's studies [43] suggest that inflammatory changes in P450s are probably independent of changes in growth hormone. In human volunteer studies, administration of LPS causes a prompt rise in growth hormone that peaks in 3h [44]. However, severely injured patients tend to have profound decreases in growth hormone [45]. Whether changes in growth hormone secretion during inflammation contributes to a gender-related difference in human P450 expression seems unlikely, but was not addressed in the current studies.

Some *in vitro* data suggest there may be gender-related differences in the inflammatory response [46]. However, the women subjects in the current study had the same clinical, cytokine, and acute phase protein responses as the men previously reported [15]. That there were also comparable decreases in drug probe clearances suggests that the intensity of the systemic and hepatic acute phase response correlates with the extent of P450 depression. For the women subjects, the lack of statistical significance (Figure 2) for correlation between extent of depression of AP clearance and the peak IL-6 and TNF responses probably reflects the small number of subjects and variability of the cytokine measurements.

In conclusion, this study demonstrates that inflammation induced by gram-negative bacterial endotoxin causes depression of cytochrome P450-mediated drug metabolism of the drug probes AP, HB, and TH in healthy women volunteers. Inflammatory depression of hepatic drug metabolism does not appear to be affected by gender, at least for the P450 isoforms assessed by these drug probes, mainly CYPs 1A2 and 2C19. Similar studies should be performed in men and women using probes for other important P450 isoforms.

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