Cytochrome P450 3A4 activity after surgical stress

Curtis E. Haas, PharmD; David C. Kaufman, MD; Carolyn E. Jones, MD; Aaron H. Burstein, PharmD; William Reiss, PharmD

Objective: **To evaluate the relationship between the acute inflammatory response after surgical trauma and changes in hepatic cytochrome P450 3A4 activity, compare changes in cytochrome P450 3A4 activity after procedures with varying degrees of surgical stress, and to explore the time course of any potential drug-cytokine interaction after surgery.**

Design: **Prospective, open-label study with each patient serving as his or her own control.**

Setting: **University-affiliated, acute care, general hospital.**

Patients: **A total of 16 patients scheduled for elective repair of an abdominal aortic aneurysm (n = 5), complete or partial colectomy (n = 6), or peripheral vascular surgery with graft (n = 5).**

Interventions: **Cytochrome P450 3A4 activity was estimated using the carbon-14 [^{14}C]erythromycin breath test (ERMBT) before surgery and 24, 48, and 72 hrs after surgery. Abdominal aortic aneurysm and colectomy patients also had an ERMBT performed at discharge. Blood samples were obtained before surgery, immediately after surgery, and 6, 24, 32, 48, and 72 hrs after surgery for determination of plasma concentrations of interleukin-6, interleukin-1β, and tumor necrosis factor-α. Clinical markers of surgical stress that were collected included duration of surgery, estimated blood loss, and volume of fluids administered in the operating room.**

Measurements and Main Results: **ERMBT results significantly declined in all three surgical groups, with the lowest value at the time of the 72-hr study in all three groups. There was a trend toward differences in ERMBT results among groups that did not reach statistical significance ($p = .06$). The nadir ERMBT result was significantly and negatively correlated with both peak interleukin-6 concentration ($r_s = -.541$, $p = .03$) and log interleukin-6 area under the curve from 0 to 72 hrs ($r_s = -.597$, $p = .014$). Subjects with a peak interleukin-6 of >100 pg/mL had a significantly lower nadir ERMBT compared with subjects with a peak interleukin-6 of <100 pg/mL (35.5% ± 5.2% vs. 74.7% ± 5.1%, $p < .001$).**

Conclusions: **Acute inflammation after elective surgery was associated with a significant decline in cytochrome P450 3A4 activity, which is predictive of clinically important changes in the metabolism of commonly used drugs that are substrates for this enzyme. (Crit Care Med 2003; 31:1338–1346)**

Key Words: cytochrome P450; surgical procedures; operative; cytokines; interleukin-6; drug metabolism; erythromycin

The term drug-cytokine interaction has been coined to describe the effects of the inflammatory response on drug metabolism (1). Extensive data from *in vitro* and animal models, and from limited data from humans, indicate that the inflammatory response to infection and tissue injury can cause significant reductions in hepatic drug metabolism by the cytochrome P450 (CYP) system (1–3). Although many mechanisms have been proposed, the predominant mechanism seems to be down-regulation of CYP gene expression. Of the inflammatory mediators, the proinflammatory cytokines interleukin (IL)-1 (IL-1α and IL-1β), IL-6, and tumor necrosis factor-α (TNFα) have been the most extensively studied and seem to play the most important role in the down-regulation of CYP activity (1–3). Clinical trials involving the administration of exogenous cytokines for the treatment of cancer or viral infections (4–11), administration of Gram-negative endotoxin to healthy volunteers (12, 13), and the study of patients with an acute febrile illness (14, 15) have also demonstrated significant reductions in hepatic drug metabolism or CYP enzyme activity.

The CYP enzymes are a large and diverse group of heme-containing enzymes responsible for the metabolism of many endogenous substances and the detoxification of exogenous compounds, including many therapeutic drugs. CYP3A4 is the most abundantly expressed CYP enzyme in the liver and gastrointestinal tract, and it is responsible for the metabolism of >150 drugs belonging to 38 therapeutic classes (16). Examples of common substrates for CYP3A4 include nifedipine, diltiazem, erythromycin, lidocaine, midazolam, quinidine, corticosteroids, carbamazepine, fentanyl, cyclosporine, lovastatin, cisapride, and terfenadine (16, 17). The hepatic content and activity of CYP3A4 is strongly influenced by environmental factors and, therefore, is susceptible to inducers and inhibitors of enzyme activity. The clinical relevance and inducible characteristics of CYP3A4 make it an excellent candidate enzyme for studies of the importance of drug-cytokine interactions.

The measurement of CYP3A4 activity *in vivo* is performed by administering an appropriate probe compound that is a selective substrate for the isoenzyme (18). An ideal probe substrate should be selec-

From the School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, The State University of New York, Buffalo, NY (CEH); the Department of Surgery, University of Rochester Medical Center, Rochester, NY (DCK, CEJ); the School of Medicine and Dentistry, University of Rochester, Rochester, NY (DCK, CEJ); the Clinical Pharmacokinetics Research Laboratory, Clinical Center Pharmacy Department, National Institutes of Health, Bethesda, MD (AHB); and the Clinical Development Department, Immunex Corporation, Seattle, WA (WR).

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tive for the enzyme being studied, be sensitive to changes in enzyme content or activity, require minimally invasive sampling, be nontoxic, and not directly affect the activity of the enzyme. The activity of the CYP3A4 isoenzyme can be estimated using the carbon-14 [^{14}C]erythromycin breath test (ERMBT), which utilizes a trace dose of intravenous [^{14}C-*N*-methyl-]erythromycin (18, 19). The *N*-demethylation of erythromycin is catalyzed by CYP3A4, and the appearance rate of $^{14}CO_2$ in the breath has been correlated with hepatic CYP3A4 content (19, 20). In addition, ERMBT results correlate with the clearance of known CYP3A4 substrates (21–23), increase with the administration of known inducers (22), and decrease after administration of known inhibitors (22, 24–28). We chose to use the ERMBT for this study because it is minimally invasive, well tolerated, and can be easily performed on multiple occasions to detect serial changes in activity over a short time period.

The hormonal, metabolic, hematologic, and immunologic response after elective surgery is collectively referred to as the stress response to surgery and is similar in nature, although usually of more moderate magnitude, to the response to other causes of physiologic stress, including severe infection and trauma. Therefore, it has been suggested that elective surgery provides an excellent model for studying the response to injury including cytokine activation and regulation (29, 30). Elective surgery, a form of programmed trauma, provides a unique opportunity to evaluate the effects of the inflammatory response on the activity of drug-metabolizing enzymes. A baseline assessment of enzyme activity can be completed before the onset of physiologic stress, the exact time of tissue injury is known, and follow-up measurements of inflammatory markers and enzyme activity can be performed at specific time points after injury. Also, by enrolling patients with varying degrees of surgical stress, the nature of the relationship between the magnitude of the inflammatory response and alterations in hepatic drug metabolism can be explored.

The objectives of this study were to evaluate the relationship between the acute inflammatory response to surgical trauma and changes in hepatic CYP3A4 activity, compare changes in CYP3A4 activity after procedures with varying degrees of surgical stress, and to explore the time course of any potential drug-cytokine interaction after surgery.

MATERIALS AND METHODS

Subjects. This study enrolled three groups of subjects scheduled for elective surgery. To be eligible for participation, subjects had to be scheduled for repair of an abdominal aortic aneurysm (abdominal aortic or aortobifemoral graft), complete or partial colectomy, or peripheral vascular surgery with graft. Subjects were excluded from study participation for a history or laboratory evidence of liver disease (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, or lactate dehydrogenase greater than two times the upper limit of normal) or a history or laboratory evidence of renal disease (serum creatinine of >2.0 mg/dL [177 μmol/L] for men or >1.8 mg/dL [159 μmol/L] for women). Subjects with a history of alcohol or substance abuse, defined as daily consumption of three or more alcoholic beverages or the chronic use of illicit drugs in the previous 5 yrs, were not eligible for study participation. Subjects consuming drugs known to inhibit or induce the expression of CYP3A enzymes were also excluded. Patients with a history of a confirmed autoimmune disease (e.g., rheumatoid arthritis, systemic lupus erythematosus) or allergy to erythromycin were ineligible for participation. For the aortic surgery group, a procedure that was anticipated to require aortic cross-clamping above the celiac artery excluded the subject due to altered hepatic blood flow during the procedure. If there was an unplanned need to cross-clamp the aorta above the celiac artery, the subject was discontinued from further study participation. The study protocol and consent form were approved by the local institutional review board and radiation safety committee, and all subjects provided written informed consent before any study-related procedures were completed.

Study Protocol. Subjects underwent complete medical history, physical exam, and laboratory studies, including a chemistry profile and a complete blood count, as part of the usual preoperative evaluation. The aortic surgery subjects were admitted to the hospital the day before their surgery, and the other groups were admitted the morning of surgery. The baseline (preoperative) ERMBT was completed the day before surgery for the aortic surgery group and the morning of admission for the colon and peripheral vascular surgery groups. An intravenous dose of 3 μCi of [^{14}C-*N*-methyl]erythromycin (Metabolic Solutions, Nashua, NH) was injected over 1 min. Using the breath collection kit provided by the manufacturer, a breath sample was collected from the subject 20 mins after completion of the labeled erythromycin injection.

Blood samples for determination of cytokine concentrations were obtained just before the baseline ERMBT. For the aortic surgery group, an additional blood sample was obtained the next morning just before the surgical procedure. The blood samples were collected using EDTA-containing tubes, immediately placed on ice, centrifuged at 4°C for 15 mins at 3000 rpm, the plasma harvested, divided into two equal volume aliquots using pyrogen-free tubes, and immediately frozen at −70°C until the time of assay.

The subject then proceeded to his or her scheduled operative procedure. Additional blood samples for cytokine concentrations were collected as early as possible after and 6 hrs after completion of surgery. Blood samples were also collected at 24, 32, 48, and 72 hrs after the start of the surgical procedure. The plasma was harvested and stored as described above.

Other clinical markers of surgical stress that were collected from the operative record of each subject included the duration of the operation, estimated blood loss, and volume of fluids administered in the operating room. At 8 am on the first, second, and third postoperative days, approximately 24, 48, and 72 hrs after surgery, repeat ERMBTs were conducted as described above. To control for the possibility of a carry-over effect from the previous dose of ^{14}C-erythromycin, a breath sample was collected just before each ERMBT.

For the aortic aneurysm and colon surgery groups, a final ERMBT was conducted on the morning of hospital discharge or on postoperative day 10, whichever occurred first. A blood sample was also obtained at this time for determination of cytokine concentrations. The peripheral vascular surgery subjects were typically discharged soon after the 72-hr ERMBT.

Measurement of $^{14}CO_2$ in Exhaled Breath. Breath samples were immediately shipped to Metabolic Solutions, where $^{14}CO_2$ content was measured using standardized procedures. Measurements were performed using a Tri-Carb 2100TR liquid scintillation counter (Packard Instrument Company, Meriden, CT). The accuracy and inter-run and intra-run precision for high and low controls all had a coefficient of variation of <0.5%. The percentage of the erythromycin dose demethylated in the first hour was calculated using a validated method (21, 31). The results of the 24-, 48-, and 72-hr measurements of $^{14}CO_2$ were corrected for the predose $^{14}CO_2$ content.

Cytokine Assays. Plasma concentrations of TNF-α, IL-6, and IL-1β were determined in duplicate using commercially available enzyme-linked immunoassay kits (Quantikine, R&D Systems, Minneapolis, MN). The standard curves ranged from 15.6 to 1000 pg/mL for TNF-α, 3.13 to 300 pg/mL for IL-6, and 3.9 to 250 pg/mL for IL-1β. All cytokine assays were completed according to the manufacturer's instructions.

Cytokine Exposure. The peak plasma concentration of each cytokine was determined by visual inspection of the data. The area under the curve from 0 to 72 hrs ($AUC_{0-72\ hrs}$) for each of the cytokines was calculated using the

linear trapezoidal rule. The peak and AUC values were used as measures of relative exposure of the subject to each cytokine.

Statistical Analysis. The results of the ERMBT, as a percentage of the baseline value, within each surgical group were compared using a one-way repeated-measures analysis of variance. If the analysis of variance indicated a statistically significant difference, multiple comparisons with the baseline value were completed using Dunnett's test. The nadir ERMBT results for each surgical group were compared using a one-way analysis of variance, with multiple comparisons performed using Tukey's test when indicated.

Peak plasma cytokine concentrations, cytokine $AUC_{0-72\ hrs}$ values, operating room time, estimated blood loss, and fluids administered in the operating room for each surgical group were compared using a one-way analysis of variance, with multiple comparisons performed using Tukey's test when indicated. Potential relationships between peak IL-6 concentration and nadir ERMBT and between IL-6 $AUC_{0-72\ hrs}$ and nadir ERMBT were evaluated using Spearman's rank-order correlation.

The subjects were then divided into two groups based on the peak IL-6 response during the first 72 hrs after surgery, a low peak IL-6 response (<100 pg/mL) and a high peak IL-6 response (>100 pg/mL). The break point was selected based on classification and regression tree analysis implemented using SYSTAT statistical software (version 8.0, SPSS, San Rafeal, CA) (32). The nadir ERMBT result for these two groups were compared using Student's *t*-test. This was a *post hoc* analysis conducted based on the observation of atypical subjects within the individual surgical groups.

All statistical tests, with the exception of classification and regression tree, were conducted using SigmaStat statistical software (version 2.03, SPSS), with $p < .05$ considered statistically significant.

RESULTS

A total of 17 subjects completed the study procedures: six subjects undergoing an abdominal aortic or aortobifemoral bypass graft (AAA group), six subjects undergoing a colon resection (colon group), and five subjects undergoing a peripheral vascular bypass graft of the lower limbs (PVD group). One subject in the AAA group was not included in the data analysis because he received intravenous diltiazem just before the 48-hr ERMBT. A second subject in the AAA group received intravenous diltiazem before the 72-hr ERMBT; however, this subject had a nadir ERMBT result at the 24-hr test, so his data were edited after the 48-hr ERMBT. Both subjects received diltiazem for the treatment of atrial fibrillation with a rapid ventricular response. Table 1 summarizes the demographic data for the three study groups.

The ERMBT results, as a percentage of the preoperative result, declined significantly in all three groups, with the lowest average value at the time of the 72-hr study in all three groups (Fig. 1). Differences in the 72-hr ERMBT results between surgical groups did not achieve statistical significance ($p = .096$). Average (±SEM) nadir ERMBT results were 34.1% ± 7.3% for the AAA group, 48.2% ± 10.9% for the colon group, and 66.8% ± 4.2% for the PVD group. There was a trend toward a difference in nadir ERMBT between groups that did not reach statistical significance ($p = .06$). One subject in the colon group had a response markedly different from the other five subjects (peak IL-6, 37 pg/mL; nadir ERMBT, 92.5%). When this subject is excluded from the analysis, the average nadir ERMBT values for the AAA group and colon group (39.3% ± 7.7%) were significantly less than the PVD group ($p < .05$).

Table 2 presents the peak IL-6 and IL-6 $AUC_{0-72\ hrs}$ data for the three groups of subjects. Although both peak IL-6 and $AUC_{0-72\ hrs}$ were higher in the AAA and colon groups compared with the PVD group, the differences did not reach statistical significance due to a high degree of variability in the results. When the atypical colon group subject is excluded from the analysis, the peak IL-6 for the colon group is significantly greater than for the PVD group ($p < .05$). Plasma concentrations of TNF-α and IL-1β were below the level of detection at all time points.

Figure 2 shows the average IL-6 plasma concentrations at each time point for the AAA, colon, and PVD groups. The peak IL-6 was observed at 24 to 32 hrs postoperatively, whereas the lowest average ERMBT was observed at approximately 72 hrs after surgery.

The nadir ERMBT result was significantly and negatively correlated with the peak IL-6 concentration ($r_s = -.541$, $p = .03$) and log IL-6 $AUC_{0-72\ hrs}$ ($r_s = -.597$, $p = .014$) by Spearman's rank-order correlation (Figs. 3 and 4). The clinical markers of surgical stress are presented in Table 3. Operating room time was significantly longer for the AAA group compared with the PVD group, and estimated blood loss and operating room fluids were significantly greater in the AAA group compared with the colon and PVD groups.

In a *post hoc* analysis, subjects were divided into two groups based on postoperative peak IL-6 response, rather than by surgical procedure, to further evaluate any potential relationship between the inflammatory response and ERMBT results. Subjects with a peak IL-6 of >100 pg/mL had a significantly lower nadir ERMBT compared with subjects with a peak IL-6 of <100 pg/mL (35.5% ± 5.2% vs. 74.7% ± 5.1%, respectively, $p < .001$) (Fig. 5).

DISCUSSION

Elective surgery serves as an exemplary inflammatory stimulus to study the effects of acute inflammation in humans. The acute-phase response after elective surgery involves activation of the hematologic, endocrine, sympathetic, inflammatory, and metabolic systems. Surgical trauma leads to elevations in plasma concentrations of catecholamines, cortisol, glucose, and proinflammatory cytokines, which seem to follow a predictable temporal pattern (30, 33). Early and transient rises in IL-1β and TNFα (34, 35) are

Table 1. Subject demographics[a]

Group	Male/Female	Age, yrs[b]	Preoperative Weight, kg[b]	Height, cm[b]	ASA[c]
AAA (n = 5)	4/1	70.2 (4.3)	81.7 (24.9)	168.6 (13.9)	3 (3)
Colon (n = 6)	5/1	68 (15.3)	83.8 (16.6)	171.8 (9.8)	2.5 (2–3)
PVD (n = 5)	2/3	60.2 (11.8)	69.6 (18.5)	167.6 (15.3)	2 (2–3)

ASA, American Society of Anesthesiology Classification.
[a]p = not significant for all variables; [b]mean (SD); [c]median (range).

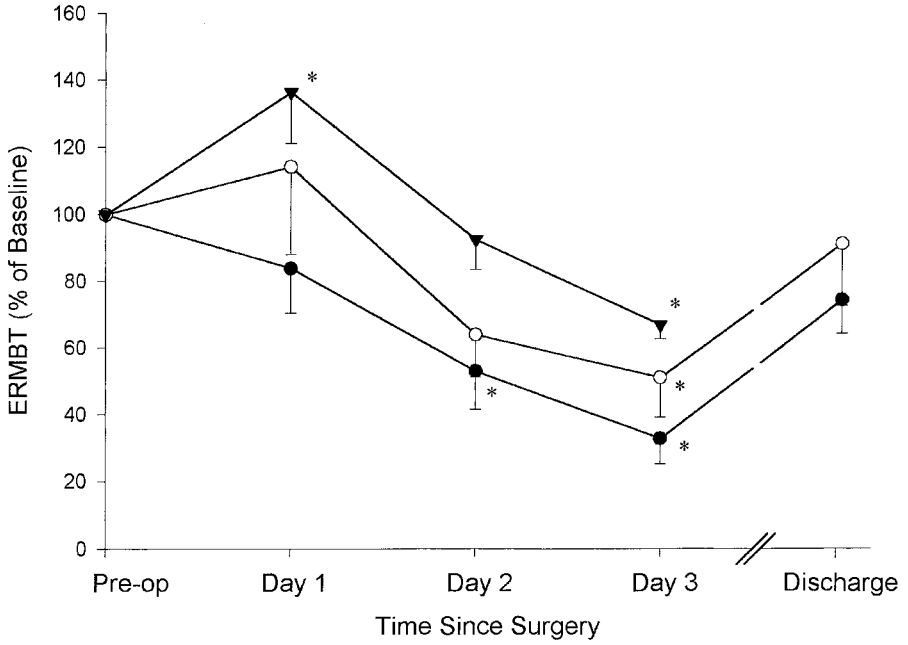


Figure 1. Mean (± SEM) carbon-14 [^{14}C]erythromycin breath test (*ERMBT*) results (percentage of baseline) for the abdominal aortic aneurysm group (*closed circle*), colon group (*open circle*), and peripheral vascular surgery with graft group (*triangle*). *$p < .05$ compared with baseline within the surgical group. SEM was calculated and reported as a two-sided test but is presented as *one-sided bars* in the figure to improve readability.

Table 2. Interleukin (IL)-6 plasma concentration data

Group	Peak IL-6, pg/mL[a,b]	IL-6 $AUC_{0-72\ hrs}$, $pg \cdot h^{-1} \cdot mL^{-1}$[a,c]
AAA (n = 5)	275.2 (34.0)	9187 (1151)
Colon (n = 6)	242 (84.3)	8843 (3406)
PVD (n = 5)	85.8 (25.6)	3705 (961)

$AUC_{0-72\ hrs}$, area under the plasma concentration vs. time curve for 0–72 hrs; AAA, abdominal aortic aneurysm; PVD, peripheral vascular surgery with graft.

[a]Mean (SEM); [b]$p = .076$; [c]$p = .192$.

followed by later and more sustained elevations in plasma IL-6 and cortisol concentrations (29, 30, 33–37). Elevations in circulating catecholamines and blood glucose concentrations occur shortly after surgery, with peak effects typically seen about 4 to 8 hrs after the procedure (33). The acute-phase proteins, such as C-reactive protein, become elevated late in the postoperative period, with peak effects typically observed at approximately 48 hrs after surgery (30, 33, 34, 36, 37). The severity of the inflammatory response, as measured by plasma IL-6 concentrations, has been positively correlated with the duration and extent of surgical trauma (34, 36, 37).

We observed increased plasma IL-6 concentrations within several hours of the surgical procedure, with peak IL-6 concentrations typically observed 24 to 32 hrs after surgery. Although the early appearance of IL-6 in the plasma is consistent with other reports, peak concentrations have typically been reported earlier, at 8 to 12 hrs after surgery (29, 34–36). However, other investigators have reported peak concentrations at approximately 24 hrs after surgery (33). Because of our sampling schedule, we may have missed the true peak IL-6 concentration. Similar to other reports (36, 37) the peak IL-6 concentration and IL-6 AUC correlated with the duration of surgery (for AUC, $r^2 = 0.46$, $p = .004$, data not shown), suggesting that the magnitude of the IL-6 response was related to the extent of tissue injury. We were unable to detect IL-1β and TNFα in any of the plasma samples from our patients. This is likely due to the transient and low level of expression of these cytokines after major and minor surgery, which is consistent with other studies (29, 30, 34).

Extensive *in vitro* and animal-model data indicate that the proinflammatory cytokines are capable of significantly down-regulating hepatocyte CYP enzyme content and activity, with resulting decreases in oxidative drug metabolism. Available clinical data relative to these proposed drug-cytokine interactions are consistent with the findings from the experimental models. Full discussions of these data, the proposed mechanisms, and the potential clinical implications for these alterations in drug metabolism have been recently reviewed (1–3). We theorize that the decreases in CYP3A4 activity observed in the current study may be due to the acute inflammatory response to elective surgery and represent a potentially clinically relevant drug-cytokine interaction. The time course of change in CYP3A4 activity after surgery, and the apparent relationship between the extent of the acute inflammatory response and the decrease in the ERMBT results, are consistent with this proposal; however, the design of this study cannot establish a cause-and-effect relationship between the increase in circulating IL-6 and changes in CYP3A4 activity.

The nadir in ERMBT results was observed at least 72 hrs after surgery for a majority of the patients participating in this study, whereas the peak IL-6 plasma concentration was observed at 24 to 32 hrs after surgery. The predominant mechanism for decreased CYP activity after exposure to proinflammatory cytokines is a down-regulation of gene expression (2, 3). Due to the indirect mechanism of this interaction, a delay in the reduction of enzyme activity is expected and is thought to be primarily dependent on the half-life of the CYP protein involved.

The administration of Gram-negative endotoxin to healthy male volunteers has been associated with signs of acute inflammation, an increase in plasma concentrations of proinflammatory cytokines, and a decrease in the clearance of antipyrine, hexobarbital, and theophylline, all drugs metabolized by various CYP enzymes (12). The decrease in drug metabolism was delayed for at least 24 hrs after endotoxin administration. Although the study design did not evaluate when maximal decreases in drug metabolism occurred, the decrease in antipyrine clearance was significantly correlated with peak IL-6 and peak TNF-α plasma concentrations (12).

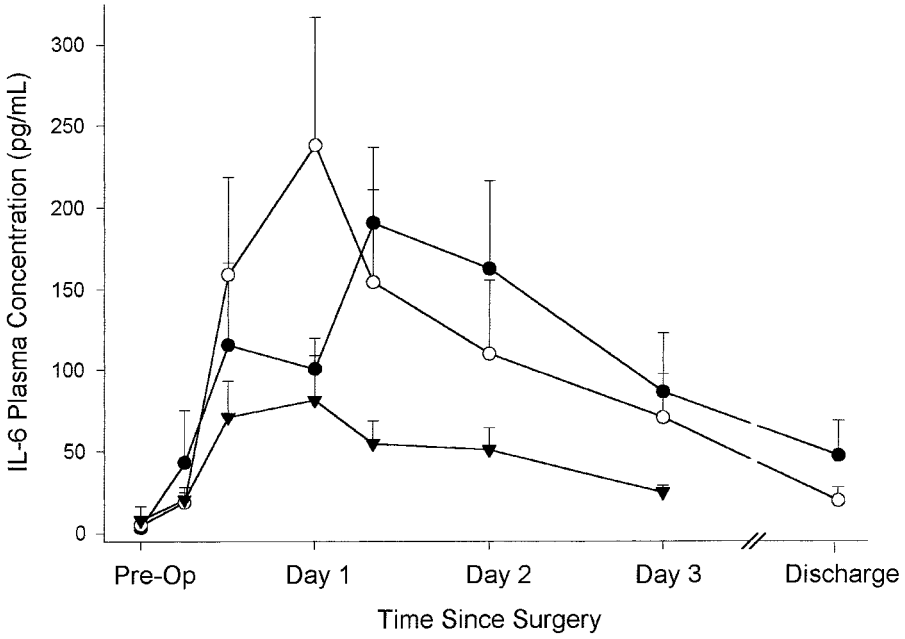


Figure 2. Mean (±SEM) plasma interleukin (*IL*)-6 concentrations for the abdominal aortic aneurysm group (*closed circle*), colon group (*open circle*), and peripheral vascular surgery with graft group (*triangle*). SEM was calculated and reported as a two-sided test but is presented as *one-sided bars* in the figure to improve readability.

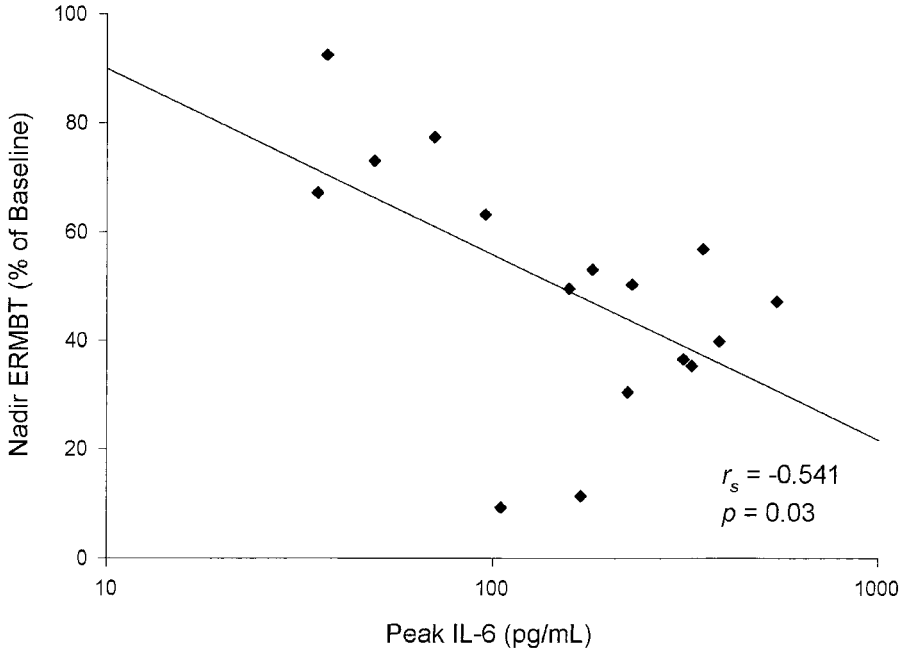


Figure 3. For illustrative purposes, the log-linear plot of peak interleukin (*IL*)-6 and Nadir carbon-14 [^{14}C]erythromycin breath test (*ERMBT*, percentage of baseline) with a simple *linear regression line* shown. The r_s and p values are from Spearman's rank-order correlation analysis.

Chen et al. (38) evaluated the effects of an acute inflammatory response, as measured by serum concentrations of cytokines and acute-phase proteins, on the pharmacokinetics of cyclosporine in six patients undergoing allogeneic bone marrow transplantation. There was a 3.60 ± 0.68–fold increase in dose-normalized cyclosporine blood concentrations an average of 15.8 days after transplantation. This increase in cyclosporine blood concentrations occurred 4.83 ± 0.95 days after the peak IL-6 concentration. The changes in these two variables were correlated (r_s = .794, p = .03), suggesting that the variations in serum IL-6 activity and cyclosporine concentrations were interdependent. The results of this study are consistent with a delayed maximal effect on CYP3A-mediated drug metabolism during acute inflammation that occurs several days after the peak expression of IL-6 activity in the blood (38).

The time course of decreases in CYP3A4 activity in our study is consistent with these previous reports. The maximal decrease in CYP3A4 activity was typically observed 36–48 hrs after the peak IL-6 plasma concentration. Because we performed the last postoperative ERMBT approximately 72 hrs after surgery, it is possible that we did not measure the true nadir in enzyme activity in all patients.

Similar to other investigators (13, 38), we observed a correlation between markers for the severity of the inflammatory response (peak IL-6 concentration and IL-6 $AUC_{0-72\ hrs}$) and decreases in CYP activity (r_s = −.597 and p = .014 for the $AUC_{0-72\ hrs}$ analysis). The strength of this correlation is moderate and may have been affected by several factors inherent to our study design. First, IL-6 plasma concentrations are a good but relatively nonspecific measure of the systemic inflammatory response and may not accurately reflect the inflammatory milieu of the hepatocyte. In addition, because of the limitations on being able to collect multiple samples and perform multiple studies in acutely ill patients, the actual peak IL-6 concentration was probably not measured, and for several patients, the true nadir in ERMBT results may have been missed. The possibility of less than optimal data introduced by the study design likely introduced a considerable degree of uncertainty into the correlation analyses. Despite these limitations, the existence of a correlation between IL-6 plasma concentrations and changes in CYP3A4 activity is apparent (Figs. 3 and 4). This potential relationship is further reinforced by the breakpoint analysis illustrated in Figure 5. The time course for changes in drug metabolism after surgical trauma and the apparent relationship between measures of the inflammatory response and changes in CYP3A4 activity are consistent with our belief that these data represent evidence of a clinically important drug-cytokine interaction.

Other factors that may have contributed to the change in drug metabolism that must be considered include the effects of general anesthesia, alterations in

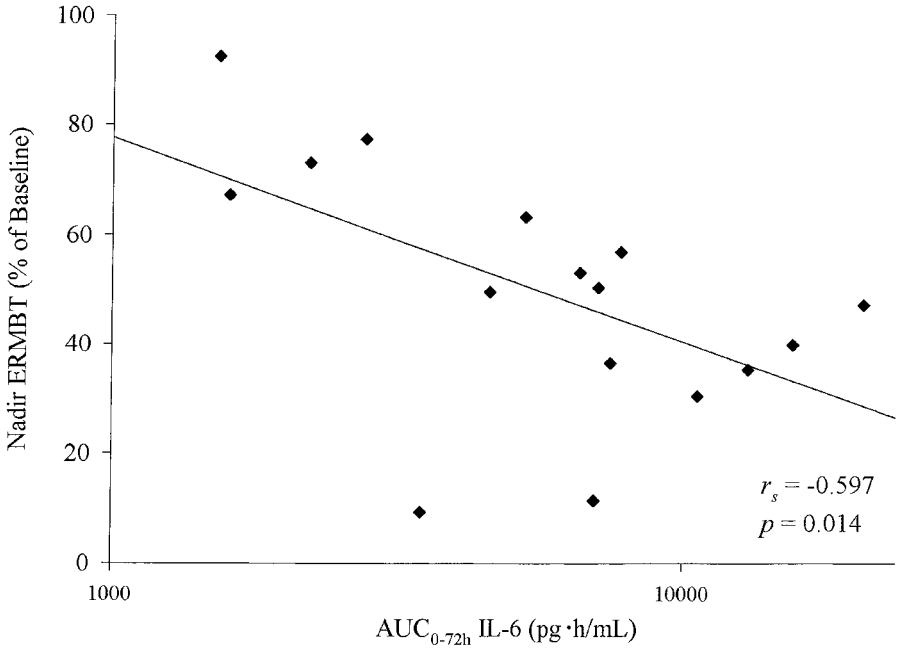


Figure 4. For illustrative purposes, the log-linear plot of interleukin (*IL*)-6 area under the curve for 0–72 hrs (AUC_{0-72h}) and Nadir carbon-14 [^{14}C]erythromycin breath test (*ERMBT*, percentage of baseline) with a simple *linear regression line* is shown. The r_s and p values are from Spearman's rank-order correlation analysis.

Table 3. Clinical markers of surgical stress

Group	OR Time, mins[a]	OR Fluids, mL[a]	EBL, mL[a]
AAA (n = 5)	228 (30)[b]	8681 (877)[c,d]	1280 (301)[c,d]
Colon (n = 6)	164 (23)	3417 (431)	308 (130)
PVD (n = 5)	110 (57)	1440 (423)	140 (98)

OR Time, duration of surgical procedure; OR Fluids, volume of fluids administered in the operating room; EBL, estimated blood loss during surgery; AAA, abdominal aortic aneurysm; PVD, peripheral vascular surgery with graft.

[a]Mean (SEM); [b]$p < .05$ vs. PVD; [c]$p < .01$ vs. Colon; [d]$p < .01$ vs. PVD.

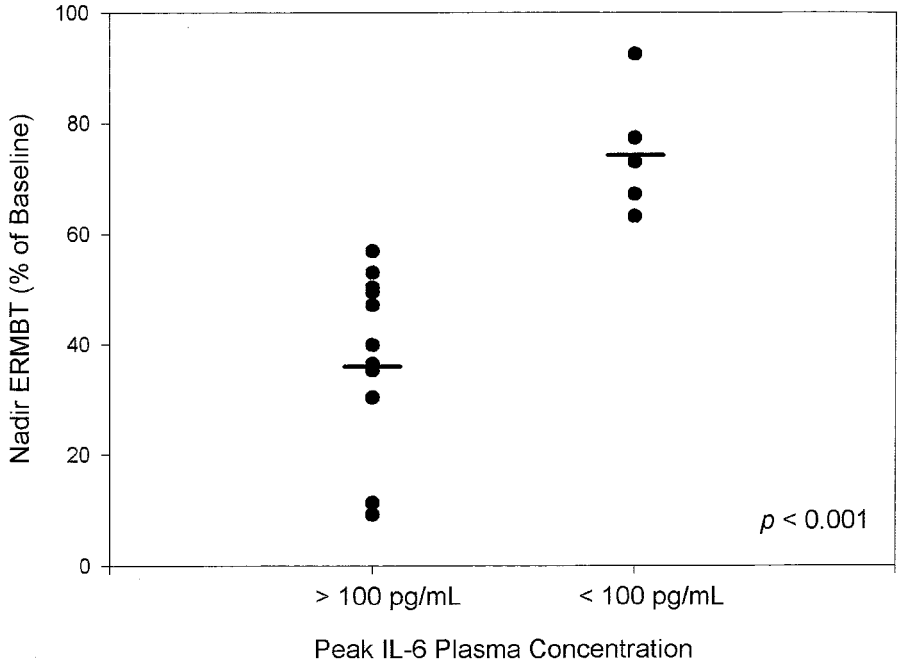
liver function as a complication of surgery, alterations in liver blood flow, and the effects of other mediators of the surgical stress response, most notably cortisol and plasma catecholamines. None of the subjects completing this study showed any clinical or biochemical evidence of acute liver dysfunction.

Volatile, halogenated anesthetics, particularly halothane, may be associated with decreased drug metabolism due to decreased hepatic blood flow, enzyme inhibition, and direct toxic effects on the liver (39). Halothane is believed to act as a suicide substrate, with its reactive metabolites reductively inactivating CYP enzymes, leading to a reduction in hepatic drug metabolism (40, 41). The majority of patients enrolled in this study received general anesthesia using nitrous oxide combined with either isoflurane (n = 9) or desflurane (n = 5). Two PVD patients received regional anesthesia. Limited available data suggest that isoflurane has less inhibitory effect on CYP activity than halothane (42, 43). Desflurane undergoes minimal metabolism (44), and based on the proposed mechanism, it should have a low propensity to cause pharmacokinetic drug interactions. Lastly, the effects of general anesthetics on hepatic drug metabolism should be maximal immediately after anesthesia, not delayed by 48 to 72 hrs, which is inconsistent with the pattern observed in this study. Because of the anesthetic agents used and the time course of suppression of CYP3A4 activity, we do not believe the changes observed in this study are explained by the administration of volatile anesthetic gases.

The decrease in CYP activity after surgical trauma could be partially caused by the altered neurohormonal response after surgery. Serum cortisol (30, 33) and plasma catecholamine (33) concentrations increase within a few hours after elective surgery, with serum cortisol concentrations remaining elevated for several days. Blunt trauma is also associated with alterations in the normal pulsatile secretion of growth hormone, decreased thyroid hormone concentrations, and in men, low serum testosterone concentrations (45). We have previously shown undetectable serum testosterone concentrations in male patients after elective AAA repair (unpublished data).

The potential effects of glucocorticoids on CYP activity are complex and not fully understood. Pharmacologic doses of glucocorticoids are associated with the induction of CYP3A4 enzyme activity (46, 47); however, lower concentrations of glucocorticoids may have different effects on drug metabolism. Shimamoto et al. (47) evaluated the roles of the sympathetic nervous system and adrenocortical system in the down-regulation of CYP enzyme content and activity after intracerebral endotoxin injection in surgical or chemical sympathectomized or adrenalectomized rats. Endotoxin injection resulted in a decrease in total CYP content and CYP-dependent drug metabolism after 24 hrs in both adrenalectomized and control rats; however, the decrease was greater in the adrenalectomized rats. Adrenalectomy effectively reduced the serum concentration of corticosterone by an average of 81% compared with rats with sham operations. In this study, adrenalectomy and subsequent low corticosterone concentrations seemed to enhance the reduction in total CYP content and activity after intracerebral endotoxin; this is not supportive of the concern that elevated glucocorticoid serum concentrations during acute inflammation have an inhibitory effect on CYP activity.

Catecholamines, cyclic–adenosine monophosphate analogs, and other activators of the cyclic-adenosine monophosphate pathway have been shown *in vitro* to result in a loss of CYP2 and CYP3 isoenzyme activity (48–50) and to inhibit the effect of known CYP enzyme inducers (51, 52). The importance of increased sympathetic activity during acute inflammation *in vivo* on CYP-mediated drug metabolism is not well described. The administration of exogenous catecholamines has also not been associated with reports of clinically important drug interactions. Shimamoto et al. (47) administered intracerebral endotoxin to



Acute inflammation after elective surgery was associated with a significant decline in cytochrome P450 3A4 activity, which is predictive of clinically important changes in the metabolism of commonly used drugs that are substrates for this enzyme.

Figure 5. Plot of nadir carbon-14 [^{14}C]erythromycin breath test (*ERMBT*, percentage of baseline) for subjects with peak interleukin (*IL*)-6 of <100 pg/mL vs. subjects with peak IL-6 of >100 pg/mL. The *lines* represent the mean values for each group.

surgically and chemically sympathectomized rats. The sympathectomy procedures reduced norepinephrine content of the liver by 85% to 88% compared with control animals. After endotoxin administration, the decrease in total liver CYP content and measures of specific enzyme activities decreased similarly in adrenalectomized and control animals. These data suggest that the sympathetic nervous system and sympathetic innervation of the liver do not play a role in the down-regulation of CYP activity during the inflammatory response after intracerebral endotoxin administration.

Alterations in growth hormone secretion (53, 54) and thyroid function (55) may also affect the regulation of CYP gene expression; however, the clinical relevance of these findings is not known. Other potential contributors to a decrease in CYP activity after an inflammatory stimulus include induction of nitric oxide synthase, heme oxygenase, and xanthine oxidase in hepatocytes and other tissues. The subsequent generation of NO, increased heme metabolism, and free radical generation, respectively, have all been implicated as potential contributors to CYP protein catabolism or degradation leading to a reduction in CYP activity (2, 3). It is possible that there are multiple and redundant pathways involved in the down-regulation of CYP enzyme content and activity after acute injury and inflammation (3) and that identifying the predominant or most important pathway during a clinical investigation is not possible. There is considerable evidence supporting an important role for the proinflammatory cytokines in this reduction in drug metabolism (1–3), but multiple other components of the inflammatory response may also be contributory.

The reduction in ERMBT activity from baseline observed in the AAA and colon groups in this study is comparable with the degree of inhibition of activity observed with well-known inhibitors of CYP3A4-mediated metabolism, including ketoconazole (26, 27), delavirdine (25), amprenavir (27, 28), and clarithromycin (28). Therefore, this change in CYP3A4 activity after elective, major surgery would be expected to result in clinically important changes in hepatic drug metabolism of many CYP3A4 substrates.

The ERMBT, as used in this study, does have some limitations. The calculations involved with the ERMBT make an assumption concerning erythromycin volume of distribution based on patient weight and height (56). Several of the AAA group patients experienced an increase in body weight during the postoperative period (0.6–13.7 kg), presumably due to extravascular fluid accumulation and edema, which may affect the relationship between total body weight and estimated volume of distribution. None of the patients in the colon and PVD groups experienced an appreciable change in body weight during the study period. In the AAA group, there was no correlation between the weight change and the ERMBT results either across the group or within individual patients. In addition, a similar IL-6 response and decrease in ERMBT response between the AAA and colon groups also argues against an inaccurate volume of distribution estimate in the AAA group as a major contributor to the results of the study.

In addition to being a CYP3A4 substrate, erythromycin is also a substrate for the multidrug-resistant transporter P-glycoprotein (P-gp) (57). If mediators of the inflammatory response also influence the content or activity of P-gp, this may have an important impact on ERMBT results. Interferon-γ has been demonstrated to up-regulate the expression and activity of P-gp in peripheral blood monocyte-derived macrophages (58), and TNF-α, IL-2, and interferon-γ have been shown to down-regulate *mdr1* gene expression (the gene encoding for P-gp) and decrease P-gp activity in human colon carcinoma cells (59, 60). The transcriptional regulator nuclear factor for IL-6 has been shown to be an important transcriptional regulator for *mdr1* and may have a potential role in *mdr1* gene induction secondary to a variety of stimuli (61). All of this evidence suggests that mediators of inflammation may have an impact on P-gp expression, although more study

in the setting of acute inflammation is needed to know the importance, direction, and time course of the effect.

For several patients in this study, there was an initial increase in CYP3A4 activity at 24 hrs after surgery, followed by an inhibition of enzyme activity. This seemed to be more common for patients with relatively low levels of IL-6 expression (PVD group). The explanation for this pattern is unclear and may be explained by multiple interacting effects on drug metabolism and transport that may follow variable time courses after initiation of an inflammatory response. In patients with a greater inflammatory response, as evidenced by higher IL-6 plasma concentrations, a more profound and immediate inhibition of CYP3A4 activity may overwhelm any other factors affecting drug metabolism, leading to a progressive decline in drug metabolism starting at the 24-hr observation point.

Concomitant drugs administered in the perioperative period could have decreased CYP3A4 activity. Patients receiving known inhibitors or inducers of CYP3A4 were excluded from study participation, and other than the previously mentioned administration of diltiazem, patients did not receive known enzyme inducers or inhibitors in the postoperative period. The colon group patients all received three doses of metronidazole, 500 mg, the evening before surgery as part of their bowel preparation regimen. We conducted a substudy involving five healthy, male volunteers to demonstrate that this metronidazole regimen had no effect on the ERMBT results (62).

CONCLUSIONS

In conclusion, hepatic CYP3A4 enzyme activity, which is predictive of clinically important changes in the metabolism of substrates for this enzyme, was significantly decreased after elective surgery. The reduction in CYP3A4 activity correlated with plasma IL-6 concentrations, a marker of the severity of the acute inflammatory response. Although we cannot establish a causal relationship between these two events, the findings are consistent with extensive experimental evidence of cytokine-mediated reductions in CYP activity. CYP3A4 substrates represent clinically important drugs commonly used in the care of critically ill patients. Alterations in drug pharmacokinetics are well recognized in the critical care setting; however, the mechanisms underlying these changes have not been well defined (63). The acute inflammatory response seems to have an important influence on hepatic CYP activity, and further study of the severity and time course of these effects in critically ill patients is needed to improve the appropriate and safe use of medications in the intensive care unit.

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