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Acetaminophen Causes an Increased International Normalized Ratio by Reducing Functional Factor VII

Ian M. Whyte,* Nicholas A. Buckley,† David M. Reith,‡ Irene Goodhew,§ Michael Seldon,§ and Andrew H. Dawson*

*Departments of *Clinical Toxicology & Pharmacology, Newcastle Mater Misericordiae Hospital, Newcastle; †Clinical Pharmacology, Royal Adelaide Hospital, Adelaide; ‡Medicine, Royal Brisbane Hospital, University of Queensland, Brisbane; §Hematology, Hunter Area Pathology Service, Newcastle, Australia*

Summary: Acetaminophen may increase International Normalized Ratio (INR) in patients taking anticoagulation medication, and in patients with acetaminophen poisoning without hepatic injury. The objective of this study was to describe and investigate the effect of acetaminophen on INR. The authors studied patients admitted to a regional toxicology treatment center with acetaminophen poisoning with INR and without potentially confounding coingestion or hepatic injury. Exposed and nonexposed (control) cohorts were recruited from admissions with acetaminophen poisoning and psychotropic drug poisoning, respectively. From 1,437 acetaminophen poisonings, after exclusions, there were 143 admissions with 205 estimations of INR. INR showed a time-dependent increase. Fifty percent of all patients and 66% of those with an extrapolated 4-hour acetaminophen concentration ≥150 mg/L had an abnormal INR at some time. Dose ingested ($p = 0.01$) and nomogram-based risk (p for trend = 0.005) were correlated with the effect. N-acetylcysteine had a protective effect. Functional factor VII was lower ($p = 0.005$) in exposed patients ($n = 30$) than controls ($n = 8$), and less than antigenic factor VII in exposed patients ($p = 0.03$). Factor IX was lower ($p = 0.02$). Factor VIIIc was not significantly different. The authors concluded that an isolated, small rise in INR is common after acetaminophen poisoning without hepatic injury. It appears to be caused by inhibition of Vitamin K–dependent activation of coagulation factors. This effect suggests a possible mechanism for the observed interaction between acetaminophen and warfarin. **Key Words:** Acetaminophen—INR—Poisoning—factor VII—Warfarin—Drug interaction.

Acetaminophen has been recommended as a safe drug to use in patients treated with warfarin. However, studies of the effects of regular maximal doses of acetaminophen in patients stabilized on a particular dose of warfarin suggest that a small but definite increase in International Normalized Ratio (INR) may occur (1,2,3). A recent case-control study suggested the odds of an INR > 6.0 in patients taking warfarin increased 10-fold (95% CI, 2.6–37.9) for those who were also taking 9,100 mg/w or more of acetaminophen (4). The mechanism for this interaction has not been determined but has been suggested to be a pharmacokinetic interaction at the level of cytochrome P450 resulting in decreased metabolism of warfarin (5). We speculated that the mechanism may be a pharmacodynamic effect on coagulation factor activation (6) and may also be seen in overdose. Acetaminophen has occasionally been noted to prolong the prothrombin time (PT) in overdoses not complicated by hepatic injury (7,8,9). This may be an important observation in two respects. First, a prolonged PT has been used as an indicator of the severity of hepatic injury, and suggested as an indication for treatment and further monitoring (9,10).

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Address correspondence and reprint requests to Dr. Ian Whyte, Department of Clinical Toxicology and Pharmacology, Newcastle Mater Misericordiae Hospital, Locked Bag 7, Hunter Regional Mail Center, New South Wales, Australia 2310.

Defining the range of PT or INR that is expected in this setting and that does not mandate prolonged monitoring is therefore necessary. Second, an understanding of the mechanism of this effect may explain the observed effect on INR in patients stabilized on warfarin. Thus the aim of our study was, in patients with a nonhepatotoxic acetaminophen overdose, to describe the range of prothrombin times (reported as INRs) seen, the time course of the abnormality, and, in a further prospective study, measure the effect on selected coagulation factor production and activation.

MATERIALS AND METHODS

Two studies were undertaken: a retrospective case series and a prospective inception cohort study. The retrospective case series examined all admissions from January 1987 to March 1999. Patients who had ingested acetaminophen and had a prothrombin time measured were included, whereas patients who had or who developed any biochemical evidence of hepatic injury (aspartate aminotransferase [AST] >35 U/L [reference range 1–35 U/L] or alanine aminotransferase [ALT] >40 U/L [reference range 1–40 U/L]) at any time were excluded. We also excluded all patients who were taking anticoagulants or who coingested substances reported to cause a prolonged PT or increased INR in overdose (11,12). All patients with poisoning admitted to our unit since 1987 have been entered onto a database that prospectively records patient demography, all drugs and doses ingested, laboratory results, details of management, and complications of the ingestion. This has been described in more detail elsewhere (13).

In the second, prospective study, 34 patients ingesting acetaminophen and a control group of eight patients ingesting psychotropic drugs were studied in detail. In this group, after informed consent, blood was drawn for coagulation factor analysis and confirmation of absence of hepatotoxicity. The Hunter Area Research Ethics Committee approved the experiments.

COAGULATION FACTOR ANALYSIS

Prothrombin times were measured using Australasian Reference Thromboplastin (Westmead Hospital; Sydney, Australia; International Sensitivity Index [ISI] of 0.98) or Innovin Thromboplastin (Dade–Behring; Brisbane, Australia; ISI of 1.00) and are reported as INRs using in-house pooled normal plasma for reference prothrombin time. Normal range for INR in our laboratory is 0.87–1.26. Functional and antigenic factor VII, factor IX and factor VIIIc were assayed. Factors VII and IX are produced in the liver by a Vitamin K–dependent mechanism, and have short (6 h) and long (24 h) half-lives, respectively (14). Factor VIIIc is produced in the liver but is not Vitamin K–dependent, so it was assayed to exclude a more generalized effect on liver synthesis of coagulation proteins. Thirty milliliters of blood were drawn into citrate, centrifuged for 15 minutes, aliquoted and frozen within 2 hours of collection, and stored at −80°C until analysis. The functional factor assays were performed on an MLA900 (Commonwealth Serum Laboratories Biosciences; Sydney, Australia) in batches with factor deficient plasma (Dade–Behring; Brisbane, Australia) using activated partial thromboplastin time for factor VIIIc (interassay CV <2%) and factor IX (CV <2%) and PT for factor VII (CV < 2%). Antigenic factor VII (CV <2%) was assayed chromogenically (Bayer Diagnostics; Leverkusen, Germany). Each assay has been standardized against known reference plasma.

STATISTICAL METHODS

Because the distribution of the continuous variables did not follow a normal pattern, medians (and ranges) are presented, and the Mann-Whitney test was used to compare groups. For dichotomous variables, Fisher's exact test and the χ^2 test for trend were performed. Odds ratios (OR) were calculated for risk factors. Statistical analyses and curve fitting were performed using GraphPad Prism version 3.00 for Windows (GraphPad Software; San Diego, CA).

RESULTS

Retrospective Case Series

The initial data set consisted of 6,423 admissions in 4,552 patients from January 13, 1987 to March 22, 1999 inclusive. Of these, 1,437 admissions involved acetaminophen ingestion. After exclusion of admissions with therapeutic or coingested anticoagulants (9 admissions) and drugs reported to cause a prolonged PT or increased INR in overdose (aspirin [74], phenytoin [11], cephalosporins [10], valproate [9], iron [6], and quinine [4]), there were 1,314 admissions. INR was measured in 220, but 11 did not have a recorded time of overdose and 66 had an AST >35 U/L or ALT >40 U/L.

The final series consisted of 143 patients without hepatic injury in whom 205 estimations of INR were made. The median age was 22 years (13 to 71), and 67.2% were female. There was a time-dependent increase in INR

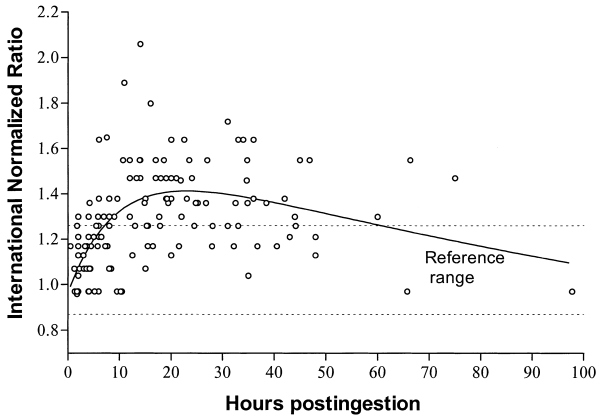

FIG. 1. Effects of acetaminophen overdose not causing hepatic injury on maximum International Normalized Ratio in 143 admissions.

TABLE 1. *Nomogram-based risk and presence of an abnormal INR at any time after acetaminophen overdose without hepatic injury*†‡§*

	Abnormal INR	Normal INR‖
Nontoxic (<150 mg/L line)	25 (34%)	48 (66%)
Possible toxicity (150 mg/L line)	24 (73%)	9 (27%)
Probable toxicity (200 mg/L line)	12 (57%)	9 (43%)
High-risk (300 mg/L line)	9 (64%)	5 (36%)
Possible or greater (≥150 mg/L)	45 (66%)	23 (34%)

INR, international normalized ratio.
* Data given is for 143 admissions.
† Values shown as n (% of total within nomogram risk group).
‡ Two admissions had initial acetaminophen concentrations drawn too late (>24 hours post-ingestion) for application of the nomogram.
§ Chi-square test for trend $\chi^2 = 7.8$, df = 1, p = 0.005.
‖ Normal range for INR 0.87–1.26.

(Fig. 1). Overall, 71 of the 143 (50%) had an abnormal INR (>1.26) at some time during admission. The effect was more common after 12 hours, with the INR being greater than 1.26 in 68% (82/121) of tests performed 12 or more hours after ingestion. The modal time period for the INR to first become abnormal was 12 to 16 hours after the overdose with a median time of 16 hours (Fig. 2). The highest INR measured was 2.1 at 14 hours after ingestion. No clinical effects of an increased INR were seen in any patient. There was a correlation between the maximum INR and the reported dose ingested ($p = 0.01$), but dose explained only 5% of the variability in peak INR ($r^2 = 0.05$). There was a significant relationship (p for trend = 0.005) between the nomogram-based risk (15) and an abnormal INR (Table 1), with an abnormal INR recorded in 66% (45/68) of patients with an extrapolated 4-hour acetaminophen concentration of 150 mg/L (possible toxicity line) or greater. The odds of an abnormal INR in this group were 3.8 (95% CI, 1.9–7.5) when compared with nontoxic exposures. The highest risk group was the possible toxicity group (73% had an abnormal INR) with no further increase in likelihood of an abnormal INR as the risk of hepatic injury increased. Our threshold for treatment with intravenous N-acetylcysteine (NAC) is the 200 mg/L line (probable toxicity group) suggesting a possible protective effect of NAC. Stratifying by NAC treatment showed that the increased risk in patients above the 150 mg/L line was entirely explained by an increased risk in those not receiving NAC. The odds for an abnormal INR in those in the possible toxicity or greater group compared with the nontoxic group in those receiving NAC was 1.4 (0.5–3.7), whereas in those not receiving NAC it was 6.5 (1.1–40.0). Coingested acute alcohol did not increase the odds of developing an abnormal INR (OR 1.4, 95% CI, 0.6–3.1), nor did the presence of alcohol abuse or dependence (OR 0.8, 95% CI, 0.2–3.2).

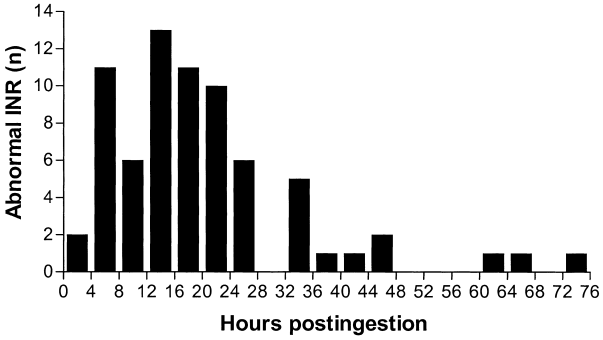

FIG. 2. Time of first abnormal International Normalized Ratio (INR) after acetaminophen overdose without hepatic injury in 71 admissions with abnormal INR at any time.

Prospective Study

Thirty-four patients admitted with acetaminophen overdose (exposed, cases) were recruited for the prospective study. An abnormal AST or ALT subsequently developed in four of these patients, and they were not analyzed further. Eight patients admitted with psychotropic drug overdose (nonexposed, controls) were also recruited. Data for the exposed group and controls are shown in Table 2. In the 30 acetaminophen overdoses, there was a similar time-dependent increase in INR to that observed in the larger group (Fig. 3), confirming that the same effect was being studied. The INR in the exposed group was significantly different from controls ($p = 0.004$). The coagulation factor most affected was factor VII, and the time course of the observed fall mirrored the rise in INR (Fig. 3) with a significant difference

TABLE 2. *Coagulation studies and characteristics of overdoses of acetaminophen (exposed) and psychotropic drugs (controls)**

	Exposed (n = 30)	Controls (n = 8)	p value§
International normalized ratio	1.36 (0.97–2.10)	1.17 (0.97–1.17)	0.004
Factor VII function (% of normal)	58 (28–180)	110 (80–200)	0.003
Factor VII antigen (% of normal)	80 (38–140)†	97 (60–160)‡	0.06
Factor IX (% of normal)	90 (60–150)	113.5 (78–155)	0.03
Factor VIIIc (% of normal)	105 (41–550)	93.5 (52–140)	0.26
Time, ingestion to admission (hours)	16.2 (1.4–66.4)	18.7 (3.1–27.5)	0.75
Time in hospital (hours)	26.0 (2–62)	16.0 (5.3–32.8)	0.25
Acetaminophen ingested (g)	20 (7–68)	0	—
Female	19 (63%)	7 (88%)	0.39
Age (years)	19 (15–71)	34 (22–49)	0.007

* Data shown as n (%) or median (range).
† n = 25 (see text).
‡ n = 7 (see text).
§ p value using Mann-Whitney test or Fisher's exact test.

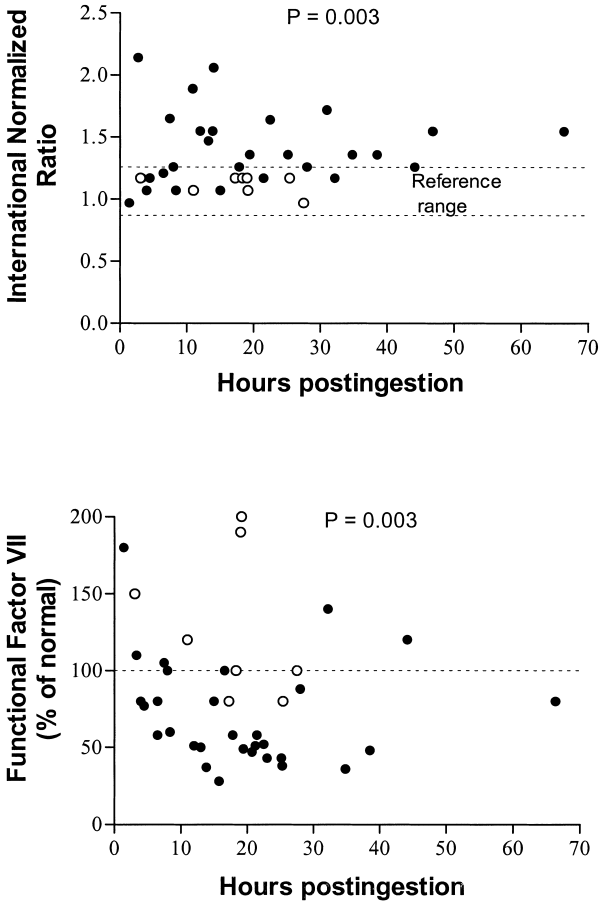

FIG. 3. International Normalized Ratio and functional factor VII in admissions with acetaminophen overdose (exposed, ●, n = 30) and psychotropic drug overdose (control, ○, n = 8). p values refer to case versus control.

between exposed and control groups (p = 0.003). Because of technical difficulties, antigenic factor VII could not be determined in one control sample and five exposed samples. Antigenic factor VII was not different in the exposed group compared with controls (p = 0.06). The functional factor VII was significantly lower than antigenic factor VII (p = 0.03) within exposed but not different within controls (p = 0.46) (Fig. 4). Functional factor IX was slightly lower in the exposed group (p = 0.03) (Fig. 5). Functional factor VIIIc did not differ between exposed and control groups (p = 0.26) (Fig. 5). Time from ingestion to admission, time in hospital, and gender were not different between exposed and control groups (Table 2). NAC was given to 23 patients in the exposed group. In 17, the INR and factor samples were drawn after the start of the NAC infusion. In six, INR and factor samples were drawn before starting NAC and in the remaining five cases no NAC was given. When results were compared between those receiving NAC (n = 17) and those not receiving NAC at the time of sampling (n = 13), there was no difference between INR (p = 0.25), functional (p = 0.10) or antigenic (p = 0.82) factor VII, factor VIIIc (p = 0.12) or factor IX (p = 0.75).

DISCUSSION

Retrospective analysis of our database shows that approximately 50% of patients with acetaminophen overdose without hepatic injury will have an abnormal INR at some time in their admission. This rise is time-, dose-, and concentration-dependent. The concentration dependence is confounded by an independent effect of NAC treatment. This could be prevention of the effect of acet-

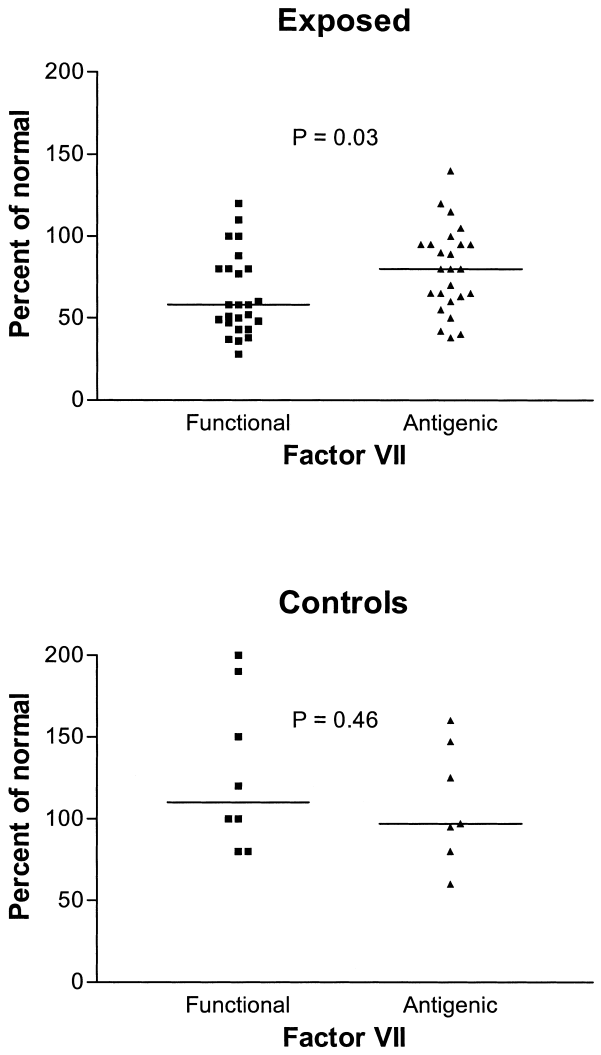

FIG. 4. Functional and antigenic factor VII in admissions with acetaminophen overdose (exposed, n = 30) and psychotropic drug overdose (control, n = 7). The line indicates the median value. p values refer to functional factor VII versus antigenic factor VII.

aminophen or a direct effect on coagulation by NAC. While NAC in supratherapeutic concentrations (0.01 mol) has been reported to prolong the clotting time in vitro (16), in patients with Adult Respiratory Distress Syndrome (17), and subsequently in normal volunteers (18) and in vitro (18), it has been shown that intravenous NAC in concentrations likely to be found during treatment (100–600 μmol/L) produces a rapid, significant, and dose-dependent decrease in PT. The mechanism is unclear but may relate to disulfide interchange occurring between this thiol amino acid and the disulfide bridges that are common in many clotting factors (16). Thus, NAC has an independent effect of reducing the INR that decreases the risk of an abnormal INR in the treated group. Perhaps because of small numbers, the prospective study did not show this effect.

The increase in INR should not present management problems for clinicians aware of this phenomenon because the changes are fewer and occur earlier (median time 16 hours, as opposed to peaking at day 3 to 4) than those observed with established acetaminophen hepatotoxicity (ALT or AST >1,000 U/L) (10). If aminotransferase levels are normal then the increased INR does not require monitoring. Conversely, a normal prothrombin time on admission has a high negative predictive value (96%) for hepatotoxicity (8). If this observation can be confirmed in larger series, this may provide a more accurate means of determining which patients require treatment if they present late or at an unknown time after ingestion.

As suggested by Malia et al (6), the mechanism of this increase in INR appears to be an inhibition of the activation of vitamin K-dependent coagulation factors. The most affected factor is factor VII, presumably because of

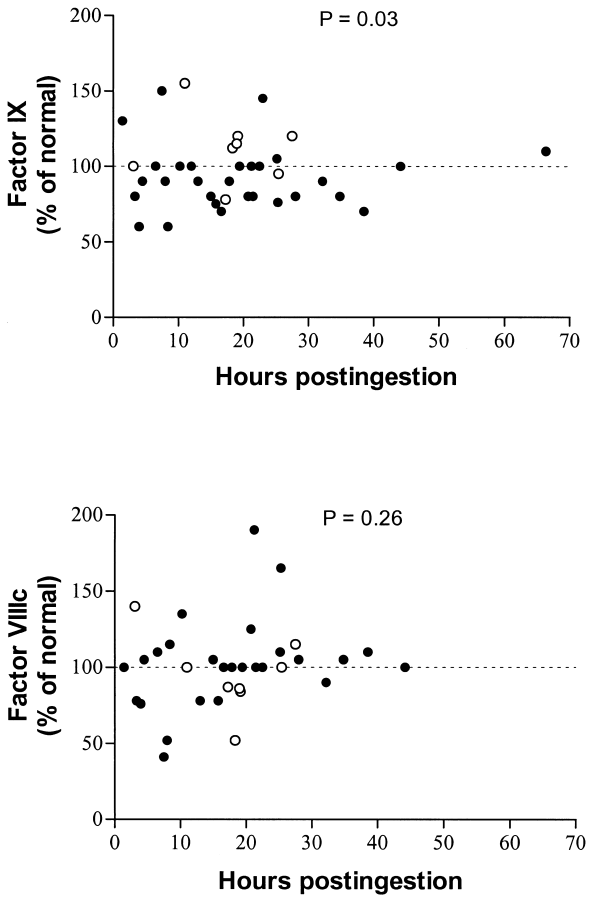

FIG. 5. Factor IX and factor VIIIc in admissions with acetaminophen overdose (exposed, ●, n = 30) and psychotropic drug overdose (control, ○, n = 8). p values refer to case versus control.

its short half-life, but factor IX is also affected. It is not caused by inhibition of general synthetic function of the liver, as demonstrated by the normal levels of factor VIIIc, which has a half-life between factors VII and IX. The effect on functional but not antigenic factor VII suggests that the mechanism is an inhibition of gamma carboxylation, the final step in activated vitamin K-dependent factor production.

In patients who are taking a stable warfarin dose, acetaminophen ingestion has been shown to increase the INR or prolong the PT in some studies. This effect is usually minor and clinically insignificant (1,2,3,19,20), but occasionally large changes have been observed (4,21). While the increases in INR demonstrated in our study caused no adverse effects in our cohort of patients who were not taking warfarin, in patients stabilized on warfarin they could easily explain the observed changes and result in clinical consequences. Our study also demonstrates a mechanism for this interaction between warfarin and acetaminophen. Warfarin reversibly inhibits Vitamin K 2,3–epoxide reductase, which leads to a reduction in availability of the reduced form of Vitamin K (Vitamin K hydroquinone), the oxidation of which is directly coupled to gamma carboxylation and activation of the Vitamin K-dependent clotting factors (II, VII, IX, X) (14). This leads to a reduction in functionally active factors and an accumulation of inactive precursors (22). We postulate that acetaminophen (or one or more of its metabolites) reduces functional levels of two Vitamin K–dependent clotting factors (VII and IX), but not antigenic levels (factor VII), by inhibiting gamma carboxylation. It is not clear whether the effect is directly on the carboxylase or via reduction of available Vitamin K hydroquinone.

The reduction in coagulation factors and increase in INR did not occur in all individuals. Known acquired and inherited differences between individuals in metabolic pathways for acetaminophen might explain this variation (23). Pharmacodynamic factors relating to coagulation factor activity may also be important (14). It has been observed that hepatotoxicity does not develop in some individuals after large acetaminophen overdoses, and some appear to have an increased susceptibility for either environmental or intrinsic reasons and hepatotoxicity develops despite "nontoxic" concentrations (24,25). Similarly, there may be significant interindividual variation in the size of the interaction of acetaminophen with warfarin. Using the INR in patients receiving chronic therapy with warfarin given acetaminophen to detect an interaction is complicated by the substantial variation in the INR because of the many other factors that may influence this result. It is even more complicated when the acetaminophen is given intermittently. By directly measuring factor VII levels during acute and chronic treatment with acetaminophen (in patients not on warfarin) finding a no-effect level that is generally applicable may be possible.

Although the use of acetaminophen in patients taking warfarin is presumably much safer than most other oral analgesics, many of which have antiplatelet and ulcerogenic effects, perhaps it is time to sound a note of caution about the use of acetaminophen in this group of patients and to reaffirm the advice that all changes to an established drug regimen should be carried out cautiously for patients on warfarin.

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