Strain-Specific Enhancement or Inhibition of Coumarin Hepatotoxicity in Mice Following Pretreatment with Two Different Liver Enzyme-Inducing Agents

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Human exposure to coumarin continues despite controversy over its hepatotoxic potential. Greater understanding of human reactions to coumarin may be achieved by studying murine interstrain differences. The metabolic basis of coumarin hepatotoxicity and its modulation by liver enzyme inducers, β-naphthoflavone (βNF) and aracol 1254 (ARO), were investigated in C3H/He and DBA/2 mice. Coumarin (200 mg/kg) was hepatotoxic to both strains, resulting in 2- to 15-fold plasma aminotransferase elevations, mild subcapsular linear hepatocyte necrosis after 24 hr, and, in some C3H/He mice, centrilobular necrosis. In this strain, βNF pretreatment caused a 2- to 3-fold further increase in plasma aminotransferases and produced perportal necrosis. In contrast, ARO-pretreated C3H/He mice tended to exhibit lower plasma aminotransferases and occasional midzonal damage. Neither pretreatment significantly altered coumarin hepatotoxicity in DBA/2 mice. In C3H/He mice, hepatic microsomal metabolism of [3-14C]-coumarin via the 3-hydroxylation pathway doubled following both βNF and ARO treatment. The contrasting nonresponsiveness of DBA/2 mice suggested that this pathway is linked to the Ah locus, which is defective in this strain. ARO treatment caused a maximal 5-fold increase in coumarin 7-hydroxylation in C3H/He mice, whereas DBA/2 mice were 30% less responsive. Potentiation of coumarin hepatotoxicity corresponded to an increase in the 3:7-coumarin hydroxylation ratio. Pretreatment-dependent shifts in the location of hepatocyte damage may be related to changes in the translobular ratio of enzymes involved in activation and detoxification of coumarin. These data highlight how genetic background, individual variation, and xenobiotic-induced alterations in enzyme profiles, factors all relevant to human risk assessment, can influence the consequence of coumarin exposure.

Coumarin (1,2-benzopyrone, cis-o-coumarinic acid lactone) is a constituent of many plants and essential oils. It is used as a perfume enhancer at concentrations varying between 0.003 and 0.2% in soap, detergent, and cosmetic preparations and is added to tobaccos and perfumes at maximum concentrations of 0.1 and 0.8%, respectively (Cohen, 1979). Due to its hepatotoxicity in rat and dog (reviewed by Cohen, 1979, and by Egan et al., 1990) and rodent carcinogenicity (National Toxicology Program, 1993), it is no longer used as a flavoring for food. However, there is renewed interest in coumarin for its anticancer properties and its potential for chemotherapeutic use in humans particularly in the treatment of malignant melanoma (Thornes et al., 1989).

Coumarin exhibits marked variation in metabolism and hepatotoxicity between different species (Fentem and Fry, 1993; Cohen, 1979), causing liver damage in rat and dog (Hagan et al., 1967; Hazleton et al., 1956) but not in Syrian hamster (Ueno and Hirono, 1981). The baboon (Evans et al., 1979) and gerbil (Fentem et al., 1992), although susceptible, appear to be less sensitive species than rat. In the mouse, there is variation in susceptibility to coumarin hepatotoxicity between different strains, C3H/He mice being more sensitive than DBA/2 mice (Endell and Seidel, 1978). While coumarin is not usually hepatotoxic in humans at therapeutic doses, idiosyncratic hepatotoxicity has been reported in clinical trials in patients with concurrent brucellosis, chronic infections, chronic fatigue syndrome, or cancer (Cox et al., 1989; Faurschou, 1982).

The basis of species and strain variation in susceptibility to coumarin hepatotoxicity has been attributed in part to differences in coumarin metabolism: in human and baboon, coumarin undergoes extensive 7-hydroxylation (Egan et al., 1990; Gangolli et al., 1974; Shilling et al., 1969; Waller and Chasseaud, 1981) whereas this route constitutes only a minor pathway in species susceptible to hepatotoxicity, viz. dog, gerbil, rat, and several strains of mouse (Cohen, 1979; Gangolli et al., 1974; Kaighen and Williams, 1961; Lake et al.,
formed in an attempt to resolve the mechanistic basis of the reported mouse strain differences in susceptibility to coumarin, and the extent of coumarin hepatotoxicity, but also the lobular site of cell damage in a way similar to that reported in the rat (Lake and Evans, 1993). Studies using mouse strains of differing susceptibility to coumarin may also be most appropriate for investigating the mechanistic basis of the infrequent episodes of human hepatotoxicity.

In humans, there is a high degree of interindividual variation in coumarin metabolism: studies in panels of human microsomes have shown up to a 30-fold individual difference in total polar metabolite formation and a 2250-fold individual variation in 7-hydroxylation (van Iersel et al., 1994a; Pearce et al., 1992). There is also evidence that the human 7-hydroxylase CYP2A6 has a genetic polymorphism (Fernandez-Salguero et al., 1995). The coumarin 3-hydroxylation pathway has also been demonstrated in human liver slices (Steenmsma et al., 1994) and in human microsomes (Fentem et al., 1991; Fentem and Fry, 1991), where its predominance over the 7-hydroxylation detoxication pathway is dependent upon substrate concentration (Lake et al., 1992a). It is plausible that the rare cases of human coumarin-related hepatotoxicity may result from the metabolic handling of coumarin in these individuals.

In parallel with the human diversity, marked interstrain differences in coumarin 7-hydroxylation have been reported in mice, both in vivo (Lush and Andrews, 1978) and in whole liver homogenates and microsomes (van Iersel et al., 1994b; Raunio et al., 1988; Wood, 1979; Wood and Conney, 1974; Wood and Taylor, 1979), and these differences are due to a single gene difference in the Cyp2a-5 gene on chromosome 7, which codes for the major mouse liver cytochrome P450 responsible for coumarin 7-hydroxylation. Thus the lower sensitivity of DBA/2 mice to coumarin hepatotoxicity relative to C3H/He mice reported by Endell and Seidel (1978) may be associated with greater coumarin 7-hydroxylation activity.

Pretreatment of rats with mixed-function oxidase inducers has been shown to modulate not only coumarin metabolism and the extent of coumarin hepatotoxicity, but also the lobular site of hepatotoxicity (Lake et al., 1992a; Lake and Evans, 1993; Peters et al., 1991). Accordingly, this study was performed in an attempt to resolve the mechanistic basis of the reported mouse strain differences in susceptibility to coumarin hepatotoxicity and to investigate whether enzyme-inducer pretreatments could alter the lobular site of cell damage in a way similar to that reported in the rat (Lake and Evans, 1993). Studies using mouse strains of differing susceptibility to coumarin may also be most appropriate for investigating the mechanistic basis of the infrequent episodes of human hepatotoxicity.

**MATERIALS AND METHODS**

Male DBA/2 and C3H/He mice (viral antibody free, 22–24 g, from B&K Universal Ltd., Grimston, North Humberside, UK) were housed in steel grid-bottomed cages under controlled temperature, lighting, and humidity (20 ± 2°C, 12:12 hr light and dark, 50 ± 10%), respectively and allowed free access to powdered (RM1 E SOC FG) diet (SDS Ltd., Wutham, Essex, UK) and tap water ad libitum. Within each strain, animals were randomly allocated to groups of at least five and allowed to acclimatize for 5 days.

Coumarin (≥99%) was from Aldrich Chemicals Ltd. (Gillingham, Dorset, UK), β-naphthoflavone (BNF, 95%) was from Sigma Chemical Co. Ltd. (Poole, Dorset, UK), food-grade corn and ground nut oils were from Safeway Supermarket, and Aroclor 1254 (ARO) was a gift from Dr. Nigel Lawrence (Robens Institute of Health and Safety, University of Surrey, Guildford, UK). Animals received BNF (80 mg/kg body wt, dissolved in ground nut oil), ARO (54, 125, or 162 mg/kg body wt, dissolved in corn oil), or the oil vehicle alone (10 ml/kg body wt) by ip injection between 10 and 11 AM on 3 consecutive days, followed 24 hr later by a single dose of coumarin, 200 mg/kg body wt (this dose was selected due to non-hepatic-related deaths that occurred at higher doses) dissolved in the same vehicle as the pretreatment or vehicle alone (10 ml/kg body wt) by oral intubation. After 24 hr, animals were killed by carbon dioxide asphyxiation. Blood was withdrawn from the inferior vena cava, placed in heparinized tubes, and mixed immediately by repeated inversion and roller mixing. The tubes were centrifuged at 1500g for 15 min and separated plasma was stored at −70°C.

At autopsy, a full macroscopic examination was performed and samples of each major lobe of the liver were fixed in 10% buffered neutral Formalin. Tissues were processed through paraffin wax for histological examination and stained with hematoxylin and eosin.

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured, as indices of hepatic damage, in once-thawed plasma samples on a COBAS BIO centrifugal analyzer using commercial kits (Roche Diagnostic Systems Ltd., Welwyn Garden City, Herts, UK, and Randox Laboratories Ltd., Ardmore, Co. Antrim, N. Ireland).

Liver microsomes were prepared from animals treated with BNF or ARO alone or their vehicles, according to the method of Mann et al. (1985). Microsomal protein (2 mg) was incubated in the presence of 50 μM [3-14C]coumarin (ICI Chemicals and Polymers, Bingham, Cleveland, UK: 0.265 μCi/tube added in 5 μl dimethyl sulfoxide). 1 mM NADPH, 5 mM MgSO4, and 50 mM Tris–HCl buffer, pH 7.4, in a final volume of 1 ml, for 10 min at 37°C. Blank incubations excluded NADPH. Incubations were terminated by the addition of 1 ml ice-cold methanol and following centrifugation the supernatants were analyzed for coumarin metabolites by HPLC (Peers et al., 1991). Metabolites were identified by comparison of their retention times with those of authentic coumarin metabolites. Covalent binding of [3-14C]coumarin metabolites to the 1200g pellet was determined by the method of Lake (1984).

Statistical analysis of data was by ANOVA and Student’s t test, and in the case of plasma enzyme levels data were log transformed prior to analysis.
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RESULTS

Coumarin treatment significantly raised the plasma aminotransferase levels in both C3H/He and DBA/2 mice (Figs. 1A and 1B), although wide interindividual variation was apparent, especially in the C3H/He mice. Neither βNF nor ARO treatment alone altered plasma enzyme levels in either mouse strain. In DBA/2 mice, neither of these agents, when given as pretreatments, significantly altered coumarin-induced elevations in plasma aminotransferases, although levels tended to be less than those with coumarin alone. In C3H/He mice, βNF pretreatment potentiated coumarin-induced increases in AST and ALT levels 2- and 3-fold, respectively. In contrast, ARO pretreatment did not significantly alter coumarin-related increases in plasma aminotransferases although plasma ALT and AST levels in these animals were generally lower than levels in those treated with coumarin alone.

Histologically (observations summarized in Table 1), coumarin treatment was characterized by a subcapsular linear hepatocyte necrosis (Fig. 2b). This was only present on the ventral surface of the caudate lobe or the dorsal aspect of the left lateral lobe, i.e., the surfaces of the lobes which are normally in closest physical contact with the stomach. This lesion was evident in all coumarin-treated animals regardless of strain or pretreatment and was normally minimal, involving only one to four hepatocytes directly under the capsule. In addition, moderate centrilobular damage was observed in three of five coumarin-treated C3H/He mice when corn oil was used as vehicle (Fig. 2c and Table 1). However, when βNF was given to C3H/He mice prior to coumarin, the majority (five of seven animals) exhibited moderate periportal hepatocyte damage (acinar Zone 1) in one or two of the four major lobes (Fig. 2d). In addition, one animal had marked panlobular damage confined to a single lobe. In contrast, coumarin administration after prior ARO treatment resulted in minimal midzonal (acinar Zone 2) necrosis in two of five C3H/He mice (Fig. 2e). After pretreatment with ARO, coumarin-treated DBA/2 mice showed no regular lobular lesion. One DBA/2 mouse exhibited marked panlobular necrosis after combined βNF and coumarin treatment. Neither βNF nor ARO treatment alone resulted in any hepatic abnormality except that the livers of some mice treated with βNF showed a mild serosal inflammatory reaction.

Microsomal fractions metabolized [3-14C]coumarin in the presence of NADPH to form several metabolites, including 7-hydroxycoumarin (7OHC), 3-hydroxycoumarin, o-hydroxyphenylethanol, o-hydroxyphenylacetaldehyde (o-HPA), o-hydroxyphenylacetic acid, and o-hydroxyphenyllactic acid as major metabolites: the latter five were considered together as products of the 3-hydroxylation pathway (Fig. 3), of which o-HPA is the major product (~80%; data not shown). Other metabolites were not individually identified but their combined proportion of the total metabolized is shown in Table 2. After repeated acid and methanol washing, some radiolabel was found to be covalently bound to microsomal proteins. The extent of this binding in baseline samples appeared vehicle dependent, with corn oil controls consistently showing one-third the activity of ground nut oil controls.

In microsomes from control animals of both strains of mouse, the 3-hydroxylation pathway was by far the major route of coumarin metabolism, accounting for ~40–60% of total metabolites; the 7OHC metabolite accounted for 5–10% and the remaining nonidentified metabolites combined made up between ~30 and 50% of the total. Generally, the basal rates of 3-hydroxy pathway metabolite formation were found to be comparable in the two strains, as was total metabolite formation, although 7-hydroxylation in DBA/2–
TABLE 1

Liver Histopathology from C3H/He and DBA/2 Coumarin-Treated Mice with or without βNF or ARO Pretreatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C3H/He</th>
<th>DBA/2</th>
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<tbody>
<tr>
<td></td>
<td>SCL</td>
<td>CL</td>
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<tr>
<td>Control'</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Coumarin</td>
<td>5</td>
<td>—</td>
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<tr>
<td>βNF</td>
<td>—</td>
<td>—</td>
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<tr>
<td>βNF/coumarin</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>Control'</td>
<td>—</td>
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<tr>
<td>Coumarin</td>
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<td>3</td>
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<tr>
<td>ARO</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ARO/coumarin</td>
<td>4</td>
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</tr>
</tbody>
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* Necrosis described as subcapsular linear (SCL), centrilobular (CL), midzonal (MZ), periportal (PP), panlobular (PL), scattered single cell (SSC). Numbers refer to number of animals exhibiting a particular lesion.

* Ordinates refer to number of mice exhibiting liver damage; denominators indicate group size.
* Controls received ground nut oil vehicle only
* Controls received corn oil vehicle only.
* One animal died prematurely and was excluded from the study.

corn oil control animals was double that in C3H/He—corn oil controls and almost double that in their DBA/2— and C3H/He—ground nut oil counterparts.

βNF treatment significantly increased the total rate of microsomal coumarin metabolism almost twofold in C3H/He mice; this was reflected in doubling of both the 3-hydroxylation pathway metabolites and the nonidentified metabolites. 7OHC formation was unaffected. In contrast, βNF had no significant effect on coumarin metabolism in DBA/2 mice although, with the exception of 7OHC, there was a tendency toward decreased metabolite formation. In C3H/He mice, ARO significantly increased total coumarin metabolism in a dose-related way by a maximum of 2.5-fold; the 7-hydroxylation pathway was the most responsive, with a 5-fold increase compared to a 3-fold increase in nonidentified metabolites and a maximum 2.5-fold increase in 3-hydroxylation pathway metabolite formation. As a percentage of total metabolism, 3-hydroxy pathway metabolites actually fell (from 61 to 53% of total). In comparison, DBA/2 mice were much more refractory to the inducing effects of ARO; 7OHC formation and nonidentified metabolites were significantly increased in DBA/2 mice receiving 3 × 125 mg/kg ARO, but when expressed as fold increase over control values, the induction was smaller than that seen in C3H/He mice receiving less than half (3 × 54 mg/kg) the dose of ARO (Table 2). In DBA/2 mice, ARO, like βNF, did not significantly alter 3-hydroxylation pathway metabolites.

Calculation of 3-hydroxylation pathway metabolites relative to 7OHC (Table 2) showed that in C3H/He mice, βNF increased the ratio while ARO had the contrary effect. Both βNF and ARO decreased the 3-/7-hydroxylation pathway ratio in DBA/2 mice but significantly so only in the latter case.

Covalent binding of [3-14C]coumarin metabolites to liver microsomal proteins was significantly increased in βNF-treated C3H/He mice but was decreased in DBA/2 mice. The relative changes in covalent binding paralleled the changes in 3-hydroxylation pathway metabolites. In C3H/He mice, but not in DBA/2 mice, ARO significantly increased covalent binding and again this was paralleled by an increase in 3-hydroxylation pathway metabolites.

DISCUSSION

Pretreatment with the enzyme inducers βNF and ARO differentially modulated the modest hepatotoxic response to a 200 mg/kg dose of coumarin in two strains of mice. Based on plasma aminotransferases and liver histopathology, in C3H/He mice βNF pretreatment potentiated coumarin hepatotoxicity and resulted in periportal necrosis. In contrast, ARO pretreatment resulted in occasional midzonal necrosis but did not significantly alter the severity of coumarin-induced damage. In DBA/2 mice, pretreatment with either βNF or ARO failed to significantly influence susceptibility to coumarin.

The modest increases in plasma aminotransferase levels seen in both C3H/He and DBA/2 mice indicate that, at the dose used, coumarin is only mildly hepatotoxic in these strains of mice although there was considerable individual variation. Coumarin hepatotoxicity was largely manifested
FIG. 2. C3H/He mouse livers showing (a) normal control, (b) subcapsular linear, and (c) centrilobular hepatocyte necrosis following coumarin treatment alone and coumarin-induced (d) peripoital and (e) midzonal hepatocyte necrosis following pretreatment with βNF and ARO, respectively. V, central vein; P, portal tract. Original magnification ×10. H&E staining.
FIG. 3. Metabolites of the coumarin 3-hydroxylation pathway: 3-hydroxycoumarin (3-HC), o-hydroxyphenyllactic acid (o-HPLA), o-hydroxyphenylacetaldehyde (o-HPA), o-hydroxyphenylethanol (o-HPE), and o-hydroxyphenylacetic acid (o-HPAA).

Histopathologically as subcapsular linear hepatocyte necrosis. This characteristic lesion is most likely to have resulted from direct diffusion of coumarin across the stomach wall, since the lobe or lobes affected were those in direct apposition to the serosal surface of the stomach. A similar lesion has been observed in rats and mice after oral dosing of another lipophillic substance, furan (Wilson et al., 1992), and has also been attributed to direct transgastric diffusion.

Endell and Seidel (1978) showed that, following an oral dose of 350 mg/kg, C3H/He mice were more sensitive to coumarin hepatotoxicity than DBA/2 mice; however, these researchers were unable to demonstrate any strain difference after a lower dose of 250 mg/kg. In contrast, our results indicate that susceptibility to coumarin hepatotoxicity does differ in these strains at a dose of 200 mg/kg, but that the choice of pretreatment/treatment vehicle and its repeated administration were determining factors in causing the difference. There was no difference in overall hepatotoxicity between the strains when ground nut oil was used as the vehicle. However, when corn oil was the vehicle, coumarin caused more marked hepatotoxicity in C3H/He mice than in DBA/2 mice. Metabolically, this difference in hepatotoxicity was consistent with a 2-fold higher rate of 7-hydroxylating activity in DBA/2 mice on corn oil in this study and in accord with earlier reports (Wood and Conney, 1974; van Iersel et al., 1994b).

The potentiation of coumarin hepatotoxicity by βNF pretreatment of C3H/He mice correlated with an increase in 3-hydroxylation pathway metabolites, which is consistent with the hypothesis that this metabolic route involves the formation of a reactive coumarin metabolite, possibly the 3,4-epoxide (Lake et al., 1992a,b, 1989b; Peters et al., 1991; Fentem et al., 1991). In βNF-pretreated DBA/2 mice, potentiation of toxicity did not occur, although a decrease in 3-hydroxylation pathway metabolites tends to suggest that βNF was weakly protective in this strain. However, it was evident that pretreatment-induced changes in coumarin 3-hydroxylation pathway metabolite formation were proportionately far greater than the more modest changes in plasma marker enzymes. This suggests that the amount of coumarin 3-hydroxylation pathway metabolites is unlikely to be the sole factor determining coumarin toxicity.

The inducibility of the 3-hydroxylation pathway by βNF in C3H/He but not DBA/2 mice suggests that it may be linked to the Ah locus, which is known to be absent in or refractory to inducing agents in DBA/2 mice (Madra and Smith, 1992). Enzymes that are Ah locus-linked include the CYP1A subfamily of cytochrome P450 isoenzymes and epoxide hydrolase, both of which could plausibly be involved in the 3-hydroxylation pathway.

In C3H/He mice, ARO induction of the 3-hydroxylation
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The pathway was accompanied by a reduction in coumarin hepatotoxicity rather than an increase. This effect is consistent with the quantitatively greater simultaneous induction of 7-hydroxylation mediated by CYP2A5 (Hahnenmann et al., 1992; Raunio et al., 1988) and thought to represent a detoxication route (Cohen, 1979). These results indicate that the ratio of the 3-7-hydroxylation pathways has more bearing on the resulting coumarin hepatotoxicity than do changes in 3-hydroxylation activity alone. This is also consistent with the vehicle-related differences in coumarin metabolism and hepatotoxicity. However, there was a reduced 3-7-hydroxylation ratio in ARO-treated C3H/He mice associated with protection against coumarin hepatotoxicity.

It should be noted that formation of the other nonidentified metabolites, which accounted for >30% of the total coumarin metabolites in vitro, was also increased by both βNF and ARO treatment of C3H/He mice. The changes in these “other” metabolites paralleled those of 3-hydroxylation product formation and since the metabolic pathways of coumarin have not all been fully elucidated, their involvement in coumarin hepatotoxicity should not be discounted. Changes in covalent binding of coumarin metabolites to microsomal proteins correlated strongly with changes in nonidentified and 3-hydroxylation pathway metabolite formation. Thus, as Lake et al. (1992a) have indicated, covalent binding per se cannot be considered an index of hepatotoxicity.

Coumarin caused Zone 1 necrosis in βNF-pretreated C3H/He mice, which has not previously been reported. Thus, βNF pretreatment increased the sensitivity of periportal hepatocytes while centrolobular cells remained protected. A similar modulation of coumarin toxicity was observed in 20-methylcholangthrene- and ARO-pretreated rats (Lake and Evans, 1993). The lobular location of the liver damage is likely to be related to the relative modulation of enzymes involved in activation and detoxication. Induction of cytochromes P450 most frequently occurs within the same cells where it is highly constitutively expressed, i.e., the centrolobular zone (Buhler et al., 1992; Horsmans et al., 1992), although there are exceptions, such as the peripheral portal expression of CYP2B in rats repeatedly given butylated hydroxytoluene (Powell et al., 1986), in phenobarbitol-induced mouse (Charles and Powell, 1994), and the reports of stronger periportal induction of several P450 isoforms resulting in their even lobular distribution (Buhler et al., 1992; Baron et al., 1982).

In human microsomes, 7-hydroxylase CYP2A6 operates at low substrate concentration and is saturable at higher concentrations, where the 3-hydroxylation pathway products are more likely to be formed (Lake et al., 1992a; Pearce et al., 1992; Yamano et al., 1990; Raunio et al., 1988). In naïve C3H/He and DBA/2 mice, the constitutive 3-hydroxylation pathway predominates. At the dose we have used, reactive
coumarin metabolites formed are presumably handled by the glutathione and other detoxication systems present in the liver with little or no resultant damage. However, in βNF-treated C3H/He mice, the capacity for toxic coumarin metabolite formation is increased and the ensuing coumarin-induced periportal damage results from an overwhelming of the protective mechanisms in this area of the liver. ARO pretreatment also increases the 3-hydroxylation pathway in the C3H/He mouse, but since portal damage does not occur, it is plausible that a concomitant increase in the high-affinity 7-hydroxylase enzyme is sufficient to reduce substrate availability for the 3-hydroxylation pathway.

In summary, although coumarin hepatotoxicity was modest, ARO or βNF pretreatment caused a shift from the centrilobular to midzonal or periportal regions (Zone 3 to 2 or 1), respectively. The shift in location of liver damage most probably reflects modulation of the translobular ratio of enzymes involved in the activation and detoxication of coumarin and seems likely to include CYP1A and CYP2A5 in the coumarin 3- and 7-hydroxylation pathways, respectively.

The mechanisms underlying the idiosyncratic cases of coumarin-related hepatotoxicity are poorly understood and the lobular site of damage is not yet characterized. However, the metabolic handling of coumarin in humans is highly variable and shifts in the balance of the different pathways of metabolism are likely to be influenced by environmental and pathological factors and, as in the mouse, previous drug exposure and genetic predisposition. The toxicological outcome of human coumarin exposure may therefore be determined by factors which conspire to increase the generally low ratio of 3- to 7-coumarin hydroxylation metabolites.

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