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Differential Metabolism of the Pyrrolizidine Alkaloid, Senecionine, in Fischer 344 and Sprague-Dawley Rats

Woon-Gye Chung and Donald R. Buhler

Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR 97331, U.S.A.

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The pyrrolizidine alkaloids (PAs), contained in a number of traditional remedies in Africa and Asia, show wide variations in metabolism between animal species but little work has been done to investigate differences between animal strains. The metabolism of the PA senecionine (SN) in Fischer 344 (F344) rats has been studied in order to compare to that found in the previously investigated Sprague-Dawley (SD) rats (*Drug Metab. Dispos.* **17**: 387, 1989). There was no difference in the formation of (±) 6,7-dihydro-7-hydroxy-1-hydroxymethyl-5*H*-pyrrolizine (DHP, bioactivation) by hepatic microsomes from either sex of SD and F344 rats. However, hepatic microsomes from male and female F344 rats had greater activity in the *N*-oxidation (detoxication) of SN by 88% and 180%, respectively, when compared to that of male and female SD rats. Experiments conducted at various pH showed an optimum pH of 8.5, the optimal pH for flavin-containing monooxygenase (FMO), for SN *N*-oxidation by hepatic microsomes from F344 females. In F344 males, however, a bimodal pattern was obtained with activity peaks at pH 7.6 and 8.5 reflecting the possible involvement of both cytochrome P450 (CYP) and FMO. Use of specific inhibitors (SKF525A, 1-benzylimidazole and methimazole) showed that the *N*-oxide of SN was primarily produced by FMO in both sexes of F344 rats. In contrast, SN *N*-oxide formation is known to be catalyzed mainly by CYP2C11 rather than FMO in SD rats. This study, therefore, demonstrated that there were substantial differences in the formation of SN *N*-oxide by hepatic microsomes from F344 and SD rats and that this detoxification is catalyzed primarily by two different enzymes in the two rat strains. These findings suggest that significant variations in PA biotransformation can exist between different animal strains.

Key words: Pyrrolizidine alkaloids (PAs), Metabolism, Species difference, Flavin-containing monooxygenase (FMO), CYP2C11

INTRODUCTION

Pyrrolizidine alkaloids (PAs) are constituents of certain plant species such as the families Boraginaceae, Compositae, and Leguminosae (Mattocks, 1986). A number of traditional herbal remedies used in Africa (Steenkamp *et al.*, 2001), Australia (Noller *et al.*, 2001), Sri Lanka (Arseculeratne *et al.*, 1985) and China (Roeder, 2000) contain PAs. Human exposure also can occur through the ingestion of foodstuffs such as herbal teas, milk, honey and contaminated grain in addition to herbal remedies (Coulombe, 2003). Many of these alkaloids such as monocrotaline, retrorsine and SN are toxic to both animals and humans.

A marked variation exists in the sensitivity of animal species to the toxic effects of PAs. Horses, cattle, rats and humans are known to be very susceptible but guinea pigs, rabbits and sheep are very resistant to the toxicities of the PAs (Mattocks, 1986; Debessai *et al.*, 1999). It is interesting that guinea pigs which are known to be very resistant to the toxicity of many PAs such as monocrotaline, retrorsine and SN, are surprisingly susceptible to the PA jacobine due to its low detoxification and high bioactivation capacities (Chung and Buhler, 1995).

PAs are metabolized by liver microsomal monooxygenase yielding PA pyrroles and *N*-oxides (Fig. 1, Mattocks and Bird, 1983; Huan *et al.*, 1998). PA pyrroles are considered to be the ultimate toxic metabolites (bioactivation) whereas *N*-oxides are believed to be non-toxic (detoxification). The

Correspondence to: Donald R. Buhler, Ph.D., Department of Environmental and Molecular Toxicology, ALS 1007, Oregon State University, Corvallis, OR 97331, USA
Tel: 1-541-737-1784; Fax: 1-541-737-0497
E-mail: donald.buhler@orst.edu

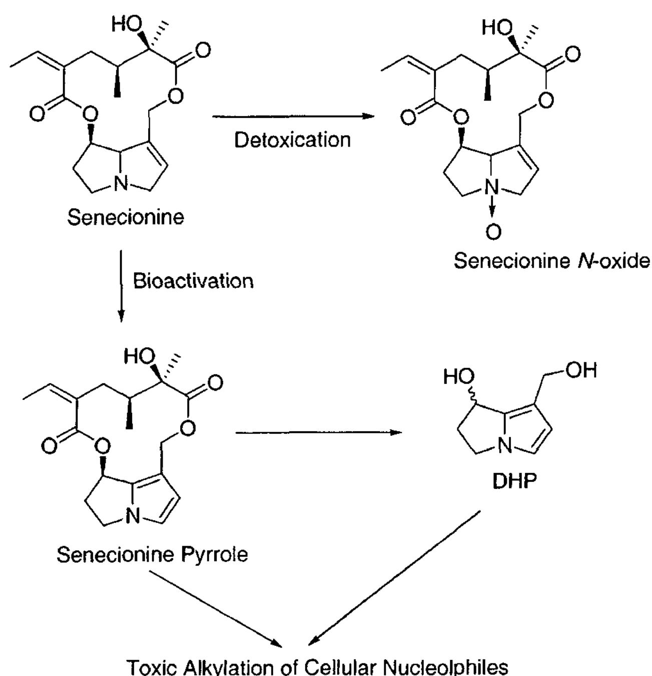

Fig. 1. Metabolic pathways of senecionine (SN). DHP denotes (±) 6,7-dihydro-7-hydroxy-1-hydroxymethyl-5*H*-pyrrolizine.

PA pyrroles either react with cellular macromolecules or are hydrolyzed to form a secondary pyrrole known as (±) 6,7-dihydro-7-hydroxy-1-hydroxymethyl-5*H*-pyrrolizine (DHP). DHP also is a strong electrophile capable of alkylating cellular nucleophiles including DNA (White and Mattocks, 1972; Chou *et al.*, 2003).

A marked variation exists in the metabolizing enzymes of the PAs in animal species and humans. In Sprague-Dawley (SD) rats (Williams *et al.*, 1989a; Stegelmeier *et al.*, 1999) and humans (Miranda *et al.*, 1991b), the microsomal formation of DHP and *N*-oxide from the PA SN were catalyzed primarily by enzymes belonging to the CYP3A subfamily. In SD rat liver microsomes, CYP2C11 was the major catalyst for SN *N*-oxidation and flavin-containing monooxygenase (FMO) accounted for no more than 20% of the PA *N*-oxidase activity (Williams *et al.*, 1989a and 1989b). However, FMO was the major catalyst of the *N*-oxidation of SN in guinea pig (Miranda *et al.*, 1991a) and CYP2B was for DHP formation in guinea pigs (Chung *et al.*, 1995). Evidence was presented that rat strains are highly polymorphic in electrophoretic recognizable isoforms of CYP (Rampersaud and Walz, 1987) and esterases (Simon *et al.*, 1985). Microsomal drug metabolism in a large number of rat inbred strains varied over an 1.3- to 7.3-fold range depending on the enzyme activity being measured (Koster *et al.*, 1989). Fischer 344 (F344) rats were found to be significantly different from SD rats in terms of the effect of cyclosporine on ethylmorphine *N*-demethylase and aniline hydroxylase activities (Augustine and Zemaitis, 1989). F344 rats developed substantial liver damage associated with the oxidative stress of diquat while SD rats produced only minimal liver necrosis (Tsokos-Kuhn, 1988). The hepatotoxicity injury of 1,2-dichlorobenzene is greater in F344 than in SD rats (Kulkarni *et al.*, 1997). Long-Evans and Han/Wistar rats exhibit an over 1000-fold difference in their LD50 values for TCDD (Anxiously *et al.*, 1993). Differences in terms of species susceptibility are well documented relative to PAs poisoning. However, little work has been done investigating strain differences in PAs metabolism. In this study, we present the evidence that different hepatic enzyme system catalyzed the *N*-oxidation of SN in different rat strains, with FMO being the major enzyme in F344 rats and CYP2C11 in SD rats.

MATERIALS AND METHODS

Chemicals

SN was purified from extracts of *Senecio jacobaea* and the DHP and *N*-oxide metabolite standards were prepared as previously described (Kedzierski and Buhler, 1986). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP+, 1-benzylimidazole, methimazole, SKF525A and thiobenzamide were obtained from the Sigma Chemical Co. (St. Louis, MO). Testosterone and its metabolites were purchased from Steroid Reference Collection (London, UK).

Microsomes preparation

F344 and SD rats (male and female, 8 weeks old from Simonson Inc., Gilroy, CA) were starved 24 h prior to sacrificing to reduce liver glucagon stores. Liver was homogenized with 0.1 mM potassium phosphate buffer, pH 7.4, containing 0.15 M KCl and 1 mM EDTA. Liver microsomes were prepared by standard differential centrifugation and stored in 0.1 M potassium phosphate buffer, pH 7.4, containing 20% glycerol and 0.1 mM EDTA at -80°C as described by Chung and Buhler (1994).

Enzyme assays

The *in vitro* metabolism of SN by microsomes from rat liver was carried out using the procedure described by Chung and Buhler (1994). A typical incubation mixtures contained 0.3 mg microsomal protein; 0.1 M potassium phosphate buffer, pH 7.6 or 8.6; 0.5 mM SN; 1 mM EDTA; and a NADPH-generating system (10 mM glucose-6-phosphate, 1.0 units/mg glucose-6-phosphate dehydrogenase and 1 mM NADP+) in a total volume of 0.5 ml. After a 1 h incubation (formations of DHP and *N*-oxide were linear up to 1 h under the assay conditions used) at 37°C, the reaction was terminated by rapid cooling on ice. The

mixture was centrifuged at 46,000 g for 45 min at 4°C and an aliquot of the supernatant was analyzed by HPLC (Kedzierski and Buhler, 1986). When inhibitors (0.1 mM 1-benzylimidazole, 0.25 mM methimazole, or 0.5 mM SKF525A) were added, the incubation mixture was first pre-incubated with the chemicals at 37°C for 20 min before the addition of SN. Microsomal protein contents were determined by the method of Lowry *et al.* (1951). Total CYP content was estimated using a spectral method (Omura and Sato, 1964). Testosterone assays were carried out by the method of Wood *et al.* (1983). Thiobenzamide S-oxide formation was measured at 370 nm by the procedure of Cashman and Hanzlik (1981) at 37°C in Tricine buffer (pH 8.4) with 2 mM NADPH in the absence of *n*-octylamine. Statistical analysis was carried out using student's *t*-test.

RESULTS

SN metabolism by microsomes

In both rat sexes, there was no significant difference in DHP formation from SN by hepatic microsomes from SD and F344 rats (Fig. 2). Male rats, however, yield higher DHP concentration than females (Fig. 2). However, in male rats 88% (0.05<p-value) more SN *N*-oxide was produced by hepatic microsomes from F344 than from the SD strain, while in females, 180% (0.05<p) more SN *N*-oxide formed by liver microsomes from the F344 strain animals than from the SD strain of rats. These results may imply that different enzyme systems and/or specificities between SD and F344 strains are responsible for SN *N*-oxidation, or more enzymes active in SN *N*-oxidation exist in F344 rats.

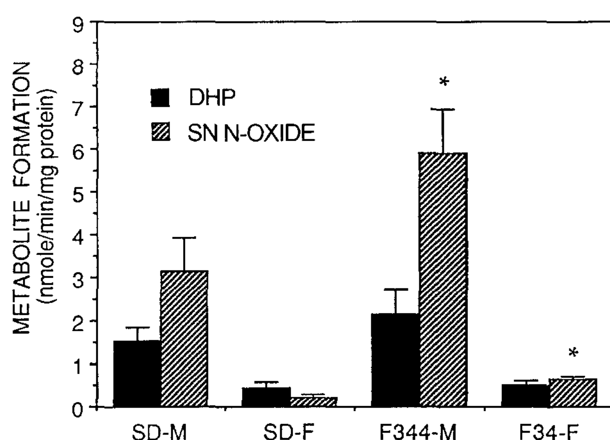

Fig. 2. Metabolism of senecionine (SN) by hepatic microsomes from Sprague-Dawley (SD) and Fischer 344 (F344) rats was studied as described in Methods. Male (M) and female (F) rats were compared. Asterisks at the top of the bars represent a significant difference from the corresponding sex of the SD rats (p<0.05).

Inhibition studies

In order to examine whether different enzyme systems existing in these two strains were responsible for SN *N*-oxide formation, we employed specific chemical inhibitors for CYPs and FMOs in SN metabolism by F344 hepatic microsomes. SKF525A and 1-benzylimidazole, specific inhibitors for total CYPs, decreased DHP formation by 81-95% (5-19% of control) in F344 males and 69-82% (18-31% of control) in F344 females, respectively. However, SN *N*-oxidation was inhibited only 0-30% by CYP inhibitors in male and 40-45% in female F344 rats (Table I). Methimazole, a competitive inhibitor for FMO, had no inhibitory activity against DHP production by hepatic microsomes from both sexes of F344. However, methimazole decreased SN *N*-oxidation by 85 and 96% in male and female F344 animals, respectively. Therefore, we conclude that DHP formation in F344 rats of both sexes is catalyzed by CYP enzymes while SN *N*-oxide is primarily produced by FMO.

Optimum pH for DHP and SN *N*-oxide formation

We carried out a determination of the optimum pH for F344 rat microsomal PA metabolism since CYP (pH 7.2-7.6) and FMO (pH 8.4-9.2) were known to have different optimum pH for maximum activities. Maximum formation of DHP occurred at a pH of 7.6-7.8 in both sexes of F344 rats (Fig. 3A and 3B). However, the maximum for SN *N*-oxide production in female F344 (Fig. 3B) was pH 8.5 whereas SN *N*-oxide showed a bimodal pattern with peaks at pH 7.6 and 8.5, with a 10% greater *N*-oxidation than that observed at pH 7.6 in male F344 (Fig. 3A). These results are also supportive of the conclusion that DHP formation was catalyzed by CYPs in both sexes of F344 rats and that SN *N*-oxide was primarily produced by FMO in female F344 rats. The optimum pH study reflected the possible involvement of both FMO and CYP in SN *N*-oxidation in male F344 rats.

Table I. Effect of inhibitors on the senecionine metabolism by hepatic microsomes from Fischer 344 rats

(Enzyme) Inhibitor (mM)	Specific Metabolism Rate, nmole/min/mg protein			
	Male		Female	
	DHP	SN NO	DHP	SN NO
None	2.13 ± 0.3 (100%)	5.91 ± 0.8 (100%)	0.50 ± 0.1 (100%)	0.64 ± 0.1 (100%)
(CYP) SKF525A (0.5)	0.40 ± 0.06 (19%)	7.32 ± 0.95 (124%)	0.16 ± 0.02 (31%)	0.38 ± 0.06 (60%)
(CYP) 1-Benzylimidazole (0.1)	0.01 ± 0.01 (5%)	4.20 ± 0.6 (71%)	0.09 ± 0.01 (18%)	0.35 ± 0.05 (55%)
(FMO) Methimazole (0.25)	2.49 ± 0.32 (117%)	0.89 ± 0.13 (15%)	0.50 ± 0.08 (100%)	0.03 ± 0.01 (4%)

Data were expressed as mean ± SD from four determinations.

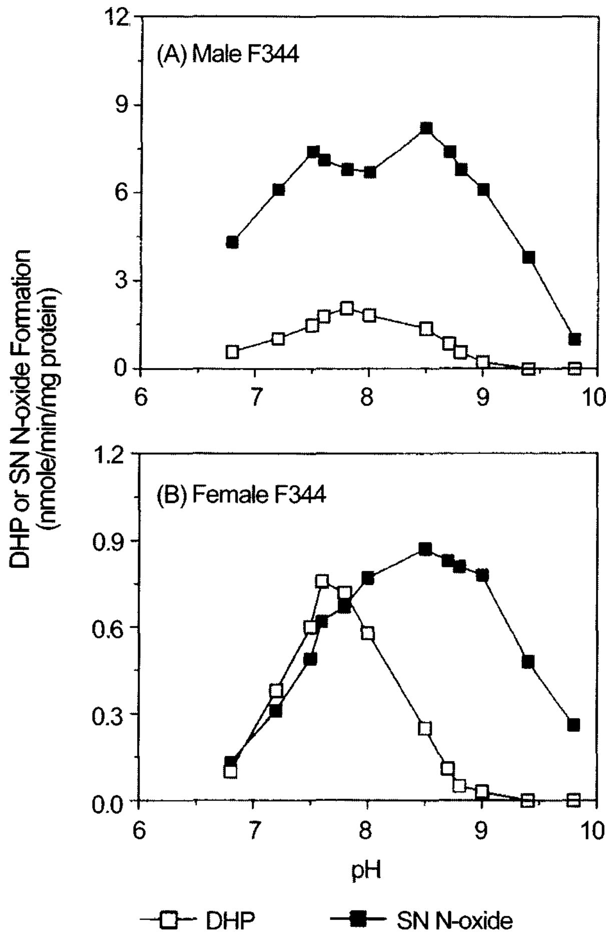

Fig. 3. Effect of pH on DHP and SN *N*-oxide formation by liver microsomes. Microsomes (0.3 mg) from F344 rats were employed in SN (0.5 mM) metabolism. Reactions were carried out using liver microsomes (0.3 mg) in potassium phosphate (pH 6.8 to 8.2), Tricine (pH 8.4 to 8.8) or glycine (pH 9.0 to 9.8) buffer. Analyses were conducted as described in Methods.

Thiobenzamide *S*-oxidation assay

We assayed thiobenzamide *S*-oxidation for further evaluation and comparison of FMO activity in SD and F344 rats. Thiobenzamide *S*-oxidation is a specific assay for non-specific FMO isozymes (Cashman and Hanzlik, 1981). Hepatic microsomes from male F344 rats produced 56% more thiobenzamide *S*-oxide than that by male SD rats while there was no difference between female rats of the two strains (Fig. 4). These results imply that higher FMO activity in male F344 rats may result in higher (72%) SN *N*-oxidation, compared to that of male SD rats.

Testosterone hydroxylase activity

We measured testosterone hydroxylase as an indicator substrate to estimate the relative contributions of different CYP isozymes. Testosterone 6β-hydroxylase activity, which is mostly catalyzed by CYP3A2 in rats (Guengerich *et al.*,

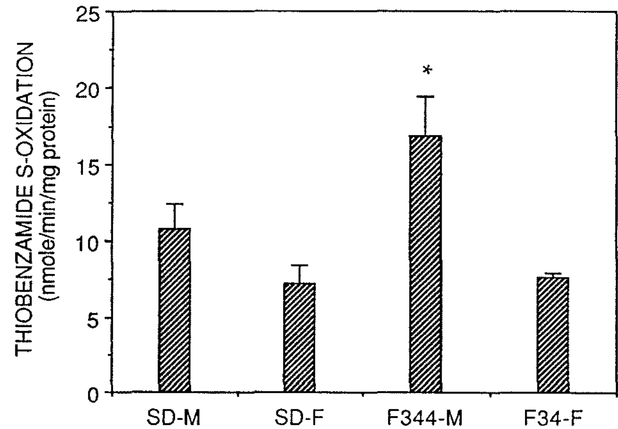

Fig. 4. Metabolism of thiobenzamide by hepatic microsomes from Sprague-Dawley (SD) and Fischer 344 (F344) rats was studied as described in Methods. Male (M) and female (F) rats were compared. Asterisk at the top of the bar represents a significant difference from the corresponding sex of the SD rats ($p<0.05$).

1982), is 50% higher in male SD than that in male F344 rats (Fig. 5). On the other hand, testosterone 16α- and 16β-hydroxylase activities, in which CYP2C11 and CYP2B1 participates (Maxman, 1984), are 120% and 84% higher in male F344 than in male SD rats, respectively. These results suggest that the activity of CYP3A2, which is an important enzyme for DHP formation in SD rats (Williams *et al.*, 1989a), is higher in male SD rats than in male F344 rats while the activity of CYP2C11, which is responsible for SN *N*-oxidation in SD rats (Williams *et al.*, 1989a), is higher in F344 strain than in SD strain.

In female rats, testosterone 7α-hydroxylase (CYP2A1) and 6β-hydroxylase (CYP3A2) activities were 80% and 1500% higher in F344 rats than those in SD rats, respec-

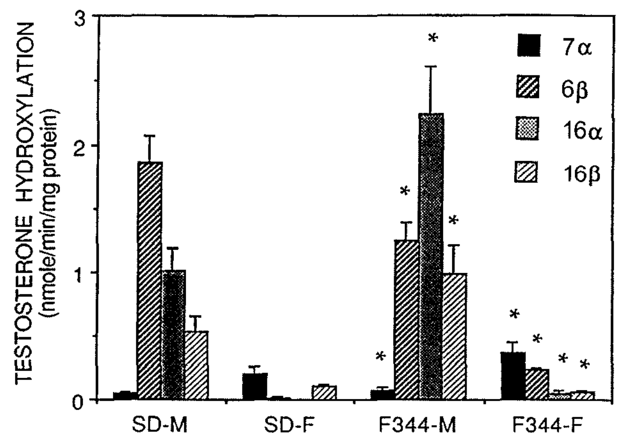

Fig. 5. Metabolism of testosterone by hepatic microsomes from Sprague-Dawley (SD) and Fischer 344 (F344) rats was studied as described in Methods. Male (M) and female (F) rats were compared. 7α, 6β, 16α and 16β denoted hydroxylated positions of testosterone. Asterisks at the top of the bars represent a significant difference from the corresponding sex of the SD rats ($p<0.05$).

tively, whereas 16β-hydroxylase (CYP2B1) was 75% higher in SD than that in F344. Testosterone 16α-hydroxylase (CYP2C11) activity was not observed in female SD rats whereas some activity was present in female F344 rats. These results imply that the male specific monooxygenase activities, such as CYP3A2 and CYP2C11, are much higher in female F344 rats while the female specific monooxygenase activity like CYP2A1 is higher in female SD than in female F344 rats. Thus, the higher activity of CYP3A and CYP2C11 which were known to be important enzymes for SN metabolism may contribute to SN metabolism together with FMO in female F344 rats.

DISCUSSION

There are several possibilities to account for species differences in susceptibility and resistance of some animals to PA toxicity. These include differences in absorption, degradation, hepatic formation of pyrroles or PA *N*-oxides, or conjugation and excretion of PAs and pyrroles (Mattocks, 1986). Strain differences in PA toxicity may be explained in a similar fashion.

We have assayed microsomal activities toward SN in order to verify the hypothesis that there are strain differences in PAs metabolism between F344 and SD rats. While there were no differences in DHP formation by hepatic microsomes in both male and female of SD and F344 rats, both sexes of F344 rats showed a greater *N*-oxidation of the PA SN than that observed in SD rats. Pan *et al.* (1993) demonstrated that F344 rats were resistant to the cardiovascular toxicity of a reactive PA metabolite, monocrotaline pyrrole, at doses that cause severe damage in SD rats, perhaps due to the presence of more refractory pulmonary endothelial cells in F344 rats. It is, therefore, possible that F344 rats may be unusually resistant to the toxicities of PAs, both because of the faster detoxication (higher rates of SN *N*-oxidation) that we have observed, and in addition, having tissues more refractory to PA intoxication as described by Pan *et al.* (1993).

Methimazole was reported to inhibit human CYP2C9, CYP2C19 and CYP3A4 activities as well as FMO (Guo *et al.*, 1997). However, methimazole can be employed as a safe inhibitor of FMO if it used with total CYP inhibitors like SKF525A and 1-benzylimidazole. The optimum pH for CYP is known to be between 7.2 and 7.6 (Sato and Omura, 1978). This contrasts with FMO which possesses a pH optimum usually around 8.4-9.2 depending on the species and tissue (Ziegler, 1988). Using this information, the inhibition of enzyme activity by chemicals and optimum pH experiment were conducted to discriminate the role of CYP and FMO in DHP and SN *N*-oxide formations. Evidence based on enzyme inhibition data and optimal pH suggests that hepatic microsomal DHP formation was catalyzed primarily by CYP systems in both sexes of F344 rats as previously demonstrated in SD rats (Williams *et al.*, 1989a) and in guinea pigs (Miranda *et al.*, 1991a, Chung *et al.*, 1995). SN *N*-oxidation was primarily catalyzed by FMO in both sexes of F344 rats as reflected by an observed 8.5 pH optimum for *N*-oxide formation.

The presence of a second optimum at pH 7.6 suggests that in the male F344 rats CYP2C11 likely also contributed to the PA *N*-oxide production. However, inhibition studies ruled out the possibility of involvement of the CYP enzyme as a major factor in the SN *N*-oxidation in male F344 rats. This contrasts with SD rats where SN *N*-oxidation is carried out primarily by CYP2C11 (Williams *et al.*, 1989a). The results obtained with F344 rats are very similar to the situation in guinea pigs (Miranda *et al.*, 1991a) which are well known to be very resistant to PA intoxication (Chesney and Allen, 1973; McLean, 1970; White *et al.*, 1973).

Lawton and Philpot (1993a) suggest that all five FMO genes (FMO1, 2, 3, 4 and 5) present in the rabbit are also present in humans, guinea pig, hamsters, rats and mice based on blot analysis of genomic DNA. With respective to substrate specificities of FMOs, the overall size of the nucleophile appears to be a major factor limiting access to the 4a-hydroperoxyflavin in different FMO isoforms (Nagata *et al.*, 1990; Ziegler, 1993). From the study of the substrate specificities of four FMO isoforms in the SN metabolism using FMO expressed microsomes (FMO1, 2, 3 and 4), which showed distinct substrate specificities against methimazole and *n*-octylamine (Lawton and Philpot, 1993b), only FMO1 expressed microsomes exhibited some SN *N*-oxidation activities (Woon-Gye Chung, Michael P. Lawton, Richard M. Philpot and Donald R. Buhler, unpublished data). Therefore, not all FMO isozymes may be active against SN. This finding may imply that F344 rats, which expressed FMO1 in liver (Larsen-Su and Williams, 1996), have different major hepatic isoform or different specificity of FMO, compared to those of SD rats.

Thiobenzamide *S*-oxidation assay, a non-specific FMO activity, showed that male F344 rats had greater FMO activity compared to that of male SD rats. The greater FMO activity in male F344 rats may also contribute to greater SN *N*-oxidation than that of SD rats. However, the interpretation of thiobenzamide *S*-oxidase activity is rather equivocal since there was no difference in FMO activity between females of these two strains, even though FMO appeared to be responsible for SN *N*-oxidation in female F344 rats. Therefore, we suggest that F344 rats, at least the females, may have distinct FMO isoforms, which are highly active against the PA SN, from SD rats. Final resolution of this possibility may require cloning and expression or purification of the F344 FMOs and confirmation of substrate specificity toward PAs in both rat strains.

The total amount of two metabolites, DHP and SN *N*-oxide, formed from the PA SN in male SD rats was only 58% of that found in male F344 rats. Thus, higher CYP3A2 levels as reflected by the high testosterone 6β-hydroxylase activity in male SD rats may contribute to the fact that no significant difference of DHP formation occurred between two strains. The testosterone 16α-hydroxylase activity (CYP2C11) is higher in male F344 than in male SD rats. If FMO cannot catalyze SN *N*-oxidation exclusively and CYP plays even a minor role, the higher activity of CYP2C11 may partially explain the higher SN *N*-oxidation found in F344 rats.

The combined results of the thiobenzamide and testosterone assays suggest that F344 rats have a higher capacity for SN *N*-oxide formation, resulting in detoxification, and that SD rats have higher rates of DHP production, resulting in bioactivation. The results from the examination of SN *N*-oxidation supported this expectation (Fig. 2).

In conclusion, in F344 rats FMO was the major catalyst for SN *N*-oxidation whereas CYP systems catalyzed formation of pyrrolic metabolites. Unlike SD rats, F344 rats exhibited SN oxidation that was catalyzed by FMO, an observation similar to that found with guinea pigs (Miranda *et al.*, 1991a), a species that is resistant to PA toxicity. This study, therefore, demonstrated that substantial differences exist in the enzymes involved and amounts of detoxified metabolite formed in the *N*-oxidation of the PA SN between F344 and SD rats. This study also demonstrates that the abundant differences in PA metabolism can exist between animal strains.

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REFERENCES

Anxiously, M., Pohjanvirta, R., Honkakoski, P., Torronen, R., and Tuomisto, J. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) induced ethoxyresorufin-*O*-deethylase (EROD) and aldehyde dehydrogenase (ALDH3) activities in the brain and liver. A comparison between the most TCDD-susceptible and the most TCDD-resistant rat strain. *Biochem. Pharmacol.*, 46, 651-659 (1993).

Arseculeratne, S. N., Gunatilaka, A. A., and Panabokke, R. G. Toxicity of some traditional medicinal herbs. *J. Ethnopharmacol.,* 13, 323-335 (1985).

Augustine, J. A. and Zemaitis, M. A. A comparison of the effects of cyclosporine on the hepatic microsomal drug metabolism in three different strains of rat. *Gen. Pharmacol.*, 20, 137-141 (1989).

Cashman, J. R. and Hanzlik, R. P. Microsomal oxidation of thiobenzamide: A photometric assay for the flavin-containing monooxygenase. Biochem. *Biophys. Res. Commun.*, 98, 147-153 (1981).

Chesney, C. F. and Allen, J. R. Resistance of the guinea pig to pyrrolizidine alkaloid intoxication. *Toxicol. Appl. Pharmacol.*, 26, 385-392 (1973).

Chou, M. W., Wang, Y. P., Yan, J., Yang, Y. C., Beger, R. D., Williams, L. D., Doerge, D. R., and Fu, P. P. Riddellineoxide is a phytochemical and mammalian metabolite with genotoxic activity that is comparable to the parent pyrrolizidine alkaloid riddelline. *Toxicol. Lett.*, 145, 239-247 (2003).

Chung, W. G. and Buhler, D. R., The effect of spironolactone treatment on the cytochrome P450-mediated metabolism of the pyrrolizidine alkaloid senecionine by hepatic microsomes from rats and guinea pigs. *Toxicol. Appl. Pharmacol.*, 127, 314-319 (1994).

Chung, W. G. and Buhler, D. R., Major factors for the susceptibility of guinea pig to the pyrrolizidine alkaloid jacobine. *Drug Metab. Dispos.*, 23,1263-1267 (1995).

Chung, W. G., Miranda, C. L., and Buhler, D. R., A cytochrome P4502B form is the major bioactivation enzyme for the pyrrolizidine alkaloid senecionine in guinea pig. *Xenobiotica* 25, 929-939 (1995).

Coulombe, R. A. Jr., Pyrrolizidine alkaloids in foods. *Adv. Food Nutr. Res.*, 45, 61-99 (2003).

Debessae, W. T., Huan, J., and Cheeke, P. R., Interactions in sheep between tall fescue ergot alkaloids and hepatotoxic carbon tetrachloride and Senecio pyrrolizidine alkaloids. *Vet. Hum. Toxicol.*, 41, 129-133 (1999).

Guengerich, F. P., Dannan, G. A., Wright, S. T., Martin, M. V., and Kaminsky, L. S., Purification and characterization of microsomal cytochrome P450s. *Xenobiotica*, 12, 701-716 (1982).

Guo, Z., Raeissi, S., White, R. B., and Stevens, J. C., Orphenadrine and methimazole inhibit multiple cytochrome P450 enzymes in human liver microsomes. *Drug Metab. Dispos.*, 25, 390-393 (1997).

Huan, J. Y., Miranda, C. L., Buhler, D. R., and Cheeke, P. R., Species differences in the hepatic microsomal enzyme metabolism of the pyrrolizidine alkaloids. *Toxicol. Lett.* 99, 127-137 (1998).

Kedzierski, B. and Buhler, D. R., Method for determination of pyrrolizidine alkaloids and their metabolites by high-performance liquid chromatography. *Anal. Biochem.*, 152, 59-65 (1986).

Koster, A. S., Nieuwenhuis, L., and Frankhuijzen-Sierevogel, A. C., Comparison of microsomal drug-metabolizing enzymes in 14 rat inbred strains. *Biochem. Pharmacol.*, 38, 759-765 (1989).

Kulkarni, S. G., Warbritton, A., Bucci, T. J., and Mehendale, H. M.m Antimitotic intervention with colchicine alters the outcome of *o*-DCB-induced hepatotoxicity in Fischer 344

rats. *Toxicology*, 27, 79-88 (1997).

Larsen-Su, S. and Williams, D. E., Dietary indole-3-carbinol inhibits FMO activity and the expression of flavin-containing monooxygenase form 1 in rat liver and intestine. *Drug Metab. Dispos.*, 24, 927-931 (1996).

Lawton, M. P. and Philpot, R. M., Molecular genetics of flavin-dependent monooxygenases. *Pharmacogenetics*, 3, 40-44 (1993a).

Lawton, M. P. and Philpot, R. M., Functional characterization of flavin-containing monooxygenase 1B1 expressed in *Saccharomyces cerevisiae* and *Escherichia coli* and analysis of proposed FAD- and membrane-binding domains. *J. Biol. Chem.*, 268, 5728-5734 (1993b).

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.,* 193, 265-275 (1951).

Mattocks, A. R. and Bird, I., Pyrrolic and *N*-oxide metabolites formed from pyrrolizidine alkaloids by hepatic microsomes *in vitro*: relevance to *in vivo* hepatotoxicity. *Chem. Biol. Interact.*, 43, 209-222 (1983).

Mattocks, A. R., Chemistry and toxicology of pyrrolizidine alkaloids. Academic Press, New York (1986).

McLean, E. K., The toxic actions of pyrrolizidine (Senecio) alkaloids. *Pharmacol. Rev.* 22, 249-483 (1970).

Miranda, C. L., Chung, W. G., Reed, R. L., Zhao, X., Henderson, M. C., Wang, J. L., Williams, D. E., and Buhler, D. R., Flavin-containing monooxygenase: A major detoxifying enzyme for the pyrrolizidine alkaloid senecionine in guinea pig tissues. *Biochem. Biophys. Res. Commun.*, 178, 546-552 (1991a).

Miranda, C. L., Reed, R. L., Guengerich, F. P., and Buhler, D. R., Role of cytochrome P450 IIIA4 in the metabolism of the pyrrolizidine alkaloid senecionine in human liver. *Carcinogenesis*, 12, 515-519 (1991b).

Nagata, T., Williams, D. E., and Ziegler, D. M. Substrate specificities of rabbit lung and porcine liver flavin-containing monooxygenase: difference due to substrate size. *Chem. Res. Toxicol.*, 3, 372-376 (1990).

Noller, B. N., Myers, S., Abegaz, B., Singh, M. M., Dronenberg, F., and Bodeker, G. Global forum on safety of herbal and traditional medicine: July 7, 2001, Gold Coast, Australia. *J. Altern. Complement. Med.*, 7, 683-601 (2001).

Omura, R. and Sato, T., The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.*, 239, 2370-2374 (1964).

Pan, L. C., Wilson, D. W., and Segall, H. J., Strain differences in the response of Fischer 344 and Sprague-Dawley rats to monocrotaline induced pulmonary vascular disease. *Toxicology*, 79, 21-35 (1993).

Rampersaud, A. and Walz, F. G., Polymorphisms of four hepatic cytochrome P450 in twenty-eight inbred strains of rat. *Biochem. Gen.*, 25, 527-534 (1987).

Roeder, E., Medicinal plants in China containing pyrrolizidine alkaloids. *Pharmazie*, 55, 711-726 (2000).

Sato, R. and Omura, T., Cytochrome P450. Academic Press, New York, p233 (1978).

Simon, B., De Looze, S., Ronai, A., and Von Deimling, O., Identification of rat liver carboxylesterase isozymes (EC 3.1.1.1) using polyacrylamide gel electrophoresis and isoelectric focusing. *Electrophoresis*, 6, 575-582 (1985).

Steenkamp, V., Stewart, M. J., van der Merwe, S., Zuckerman, M., and Crowther, N. J., The effect of *Senecio latifolius*, a plant used as a South African traditional medicine, on a human hepatoma cell line. *J. Ethnopharmacol.*, 78, 51-58 (2001).

Stegelmeier, B. L., Edgar, J. A., Colegate, S. M., Gardner, D. R., Schoch, T. K., Coulombe, R. A., and Molyneux, R. J. Pyrrolizidine alkaloid plants, metabolism and toxicity. *J. Nat. Toxins*, 8, 95-116 (1999).

Tsokos-Kuhn, J. O., Lethal injury by diquat redox cycling in an isolated hepatocyte model. *Arch. Biochem. Biophys.,* 265, 415-424 (1988).

Waxman, D. J., Rat hepatic cytochrome P-450 isoenzyme 2c. *J. Biol. Chem.*, 259, 15481-15490 (1984).

White, I. H. N. and Mattocks, A. R., Reaction of dihyropyrrolizines with deoxyribonucleic acids *in vitro. Biochem. J.*, 129, 291-297 (1972).

White, I. N. H., Mattocks, A. R., and Butler, W. H., The conversion of the pyrrolizidine alkaloid retrorsine to pyrrolic derivatives *in vivo* and *in vitro* and its acute toxicity to various animal species. *Chem. Biol. Interact.*, 6, 207-218 (1973).

Williams, D. E., Reed, R. L., Kedzierski, B., Dannan, G. A., Guengerich, F. P., and Buhler, D. R., Bioactivation and detoxification of the pyrrolizidine alkaloid senecionine by cytochrome P450 enzymes in rat liver. *Drug Metab. Dispos.*, 17, 387-392 (1989a).

Williams, D. E., Reed, R. L., Kedzierski, B., Ziegler, D. M., and Buhler, D. R., The role of flavin-containing monooxygenase in the *N*-oxidation of the pyrrolizidine alkaloid senecionine. *Drug Metab. Dispos.*, 17, 380-386 (1989b).

Wood, A. W., Ryan, D. E., Thomas, P. E., and Levin, W., Regio- and stereo-selective metabolism of two C19 steroids by five highly purified and reconstituted rat hepatic cytochrome P450 isozymes. *J. Biol. Chem.*, 258, 8839-8847 (1986).

Ziegler, D. M., Flavin-containing monooxygenase: catalytic mechanism and substrate specificities. *Drug Metab. Rev.*, 19, 1-32 (1988).

Ziegler, D. M., Recent studies on the structure and function of multisubstrate flavin-containing monooxygenases. *Annu. Rev. Pharmacol. Toxicol.*, 33, 179-199 (1993).