

Comparative Toxicity of *N*-Hydroxy-2-acetylaminofluorene in Several Strains of Rats¹

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SUMMARY

Male Sprague-Dawley rats were 6 or 7 times more susceptible than females to the acute toxic effects of a single i.p. injection of *N*-hydroxy-2-acetylaminofluorene. The *N*-hydroxy compound was equally toxic in male and female Fischer rats and about twice as toxic to male as to female Wistar rats. A negative correlation between the 50% lethal dose of *N*-hydroxy-2-acetylaminofluorene and hepatic *N*-hydroxy-2-acetylaminofluorene sulfotransferase activity was found. These data substantiate earlier indications that the level of the liver sulfotransferase is an important factor in determining the degree of toxicity of *N*-hydroxy-2-acetylaminofluorene. It is suggested that the reported sex difference in the hepatocarcinogenicity of *N*-hydroxy-2-acetylaminofluorene might be peculiar to the Sprague-Dawley rat.

INTRODUCTION

It is generally assumed that there is a sex difference in the response of male and female rats to liver tumor induction by AAF² and *N*-hydroxy-AAF (for a general review of this topic, see Ref. 11). However, most of the studies upon which this assumption is based have been done with Sprague-Dawley rats. Gutmann *et al.* (4) have shown that female Fischer rats are likewise more resistant than male Fischer rats to hepatocarcinogenesis by AAF. However, female and male Fischer rats were found to be equally susceptible to the induction of liver cancer by *N*-hydroxy-AAF. Since it has always been tacitly assumed that there was a true sex difference in the response of male and female rats to liver carcinogenesis by *N*-hydroxy-AAF, results of the experiments of Gutmann *et al.* cast doubt on the validity of this assumption.

Although the mechanism involved in the differential response of male and female rats to liver tumor induction by *N*-hydroxy-AAF is unknown, it has been postulated to be

due to differences in hepatotoxicity in the sexes (2, 7, 8). Current evidence suggests that the hepatotoxicity and hepatocarcinogenicity of *N*-hydroxy-AAF are each markedly dependent upon the levels of *N*-hydroxy-AAF sulfotransferase activity in liver (2, 3, 7, 14). Because of the lack of comparative data in the literature, levels of hepatic *N*-hydroxy-AAF sulfotransferase in other strains of rats were determined. In addition, despite the fact that the metabolism and carcinogenicity of *N*-hydroxy-AAF have been studied extensively since 1960, there are no data in the literature on the LD₅₀ of this carcinogen. Consequently, the LD₅₀ of *N*-hydroxy-AAF in several strains of rats of both sexes has been determined and compared with the data obtained for *N*-hydroxy-AAF sulfotransferase activity.

MATERIALS AND METHODS

Animals were obtained from the following sources: Holtzman albino rats (Holtzman Company, Madison, Wis.); Carworth Sprague-Dawley-derived (CFE) and Wistar-derived (CFN) rats (Carworth, New City, N. Y.); and Fischer (F344) rats (Charles River, Wilmington, Mass.). At the time of use the Holtzman, Carworth Sprague-Dawley, and Carworth Wistar rats weighed 140 to 180 g (female) and 150 to 200 g (male). The Fischer rats weighed 120 to 130 g (female) and 160 to 180 g (male). All animals were maintained on Purina laboratory chow and water *ad libitum*.

N-Hydroxy-AAF was synthesized by published procedures (9, 10). For the toxicity studies, *N*-hydroxy-AAF was dissolved in dimethyl sulfoxide and diluted with 4 volumes of corn oil. At the higher doses used, the compound did not remain in solution and was injected as a suspension which was stirred vigorously (Vortex mixer) immediately before administration. All animals were given the *N*-hydroxy-AAF i.p. in a volume of 7.5 ml/kg body weight. Control rats were given 20% dimethylsulfoxide in corn oil (v/v) at a dose of 7.5 ml/kg of body weight; the injection vehicle showed no toxic effects.

The acute toxicity of *N*-hydroxy-AAF was determined by the method of Weil (12) and was expressed as the LD₅₀. Groups of 16 rats were used for each LD₅₀ determination; each group contained 4 animals per dosage level with 4 dosage levels of *N*-hydroxy-AAF. Almost all of the rats that died did so within 2 to 4 days after injection of the *N*-hydroxy-AAF. Although cause of death was not determined in these studies, the animals probably died of acute

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² The abbreviations used are: AAF, 2-acetylaminofluorene (*Chemical Abstracts* nomenclature: *N*-2-fluorenylacetylamine); LD₅₀, 50% lethal dose.

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Table 1
Hepatic N-hydroxy-AAF sulfotransferase activity and acute toxicity (LD₅₀) of N-Hydroxy-AAF in various strains of rats

Strain of rat	Source	N-Hydroxy-AAF sulfotransferase		LD ₅₀ of N-hydroxy-AAF		
		Activity ^a	Ratio, ♂/♀	mg/kg	Ratio, ♀/♂	
Sprague-Dawley	Female	Holtzman	13 ± 2 ^b (14) ^c	7.6	315 (241-413) ^d	6.3
	Male					
Sprague-Dawley	Female	Carworth CFE	31 ± 4 (6)	5.5	506 (372-689)	7.4
	Male					
Wistar	Female	Carworth CFN	69 ± 4 (6)	1.7	116 (76-175)	2.2
	Male					
Fischer 344	Female	Charles River	77 ± 4 (6)	1.9	52 (34-80)	0.9
	Male					

^a The nmoles of *o*-methylmercapto-AAF formed per 30 min per mg of protein.

^b Mean ± S.E.

^c Number of animals.

^d These numbers in parentheses, the 95% confidence limits of the calculated LD₅₀.

hepatotoxicity resulting from massive periportal necrosis, as reported by others (3).

N-Hydroxy-AAF sulfotransferase activity was determined in the 105,000 × *g* supernatant of liver homogenate by the procedure in which the unstable AAF-N-sulfate that is formed is trapped by reaction with methionine (2, 6).

RESULTS AND DISCUSSION

The data on hepatic N-hydroxy-AAF sulfotransferase activity and LD₅₀'s are shown in Table 1. In confirmation of previous results obtained by DeBaun *et al.* (2) and in our laboratory (7), male Holtzman rats had a much higher level of sulfotransferase activity, with a male/female ratio of 7.6. The higher level of hepatic sulfotransferase activity in male rats was also observed for the Carworth CFE strain of rat, the male/female ratio being 5.5. However, in the other 2 strains of rats studied, the Carworth CFN and the Fischer 344, the male/female ratios of liver sulfotransferase were much lower (less than 2). In all cases, liver sulfotransferase differences between male and female rats of a particular strain or source were statistically significant (Student's *t* test; *p* value, <0.001). The following combinations of differences were also significant: Holtzman female rats compared to females of other strains, including Carworth CFE (*p* < 0.001); Holtzman male compared to Fischer and Carworth CFE males (*p* < 0.001) or to Carworth CFN males (*p* = 0.01); and Carworth CFE females compared to Fischer and Carworth CFN females (*p* < 0.001).

As suggested by the limited data available on the comparative toxicity of i.p.-injected N-hydroxy-AAF in male and female rats (3), an inverse correlation between the level of hepatic N-hydroxy-AAF sulfotransferase and the LD₅₀ of a single i.p. injection of N-hydroxy-AAF was found

(Table 1). The female/male ratios of LD₅₀'s for the Holtzman and the Carworth CFE rats were 6.3 and 7.4, respectively, whereas the female/male ratios for the Carworth CFN and Fischer 344 rats were 2.2 and 0.9. The coefficient of correlation (1) between the mean hepatic N-hydroxy-AAF sulfotransferase and the LD₅₀ of N-hydroxy-AAF for all strains and sexes (Table 1) was -0.74. The low correlation coefficient may be attributable to the fact that the toxicity of N-hydroxy-AAF is influenced by variable factors in addition to N-hydroxy-AAF sulfotransferase activity: rate of absorption; detoxification reactions, such as reduction to AAF and conjugation as the glucuronide; and the extent of enterohepatic circulation and metabolism in the gut (reviewed in Refs. 5 and 13). Nevertheless, the data presented in Table 1 point out that the level of N-hydroxy-AAF sulfotransferase is of considerable importance in determining the degree of hepatotoxicity of N-hydroxy-AAF.

Assuming a relationship exists between hepatotoxicity and hepatocarcinogenicity of N-hydroxy-AAF (2, 3, 7, 14), it appears that the sex difference reported for the hepatocarcinogenicity of N-hydroxy-AAF may be of more of a strain difference than a true sex difference; at least, the sex difference may be peculiar to the Sprague-Dawley strain of rats. For example, Gutmann *et al.* (4) reported that the female Fischer rat was quite susceptible to liver tumor induction by N-hydroxy-AAF. The data in Table 1 lead one to believe that the female Carworth CFN (Wistar-derived) rat might also be moderately susceptible to hepatocarcinogenesis by N-hydroxy-AAF.

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