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SHORT COMMUNICATIONS

Sex-related differences in the impairment of hepatic drug-metabolizing enzymes in rats with adjuvant disease

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The polyarthritic syndrome, induced by the subdermal injection of heat-killed mycobacteria dispersed in oil, is a form of chronic arthritis in rats. The model system is extensively used in the routine testing of potential anti-inflammatory substances [1, 2]. Animals of either sex have been utilized for the induction of adjuvant disease in the rat [3–5], and the incidence of arthritis occurred as readily in the female as in the male rat [5]. However, studies on the decreases of hepatic microsomal mono-oxygenases in this model system have primarily employed the male rat [6–10]. Since sex-related differences in the hepatic drug-metabolizing enzymes (HDME) of the rat have been well established [11, 12], the present study was undertaken to delineate the differences in the extent of impairment, if any, in HDME of male vs female rats with adjuvant disease.

Sprague–Dawley male and female rats, weighing 137–160 g (6 to 8-weeks-old), were obtained from ARS/Sprague–Dawley, Madison, WI; they were housed in groups of three animals per cage throughout the experiment. Adjuvant disease was induced by the subdermal injection of 0.1 ml adjuvant in the tail. The adjuvant contained 0.5% of heat-killed *Mycobacterium butyricum* (Difco Labs., Detroit, Mich.) suspended in light mineral oil.

Animals were sacrificed by nitrogen asphyxiation on days 0, 14 and 24 after adjuvant administration. Liver homogenates (20%, w/v) were prepared from tissues of two to three rats in 0.1 M phosphate buffer, pH 7.4. The methods for the preparation of microsomes, measurements of aminopyrine *N*-demethylase and aniline hydroxylase activities, and the protein concentration have been previously described [13, 14]. Data were analyzed by the two tail Student's *t*-test at the 2.5 per cent level [15].

Table 1 summarizes the differences in the activities *in vitro* of two HDME, aniline hydroxylase and aminopyrine *N*-demethylase, in the normal rat and in rats with adjuvant disease. In the control group, the activity of aniline hydroxylase was similar in either sex group, while the *N*-demethylating enzyme activity was 2.2 to 3.5 times higher in the male rat. The results are in general agreement with those of other investigators [11, 12]. No significant differences were observed in the HDME activities in the control groups of rats receiving distilled water or subdermal injection of the adjuvant vehicle (light mineral oil).

Table 1. Sex-related differences in the impairment of hepatic microsomal drug-metabolizing enzyme activities in rats with adjuvant disease*

Group	Sex	Body wt at sacrifice (g)	Liver wt (g/100 g body wt)	Microsomal proteins (mg/g)	Aniline hydroxylase (nmoles of *p*-aminophenol formed/15 min/mg protein)	Aminopyrine *N*-demethylase (nmoles of 4 amino-antipyrine formed/30 min/mg protein)
			Day 0 post-adjuvant			
Controls	M	148 ± 2	3.77 ± 0.11	27.5 ± 0.9	7.60 ± 0.30	5.85 ± 0.26
Controls	f	144 ± 3	3.50 ± 0.10	24.9 ± 2.3	7.63 ± 1.94	2.65 ± 0.57†
			Day 14 post-adjuvant			
Controls	M	251 ± 4	3.22 ± 0.10	36.2 ± 0.3	6.15 ± 0.42	6.37 ± 1.07
Adjuvant	M	218 ± 7‡	3.37 ± 0.13	31.7 ± 1.3‡	1.82 ± 0.13‡	1.44 ± 0.16‡
Controls	F	184 ± 3†	3.01 ± 0.03	27.2 ± 0.5†	10.34 ± 0.19	2.58 ± 0.11†
Adjuvant	F	181 ± 2	3.36 ± 0.05	30.6 ± 2.8	4.24 ± 0.55‡	1.59 ± 0.21‡
			Day 24 post-adjuvant			
Controls	M	279 ± 7	3.06 ± 0.09	34.3 ± 0.2	8.34 ± 0.80	8.01 ± 1.10
Adjuvant	M	215 ± 4‡	3.15 ± 0.11‡	30.1 ± 2.2	4.72 ± 0.46‡	2.04 ± 0.57‡
Controls	F	201 ± 8†	2.97 ± 0.10	28.0 ± 1.3†	10.86 ± 1.39	2.30 ± 0.31†
Adjuvant	F	164 ± 9‡	3.24 ± 0.07‡	28.4 ± 1.7	7.21 ± 0.79	1.49 ± 0.14

* All values are means ± S.E. for 6–8 separate determinations from three microsomal preparations. Each microsomal preparation was made from pooled livers of two to three rats. The body and liver weights represent six to nine animals per group. All animals were deprived of food 16–18 hr prior to sacrifice but had access to drinking water at all times.

† $P < 0.025$ control male vs control female group of the same day.

‡ $P < 0.025$ control vs adjuvant group of the same day and same sex.

Significant reductions in hydroxylating and *N*-demethylating activities were observed 14 days after adjuvant administration to rats of either sex. However, the magnitude of the decrease of HDME was greater in the male rat, particularly for *N*-demethylation of aminopyrine. There was a partial reversal for the loss in HDME in female

animals at 24 days post-adjuvant ($P > 0.05$ control vs adjuvant). These experiments also indicate some reversal in the activity of aniline hydroxylase of male rats on day 24 after adjuvant insult; however, additional studies demonstrated significant reduction in the activity of this enzyme up to 40 days post-adjuvant [9, 10].

The loss in the activities of aniline hydroxylase and ethylmorphine *N*-demethylase has been shown to be associated with the conversion of microsomal cytochrome P-450 to cytochrome P-420 [16, 17]. Recently, a temporal relationship between the administration of adjuvant to male rats and the progressive decline in the metabolism *in vitro* of aniline, aminopyrine, *p*-nitroanisole and NADPH-cytochrome *c* reductase activity has been demonstrated [9, 10]. This reduced rate of drug metabolism paralleled the decrease in cytochrome P-450 with a concomitant appearance of cytochrome P-420 in the hepatic microsomes [9, 10]. Reductions in cytochrome P-450 in female rats with established adjuvant disease (day 14) have also been observed (P. P. Mathur, unpublished observations). Thus, this may underline the mechanism responsible for the impairment of the drug-metabolizing enzyme system observed in our present study in the livers of rats with adjuvant disease. Alternatively, the reversal of the impaired enzymes may be associated with improvement in cytochrome P-450 content. Another explanation for the reversal of HDME observed in female polyarthritic rats may be substrate related, with more pronounced affinity for aniline (Type II substrate) than for aminopyrine (Type I substrate).

Although paw edema was not monitored in these animals, the severity of the disease appeared to be similar in rats of either sex, as assessed by lesions in the tail, inflammation of the snout, adhesion of abdominal tissues to the peritoneum or to each other, and alopecia. A similar lack of sex differences in either the incidence or the severity of adjuvant-induced arthritis in many strains of rats has been confirmed by others [5, 18]. In this regard, Ryzewski [19] observed some sex-related differences in the severity of arthritic lesions in the advanced phases of the disease (between days 30 and 90 post-adjuvant). In an August strain of rats there was a more intense and prolonged acute polyarthritis in the male rat than in the corresponding strain of female rat. However, these differences were either less marked or absent between male and female rats of the Wistar strain [19]. This was substantiated by Dr L. F. Sancilio (personal communication), who observed no difference in the response between male and female Charles River–Lewis Wistar rats. The change in limb volume of the uninjected foot from days 18 to 29 was similar in both groups ($N = 8$ animals per group: $\bar{x} \pm$ S.E. was 1.07 ± 0.19 ml for male rats and 0.94 ± 0.16 ml for female rats).

Beck and Whitehouse [6] have shown varying effects on HDME in rats with arthritic lesions produced by different types of adjuvant. Female rats exhibited a lesser degree of impairment in *N*-demethylase activity than the corresponding male animals with *Mycoplasma arthritidus*-induced arthritis [20]. Evidence presented in this report demonstrates the presence of sex differences in HDME in the adjuvant rat. These results suggest that the female rat is more resistant to the impairment of *N*-demethylating enzymes after adjuvant administration. Furthermore, there is a slight amelioration in the activities of both hydroxylase and *N*-demethylase with prolongation of the disease in the female rat. This could be related to the secretion of steroidal hormones in the female rats and their subsequent effects on the hepatic microsomal enzyme activity, with restoration of hemoprotein (cytochrome P-450) levels. These steroids may also help in the stabilization of microsomal membranes, resulting in less damage to microsomal structures.

In the present study, the female rats gained less body weight than the males; however, the experiments of Cawthorne *et al.* [20] have demonstrated a lack of this effect on aminopyrine demethylase activity in rats restricted in body-weight gain by pair-feeding the animals to the corresponding group of adjuvant-treated rats.

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REFERENCES

1. B. B. Newbould, *Br. J. Pharmac. Chemother.* **21**, 127 (1963).
2. C. A. Winter, A. E. Risley and G. W. Nuss, *Proc. Soc. exp. Biol. Med.* **111**, 544 (1962).
3. B. H. Waksman, C. M. Pearson and J. T. Sharp, *J. Immun.* **85**, 503 (1960).
4. J. T. Sharp, B. H. Waksman, C. M. Pearson and S. Madoff, *Arthritis Rheum.* **4**, 169 (1961).
5. E. M. Glenn and J. Gray, *Am. J. vet. Res.* **26**, 1180 (1965).
6. F. J. Beck and M. W. Whitehouse, *Biochem. Pharmac.* **22**, 2453 (1973).
7. F. J. Beck and M. W. Whitehouse, *Proc. Soc. exp. Biol. Med.* **145**, 135 (1974).
8. D. M. Morton and D. H. Chatfield, *Biochem. Pharmac.* **19**, 473 (1970).
9. P. P. Mathur, R. D. Smyth, C. Witmer, G. W. Nuss and G. Carr, *Fedn Proc.* **35**, 537 (1976).
10. P. P. Mathur, R. D. Smyth, C. Witmer, G. Carr and N. H. Reavey-Cantwell, *J. Pharmac. exp. Ther.*, **203**, 169 (1977).
11. R. Kato, in *Drug Metabolism Rev.* Sex-Related differences in Drug Metabolism (Ed. F. J. Dicarlo), Vol. 3, p. 1. Marcel Dekker, New York (1974).
12. S. E. D. El Masry, G. M. Cohen and G. J. Mannering, *Drug Metab. Dispos.* **2**, 267 (1974).
13. P. P. Mathur, R. D. Smyth, T. Herczeg and N. H. Reavey-Cantwell, *J. Pharmac. exp. Ther.* **196**, 204 (1976).
14. P. P. Mathur, R. D. Smyth, C. Witmer, G. Carr and N. H. Reavey-Cantwell, *Curr. Ther. Res.* **21**, 550 (1977).
15. G. W. Snedecor, in *Statistical Methods*, 5th Edn, p. 45. Iowa State College Press, Ames (1959).
16. K. G. Symms and M. R. Juchau, *Drug Metab. Dispos.* **2**, 194 (1974).
17. A. P. Alvares, J. Cohn and A. Kappas, *Drug Metab. Dispos.* **2**, 259 (1974).
18. K. F. Swingle, in *Anti-inflammatory Agents* (Eds R. A. Scherrer and M. W. Whitehouse), Vol. 11, p. 34. Academic Press, NY (1974).
19. J. Ryzewski, *Reumatologia* (Poland) **7**, 195 (1969).
20. M. A. Cawthorne, E. D. Palmer and J. Green, *Biochem. Pharmac.* **25**, 2683 (1976).

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