Biochemical Pharmacology, Vol. 42, No. 1, pp. 181–184, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

SHORT COMMUNICATIONS

Inhibition of hepatic drug metabolism and carbon tetrachloride toxicity in Fischer-344 rats by exercise*

(*Received* 22 *October* 1990; *accepted* 23 *January* 1991)

Clinical studies have appeared in the literature suggesting that exercise affects the pharmacokinetics of numerous drugs (see Van Baak [1] and Somani *et al.* [2] for recent comprehensive reviews). This is not unexpected since many of the factors that can affect the kinetics of drugs are altered during exercise (e.g. body temperature, G.I. motility, blood pH, and blood flow). Since blood flow to the liver is decreased during exercise, hepatic clearance of certain flow-dependent (high extraction) drugs would similarly be expected to be altered. However, this anticipated effect has not been proven. In this regard, early investigations were primarily directed at identifying effects of exercise on hepatic clearance using a common marker of hepatic blood flow, indocyanine green. Although acute exercise decreased liver blood flow 30–60% depending on exercise intensity [3, 4], the anticipated change in the clearance of flow-dependent compounds such as verapamil and propranolol was not found [5, 6]. Conversely, in a paper commonly referenced in discussions on this topic, Sweeney [7] did find a reduced clearance of lidocaine during exercise. However, numerous criticisms of Sweeney's data have been presented [1], including the fact that only a single subject was used.

In contrast to conflicting data with flow-limited drugs, evidence supports the conclusion that the clearance of capacity (metabolism)-limited (low clearance) drugs such as antipyrine, diazepam and amylobarbitone is not changed during acute exercise [8–10]. Acute exercise represents a single bout of physical activity in a trained or untrained individual, while chronic exercise is defined by a more regimented program of exercise over an extended period of time. When results with chronic exercise on capacity-limited hepatic clearance are reviewed, evidence is inconclusive. Aminopyrine metabolism was found to be unchanged [11] or slightly greater [10, 12] in physically fit (chronically trained) individuals. In subjects used as their own controls, several months of physical exercise produced only a slight (12%) increase in the metabolism of antipyrine. This latter effect correlated with changes in maximum O_2 uptake (Vo_2 max), an index of physical fitness [10].

While the above discussion raises the question that there may be changes in the hepatic clearance of drugs during chronic exercise, the minimal number of carefully-controlled studies, the use of only a few substrates, and the lack of specific hepatic biochemical (enzymatic) determinations prevent definite conclusions from being drawn at this time. In light of these facts, a series of experiments were designed to characterize and quantify the effects of exercise on components of the hepatic drug-metabolizing systems in animals. To accomplish this, whole cells and subfractions of livers from Fischer-344 rats that were chronically exercised on a treadmill were used. In the course of initial exercise studies on levels of cytochrome P450 (P450), oxidative and reductive metabolism of xenobiotics, and related toxicity, it became apparent that a protective effect occurred with the metabolism/toxicity of one substrate, carbon tetrachloride, (CCl_4†). This protective effect of exercise on CCl_4 toxicity and data which illustrate the possibility that exercise specifically affects specific isozymes are presented.

Methods

Barrier-reared male Fischer-344 rats, 10 months of age, were obtained from colonies maintained by the National Institute on Aging (Bethesda, MD). Animals were housed in a facility controlled for temperature (21–22%) and humidity (40–50%) and were exposed to a 12-hr light/dark cycle. Food (Purina Rodent Chow) and water were freely available. Following a 1-week acclimation period, rats were classified as exercised or sedentary. Exercised rats were run on a motorized treadmill (Omni-Pacer, model PTM; Omnitech Electronics, Inc., Columbus, OH) at a 10% grade. The protocol followed was similar to work of McMaster and Carney [13] with some modifications. Exercise sessions took place once a day 4 days per week for 8 weeks. In week 1, rats were run at 8 meters/min (m/min) for 30 min to familiarize them with the exercise procedure. Thereafter, treadmill speed was increased 1 m/min each day of exercise and running time was increased 5 min every other exercise day up to 20 m/min for 60 min by the end of week 4. This rate and time were maintained for the duration of the 8-week training period. Sedentary rats were placed on the treadmill two times per week at a rate of 8 m/min for 5 min to maintain familiarity with the treadmill and handling. Control rats that were not handled or exposed to the treadmill were studied and found to have no significant differences in any of the parameters investigated as compared to sedentary rats.

Twenty to twenty-four hours after the last exposure to the treadmill, rats were lightly anesthetized under ether and hepatocytes were isolated as previously described [14]. Cell viability was assessed by trypan blue exclusion [14]. Cell viability was used as an indicator for CCl_4-induced toxicity. The initial viability of the hepatocytes was at least 90% or they were discarded. Hepatocytes were diluted to 2×10^6 live cells/mL, and 10-mL samples were incubated in 50-mL round bottom flasks at 37° in a Dubnoff metabolic shaking bath (100 oscillations/min) under an atmosphere of 95% O_2/5% CO_2 (carbogen). Each flask received either saline or CCl_4 (1, 5 or 10 mM final concentrations), and each incubation was performed in duplicate. Aliquots (200 μL) were removed at 1, 2 and 3 hr, and viability was measured. In addition to hepatocyte assays, microsomal P450 content and *p*-nitroanisole (pNA) *O*-demethylase, aniline hydroxylase and ethoxyresorufin *O*-deethylase activites were determined. After the initial perfusion with Krebs buffer (pH 7.4), a single lobe of each liver was ligated and removed. Upon removal, the lobe was blotted, weighed and immediately frozen in liquid nitrogen for

* Presented at the annual meeting of the Federation of the American Societies of Experimental Biology in Washington, DC, April 1–5, 1990.

† Abbreviations: CCl_4, carbon tetrachloride; pNA, *p*-nitroanisole; pNP, *p*-nitrophenol; and pAP, *p*-aminophenol.

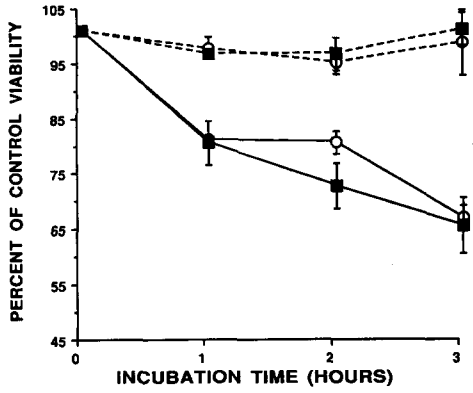

Fig. 1. Effect of CCl_4, 1 mM (○) and 5 mM (■), on hepatocyte viability in sedentary (——) and exercised (---) rats. Control viability represents hepatocytes incubated with saline. The mean viability of saline controls for both exercised and sedentary groups was 93% at 0 time. Each point is the mean ± SEM of eight animals. Values for exercised rats were significantly greater ($P < 0.05$) from those of sedentary rats at all time periods.

future analysis. Microsomes were isolated from each lobe as previously described [15].

The content of P450 was determined from the reduced carbon monoxide difference spectrum according to the method of Omura and Sato [16]. The metabolism of pNA was determined by measurement of the demethylated product, *p*-nitrophenol (pNP) [14]. The metabolism of ethoxyresorufin was determined by measurement of the fluorescent metabolite, resorufin, as outlined by Pohl and Fouts [17]. Aniline hydroxylase activity was determined through measurement of the metabolite, *p*-aminophenol (pAP) [18]. Protein content was determined by the method of Lowry *et al.* [19] using bovine serum albumin as the standard. Statistical analysis utilized Student's *t*-test or analysis of variance (ANOVA) as appropriate ($P < 0.05$).

Results and Discussion

Exercise had no effect on body weight, liver weight, liver to body weight ratio, hepatic microsomal protein levels or hepatocyte viability. There was no difference in the viability of hepatocytes from sedentary or exercised rats when comparing the saline controls at any time interval. The mean viability of saline controls for both exercised and sedentary groups was 93% at 0 time and was 72% at 3 hr. During the 3-hr incubation of 1 mM CCl_4 with hepatocytes from sedentary rats, a decline in cell viability to 46% (representing 64% of control) was measured, with maximal toxicity reached at 3 hr (Fig. 1). In contrast, hepatocytes from exercised rats had no decline in cell viability at any time interval (0–3 hr) with 1 mM CCl_4 as compared to the saline control. Concentrations of 5 and 10 mM CCl_4 were incorporated into the study to determine if additional toxicity could be induced and if the cells exhibiting protection could be overwhelmed. No additional toxicity was found in either exercised or sedentary rats. For clarity, Fig. 1 only shows 1 and 5 mM CCl_4 since little additional effect was found with 10 mM CCl_4. This maximal result with these doses is similar to the effects of comparative concentrations (2.5 to 12.5 mM) of CCl_4 used in a previous study on lipid peroxidation and on viability indices of hepatocytes comparing carbogen to air [20].

The results for the microsomal studies are presented in Table 1. P450 levels declined 30% in exercised rats, from

Table 1. Effect of exercise on cytochrome P450 content and aniline, pNA and ethoxyresorufin metabolism

Group	Cytochrome P450 (nmol/mg protein)	Aniline hydroxylase (nmol pAP/mg protein/min)	pNA *O*-demethylase (nmol pNP/mg protein/min)	Ethoxyresorufin *O*-deethylase (pmol/mg protein/min)
Sedentary	0.900 ± 0.06 (9)	0.550 ± 0.073 (7)	2.57 ± 0.18 (6)	4.05 ± 0.27 (4)
Exercised	0.607 ± 0.04* (11)	0.433 ± 0.028* (8)	1.78 ± 0.16* (7)	3.66 ± 0.38 (4)

Rats were exercised as described in Methods. Results are means ± SEM. The number of rats in each group is indicated in parentheses.
* Significant change from sedentary value, $P < 0.05$.

0.900 to 0.607 nmol/mg protein. Sedentary rats metabolized pNA at a rate of 2.57 nmol pNP/mg protein/min, whereas the rate of metabolism of pNA in exercised rats was 1.78, a decline of 31%. No significant difference in metabolism of ethoxyresorufin between exercised (3.66 pmol/mg protein/min) and sedentary (4.05 pmol/mg protein/min) rats was found. Exercise caused a decline of 21% in the microsomal activity of aniline hydroxylase, with rates declining from 0.550 nmol pAP/mg protein/min in sedentary rats to 0.433 in exercised rats.

These results indicate that exercise is capable of inhibiting the metabolism of specific substrates. Along with decreased levels of P450, the metabolism of pNA and aniline was inhibited, whereas ethoxyresorufin metabolism was unaffected. Since exercise resulted in complete protection of hepatocytes at all CCl_4 concentrations, it can be reasoned that the effect may be related to a decreased formation or increased elimination of the toxic metabolite. The mechanism for the activation of toxicity of CCl_4 has been determined to be a P450-mediated reduction to yield a trichloromethyl radical ($\cdot CCl_3$), a short-lived reactive intermediate which covalently binds to macromolecules [21, 22]. Thus, since exercise was found to inhibit P450 by 30% as well as inhibiting by 21% the metabolism of aniline (a substrate metabolized by the same P450 isozyme as CCl_4 [23]), it is possible that the activation of CCl_4 to its toxic radical was decreased to a level which could be subsequently managed by intracellular free radical scavengers (e.g. superoxide dismutase and catalase) or by antioxidants (e.g. glutathione and vitamin E). Another possibility which cannot be overlooked is that exercise may stimulate the above described intracellular protective mechanisms. In this regard, it has been shown that acute bouts of exercise in trained or untrained rats can increase significantly hepatic catalase [24]. Since there was a complete reversal of the decreased viability usually associated with CCl_4 hepatotoxicity, it is likely that several factors are involved in the protection.

In other studies which have found that external factors, such as aging, diet, or smoking, could selectively modify xenobiotic metabolism [25–27], it was suggested that the effects were related to selective modification of specific isozymes of P450. The present results with exercise are not unlike those previous findings and indicate that exercise may selectively affect isozymes of P450. In this regard, ethoxyresorufin has been shown to be metabolized by P450IA1 [28], whereas aniline and CCl_4 are primarily metabolized by P450IIE1 [23]. Thus, P450IIE1 may be one of the more sensitive isozymes which responds to the effects of exercise. In addition, other isozymes may be involved since the metabolism of pNA was decreased (31%). The hypothesis that exercise may selectively inhibit specific isozymes of P450 is currently being investigated through quantification of specific forms of P450 mRNA following exercise.

In summary, a substrate selective inhibition of hepatic metabolism was found in Fischer-344 rats which were exercised on a chronic basis. Microsomal metabolism of pNA and aniline was decreased, whereas metabolism of ethoxyresorufin was unaffected. Furthermore, chronic exercise using treadmill running provided protection from CCl_4 toxicity in hepatocytes incubated with this solvent. Although it was not directly determined, it appears that the metabolism of CCl_4 was decreased since the metabolism of aniline, a similar P450 isozyme substrate, was inhibited.

Acknowledgements—The authors are extremely grateful to Mr. Michael Gentry for his technical support, to Michelle Centra and Ted Piatkowski for laboratory assistance, and to the National Institute of Aging for supplying animals used in this study. This research was supported by a Biomedical Research Support Grant and by a Designated Research Initiative Fund, University of Maryland at Baltimore.

Department of Pharmacology and Toxicology
School of Pharmacy
University of Maryland at Baltimore
Baltimore
MD 21201, U.S.A.

WESLEY W. DAY
MYRON WEINER*

* Correspondence: Myron Weiner, Ph.D., Department of Pharmacology and Toxicology, University of Maryland at Baltimore, School of Pharmacy, 20 North Pine St., Baltimore, MD 21201.

REFERENCES

1. Van Baak MA, Influence of exercise on the pharmacokinetics of drugs. *Clin Pharmacokinet* **19**: 32–43, 1990.
2. Somani SM, Gupta SK, Frank S and Corder CC, Effect of exercise on drug disposition and pharmacokinetics of drugs. *Drug Dev Res* **20**: 251–275, 1990.
3. Rowell LB, Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev* **54**: 75–159, 1974.
4. Swartz RD, Sidell FR and Cucinell SA, Effects of physical stress on the disposition of drugs eliminated by the liver in man. *J Pharmacol Exp Ther* **188**: 1–7, 1974.
5. Mooy J, Arends B, Van Kemenade J, Bohm R, Rahn KH and Van Baak MA, Influence of prolonged submaximal exercise on the pharmacokinetics of verapamil in humans. *J Cardiovasc Pharmacol* **8**: 940–942, 1986.
6. Arends B, Bohm R, Van Kemenade J, Rahn KH and Van Baak MA, Influence of physical exercise on the pharmacokinetics of propranolol. *Eur J Clin Pharmacol* **31**: 375–377, 1986.
7. Sweeney GD, Drugs—Some basic concepts. *Med Sci Sports Exerc* **13**: 247–251, 1981.
8. Klotz U and Lucke C, Physical exercise and the disposition of diazepam. *Br J Clin Pharmacol* **5**: 349–350, 1978.
9. Balasubramaniam K, Mawer GE and Simmons PJ, The influence of dose on the distribution and elimination of amylobarbitone in healthy subjects. *Br J Pharmacol* **40**: 578–579, 1970.
10. Boel J, Anderson LB, Rasmussen B, Hansen SH and Døssing M, Hepatic drug metabolism and physical fitness. *Clin Pharmacol Ther* **36**: 121–126, 1984.
11. Ducry J, Howald H, Zysset T and Bircher J, Liver function in physically trained subjects: Galactose elimination capacity, plasma disappearance of indocyanine green, and aminopyrine metabolism in long distance runners. *Dig Dis Sci* **24**: 192–196, 1979.
12. Gikalovand I and Bircher J, Dose dependence of the ^{14}C-aminopyrine breath test: Intrasubject comparison of tracer and pharmacological doses. *Eur J Clin Pharmacol* **12**: 229–233, 1977.
13. McMaster SB and Carney JM, Changes in drug sensitivity following acute and chronic exercise. *Pharmacol Biochem Behav* **23**: 191–194, 1985.
14. Eacho PI and Weiner M, Metabolism of *p*-nitroanisole and aniline in isolated hepatocytes of streptozotocin-diabetic rats. *Drug Metab Dispos* **8**: 385–389, 1980.
15. Weiner M and Olson J, Inhibition of hepatic drug metabolism in VX_2 carcinoma-bearing rabbits. *Drug Metab Dispos* **8**: 139–142, 1980.
16. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its

hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
17. Pohl RJ and Fouts JR, A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Anal Biochem* **107**: 150–155, 1980.
18. Mieyl J and Blumer J, Acceleration of the autooxidation of human oxyhemoglobin by aniline and disrelation to hemoglobin-catalyzed aniline hydroxylation. *J Biol Chem* **251**: 3442–3446, 1976.
19. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
20. Stacey NH, Ottenwalder H and Kappus H, CCl_4-induced lipid peroxidation in isolated rat hepatocytes with different oxygen concentrations. *Toxic Appl Pharmacol* **62**: 421–427, 1981.
21. Noguchi T, Fong KL, Lai EK, Alexander SS, King MM, Olsen L, Poyer JL and McCay PB, Specificity of a phenobarbital-induced cytochrome P-450 for metabolism of CCl_4 to the trichloromethyl radical. *Biochem Pharmacol* **31**: 615–624, 1982.
22. Cheeseman KH, Albano EF, Tomasi A and Slater TF, Biochemical studies on the metabolic activation of halogenated alkanes. *Environ Health Perspect* **64**: 85–101, 1985.
23. Koop DR, Laethem CL and Tierney DJ, Utility of *p*-nitrophenol hydroxylation in P-450IIE1 analysis. *Drug Metab Rev* **20**: 541–551, 1989.
24. Ji LL, Stratman FW and Lardy HA, Antioxidant enzyme systems in rat liver and skeletal muscle. *Arch Biochem Biophys* **263**: 150–160, 1988.
25. Rikans LE, Influence of aging on chemically induced hepatotoxicity: Role of age-related changes in metabolism. *Drug Metab Rev* **20**: 87–110, 1989.
26. Vestal RE, Norris AH, Tobin JD, Cohen BH, Shock NW and Andres R, Antipyrine metabolism in man: Influence of age, alcohol, caffeine, and smoking. *Clin Pharmacol Ther* **18**: 425–431, 1975.
27. Roberts J and Turner N, Age and diet effects in drug action. *Pharmacol Ther* **37**: 111–115, 1988.
28. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T and Mayer RT, Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: A series of substrates to distinguish between different induced cytochromes P-450. *Biochem Pharmacol* **34**: 3337–3345, 1985.

Biochemical Pharmacology, Vol. 42, No. 1, pp. 184–188, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Comparison of the effects of selected monocarboxylic, dicarboxylic and perfluorinated fatty acids on peroxisome proliferation in primary cultured rat hepatocytes

(*Received* 28 *September* 1990; *accepted* 22 *January* 1991)

Administration of hypolipidemic agents and phthalate ester plasticizers to rodents increases hepatic peroxisome numbers and peroxisome-associated enzyme activities [1]. High fat diets [2] also cause peroxisome proliferation with an induction of the peroxisomal fatty acid β-oxidation system, although the extent of the induction compared to xenobiotics is low. In contrast, metabolically stable perfluorinated carboxylic acids [3] and non-β-oxidizable sulfur-containing fatty acid analogues [4] are potent peroxisome proliferators *in vivo*. Hepatic peroxisomal responses to xenobiotics may be mediated by interaction with a specific receptor(s) leading to increased synthesis of enzymes of the peroxisomal fatty acid β-oxidation system [1, 5] or through a perturbation in lipid metabolism which initiates a cellular adaptive response leading to a proliferation of peroxisomes [6, 7]. Microsomal cytochrome P450IVA1 which mediates ω- and (ω-1)-hydroxylation of lauric acid (laurate hydroxylase, LH) is co-induced along with peroxisomal fatty acyl-CoA oxidase (FACO) by agents which cause peroxisome proliferation [8–10]. Dicarboxylic acids, products of the microsomal LH system, may be the proximal stimulus for hepatic peroxisome proliferation [6, 7].

To further our understanding of structural requirements for fatty acids in mediating peroxisome proliferation, we have evaluated a series of monocarboxylic, dicarboxylic and metabolically stable perfluorinated fatty acids of a chain length of 4, 8 and 10 carbons (C_4, C_8 and C_{10}, respectively) for their induction of peroxisome-associated enzymes (LH and FACO) in cultured adult rat hepatocytes. Perfluorinated octanol was also examined to determine whether a carboxylic acid function is required for hepatic peroxisome proliferation. Clofibric acid was included as a positive standard for peroxisome proliferation [9, 11]. Structures and abbreviations of the fatty acid analogues used in this study are given in Table 1. A preliminary report of this work has appeared elsewhere [12].

Materials and Methods

Materials. Biochemicals used were: clofibric acid (CPIB), DB, DO and DD (Sigma Chemical Co., St. Louis, MO), OA, DA, PFBA, PFOA, PFDA and PFOL (Aldrich Chemical Co., Milwaukee, WI), BA (Fischer Scientific, Cincinnati, OH), collagenase type IV (Cooper Biochemical, Malvern, PA), [1-^{14}C]lauric acid (58 mCi/mmol) (Amersham, Arlington Heights, IL), Nu-Serum (Collaborative Research Inc., Lexington, MA), Vitrogen (Collagen Corp., Palo Alto, CA) and Williams Medium E (Gibco, Grand Island, NY). Other biochemicals were obtained from the Sigma Chemical Co. Male Sprague–Dawley rats (225–325 g) were obtained from Harlan Sprague–Dawley, Inc. (Indianapolis, IN).

Primary culture of hepatocytes. Hepatocytes were isolated from adult rats by the collagenase perfusion method as