

Acta Pharmacologica Sinica 1987 Jan, 8 (1) : 53-57

Effects of 1-nitropyrene pretreatment on the mixed-function oxidases of rat liver

HE Shao-Xiong¹, Francis C P LAW

(*Environmental Toxicology Program, Dept Biological Sciences, Simon Fraser University, Burnaby BC, Canada V5A 1S6*)

ABSTRACT The inducibility of hepatic mixed-function oxidases (MFO) was examined in adult rats following ip or dermal applications of 1-

nitropyrene (1-NP) at a daily dose of 30 mg/kg for 3 d. The liver weights, hepatic microsomal cytochrome p-450 and b₅ contents, and 7-ethoxycoumarin O-deethylase and benzopyrene hydroxylase activities of rats treated with 1-NP ip were induced significantly. In contrast, hepatic aminopyrine N-demethylase activity was unaffected. Pretreatment of rats with 1-NP applied on

Received 1986 Jan 21 Revised 1986 Jul 2

¹Visiting Scientist from the Tianjin Institute of Materia Medica, Tianjin 300070, The People's Republic of China

the skin did not alter significantly the liver weights and hepatic MFO activities. These results indicate that 1-NP is a 3-methylcholanthrene-type inducer of hepatic MFO but it is not absorbed by the skin of adult rats.

KEY WORDS 1-nitropyrene; mixed-function oxidoreductases; 3-methylcholanthrene-type inducers; cytochrome p-450; cytochrome b₅; 7-ethoxycoumarin O-deethylase; benzopyrene hydroxylase; aminopyrine N-demethylase

Nitroarenes are by-products of incomplete combustion processes and have been found in extracts of diesel and gasoline emissions, fly ash particles, carbon blacks, cigarette smoke condensates and urban atmosphere⁽¹⁾. 1-Nitropyrene (1-NP) is the most abundant nitroarene in the environment⁽²⁾. It has mutagenic activity in the *Salmonella typhimurium* test⁽³⁾, induces sister chromatid exchanges in Chinese hamster lung and ovary cells⁽⁴⁾ and is carcinogenic to male rats⁽⁵⁾.

Although a major route of human exposure to nitroarenes is *via* the skin, little is known about the percutaneous absorption of 1-NP. A recent study has demonstrated that a single topical application of a mixture of 1-NP and dinitropyrenes on neonatal rats induces significantly the hepatic MFO activities⁽⁶⁾. Since the skin of mature animals is less permeable to chemicals than the skin of newborn animals⁽⁷⁾, the purpose of this investigation was to examine the effects of 1-NP administered *ip* and *dermally* on the hepatic MFO in adult rats.

MATERIALS AND METHODS

Chemicals 1-NP was purchased from Aldrich Chemical Co. and purified by TLC as previously described⁽⁸⁾. The purity of 1-NP was determined to exceed 99.5% by HPLC. Aminopyrine was purchased from Aldrich Chemical Co. 7-Ethoxycoumarin,

7-hydroxycoumarin and N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) were obtained from Sigma Chemical Co. [³H]Benzopyrene was purchased from New England Nuclear. NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were products of Boehringer Mannheim Canada Ltd. All other chemicals utilized were of the highest purity commercially available.

Animals Male Wistar rats weighing about 250 g were purchased from the Animal Care Centre, the University of British Columbia, Vancouver. The rats, housed one per stainless steel cage, were maintained on a 12-h light/12-h dark photoperiod in the Animal Care Facility, Simon Fraser University. The rats were allowed food and water *ad lib*, but food was withdrawn from the rat overnight before they were sacrificed.

Rats were treated *ip* or *dermally* for 3 d with 1-NP (30 mg/kg) dissolved in peanut oil (15 mg/ml). Control rats were treated with the same volume of peanut oil. The rats were decapitated on the next day after the last treatment. Pretreatment of rats *dermally* with 1-NP was carried out on a shaved area (about 4 cm²) of the midback of rats. 1-NP was applied to the skin of each rat with a microliter pipet. The treated area was covered by gauze which was wrapped securely by bandage.

Preparation of liver microsomes The rat liver was rinsed in an ice-cold 1.15% KCl-HEPES 0.05 mol/L (pH 7.3) solution. The liver was homogenized with KCl-HEPES in a Potter-Elvehjem homogenizer as described by Bend *et al.*⁽⁹⁾. The homogenate was centrifuged at 10 000 × g for 20 min in a Sorvall RC-5 B Refrigerated Super-speed Centrifuge. The post-mitochondrial supernatant was removed and recentrifuged at 105 000 × g for 1 h in a Sorvall ODT75B Ultracentrifuge to obtain the microsomes. Microsomal pellets were resuspended in KCl-HEPES. Protein concentrations were

Tab 1. Effects of intraperitoneally administered or dermally applied 1-NP on hepatic microsomal MFO activities of rats ($\bar{x} \pm SD$)

	Intraperitoneally injected (n = 6)			Dermally applied (n = 3)		
	Control	Treated	p	Control	Treated	p
Cytochrome p-450/448 (nmol/mg protein)	0.47 \pm 0.16	0.69 \pm 0.18	<0.05	0.57 \pm 0.08	0.60 \pm 0.04	>0.05
Cytochrome b ₅ (nmol/mg protein)	0.38 \pm 0.02	0.49 \pm 0.06	<0.01	0.38 \pm 0.02	0.41 \pm 0.09	>0.05
Aminopyrine N-demethylase [nmol HCHO formed/ (mg protein·min)]	5.39 \pm 0.57	5.48 \pm 1.14	>0.05	5.07 \pm 0.35	5.17 \pm 0.36	>0.05
7-Ethoxycoumarin O-deethylase [nmol product formed/ (mg protein·min)]	0.55 \pm 0.10	0.90 \pm 0.09	<0.01	0.67 \pm 0.01	0.66 \pm 0.06	>0.05
Benzopyrene hydroxylase [nmol product formed/ (mg protein·min)]	0.43 \pm 0.03	0.87 \pm 0.09	<0.01	0.52 \pm 0.11	0.57 \pm 0.22	>0.05

determined by the method of Lowry *et al.*,⁽¹⁰⁾ using bovine serum albumin as the standard.

Hepatic microsomal MFO assays
Microsomal cytochrome P-450 and b₅ contents were measured by the methods of Omura and Sato⁽¹¹⁾.

A typical incubation mixture contained 1.5 mg microsomal protein, 0.25 ml HEPES 1.0 mol/L buffer (pH 7.4), 0.3 mg MgSO₄, 4.3 mg NADP, 4.5 mg glucose-6-phosphate, 2 units of glucose-6-phosphate dehydrogenase and the substrate. The mixture was adjusted to a final volume of 2 ml with distilled water. Incubations were carried out in 20-ml glass liquid scintillation vials at 37°C for 15 min. The mixtures were preincubated for 5 min before substrate addition. Substrate concentrations were aminopyrine 5 μ mol/L; 7-ethoxycoumarin 0.2 μ mol/L; and benzopyrene 0.16 μ mol/L. Aminopyrine N-demethylase activity was measured by the formation of formaldehyde⁽¹²⁾. 7-Ethoxycoumarin O-deethylase activity was determined by measuring the formation of 7-hydroxycoumarin⁽¹³⁾.

RESULTS

Effects of 1-NP on the hepatic MFO of

rats following ip administration Pretreatment of rats with 1-NP ip resulted in increases in their liver weights. The liver to body weight ratios of treated and control rats were 3.25 \pm 0.31 g and 2.98 \pm 0.16 g ($\bar{x} \pm SD$), respectively.

Table 1 summarizes the effects of 1-NP on the hepatic MFO of rats. Microsomal 7-ethoxycoumarin O-deethylase and benzopyrene hydroxylase activities and cytochrome P-450 and b₅ contents of the rat were induced significantly by 1-NP pretreatment. In contrast, 1-NP pretreatment appeared to have no effect on the hepatic aminopyrine N-demethylase activity of rats.

Effects of 1-NP on the hepatic MFO of rats following dermal applications The liver weights of rats pretreated with 1-NP dermally were not altered significantly. The liver to body weight ratios of the treated and control rats were 2.95 \pm 0.05 g and 2.89 \pm 0.05 g, respectively.

Table 1 summarizes the effects of 1-NP on the hepatic MFO of rats. Neither microsomal P-450 and b₅ contents nor microsomal aminopyrine N-demethylase, 7-ethoxycoumarin O-deethylase and benzopyrene hydroxylase activities were altered

significantly by 1-NP pretreatment.

DISCUSSION

Our results indicate that 1-NP is a 3-methylcholanthrene-type (3-MC-type) inducer of hepatic MFO of rats. This conclusion is based on the observation that hepatic 7-ethoxycoumarin *O*-deethylase and benzopyrene hydroxylase activities are induced significantly by ip 1-NP to rats whereas hepatic aminopyrine *N*-demethylase activity is unaffected (Tab 1). Benzopyrene hydroxylation is mainly catalyzed by cytochrome P-448 whereas deethylation of 7-ethoxycoumarin is metabolized by both cytochrome P-450 and P-448. Therefore, a 3-MC-type inducer of hepatic MFO enhances both 7-ethoxycoumarin-*O*-deethylase and benzopyrene hydroxylase activities⁽¹⁵⁾. *N*-demethylation of aminopyrine is catalyzed by cytochrome P-450 which is enhanced mainly by phenobarbital-type inducers of hepatic MFO⁽¹⁵⁾. Since 1-NP has no effect on hepatic aminopyrine *N*-demethylase activity but enhances both 7-ethoxycoumarin-*O*-deethylase and benzopyrene hydroxylase activities, it is a 3-MC-type inducer of hepatic MFO.

Our study does not show that 1-NP is absorbed by the skin of mature rats since the hepatic MFO activity is not altered significantly following dermal applications of 1-NP (Tab 2). This result is contradictory to the report that hepatic MFO is induced by a single topical application of a mixture of nitroarenes to the skin of newborn rats⁽⁶⁾. A plausible explanation for the discrepancy may be that nitroarenes are not absorbed by the skin of adult rats or the amount absorbed is not significant enough to affect the hepatic MFO. Another possibility may be that the induction of hepatic MFO activity reported by Asokan *et al*⁽⁶⁾ is due to the percutaneous absorption of nitroarenes other than 1-NP or is the result of a combined effect of several nitroarenes.

In summary, our data demonstrate that 1-NP is a potent 3-MC-type inducer of hepatic MFO of rats. However, it does not appear to be absorbed appreciably by the skin of adult rats.

ACKNOWLEDGMENTS This study was supported by a grant from the Canadian International Development Agency/Natural Science and Engineering Research Council (CIDA/NSERC). HE Shao-Xiong was the recipient of a CIDA/NSERC Research Associateship.

REFERENCES

- 1 Rosenkranz HS. Direct-acting mutagens in diesel exhausts; magnitude of the problem. *Mutat Res* 1982; 101 : 1
- 2 Pitts JN Jr, Lokensganđ DM, Harger W, *et al*. Mutagens in diesel exhaust particulates, identification and direct activities of 6-nitrobenzo(a) pyrene, 9-nitroanthracene, 1-nitrorene and 5 H-phenanthra (4, 5, -6 cd) pyran-5-one. *Ibid* 1982; 103 : 241
- 3 Mermelstein R, Kiriazides DK, Butler M, McCoy EC, Rosenkranz HS. The extraordinary mutagenicity of nitropyrenes in bacteria. *Ibid* 1981; 98 : 187
- 4 Nachtman JP, Wolff S. Activity of nitro-polynuclear aromatic hydrocarbons in the sister chromatid exchange assay with and without metabolic activation. *Environ Mutagen* 1982; 4 : 1
- 5 Ohgake H, Matsukura N, Morinao K, *et al*. Carcinogenicity in rats of the mutagenic compounds 1-nitro pyrene and 3-nitrofluoranthone. *Cancer Lett* 1982; 15 : 1
- 6 Asokan P, Das M, Rosenkranz HS, Bickers DR, Mukhtar H. Topically applied nitropyrenes are potent inducers of cutaneous and hepatic monooxygenases. *Biochem Biophys Res Commun* 1985; 129 : 134
- 7 Hayes WJ. *Toxicology of pesticides*. 1st ed. Baltimore, Williams & Wilkins, 1975 : 147
- 8 Pederson TC, Siak JS. The role of nitroaromatic compounds in the direct-acting mutagenicity of diesel particle extracts. *J Appl Toxicol* 1981; 1 : 54
- 9 Bend JR, Hook GE, Easterling RE, Gram TE, Fouts JR. A comparative study of the hepatic and pulmonary microsomal mixedfunction oxidase systems in the rabbit.

- J Pharmacol Exp Ther* 1972; 183 : 206
- 10 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193 : 265
 - 11 Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes 1. Evidence for its hemoprotein nature. *Ibid* 1964; 239 : 2370
 - 12 Sladek NE, Mannering GL. Induction of drug metabolism 1. Differences in the mechanisms by which polycyclic hydrocarbons and phenobarbital produce their inductive effects on microsomal *N*-demethylating systems. *Mol Pharmacol* 1969; 5 : 174
 - 13 Creaven PJ, Parke DV, Williams RT. A spectrofluorimetric study of the 7-hydroxylation of coumarin by liver microsomes. *Biochem J* 1965; 96 : 390
 - 14 DePierre JW, Moron MS, Johannesen KAM, Ernster L. A reliable, sensitive, and convenient radioactive assay for benzopyrene monooxygenase. *Anal Biochem* 1975; 63 : 470
 - 15 Ioannides C, Lum PY, Parke DV. Cytochrome P-448 and the activation of toxic chemicals and carcinogens. *Xenobiotica* 1984; 14 : 119

中国药理学报 1987年1月; 8(1): 53-57

1-硝基苊对大鼠肝混合功能氧化酶的作用

何绍雄¹, Francis C P LAW (Environmental Toxicology Program, Dept Biological Sciences, Simon Fraser University, Burnaby B C, Canada V5A 1S6)

提要 将1-硝基苊 30 mg/(kg·d) 给成年大鼠 ip 或涂皮共 3 天, 然后测定其对肝混合功能氧化酶的诱导作用。ip 给药后能显著诱导肝脏重量, 肝微粒体细胞色素 P-450 和 b₅ 的含量以及 7-乙氧基香豆素 O-去乙酰酶和苯并苊羟化酶的活性, 但对氨基比林-N-去甲基酶的活性则无作用。皮肤给药后并不显著改变肝重及

混合功能氧化酶的活性。上述结果表明, 1-硝基苊是一种 3-甲基胆蒎类的肝混合功能氧化酶的诱导剂, 且在此剂量下不被成年大鼠的皮肤所吸收。

关键词 1-硝基苊; 混合功能氧化还原酶; 3-甲基胆蒎类诱导剂; 细胞色素 P-450; 细胞色素 b₅; 7-乙氧基香豆素 O-去乙酰酶; 苯并苊羟化酶; 氨基比林 N-去甲基酶

¹ 天津药物研究所 CIDA/NSERC 计划访问学者。