Biochemical Pharmacology, Vol. 58, pp. 461–463, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00111-2

SHORT COMMUNICATION

Acetone Catabolism by Cytochrome P450 2E1: Studies with CYP2E1-Null Mice

Flordeliza Y. Bondoc, Ziping Bao,* Wen-Yu Hu,* Frank J. Gonzalez,† Yongyu Wang,* Chung S. Yang* and Jun-Yan Hong*‡*

*Laboratory for Cancer Research, College of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854; and †Laboratory of Metabolism, NCI, National Institutes of Health, Bethesda, MD 20892, U.S.A.

ABSTRACT. Previous experiments *in vitro* have suggested that cytochrome P450 2E1 (CYP2E1) is involved in acetone catabolism by converting acetone to acetol and then to methylglyoxal, both intermediates in the gluconeogenic pathway. In the present study, CYP2E1-null mice were used to demonstrate the role of CYP2E1 in acetone catabolism *in vivo*. The blood acetone level in male CYP2E1-null mice was 3.3 ± 0.9 μg/mL, which was similar to levels of their sex- and age-matched parental lineage strains C57BL/6N (2.3 ± 0.2 μg/mL) and 129/Sv (3.5 ± 0.3 μg/mL) mice (both are CYP2E1wild-type). After fasting for 48 hr, the blood acetone levels in the CYP2E1 wild-type mice were increased by 2.5- to 4.4-fold, but that in the CYP2E1-null mice increased 28-fold. These results clearly demonstrate that CYP2E1 plays a vital role in the catabolism of acetone under fasting conditions. BIOCHEM PHARMACOL **58**;3:461–463, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. acetone metabolism; fasting; cytochrome P450 2E1; 2E1 knockout mice

Cytochromes P450 are a superfamily of hemoprotein monooxygenases that play a critical role in the biotransformation of numerous endogenous and exogenous compounds. CYP2E1§, which is highly conserved in mammals, has been shown to catalyze the oxidation of many low molecular weight compounds, including alcohols and industrial solvents [1–3]. CYP2E1 is constitutively expressed in various tissues, with the liver having the highest level [2].

Acetone, produced under conditions of fasting or diabetes, is both an inducer and a substrate of CYP2E1 [2, 4]. The utilization of acetone in mammalian glucose metabolism has been established [5, 6]. It has been demonstrated *in vitro* that CPY2E1 catalyzes the conversion of acetone to acetol and then to methylglyoxal, both intermediates in the gluconeogenesis pathway [7–9]. In both humans and rats, administration of disulfiram, a potent inhibitor of CYP2E1, causes a significant increase in the blood acetone level [10, 11]. In rats treated with diallyl sulfide, another selective inhibitor of the enzyme, elevation of the blood acetone level also has been observed and was attributed to the decrease of acetone catabolism [12].

Although the importance of CYP2E1 in acetone catabolism *in vivo* was strongly implicated in the inhibition studies with disulfiram and diallyl sulfide, the specificity of these inhibitors is always of concern. Recently, a transgenic mouse line lacking CYP2E1 has been developed by gene targeting [13]. This mouse line provides a unique model to study the physiological and toxicological roles of CYP2E1 [13, 14]. Thus, using the CYP2E1-null mice, the role of CYP2E1 in the catabolism of acetone *in vivo* was investigated. Sex- and age-matched C57BL/6N and 129/Sv mice, both of which are wild-type and the parental lineage strains of the CYP2E1-null mice, were used as controls.

MATERIALS AND METHODS

Animals and Treatments

CYP2E1-null mice were produced as described [13]. Embryonic stem cells of 129/Sv mouse origin were used for gene targeting and were microinjected into the blastocysts of C57BL/6N mice. Breeding pairs of the CYP2E1-null mice were shipped to the Animal Facility at Rutgers University from the National Cancer Institute, and the offspring of these mating pairs were used to establish the CYP2E1-null mouse lines. C57BL/6N and 129/Sv mice were purchased from Taconic Farms. Male CYP2E1-null, C57BL/6N, and 129/Sv mice at 22 weeks of age were deprived of food for 48 hr, and their corresponding control groups were given commercial laboratory chow. All the mice received water *ad lib*. Prior to euthanasia, blood was taken from the orbital sinus for the determination of acetone level. All experiments were done in accordance with the Animal Care and Use guidelines of Rutgers University.

‡ Corresponding author: Dr. Jun-Yan Hong, Laboratory for Cancer Research, College of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854-8020. Tel. (732) 445-5358; FAX (732) 445-0687; E-mail: jyhong@rci.rutgers.edu

§ *Abbreviations*: CYP2E1, cytochrome P450 2E1; and NDMA, *N*-nitrosodimethylamine.

Received 10 September 1998; accepted 15 December 1998.

Blood Acetone Determination

Acetone in the blood was analyzed using head-space gas chromatography as previously described [12] with some modifications. In brief, 150 μL of heparinized blood was added to 0.5 mL of cold 0.72 N $HClO_4$. The precipitated protein was removed by centrifugation at 14,000 *g* at 4° for 10 min. Two hundred microliters of the supernatant was neutralized with 50 μL of 4 N NaOH and sealed in a septum vial with nitrogen on top of the solution. Acetone was measured immediately in a Perkin Elmer (model 8500) gas chromatograph with a Tenax 60/80 mesh column (1/8 in. × 6 ft, Alltech Inc.) and an HS-101 head-space autoinjector. The injector and flame ionization detector were at 160° and 180°, respectively, while the oven temperature was 120°. Statistical analysis was performed using ANOVA with Statview 4.5 software (Abacus Concepts, Inc.).

RESULTS AND DISCUSSION

To confirm that the CYP2E1-null mice lacked CYP2E1 expression and that this P450 was the major form catalyzing NDMA demethylation, liver microsomes from the CYP2E1-null mice and their parental lineage strains, C57BL/6N and 129/Sv mice, were measured for NDMA demethylase activity. The activity in the liver microsomes from the C57BL/6N and 129/Sv mice was 228 ± 25 and 233 ± 30 pmol/min/mg, respectively. In contrast, NDMA demethylase activity was not detectable in the liver microsomes from the CYP2E1-null mice. This result is consistent with the previous report that CYP2E1 protein and mRNA are not expressed in the livers of CYP2E1-null mice [13].

It has been reported previously that fasting, diabetes, and consumption of ethanol result in an elevation of ketone bodies in the blood [15], one of which is acetone. To assess the role of P450 2E1 in the catabolism of acetone under fasting conditions, the blood acetone levels were measured in non-fasted mice and in mice after 48 hr of fasting. A representative chromatogram of the blood acetone analysis in non-fasted and fasted CYP2E1-null mice is shown in Fig. 1. There was no significant difference in the blood acetone levels among the non-fasted CYP2E1-null, C57BL/6N, and 129/Sv mice (Table 1). In the CYP2E1-null mice, the acetone level was 3.3 ± 0.9 μg/mL in the non-fasted group but was elevated significantly (92.3 ± 41.6 μg/mL; by 28-fold) in the fasting group (Table 1). Although the blood acetone levels in the fasted CYP2E1-null mice varied widely, ranging from 34 to 125 μg/mL, the lowest level was still significantly higher than that of the fasted C57BL/6N and 129/Sv mice. Only 2- and 4-fold increases in the blood acetone levels were observed for the fasted C57BL/6N and 129/Sv mice, respectively (Table 1), in comparison with their corresponding non-fasted controls.

The blood level of acetone in an animal depends on the balance of its production and elimination. Acetone is produced under ketogenic conditions through the nonenzymatic decarboxylation of acetoacetate [16]. The elimination of acetone involves enzymatic oxidation and exhalation [12, 17]. Two gluconeogenic pathways have been proposed for the metabolism of acetone, in which the initial step in both pathways involves the oxidation of acetone to acetol [7]. One pathway involves the conversion of acetol to methylglyoxal, and the other involves the conversion of acetol to L-1,2-propanediol. In the initial hydroxylation and in the conversion of acetol to methylglyoxal, the involvement of a monooxygenase system has been suggested [8]. Previous studies have implicated CYP2E1 as a major enzyme involved in catalyzing the initial steps in acetone metabolism [7–10, 12]. Consistent with the reports that acetone oxidation is catalyzed by CYP2E1 *in vitro*, our present study demonstrated a significant elevation of the blood acetone level in CYP2E1-null mice, suggesting the inability of the mice to catabolize the excess acetone

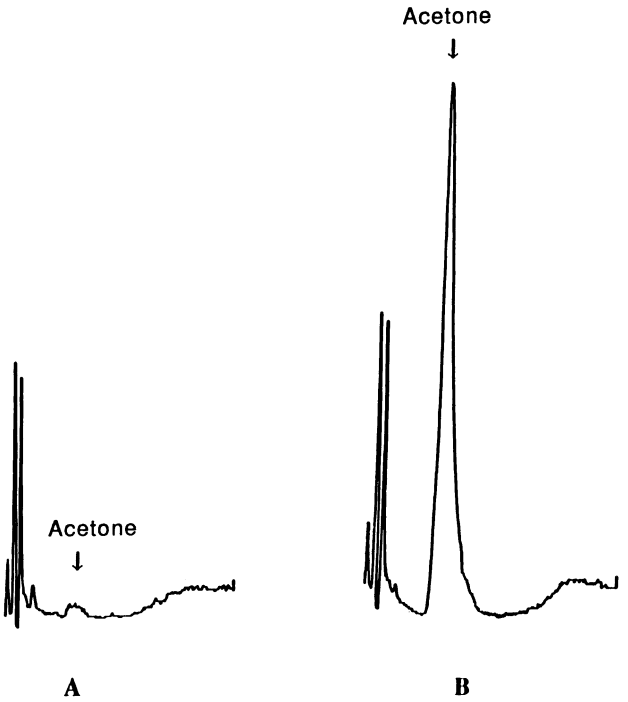

FIG. 1. Representative chromatogram of the blood acetone levels in non-fasted (A) and 48-hr fasted (B) CYP2E1-null mice. Blood was collected from the orbital sinus into a heparinized tube, and the blood acetone level was determined by head-space gas chromatography as described in Materials and Methods.

TABLE 1. Effect of fasting on blood acetone levels in CYP2E1-null mice and their parental lineage strains

	Blood acetone (μg/mL)		
Treatment	**CYP2E1-null**	**C57BL/6N**	**129/Sv**
Control	3.3 ± 0.9	2.3 ± 0.2	3.5 ± 0.3
Fasting	92.3 ± 41.6*	5.7 ± 1.8	15.4 ± 1.9

Values are means ± SD (N = 3–5).

*Significantly different from all the control groups and from the fasted C57BL/6N and 129/Sv groups ($P < 0.0001$).

produced during fasting. It is noteworthy that fasting and acetone are known to induce CYP2E1 in animals [18, 19], which is expected to further increase the capability of animals to metabolize acetone. The absence of both constitutive and inducible CYP2E1 in the null mice may account for the large difference in the blood acetone levels between the fasted CYP2E1-null and wild-type mice. These results clearly demonstrate that CYP2E1 plays a vital role in the catabolism of acetone under fasting conditions. Gluconeogenesis during prolonged starvation, utilizing the metabolites of acetone and other ketone bodies, is important as a source for providing energy to the body, especially the brain. Therefore, the CYP2E1-catalyzed acetone catabolism could have significant impact on homeostasis in fasted animals. The catabolism may also help eliminate excess acetone in the body to prevent acetone toxicity.

We thank Drs. Robert Harris and Stephen Curtis for their help in establishing the CYP2E1-null mouse colony at the Nelson Animal Facility at Rutgers University, and Dr. Theresa Smith for her critical reading of this manuscript. This work was supported by grants from NIH (ES03938) and the Health Effects Institute (#96–2), and an NIEHS Center Grant (ES05022).

References

1. Guengerich FP, Kim D-H and Iwasaki M, Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem Res Toxicol* **4:** 168–179, 1991.
2. Koop DR, Oxidative and reductive metabolism by cytochrome P450 2E1. *FASEB J* **6:** 724–730, 1992.
3. Wang M-H, Wade D, Chen L, White S and Yang CS, Probing the active sites of rat and human cytochrome P450 2E1 with alcohols and carboxylic acids. *Arch Biochem Biophys* **317:** 299–304, 1995.
4. Yang CS, Yoo JSH, Ishizaki H and Hong J-Y, Cytochrome P450IIE1: Roles in nitrosamine metabolism and mechanism of regulation. *Drug Metab Rev* **22:** 147–159, 1990.
5. Borek E and Rittenberg D, The metabolism of acetone by surviving rat liver. *J Biol Chem* **179:** 843–845, 1949.
6. Price TD and Rittenberg D, The metabolism of acetone. I. Gross aspects of catabolism and excretion. *J Biol Chem* **185:** 449–459, 1950.
7. Casazza JP, Felver ME and Veech RL, The metabolism of acetone in rats. *J Biol Chem* **259:** 231–236, 1984.
8. Casazza JP and Fu JL, Measurement of acetol in serum. *Anal Biochem* **148:** 344–348, 1985.
9. Koop DR and Casazza JP, Identification of ethanol-inducible P-450 isozyme 3a as the acetone and acetol monooxygenase of rabbit microsomes. *J Biol Chem* **260:** 13607–13612, 1985.
10. Stowell A, Johnsen J, Ripel A and Morland J, Disulfiram-induced acetonaemia. *Lancet* **1:** 882–883, 1983.
11. DeMaster EG and Nagasawa HT, Disulfiram-induced acetonaemia in the rat and man. *Res Commun Chem Pathol Pharmacol* **18:** 361–364, 1977.
12. Chen L, Lee MJ, Hong J-Y, Huang W-Q, Wang EJ and Yang CS, Relationship between cytochrome P450 2E1 and acetone catabolism in rats as studied with diallyl sulfide as an inhibitor. *Biochem Pharmacol* **48:** 2199–2205, 1994.
13. Lee SS, Buters JT, Pineau T, Fernandez-Salguero P and Gonzales FJ, Role of CYP2E1 in the hepatotoxicity of acetaminophen. *J Biol Chem* **271:** 12063–12067, 1996.
14. Hong J-Y, Wang Y-Y, Bondoc FY, Yang CS, Gonzales FJ, Pan Z, Cokonis C and Bao ZP, Metabolism of methyl *tert*-butyl ether and other gasoline ethers in mouse liver microsomes lacking cytochrome P450 2E1. *Toxicol Lett* **105:** 83–88, 1999.
15. Song BJ, Gelboin HV, Park SS, Yang CS and Gonzales FJ, Complementary DNA sequences of ethanol-inducible rat and human cytochrome P-450s: Transcriptional and post-transcriptional regulation of the rat enzyme. *J Biol Chem* **261:** 16689–16697, 1986.
16. Stryer L, *Biochemistry*, pp, 478–480. W. H. Freeman, New York, 1988.
17. Owen OE, Trapp VE, Skutches L, Mozzoli MA, Hoeldtke RD, Boden G and Reichard GA Jr. Acetone metabolism during diabetic ketoacidosis. *Diabetes* **31:** 242–248, 1982.
18. Hong J-Y, Pan J, Gonzales FJ, Gelboin HV and Yang CS, The induction of a specific form of cytochrome P-450 (P-450j) by fasting. *Biochem Biophys Res Commun* **142:** 1077–1083, 1987.
19. Dong Z, Hong J-Y, Ma Q, Li D, Bullock J, Gonzales FJ, Park SS, Gelboin HV and Yang CS, Mechanism of induction of cytochrome $P\text{-}450_{ac}$ ($P\text{-}450_j$) in chemically induced and spontaneously diabetic rats. *Arch Biochem Biophys* **263:** 29–35, 1988.