Differences in the Induction of Carboxylesterase RL4 in Rat Liver Microsomes by Various Perfluorinated Fatty Acids, Metabolically Inert Derivatives of Fatty Acids

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Differences in the ability of metabolically inert peroxisome proliferators [perfluoro-n-decanic acid (PFDA, C₁₀), perfluoro-n-octanoic acid (PFOA, C₈), perfluoroctane sulfonic acid (PFOS, C₈) and 1H,1H-pentadecafluoro-n-octanol (PFOLAN, C₈)] to induce liver microsomal carboxylesterase RL4 in male rats were studied by evaluating changes in the RL4 content by immunoblot analysis with a specific antibody. The administration of PFOA, PFOS and PFOLAN markedly increase the content of carboxylesterase RL4. On the other hand, PFDA decreases PNPA, BUTA, and ISOC hydrolase activity, and slightly increases the carboxylesterase RL4 content.

Key words carboxylesterase; induction; peroxisome proliferator; perfluorinated fatty acid; rat; liver

Because carboxylesterases (CES) play an important role in the metabolic activation of prodrugs and detoxification of xenobiotics, differences in substrate specificity and immunological properties of the enzymes are important when choosing a suitable laboratory animal for evaluating the biotransformation and toxicity of drugs. Multiple isozymes of hepatic microsomal CESs exist in various animal species and humans. We have also suggested that changes in hepatic CES isoforms occur during hepatocarcinogenesis. On the other hand, we and other investigators have reported that hepatic microsomal CESs are induced by exogenous compounds such as phenobarbital, Aroclor 1254, synthetic glucocorticoid, pregnenolone 16α-carbonitrile, clofibrate and di(2-ethylhexyl)phthalate. Recently, we have reported the purification and characterization of a novel CES isozyme, RL4, from di(2-ethylhexyl)phthalate-treated rat liver microsomes, and also reported that the CES RL4 was strongly induced by dietary administration of di(2-ethylhexyl)phthalate. The phthalate ester is a widely used plasticizer in our environment, and also a well-known peroxisome proliferator. Consequently it is of interest to know whether this is a common property of peroxisome proliferators, since their chemical structures are extremely diverse and include a wide spectrum of chemicals such as hypolipidemic drugs, phthalate ester plasticizers, and some industrial solvents. However, little is known about the influence of other peroxisome proliferators on liver microsomal CESs. Perfluorinated fatty acids are used commercially in plating systems, as wetting agents and as corrosion inhibitors. Ikeda et al. observed that the perfluorinated fatty acids are potent peroxisome proliferators.

The purpose of the present study, therefore, was to examine the effects of various perfluorinated fatty acids which are industrial solvents and also peroxisome proliferators, on liver microsomal CES RL4.

MATERIALS AND METHODS

Perfluorodecanoic acid (PFDA), perfluoroctanoic acid (PFOA), perfluoroctane sulfonic acid (PFOS) and 1H,1H-pentadecafluoro-n-octanol (PFOLAN) were kindly supplied by Dr. Toshikiko Ikeda, Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., Hiromachi, Shinagawaku, Tokyo, Japan. Butanilicaine (N-butylnaoyctyl-2-chloro-6-methylanilide) and isocarboxazid were donated by Hoechst AG, Frankfurt, Germany and Nippon Roche Research Center, Kamakura, Japan, respectively. Other chemicals were reagent grade products from commercial sources.

Throughout the present study, adult male rats of the F344 strain obtained from Japan SL Inc., Shizuoka, Japan, were used. Animals were fed a laboratory animal chow (CE-2, CLEA Inc.) and water ad libitum and were housed in wire-bottomed cages at constant temperature (22–24°C) and humidity (50–60%) under a 12 h light-dark cycle (7:00 a.m. to 7:00 p.m.). All chemicals were dissolved in corn oil. Groups of four male rats, 10 weeks of age, were each given intraperitoneal injections of perfluorinated compounds (PFDA, PFOA, PFOS or PFOLAN) at 30 mg/kg. Rats were sacrificed at 3d after intraperitoneal administration by decapitation and their livers were removed, weighed and perfused with 1.15% (w/v) KCl. Microsomal fractions were isolated by differential centrifugation as previously described. All subsequent procedures were performed at 0–4°C, unless otherwise stated.

CES activities towards three substrates, i.e., p-nitrophenyl acetate (PNPA), isocarboxazid (ISOC) and butanilicaine (BUTA) were determined according to published procedures. Specificity of antibody to CES RL4 was checked by immunochromatography analysis for immunochromatography quantitation (data not shown). For quantitative analysis, the intensity of the enzyme protein bands seen on blots was determined by densitometry. Protein was determined by the method of Lowry et al.

All data in this study were statistically analyzed by using Student’s t-test.

RESULTS AND DISCUSSION

The effects of PFDA, PFOA, PFOS and PFOLAN on CES activities towards PNPA, ISOC and BUTA, believ-
ed to be specific substrates for the three constitutive CES isozymes, RL1, RL2 and RH1, respectively.2,3) are shown in Table 1. PNPA hydrolyase activity was significantly increased by treatment with PFOA (1.10-fold), PFOS (1.25-fold) and PFOL (1.32-fold). However, PNPA hydrolyase activity was significantly decreased (0.83-fold) by treatment with PFDA. On the other hand, PFOA, PFOS and PFOL increase the hydrolyase activity towards BUTA, 1.28-fold, 1.12-fold and 1.34-fold, respectively. Liver microsomal ISOC hydrolyase activity was significantly increased by PFOA, PFOS and PFOL treatment, 1.12-fold, 1.17-fold and 1.23-fold, respectively. However, the administration of PFDA significantly decreased the activity towards all three substrates. Recently, we have reported the purification of a novel inducible form of CES RL4 and the preparation of a specific antibody to this isozyme.5) In the present study, we also determined the amount of CES RL4 using an immunoblot analysis. The results of the immunoblot analysis for CES RL4 reacting with specific antibody are shown in Figs. 1 and 2. The administration of PFDA slightly, but not significantly, increased the amount of CES RL4 (1.38-fold). On the other hand, CES RL4 was markedly induced by administration of PFOA, PFOS and PFOL, 3.67-fold, 5.41-fold and 2.16-fold, respectively. However, a poor correlation was found between the CES RL4 content and liver microsomal hydrolyase activity towards PNPA, BUTA and ISOC (data not shown).

In previous papers, we employed immunochemical methods to demonstrate the induction of the constitutive form of CES isozymes in rat liver microsomes by several peroxisome proliferators.5,7) It seems that induction of CES isozymes may be a common property of peroxisome proliferators, despite their extremely diverse chemical structures. To extend the above work, we investigated...
the induction of the constitutive form of CES isozymes by perfluorinated fatty acids, which are metabolically inert and used as wetting agents, corrosion inhibitors and for importing oil resistance to paper, as well as also peroxisome proliferators. In this study, we found that CES RL4 was strongly induced by administration of PFOA(C₈). Conversely, the CES RL4 contents was slightly increased by PFDA(C₁₀) treatment. This marked difference in the activity of PFOA and PFDA, despite the rather small structural difference between them, is consistent with the finding of Olson and Anderson that the toxicity of PFDA is much higher than that of PFOA. Like PFOA, PFOS induced CES RL4 in rat liver microsomes (Table 1). Ikeda et al. demonstrated that catalase, the acyl-CoA oxidation system and carnitine acetyltransferase activity were all induced by administration of PFOS. Kozuka et al. also reported that carnitine acetyltransferase and fatty acyl-CoA dehydrogenase activity is induced by treatment with PFOS. Since PFOS has no carboxylic acid, these results suggest that a negatively charged group rather than a carboxylic acid group is important for peroxisome proliferation and enzyme induction. Detailed studies of the peroxisomal β-oxidation enzyme gene have indicated the presence of a peroxisome proliferator response element (PPRE), located upstream from the initiation site of transcription, but it is not clear whether the PPRE is involved in the regulation of CES isozymes or not. When PFOL was administered to rats, the CES RL4 content significantly increased (Figs. 1, 2). Similar inductive responses have been reported for some peroxisomal enzymes. PFOL has two hydrogen atoms around the hydroxylated carbon atom and may be metabolized to an active compound, PFOA, via perfluorooolan. However, Intraksuki and Feller demonstrated that peroxisomal fatty acyl-CoA oxidase and microsomal dehydrogenase activity was not induced by PFOL in cultured hepatocytes, and they suggested that either the oxidation of PFOL occurs to a significant degree only in vivo or PFOL may act through an indirect mechanism independent of the liver.

In conclusion, we have demonstrated that metabolically inert perfluorinated fatty acids induce liver microsomal CES RL4, as determined by immunoblot analysis using a specific antibody. This is the first report that perfluorinated fatty acids affect the CES RL4 in rat liver microsomes; and our data also emphasize the importance of peroxisome proliferators in the hepatic metabolism of xenobiotics.

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REFERENCES