Inhibition by Retinoids of Benzo(A)pyrene Metabolism Catalyzed by 3-Methylcholanthrene–Induced Rat Cytochrome P-450 1A1

Dong-Yang Huang, Taira Ohnishi, Houbo Jiang, Aizo Furukawa, and Yoshiyuki Ichikawa

Benzo(a)pyrene, a well-known procarcinogen, is believed to be activated by microsomal cytochrome P-450 1A1 (CYP 1A1). We recently reported that rat CYP 1A1 induced by 3-methylcholanthrene (3-MC) catalyzed the conversion of retinal to retinoic acid. In this study, we investigated retinoid inhibition of the metabolism of benzo(a)pyrene and 7-ethoxyresorufin, another specific substrate of CYP 1A1, using liver microsomes prepared from control and 3-MC–pretreated rats as the enzyme source. In 3-MC–treated rats, retinal and retinol, but not retinoic acid, inhibited benzo(a)pyrene metabolism. The 50% inhibitory concentration (IC_{50}) of retinal was about 11.5 μmol/L and the inhibition was competitive, with a K_i value of 5.8 μmol/L. Retinol is a more potent inhibitor than retinal. The IC_{50} was about 5 μmol/L and the inhibition was mixed, with a K_i value of 19.2 μmol/L and a K_i' value of 4.2 μmol/L. Almost the same results were obtained for the reaction of 7-ethoxyresorufin deethylation. In contrast, the metabolic activity of both benzo(a)pyrene and 7-ethoxyresorufin was much lower in untreated versus 3-MC–treated rats, and only weak inhibition by the retinoids was observed. The results suggest that retinoids inhibit the activation of benzo(a)pyrene and that the substrate specificity of cytochrome P-450 isozymes associated with retinoid metabolism should be taken into account when studying the anticarcinogenic action of retinoids.

IT IS WELL ESTABLISHED that most chemical carcinogens require metabolic activation before they can exert carcinogenic effects. Various studies using purified rat and rabbit cytochrome P-450 have established a role for cytochrome P-450 1A (CYP 1A) enzymes in the activation of procarcinogenic xenobiotics, including benzo(a)pyrene and various heterocyclic amines. The first step of benzo(a)pyrene metabolism is mainly catalyzed by CYP 1A1,[1-4] resulting in the production of a variety of reactive arene oxides or epoxides, which are further transformed into more polar hydrosoluble metabolites through both enzymatic and nonenzymatic processes. These epoxides are most likely the ultimate or proximate carcinogens.[1]

Retinal is an intermediate of the biosynthetic pathway for retinoic acid starting from retinol (vitamin A), and is also produced with the cleavage of β-carotene. The details of the biosynthetic pathway for retinoic acid vary with the species. Generally, retinal produced from retinol by cytosolic or microsomal dehydrogenases[5,6] is converted to retinoic acid by cytosolic NAD-dependent dehydrogenases, flavin adenine dinucleotide–linked retinal oxidase, and a microsomal cytochrome P-450–linked monoxygenase (CYP-450).[7,8] Recently, we demonstrated that the activity of CYP-450 in retinoic acid synthesis in rat liver microsomes is induced by 3-methylcholanthrene (3-MC), and the main enzyme responsible for this activity is CYP 1A1.[9]

Vitamin A (retinol) and β-carotene (provitamin A) protect animals treated with benzo(a)pyrene from carcinogenesis.[10] Taking into account the metabolism of retinoids and benzo(a)pyrene by CYP 1A1, it can be speculated that the preventive effect of retinoids on benzo(a)pyrene-induced carcinogenesis may be partially due to the competitive metabolism of retinoids and benzo(a)pyrene by CYP 1A1. To clarify this, we studied the inhibitory effects of retinoids on benzo(a)pyrene metabolism and 7-ethoxyresorfin deethylation using liver microsomes prepared from 3-MC–treated rats as a source of CYP 1A1, and we also performed experiments in parallel using liver microsomes prepared from untreated rats.

MATERIALS AND METHODS

Chemicals

Benzo(a)pyrene, 3-MC, all-*trans*-retinal, all-*trans*-retinol, all-*trans*-retinoic acid, ethoxyresorufin, and resorufin were obtained from Sigma Chemical (St Louis, MO), and [1,3,6-^{3}H]-benzo(a)pyrene was obtained from DuPont NEN (Boston, MA). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and NADPH were obtained from Oriental Yeast (Osaka, Japan). All other chemicals were of the highest grade commercially available.

Preparation of Liver Microsomes From Rats Treated and Untreated With 3-MC

Male Sprague-Dawley rats (300 to 350 g) fed a standard commercial laboratory diet were obtained from the animal laboratory of our university. The rats were treated with 3-MC, which was dissolved in corn oil, at a dose of 30 mg/kg/d intramuscularly for 6 days. Control rats were treated with corn oil alone intramuscularly for 6 days. Hepatic microsomes from control and 3-MC–treated rats were prepared by successive centrifugation and ultracentrifugation according to the method of Mitoma et al.[11] The microsomal pellets were suspended in 50 mmol/L Tris · HCl buffer (pH 7.5) at a protein concentration of 6.7 mg/mL for 3-MC–treated rats and 10 mg/mL for untreated rats and then kept at −80°C.

Assay of Benzo(a)pyrene Metabolism

Assays were performed in duplicate or triplicate for kinetic analysis (variation, <10%, on average), under red illumination. The standard reaction mixture contained 50 mmol/L Tris · HCl buffer (pH 7.5), 4 mmol/L $MgCl_2$, 5 mmol/L glucose-6-phosphate, and 0.5 IU glucose-6-phosphate dehydrogenase, in a final volume of 0.5 mL. ^{3}H-benzo(a)pyrene was diluted to 0.1 mmol/L with nonradioactive benzo(a)pyrene to a radioactivity level of 7.6×10^7 dpm/mL. Microsomes from 3-MC–treated rats (1 to 10 μg) or untreated rats (10 to 100 μg) and ^{3}H-benzo(a)pyrene (0.1 to 0.5 μmol/L) were added to the standard reaction mixture in the absence and presence of 2 to 30 μmol/L retinoids. The retinoids were dissolved in dimethyl sulfoxide and then added to the reaction mixtures at a final concentration of less than 1%

From the Department of Biochemistry, School of Medicine, Kagawa Medical University, Kagawa, Japan.

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Address reprint requests to Dong-Yang Huang, Department of Biochemistry, School of Medicine, Kagawa Medical University, Miki-cho, Kita-gun, Kagawa 761-07, Japan.

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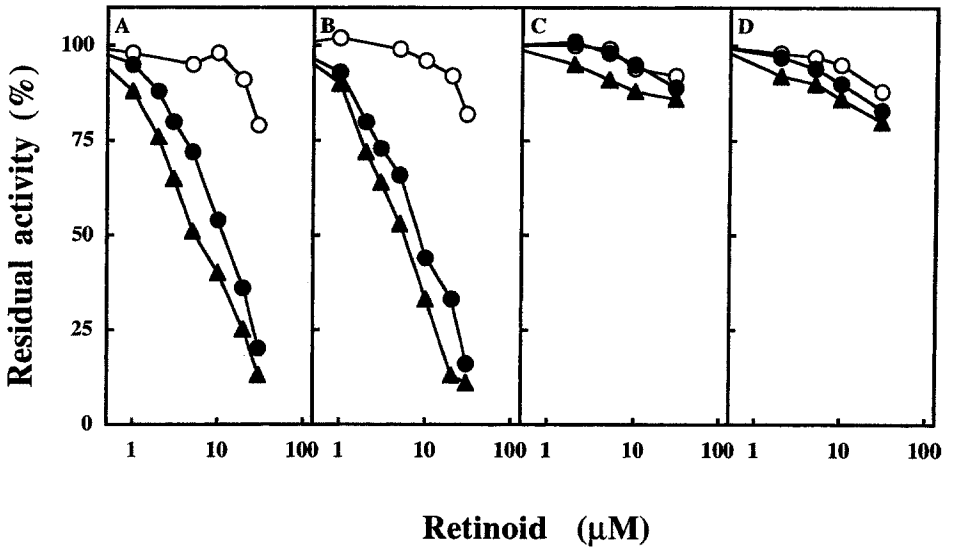

Fig 1. Inhibitory effects of retinal (●), retinol (▲), and retinoic acid (○) on benzo(a)pyrene metabolism and 7-ethoxyresorufin deethylation. Benzo(a)pyrene 0.2 μmol/L and 1 μg microsomes from 3-MC–treated rats (A) or 10 μg microsomes from untreated rats (C) were used. 7-ethoxyresorufin 0.5 μmol/L and 2 μg microsomes from 3-MC–treated rats (B) or 80 μg microsomes from untreated rats (D) were used.

(vol/vol). The reaction was initiated by the addition of 0.2 mmol/L NADPH and continued for 2 to 5 minutes at 37°C. The metabolic products of benzo(a)pyrene were analyzed by a method reported previously.[12] After the unmetabolized substrate was extracted with hexane, the polar products in the aqueous phase were counted by liquid scintillation.

Assay of 7-Ethoxyresorufin Deethylation

The assays were performed in 1 mL standard reaction mixture as described before in the absence and presence of 2 to 30 μmol/L retinoids. The activity of 7-ethoxyresorufin (0.5 to 10 μmol/L) was measured fluorometrically with 2 μg 3-MC–treated microsomal protein or 80 μg untreated microsomal protein.[13]

Data Analysis

All assays were performed in duplicate or triplicate. Kinetic parameters were calculated using a minimal square linear regression program based on double reciprocal plots. To calculate the K_i value, we assumed K_i to be the dissociation constant between the enzyme and inhibitor, and K_i' to be the constant between the enzyme-substrate complex and inhibitor.

RESULTS

Benzo(a)pyrene metabolism and 7-ethoxyresorufin deethylation, both of which are catalyzed mainly by CYP 1A1, were measured in the presence of retinal, retinol, and retinoic acid using microsomes from 3-MC–treated rats and untreated rats. Retinal and retinol, but not retinoic acid, inhibited both metabolic reactions catalyzed by the microsomes of 3-MC–treated rats. The 50% inhibitory concentration (IC_{50}) of retinal for benzo(a)pyrene metabolism was 11.5 ± 1.4 μmol/L (mean ± SE, n = 3) at a substrate concentration of 0.2 μmol/L. Retinol was a more potent inhibitor than retinal, with an IC_{50} of 5.0 ± 1.0 μmol/L (n = 3) (Fig 1). A similar extent of inhibition of the activity of 7-ethoxyresorufin deethylation was observed, with an IC_{50} for retinal and retinol of 9.0 ± 1.8 and 5.5 ± 1.3 μmol/L (n = 3), respectively, at a substrate concentration of 0.5 μmol/L. Benzo(a)pyrene 0.2 μmol/L and 7-ethoxyresorufin 0.5 μmol/L were in their respective K_m range, and the specific activity at these concentrations was 1.62 ± 0.3 and 1.58 ± 0.4 nmol/min/mg protein (n = 3), respectively. In contrast, microsomes from untreated rats were much less active in terms of benzo(a)pyrene metabolism and 7-ethoxyresorufin deethylation, and inhibition by the retinoids was weak. The specific activity of benzo(a)pyrene metabolism was 0.14 ± 0.04 nmol/min/mg protein (n = 3) in the absence of retinoids, and the maximum inhibition by 30 μmol/L retinol was about 24%. The specific activity of 7-ethoxyresorufin deethylation was 0.037 ± 0.007 nmol/min/mg (n = 3) in the absence of retinoids, and the maximum inhibition by retinol was also 20% (Fig 1).

Figure 2 A and B shows Lineweaver-Burk plots for benzo(a)pyrene metabolism and 7-ethoxyresorufin deethylation with microsomes from 3-MC–treated rats. Double reciprocal plots are shown for the initial velocity versus substrate concentration in the absence and presence of retinal or retinol. The apparent K_m for benzo(a)pyrene metabolism is 0.18 μmol/L, and for 7-ethoxyresorufin deethylation, 0.43 μmol/L. In both metabolic assays, the inhibition by retinal was competitive and the inhibition by retinol was mixed. K_m, K_i, and K_i' values are summarized in Table 1.

Table 1. Kinetic Parameters for the Effects of Retinoids on Benzo(a)pyrene and 7-Ethoxyresorufin Metabolism

		Retinol			Retinal		
Substrate	K_m	Type	K_i	K_i'	Type	K_i	K_i'
Benzo(a)pyrene	0.18 ± 0.04	m	19.2 ± 3.1	4.2 ± 0.7	c	5.8 ± 1.0	—
7-Ethoxyresorufin	0.43 ± 0.09	m	3.5 ± 0.6	12.2 ± 2.1	c	4.6 ± 1.1	—

NOTE. Values (μmol/L) are the mean ± SE for 3 determinations each with microsomes obtained from a different preparation.
Abbreviations: c, competitive inhibition; m, mixed inhibition.

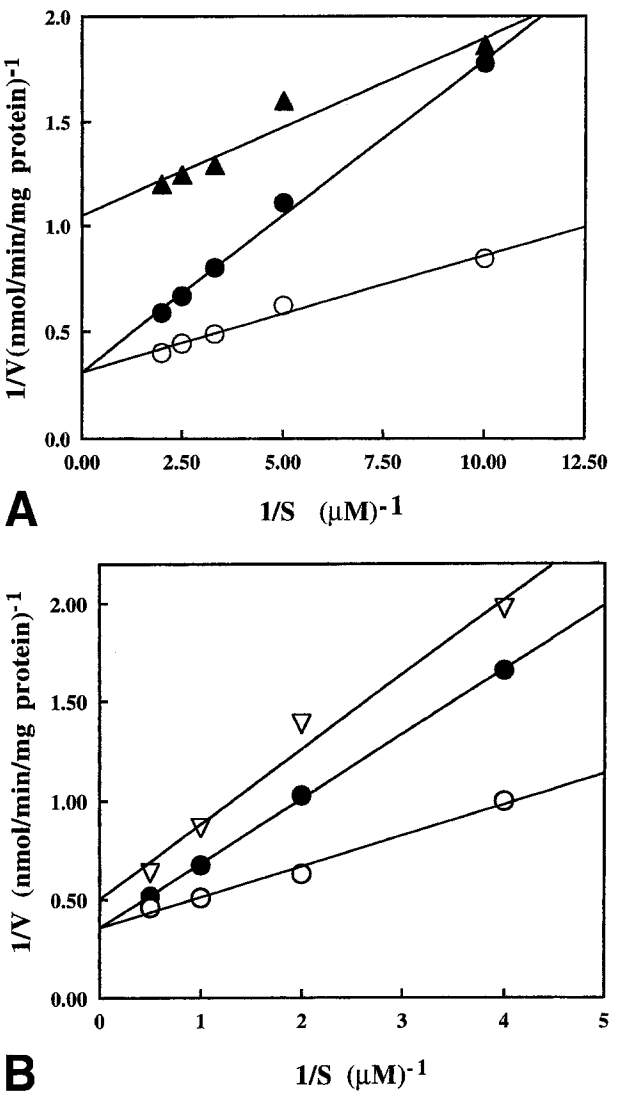

Fig 2. Lineweaver-Burk plots of benzo(a)pyrene metabolism (A) in the absence of retinoids (○) and the presence of 10 μmol/L retinal (●) and 10 μmol/L retinol (▲), and of 7-ethoxyresorufin deethylation (B) in the absence of retinoids (○), and the presence of 5 μmol/L retinal (●) and 5 μmol/L retinol (▲).

DISCUSSION

Polyaromatic hydrocarbons (PAHs) are the most common carcinogens and widespread environmental contaminants. PAHs are well known to induce the CYP 1A family of cytochrome P-450s and to be metabolized by CYP 1A.[4,14] Moreover, the carcinogenicity of PAHs appears after they are metabolized by CYP 1A.[2,14,15] Benzo(a)pyrene is one of the most well-known PAHs and is mainly metabolized by CYP 1A1.[2-4] The competitive inhibition of benzo(a)pyrene metabolism by retinal agrees with our recent finding that CYP 1A1 is the major cytochrome P-450 isozyme responsible for retinoic acid synthesis. 7-Ethoxyresorufin is a preferential specific substrate of CYP 1A1. 3-MC–treated rats exhibited a 11.6-fold increase in benzo(a)pyrene metabolism and a 42.7-fold increase in 7-ethoxyresorufin deethylation activity due to the induction of CYP 1A1. The activities in untreated rats can be accounted for primarily by the contribution of the low-affinity components of benzo(a)pyrene metabolism and 7-ethoxyresorufin deethylation.

The chemoprevention of cancer is a crucial issue in cancer research, and retinoids are considered important candidates for the chemoprevention of cancer. Experimental models of carcinogenesis have demonstrated the efficacy of pharmacologic levels of retinol in preventing cancers of the skin, oral cavity, lung, mammary gland, prostate, bladder, liver, and pancreas in animals exposed to carcinogenic agents.[10] The human lung cancer risk, which is correlated with exposure to PAHs in cigarette smoke, appears to be reduced by vitamin A or β-carotene.[16,17] Kellerman et al[18] suggested that the susceptibility to lung cancer may be linked to the polymorphic inducibility of (lymphocyte) benzo(a)pyrene hydroxylation, apparently catalyzed by CYP 1A1, and a correlation does seem to exist between the basal level of the enzyme and the incidence of lung cancer in smokers.[19] Exposure to benzo(a)pyrene promotes vitamin A depletion in exposed tissues.[20] However, to elucidate the chemopreventive effect of retinoids, most reports have concentrated on the cellular response to retinoic acid, that is, the binding of retinoic acid to the retinoic acid receptor, which acts as a transcription factor that regulates the proliferation and differentiation of tumor.[10] Although Hill and Shih[21] indicated that vitamin A compounds and analogs inhibit mixed-function oxidases that metabolize carcinogenic polycyclic hydrocarbons and other compounds, little attention has focused on this topic.

The results of the present study suggest that the preventive effect of retinoids in benzo(a)pyrene-induced carcinogenesis may be at least partially due to the specific inhibition by retinoids of benzo(a)pyrene activation catalyzed by CYP 1A1. The present results may also be applicable to other carcinogenic polycyclic hydrocarbons, which must first be activated to exert effects. Thus, this study provides new insight into the anticarcinogenic action of retinoids.

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