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EVIDENCE THAT COAL TAR IS A MIXED INDUCER OF MICROSOMAL DRUG-METABOLIZING ENZYMES

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SUMMARY

Topical application of coal tar (U.S.P.) to neonatal rats resulted in the induction of hepatic cytosolic glutathione-*S*-transferase and microsomal epoxide hydrolase and aminopyrine *N*-demethylase activities. Analogous to the effect of the polychlorinated biphenyl Aroclor 1254, treatment of neonatal rats with coal tar resulted in a one nm shift to the blue region in the wavelength maximum of the hepatic microsomal hemoprotein cytochrome P-450. These results demonstrate that therapeutic coal tar contains both type I and type II inducers of hepatic drug-metabolizing enzymes.

INTRODUCTION

Coal tar is a dirty brown black aromatic material obtained during the incomplete combustion of coal. It is an extremely complex mixture containing several thousand chemical moieties only a few of which have been characterized. Defined constituents include benzo(a)pyrene which is present in most coal tar preparations in concentrations ranging from 0.1–0.5 mg/g of tar. Coal tar is widely used as a therapeutic agent in dermatologic practice, particularly in the treatment of chronic dermatoses such as eczematous dermatitis and psoriasis [1, 2].

Topical application of coal tar to the skin of humans [3] and neonatal rats [4] results in the induction of AHH activity. In general, cytochrome P-450 dependent mixed-function oxidase activities, including AHH, are induced by pretreatment of animals with various xenobiotics. These mixed-function oxidase inducers can be broadly classified as type I or type II [5]. A prototype of type I is phenobarbital and

Abbreviation: AHH, aryl hydrocarbon hydroxylase.

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type I inducers have little or no induction effect on skin and liver AHH activity when applied topically or injected intraperitoneally [6, 7]. A prototype of type II inducers includes certain polychlorinated biphenyls and polycyclic aromatic hydrocarbons. These have been shown to induce skin and liver AHH activity in certain rodents including the neonatal rat [8]. Our finding that application of coal tar caused induction of skin AHH activity was of interest, though in retrospect not surprising, since coal tar contains several defined polycyclic aromatic hydrocarbons such as benzo(a)pyrene.

Another microsomal enzyme that is independent of cytochrome P-450 is known as epoxide hydrolase which functions in the conversion of epoxides to dihydrodiols [8–10]. Glutathione-*S*-transferase(s) participates in the detoxification of a variety of reactive metabolites including epoxides of polycyclic aromatic hydrocarbons [11–14]. Microsomal epoxide hydrolase activity in rat liver is not induced by pretreatment with type II inducers [9] whereas cytosolic glutathione-*S*-transferase(s) is induced by treatment with type I and type II inducers [11, 12]. By taking advantage of the differential induction of these two epoxide metabolizing enzyme activities and the known features of hepatic cytochrome P-450 we have found that coal tar contains constituents that possess both type I and type II induction properties for hepatic drug-metabolizing enzyme activities.

MATERIALS AND METHODS

[^{3}H] Benzo(a)pyrene-4,5-oxide (spec. act. 282 mCi/mmol) and unlabelled benzo(a)-pyrene-4,5-oxide were provided by the Cancer Research Program of the National Cancer Institute, Division of Cancer Cause and Prevention, Bethesda, MD. [8-^{14}C]. Styrene oxide (spec. act. 0.582 mCi/mmol) was purchased from New England Nuclear, Boston, MA, and unlabelled styrene oxide was a product of Eastman Organic Chemicals, Rochester, NY. The oxides were purified further before use and were > 98% pure. Glutathione, NADP, NADH and NADPH were obtained from Sigma Chemical Co., St. Louis, MO. Benzo(a)pyrene (gold label), 7-ethoxycoumarin (gold label) and 7-hydroxycoumarin (gold label) were product of Aldrich Chemical Co., Milwaukee, WI. Coal tar used was of USP grade.

Pregnant rats were obtained from Holtzman Rat Farm, Madison, WI. Newborn rats were allowed to suckle until day 4 after birth. The animals were withdrawn from their mothers and treated with skin application of 100 μl of coal tar (USP) solution or the inducers as described in the tables. Control rats were treated with acetone. 24 h after treatment, the rats were killed and skin and liver microsomes and cytosols prepared as described previously [8]. Glutathione-*S*-transferase activity of hepatic and cutaneous cytosols was assayed using styrene oxide and benzo(a)pyrene 4,5-oxide as substrates by quantitating product formation as described by James et al. [13]. Microsomal epoxide hydrolase towards styrene oxide and benzo(a)pyrene 4,5-oxide as substrates was assayed by the thin-layer chromatographic technique of

Jerina et al. [10]. The details of these assay procedures were described earlier [14, 15]. AHH activity was determined by a modification of the method of Nebert and Gelboin [16] as previously described [3]. The quantitation of phenolic BP metabolites was based on comparison of fluorescence to a standard of 3-OH-BP. 7-Ethoxycoumarin-*O*-deethylase activity was determined according to a slight modification of the procedure of Greenlee and Poland [17] the details of which were described earlier [8]. Aminopyrine *N*-demethylase activity was determined according to the radiometric procedure of Poland and Nebert [18], the details of which have been reported elsewhere [19]. Cytochrome P-450 was quantitated by the method of Omura and Sato [20] using a DW 2a dual beam spectrophotometer equipped with a Midan microprocessor. Protein was estimated according to Lowry et al. [21] using bovine serum albumin as reference standard.

RESULTS AND DISCUSSION

The data given in Table I compare the level of cytosolic glutathione-*S*-transferase activity (toward styrene oxide and benzo(a)pyrene 4,5-oxide as a second substrate) and microsomal AHH, 7-ethoxycoumarin *O*-deethylase, and epoxide hydrolase (toward benzo(a)pyrene 4,5-oxide and styrene oxide as substrates) activities in liver and skin of control and coal tar-treated neonatal rats. Following topical application

TABLE I

EFFECT OF A SINGLE TOPICAL APPLICATION OF COAL TAR SOLUTION (U.S.P.) TO NEONATAL RATS ON MICROSOMAL MIXED-FUNCTION OXIDASES, EPOXIDE HYDROLASE AND CYTOSOLIC GLUTATHIONE-*S*-TRANSFERASE IN LIVER AND SKIN

Data represent mean ± S.D. of four values. For each determination tissues from 6–8 neonatal rats were pooled. SO, Styrene oxide; 4,5-BPO, benzo(a)pyrene-4,5-oxide.
4-day-old rats were treated topically with (100 μl) of either Coal Tar Solution (U.S.P.) or with acetone (control). Rats were killed 24 h after treatment. Mixed-function oxidase and epoxide hydrolase (with SO and 4,5-BPO as substrates) in microsomes and glutathione-*S*-transferase (with SO and 4,5-BPO as substrates) in cytosolic fraction were assayed as described in the text.

Parameters	Skin		Liver	
	Control	Coal Tar	Control	Coal Tar
Aryl hydrocarbon hydroxylase[a]	1.12 ± 0.12	11.26 ± 1.05[b]	43.12 ± 2.51	268.21 ± 8.59[b]
7-Ethoxycoumarin deethylase[a]	0.40 ± 0.07	3.86 ± 0.42[b]	1.23 ± 0.14	3.59 ± 0.42[b]
Epoxide hydrolase (4,5-BPO)[c]	0.12 ± 0.03	0.13 ± 0.03	2.28 ± 0.16	4.00 ± 0.32[b]
Epoxide hydrolase (SO)[c]	0.15 ± 0.03	0.14 ± 0.03	2.58 ± 0.31	4.94 ± 0.47[b]
Glutathione-*S*-transferase (4,5-BPO)[c]	0.72 ± 0.03	0.82 ± 0.09	4.51 ± 0.52	7.36 ± 0.42[b]
Glutathione-*S*-transferase (SO)[c]	3.21 ± 0.24	3.18 ± 0.26	23.42 ± 1.21	41.32 ± 0.62[b]

[a]pmol product/min/mg protein.
[b]Statistically different ($P < 0.05$) from control.
[c]nmol product/min/mg protein.

of coal tar there was an approx. 10-fold induction of AHH and 7-ethoxycoumarin *O*-deethylase activities in skin. Hepatic AHH and 7-ethoxycoumarin *O*-deethylase activities were induced 6.2 and 2.9-fold, respectively. These observations are consistent with our earlier findings [4] and were expected because therapeutic coal tar contains a number of polycyclic aromatic hydrocarbons which are inducers of AHH (Mukhtar et al., in preparation). Coal tar application to the skin of neonatal rats also resulted in the induction of hepatic cytosolic glutathione-*S*-transferase activities. These enzymes are known to be induced by phenobarbital and 3-methylcholanthrene [1] and by certain polychlorinated biphenyls [8] which are mixed inducers of microsomal monooxygenases [22]. Pretreatment of rats with type I inducers also enhances epoxide hydrolase in liver [23]. In contrast type II inducers are ineffective in producing any effect on hepatic epoxide hydrolase [9, 24]. The induction of hepatic epoxide hydrolase by coal tar application to skin (Table I) suggests that coal tar contains type I inducers of microsomal mixed-function oxidases. This is further substantiated by the results shown in Table II. The liver weights, the liver: body weight ratios and the microsomal protein level were each increased by coal tar treatment. The absorption maximum of the CO-difference spectra from control neonatal rat liver was at 452 nm which is consistent with that reported previously [25]. A shift of 2 nm to the blue region was observed in 3-methylcholanthrene-treated neonatal rats. Treatment of the neonatal rats with Aroclor 1254 and with coal tar resulted in a 1 nm shift in the wavelength maximum of the hepatic hemoprotein. Aroclor 1254 treatment to adult rats has previously

TABLE II

EFFECT OF A SINGLE TOPICAL APPLICATION OF COAL TAR, AROCLOR 1254 AND 3-METHYLCHOLANTHRENE TO NEONATAL RATS ON LIVER WEIGHT, MICROSOMAL PROTEIN, CYTOCHROME P-450 CONCENTRATIONS, AND ABSORPTION MAXIMA OF THE HEME-PROTEIN

4-day-old neonatal rats (9 ± 1 g) were treated with topically applied acetone (control) or coal tar (U.S.P.) (100 μl), or with 3-methylcholanthrene or Aroclor 1254 (1 mg/10 g body weight) in 100 μl acetone. Rats were killed 24 h after the treatment. Values in the table are mean ± S.D. of four individual determinations.

	Liver wt. (mg)	Liver wt (mg/10 g) body wt)	Microsomal Protein (mg/g fresh liver)	Cytochrome-450	
				λmax (nm)[a]	nmol/mg protein
Control	221 ± 11	238 ± 12	4.10 ± 0.17	452	0.33 ± 0.01
Aroclor 1254	253 ± 8[b]	273 ± 14[b]	4.49 ± 0.14[b]	451	0.52 ± 0.02[b]
3-Methylcholanthrene	242 ± 9[b]	269 ± 13[b]	4.87 ± 0.14[b]	450	0.48 ± 0.03[b]
Coal tar	262 ± 13[b]	285 ± 12[b]	4.67 ± 0.12[b]	451	0.57 ± 0.03[b]

[a] The absorption maximum may deviate from these values by no more than ± 0.2 nm.
[b] Statistically significant ($P < 0.05$) from control.

been shown to result in a 1 nm blue shift [22]. This 1 nm shift has been shown to be a result of the presence of both type I and type II inducers in Aroclor 1254 which is a complex mixture of halogenated hydrocarbons. Coal tar appears to resemble Aroclor 1254 in that it contains both type I and type II inducers.

Aminopyrine is a typical type I substrate of cytochrome P-450 and its metabolism is known to be induced by the phenobarbital type of inducers [5, 18]. We have recently reported that skin microsomes possess low levels of aminopyrine *N*-demethylase activity [19]. A single topical application of coal tar to neonatal rats results in 1.3-fold induction of hepatic aminopyrine *N*-demethylase activity (Table III). The observed induction though small was statistically significant. These data clearly establish that coal tar also possesses phenobarbital type of inducers of drug metabolizing enzymes.

The results shown in Tables II and III also indicate that the induction response of the hepatic microsomal heme-protein, cytochrome P-450, is similar in neonatal and adult rats. Furthermore it is also clear from the data reported here that skin application of inducers of mixed-function oxidases present in coal tar penetrate the cutaneous barrier and gain entry into the circulation to produce effects on hepatic drug-metabolizing enzyme activities.

The data in this paper suggest that cutaneous application of therapeutic coal tar induces skin AHH and 7-ethoxycoumarin deethylase and liver epoxide-metabolizing enzyme activities aminopyrine *N*-demethylase activity and the heme protein cytochrome P-450 and that type I and type II inducers of drug-metabolizing enzyme-activity are present in coal tar; they do not imply that the responses observed are due to a single constituent or to multiple constituents of coal tar inasmuch as the response may be due wholly or in part to unidentified factor(s) acting alone or in combination. It is also likely that the observed response of drug-metabolizing enzyme activities to coal tar may be the result of synergism between several constituents whose individual concentrations might be insufficient to induce

TABLE III

EFFECT OF A SINGLE TOPICAL APPLICATION OF COAL TAR SOULATION (U.S.P.) TO NEONATAL RATS ON HEPATIC AND CUTANEOUS MICROSOMAL AMINOPYRINE *N*-DEMETHYLASE ACTIVITY

Data represent mean ± S.D. of four values. For each determination tissues from 20 neonatal rats were pooled. Treatment details as given under Table I.

	Aminopyrine *N*-demethylase pmol HCHO/min/mg protein		Treated/Control
	Control	Coal tar	
Skin	5.9 + 1.5	6.1 + 1.8	1.0
Liver	811 + 32	1059 + 72 [a]	1.3

[a]Statistically different ($P < 0.05$) from control.

the observed responses. These data further emphasize the need to more precisely define the inducers of mixed-function oxidases present in therapeutic coal tar and other complex mixtures of chemicals present in the environment.

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