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Induction of Neonatal Rat Skin and Liver Aryl Hydrocarbon Hydroxylase by Coal Tar and Its Constituents

DAVID R. BICKERS, M.D., DAVID WROBLEWSKI, TAPU-DUTTA-CHOUDHURY, M.S., AND HASAN MUKHTAR, PH.D.

Department of Dermatology, Case Western Reserve University School of Medicine, University Hospitals of Cleveland, Cleveland Veterans Administration Hospital, Cleveland, Ohio, U.S.A.

Topical application of coal tar solution (USP) to neonatal rats resulted in the induction of skin and liver aryl hydrocarbon hydroxylase (AHH) activities. Furthermore indirect exposure of the animals to coal tar vapors resulted in induction of the enzyme in skin and liver. Cutaneous application of coal tar to pregnant rats resulted in induction of skin and liver AHH activity in both mothers and prenatal rats. Among several defined constituents of coal tar tested benzo(a)pyrene (BP), anthracene and acridine were found to have measurable induction effects on neonatal rat skin and liver AHH. These studies indicate that therapeutic coal tar solution as well as selected defined chemical constituents of coal tar are capable of altering the activity of AHH in skin and liver.

The combustion of fossil fuels is responsible for the generation of a wide variety of substances, some of which have significant toxic effects. For example, when coal undergoes combustion a number of polyaromatic hydrocarbons are produced among them benzo(a)pyrene (BP). Some of these hydrocarbons have carcinogenic and mutagenic effects in biological systems [1–3].

Coal tar is a brown-black material also generated during the incomplete combustion of coal and is an extremely complex mixture containing several thousand chemical moieties only a few of which have been characterized. Defined constituents include BP which is present in most coal tar preparations in concentrations ranging from 0.1–0.5 mg/gm of tar [1]. Coal tar is also a widely used therapeutic agent in dermatologic practice, particularly in the treatment of chronic dermatoses such as eczematous dermatitis and psoriasis [4,5].

Polycyclic hydrocarbons such as BP and 7,12-dimethylbenz(a,h)anthracene (DMBA) are carcinogenic for the skin of experimental animals. Aryl hydrocarbon hydroxylase (AHH) is one of the cytochrome P450-dependent monooxygenases which is present in skin [6–8] and functions in the metabolism of various polycyclic aromatic hydrocarbons. This enzyme may play a critical role in carcinogenic responses to these compounds since the enzyme is capable of converting them into reactive metabolic species such as diol-epoxides that may initiate tumor formation. The current study was undertaken to assess the effects of coal tar and selected, defined, purified constituents of therapeutic coal tar on skin and liver AHH activity of neonatal rats.

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Reprint requests to: David R. Bickers, M.D., Professor and Chairman, Department of Dermatology, University Hospitals of Cleveland, 2074 Abington Road, Cleveland, Ohio 44106.

Abbreviations:
AHH: aryl hydrocarbon hydroxylase
BP: benzo(a)pyrene
DMBA: 7,12-dimethyl-benz(a,h)anthracene

MATERIALS AND METHODS

Animals and Treatment

Pregnant rats were obtained from Holtzman Rat Farm, Madison, Wisconsin. Neonatal rats were allowed to suckle until the day of experiment (4–6 days after birth). At that time 6–8 neonates were treated with a single application of 100 μl of coal tar solution (USP) and sacrificed 24 hr later. The animals were pooled for the studies of enzyme activity. For studies of maternal and prenatal enzymes 48 hr prior to the expected date of delivery the backs of pregnant animals were shaved and coal tar solution (USP) was applied to the shaved area. Treatment and other details are given in the appropriate tables.

Chemicals

Standard coal tar solution (USP) was used. Defined constituents of coal tar were purchased in the highest purity commercially available from Aldrich Chemical Co., Milwaukee, Wisconsin, and were recrystallized from hot acetone or benzene in a rotary evaporator to assure maximum purity.

Enzyme Assay

After the desired period of treatment with coal tar and its constituents, animals were killed by decapitation. In each experiment tissues from 6 animals were pooled for single determinations. Skin and liver were removed. Epidermal-dermal serparation was obtained by incubating whole skin in 0.1 M phosphate buffer pH 7.40 containing 10 mM dithiothreitol according to the procedure of Epstein, Munderloh, and Fukuyama [9]. No cross-contamination of epidermis and dermis occurs using this method. 9,000 *xg* supernatant fractions were prepared according to established techniques [10] and used as the source of enzyme. AHH activity was assayed according to Nebert and Gelboin [11] as previously described [12]. Specific enzyme activity is expressed as p moles of 3 hydroxy BP formed per minute per mg protein. Protein was measured according to Lowry et al [13] using bovine serum albumin as reference standard.

RESULTS

Application of coal tar solution to neonatal rats 24 hr prior to sacrifice induced skin and liver AHH 15- and 8-fold respectively. AHH induction in isolated epidermis and dermis was 10 and 18-fold over the corresponding control values (Table I). At the outset of these experiments control and coal tar treated animals were housed in separate but adjacent cages in the animal facility. We initially observed wide variations in the AHH activity of the skin of control animals. This degree of variation was initially unexplainable and was not observed in our other ongoing experiments in which chemicals other than coal tar were being tested. Because of the inherent volatility of coal tar it was suspected that vapors from the coal tar solution applied to the experimental animals might be inducing cutaneous AHH activity in the controls. Data supporting this hypothesis are also presented in Table I. Coal tar fumes induced skin and liver enzyme activities in the control animals housed adjacent to the animals to which coal tar was applied. Although the induction response was statistically significant it was considerably less than that observed in the directly treated animals. It is unlikely that this enzyme induction effect in skin was due to inhalation and subsequent delivery to skin since skin enzyme activity increased before any measurable increases occurred in the lungs (data not shown). However, it is possible that the kinetics of induction of AHH by coal tar differs in various tissues. On the

basis of these findings all further experiments were conducted using animals housed in separate rooms. When pregnant rats were treated with topically applied coal tar solution the skin and liver AHH activity of mothers was induced to a higher extent (3.8 and 4.8-fold for skin and liver respectively) than that achieved in the fetuses (2.0 and 1.9-fold for skin and liver respectively) (Table II). In further studies an effort was made to identify specific chemical constituents of coal tar that might induce AHH. The data in Table III demonstrate that some coal tar constituents such as benzene and naphthalene had no induction effects on liver and skin AHH whereas acridine had a significant induction effect only on the skin enzyme (2.2-fold). Anthracene and BP had greater induction effects than any of the other constituents in skin.

TABLE I. *Effect of cutaneous application of coal tar solution (USP) to neonatal rats on skin and liver AHH*

Treatment	AHH p mole 3-OH BP/ min/ mg protein			
	Whole skin	Epidermis	Dermis	Liver
Control[a]	0.24 ± 0.03	0.35 ± 0.02	0.42 ± 0.03	23.22 ± 1.41
Coal tar[b]	3.69 ± 0.42[d]	3.58 ± 0.51[d]	7.82 ± 0.81[d]	192.73 ± 5.82[d]
Coal tar fumes[c]	0.51 ± 0.06[d]	0.62 ± 0.04[d]	0.86 ± 0.06[d]	39.47 ± 1.57[d]

[a] Four-day-old neonatal rats were treated with topically applied acetone (100 μl) (and kept in a room separate from other experimental animals).

[b] Animals were treated with 100 μl of coal tar solution (USP) 24 hr prior to sacrifice.

[c] Animals were treated with 100 μl acetone and housed in cages adjacent to coal tar treated animals 24 hr prior to sacrifice. Data represents mean ± SD of 3 experiments.

[d] Results are significantly different from respective controls ($p < 0.05$).

TABLE II. *Effect of cutaneous application of coal tar solution (USP) to pregnant rats on maternal and fetal skin and liver AHH*

	Skin		AHH (p mole 3-OH BP/ min/ mg protein) Liver	
	Control	Treatment	Control	Treated
Mother	1.01 ± 0.11	3.82 ± 0.26[a]	3.24 ± 0.31	15.72 ± 0.86[a]
Prenatal (−16 hr)	0.24 ± 0.01	0.47 ± 0.03[a]	0.45 ± 0.05	0.85 ± 0.07[a]

Sperm positive pregnant rats at 19 days of gestation (2 days before expected delivery) were shaved and treated with 500 μl coal tar solution (USP). 24 hr later (16 hr before expected delivery) mothers were killed by decapitation. Unborn rats from control and coal tar-treated mothers were removed and washed thoroughly. Skin and liver 9,000 *xg* supernatant fraction of mother and prenatal rats were prepared and used as the enzyme source. Data represent mean ± SD of 3 experiments in each of which one mother and a minimum of 8 neonates was used.

[a] Results statistically different from controls ($p < 0.05$).

DISCUSSION

Although the precise relationship between AHH activity, inducibility of the enzyme in target tissues and susceptibility to chemical carcinogenesis by polycyclic aromatic hydrocarbons remains controversial [14,15], some believe that enzyme activity is a major determinant of carcinogenic risk [16]. The importance of the metabolism of BP in inducing tumors in experimental animals is clear from studies showing that BP-7,8-dihydrodiol is a precursor for isomeric diol-epoxides which are thought to be the ultimate carcinogenic species of BP [17]. Diol-epoxides of BP have tumorigenic effects in skin equal to or exceeding that of the parent compound [18]. It should be emphasized however that other enzymes are involved in the metabolic transformation of polycyclic hydrocarbons. These include epoxide hydrolase and glutathione S-transferases which could also influence the susceptibility of the skin to chemical carcinogenesis [19,20]. Since the latter enzymes appear to be, in general, noninducible in skin, AHH must play a major role in the generation of tumorigenic metabolic species in skin.

The use of medications containing coal tar has been associated with skin cancer in human populations [21,22]. Furthermore coal tar is a potent skin carcinogen in experimental animals [23]. BP has been suspected as the major constituent in coal tar responsible for tumorigenic activity. Poel and Kammer [24] applied different coal tar fractions to mouse skin and found that fractions without detectable BP were also tumorigenic. These studies suggested that the carcinogenicity of coal tar is not simply related to BP content but that other chemical constituents could also be tumorigenic. Coal tar is a complex mixture containing at least 10,000 constituents less than 500 of which have been identified structurally. Our data indicate that topical application of coal tar or exposure of the skin to volatile coal tar vapors results in the induction of liver and skin AHH in neonatal rats. In addition application of coal tar to pregnant animals caused induction of AHH activity in both maternal and prenatal skin and liver. These findings indicate that coal tar is capable of crossing the feto-placental barrier in the rat. Since vapors from topical applied coal tar result in the induction of AHH activity in liver and skin of otherwise untreated animals it can be presumed that other tissues could be at risk for oncogenesis as well. For example the inhalation of coal tar vapors could initiate tumor formation in the lung.

Of the several known constituents of coal tar tested, acridine produced no induction effects on liver AHH, but induced the skin enzyme almost 3-fold. Anthracene produced comparable induction effects (2.7-fold) on liver and skin AHH. BP was the most potent inducer of the skin enzyme studied here.

Our studies indicate that for therapeutic purposes it would be desirable to remove those constituents of coal tar with potentially toxic/carcinogenic effects such as BP. Ideally those would be selectively extracted while maintaining the therapeutic efficacy of the coal tar. However, prior attempts to remove such chemicals have resulted in a dramatic decrease in therapeutic efficacy [25]. These studies further emphasize that tis-

TABLE III. *Effect of cutaneous application of several defined constituents of coal tar on skin and liver AHH activity in neonatal rats*

Constituent[a]	Skin		AHH (p mole 3-OH BP/ min/ mg protein) Liver	
	Control	Treated	Control	Treated
Benzene	0.51 ± 0.03	0.54 ± 0.04	22.15 ± 4.12	24.81 ± 5.12
Naphthalene	0.53 ± 0.05	0.57 ± 0.07	23.12 ± 3.12	25.89 ± 4.81
Acridine	0.57 ± 0.05	1.23 ± 0.02[b]	23.17 ± 3.33	27.80 ± 6.67
Anthracene	0.53 ± 0.04	1.43 ± 0.11[b]	21.16 ± 1.67	58.67 ± 9.67[b]
Benzo(a)pyrene	0.59 ± 0.04	5.23 ± 0.40[b]	27.83 ± 8.33	214.50 ± 9.33[b]

[a] Each was dissolved in acetone or benzene (once benzene itself was shown to be ineffective) and administered topically to 4-day-old rats in a single dose (100 mg/kg body weight). Animals were sacrificed 24 hr later. Data represent mean ± SD of 3–4 experiments in each of which a minimum of 6 neonates was studied.

[b] Results are significantly different from respective controls ($p < 0.05$).

sues such as skin which function as interfaces between the body and its environment possess enzyme activity that can be directly influenced by exposure to various exogenous agents, including topically applied drugs.

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