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SHORT COMMUNICATIONS

The effect of chloramphenicol on the metabolism of 7,12-dimethylbenz[*a*]-anthracene in rat adrenal and liver microsomes

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7,12-Dimethylbenz[*a*]anthracene (DMBA) is one of the most potent synthetic carcinogens and mutagens known. DMBA also causes cell destruction in the adrenal cortex and testis of adult rats. DMBA is converted to several metabolites by the cytochrome P-450 dependent monooxygenase system, and it is commonly accepted that metabolic activation is required for the mutagenic and tumorogenic effects of the compound. On the other hand, the role of biotransformation in the adrenocorticolytic effect of DMBA has not been elucidated, and in particular the relative importance of the hepatic and adrenal metabolism of the compound for the adrenal toxicity is unclear. Pretreatment of rats with 3-methylcholanthene has been shown to induce the hepatic but not the adrenal metabolism of DMBA and to protect against the adrenocorticolytic effect (for review see Refs 1 and 2).

The antibiotic chloramphenicol has also been shown to prevent the development of adrenal necrosis caused by DMBA [3], but the effect of chloramphenicol on the adrenal and hepatic metabolism of DMBA has not been investigated. Chloramphenicol has been shown to protect against the toxicity and tumorigenicity of a number of different xenobiotics including polycyclic aromatic hydrocarbons, polychlorinated hydrocarbons, and nitroso compounds [4–7]. The protection by chloramphenicol against the hepatotoxicity of such compounds as carbon tetrachloride is most likely due to the ability of chloramphenicol to act as a suicide substrate of rat liver cytochrome P-450 [8]. However, the molecular mechanism of the protection by chloramphenicol against extrahepatic toxicity is still obscure. In the present report the effect of chloramphenicol *in vivo* and *in vitro* on the metabolism of DMBA in rat liver and adrenal microsomes has been investigated.

Preparation of rat adrenal and liver microsomes. Inbred female Sprague–Dawley rats weighing 180 g (49–50 days old) were used in all experiments. At 1.5 or 48 hr after treatment with chloramphenicol, DMBA or vehicle, the animals were killed by decapitation and the adrenal glands and livers from three or four animals were weighed and pooled. Microsomes were prepared essentially according to Ogle [9] and Ernster *et al.* [10], respectively, and were stored at −20° until use. The protein concentration was determined according to the method of Lowry *et al.* [11].

Assays. The incubation medium for assaying DMBA metabolism contained 2 mM isocitrate, 6 mM $MgCl_2$, 30 μg isocitrate dehydrogenase, 25 or 50 μM [^{14}C]DMBA (5.1–40.6 mCi/mmole), 2 mM EDTA, 15 mM KCl, 5 mM K_2PO_4, 50 mM Tris–HCl (pH 7.0) and 0.05–1 mg of microsomal protein in a final volume of 0.5 ml. After preincubation for 5 min at 30° the reaction was started by the addition of 0.5 mM NADPH and allowed to proceed for 60 min. Under these conditions activity was linearly dependent on protein concentration and time [2]. The incubation was stopped with potassium hydroxide in dimethylsulfoxide and the unreacted substrate extracted with *n*-hexane [2]. Alternatively, the incubations were stopped by the addition of 4 ml ethyl acetate containing 0.04% (w/v) butylated hydroxytoluene. After an additional extraction with 4 ml of the ethyl acetate, the ethyl acetate phases were combined and analyzed for DMBA metabolites by HPLC as described earlier [2]. Covalent binding was estimated after adsorption of protein to filter paper discs [2].

Effect of chloramphenicol in vivo *on DMBA metabolism.* Doses of chloramphenicol above 100 mg/kg have been shown previously to protect completely against adrenal necrosis when administered 1.5–2 hr prior to an otherwise toxic dose of DMBA. After 48 hr, however, even doses as high as 1000 mg/kg chloramphenicol provided only partial protection [6]. The effect of treatment of rats for 1.5 or 48 hr with 300 mg/kg chloramphenicol and/or 100 mg/kg DMBA on the liver and adrenal microsomal metabolism of DMBA to both soluble and covalently-bound products was therefore examined. As can be seen in Table 1, the rate of metabolism of DMBA by adrenal microsomes from rats pretreated with chloramphenicol for 48 hr was only slightly decreased in comparison with control microsomes; no effect was seen after 1.5 hr pretreatment. The same results were obtained with pretreatment with DMBA or a mixture of chloramphenicol and DMBA where chloramphenicol was added before DMBA. Liver metabolism of DMBA was markedly decreased by pretreatment with chloramphenicol for 48 hr but not for 1.5 hr. Pretreatment with DMBA for 48 hr led to the expected 30-fold increase in DMBA metabolism, which was not influenced by the additional pretreatment with chloramphenicol.

The effect of the various treatments on the covalent binding of DMBA metabolites to adrenal and liver microsomal protein *in vitro* is shown in Table 2. It may be seen that, in the adrenal 1.5 hr after chloramphenicol administration, there was no difference between the chloramphenicol-treated and control groups, whereas after 48 hr of chloramphenicol pretreatment there was a slight decrease in covalent binding both in the chloramphenicol and chloramphenicol plus DMBA pretreated groups. In the liver, pretreatment with chloramphenicol for 1.5 hr had no effect on covalent binding whereas pretreatment for 48 hr inhibited binding completely. Pretreatment with DMBA for 48 hr gave the expected 20-fold increase in covalent binding as compared to the control. The results in Tables 1 and 2 are consistent with previous findings that the monooxygenase system responsible for DMBA metabolism in the rat adrenal is not inducible by polycyclic hydrocarbons [12, 13].

In view of the quantitative changes in DMBA metabolism and covalent binding caused by chloramphenicol treatment, it was considered of interest to examine whether the DMBA metabolite profiles in liver or adrenal microsomes were altered by the treatment with chloramphenicol. However, analysis of ethyl acetate extracts by HPLC revealed no significant differences between the chloramphenicol-treated and control groups (not shown).

In vitro *studies with chloramphenicol.* Using ^{14}C-labeled chloramphenicol (11.7 mCi/mmole) at a concentration of 75 μM, no NADPH-dependent metabolism or covalent binding of chloramphenicol in adrenal microsomes or mitochondria was observed. In accordance with these results, preincubation of adrenal microsomes with 500 μM chloramphenicol for 30 min prior to the addition of 50 μM DMBA, or simultaneous incubation with chloramphenicol and DMBA for 1 hr, did not alter the rate of DMBA

Table 1. Metabolism of DMBA by rat liver and adrenal microsomes*

Conditions	Time of pretreatment (hr)	Activity† (pmoles/min · mg protein) Liver	Adrenal
Expt. 1			
Control		16.4 ± 1.5	307 ± 5
+ chloramphenicol	1.5	19.8 ± 0.3	304 ± 18
Expt. 2			
Control		17.6 ± 5.6	211 ± 2
+ chloramphenicol	48	1.52 ± 5.9	168 ± 3
+ DMBA	48	549 ± 47	173 ± 3
+ chloramphenicol + DMBA	48	542 ± 27	162 ± 5

* In Expt. 1 three or four female rats (180 g) were injected i.p. with 300 mg/kg chloramphenicol dissolved in 0.3 ml 1,2-propanediol; the controls received only the solvent. 1.5 hr later the rats were killed. In Expt. 2 the same number of rats were treated with chloramphenicol or DMBA and killed 48 hr later; alternatively, the rats were treated with chloramphenicol for 1.5 hr followed by 48 hr of DMBA treatment. Assays were performed as described in the text and Ref. 2.

† Mean ± S.D. of three determinations on microsomes pooled from three or four animals per group.

Table 2. Covalent binding of DMBA metabolites to liver and adrenal microsomal protein *in vitro**

Conditions	Time of pretreatment (hr)	Covalent binding† (pmoles/min · mg protein) Liver	Adrenal
Expt. 1			
Control		0.71 ± 0.08	4.88 ± 0.44
+ chloramphenicol	1.5	0.66 ± 0.06	5.36 ± 0.28
Expt. 2			
Control		0.60 ± 0.06	5.83 ± 0.26
+ chloramphenicol	48	0	4.77 ± 0.54
+ DMBA	48	11.3 ± 2.7	5.43 ± 0.41
+ chloramphenicol + DMBA	48	15.5 ± 2.50	4.84 ± 0.53

* Female rats (180 g) were treated as described in Table 1. Covalent binding of DMBA metabolites was measured as described in the text [2].

† Mean ± S.D. of four determinations on microsomes pooled from three or four animals per group.

metabolism (not shown). These results are in contrast to the known effect of chloramphenicol on the liver microsomal metabolism of a number of substrates [8].

Conclusions. The results of the present investigation suggest that the previously reported protection by chloramphenicol against the adrenocorticolytic effect of DMBA may be mediated by a different mechanism than inhibition or induction of DMBA metabolism. Thus, 1.5 hr after chloramphenicol adminstration *in vivo*, no effect was observed on the *in vitro* metabolism of DMBA by adrenal or liver microsomes, nor did chloramphenicol inhibit the induction by DMBA of its own metabolism in the liver. Although there was a slight effect of treatment with chloramphenicol for 48 hr on the adrenal metabolism of DMBA, this is in conflict with the observation that the protective effect of chloramphenicol against adrenal necrosis is diminished after 48 hr as compared to 1.5 hr [3]. In the induced liver, but presumably not in the untreated liver, the bulk of the DMBA metabolism appears to be catalyzed by a hydroxylase which is different from that metabolizing chloramphenicol, and chloramphenicol is probably not metabolized at all in the adrenals. Thus, although the protection by chloramphenicol against the hepatotoxic effects of certain compounds is most likely due to enzyme inhibition (cf. also Ref. 8), the prevention of liver carcinogenesis of 3′-methyl-4-dimethyl-aminoazobenzene by chloramphenicol could not be explained on the basis of diminished metabolic activation of the carcinogen [5]. Thus, chloramphenicol may protect against different forms of toxicity by different mechanisms.

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Effect of ACTH on cytochrome P-450 content and DMBA metabolism in immature rat adrenal

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The aromatic hydrocarbon 7,12-dimethylbenz-[*a*]anthracene (DMBA)* is one of the most potent chemical carcinogens known. It causes cancer in several organs, e.g. the liver, lung, skin, ovary and mammary gland [1]. In rat adrenal cortex and testis DMBA but not BP or other polycyclic aromatic hydrocarbons causes necrosis. The two inner zones of the adrenal cortex are destroyed and this effect is dependent on endogenous factors, e.g. ACTH [1] and estradiol [2]. Protection against necrosis may be obtained by certain cytochrome P-450 inhibitors and inducers [3].

However, the adrenals are unaffected if DMBA is administered to immature or hypophysectomized mature rats, but necrosis is induced if ACTH is co-administered [4]. In mature rats this has been shown to be related to an ACTH-dependent cytochrome P-450 content and BP hydroxylase activity [5]. However, the consequences of ACTH administration to immature rats have not been investigated with regard to changes in enzyme levels involved in detoxification.

Treatment of rats in vivo *with ACTH and DMBA.* Experiments were carried out with juvenile (20 days old, 40 g) and adult (50 days old, 180 g) female Sprague–Dawley rats. Both classes of rats were divided into four groups (control, ACTH, ACTH plus DMBA, and DMBA groups) each containing five rats. Juvenile rats were treated with ACTH for 10 consecutive days prior to decapitation. The ACTH and the ACTH plus DMBA groups were injected intramuscularly with ACTH (8 I.U./day) alone or plus a single intraperitoneal injection of DMBA (25 mg/kg, dissolved in corn oil) 3 days before decapitation. ACTH was dissolved as a lyophilized powder in an acidic saline buffer (pH 2). In the case of the DMBA group only DMBA was administered in this manner. Control and ACTH groups received corn oil (2.8 ml/kg). Treatment of adult rats was carried out in a similar way except that the rats received 4 I.U. of ACTH/day for seven consecutive days. The intraperitoneal dose of DMBA was 50 mg/kg in this case.

Adrenal microsomes from each group of five rats were pooled and prepared as described by Ogle [6]. Determination of DMBA metabolism was carried out with HPLC analysis and by a distribution assay [7]. The content of cytochrome P-450 was estimated according to Omura and Sato [8].

ACTH (Synacten Depot type) was generously donated by Ciba-Geigy (Basel, Switzerland). DMBA and [^{14}C]dimethyl-DMBA were purchased from Sigma Chemical Co. (St. Louis, MO) and NEN (Dreieichenbahn, F.R.G.), respectively.

Effect of ACTH on cytochrome P-450 and AHH. Adrenal microsomes isolated from control immature female Sprague–Dawley rats converted DMBA at a rate of 48.3 pmoles/min/mg protein (Table 1). Administration of ACTH increased this activity almost two-fold. Typically, the content of microsomal cytochrome P-450 showed a similar increase following ACTH administration (Table 1). In absolute terms the values of the control rats were considerably higher than those reported by Jellinck *et al.* [9] who found a complete lack of AHH activity with DMBA as substrate in 10–15-day-old untreated rats. This discrepancy may be due to the fact that 30-day-old rats were used in the present investigation. Table 1 shows the cytochrome P-450 content and AHH activity of adrenal microsomes isolated from adult female Sprague–Dawley rats. In this case the AHH activity was 86.3 pmoles/min/mg protein with DMBA as the substrate; administration of ACTH did not change cytochrome P-450 content or AHH activity significantly.

DMBA administration caused a slight decrease in DMBA metabolism in both ACTH-treated as well as untreated immature or mature rats (Table 1). This lower activity may be due to the presence of unmetabolized DMBA in the microsomal membranes during the assay, derived from injected DMBA, or reflect inactivation of the DMBA-metabolizing enzymes.

Conclusion. The present results show that adrenal microsomal cytochrome P-450 and AHH are lower in immature female rats than in mature ones and that ACTH administration markedly decreases this difference by inducing cytochrome P-450 and AHH in the immature rats. It is thus conceivable that the low and ACTH-dependent levels of

* Abbreviations: AHH, aryl hydrocarbon hydroxylase; DMBA, 7,12-dimethylbenz[*a*]anthracene; BP, benz-[*a*]pyrene; ACTH, adrenocorticotropic hormone.