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BIOCHEMICAL BASIS FOR THE RESISTANCE OF GUINEA PIGS TO CARCINOGENESIS BY 2-ACETYLAMINOFLUORENE

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SUMMARY

Studies were made on why guinea pigs are resistant to carcinogenesis by 2-acetylaminofluorene. Cytochrome P-448 and arylhydrocarbon hydroxylase were not induced in either the microsomes and nuclei of guinea pigs by 3-methylcholanthrene treatment. 3-Methylcholanthrene treatment caused only 2-fold increase in the binding of 2-acetylaminofluorene to DNA in nuclei isolated from guinea pigs, while it caused 17-fold increase in the binding in rat nuclei. Microsomes from 3-methylcholanthrene treated rats had 5 times more effect than microsomes from 3-methylcholanthrene treated guinea pigs on the binding of 2-acetylaminofluorene to DNA of nuclei from untreated guinea pigs. N-Hydroxy-2-acetylaminofluorene combined equally well with the DNA of rats and guinea pigs. In guinea pigs, there was a good correlation between the low inducibility of cytochrome P-448 and the low binding of 2-acetylaminofluorene to DNA. Our results clearly showed that guinea pigs are resistant to tumor induction by 2-acetylaminofluorene through inability to carry out the first step of activation of 2-acetylaminofluorene.

INTRODUCTION

2-Acetylaminofluorene(AAF)* is a potent carcinogen in several species of animals(1,2) and it requires two steps of activation, the first step being N-hydroxylation with cytochrome P-450 dependent monooxygenase systems(3). Guinea pigs do not develop tumors when treated with AAF(2). However, as they do develop tumors when treated with N-OH-AAF, a proximate form of AAF, and as very small amounts of N-OH-AAF have been identified as a urinary metabolite of animals treated with AAF (2), guinea pigs may be deficient in the enzyme necessary for the first step of activation of AAF(4).

Previously we found that AAF is activated and bound to nuclear DNA in two different ways in rats(5): one way is direct activation and binding to DNA in the nuclei, and the other is microsome-dependent activation followed by intracellular

*Abbreviations: AAF, 2-acetylaminofluorene; AHH, arylhydrocarbon hydroxylase; 3-MC, 3-methylcholanthrene; N-OH-AAF, N-hydroxy-2-acetylaminofluorene.

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transfer of activated AAF to nuclei. If interaction of AAF with nuclear DNA is a critical event in carcinogenesis, there should be a difference in the binding of AAF to DNA in animals that are susceptible and resistant to AAF carcinogenesis.

Therefore, we investigated the binding of AAF to nuclear DNA of nuclei isolated from untreated or 3-MC-treated guinea pigs. We found that 3-MC treatment did not increase the binding of AAF to DNA or induce cytochrome P-448 in either the microsomes or nuclei of guinea pigs. On the other hand, N-OH-AAF bound equally well to DNA in nuclei from guinea pigs and rats. These results suggest a relation between the ability of AAF to bind to DNA and its carcinogenicity

MATERIALS AND METHODS

Preparation of nuclei and microsomes from guinea pigs and rats: Male guinea pigs(Hartley) and Sprague-Dawley rats weighing 180-200 g were used. 3-MC was injected intraperitoneally into animals 40 hours before killing them(30 mg/kg and 10 mg/kg to guinea pigs and rats, respectively). The procedures for preparation of nuclei and microsomes from untreated or 3-MC-treated animals were as described previously(5).

Binding of AAF to nuclear DNA: For in vitro binding of AAF to DNA the incubation mixture contained nuclei from untreated or 3-MC-treated animals(9 mg of protein) and 2 mg of NADPH adjusted to a total volume of 4 ml with 0.25 M sucrose-3 mM $CaCl_2$. The reaction was started by adding 3 μCi of ^{14}C-AAF, and after incubation for the indicated time at 37°C, the reaction mixture was cooled in ice-cold water, nuclei were precipitated, and DNA was purified as described by Marmur(6).

Analytical procedures: The contents of cytochrome b_5 and P-450 in nuclei and microsomes were determined as described by Omura and Sato(7). NADH- and NADPH-cytochrome c reductases were assayed as described by Omura and Takesue(8). AHH was measured by the method of Nebert and Gelboin(9). Protein was determined by the method of Lowry et al.(10). Phospholipid-phosphorus was determined by Allen's procedure(11) and values obtained were multipled by 25 to obtain the amount of phospholipid. DNA was estimated by measuring the absorption at 259 nm (1 A_{259}= 50 μg/ml).

Chemicals: [9-^{14}C]-AAF(46.16 mCi/mmole) was purchased from New England Nuclear, Boston, U.S.A; [9-^{14}C]-N-OH-AAF(32 mCi/mmole) was from ICN Pharmaceutical Inc; NADPH was from Oriental Yeast Co., Tokyo; and cytochrome c, RNase A, and RNase T_1 were from Sigma Chemical Co., St. Louis. Other chemicals were standard products of reagent grade.

RESULTS

Figure 1 shows the binding of AAF to nuclear DNA of rats and guinea pigs. The binding of AAF to DNA of the nuclei from untreated rats and guinea pigs was identical. However, previous treatment of the animals with 3-MC induced 17-fold increase in the binding to nuclear DNA of rats, but only 2-fold increase in the

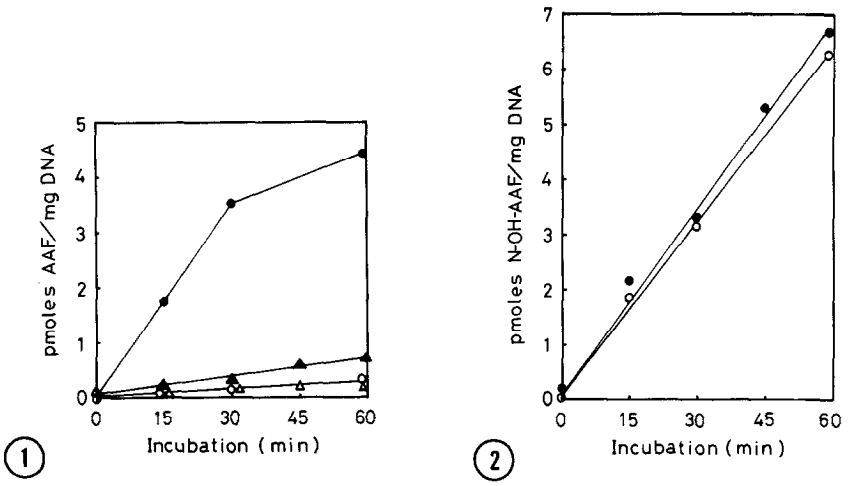
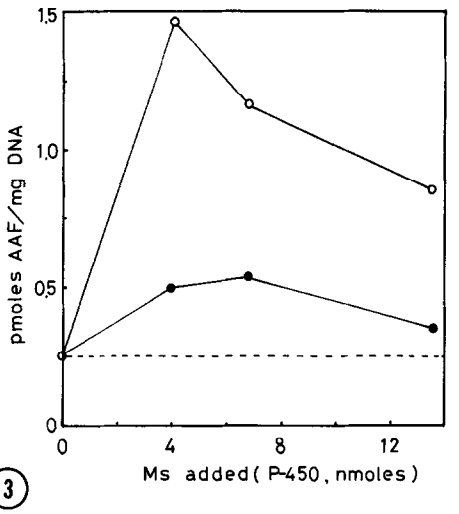


Figure 1. Effect of incubation time on the binding of AAF to DNA. Incubation was carried out as described in the MATERIALS AND METHODS. The radioactivity of AAF covalently bound to DNA was counted. (▲) nuclei from 3-MC-treated guinea pigs, (△) nuclei from untreated guinea pigs, (●) nuclei from 3-MC-treated rats, (○) nuclei from untreated rats.

Figure 2. Binding of N-OH-AAF to nuclear DNA of nuclei isolated from rats and guinea pigs. Incubation was carried out as in the experiment of figure 1 except that 2 μCi of ^{14}C-N-OH-AAF was added and NADPH was omitted. (○) nuclei from 3-MC-treated rats, (●) nuclei from 3-MC-treated guinea pigs.

binding to that of guinea pigs. Figure 2 shows that the binding of N-OH-AAF, a proximate form of AAF, to DNA of the nuclei of rats and guinea pigs was identical. Figure 3 shows the effects of microsomes from 3-MC-treated rats and guinea pigs on the binding of AAF to DNA with nuclei of untreated guinea pigs. The binding of AAF increased on addition of small amounts of microsomes, but decreased on addition of large amounts of microsomes from either species. These results agree with previous observations(5). Microsomes of 3-MC-treated rats induced about 5 times more binding than those of 3-MC-treated guinea pigs. Thus the first activation step of AAF seems to be different in rats and guinea pigs.

Table I shows the oxidoreductase activities of microsomes and nuclei from untreated and 3-MC-treated guinea pigs. On the basis of phospholipid, the specific activities of these enzymes in the nuclear fraction were about 50 % of those in the microsomal fraction. Treated of guinea pigs with 3-MC did not affect the amount of cytochrome P-448 in either the microsomes or nuclei(Figure 4), but



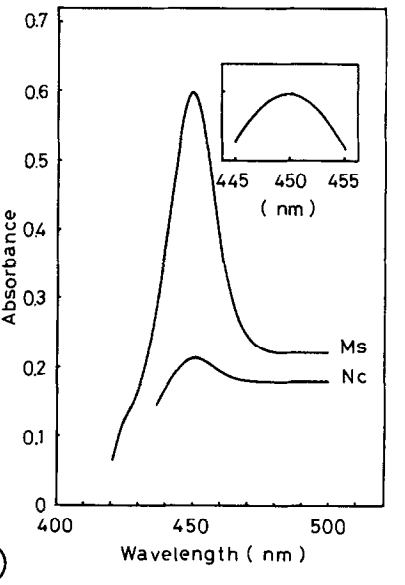


Figure 3. Binding of AAF to nuclear DNA in the presence of microsomes. Various amounts of microsomes from 3-MC-treated guinea pigs(●) or rats(o) were added to assay mixtures containing constant amounts of nuclei(9 mg of protein) from untreated guinea pigs. The contents of cytochrome P-450 in microsomes isolated from 3-MC-treated rats and guinea pigs were 1.5 nmoles/mg of protein and 1.7 nmoles/mg of protein, respectively. Nuclei and microsomes were separated by centrifugation at 3,000 rpm for 10 minutes. The other experimental procedures were as described in the MATERIALS AND METHODS.

Figure 4. Carbon monoxide-difference spectra of microsomal and nuclear fractions from the livers of 3-MC-treated guinea pigs. The abscissa of the figure indicates the wavelength, and the ordinate the absorbancy, The inset shows an expansion of absorption between 455 nm and 445 nm of the microsomal fraction. Standard assay conditions used were: microsomal fraction(Ms, 2.4 mg protein/ml), nuclear fraction(Nc, 3.0 mg protein/ml).

Table I. Enzyme Activities of Microsomes and Nuclei of Guinea pig Liver Cells.

Treatment	untreated		3-MC-treated	
Enzyme Activity	Ms	Nc	Ms	Nc
NADH-cytochrome c reductase(U/mg PL*)	0.85	0.56	0.81	0.44
Cytochrome b_5(nmoles/mg PL)	0.90	0.46	1.19	0.72
NADPH-cytochrome c reductase(U/mg PL)	0.18	0.11	0.27	0.10
Cytochrome P-450(nmoles/mg PL)	1.97	0.81	3.37	1.28
AHH(mU/mg PL)	2.55	1.25	5.44	2.80

PL* = Phospholipid; Ms, Microsomes; Nc, Nuclei
Data shown in this table represent the average values in 3 separate experiments.

increased the contents of cytochrome P-450 about 70 % in the microsomes and 60 % in the nuclei. 3-MC treatment caused only about 2-fold increase in the AHH activity in the two fractions.

DISCUSSION

To investigate the mechanism of resistance of guinea pigs to carcinogenesis by AAF, we examined the induction of cytochrome P-448 by 3-MC and the binding of AAF to DNA of isolated nuclei from guinea pigs. Thorgeirsson et al.(12) showed that cytochrome P-448 participates in mutagenesis by AAF in mice, while Lotlikar et al.(13) found that it is involved in the formation of N-OH-AAF from AAF in reconstituted microsomal monooxygenase systems. Thus we hoped to obtain further information on the correlation between the inducibility of cytochrome P-448 and tumor induction by AAF by experiments on guinea pigs. We found that in guinea pigs, cytochrome P-448 in the microsomes and nuclei of liver was not induced by 3-MC treatment(Table I, Figure 4), indicating a correlation of deficiency of cytochrome P-448 with resistance to carcinogenesis by AAF in guinea pigs. These findings are consistent with reports of the low inducibility of formation of N-OH-AAF by 3-MC treatment in guinea pigs(14), and of the non-inducibility of mutagenesis by AAF using the S-9 fraction from guinea pigs treated with β-naphthoflavone and phenobarbital(15).

The non-inducibilities of cytochrome P-448 in the microsomes and nuclei of guinea pigs by 3-MC treatment are reflected in the low covalent binding of AAF to nuclear DNA; both direct activation of AAF in the nuclei followed by its binding to DNA in situ(Figure 1) and intracellular transfer of hydroxylated AAF from the microsomes to nuclear DNA(Figure 3) were less in guinea pigs than in rats. On the other hand, there was no difference between the binding of N-OH-AAF to nuclear DNA in rats and guinea pigs(Figure 2). These findings show guinea pigs lack the enzymes necessary for the first step of activation of AAF, but that the following activation step of AAF is present in guinea pigs as well as in rats(5,16). A good relation was found between the binding of AAF to nuclear DNA and its carcinogenicity.

Recently, Thorgeirsson et al.(17) reported that in cotton rats, a species resistant to AAF-induced carcinogenesis, cytochrome P-448 and N-hydroxylation of AAF were induced by 3-MC treatment. These results suggest that cotton rats may be deficient of the enzymes necessary for the second step of activation of AAF. If so, it seems that in different species different mechanisms are responsible for resistance to carcinogenesis by AAF. Thus, it seems useful for elucidation of mechanism of chemical carcinogenesis to compare the mechanisms of action of carcinogens.

Gutmann et al.(18) reported that a single injection of AAF into rats increased the N-hydroxylation of AAF by hepatic microsomes without changing the content of microsomal cytochrome P-450, and they also showed that the induction ratio was identical to that by 3-MC treatment. Cameron et al.(19) reported that administration of AAF to rats did not induce the cytochrome P-448 in microsomes. At present it is unknown whether a single molecular species of cytochrome P-450 participates in the N-hydroxylation of AAF. For clarification of the mechanism of activation of chemical carcinogens in their target organs, it seems important to determine the organ distribution of the molecular species of cytochrome P-450 necessary for activation of the parent carcinogens.

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