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PREVENTION OF 2-ACETYLAMINOFLUORENE-INDUCED LOSS OF NUCLEAR ENVELOPE CYTOCHROME P450 BY THE SIMULTANEOUS ADMINISTRATION OF 3-METHYLCHOLANTHRENE*

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Abstract—Rats fed a basal diet containing 0.05% (w/w) 2-acetylaminofluorene (AAF) for 3 weeks showed a 50% loss of hepatic nuclear envelope cytochrome P450, whereas microsomal P450 remained at control levels. A similar dietary treatment with 0.004% (w/w) 3-methylcholanthrene (MC) caused moderate losses (20–25%) of cytochrome P450 in both nuclear envelopes and microsomes. Administration of the basal diet supplemented with a mixture of AAF (0.05%) plus MC (0.004%) resulted in a preservation of control levels of nuclear envelope cytochrome P450 and a 30% elevation of microsomal P450. Immunoblot analysis revealed that AAF alone, or in concert with MC, induced comparable levels of the P450d form. Induction of cytochrome P450c by dietary MC was detected only when MC was fed together with AAF. As previously found for butylated hydroxytoluene (BHT), the protective effect of dietary MC against hepatocarcinogenesis in AAF-fed rats correlated with a preservation of nuclear envelope cytochrome P450 content and with the induction of cytochrome P450c.

Prolonged dietary administration of 2-acetylaminofluorene (AAF‖) over a period of several months leads to the sequential formation of hyperplastic liver nodules and hepatomas in male rats [1].

Our biochemical studies during early stages of AAF hepatocarcinogenesis showed that a relatively short-term feeding (3 weeks) of AAF (0.05%, w/w) has a significant effect on hepatic drug-metabolizing enzymes, before histopathological alterations can be detected in the livers of these animals [2]. A markedly decreased content of cytochrome P450 was observed in nuclear envelopes whereas the microsomal P450 content remained at control levels. Since cytochrome P450 plays a role in both the activation (N-hydroxylation) and the detoxification (ring-hydroxylation) of AAF [3], these observations suggested the hypothesis that this early loss of nuclear envelope cytochrome P450 may reflect the loss of a critical line of defense of the nuclear genetic material against the attack by mutagenic metabolites generated by an undiminished microsomal cytochrome P450.

We have shown recently that dietary administration of the phenolic antioxidant butylated hydroxytoluene (BHT; 0.3%, w/w), which is known to exert a protective effect [4] against AAF carcinogenesis, also results in a protective effect on nuclear envelope P450 [5, 6], a finding which is consistent with the above hypothesis. Since dietary administration of 3-methylcholanthrene (MC) has a marked inhibitory effect on AAF hepatocarcinogenesis [7], we decided to investigate if this protective effect is also correlated with a preservation of nuclear envelope cytochrome P450. An abstract describing the initial experiments has been published [8].

MATERIALS AND METHODS

Male Sprague–Dawley rats (90–100 g body weight) were purchased from SASCO (Omaha, NE). Upon arrival, the animals were placed on a commercial (Purina) rat chow for a 48-hr period of adaptation to the new environment, and then they were divided into four groups. Control rats were fed the basal semipurified diet of Medes *et al.* [9], and rats in the three experimental groups were fed basal diet supplemented with 0.05% (w/w) AAF, 0.004% (w/w) MC, or a mixture of 0.05% AAF + 0.004% MC. The basal diet was obtained from the Life Science Division of ICN Nutritional Biochemicals (Cleveland, OH). Addition of AAF and/or MC to the diet was carried out as follows: the carcinogens were triturated in a small amount of basal diet with mortar and pestle, added to the appropriate bulk of basal diet, and mixed thoroughly using a commercial Hobart Food Mixer. The diets were stored in sealed plastic containers at 4°. All operations using AAF and/or MC were conducted following strict institutional and federal safety regulations [10]. The rats were fed *ad lib.* with free access to drinking water, and killed by decapitation

* The authors dedicate this paper to Dr. Raúl E. Trucco on the occasion of his 75th birthday, September 2, 1990.

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‖ Abbreviations: AAF, 2-acetylaminofluorene; MC, 3-methylcholanthrene; and BHT, butylated hydroxytoluene.

Table 1. Effect of dietary AAF and MC on the cytochrome P450 content of rat liver microsomes and nuclear envelopes

Dietary treatment	Nuclear envelopes (nmol cytochrome P450/mg protein)	Microsomes (nmol cytochrome P450/mg protein)
Basal (control)	0.043 ± 0.004	0.634 ± 0.023
AAF	0.022 ± 0.002*	0.651 ± 0.046
MC	0.034 ± 0.002†§	0.469 ± 0.049‡§
AAF + MC	0.043 ± 0.006§	0.832 ± 0.049*‖

Results are the means ± SEM of 3–6 determinations with different liver specimens. Concentrations: AAF, 0.05%; and MC, 0.004%.
Significantly different from control: * $P < 0.005$, † $P < 0.05$ and ‡ $P < 0.01$.
Significantly different from AAF-fed group: § $P < 0.025$ and ‖ $P < 0.05$.

at the end of week 3. To assess the acute effects of MC on cytochrome P450 isozymes, rats fed control diet for 3 weeks were given a single intraperitoneal injection of a solution of MC (20 mg/kg) in trioctanoin 24 hr before being killed [11].

Highly purified nuclei were isolated from whole-liver homogenates by sucrose-density ultracentrifugation as described by Kasper [12]. The washed nuclei were then treated with DNAase I, and the nuclear envelopes were isolated as described by Kay *et al.* [13]. The nuclear membranes were treated with a lysis buffer containing detergents (sodium cholate, Renex 690) and glycerol [14], and their cytochrome P450 content was determined by the CO-reduced versus reduced difference spectrum [15] as previously described [5]. Immunochemical analysis of cytochrome P450 was performed by an immunoblot technique using rabbit antibody that recognizes both P450c and P450d as previously described [16]. Protein was determined by the method of Lowry *et al.* [17] using crystalline bovine serum albumin as the standard. All data are expressed as means ± SEM. The significance of the differences between groups was assessed by Student's *t*-test.

RESULTS

Rats fed basal diet containing 0.05% AAF for 3 weeks underwent a 50% loss ($P < 0.005$) of hepatic nuclear envelope cytochrome P450, whereas the microsomal P450 remained at control levels (Table 1).

In rats fed basal diet containing 0.004% MC there was a smaller (21%) yet statistically significant ($P < 0.05$) loss of nuclear envelope cytochrome P450. In contrast with the rats fed AAF, a 26% loss ($P < 0.01$) of microsomal P450 was also observed after 3 weeks of dietary treatment with MC.

The livers of rats fed basal diet supplemented with both AAF (0.05%) and MC (0.004%) retained control levels of nuclear envelope cytochrome P450 and showed a 31% elevation ($P < 0.005$) of microsomal cytochrome P450.

Examination of the data in Table 1 reveals additional interesting features of the effects of these xenobiotics. A comparison of the cytochrome P450 levels in MC-fed versus AAF-fed rats showed that the animals in the MC-fed group had significantly higher ($P < 0.025$) nuclear envelope P450 and significantly lower ($P < 0.025$) microsomal P450 than the AAF-fed rats. On the other hand, rats fed the diet containing MC plus AAF had significantly higher levels of both nuclear envelope ($P < 0.025$) and microsomal ($P < 0.05$) cytochrome P450 than the rats fed AAF alone.

The effects of the various dietary treatments on the expression of two carcinogen-metabolizing forms of cytochrome P450, P450c and P450d, are shown in Fig. 1. Subcellular fractions from the livers of rats treated intraperitoneally with MC were included as markers of these two forms of cytochrome P450 [16]; the immunoblot showed a clear induction of cytochrome P450c (lanes 5, 10, 15) following parenteral administration of MC. On the other hand, fractions from rats subjected to dietary treatment with MC (lanes 3, 8, 13) revealed only P450d, thus resembling the fractions from control rats (lanes 1, 6, 11). However, when MC was fed in conjunction with AAF (lanes 4, 9, 14), there was an induction of cytochrome P450c. AAF by itself (lanes 2, 7, 12) induced P450d to levels similar to those observed with the mixture of AAF plus MC (lanes 4, 9, 14).

DISCUSSION

A marked decrease in the cytochrome P450 content of microsomes isolated from hyperplastic liver nodules of male rats subjected to prolonged AAF feeding has been reported by several laboratories [2, 18, 19]. Low levels of microsomal cytochrome P450, aryl hydrocarbon hydroxylase and NADPH-cytochrome *c* reductase in these preneoplastic lesions [18] suggest a decrease in the activation of hepatocarcinogens and hepatotoxins which would be consistent with a relative resistance to carcinogen cytotoxicity in hyperplastic nodules [20]. Short-term (3 week) AAF feeding, on the other hand, does not affect microsomal cytochrome P450 but causes a marked depression of P450 content in nuclear envelopes [2].

Cytochrome P450 plays a role in the activation as well as in the detoxification of AAF [3]. Acute AAF treatment by intraperitoneal injection causes a marked increase in the microsomal N-hydroxylation of AAF [21]; therefore, the presence of control

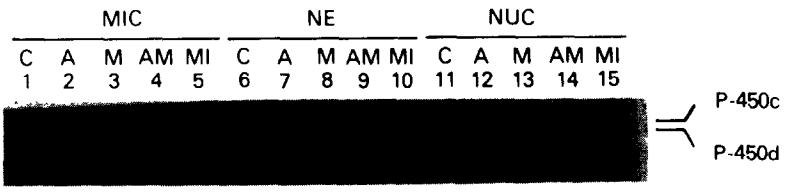

Fig. 1. Immunoblot analysis of subcellular liver fractions from rats fed a diet with or without AAF (0.05%) and/or MC (0.004%) supplementations for 3 weeks. Lanes 1–4 contained 2 μg of microsomal protein (MIC) from control (C) rats, and rats fed AAF (A), MC (M), and a combination of AAF plus MC (AM), respectively. Lane 5 contained 0.1 μg microsomes from rats treated intraperitoneally with MC (MI). Lanes 6–10 and 11–15 contained nuclear envelope (NE) and nuclear (NUC) fractions in the same sequence as those in lanes 1–5, but 20-fold greater amounts of material was applied to the gel.

amounts of microsomal cytochrome P450 after 3 weeks of AAF (Table 1) suggests that the microsomal activation of AAF probably remains elevated during this early stage of AAF hepatocarcinogenesis. On the other hand, if nuclear envelope cytochrome P450 plays a role in the detoxification of active AAF metabolites, the early loss of nuclear envelope cytochrome P450 could compromise its ability to block the attack on nuclear macromolecules (initiation) by mutagenic metabolites generated through microsomal activation of AAF.

Dietary administration of BHT, a synthetic phenolic antioxidant which is known to protect against AAF hepatocarcinogenesis [4], was found previously to preserve nuclear envelope cytochrome P450 content in AAF-fed rats [5]. This antioxidant also causes a significant induction of P450 content in nuclear envelopes and microsomes of rats fed AAF-free diet [5] and enhances the induction of cytochrome P450c in AAF-fed rats [16].

In this report we have shown that rats fed a mixture of AAF and MC retained control levels of nuclear envelope cytochrome P450. When fed by itself, the effect of MC was different from that of AAF and of BHT. While AAF caused a marked loss of nuclear envelope cytochrome P450 (Table 1) and BHT causes a significant induction [5], MC caused a moderate, yet statistically significant ($P < 0.05$) loss of nuclear envelope P450. Although after 3 weeks of AAF-feeding microsomal cytochrome P450 remained at control levels (Table 1), MC-fed rats showed a significant ($P < 0.01$) loss of microsomal P450 and rats fed the mixture AAF + MC showed a clear induction ($P < 0.005$) of microsomal P450 (Table 1).

Consistent with our previous report [16], the immunoblot data show that changes in the levels of spectrally measured cytochrome P450 content do not always correlate with those of individual P450 forms. Thus, the decrease in the level of nuclear envelope cytochrome P450 content following AAF feeding (Table 1) was accompanied by an induction of cytochrome P450d (Fig. 1). These results suggest simultaneous selective induction and depression of different forms of cytochrome P450, whereas the loss of P450 content in nuclear envelope but not in microsomes provides additional evidence for independent regulation of the drug-metabolizing system in these subcellular fractions.

It should also be noted that acute effects following intraperitoneal injections of AAF or MC are different from those observed after 3 weeks of dietary administration. For example, intraperitoneal injections of AAF for 5 consecutive days [22] causes a 50% increase in the microsomal P450 content, and 24 hr after a single intraperitoneal injection of MC there is about a 2-fold elevation of the P450 content in microsomes as well as nuclear envelopes [23].

The conclusions derived from our data can be summarized as follows: (a) the protective effect of dietary BHT and MC against AAF hepatocarcinogenesis correlates with a preservation of nuclear envelope cytochrome P450 content and with the induction of cytochrome P450c; (b) differences in the pattern of response of nuclear envelope and microsomal cytochrome P450 content to the metabolic stress resulting from the feeding of these xenobiotics provide additional evidence for an independent regulation of the nuclear and microsomal drug-metabolizing systems; and (c) the enzymatic changes observed following xenobiotic feeding often differ from those observed following acute administration, suggesting a metabolic adaptation designed to cope with the chronic administration of these foreign compounds during dietary treatment.

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