2-Amino-3,4-dimethylimidazo[4,5-f]quinoline Induces and Inhibits Cytochrome P450 from the IA Subfamily in Chick and Rat Hepatocytes


VA Medical Center, White River Junction, Vermont 05009; Departments of Biochemistry, Pathology, and Radiology, Dartmouth Medical School, Hanover, New Hampshire 03755-3844; and Department of Radiologic Health Sciences, Colorado State University, Fort Collins, Colorado 80523

ABSTRACT

Several heterocyclic amines, found in cooked food, are powerful mutagens in the Ames Salmonella mutagenicity test system. One of these, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) is one of the most potent mutagens yet tested. In chick and rat hepatocytes, MeIQ, by itself, induced cytochrome P450 from the IA subfamily but was a weak inducer compared to 3-methylcholanthrene. However, in both chick and rat hepatocytes in culture, MeIQ decreased the amount of 3-methylcholanthrene-induced ethoxyresorufin deethylase activity, which is catalyzed by cytochrome P450 IA. The protein moiety of cytochrome P450 IA was decreased at MeIQ concentrations of 2.5 µg/ml or greater in chick hepatocytes and 25 µg/ml in rat hepatocytes. In hepatic microsomes from methylcholanthrene-treated chicks and rats, MeIQ was a competitive inhibitor of both ethoxyresorufin deethylase activity, a reaction catalyzed mainly by rodent cytochrome P450 IA1, and uroporphyrinogen oxidation, a reaction catalyzed by rodent P450 IA2. In cultured chick hepatocytes, MeIQ also decreased cytochrome P450-mediated oxidation of uroporphyrinogen by intact cells. The ability of MeIQ to inhibit as well as to induce cytochrome P450 IA of the IA subfamily may be important in assessing the mutagenic and carcinogenic effects of MeIQ in mammals.

INTRODUCTION

Diet is an important environmental factor associated with the development of human cancers (1). Potential carcinogens and mutagens have been detected in several types of diets (2-5). Many chemicals are carcinogenic only after activation by particular forms of cytochrome P450 (for review see Ref. 42). A short-term bioassay for mutagenicity that includes induced P450 from rodent liver, referred to as the Ames Salmonella mutagenicity test system (6), has been widely used to screen potential carcinogens, under the assumption that most carcinogens act through mutational events. After heating protein-rich foods above 150°C, a number of heterocyclic primary amines are formed that are converted to potent mutagens in the Ames Salmonella mutagenicity test system (for review see Refs. 7 and 8). One of these, MeIQ, is one of the most potent mutagens yet tested (9-13). In either case, the potent mutagenicity of MeIQ raises concern about its carcinogenic potential. MeIQ is a carcinogen in rodents and primates (14).

Several forms of P450 have been examined for their activity in converting MeIQ to mutagens detected in the Ames system. Purified rodent cytochrome P450 IA2 is highly active in the conversion of MeIQ to a mutagen, whereas other forms of cytochrome P450 from the IA1, II, and III families have little or no activity (for review see Refs. 7 and 8). This finding was confirmed with human forms of P450 expressed in COS cells in which P450 IA2 was found to be highly active in the conversion of MeIQ to a mutagen, whereas P450 IA1 was inactive (13). Cytochrome P450 IA2 has been found in the liver of many species and is increased after exposure of humans and experimental animals to polycyclic aromatic hydrocarbons (for review, see Ref. 15).

Three other heterocyclic quinolines, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, and 2-amino-3-methylimidazo[4,5-f]quinoline are also formed during cooking of food. These chemicals are mutagenic in the Ames assay and cause a weak induction of P450 from the IA subfamily in the livers of intact rats (16-19). MeIQ itself has not been tested in intact rats for its ability to induce P450. Therefore, we investigated whether MeIQ induces P450 from the IA family in cultured chick and rat hepatocytes. Use of cultured hepatocytes for studying the effects of MeIQ on hepatic P450 enables investigations of (a) direct effects on the liver and (b) the mechanism of the response in a more controlled environment than that of the whole animal. However, in order to study the effects of various chemicals on P450 in cultured hepatocytes, it is essential that the cultured hepatocytes maintain the in vivo response of P450 inducibility. We and others have developed conditions whereby several forms of P450 are inducible in chick and rat hepatocytes in culture at the level induced in the liver of intact animals (20-24).

We found that MeIQ was a weak inducer of P450 IA1 and IA2 in cultured rat and chick hepatocytes. However, MeIQ decreased MC-induced P450 IA-mediated enzyme activities in cultured hepatocytes from both species. In microsomes from MC-treated animals, MeIQ was a competitive inhibitor of both microsomal EROD activity, an activity mainly catalyzed by P450 IA1, and uroporphyrinogen oxidation, an activity catalyzed by P450 IA2. Altogether, the data suggest that the extent of activation of MeIQ to a mutagen in vivo probably results from a balance of metabolism by versus inhibition of cytochrome P450.

MATERIALS AND METHODS

Chemicals. Porcine insulin, NADPH, NADP+, Trizma base, dexmethasone, 3,3',5-triiodothyronine, and MC were obtained from Sigma Chemical Company, St. Louis, MO. Dexamethasone phosphate (injectable) was from Elkins-Sinn, Cherry-Hill, NJ. 4-(2-Hydroxyethyl)-1-piperazinethanesulfonic acid was from Calbiochem, La Jolla, CA. Penicillin G/streptomycin and Williams E medium were from Gibco Laboratories, Grand Island, NY. Ethoxyresorufin was from Molecular Probe, Eugene, OR. Resorufin was from Pfaltz & Bauer, Stamford,
ACTION OF MelQ ON HEPATOCYTE CYTOCHROME P450

MelQ was a gift from Hoffman-LaRoche, Nutley, NJ. Uroporphyrin was obtained from Porphyrin Products Inc., Logan, UT. Desferrioxamine mesylate was from Ciba Geigy, Summit, NJ. A polyclonal goat antibody that detects the major MC-induced form of chicken P450 was kindly provided by Dr. Steve Wrightson. We prepared a polyclonal rabbit antibody that detects the major MC-induced form of chicken P450 (21, 25).

Treatment of Chick Embryos and Rats. Chicken embryos (16 days old) were exposed to MC for 48 h (0.5 mg/egg in 0.2 ml DMSO, injected into the fluid surrounding the embryo). Male Fischer rats (300 g) were given i.p. injections of MC (50 mg/kg, dissolved in corn oil) and killed 48 h later, by decapitation. Hepatic microsomes were prepared as described (26).

Preparation and Treatment of Cultured Hepatocytes. Chicken hepatocytes were prepared from 16-day-old embryos, as described (27). Hepatocytes were plated in Williams E medium containing insulin (10^{-7} M), dexamethasone phosphate (8 \times 10^{-7} M), and 3,3',5-triiodothyronine (1.8 \times 10^{-8} M). After 24 h in culture, cells were maintained in Williams E medium containing dexamethasone and 3,3',5-triiodothyronine, but no insulin. Rat hepatocytes were prepared from male Fischer rats as described (23). Hepatocytes were inoculated and maintained in Williams E medium containing 1.7 \times 10^{-7} M insulin and 10^{-7} M dexamethasone phosphate. MC and MelQ were dissolved in DMSO and added to the cultured cells such that the final amount of DMSO was 2 \mu M/ml medium. This amount of DMSO alone had no effect. PIA was dissolved in 50% aqueous ethanol, with the final concentration of ethanol being 50\% in the culture medium. DES was dissolved in water. The details of individual treatments are presented in the figure legends.

For measurement of uroporphyrinogen oxidation by intact cells, the chick hepatocytes were pretreated with MC (250 ng/ml) to induce P450 IA and then exposed to TCB (1 \mu M) and 5-aminolevulinate (25 \mu M/ml) for 6 h as described (28). MelQ (10 \mu M) was added as indicated 1 h prior to the addition of TCB and 5-aminolevulinate. After 6 h, perchloric acid-methanol was added to extract the porphyrins, and uroporphyrinogen as well as total porphyrins were determined by a spectrofluorometric method (28). The inclusion of 5-aminolevulinate overcomes the rate-limiting step for generation of porphyrinogens, namely induction of 5-aminolevulinate synthase (28). TCB is required for marked enhancement of uroporphyrinogen oxidation in chick hepatocytes (28, 29).

EROD Activity. For measurement of activity in sonicates of cultured chick hepatocytes, cells were harvested in 20 mM Tris-Cl (pH 7.8), homogenized with a Potter-Elevehjem homogenizer, and sonicated for 4 s at position 2 using an Ultrasonix Sonifier cell disruptor equipped with a microtip. The final reaction mixture (150 \mu l total volume) contained: 20 mM sodium isocitrate, 0.03 unit isocitrate dehydrogenase, 13 mM nicotinamide, 12.5 mM MgCl\(_2\), 200 \mu g bovine serum albumin, 20 mM Tris-Cl (pH 7.8), and 0.25 \mu M NADPH. The reaction was initiated by the addition of 3 nmol ethoxyresorufin and incubated at 37°C for 15 min. The reaction was terminated by the addition of 1 ml cold methanol. The product, resorufin, was measured fluorimetrically, with both MC (250 ng/ml) and MelQ (10 \mu M); and (b) overnight pretreatment with MC, followed by a 9-h exposure to MelQ in the presence of PIA plus DES. Porphyrins were measured in cells plus medium after extraction into perchloric acid-methanol, as described (28).

Additional Assays. Proteins were analyzed by the procedure of Lowry et al. (37), using bovine serum albumin as a standard. Lactate dehydrogenase was measured in the culture medium, as described (38).

RESULTS

Induction of Cytochrome P450 by MelQ or MC in Cultured Hepatocytes

Total cytochrome P450, as measured spectrophotometrically (26), did not change after treatment of chicken or rat hepatocytes with MelQ up to 200 \mu g/ml (results not shown). However, treatment with MelQ at 10 \mu g/ml resulted in a small increase in EROD activity, as well as the immunoreactive proteins that are also induced by MC (Fig. 1). In rats, MC induces 2 forms of P450 IA and IA2, that catalyze EROD. In hepatic microsomes from MC-treated rats, 80\% of the EROD activity is catalyzed by P450 IA1 and 20\% is catalyzed by P450 IA2, as shown by immunoinhibition studies (25, 39). Although P450 IA2 is present in livers of untreated rats (15), neither form is present in untreated rat hepatocytes in culture (Fig. 1A). MelQ treatment of cultured rat hepatocytes resulted in induction of immunoreactive P450 IA1 and a lesser increase in P450 IA2. In chick embryos, MC appears to induce only one form of P450 that catalyzes EROD activity (21, 25). In cultures of chick hepatocytes, this P450 is detected immunologically after exposure to MelQ or MC (Fig. 1A). In both systems the induction of these forms of P450 by MelQ was much less than by MC.
Effect of MelQ on MC-induced EROD Activity in Cultured Hepatocytes

Fig. 2 shows that MelQ decreased MC-induced EROD in a dose-dependent manner in both chick and rat hepatocytes in culture. In cultured rat hepatocytes, immunoreactive P450 IA protein did not decrease at concentrations of MelQ that caused maximal decrease in enzyme activity (Fig. 3B). However, immunoreactive P450 IA decreased in rat hepatocytes at 25 µg MelQ/ml (Fig. 3B, Lane 6 versus Lane 1). In chick hepatocytes, immunoreactive P450 IA was not significantly decreased at concentrations of MelQ that caused a 60% decrease in enzyme activity (1 µg/ml; Fig. 3A, Lane 2 versus Lane 1). However, treatment with higher concentrations of MelQ (≥2.5 µg/ml) was associated with decreases in both immunoreactive protein and enzyme activity (Figs. 2 and 3A). To see whether a short-term exposure to these high concentrations of MelQ also affected immunoreactive P450 IA in chick hepatocytes, we treated the cells with MC for 16 h and then added 10 µg MelQ/ml (47 µM) for 2 h before harvesting the cells. Both immunoreactive chick P450 IA (Fig. 3) and EROD activity (results not shown) were decreased compared to MC alone.

Determination of Possible Toxicity of MelQ in the Cultured Hepatocytes

To investigate whether the inhibitory effects of MelQ on EROD and uroporphyrinogen oxidation in MC-treated cells were due to nonspecific toxicity, we analyzed the effect of MelQ...
in MC-treated cells on (a) the induction of another enzyme, ALA-S and (b) the release of the enzyme LDH from cells. Fig. 4A shows that concentrations as high as 20 \( \mu \)g MeIQ/ml caused no increase in LDH release. MeIQ also had no effect on induction of ALA-S, regardless of whether MeIQ was added either simultaneously with MC (Fig. 4B) or after an overnight preexposure to MC (results not shown). Therefore, by both these criteria MeIQ was not toxic.

**Effect of MeIQ on the Kinetics of Microsomal EROD Activity**

In hepatic microsomes prepared from MC-treated chick embryos or rats, the 50 for ethoxyresorufin was similar, 62 nm for the former and 82 nm for the latter. However, the Vmax for EROD was greater in rat than in chick microsomes when expressed per mg protein (7.19 versus 1.68 nmol/min/mg protein, respectively) or per nmol P450 (8.4 versus 3.4 nmol, respectively). Since treatment of cultured hepatocytes with MeIQ decreased MC-induced EROD activity, we investigated whether MeIQ would also inhibit EROD activity in hepatic microsomes prepared from both chicken embryos and rats treated with MC. We found that MeIQ almost completely inhibited EROD activity in hepatic microsomes from MC-treated rats (Fig. 5, C and D) or chicken embryos (Fig. 5, A and B).
ACTION OF MelQ ON HEPATOCYTE CYTOCHROME P450

Fig. 6. Inhibition of microsomal uroporphyrinogen oxidation by MelQ. Hepatic microsomes were prepared from rats and chick embryos that had been treated with MC, and the inhibition of microsomal uroporphyrinogen oxidation was analyzed, as described in “Materials and Methods.” D, A, 2.5 µM uroporphyrinogen; •, A, 5 µM uroporphyrinogen. A, C, V versus 1 plot; B, D, Dixon plot.

B). The inhibition was shown to be competitive. The $K_i$ of MelQ for inhibition of microsomal EROD activities was 1 µM for both rats and chicks.

Effect of MelQ on MC-induced Uroporphyrinogen Oxidation (UROX) in Rat and Chick Microsomes and in Cultured Chick Hepatocytes

Rat and Chick Microsomes. Uroporphyrinogen is oxidized by MC-induced forms of cytochrome P450 to uroporphyrin in liver microsomes from both chicken embryos and rodents (25). In hepatic microsomes from MC-treated animals, uroporphyrinogen oxidation is catalyzed by rodent P450 IA2 but not P450 IA1 (25). In chickens, one form of P450 accounts for both uroporphyrinogen oxidation and EROD catalyzed by microsomes from MC-treated animals (25). For unknown reasons, avian but not rodent uroporphyrinogen oxidation activity requires addition of a planar halogenated biphenyl such as TCB (25, 29, 32). In hepatic microsomes from MC-treated rats or chickens, MelQ competitively inhibited microsomal uroporphyrinogen oxidation. The $K_i$ was about 5 µM for both species (Fig. 6). Inhibition of uroporphyrinogen oxidation was almost complete at MelQ concentrations of 40 µM or greater.

Cultured Chick Hepatocytes. Having shown that MelQ inhibits UROX in hepatic microsomes, we investigated whether MelQ would inhibit uroporphyrinogen oxidation by intact hepatocytes. We have previously shown that in cultured chick hepatocytes preinduced for P450 IA, the oxidation of uroporphyrinogen is increased in cells also exposed to TCB and 5-aminolevulinate (ALA), the precursor of uroporphyrinogen (28). Uroporphyrin, the oxidation product of uroporphyrinogen, is not further metabolized and accumulates in the cells. In hepatocytes treated with ALA alone, uroporphyrinogen is further metabolized and protoporphyrin is the major porphyrin accumulating (28).

As shown in Fig. 7, MelQ selectively inhibited the accumulation of uroporphyrin by intact cells, in both the absence and the presence of TCB. MelQ did not affect the accumulation of total porphyrin. These results suggested that MelQ inhibited P450 IA-mediated oxidation of uroporphyrinogen in the intact hepatocyte, consistent with our observations with microsomes. We could not investigate the effect of MelQ on inhibition of uroporphyrinogen oxidation in cultured rat hepatocytes, since MC-treated rat hepatocytes in culture do not accumulate uroporphyrin (32, 34). This is probably due to the finding that MC is a poor inducer of P450 IA2 in cultured rat hepatocytes relative to that induced in vivo (Ref. 34 versus Fig. 1A).

DISCUSSION

In this study, using primary cultures of chick and rat hepatocytes, we found that MelQ was a relatively weak inducer of cytochrome P450 from the IA subfamily compared to MC, as shown by small changes in immunoreactive protein and EROD activity (Fig. 1). Other heterocyclic aromatic primary amines isolated from cooked foods have been shown to be weak inducers of P450 IA in the liver of intact rats (16–19). Surprisingly,
MeIQ decreased EROD activity induced by the prototypic inductor of this activity, MC, in cultured chick and rat hepatocytes, as well as uroporphyrinogen oxidation in cultured chick hepatocytes.

In hepatic microsomes from MC-treated rats, MeIQ competitively inhibited both EROD and UROX. These findings indicate that MeIQ inhibited both P450 IA1 and P450 IA2, since, in MC-induced microsomes, EROD is catalyzed mainly by P450 IA1 (25, 39) and UROX is catalyzed mainly by P450 IA2 (25). These results indicate that MeIQ binds to the substrate binding sites of both P450 IA1 and P450 IA2 and suggest that both forms of P450 metabolize MeIQ. Nevertheless, P450 IA2 is more active than P450 IA1 in converting MeIQ to a metabolite that is a bacterial mutagen (9, 13).

In rat hepatocytes in culture, MeIQ at concentrations up to 10 µg/ml decreased EROD activity by 50% with no decrease in the protein moiety of P450 (Fig. 3B). This lack of effect on immunoreactive P450 IA1 of concentrations of MeIQ that inhibit enzyme activity also indicates that MeIQ did not affect the MC-mediated induction of P450 IA. In the chick hepatocytes, MeIQ at both 1 and 2.5 µg/ml caused 60% decreases in EROD activity (Fig. 3A). At 10 µg MeIQ/ml, the extent of the decrease in chick P450 IA was greater than expected from the half-life of this protein in culture (40). The decrease in immunoreactive P450 IA protein could result from increased degradation of the MC-induced forms of P450 mediated by an activated metabolite(s) of MeIQ. Similar effects are observed with other suicidal inhibitors of cytochrome P450 (41). If the MeIQ-derivative suicidal inactivator of P450 proves to be identical to the mutagenic metabolite(s) of MeIQ, then P450 IA2 would be more susceptible to proteolytic degradation than P450 IA1 since P450 IA2 is more active at generating the mutagenic metabolite (9, 13). Chick P450 IA, which has the substrate specificities of both mammalian P450 IA1 and IA2 (25), was more susceptible to degradation after treatment with MeIQ than rat P450 IA1 (Fig. 3). The results suggest that chick P450 IA generates more of the activated metabolite of MeIQ than rat P450 IA1.

The MeIQ-mediated decrease in MC-induced P450 in culture was not due to general toxicity, since the induction of another hallmark of cell damage.

Note Added in Proof


ACKNOWLEDGMENTS

We thank Richard Lambrecht and Joseph Williams for fruitful discussions.

REFERENCES


20. Sinclair, J., Wood, S., Smith, E., Sinclair, P., and Koop, D. Comparison of the 5-aminolevulinic acid synthase, was not affected (Fig. 4B). In addition, MeIQ treatment did not increase the release of the intracellular enzyme LDH (Fig. 4A), the release of which is a hallmark of cell damage.

21. In summary, we have found, in cultured rat and chick hepatocytes, that treatment with MeIQ of itself is a weak inducer of P450 IA and inhibits P450 IA induced by MC in these cells. In microsomes from MC-treated rats and chickens, MeIQ was a competitive inhibitor of P450 IA-mediated EROD activity and uroporphyrinogen oxidation, indicating that MeIQ inhibited both P450 IA1 and P450 IA2. The ability of MeIQ to inhibit P450 from the IA subfamily may relate to the potency of MeIQ to bind to this form of P450 and be metabolized to a mutagen.
2-Amino-3,4-dimethylimidazo[4,5-f]quinoline Induces and Inhibits Cytochrome P450 from the IA Subfamily in Chick and Rat Hepatocytes


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/52/13/3615

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.