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The suppression of hepatic cytochrome P4504A mRNA mediated by the interferon inducer polyinosinic acid·polycytidylic acid

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Abstract—Interferon and interferon inducers are well known to depress the cytochrome P450-dependent hepatic mixed-function oxidase system and cause a decrease in the capacity of the liver to metabolize drugs and xenobiotics. In this study we have shown that the interferon-mediated changes in an induced form of hepatic cytochrome P450 (CYP4A) are mediated via a depression in the levels of mRNA as assessed by Northern blot and slot blot analyses using a 20-base synthetic oligodeoxyribonucleotide hybridization probe. Rats were pretreated with clofibrate to maximize CYP4A mRNA levels prior to the administration of polyinosinic acid·polycytidylic acid (poly IC), an α/β interferon inducer. Hepatic CYP4A mRNA levels were decreased by 49 and 30% at 6 and 24 hr, respectively, following poly IC administration. In hepatic microsomes cytochome P450 and functional CYP4A as measured by lauric acid hydroxylation, were not affected at 6 hr, but were depressed by 39 and 27%, respectively, 24 hr following poly IC administration. These results suggest that interferon depresses induced levels of hepatic drug metabolism by lowering the level of cytochrome P450 mRNAs and subsequent synthesis of cytochrome P450 apoproteins.

In 1976, two laboratories [1, 2] simultaneously reported that the α/β interferon inducer tilorone depressed hepatic cytochrome P450 and related biotransformation. Subsequently it was shown that twelve different α/β interferon inducers, representing a wide variety of structures and molecular weights, depressed the microsomal cytochrome P450 system in rats and it was suggested that cytochrome P450 depression was a general property of interferon inducers [3]. Singh *et al.* [4] first reported that recombinant human α interferon depressed cytochrome P450 in mice and this has been confirmed for α, β and λ interferon [5–7]. It appears that the action of these recombinant interferons on cytochrome P450 is inseparable from the antiviral and antitumour actions [5, 8, 9]. Although it has been suggested that the loss in cytochrome P450 results from a loss in heme synthesis [10], several studies have now suggested that interferon or its inducers can depress the levels of constitutive hepatic microsomal cytochrome P450 by decreasing the synthesis of the cytochrome P450 apoprotein [11–15]. To date it appears that the synthesis and expression of induced forms of cytochrome P450 are resistant to this effect.

In this study we have shown, using a synthetic oligodeoxyribonucleotide hybridization probe, that the level of hepatic mRNA coding for the induced cytochrome P450 form CYP4A is decreased in clofibrate-treated rats receiving the α/β interferon inducer polyinosinic acid·polycytidylic acid (poly IC). This demonstrates that the interferon-mediated decrease of an induced form of cytochrome P450 is a pre-translational event resulting in a decrease in the synthesis of the cytochrome P450 apoprotein.

Materials and Methods

Bovine serum albumin, clofibrate, Ficoll (type 400), formamide, guanidine-HCl, lambda DNA Hind III digest, 3-[*N*-morpholino]propanesulfonic acid, polyvinylpyrrolidone (PVP-360), and salmon sperm DNA were purchased from the Sigma Chemical Co., St. Louis, MO. Spermidine trihydrochloride was purchased from the Aldrich Chemical Co. Inc., Milwaukee, WI. Poly IC was purchased from Calbiochem, La Jolla, CA. T4 polynucleotide kinase was purchased from Pharmacia Inc., Dorval, Quebec. Agarose was obtained from Bio-Rad Laboratories Ltd., Mississauga, Ontario. GeneScreenPlus® hybridization transfer membrane was purchased from Du Pont Canada Inc., Dorval, Quebec. [^{32}P]ATP was purchased from either ICN Biomedicals Canada Ltd., Montreal, Quebec (7000 Ci/mmol) or Dupont Canada Inc., Dorval, Quebec (6000 Ci/mmol). [1-^{14}C]Lauric acid (10–30 mCi/mmol) was purchased from Amersham Canada Ltd., Oakville, Ontario.

Male Sprague–Dawley rats (200–350 g) obtained from Canadian Hybrid Farms, Kentville, N.S., were housed in wire-bottom cages and fed Purina rat chow and water *ad lib*. They were allowed to acclimatize in our facilities for at least 4 days before receiving drug treatment. In experiments designed to assess the effects of poly IC, rats were induced with clofibrate (250 mg/kg in corn oil) i.p. for 4 days to increase CYP4A and its mRNA [6]. They were killed 24 hr after the last clofibrate injection. Poly IC (10 mg/kg in saline) or an equivalent volume of saline was given i.p. to clofibrate-induced rats 6 or 24 hr before they were killed.

Previously described methods were used for the preparation of hepatic microsomes [17] and for the determination of microsomal protein [18], cytochrome P450 and lauric acid hydroxylation [20]. A portion of the liver was removed for microsome preparation and the remainder was quick frozen in liquid nitrogen prior to the isolation of RNA using the method outlined by Protter *et al.* [21]. All RNA samples prepared had a 260 to 280 nm absorbance ratio of greater than 1.9.

The sequence used for the oligomer was based on the rat cDNA sequence for CYP4A published by Hardwick *et*

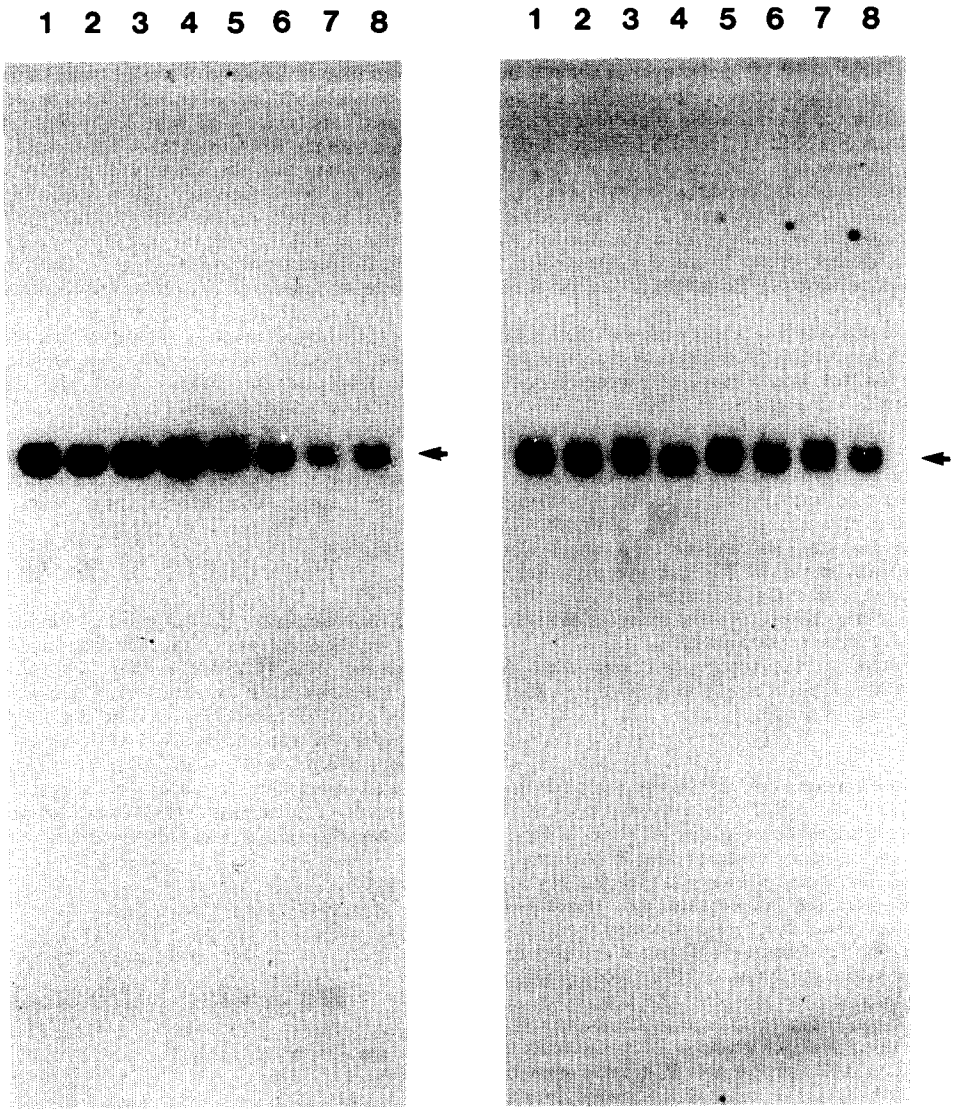

Fig. 1. Left panel: Northern blot analysis of hepatic CYP4A mRNA 6 hr following poly IC or saline administration in clofibrate-induced rats. Total hepatic RNA (20 μg/lane) from individual rats was subjected to Northern blot analysis using the CYP4A oligomer [^{32}P-labelled] as described in Materials and Methods. Lanes 5–8 contain RNA from clifobrate-induced rats given poly IC (10 mg/kg) 6 hr before they were killed. Lanes 1–4 contain RNA from clofibrate-induced rats given saline 6 hr before they were killed. The location of CYP4A mRNA is indicated by an arrow. Right panel: Northern blot analysis of hepatic CYP4A mRNA 24 hr following poly IC or saline administration in clofibrate-induced rats. Lanes 5–8 contain RNA from clofibrate-induced rats given poly IC (10 mg/kg) 24 hr before they were killed. Lanes 1–4 contain RNA from clofibrate-induced rats given saline 24 hr before they were killed. The location of CYP4A mRNA is indicated by an arrow.

al. [16]. The area corresponding to nucleotides 768–787 of this sequence was chosen for construction of the probe. The complement of this cDNA strand, 5′-d[GGGCAAGTTGACAAGCACGG]-3′, was synthesized and purified by the Regional DNA Synthesis Laboratory, University of Calgary, Calgary, Alberta. The 5′-OH ends of the oligomer were labelled with ^{32}P by a modification of the method of Maxam and Gilbert [22]. Approximately 65% of the [^{32}P]ATP was incorporated into the oligomer.

For Northern blot analysis, total hepatic RNA was separated in a 1.3% agarose gel containing 2.2 M formaldehyde, transferred to GeneScreen nylon membrane (Dupont Cat No. NEN-976), and baked at 80° for 2 hr. The blot was prehybridized at 42° overnight in 6 SSPE, 1% sodium dodecyl sulfate (SDS), 5 Denhardt's and 100 μg/mL sheared salmon sperm DNA (1 SSPE contains 0.15 M NaCl, 10 mM sodium phosphate, 1 mM EDTA, pH 7.4; 1 Denhardt's solution contains 0.02% each of Ficoll, polyvinylpyrrolidone and bovine serum albumin).

The prehybridization solution was replaced with hybridization solution (6 SSPE, 1% SDS, 0.1% Ficoll, 0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone) and 5 μL of ^{32}P-labelled oligomer at 42° for 20–24 hr. The blot was removed from the hybridization solution and washed with four changes of 6 SSPE, 0.1% SDS (10 min/change), two at room temperature followed by two at 54°. The synthetic oligodeoxyribonucleotide probe hybridized to a clofibrate-inducible RNA which was ~2.1 kb long, and low levels were present in non-induced rats. This verified that the CYP4A oligomer probe hybridized specifically to CYP4A mRNA because (1) the length of this RNA was within 5% of the published size of the mRNA, (2) the CYP4A gene is constitutively expressed, and (3) CYP4A and its mRNA are selectively induced by clofibrate [16]. To carry out the slot blot, a range of concentrations of each RNA sample was prepared by 2-fold serial dilutions with water and loaded into the wells of a slot blot apparatus (Bio-Rad Bio-Dot® SF apparatus) using GeneScreenPlus nylon membranes. Prehybridization and hybridization of the slot blots were performed as described for the Northern blots. For each sample a range of RNA concentrations was analyzed that permitted calculation of a linear slope value (absorbance per μg of RNA analyzed) following autoradiography and scanning densitometry. An oligo(dT)$_{18}$ probe was utilized to ensure that the amounts of mRNA applied to each blot were identical [23].

Student's *t*-test for unpaired data was used for statistical comparison between the treated group and the corresponding control group.

Results

Northern blot analysis of the RNA isolated from clofibrate-induced rats killed 6 hr following poly IC or saline administration is shown in the left panel of Fig. 1. The intensity of the bands obtained with RNA from the poly IC-treated rats (lanes 5–8) was less than that obtained with the RNA from the saline-treated rats (lanes 1–4), indicating that the quantity of mRNA coding for CYP4A was decreased 6 hr following poly IC treatment. Slot blot analysis indicated that the amount of RNA that hybridized with the oligomer was decreased in the poly IC-treated rats by 49% (Fig. 2). The hybridization to an oligo(dT)$_{18}$ probe was identical for each group (1.24 ± 0.10 units for controls vs 1.27 ± 0.12 units for poly IC-treated animals), indicating that the levels of total mRNA loaded onto the blots were identical. Northern blot analysis of the RNA isolated from rats killed 24 hr following poly IC or saline administration is shown in the right panel of Fig. 1. The bands obtained with the RNA from three of the four poly IC-treated rats (lanes 6–8) were less intense than those obtained with the RNA from the saline-treated rats (lanes 1–4), indicating that the quantity of mRNA coding for CYP4A was also decreased 24 hr following poly IC treatment. Slot blot analysis indicated that the amount of RNA that hybridized with the oligomer was decreased in the poly IC-treated rats by 30% (Fig. 2). The hybridization of samples to an oligo(dT)$_{18}$ probe was identical for each group (0.78 ± 0.07 units for controls vs 0.79 ± 0.12 units for poly IC-treated animals), indicating that the levels of total mRNA loaded onto the blots were identical.

The level of cytochrome P450 and lauric acid hydroxylation activity in hepatic microsomes prepared from rats treated with poly IC for 6 hr were identical to those observed in control animals (Fig. 3). In microsomes from rats given poly IC 24 hr before they were killed, cytochrome P450 and lauric acid hydroxylation were decreased significantly by 39 and 27%, respectively, compared to saline controls.

Discussion

The results indicate that the interferon inducer poly IC depressed the expression of the clofibrate-induced form of cytochrome P450 (CYP4A) within 24 hr of treatment. CYP4A mRNA levels were decreased significantly within 6 hr of poly IC administration but the levels of total cytochrome P450 and lauric acid hydroxylation in the liver were not affected at this time. All three parameters were decreased significantly by 24 hr. This sequence of events indicates that the decrease in CYP4A mRNA preceded the loss of hemoprotein and was responsible for the decrease

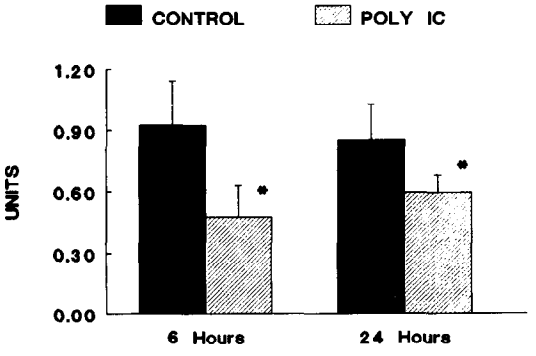

Fig. 2. Slot blot analysis of hepatic CYP4A mRNA 6 and 24 hr following poly IC or saline administration in clofibrate-induced rats. Total hepatic RNA from individual rats was subjected to slot blot analysis using the CYP4A oligomer [^{32}P-labelled] as described in Materials and Methods. The results are expressed as the means ± SEM (N = 4) of the units of slope (absorbance/μg RNA) as determined from the autoradiographs of the slot blots. Key: (*) significantly different from corresponding control, $P < 0.05$.

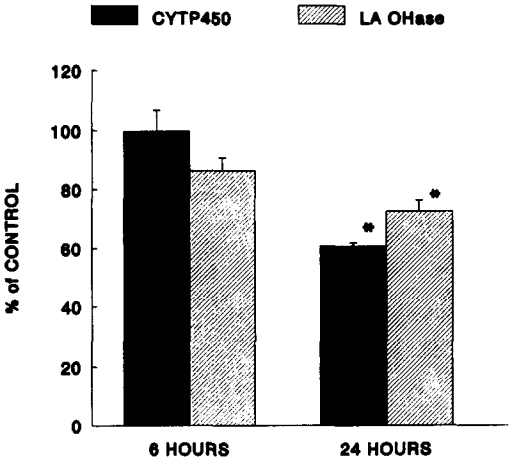

Fig. 3. Effect of poly IC on hepatic microsomal cytochrome P450 and lauric acid hydroxylase in clofibrate-induced rats. Data are expressed as means ± SEM from four individual rats. The levels of cytochrome P450 in controls were 0.94 ± 0.07 and 1.137 ± 0.07 nmol/mg protein at 6 and 24 hr respectively. The activities of lauric acid hydroxylase in control animals were 22.78 ± 1.76 and 30.75 ± 1.28 nmol 11/12 hydroxy-lauric acid formed/min/mg protein at 6 and 24 hr respectively. All rats received clofibrate (250 mg/kg) for 4 days. Poly IC (10 mg/kg) or saline was given 6 or 24 hr before the rats were killed. Key: (*) significantly different from corresponding control, $P < 0.05$.

in functional CYP4A. Although the decrease in CYP4A mRNA levels would result in a decrease in the rate of synthesis of the CYP4A apoprotein by 6 hr, the loss in functional CYP4A would not be apparent until degradation of existing CYP4A hemoprotein had occurred. The order of these findings supports the idea that interferon or its inducers exert their effect at pre-translational sites of action. These experiments do not allow us to determine if the effect is directly on gene expression or at another potential site in the induction process.

It has now become well established that during the activation of host defence mechanisms the levels of different cytochrome P450 families are depressed and a number of investigators have suggested that the interferon-mediated losses occur at a pre-translational step via the depression in mRNA levels [9, 14, 24, 25]. Only constitutive forms of cytochrome P450, namely CYP2C12 [12], CYP2C11 [14], CYP3A2 [9], CYP1A2 [25], and CYP2C6 [25], appear to be affected in this manner. The mRNA levels and subsequent proteins of some induced forms of cytochrome P450, namely CYP3A1 [9], CYP1A1 [25], CYP1A2 [25], CYP2C6 [25], and CYP2B1 [25], do not appear to be affected by interferons or their inducers. The present data on CYP4A are the first report indicating that an inducible form of cytochrome P450 and its corresponding mRNA are depressed by an interferon inducer.

Stanley *et al.* [26] demonstrated that during an inflammatory response evoked by endotoxin members of some cytochrome P450 families are depressed while in others the induction of the cytochrome is actually enhanced. The levels of mRNA are depressed for CYP2C but remain unchanged for the induced CYP2B and CYP1A families. During the administration of endotoxin to rats, Wright and Morgan [27] observed a loss of constitutive CYP2C12 apo-enzyme and its mRNA with interleukin-1 and dexamethasone, but not interleukin-6, mimicking the effects of this inflammatory response. A recent report by Williams *et al.* [28] indicated that interleukin-6, a major mediator of the acute phase response, depresses CYP2B1/2 in cultured hepatocytes and prevents the phenobarbital induction of CYP2B mRNA. Collectively these results indicate that although the levels of constitutive cytochromes P450 families are consistently depressed following host defence stimulation, the responses of induced forms of the enzyme system are variable and depend on the form of cytochrome P450 and the conditions under which it is studied.

The studies presented in this paper demonstrate that poly IC lowered the level of an induced form of hepatic cytochrome P450 mRNA and this occurred prior to the observed decrease in the cytochrome P450 hemoprotein. Thus, it can be concluded that a decrease in the synthesis of the CYP4A apoprotein was responsible for the depression of the induced levels of CYP4A hemoprotein mediated by poly IC. This mechanism is similar to that reported by others for constitutive forms of the enzyme. It is therefore likely that at least some, but not all, of the induced forms of cytochrome P450 are decreased by interferon following a depression in the corresponding mRNA.

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