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Interferon-Mediated Depression of Cytochrome P-450-Dependent Drug Biotransformation

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SUMMARY

A general property of interferon-inducing agents is depression of cytochrome P-450-dependent drug biotransformation in liver. Evidence for a direct involvement of interferon is suggested by experiments carried out in inbred strains of mice carrying four distinct genetic loci which influence the levels of circulating interferon produced by specific viruses. For Newcastle disease virus (NDV), one autosomal locus (IF-1) determines a 10-fold difference in serum interferon levels. We utilized strains of mice carrying the high (IF-1^h) or low (IF-1^l) production allele at the IF-1 locus to demonstrate that depression of hepatic cytochrome P-450 can be correlated with circulating interferon levels. In C57BL/6J mice containing the *h*-allele at IF-1, cytochrome P-450 and aminopyrine *N*-demethylase were decreased by 35% and 48%, respectively, 24 hr after the injection of NDV. The mean circulating level of interferon was 2443 PRD_{50} units/ml (PRD_{50} unit = amount of interferon required for 50% plaque reduction). In C_3H/HeJ mice containing the *l*-allele at IF-1, no significant change was observed in cytochrome P-450 levels or in aminopyrine *N*-demethylase activities following injection of NDV. The circulating levels of interferon in this strain were below our lowest limits of detection. Poly(rI·rC), which induces interferon via loci other than IF-1, provided high levels of interferon and depressed cytochrome P-450 and aminopyrine *N*-demethylase in both strains of mice. It is concluded that an impairment of cytochrome P-450-dependent drug biotransformation can occur via an interferon-mediated interaction.

INTRODUCTION

The level of hepatic cytochrome P-450 and the capacity of liver to metabolize drugs is altered by many chemicals, physiological factors, and pathological states (1). Renton and Mannering (2) and Leeson *et al.* (3) demonstrated that cytochrome P-450 and the activity of several representative drug oxidations was decreased in rats following the administration of the interferon-inducing agent tilorone. As this effect was subsequently shown to be a common property of most interferon-inducing agents (4), it was suggested that interferon or interferon production had a deleterious effect on the normal steady-state levels of cytochrome P-450 in liver (5). However, because interferon inducers have a number of other actions and the administration of crude fibroblast interferon preparations had no effect on cytochrome P-450, it was impossible to determine clearly whether this depression of cytochrome P-450 was due directly to interferon.

DeMaeyer and DeMaeyer -Guignard (6) have recently described four distinct genetic loci which influence the levels of circulating interferon in response to specific viruses. For NDV,[3] one autosomal locus (IF-1) determines a 10-fold difference in serum interferon levels in different mouse strains. In this paper we have utilized strains of mice carrying the high (IF-1^h) or low (IF-1^l) production allele at the IF-1 locus to demonstrate that depression of hepatic cytochrome P-450 by NDV can be correlated with circulating interferon levels.

MATERIALS AND METHODS

Materials. NDV was a gift from Dr. S. Lee, Department of Microbiology, Dalhousie University. Poly(rI·rC) had a minimum molecular weight of 100,000 and was purchased from Sigma Chemical Company (St. Louis, Mo.).

Animals. Inbred stains of C_3H/HeJ and C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, Me.). Animals were allowed to acclimatize in our

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[3] The abbreviations used are: NDV, Newcastle disease virus; IF, interferon; IF-1^h and IF-1^l, autosomal loci carrying the high- and low-production allele, respectively; PRD_{50} unit, amount of interferon required for 50% plaque reduction.

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TABLE 1

Circulating levels of interferon in inbred strains of mice following the administration of NDV or poly(rI·rC)

Each value is the serum interferon level in an individual mouse. Levels were determined 24 hr after the administration of NDV (5×10^7 plaque-forming units) or poly(rI·rC) (10 mg/kg).

Strain	Treatment	Allele	Interferon
			PRD_{50} units/ml
C57BL/6J	Saline	IF-1^h	<40, <40, <40
C57BL/6J	NDV	IF-1^h	1448, 1702, 4178
C57BL/6J	Poly(rI·rC)	IF-1^h	1742, 2010
C_3H/HeJ	Saline	IF-1^l	<40, <40, <40
C_3H/HeJ	NDV	IF-1^l	<40, <40, <40
C_3H/HeJ	Poly(rI·rC)	IF-1^l	2118, 4376

own facility for at least 6 days before use and were provided water and standard diet ad libitum. Pretreated animals received i.p. injections of NDV (5×10^7 plaque-forming units/mouse) or poly(rI·rC) (10 mg/kg) 24 hr before they were decapitated. Control animals received injections of normal saline at the same time.

Microsomes. Hepatic microsomes were prepared as described by El Defrawy El Masry *et al.* (7) and were used on the day they were prepared. Microsomal protein levels were determined by the method of Lowry *et al.* (8), using bovine serum albumin as a standard. Cytochrome P-450 and cytochrome b_5 levels in microsomes were determined by the method of Omura and Sato (9). Microsomal *N*-demethylation was determined as described by Sladek and Mannering (10).

Interferon. Twenty-four hours after treatment with NDV, poly(rI·rC), or saline, animals were anesthetized with ether and blood samples were obtained from the brachial plexus. Serum interferon was then determined by the plaque-reduction method in monolayers of mouse L-929 cells using vesicular stomatitis virus as the challenge virus (11). The amount of interferon required for 50% plaque reduction was defined as one PRD_{50} unit.

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

RESULTS

Effect of NDV on interferon levels and drug biotransformation. Twenty-four hours after the administration of NDV to C57BL/6J mice, which carry the allele for the high production of interferon at the IF-1 locus, the mean circulating level of interferon was 2443 PRD_{50} units/ml (Table 1). At the same time, cytochrome P-450 levels and aminopyrine *N*-demethylase activities were depressed by 35% and 48%, respectively, in hepatic microsomes (Figs. 1 and 2). In C_3H/HeJ mice, which carry the allele for the low production of interferon at the IF-1 locus, the circulating levels of interferon were below our lowest limit of detection (40 PRD_{50} units/ml) 24 hr after treatment with NDV (Table 1). In this strain, NDV had no effect on the levels of cytochrome P-450 or aminopyrine *N*-demethylase activity in hepatic microsomes (Figs. 1 and 2). In both strains, cytochrome b_5 was unchanged following NDV treatment.

Effect of poly(rI·rC) on interferon levels and drug biotransformation. Twenty-four hours after administration of poly(rI·rC), which induces the formation of interferon via loci other than IF-1, high concentrations of interferon were observed in the serum of both strains of mice (Table 1). In C57BL/6J mice, cytochrome P-450 levels and aminopyrine *N*-demethylase activities were decreased by 44% and 50%, respectively, 24 hr after the administration of poly(rI·rC) (Figs. 3 and 4). In C_3H/HeJ mice, cytochrome P-450 levels and aminopyrine *N*-demethylase activities were decreased by 33% and 38% following the administration of poly(rI·rC) (Figs. 3 and 4). In both strains, cytochrome b_5 was unchanged following poly(rI·rC) treatment.

DISCUSSION

In these experiments, NDV depressed cytochrome P-450 levels and aminopyrine *N*-demethylase activity only

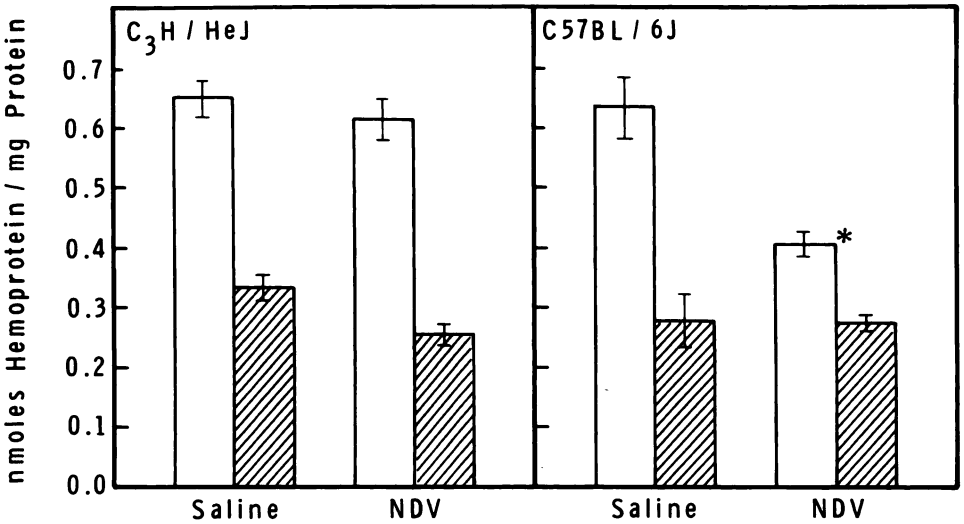

FIG. 1. *Effect of NDV on cytochrome P-450 and cytochrome b_5 levels in hepatic microsomes prepared from inbred strains of mice*

C57BL/6J carry the high production allele at IF-1 and C_3H/HeJ carry the low production allele at IF-1. The animals were killed 24 hr after the administration of NDV. The *open bars* represent cytochrome P-450 levels and the *shaded bars* the cytochrome b_5 levels. Each value is the mean ± standard error of six individual mice. * Significantly different from control ($p < 0.01$).

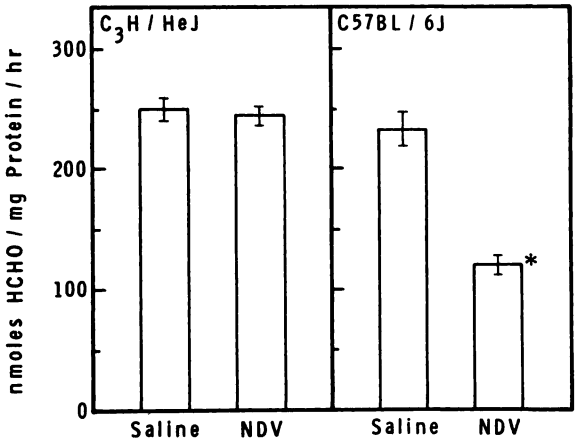

FIG. 2. *Effect of NDV on aminopyrine N-demethylase activity in hepatic microsomes prepared from inbred strain of mice*

The animals were killed 24 hr after the administration of NDV. Activity is expressed as the amount of formaldehyde formed per milligram of protein per hour. Each *bar* represents the mean ± standard error of six individual mice. * Significantly different from control ($p < 0.01$).

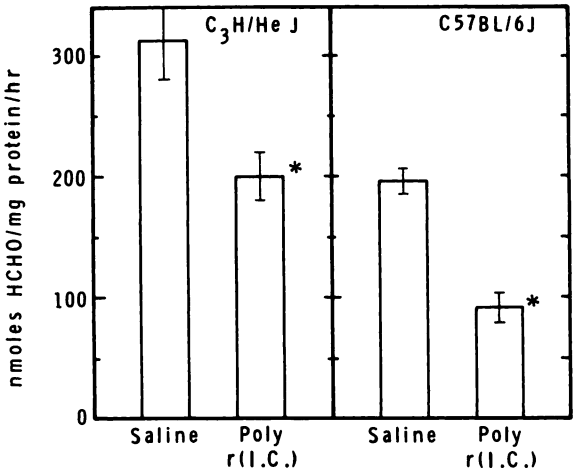

FIG. 4. *Effect of poly(rI·rC) on aminopyrine N-demethylase activity in hepatic microsomes prepared from inbred strains of mice*

The animals were killed 24 hr after the administration of poly(rI·rC). Activity is expressed as the amount of formaldehyde formed per milligram of protein per hour. Each *bar* represents the mean ± standard error of six individual mice. *Significantly different from control ($p < 0.01$).

in C57BL/6J mice. These mice carry the IF-1^h allele and produced high levels of interferon when challenged with NDV. In C_3H/HeJ mice, which carry the IF-1^l allele, NDV had no effect on cytochrome P-450 or drug biotransformation. In this strain circulating interferon concentrations were below our lowest detectable level. The differences in the circulating interferon levels between the two strains was at least 40-fold, which is slightly greater than the differences in strains of mice carrying the two alleles as originally described by DeMaeyer and DeMaeyer-Guignard (12). Although a more extensive analysis using back-crosses of the two strains might constitute direct genetic proof, these experiments suggest that the depression of cytochrome P-450 in response to NDV is dependent on high circulating levels of interferon.

In contrast to the results with NDV, treatment of C57BL/6J or C_3H/HeJ mice with poly(rI·rC) produced high concentrations of interferon in the serum of both strains. Both hepatic microsomal cytochrome P-450 and aminopyrine *N*-demethylase were significantly depressed in both strains. Poly(rI·rC) is a synthetic polynucleotide which induces the formation of interferon at loci other than IF-1 (6). This experiment indicates that the cytochrome P-450 system in C_3H/HeJ mice has the ability to respond when high levels of circulating interferon are produced at other gene loci. The equal response to poly(rI·rC) in the two mouse strains strengthens the argument derived from the differential response to NDV.

Previously Renton and Mannering (4) and Mannering *et al.* (5) demonstrated that depression of drug metabolism was a common property of interferon-inducing agents, but they were unable to determine clearly whether this effect was due to interferon itself or to another common property of these agents. Because interferon inducers also modulate the immune response, it was not known whether the cytochrome P-450 depression was caused by interferon or was due to the effect of interferon-inducing agents on other aspects of host defense. Other workers (13–16) have demonstrated similar effects on cytochrome P-450 following the stimulation of host defense mechanisms, but it was unclear whether individual factors such as immune enhancement, reticuloendothelial cell stimulation, interferon production, or a combination of these was involved in the observed effects. Recent studies by Sonnenfeld *et al.* (17) have shown that the administration of crude preparations of immune type interferon (IF-γ, Type II) can also depress drug biotransformation in liver. These workers also reported that fibroblast interferon had no effect on cytochrome P-450, which confirms experiments carried out in our own laboratory. The interferon produced following the administration of NDV is derived from lymphocytes and macrophages and can be compared with human leukocyte interferon (Type I) (18). The results reported here using strains of mice producing different circulating levels of interferon at a specific gene locus provide strong

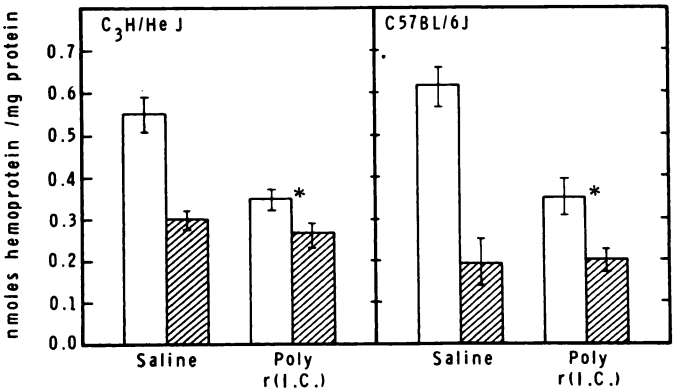

FIG. 3. *Effect of poly(rI·rC) on cytochrome P-450 and cytochrome b_5 levels in hepatic microsomes prepared from inbred strains of mice*

The animals were killed 24 hr after the administration of poly(rI·rC). The *open bars* represent cytochrome P-450 levels and the *shaded bars* the cytochrome b_5 levels. Each value is the mean ± standard error of six individual mice. *Significantly different from control ($p < 0.01$).

evidence that Type I interferon or the process which produces interferon can depress the level of cytochrome P-450 in liver.

Various preparations of interferon are being tested throughout the world for their antitumor effects. We can therefore predict that impairment of cytochrome P-450 by interferon may lead to changes in the pharmacokinetics and metabolite formation of other drugs used at the same time. Interferon is also likely to be a factor in depressing drug biotransformation during natural infections. Examples have already been reported for the elimination rate of the theopylline, which is impaired during influenza infection (19) and following the administration of influenza vaccine (20).

We conclude that an impairment of cytochrome P-450-mediated drug biotransformation occurs via an interferon-mediated interaction and that this effect may enhance the toxicity of drugs and exogenously administered chemicals.

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