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DEPRESSION OF HEPATIC CYTOCHROME P-450-DEPENDENT MIXED FUNCTION OXIDASES DURING INFECTION WITH ENCEPHALOMYOCARDITIS VIRUS*

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Abstract—Infection of mice with encephalomyocarditis virus had a marked effect on drug biotransformation in the liver. The levels of cytochrome P-450 and cytochrome b_5 and aminopyrine *N*-demethylase activity were significantly decreased 3 days after the administration of lethal and sublethal doses of virus. Interferon was detected in serum at doses of virus that produced decreased drug biotransformation. Cytochrome P-450 content and aminopyrine *N*-demethylase activity remained at normal levels for the first 2 days following infection before reaching a maximum decrease on days 3 and 4. Concentration of interferon in the serum of infected mice appeared on day 2 of infection and reached peak levels on day 3. Increased heme oxygenase activity was associated with the decrease in cytochrome P-450 level during the infection. These studies indicate the existence of an interaction between virus infection and cytochrome P-450-dependent drug biotransformation. This may cause adverse toxic effects during the use of drugs that depend on the hepatic mixed function oxidases for elimination.

The cytochrome P-450-dependent drug oxidizing system located in hepatic endoplasmic reticulum can be affected by a large number of endogenous and exogenous factors [1]. Several agents that stimulate certain aspects of the immune system [2] and agents that induce the formation of interferon [3] have been shown to depress hepatic cytochrome P-450 levels and related drug biotransformation. Though both of these processes are involved in the response of the host to viral infections, little information exists on drug biotransformation during episodes of such infections. The present study was designed to determine if hepatic cytochrome P-450-dependent drug oxidation can be altered during the course of viral infection.

MATERIALS AND METHODS

Male Swiss random bred mice (20–30 g) were obtained from Bio-Breeding Laboratories, Ottawa, Ontario, and were allowed to acclimatize in our facilities for at least 10 days before use. Encephalomyocarditis (EMC) virus was obtained from Dr. S. Lee, Department of Microbiology, Dalhousie University, Halifax, Nova Scotia, and was stored at −75°. The stock virus used in these experiments was assayed by plaque formation using mouse L-929 cells, and contained 1.8×10^7 p.f.u./ml.

Hepatic microsomes were prepared as described by El Defrawy El Masry *et al.* [4] and utilized on the day of preparation. Protein was determined by the method of Lowry *et al.* [5] 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 [6] using extinction coefficients of 91 $mM^{-1}\,cm^{-1}$ and 171 $mM^{-1}\,cm^{-1}$ respectively. Aminopyrine *N*-demethylation was determined by the method described by Sladek and Mannering [7]. Heme oxygenase activity was determined by the method of Tenhunen *et al.* [8]. Interferon was assayed in L-929 cell monolayers by the plaque reduction method using vesicular stomatitis virus (Indiana strain) as the challenge virus [9]. The amount of interferon required for 50 per cent plaque reduction was defined as 1 plaque reduction dose (1 PRD_{50} unit).

RESULTS

The lethality of the batch of EMC virus used is illustrated in Fig. 1. No animals died at any dose of virus during the first 3 days or at anytime following the injection of doses of virus of 1.8×10^{-3} p.f.u. or less. Hepatic cytochrome P-450 and cytochrome b^5 levels and aminopyrine *N*-demethylase activities in animals infected for 3 days with various doses of EMC virus are shown in Fig. 2. All three indices were decreased significantly at doses of virus of 1.8×10^{-2} p.f.u. and greater. Body weight, liver weight, and microsomal protein levels were unchanged at all doses of virus. EMC virus doses greater than 1.8×10^{-2} p.f.u. produced significant levels of interferon in the serum of mice infected for 3 days (Table 1). The dose of virus selected for the remaining experiments was 1.8×10^{-1} p.f.u. which produced 33 percent deaths within 5 days of virus administration.

The time course of the changes in the hepatic microsomal mixed function oxidase system and the appearance of interferon in the serum following infection of Swiss strain mice by EMC are illustrated in Fig. 3. Cytochrome P-450 and aminopyrine *N*-demethylase remained at normal levels for 2 days

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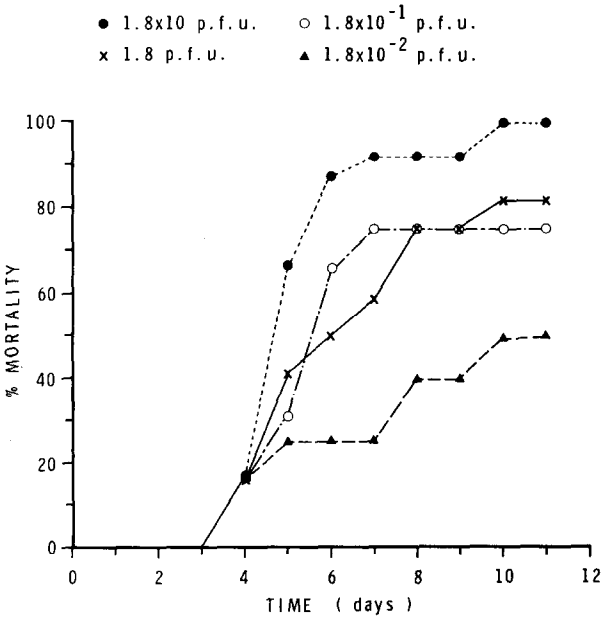

Fig. 1. Mortality of Swiss strain mice during infection with encephalomyocarditis virus. Results are expressed as cumulative mortality on a daily basis following the administration of virus (n = 20, each group).

Table 1. Serum interferon levels in mice infected with encephalomyocarditis virus for 3 days*

EMC (p.f.u./mouse)	Interferon (PRD_{50} units/ml)
Control	<13, <13, <13
1.8×10	432, 234, 100
1.8	120, 320
1.8×10^{-1}	180, 520, 180, <20
1.8×10^{-2}	78, <26
1.8×10^{-3}	<13, <13, <13

* Each value is the serum interferon level determined in an individual mouse.

follwing virus administration and then decreased by more than 40 percent on day 3, compared to control noninfected animals killed on the same day. By day 5 the levels appeared to be recovering toward normal values. Interferon levels in the serum were below detectable levels in serum until day 2 of infection and reached a maximum level of 214 ± 44 PRD_{50} units/ml on day 3.

Heme oxygenase activity in hepatic microsomes prepared from Swiss mice infected with 1.8×10^{-1} p.f.u. of EMC virus for various times is illustrated in Fig. 4. Heme oxygenase activity was similar to that found in the control animals for 2 days following

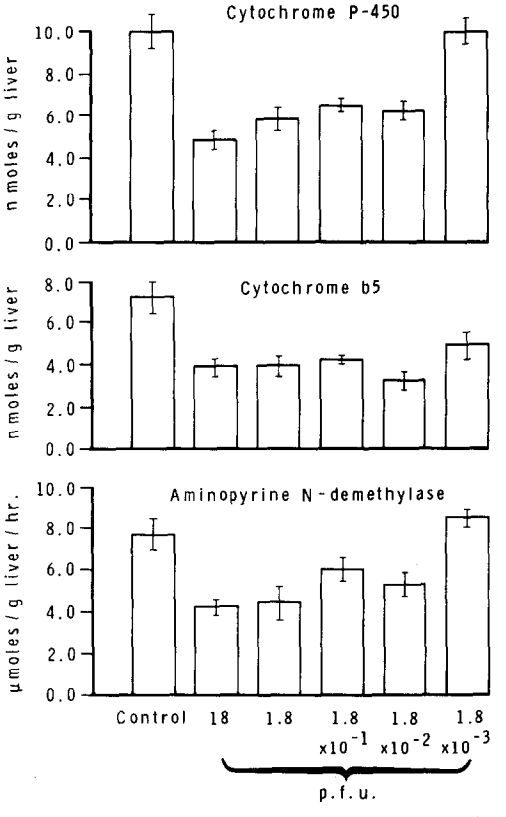

Fig. 2. Cytochrome P-450 and cytochrome b_5 concentrations and aminopyrine *N*-demethylase activity in hepatic microsomes prepared from Swiss strain mice infected with various doses of encephalomyocarditis virus. Determinations were carried out 72 hr after administration of the virus. Each value is the mean ± S.E. of six individual mice.

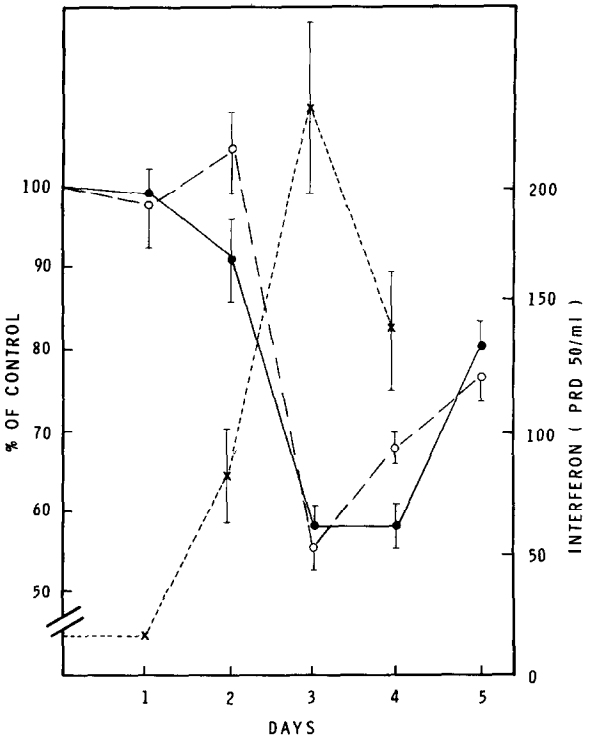

Fig. 3. Cytochrome P-450 (% of control) and aminopyrine *N*-demethylase activity in hepatic microsomes, and interferon concentration in serum, at various times following the infection of Swiss mice with 1.8×10^{-1} p.f.u. encephalomyocarditis virus. Each value is the mean ± S.E. of eight individual mice. Means of control values were as follows: cytochrome P-450 (○– –○) = 0.652 nmole/mg protein; aminopyrine *N*-demethylase (●——●) = 430 ± 28 nmoles HCHO/mg protein·hr^{-1}. Serum interferon levels (×– –×) are expressed as PRD_{50} units/ml.

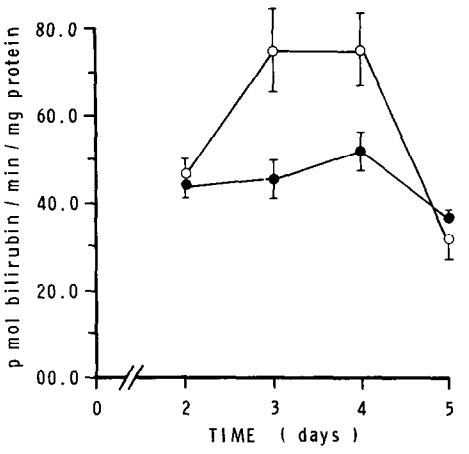

Fig. 4. Heme oxygenase activity in hepatic microsomes prepared at various times following the infection of Swiss strain mice with 1.8×10^{-1} p.f.u. encephalomyocarditis virus (○—○). Control mice (●—●) were maintained under identical conditions and killed on the same day. Each value is the mean ± S.E. of eight individual mice.

administration of the virus but was significantly increased on days 3 and 4. On day 5 heme oxygenase activity had returned to control levels.

DISCUSSION

This study demonstrates that decreased cytochrome P-450 levels and diminished drug oxidation capacity occur in liver microsomes during the course of an infection with EMC virus in the mouse. These results are similar to those reported by Renton and Mannering [3] who reported that, in the rat, drug oxidation was depressed following the administration of lethal doses of Mengo virus which is antigenically indistinguishable from the EMC virus used in the present studies. Previously, other workers have demonstrated that murine hepatitis virus decreases hexobarbital oxidation [10] and cytochrome P-450 levels [11] in the liver. In the duck, hepatitis virus depresses certain drug oxidation reactions and also enhances induction of cytochrome P-450-mediated oxidations by 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT) [12,13]. In humans, hepatitis virus has been reported to have a variable effect on drug biotransformation and can increase, decrease, or leave unchanged the half-life of a number of different drugs [14]. The present results support the idea that cytochrome P-450 and drug biotransformation can be altered by viral infections other than hepatitis virus. However, even though the primary pathological changes occur in the CNS and heart during infection with EMC virus, the cells in most other tissues including the liver are infected with this virus [15]. EMC virus titres achieved in the liver are comparable to most other tissues and can be as high as those found in the brain or heart.

The decrease in cytochrome P-450 content and in drug oxidation during infection with EMC virus is similar to that reported when animals are treated with a number of other agents that can stimulate host defense mechanisms. These agents have included interferon inducers [3], Bacillus Calmette-Guérin (BCG) [16], *Corynebacterium parvum* [17], *Escherichia coli* endotoxin [18], *Plasmodium berghei* [19] and pyran copolymers [20]. Renton and Mannering [3] have suggested that these reductions may result from interferon production or from a process involved in the antiviral action of interferon. In the present experiments, interferon levels in serum from mice were increased at doses of virus causing decreased cytochrome P-450 levels. Further, the time course of interferon production corresponded to the times when cytochrome P-450 and drug biotransformation were reduced. These results, therefore, are consistent with the idea that a relationship exists between the two. It is still unclear, however, if individual factors, such as interferon production, immune stimulation, reticuloendothelial cell stimulation or a combination of more than one of these are involved in the decrease in drug biotransformation that is observed during the viral infection.

Heme oxygenase activity increased on day 3 of infection and corresponded to the time when cytochrome P-450 decreased. Increased heme oxygenase activity associated with a decrease in cytochrome P-450 in the liver has been described for a number of different agents [21–24]. In a recent paper, El Azhary and Mannering [24] suggested that interferon-inducing agents cause an increase in the magnitude of the regulatory heme pool by increasing heme dissociation from hemoproteins or by depressing synthesis of the apoprotein P-450. An increase in the heme pool results in an induction of heme oxygenase by the excess heme. The increase in heme oxygenase during viral infection may be caused by a similar interferon-mediated mechanism. This depressant effect is not confined to cytochrome P-450 alone. Another heme-containing enzyme, tryptophan 2,3-dioxygenase, is also decreased by interferon-inducing agents [24] and during infection with Sindbis Virus [25].

These studies raise the important possibility that drug biotransformation capacity for certain drugs metabolized by the cytochrome P-450-dependent mixed function oxidase system may be impaired during episodes of viral infection in man. The occurrence of such an effect is suggested by the work of Chang *et al.* [26] who described increased theophylline half-life in six patients with upper respiratory tract viral infections, and by the report of Fleetham *et al.* [27] who described increased theophylline levels in a single patient during infection with influenza virus. More recently, Woo *et al.* [28] described several cases of theophylline toxicity during respiratory infections. In our own laboratory, we have demonstrated increased theophylline levels in three patients receiving chronic theophilline therapy following vaccination with influenza vaccine [29]. In the same study the half-life of a single dose of theophylline was increased by more than 100 percent in four normal volunteers 24 hr after vaccination with influenza virus vaccine. An influenza virus (RP-8-34-60) has also been shown to depress lung benzo[*a*]pyrene activity in the mouse [30].

Several workers have reported that the induction of drug biotransformation in animals by certain

chemical agents can be enhanced during a concomitant viral infection [10,11]. Doshi *et al.* [31] demonstrated the occurrence of hyperplasia of the endoplasmic reticulum and an increase in pentobarbital hydroxylase activity during the regenerative phase of acute viral hepatitis in man. Recently, Leppik *et al.* [32] reported a single case in which phenytoin elimination was increased in a patient with infectious mononucleosis. Each of these reports concerning increased drug oxidation during a viral infection involves a situation in which proliferation of the endoplasmic reticulum occurs. Drug biotransformation appears to be depressed in situations where the endoplasmic reticulum is normal, as in the study reported here. These studies indicate the existence of an interaction between drugs and viral illness which has the potential to cause adverse toxic effects during the use of certain drugs.

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