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Pharmacological reversal of cholestasis-associated decrease in hepatic cytochrome P-450

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The liver has an important role in the metabolism of a wide variety of endogenous and exogenous substances [1], and hepatic disease may adversely affect this function. We have recently demonstrated in the rat that mild cholestatis induced by ethinyl estradiol administration as well as severe cholestasis produced by bile duct ligation is associated with a significant reduction in hepatic cytochrome P-450, the major component and terminal oxidase of the microsomal drug-metabolizing system [2, 3]. In both cholestatic models, the rate of synthesis of the apoprotein of cytochrome P-450 was reduced. This effect of cholestasis on the turnover of cytochrome P-450 appeared to be the inverse of that seen with the pharmacological-inducing agent, phenobarbital [4]. We have, therefore, investigated the potential of the established microsomal-inducing agents, phenobarbital and 3-methylcholanthrene (3-MC), for reversing the decrease in the components of the hepatic microsomal drug-metabolizing system associated with bile duct ligation and ethinyl estradiol treatment.

Male Sprague–Dawley rats (200–250 g) were housed in wire-bottomed cages and fed laboratory chow and water *ad lib*. Cholestasis was produced either by double ligation and transection of the common bile duct under ether anesthesia, or by the administration of ethinyl estradiol, 5 mg/kg/day, in propylene glycol by i.m. injection. Phenobarbital, 80 mg/kg/day, in normal saline and 3-MC, 15 mg/kg/day, in corn oil were administered i.p. To test the pharmacologic reversibility of the cholestasis-associated decrease in mixed function oxidase system components, either phenobarbital or 3-MC was administered daily as described above, simultaneously with the first injection of ethinyl estradiol or immediately after bile duct ligation. Livers from 12-hr fasted animals were homogenized in 0·25 M sucrose (20% w/v) and a microsomal fraction was collected by differential centrifugation [2]. The microsomal cytochromes P-450 and b_5 were assayed by the method of Omura and Sato [5], while NADPH cytochrome *c* reductase was determined by the method of Baron and Tephly [6]. Protein was assayed by the Folin-phenol reaction [7], and data were analyzed for significance by the Student *t*-test.

Both bile duct ligation and ethinyl estradiol were associated with a significant ($P < 0.001$) decrease in cytochromes P-450 and b_5 and in NADPH cytochrome *c* reductase (Table 1). The administration of phenobarbital or 3-MC increased the level of cytochrome P-450, but in contrast to phenobarbital, 3-MC did not increase NADPH cytochrome *c* reductase. When ethinyl estradiol and phenobarbital were administered simultaneously daily for 5 days, the level of cytochrome P-450 was slightly greater than that of control ($P < 0.05$), but neither b_5 nor reductase differed significantly from control values. When 3-MC, which by itself increases only cytochrome P-450 (or P-448) [8], was administered with ethinyl estradiol for 5 days, only cytochrome P-450 did not differ significantly from control, while both cytochrome b_5 and reductase were significantly lower than control ($P < 0.001$). These latter values did not differ significantly from those seen with ethinyl estradiol alone. The potential of phenobarbital for reversing the cholestasis-associated decrease in all three components of the mixed function oxidase system was further demonstrated in the bile duct ligated animals. Thus, when phenobarbital was administered daily for 3 days after bile duct ligation, the levels of the three components were all significantly greater than those seen after ligation alone and indeed did not differ from the control.

Both bile duct ligation and ethinyl estradiol were associated with a significant ($P < 0.001$) decrease in the specific activity of the microsomal marker enzyme glucose 6-phosphatase which was assayed by the method of deDuve *et al.* [9]. A comparable reduction in glucose 6-phosphatase activity was observed in the phenobarbital-treated animals. Simultaneous administration of phenobarbital with ethinyl estradiol resulted in a lower activity of this enzyme than that seen with either agent alone.

The effect of bile duct ligation and ethinyl estradiol administration on the turnover of cytochrome P-450 apo-

Table 1. Effect of cholestasis and phenobarbital or 3-methylcholanthrene treatment on components of the hepatic mixed function oxidase system*

	Cytochrome P-450 (nmoles/mg protein)	Cytochrome b_5 (nmoles/mg protein)	NADPH cytochrome *c* reductase (nmoles cytochrome *c* reduced/mg protein/hr)
Control	1·08 ± 0·08	0·76 ± 0·07	87 ± 8
Ethinyl estradiol†	0·67 ± 0·13	0·45 ± 0·13	43 ± 8
Bile duct ligation‡	0·48 ± 0·10	0·41 ± 0·11	46 ± 10
Phenobarbital†	2·56 ± 0·13	0·94 ± 0·04	170 ± 6
3-Methylcholanthrene†	1·63 ± 0·14	0·72 ± 0·07	83 ± 7
Ethinyl estradiol plus phenobarbital†	1·29 ± 0·05	0·67 ± 0·11	84 ± 6
Ethinyl estradiol plus 3-methylcholanthrene†	1·12 ± 0·05	0·47 ± 0·10	40 ± 7
Bile duct ligation plus phenobarbital‡	1·06 ± 0·05	0·71 ± 0·06	79 ± 9

* Each value represents mean ± 1 S.D.
† Each given for 5 days.
‡ After three days of bile duct ligation.

protein appears to be the inverse of the effect of phenobarbital [2, 4]. From the data presented above, it is apparent that these opposing effects where acting in concert result in a balance with the level of cytochrome P-450 remaining near the control value. The same argument may well apply to the other components of the mixed function oxidase system stimulated or induced by phenobarbital. Further support for this interpretation is provided by the effect of 3-MC in the ethinyl estradiol-treated rat. Thus, 3-MC alone increases the level of cytochrome P-450 (P-448) but does not affect cytochrome b_5 or NADPH cytochrome *c* reductase. In combination with ethinyl estradiol, 3-MC apparently reverses the effect of estradiol in decreasing P-450 levels, but cytochrome b_5 and NADPH cytochrome *c* reductase remain at the low levels seen with estradiol treatment alone. We, therefore, conclude that the pharmacological reversal of the cholestasis-associated changes in microsomal proteins is specific, and only those proteins stimulated by the pharmacological agent are affected.

While the role of the decrease in mixed function oxidase system components and activity in the pathogenesis of cholestasis remains undefined, the significance and clinical applicability of the pharmacologic reversibility are not clear. However, the comparable degree of impairment of this system in mild intrahepatic cholestasis and severe extraheptatic cholestasis suggests that this may not merely be a secondary phenomenon but more integrally related to the pathogenesis of bile secretory failure or cholestasis.

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