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SUPPRESSION OF THE INDUCTIVE EFFECTS OF PHENOBARBITAL AND 3-METHYLCHOLANTHRENE ON ASCORBIC ACID SYNTHESIS AND HEPATIC CYTOCHROME P-450-LINKED MONOOXYGENASE SYSTEMS BY THE INTERFERON INDUCERS, POLY rI·rC AND TILORONE

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SUMMARY. The interferon inducing agents, poly rI·rC and tilorone, cause a marked depression of hepatic cytochrome P-450-linked monooxygenase systems. Ascorbate synthesis and hepatic monnoxygenase systems are induced by phenobarbital and 3-methylcholanthrene. Poly rI·rC and tilorone suppressed the induction of ascorbate synthesis, P-450 and monooxygenase activity (ethylmorphine N-demethylase and benzo[a]pyrene hydroxylase) by phenobarbital. 3-Methylcholanthrene-induced ascorbate synthesis was suppressed by poly rI·rC, but equivocal results were obtained with tilorone. Induction of P-450 by 3-methylcholanthrene was suppressed completely by poly rI·rC or tilorone, but that of benzo[a]pyrene hydroxylase was lowered by only 40%, thus demonstrating the selective depressive action of interferon inducing agents on different species of P-450.

INTRODUCTION. Many drugs and other xenobiotics which induce hepatic P-450-linked monooxygenase systems also induce the biosynthesis of ascorbic acid in rats (1,2). This might suggest that a common mechanism is involved in the maintenance of steady states of these monooxygenase systems and the synthesis of ascorbate. If this is the case, factors which suppress the induction of one system should also suppress the induction of the other. A variety of interferon inducing agents, including poly rI·rC[2] and tilorone, cause a marked depression of hepatic monooxygenase systems when administered to rats or mice (3). The present study explored the possibility that poly rI·rC and tilorone might suppress phenobarbital- and 3-methylcholanthrene-induced P-450 systems and ascorbic acid synthesis in rats.

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[2]Abbreviations: Poly rI·rC or IC, polyriboinosinic acid - polyribocytidylic acid; MC, 3-methylcholanthrene; PB, phenobarbital; BP, benzo[a]pyrene; EM, ethylmorphine.

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MATERIALS AND METHODS. Chemicals. Tilorone (2,7-bis[2-(diethylamino)ethoxy] fluoren-9 one dihydrochloride) was a gift from Richardson-Merrell, Inc., Cincinnati, OH. Poly rI·rC, 3-methylcholanthrene (MC), benzo[a]pyrene (BP) and ascorbic acid were purchased from Sigma Chemical Co., St. Louis, Mo. Sodium phenobarbital (PB) was obtained from J. T. Baker Chemical Co., St. Louis, Mo., and ethylmorphine (EM) hydrochloride from Merck Chemical Div., Rahway, N. J.

Animals. Male, Simonson strain rats (170-200 g) were used in all experiments. When urinary ascorbic acid excretion was studied, rats were housed in metabolic cages (3 per cage) and fed water and a standard laboratory chow ad libitum. The combined urine from 3 rats was collected every 24 hr into bottles immersed in ice. The following materials were administered singly or in various combinations for one or more days (per kg of body weight): saline (2 ml, i.p., or 10 ml, p.o.); corn oil (2.5 ml, i.p.); PB (40 mg in saline, i.p.); MC in corn oil (20 mg, i.p.); poly rI·rC in saline (10 mg, i.p.); tilorone in saline (50 mg, p.o.). When monooxygenase systems were studied, the same procedures were used with the following exceptions: rats were housed in individual cages, urine was not collected, all agents were administered for 4 days only, rats were killed 24 hr after the last administration of an agent, the livers were removed and hepatic microsomes were prepared as described previously (4).

Assays. The ascorbate content of the urine was determined daily using the method of Vann (5), which was modified by deleting the chloroform extraction. Chloroform extraction increases color stability, but is unnecessary when samples are read within 10 min of color development. Microsomal EM N-demethylase and BP hydroxylase activities were determined as described previously (4). Microsomal cytochromes P-450 and b_5 were determined as described by Omura and Sato (6).

RESULTS. Fig. 1A shows the effects of PB and poly rI·rC administration, singly or in combination, on the daily urinary excretion of ascorbate by the rat. In the absence of poly rI·rC, PB caused a gradual increase in the excretion of ascorbate until the fifth day when values were about 7-fold greater than those seen with control rats. The increased rate of ascorbate excretion remained about the same throughout the rest of the experiment. Induction of ascorbate excretion by PB was almost completely prevented by poly rI·rC throughout the 10-day experimental period. During this time the weights of control, PB-treated, poly rI·rC-treated and PB- + poly rI·rC-treated rats increased 19, 24, 20 and 12 g, respectively.

Fig. 1B shows the effects of PB and tilorone administration, singly or in combination, on the daily urinary excretion of ascorbate. The effect of tilorone on PB-induced ascorbate excretion was similar to that of poly rI·rC, but not as complete or as continuous. During this experiment, the growth of control, PB-treated, tilorone-treated or PB- plus tilorone-treated rats

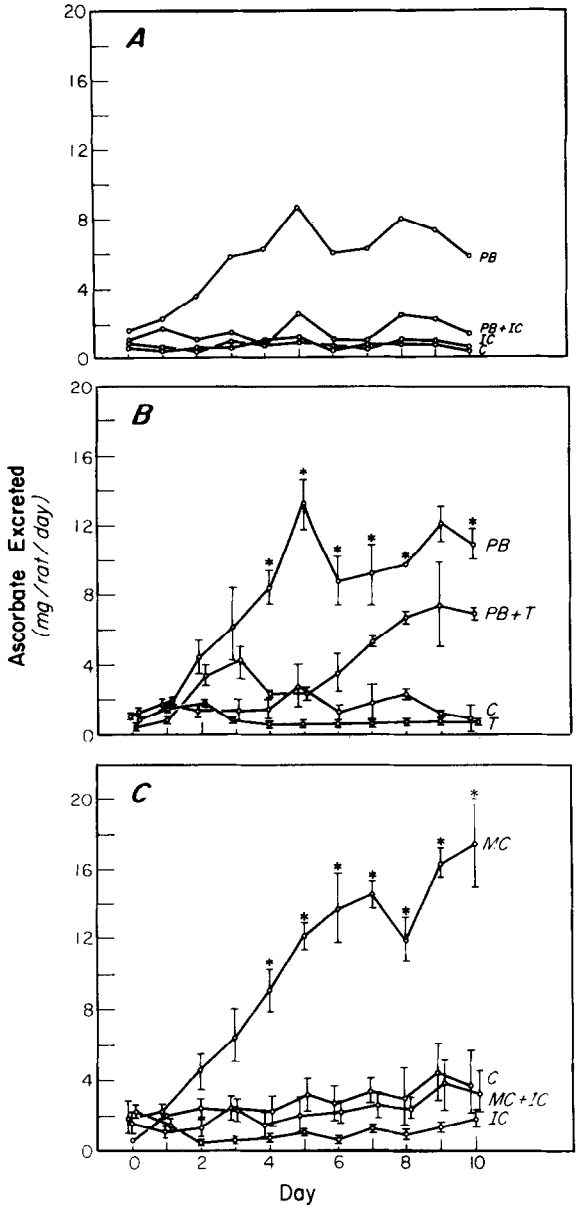

Fig. 1: Effects of poly IC (IC) and tilorone (T) on the urinary excretion of ascorbate induced by treatment of rats with phenobarbital (PB) or 3-methylcholanthrene (MC). A. Rats were given saline (C), i.p.; PB, i.p. (40 mg/kg. day); IC, i.p., (10 mg/kg/day) or the same doses of PB + IC for 10 days. Daily urine collections from groups of 3 rats were analyzed daily for ascorbate content. Values are the means of 2 experiments. B. As in A, but tilorone, p.o. (50 mg/kg/day) was substituted for IC and saline was given p.o. Bars prepresent the mean ± S.E.; N=4. C. Rats were given corn oil (C) i.p., for 10 days; MC, i.p. (20 mg/kg/day), for the first 4 days only; IC, p.o. (10 mg/kg/day) for 10 days; or the same doses of MC (4 days) + IC (10 days). Urine was collected and analyzed for ascorbate as in A. Bars represent means ± S.E.; n = 4.

was 19, 24, 20 and -1g, respectively. The difference between the growth of tilorone-treated and PB + tilorone-treated rats was statistically significant ($p<0.05$).

Fig. 1C shows the effects of MC and poly rI·rC, singly or in combination, on the daily renal excretion of ascorbate. The magnitude and temporal aspects of the induction of ascorbate excretion by MC were similar to those produced by PB except that with PB the effect was maximal within 5 days, whereas with MC the effect increased throughout the 10-day experiment. Poly rI·rC completely reversed the inductive effect of MC. Moreover, poly rI·rC decreased the excretion of ascorbic acid by rats that had not received MC. The weights of control, MC-treated, poly rI·rC-treated, and MC-plus poly rI·rC-treated rats increased 19, 20, 25 and 4 g, respectively, during the experiment.

The effect of tilorone on MC-induced ascorbate excretion was not consistent (data not shown). In some experiments, tilorone caused a lesser depression of the inductive effect than that seen with poly rI·rC; in other experiments, tilorone produced no statistically significant depression of the induction.

Fig. 2 shows the effects of poly rI·rC and tilorone on the hepatic P-450 levels and monooxygenase activities induced by PB and MC. The respective 137 and 270% increases in P-450 and EM N-demethylase activity induced by PB were lowered to 23 and 80%, i.e., 83 and 70% of the inductions were suppressed (Fig. 2A). Tilorone caused corresponding losses of induction of 68 and 51% (Fig. 2C). BP hydroxylase activity was not increased by PB. The 42% increase in P-450 induced by MC was decreased by poly rI·rC to a level 52% below that of controls (Fig. 2B); the 377% increase in BP hydroxylase activity induced by MC was lowered to 227% (Fig. 2B), a decrease of only 40%. Tilorone lowered the induction of P-450 by MC (93%) to a level 58% below the control level (Fig. 2D); the 455% increase in BP hydroxylase activity induced by MC was lowered by tilorone to 266% (Fig. 2D), a decrease of 41%. EM N-demethylase activity was not induced by MC (Figs. 2A and 2C).

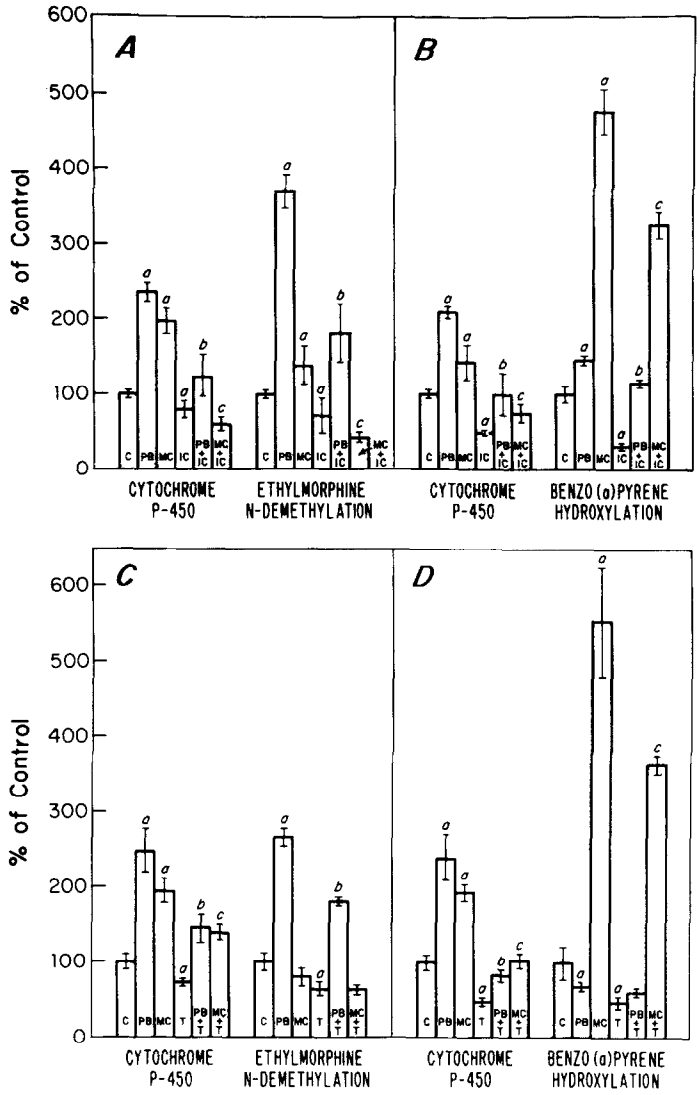

Fig. 2, A and B: Effects of poly rI·rC (IC) on the microsomal P-450, ethylmorphine (EM) demethylase and benzo[a]pyrene (BP) hydroxylase induced by phenobarbital (PB) or 3-methylcholanthrene (MC). Rats were given (i.p.) saline or corn oil (C); PB (40 mg/kg/day); MC (20 mg/kg/day); IC (10 mg/day), or combinations of the same doses of IC + PB or IC + MC. Injections were given daily for 4 days, the animals were killed 24 hr after the last injection and microsomes were prepared from their livers. 100% (C) values (per mg of microsomal protein): P-450, 0.80 ± 0.03 (A) and 0.95 ± 0.04 (B) nmoles; EM N-demethylase activity, 5.0 ± 0.42 nmoles HCHO formed/min; BP hydroxylase activity, 0.46 ± 0.02 nmoles BP hydroxylated/min. C and D: As in A and B, but tilorone (T), p.o. (50 mg/kg/day), was substituted for IC. 100% (C) values (per mg of microsomal protein): P-450, 0.79 ± 0.05 (C) and 0.08 nmoles (D); EM N-demethylase activity, 3.5 ± 0.37 nmoles HCHO formed/min; BP hydroxylase activity, 0.40 ± 0.02 nmoles hydroxylated BP formed/min. Bars represent ± S.E., N=4. [a]Different than C ($p<0.05$). [b]Different than PB ($p<0.05$). [c]Different than MC ($p<0.05$).

DISCUSSION. The concomitant induction of hepatic P-450-linked monooxygenase systems and ascorbic acid synthesis by the same agents suggests that these two seemingly unrelated processes may be controlled by a common regulatory mechanism. This suggestion is reinforced by the current observation that poly rI·rC and tilorone depress both the induction of monooxygenase systems and the induction of ascorbate synthesis. The depression of hepatic monooxygenase systems by poly rI·rC resembles that produced by endotoxin, which is also a potent interferon inducer (7). Endotoxin causes a dissociation of heme from P-450 (8,9). In theory, this heme is added to an unassigned heme pool which depresses the synthesis of -aminolevulinic acid synthetase (ALA-S) - the rate limiting enzyme in heme synthesis - by a negative feedback mechanism. The enlarged heme pool induces heme oxygenase, which contributes to the regulatory mechanism by breaking down heme. We have observed recently that poly rI·rC and tilorone also depress ALA-S and induce heme oxygenase (10,11). The possibility that this regulatory heme pool may be involved in the synthesis of ascorbate is not particularly attractive because none of the enzymes directly involved in ascorbate synthesis are hemoproteins. However, the synthesis of certain non-heme erythrocytic proteins is thought to be regulated by heme (12,13) and the possibility must therefore be considered that heme may regulate the synthesis of the enzymes involved in the synthesis of ascorbate. Ascorbate may be essential for the synthesis of the heme of P-450 (14-17).

MC induces high concentrations of cytochrome P_1-450 in hepatic microsomes. P_1-450 has a high substrate specificity for BP and a low substrate specificity of ethylmorphine (18). Ethylmorphine N-demethylase is depressed to a greater extent that BP hydroxylation by poly rI·rC and tilorone. This is interpreted to mean that P_1-450 is less vulnerable to the suppressive effects of these agents than other species of P-450 that can metabolize ethylmorphine and BP. The co-administration of poly rI·rC and MC provides a means of increasing the concentration of P_1-450 relative to other species of P-450 in microsomes than can be achieved with MC alone.

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