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EFFECTS OF THE INTERFERON INDUCING AGENTS TILORONE AND POLYRIBOINOSINIC ACID·POLYRIBOCYTIDYLIC ACID (POLY IC) ON THE HEPATIC MONOOXYGENASE SYSTEMS OF THE DEVELOPING NEONATAL RAT*

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Abstract—This paper describes the effects of the interferon inducing agents tilorone and polyriboinosinic acid·polyribocytidylic acid (poly IC) on the postnatal development of hepatic cytochrome P-450-linked monooxygenase systems of male rats from birth through early adolescence. The administration of tilorone to rats on days 1 and 2 postpartum modified the changes in the activities of hepatic monooxygenase systems that occur normally during the first four days postpartum. Thus, aniline hydroxylase activity, which develops very rapidly during the first 2 days postpartum, was depressed markedly by tilorone, ethylmorphine *N*-demethylase activity was depressed moderately, and benzo[*a*]pyrene hydroxylase, normally the slowest of the three monooxygenase activities to develop, was induced. These changes in monooxygenase activities occurred without a significant change in the cytochrome P-450 content. These observations suggest that not all species of neonatal cytochrome P-450 are affected equally by tilorone administration. By day 7 postpartum, the cytochrome P-450 content and all three monooxygenase activities were depressed in rats that had received tilorone on days 1 and 2 postpartum. All three monooxygenase systems were depressed by the administration of a single dose of poly IC (10 mg/kg) in 1-, 2-, 21-, 28- and 56-day-old rats. The length of the period between maximal depression and complete recovery of cytochrome P-450 systems was shown to be a function of the age of the rat; it increased from about 6 hr in 1-day-old rats to 48 hr in 56-day-old rats. Protein is synthesized more rapidly and degraded more slowly in neonate than in adult animals; this may account for the more rapid recovery of poly IC-induced depression of monooxygenase systems in neonates.

The liver undergoes dramatic morphological and biochemical changes during the perinatal period as it assumes many functions previously performed by the maternal liver [1–6]. For example, the rat fetus is essentially devoid of hepatic cytochrome P-450-dependent monooxygenase activity almost until parturition. These systems develop very rapidly after parturition and attain adult levels within a few weeks (see Ref. 7 for review). The rapid postnatal development of the hepatic monooxygenase systems corresponds with the morphological differentiation of the hepatocyte [3, 4]. At 4 days prepartum, the fetal rat liver contains about 60% hematopoietic tissue; by 5 days postpartum this has declined to 10%. Interferon is a potent hepatotoxin during this perinatal period. Electrophoretically pure mouse interferon administered to newborn mice for 8 days produces almost total disruption of the liver architecture with diffuse and extensive cell necrosis; hepatotoxicity is not observed when interferon administration is initiated after the mice are 7 days old [8]. These observations predict that tilorone and poly IC§ should alter the development of hepatic monooxygenase systems in neonate rats if significant amounts of interferon are induced by these agents. This paper describes the effects of tilorone and poly IC on the postnatal development of hepatic cytochrome P-450-dependent monooxygenase systems of rats from birth through early adolescence.

MATERIALS AND METHODS

Animals, materials and assays for microsomal ethylmorphine (EM) *N*-demethylase, benzo[*a*]pyrene (BP) hydroxylase and aniline hydroxylase activities and cytochrome P-450 and protein contents have been described previously [9, 10]. Male Sprague–Dawley rats (Bio-Lab Co., St. Paul, MN) were used in all experiments. Animals were weaned at 21 days after birth. Hepatic microsomes (S-microsomes) from rats of all ages were prepared by using a

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§ Abbreviations: poly IC, polyriboinosinic acid·polyribocytidylic acid; EM, ethylmorphine; BP, benzo[*a*]pyrene; and AN, aniline.

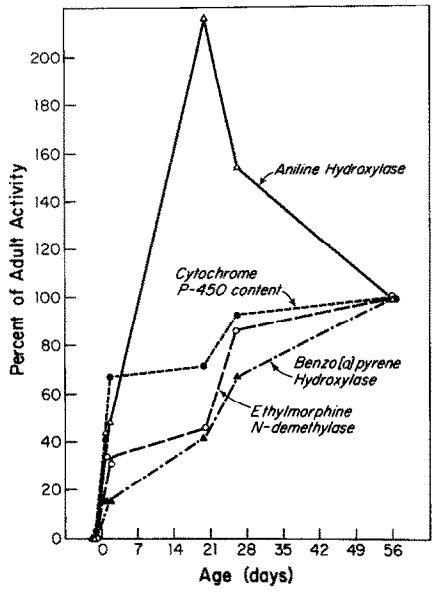

Fig. 1. Development of hepatic cytochrome P-450 systems. S-microsomes were prepared from rats aged 1 day prepartum to 56 days postpartum and assayed for cytochrome P-450 content and EM *N*-demethylase, BP hydroxylase and AN hydroxylase activities. One hundred percent adult (56-day-old) values were: cytochrome P-450, 14.9 ± 0.11 nmoles P-450/g liver; EM *N*-demethylase activity, 103.0 ± 12.4 nmoles CH_2O formed/g liver/min; BP hydroxylase activity, 4.91 ± 0.69 nmoles 3-OH BP/g liver/min, and AN hydroxylase 13.3 ± 0.97 nmoles p-OH AN formed/g liver/min. N = 8 for each age group.

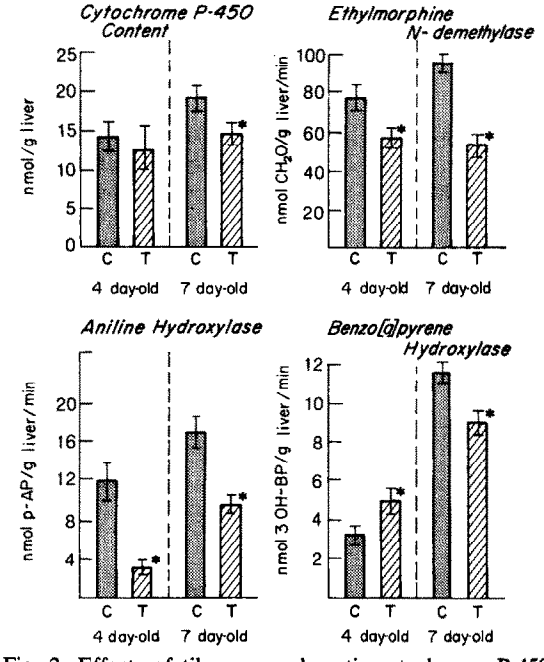

Fig. 2. Effects of tilorone on hepatic cytochrome P-450 systems of newborn rats. Male rates were injected with 50 mg tilorone/kg/day on days 1 and 2 postpartum. Rats were killed on day 4 or 7 postpartum, and S-microsomes were prepared and assayed for cytochrome P-450 content and EM *N*-demethylase, AN hydroxylase and BP hydroxylase activities. N = 8 for each age group. Key: C, control (saline-treated); T, tilorone-treated; *p*-AP, aminophenol; and (*) significantly different from control ($P < 0.05$).

modified homogenization procedure and gel filtration as described previously [9].

Data were adjusted to accommodate 90% recovery of microsomes by gel filtration [9] and analyzed for statistical significance at the 0.05 level as described previously [9].

RESULTS

Development of hepatic microsomal cytochrome P-450 and monooxygenase activities in rats from 1 day prepartum to 56 days postpartum. The cytochrome P-450 content and ethylmorphine *N*-demethylase, benzo[*a*]pyrene hydroxylase and aniline hydroxylase activities of S-microsomes from 1-day prepartum, the day of birth, 1-day-old, 21-day-old, 28-day-old and 56-day-old male rats are shown in Fig. 1. The immediate rapid increases in the three monooxygenase activities that occurred after birth varied considerably. By the first day, the level of EM *N*-demethylase activity was about twice that of the BP hydroxylase activity, which in turn was only about half that of the aniline hydroxylase activity. Increases in the former two activities were more gradual thereafter and tended to level off during puberty. On the other hand, development of aniline hydroxylase activity was biphasic, rising to a very high level by 21 days and dropping off precipitously thereafter to an intermediate level by the time the rats were 56-days-old. The results are generally quite similar to those performed previously with 9000 *g* supernatant fraction from 21- to 56-day-old rats [10]. These studies not only show differences in the development of monooxygenase activities, which presumably reflect differences in the development of those species of cytochrome P-450 primarily involved in these reactions, but they also demonstrate the feasibility of using S-microsomal preparations throughout an extended developmental period.

Effects of tilorone on the development of cytochrome P-450 systems. Male rats were injected with tilorone on days 1 and 2 postpartum, and S-microsomes were prepared from their livers on day 4 or 7 postpartum. The cytochrome P-450 content and monooxygenase activities of these microsomes are shown in Fig. 2. At 4 days after birth, cytochrome P-450 was not altered, but EM *N*-demethylase and AN hydroxylase activities were depressed 26 and 70% respectively. BP hydroxylase activity was increased by 53%. However, at 7 days after birth, cytochrome P-450 and all three monooxygenase activities were depressed—cytochrome P-450 by 30%, EM *N*-demethylase activity by 43%, AN hydroxylase activity by 44% and BP hydroxylase by 23%. The cytochrome P-450 contents and EM *N*-demethylase, AN hydroxylase and BP hydroxylase activities of 21-day-old rats that had received the same dose of tilorone on the first and second days after birth were still somewhat depressed (17, 27, 19 and 18% respectively), but all values had returned to normal by day 56 (Table 1).

Time course of poly IC-induced losses and recoveries of cytochrome P-450 systems in newborn, weanling and adult rats. Time courses of losses and recoveries of microsomal cytochrome P-450, EM *N*-demethylase, AN hydroxylase and BP hydroxylase

Table 1. Levels of hepatic cytochrome P-450 and monooxygenase activities in 21- and 56-day-old rats that had received tilorone on days 1 and 2 postpartum*

Age and treatment	Animal wt (g)	Liver wt (g)	Cytochrome P-450 (nmoles/g liver)	EM *N*-demethylase (nmoles CH_2O formed/g/min)	AN-hydroxylase (nmoles *p*-OH AN formed/g/min)	BP hydroxylase (nmoles 3-OH BP formed/g/min)
21-Day-old						
Saline	65 ± 8	2.8 ± 0.3	10.06 ± 0.34	56.3 ± 4.26	15.20 ± 1.33	2.14 ± 0.17
Tilorone	57 ± 5	2.6 ± 0.3	8.32 ± 0.52†	41.3 ± 6.11†	12.16 ± 0.49†	1.73 ± 0.11†
56-Day-old						
Saline	263 ± 19	11.3 ± 1.0	13.8 ± 0.60	117.6 ± 8.7	14.83 ± 1.70	4.33 ± 0.43
Tilorone	217 ± 27	10.0 ± 0.8	14.2 ± 1.0	117.4 ± 7.0	14.96 ± 1.37	4.10 ± 0.40

* Male rats received 50 mg of tilorone/kg/day, i.p., on days 1 and 2 postpartum. They were killed 21 and 56 days after birth, and S-microsomes were prepared. Values represent the mean ± S.E.; N = 4.
† Significantly different from saline control ($P < 0.05$).

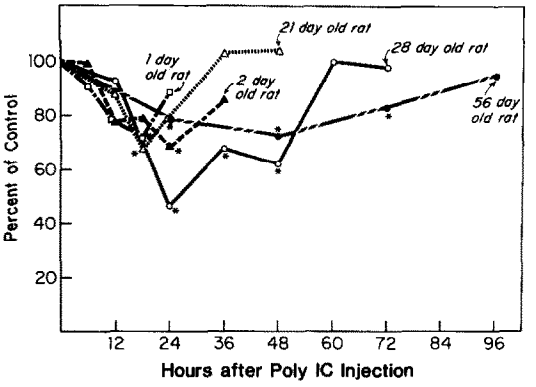

Fig. 3. Time course of the depressant effect of poly IC on the cytochrome P-450 content of hepatic microsomes from newborn, weanling and adult male rats. S-microsomes were prepared from rats killed at indicated times after a single injection (i.p.) of 10 mg/kg of poly IC. One hundred percent control (saline-treated) values for 1-, 2-, 21-, 28- and 56-day-old rats were: 8.9 ± 0.3, 9.8 ± 0.4, 19.6 ± 0.8, 24.9 ± 1.1 and 21.8 ± 1.2 nmoles of P-450/g liver. N = 6 for each time point. Key: (*) significantly different from control ($P < 0.05$).

activities after a single injection of poly IC to 1-, 2-, 21-, 28- or 56-day-old rats are shown in Figs. 3–6. Respective maximal losses of cytochrome P-450 of 30, 35, 35, 57 and 30% (Fig. 3) occurred at 18, 24, 18, 24 and 48 hr; respective times of recovery were 24, 36, 36, 60 and 96 hr. Thus, the length of the period between the loss and recovery of cytochrome P-450 was a function of age, whereas the magnitude of the depressant effect was independent of age. The time courses for the losses and recoveries of monooxygenase activities (Figs. 4–6) paralled those for cytochrome P-450 (Fig. 3). In 1-, 2-, 21-, 28- and 56-day-old rats, respective maximal losses of EM *N*-demethylase activity were 55, 45, 45, 60 and 50%;

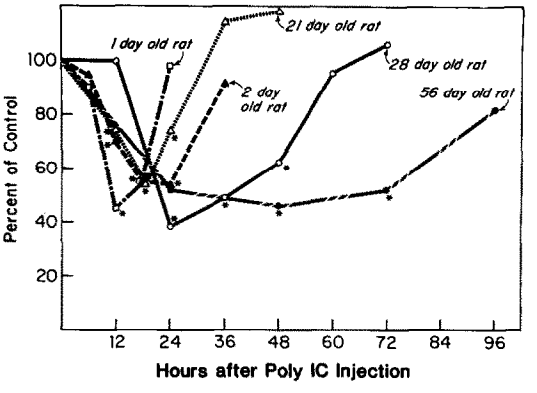

Fig. 4. Time course of the depressant effect of poly IC on the ethylmorphine (EM) *N*-demethylase activity of hepatic microsomes from newborn, weanling and adult male rats. S-microsomes were prepared from rats killed at indicated times after a single injection (i.p.) of 10 mg/kg of poly IC. One hundred percent control (saline-treated) values for 1-, 2-, 21-, 28- and 56-day-old male rats were: 90.7 ± 10.0, 101 ± 5.9, 135 ± 4.8, 255 ± 11 and 296 ± 22 nmoles CH_2O formed/g liver/min respectively. N = 6 for each time point. Key: (*) significantly different from control ($P < 0.05$).

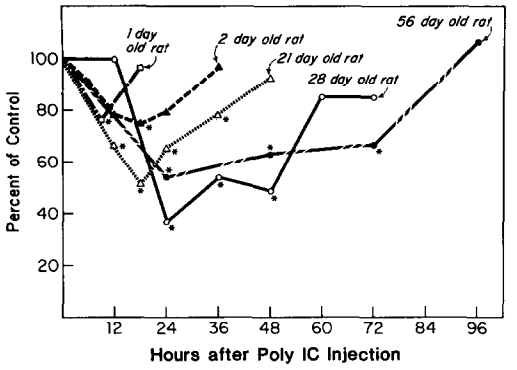

Fig. 5. Time course of the depressant effect of poly IC on the aniline (AN) hydroxylase activity of hepatic microsomes from newborn, weanling and adult male rats. S-microsomes were prepared from rats killed at indicated times after a single injection (i.p.) of 10 mg/kg of poly IC. One hundred percent control (saline-treated) values for 1-, 2-, 21-, 28- and 56-day-old male rats were: 6.9 ± 0.4, 7.5 ± 0.7, 33.7 ± 1.1, 23.7 ± 1.3 and 15.6 ± 0.6 nmoles *p*-OH AN formed/g liver/min respectively. N = 6 for each time point. Key: (*) significantly different from control ($P < 0.05$).

corresponding maximal losses of AN hydroxylase and BP hydroxylase activities were 22, 23, 47, 62 and 45% and 18 (not significant), 25, 40, 53 and 50% respectively. Thus, the poly IC-induced losses of EM *N*-demethylase activity were quite similar in rats of all ages, whereas AN and BP hydroxylase activities were depressed to a lesser extent relative to that of EM demethylase activity in 1- and 2-day-old animals. This suggests that not all species of cytochrome P-450 are affected equally by poly IC and that this unequality is age related.

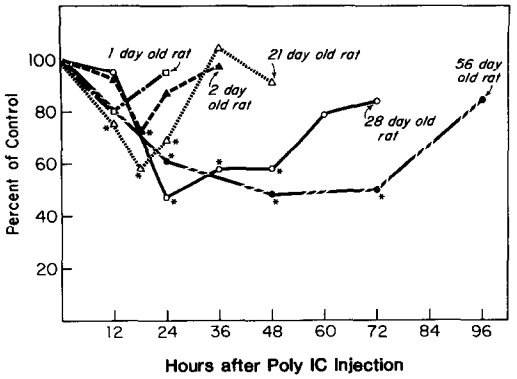

Fig. 6. Time course of the depressant effect of poly IC on the benzo[*a*]pyrene (BP) hydroxylase activity of hepatic microsomes from newborn, weanling and adult male rats. S-microsomes were prepared from rats killed at indicated times after a single injection (i.p.) of 10 mg/kg of poly IC. One hundred percent control (saline-treated) values for 1-, 2-, 21-, 28- and 56-day-old male rats were: 4.8 ± 0.2, 4.8 ± 0.6, 13.7 ± 1.1, 28.0 ± 0.6 and 33.3 ± 2.2 nmoles 3-OH BP formed/g liver/min respectively. N = 6 for each time point. Key: (*) significantly different from control ($P < 0.05$).

DISCUSSION

The administration of tilorone to rats on days 1 and 2 postpartum modified the changes in the activities of hepatic monooxygenase systems that occur normally during the first 4 days postpartum. Thus, AN hydroxylase activity, which develops very rapidly during the first 2 days postpartum, was depressed markedly, EM *N*-demethylase activity, which develops at a rate about half that of AN hydroxylase activity, was depressed moderately, and BP hydroxylase activity, which is the slowest of the three monooxygenase activities to develop during this early neonatal period, was induced rather than depressed (Figs. 1 and 2). One might speculate from this observation that tilorone is most effective on those monooxygenases which are being synthesized most rapidly; however, this speculation does not accommodate the induction of BP hydroxylase activity that occurred. The effect of the interferon inducing agent on the hypothetical maternal repressor substance [9, 11, 12] might explain this paradox. In theory, the cytochrome P-450 systems of the fetus are repressed by a substance of maternal origin. Parturition removes the newborn from the source of this repressor. However, if some residual repressor substance were to remain in the newborn during day 1 or 2 postpartum, development of the cytochrome P-450 systems might still be partially impaired. If the interferon inducing agent were to reverse the action of the repressor substance [9] as well as depress cytochrome P-450 systems, the net effect of the agent on a given cytochrome P-450 system could be either depression or an apparent induction, depending upon the relative effectiveness of each of these opposing factors on that system. In accordance with this concept, the net effect would be depression in the cases of EM *N*-demethylase and AN hydroxylase systems and an apparent induction in the case of the BP hydroxylase system. By day 7 postpartum, the residual repressor would have disappeared from the neonate and the net effect of the interferon inducing agent would be depression in the cases of all three monooxygenase systems (Fig. 2).

In any event, it is apparent that in the 4-day-old neonate, tilorone does not affect all monooxygenase systems equally. Tilorone induced a marked depression of AN hydroxylase without a significant depression of cytochrome P-450. This is interpreted to mean that, in these very young rats, almost all of the AN hydroxylase activity is associated with only a small amount of the total cytochrome P-450.

At 7 days, the cytochrome P-450 content and all three monooxygenase activities were depressed in rats that had received tilorone on days 1 and 2 postpartum. No change in the levels of cytochrome P-450 or EM *N*-demethylase activity occurred in tilorone-treated animals between days 4 and 7; however, AN hydroxylase and BP hydroxylase activities increased appreciably, although not to the levels observed in untreated rats. Thus, the depressant effect of tilorone on different monooxygenase systems was again shown not to be uniform in these early neonates.

Depression of cytochrome P-450 and monooxygenase activities induced by administration of tilorone on days 1 and 2 postpartum persisted for as

long as 3 weeks (Table 1), whereas values returned to normal within 10 days when single injections of tilorone were administered to adult rats [13]. These observations are not strictly comparable because the neonates received two doses of the drug and the adults only one; however, it may be safe to conclude that recovery is much slower in the neonate than in the adult. In fact, the recovery resembles that seen in adult rats given daily doses of tilorone over a 3-week period [14]. The time required for the recovery of the tilorone-induced depression of cytochrome P-450 systems is much longer than that required for the recovery of poly IC-induced depression of these systems (Figs. 3–6), possibly because tilorone has a long (10–12 days) half-life [15]. Interferon reaches a peak level in the serum of the rat about 12 hr after the administration of tilorone; it is not detectable 18 hr later [16]. However, serum levels of interferon do not always correspond with tissue levels of interferon [17]; in fact, 30 hr after the administration of tilorone no interferon was detectable in the serum of rats but the interferon titre of the spleen was still about 35% of the peak titre observed at 18 hr [17]. The possibility is therefore considered that the extended period of depression of cytochrome P-450 systems may be due to a persistent low level induction of interferon in the liver or other tissues through the slow but continuous involvement of new cells capable of producing interferon in response to tilorone. Alternatively, tilorone may depress cytochrome P-450 systems by a mechanism other than, or in addition to, the induction of interferon, e.g. tilorone or a metabolite of tilorone may act as an inhibitor of the cytochrome P-450 reactions employed in this study.

The length of the period from maximal depression to complete recovery of cytochrome P-450 systems after the administration of a single dose of poly IC was shown to be a function of the age of the rat. This period increased from about 6 hr in 1-day-old rats to 48 hr in 56-day-old rats. This observation might have been anticipated from the studies of Conde and Scornik [18], who showed that the increase in protein in growing livers of 4-day-old mice is due to faster synthesis and slower degradation of protein than occur in livers of adult mice. An enhanced rate of protein synthesis in the newborn rat liver could account for the rapid recovery of the monooxygenase systems after a single dose of poly IC.

Losses and recoveries of hepatic hemoproteins that occur after poly IC administration relate to their half-lives. Thus, the period between loss and recovery of tryptophan 2,3-dioxygenase, an enzyme with a relatively short half-life, was significantly less than those of catalase and cytochrome P-450, which have substantially longer half-lives [19]. Levin and Kuntzman [20] demonstrated that two populations of cytochrome P-450 exist in hepatic microsomes, one with a relatively short half-life and a second with a considerably longer half-life; more of the rapidly turning-over cytochrome P-450 is present in immature animals than in adults [21]. El Azhary and Mannering [19] observed that single injections of tilorone or poly IC cause a depression of the short-lived population whereas the more slowly turning over population was unaffected. These findings support our observation that the time course of loss and recovery of monooxygenase systems is shorter in immature than in mature animals.

Schimke *et al.* [22] proposed that the time required for a change in the steady-state level of an enzyme is dependent solely on the rate of degradation. Conde and Scornik [18] have determined that the accumulation of protein in the neonatal mouse liver is due more to a slowed rate of degradation than to an enhanced rate of synthesis. Since the hepatic cytochrome P-450 level is not at steady state in newborn rats, but rapidly increasing, it is difficult to ascertain whether the more rapid loss and recovery from the effects of poly IC were due to an altered rate of protein synthesis or to an altered rate of degradation.

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