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Report

Effect of the Immunomodulator Tilorone on the *in Vivo* Acetylation of Procainamide in the Rat

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Interferon and interferon inducers have been found to inhibit cytochrome P-450-dependent metabolism in animals and man. The effect of these agents on the acetylation of drugs has not been previously reported. Since these agents stimulate the reticuloendothelial system, together with the abundance of *N*-acetyltransferase in the reticuloendothelial system, it was hypothesized that these immunomodulators may affect drug acetylation. To test this hypothesis, the effect of tilorone (a synthetic interferon inducer) on the *in vivo* acetylation of procainamide was examined in the rat. Pretreatment with tilorone hydrochloride (50 mg/kg) 48 hr prior to the administration of procainamide hydrochloride (50 mg/kg) resulted in a 32% increase in the urinary recovery of *N*-acetylprocainamide and a 35% increase in the metabolic clearance of procainamide to *N*-acetylprocainamide. These data indicate that interferon inducers increase the *N*-acetylation of drugs *in vivo*.

KEY WORDS: acetylation; immunomodulators; interferon inducers; pharmacokinetics; procainamide; tilorone.

INTRODUCTION

A wide variety of immunomodulating agents have been found to inhibit cytochrome P-450-dependent drug metabolism in both animals and man (1,2). While the exact mechanism of this interaction has not been elucidated, it appears to be secondary to the induction of interferon. Indeed, one common factor which all immunomodulators inhibiting oxidative drug metabolism have is the ability to induce interferon. Moreover, the administration of exogenous interferon has been shown to reduce oxidative metabolism in both animals and man (3,4).

In contrast to the well-studied effect of these agents on Phase I drug metabolism, there has been very little research on the effects of immunomodulators on Phase II processes. The conjugation of acetylsalicylic acid with glycine or glucuronic acid was not significantly altered in animals pretreated with the immunomodulator poly(rI–rC) (5). These same investigators also found that this agent had no significant effect on the glucuronidation or sulfation of acetaminophen in mice (6).

Since *N*-acetyltransferase is abundant in the reticuloendothelial system (RES), together with the fact that a number of these agents stimulate the RES (7–10), it appears likely that immunomodulators may alter drug acetylation. Investigations examining immunostimulants of bacterial origin have, however, yielded conflicting results. Zidek *et al.* (11) observed that the ratio of acetylsulfamethazine to the total sulfamethazine eliminated in the urine of rats was significantly increased 21 days after the administration of Freund's adjuvant. Their observation may have been secondary to changes in acetylating capacity or the renal excretion of the parent compound. Subsequently, duSouich and Courteau (12) reported that pretreatment with Freund's adjuvant increased the sulfamethazine metabolic rate constant in fast- and slow-acetylating rabbits by 60 and 135%, respectively. This observation suggests that the acetylating capacity in the rabbit is induced by Freund's adjuvant. In contrast, Lapka *et al.* (13) found that endotoxin [which induces interferon and inhibits cytochrome P-450-dependent metabolism (1)] at low doses (2 and 20 μg/kg) reduced the portion of acetylated sulfamethazine excreted in the urine of rabbits. Zymosan, an immunostimulant of yeast origin, has been shown to enhance the acetylation of sulfamethazine in the rat (14).

The effect of interferons or interferon inducing agents per se on acetylation has not been previously reported. To determine the effect of these agents on acetylation, we examined the influence of the immunomodulator tilorone on the acetylation of procainamide *in vivo* in the rat. Tilorone is a synthetic interferon inducer which has been shown to reduce oxidative metabolism in pretreated animals both *in vitro* and *in vivo* (15,16).

MATERIALS AND METHODS

Materials. Procainamide hydrochloride was purchased from Aldrich Company (Milwaukee, Wis.). Analytical standards of procainamide and *N*-acetylprocainamide were gifts

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from E. R. Squibb & Sons, Inc. (Princeton, N.J.). These analytical standards were used for standard curves. *N*-proprionylprocainamide was purchased from Sigma Chemical Company (St. Louis, Mo.). Tilorone hydrochloride was a gift from Merrell Dow Research Institute (Cincinnati, Ohio). Acetonitrile, chloroform, methanol, and sodium acetate were purchased from Fisher Chemical Company (Livonia, Mich.). All chemicals were used as received.

Animals and Treatment. Male Sprague–Dawley rats (Charles River, Wilmington, Mass.) weighing 191 to 229 g had an indwelling cannula implanted in the right jugular vein with the animals under light ether anesthesia 1 day prior to procainamide administration. Forty-eight hours prior to receiving procainamide, animals were administered a single dose of isotonic saline or tilorone hydrochloride (50 mg/kg) dissolved in isotonic saline (final concentration, 20 mg/ml) by gastric intubation between 8 and 9 AM. This pretreatment regimen has been shown to reduce antipyrine clearance in the rat by 39% (12). On the morning of the study, animals were individually housed in plastic metabolism cages and procainamide hydrochloride (50 mg/kg) dissolved in isotonic saline (final concentration 20 mg/ml) was infused through the cannula at a rate of 0.34 ml/min between 9 and 10 AM. An aliquot of the dosing solution was frozen and assayed for procainamide content. Serial blood samples (0.25 ml) were obtained through the cannula prior to and 5, 10, 15, 30, 60, 90, 120, 180, 240, and 300 min after procainamide administration. Plasma was separated by centrifugation (10 min at 540*g*) and stored in polypropylene tubes at −20°C until analyzed. Urine was collected through 52 hr and diluted with distilled water to a final volume of 100 ml. An aliquot of the diluted urine was centrifuged (10 min at 540*g*) to pellet solid material (i.e., rodent hair) and an aliquot of the supernatant was stored at −20°C pending assay. Procainamide and *N*-acetylprocainamide were quantitated by a high-performance liquid chromatographic method described previously (17). Food was withheld during the first 24 hr after procainamide dosing but available for the final 24 hr. Water was available ad libitum from 8 to 52 hr after procainamide dosing.

Data Analysis. The plasma concentration versus time data were fitted to a biexponential equation using an unweighted nonlinear least-squares regression program (RSTRIP, MicroMath, Inc., Salt Lake City, Utah) to obtain an estimate of the initial plasma concentration of procainamide (i.e., concentration at time 0). The data were then analyzed using the LAGRAN program to obtain noncompartmental pharmacokinetic parameters (18).

The percentage urinary recovery of procainamide or *N*-acetylprocainamide was calculated as the total amount (moles) of procainamide or *N*-acetylprocainamide excreted in the urine within 52 hr divided by the dose of procainamide administered (moles). The renal clearance (CLr) of procainamide (PA) and *N*-acetylprocainamide (NAPA) was calculated as

$$CL_r(PA) = \frac{\text{Total amount of PA excreted in urine}}{AUC(PA)}$$

$$CL_r(NAPA) = \frac{\text{Total amount of NAPA excreted in urine}}{AUC(NAPA)}$$

The partial metabolic clearance of procainamide to *N*-acetylprocainamide (CL_m) was calculated as the product of the percentage of the dose recovered in the urine as NAPA and the systemic clearance of procainamide.

The pharmacokinetic parameters and mean plasma concentrations between control and treatment groups were compared using the two-tailed unpaired Student *t* test. A value of $P < 0.05$ was considered statistically significant. Data are presented as mean ± 1 standard deviation.

RESULTS

The mean procainamide plasma concentration versus time profile after intravenous dosing of 50 mg/kg of procainamide hydrochloride in control animals and those pretreated with a single dose of tilorone hydrochloride 50 mg/kg is illustrated in Fig. 1. Mean data after the 120-min sample are not plotted because several animals had procainamide plasma concentrations below the limit of detection by these time points. The pharmacokinetic parameters for procainamide in control and tilorone-pretreated animals are presented in Table I. There was no significant difference in the pharmacokinetic parameters for procainamide between the two groups.

The urinary recoveries of procainamide and *N*-acetylprocainamide in the control and treatment groups are shown in Table I. The percentage urinary recovery of *N*-acetylprocainamide was increased in the tilorone-pretreated animals by 32% (from 15.3 ± 2.4 to 20.2 ± 1.7%; $P < 0.05$). In contrast, the urinary recovery of procainamide was not significantly changed by tilorone pretreatment.

As also shown in Table I, the renal clearances of pro-

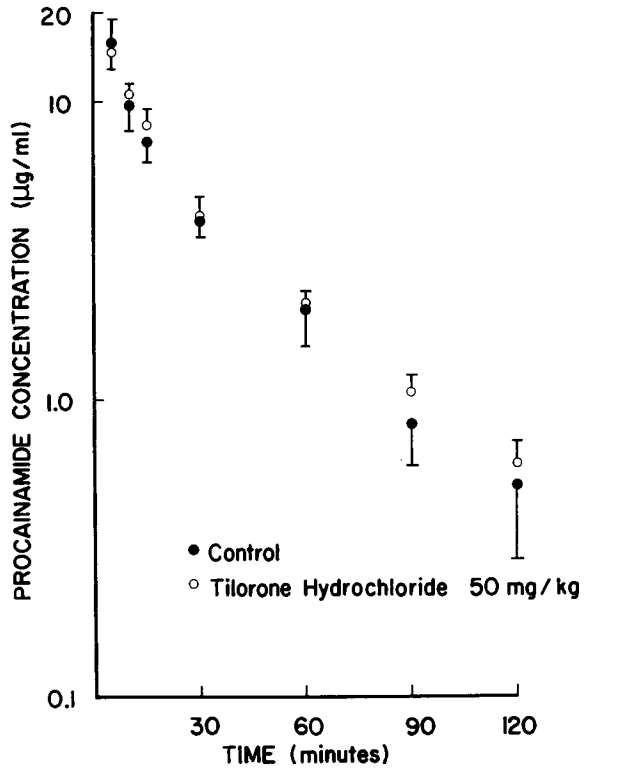

Fig. 1. Mean procainamide plasma concentration versus time profile in tilorone pretreated ($N = 5$) and control ($N = 6$) animals. Bars represent 1 SD.

Table I. Effect of Tilorone Hydrochloride Pretreatment on the Disposition on Procainamide in the Rat[a]

Pharmacokinetic parameter	Control (N = 6)	Tilorone (N = 5)
CL_s (ml/min/kg)	104 (21)	106 (13)
$t_{1/2}$ (min)	43.7 (30.9)	39.2 (4.7)
V_{ss} (liters/kg)	4.17 (2.22)	4.66 (0.99)
Urinary recovery (% of dose)		
Procainamide	36.4 (6.1)	40.2 (6.0)
N-Acetylprocainamide	15.3 (2.4)	20.2 (1.7)*
CL_r(PA) (ml/min/kg)	38.7 (12.7)	42.8 (9.3)
CL_r(NAPA) (ml/min/kg)	29.5 (5.3)	26.5 (4.4)
CL_m (ml/min/kg)	15.8 (3.5)	21.3 (2.4)*

[a] Tilorone hydrochloride (50 mg/kg) was administered as a single dose 48 h prior to procainamide hydrochloride (50 mg/kg) administration. Results are expressed as mean (±SD). CL_s, systemic clearance; $t_{1/2}$, half-life; V_{ss}, steady-state volume of distribution; CL_r(PA), renal clearance of procainamide; CL_r(NAPA), renal clearance of *N*-acetylprocainamide; CL_m, metabolic clearance of procainamide to *N*-acetylprocainamide.

* $P < 0.05$.

cainamide and *N*-acetylprocainamide did not significantly differ between control and treated animals. The partial clearance of procainamide to *N*-acetylprocainamide was increased by 35% (from 15.8 ± 3.5 to 21.3 ± 2.4 ml/min/kg; $P < 0.05$) in the tilorone-pretreated animals compared to the control group.

The mean plasma *N*-acetylprocainamide concentrations in control and tilorone-pretreated animals are shown in Fig. 2. The mean *N*-acetylprocainamide concentration in the tilorone pretreatment group was significantly higher than that in the control group from 60 to 300 min.

DISCUSSION

Compounds containing amino, hydroxyl, or sufhydryl groups may undergo biotransformation via acetylation. This pathway is a primary determinant in the elimination of several important therapeutic compounds (i.e., isoniazid, procainamide, hydralazine, and several sulfa antibacterial agents). The capacity to acetylate amino containing compounds may determine an individual's predisposition to toxicity from several agents (7). *N*-Acetyltransferase activity is particularly abundant in the reticuloendothelial system (7,8). Since stimulation of the reticuloendothelial system by immunomodulators reduces cytochrome P-450-dependent drug metabolism (9,10), it is reasonable to hypothesize that these agents may also alter the acetylation of drugs. Previous studies using immunostimulants of bacterial origin have yielded conflicting results. In an effort to resolve this conflict, we have examined the effect of a synthetic interferon inducer on the acetylation of procainamide *in vivo*.

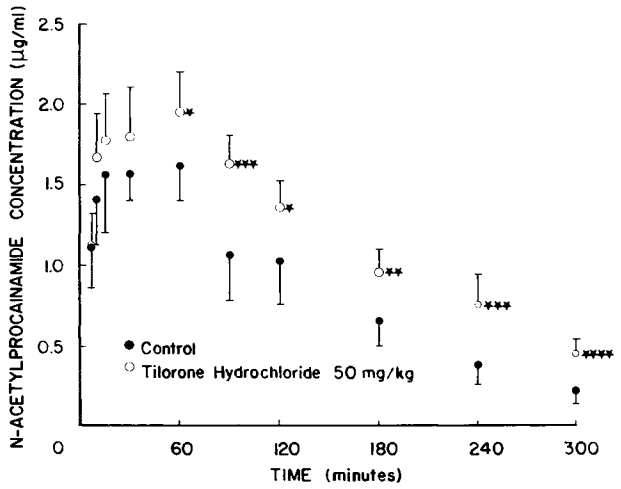

Fig. 2. Mean *N*-acetylprocainamide plasma concentration versus time profile in tilorone pretreated (N = 5) and control (N = 6) animals. Bars represent 1 SD. (*)$P < 0.05$; (**) $P < 0.02$; (***) $P < 0.005$; (****) $P < 0.002$.

The observed increases in the urinary recovery of *N*-acetylprocainamide and the partial clearance of procainamide to *N*-acetylprocainamide indicate that pretreatment with tilorone results in a stimulation of drug acetylation *in vivo*. The lack of change in the total clearance of procainamide in the presence of an increase in the partial metabolic clearance to *N*-acetylprocainamide is most likely the result of two opposing interactions by tilorone. The elimination pathways of procainamide include renal excretion of unchanged drug, acetylation, and oxidative metabolism (20). The observed increase in acetylation and the absence of an effect on the total clearance suggest that tilorone may inhibit the oxidative metabolism of procainamide. An induction of acetylation and an inhibition of oxidative metabolism, if of similar magnitude, may result in a net effect of no change in the total clearance.

Our findings are in agreement with previous reports that stimulation of the RES with Freund's adjuvant results in an increased rate of *N*-acetylation *in vivo* in both the rat (11) and the rabbit (12). Although duSouich and Courteau (12) found that pretreatment with Freund's adjuvant reduced the renal clearance of sulfamethazine, we found that tilorone pretreatment had no effect on the renal clearance of procainamide or *N*-acetylprocainamide.

The mechanism by which these agents stimulate acetylation has not been elucidated. duSouich and Courteau (12) found that colloidal carbon and cyclophosphamide (agents which inhibit RES phagocytic activity) had no effect on

acetylation. Based on these observations, they concluded that the rate of acetylation was independent of RES activity. Chronic administration of hydrocortisone in the rabbit has been shown to enhance the acetylation of sulfamethazine (12,21), apparently secondary to an increase in liver cell mass (21). This mechanism would not explain the effect of tilorone pretreatment since this agent does not alter liver weight (15). Alternative mechanisms for the observed phenomenon include increased availability of cosubstrate (i.e., acetyl-CoA), altered turnover of *N*-acetyltransferase, and enhanced activity of the native enzyme.

In summary, our results indicate that the interferon inducer tilorone increases the rate of drug acetylation *in vivo*. These data indicate that predisposition to drug toxicity from several important agents may be influenced by immunological status. Future studies will explore the mechanism of this phenomenon and its significance in the toxicity of agents which undergo biotransformation via acetylation.

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