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IMMUNOMODULATION AND DRUG ACETYLATION: INFLUENCE OF THE IMMUNOMODULATOR TILORONE ON HEPATIC, RENAL AND BLOOD *N*-ACETYLTRANSFERASE ACTIVITY AND ON HEPATIC CYTOSOLIC ACETYL COENZYME A CONTENT

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Abstract—The biochemical alteration responsible for immunomodulator enhancement of drug acetylation *in vivo* was probed *ex vivo* and *in vitro* in the rat. Rat liver or kidney cytosol, obtained by differential centrifugation, or whole blood served as the source of *N*-acetyltransferase (NAT). Addition of tilorone (0.5–8.0 mM) to incubation mixtures containing procainamide (PA, 0.6 mM) and acetyl coenzyme A (AcCoA, 0.42 mM) resulted in the inhibition of *N*-acetylprocainamide formation, while lower concentrations of tilorone had no effect. Pretreatment of rats with tilorone (50 mg/kg) administered orally 48 hr prior to sacrifice did not alter hepatic apparent K_m and V_{max} for NAT toward PA compared to control animals. Utilization of an AcCoA regenerating system in the incubation mixtures also resulted in no significant differences in the apparent Michaelis–Menten parameters obtained. Acetylation activity in kidney and whole blood also was not altered by immunomodulator pretreatment. Hepatic cytosolic AcCoA content was reduced significantly 48 hr after tilorone pretreatment (5.10 ± 2.1 vs 11.97 ± 2.2 nmol/mg protein) ($P < 0.05$). These data indicate that an increase in NAT content or activity is not the biochemical alteration responsible for immunomodulator enhancement of drug acetylation, and that the required cofactor, cytosolic AcCoA, is decreased by immunomodulator pretreatment.

Numerous studies have demonstrated that immunomodulators inhibit cytochrome P450-dependent biotransformation in both animals and humans [1–3]. The exact mechanism for this observed effect has not been determined, but appears to be secondary to induction of interferon. This conclusion is supported by the observation that administration of exogenous interferon reduces oxidative metabolism in animals and humans [3–5]. The effect of immunomodulation on other metabolic pathways has received little attention.

Recent studies have demonstrated that immunomodulators may *enhance* the rate of drug acetylation *in vivo* in laboratory animals. In particular, Zídek *et al.* [6] found that the ratio of *N*-acetylsulfamethazine (NASMZ‡) to total sulfamethazine (SMZ) eliminated in the urine of rats is increased significantly 21 days after administration of Freund's adjuvant. This result may have been due to an increase in acetylation of SMZ or an alteration in the renal elimination of NASMZ or SMZ. duSouich and Courteau [7] later showed that pretreatment with Freund's adjuvant increases the metabolic rate constant for SMZ acetylation in fast- and slow-acetylating rabbits by 60 and 135%, respectively. Studies in this laboratory have shown that pretreatment with the immunomodulator tilorone (a synthetic interferon inducer) increases procainamide (PA) acetylation *in vivo* in the rat [8]. Tilorone pretreatment 48 hr prior to the administration of PA resulted in a 32% increase in the urinary recovery of *N*-acetylprocainamide (NAPA) and a 35% increase in the metabolic clearance of PA to NAPA. The pretreatment regimen utilized has been found previously to substantially reduce cytochrome P450-dependent activity in this species [9].

The mechanism by which these immunomodulators enhance drug acetylation has not been elucidated. We hypothesize that the biochemical alteration induced by immunomodulator pretreatment may be an increase in the activity and/or content of hepatic cytosolic *N*-acetyltransferase (NAT) and/or an increase in hepatic cytosolic acetyl coenzyme A (AcCoA) content. The experiments reported in the present paper describe our efforts in elucidating the biochemical alteration induced by immunomodulators which may enhance drug acetylation.

MATERIALS AND METHODS

Chemicals. PA was purchased from the Aldrich Company (Milwaukee, WI). Analytical standards of

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‡ Abbreviations: AcCoA, acetyl coenzyme A; CS, citrate synthase; MDH, malate dehydrogenase; NAPA, *N*-acetylprocainamide; NASMZ, *N*-acetylsulfamethazine; NAT, *N*-acetyltransferase; PA, procainamide; and SMZ, sulfamethazine.

PA and NAPA were gifts from E. R. Squibb & Sons, Inc. (Princeton, NJ). These analytical standards were used for standard curves.

N-Propionylprocainamide, SMZ, hydralazine, AcCoA, acetyl carnitine, carnitine *O*-acyltransferase (EC 2.3.1.7), β-NAD, D,L-malic acid, Tris buffer, malate dehydrogenase (MDH, EC 1.1.1.37) and citrate synthase (CS, EC 4.1.3.7) were purchased from the Sigma Chemical Co. (St. Louis, MO). Tilorone hydrochloride was a gift from Merrell Dow Research Institute (Cincinnati, OH). All chemicals were used as received.

NASMZ was synthesized by incubating 1 g of SMZ in 100 mL of acetic anhydride for 24 hr at room temperature while stirring. The NASMZ was recrystallized from 95% ethanol/water (1:1, v/v). Identification of NASMZ was confirmed by melting point determination, nuclear magnetic resonance spectroscopy, and HPLC.

Animals. Male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 185–404 g were used in the studies. Animals were acclimated in a 12-hr light–dark cycle, humidity-controlled environment for at least 7 days prior to experimentation. Animals had access to rat chow and water *ad lib.* until the time they were killed.

Determination of NAT activity, in vitro. Rats were weighed and anesthetized with ether; the liver or both kidneys were removed and blotted dry, and wet weights were determined. Liver or kidney homogenates were prepared according to the method of Schneck *et al.* [10] utilizing ice-cold Sorensen phosphate buffer (0.067 M, pH 7.4). The 100,000 *g* supernatant (liver or kidney cytosol) from each animal served as the source of NAT. Cytosolic protein concentrations were determined utilizing a microprotein determination kit (procedure 690, Sigma) based on the method of Lowry *et al.* [11].

Cytosolic NAT activity was determined utilizing PA as the substrate in the presence of the required cosubstrate AcCoA (0.42 mM) based on previously described methods [10]. Initial incubation concentrations of PA ranged from 0.15 to 0.60 mM. Incubation mixtures were brought to a volume of 900 μL with Sorensen phosphate buffer and maintained at 34° by placement in a temperature-controlled water bath. Reactions were initiated by the addition of 100 μL cytosol to each incubation mixture. Aliquots (100 μL) were withdrawn from the mixtures at 5, 7.5, 10, 15, and 20 min and added to centrifuge tubes containing 200 μL of 1 M NaOH to terminate the enzyme reaction. NAPA concentrations in the aliquots were determined by an HPLC method described previously [12]. NAT activity was expressed as nanomoles NAPA formed per minute per milligram of cytosolic protein. Preliminary studies indicated that PA was not acetylated in the absence of AcCoA, nor was NAPA deacetylated in the incubation mixtures.

The determination of hepatic or renal cytosolic NAT activity was also conducted in the presence of an AcCoA regenerating system in the incubation mixtures as described by Andres *et al.* [13]. Determinations were conducted in new groups of control and tilorone-pretreated animals. The recycling system consisted of acetyl carnitine (5 mM) and carnitine *O*-acyltransferase (1 U/mL), with the initial incubation concentration of AcCoA reduced to 0.1 mM. As the velocity of NAPA formation was determined to be linear for at least 15 min in the presence of the recycling system, a single 100 μL was removed from each incubation mixture at 10 min and subsequently assayed for NAPA. From preliminary studies it was determined that NAT does not utilize acetyl carnitine as a cosubstrate for acetylation of PA nor is PA acetylated by the recycling system in the absence of NAT.

Effect of tilorone, in vitro. Tilorone was added to incubation mixtures containing PA (0.6 mM) and AcCoA (0.42 mM). Initial incubation concentrations of tilorone were 0.1 to 8.0 mM. Reactions were initiated by the addition of hepatic cytosol as described above. The velocity of NAPA formation in the incubation mixtures was determined at each concentration in duplicate.

Effect of tilorone pretreatment on NAT activity, ex vivo. Animals were pretreated via gastric gavage with saline or tilorone hydrochloride (50 mg/kg) in saline (20 mg/mL) 48 hr prior to being killed, at which time the hepatic or renal cytosol was isolated and NAT activity determined *in vitro* as described.

Tilorone was found to elute at 61.8 min in the HPLC assay utilized for PA. Consequently, it was possible to assay hepatic and renal cell cytosol from treated animals for residual tilorone and compare the results to cytosol from a control animal to which tilorone had been added.

Effect of tilorone pretreatment on hepatic cytosolic AcCoA content. Animals were pretreated with saline or tilorone in saline vehicle and exsanguinated at the time of sacrifice; liver homogenates were prepared as described. Homogenates were ultracentrifuged at 227,000 *g* for 20 hr. From each supernatant, 3 mL was ultrafiltered at 2,225 *g* for 50 min using a CENTRIFREE micropartition system (Amicon, Danvers, MA). Ultrafiltrate fractions were analyzed for protein content. The ultrafiltrate from each animal was also analyzed in duplicate for AcCoA content as described below.

AcCoA content was determined utilizing a spectrophotometric enzyme assay described by Decker [14] with minor modifications. Solutions and reagents were utilized at concentrations given by Decker with the exception of MDH and CS suspensions which were utilized at concentrations of 90 kU/L and 7.5 kU/L in ammonium sulfate solution, respectively.

The following volumes of solutions were added to cuvettes at the appropriate times: sample (or standard) + Sorensen phosphate buffer, 1.4 mL; Tris buffer (0.5 M, pH 8.1) 0.16 mL; DL-malate solution, 0.05 mL; β-NAD solution, 0.08 mL, MDH suspension, 0.02 mL; and CS suspension, 0.02 mL.

The enzyme assay was conducted as described by Decker with the following exceptions. The absorbance reading ΔA_1 was taken at 4 min following the addition of MDH suspension and ΔA_2 was taken 40 min following the addition of CS suspension. The reading of ΔA_1 and ΔA_2 were time standardized to lower the limit of detection.

AcCoA in each sample was quantitated based on a set of six AcCoA standards ranging from 0.004 to

0.1 mM. The coefficient of variation was 1.4% at 0.05 mM and 8.2% at 0.01 mM. The AcCoA concentration for each animal was taken as the average of two samples and is expressed as nanomoles AcCoA per milligram of protein.

Effect of tilorone pretreatment on acetylation activity in whole blood. Blood collected from exsanguinated animals was added to heparinized tubes. Whole blood NAT activity was determined utilizing PA or SMZ as the substrate according to the method of Lindsay and Baty [15]. Initial incubation concentrations of PA were 0.08, 0.8, and 8.0 mM and those for SMZ were 0.072, 0.72 and 7.2 mM. Following incubation, PA and NAPA were quantitated acccording to the HPLC assay of Liu *et al.* [12] and SMZ and NASMZ according to the method of Reeves *et al.* [16].

Data analysis. For each incubation of hepatic or renal cell cytosol, NAPA formation velocity (nmol NAPA formed/min/mg cytosolic protein) was taken to be equal to the slope of the linear regression line of NAPA formed (nmol/mg cytosolic protein) versus time data. For studies utilizing liver cytosol in the incubation mixtures data points consisting of NAPA formation velocity versus initial PA concentration were subsequently fitted to the Michaelis–Menten equation using PC-NONLIN (Statistical Consultants Inc., Lexington, KY) with a weight of $1/y^2$ to obtain the apparent K_m and V_{max} for NAT toward PA in hepatic cell cytosol. This analysis was repeated for each animal.

Data are presented as means ± SD, unless otherwise indicated. Statistical comparisons within treatment groups were performed by the paired *t*-test and comparisons between control and tilorone-pretreated groups were performed by the unpaired *t*-test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Effect of tilorone, in vitro. As shown in Fig. 1 tilorone when added *in vitro* at concentrations ranging from 0.5 to 8.0 mM inhibited NAPA formation by greater than 10%, whereas at concentrations of 0.1 and 0.25 mM, it had essentially no effect on NAPA formation. Tilorone added to the incubation mixtures did not alter pH.

Effect of tilorone pretreatment on NAT activity, ex vivo. Table 1 summarizes the effect of tilorone pretreatment on the apparent Michaelis–Menten parameters for hepatic cytosolic NAT activity toward PA determined in the absence and presence of an AcCoA regenerating system. From the results it can be seen that there were no significant differences in apparent K_m and V_{max} compared to control in the absence of the regenerating system. Tilorone pretreatment did not alter body weights, liver weights or cytosolic protein content from controls. Tilorone pretreatment in the group of animals utilizing an AcCoA regenerating system in the incubation mixtures also did not alter the apparent K_m and V_{max} for NAT toward PA from control when more physiologic levels of AcCoA were utilized.

As the *in vitro* studies indicated that tilorone at concentrations greater than 0.25 mM inhibited

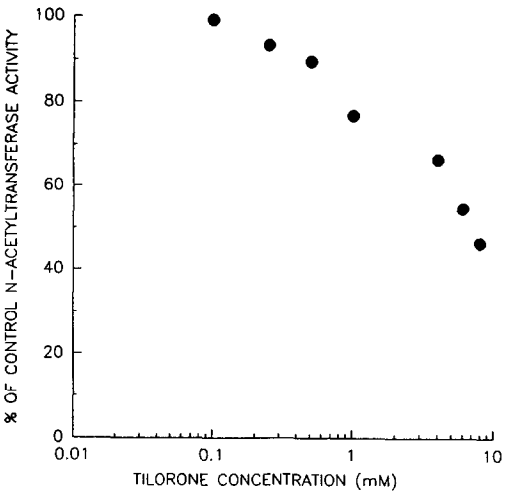

Fig. 1. Effect of addition of tilorone on *in vitro* NAPA formation by rat hepatic cytosol. Tilorone was added *in vitro* to incubation mixtures containing 0.6 mM PA, 0.42 mM AcCoA and hepatic cytosol. Each point is the average of duplicate determinations. Control NAT activity averaged 0.403 nmol/min/mg protein.

NAPA formation, residual tilorone present in hepatic or renal cell cytosol may have interfered with the ability to assess accurately NAPA formation rates *ex vivo*. Fortunately, tilorone was found to elute in the assay for NAPA at 61.8 min. Therefore, liver or kidney cytosol from tilorone-pretreated animals was assayed, and chromatograms were monitored for possible evidence of residual tilorone by elution of a peak at 61.8 min. These chromatograms were compared to liver cytosol which was spiked with tilorone at a concentration of 0.25 mM. The results of the analysis for residual tilorone showed that if tilorone is present in hepatic or renal cell cytosol, it is present at a concentration which is below that found to have no significant effect on NAT activity *in vitro*.

According to Schneck *et al.* [10], the kidneys are also a significant site for NAT activity. Therefore, in two experiments, NAT activity was also determined utilizing renal cell cytosol as the source of NAT in incubation mixtures containing PA (0.6 mM) and AcCoA (0.42 mM), or 0.6 mM PA and 0.1 mM AcCoA in the presence of the AcCoA regenerating system. Figure 2 shows that NAPA formation velocity, expressed as nmol/min/mg protein, determined in the presence or absence of the AcCoA regenerating system, was not altered by tilorone pretreatment. Expressed as nmol/min/g kidney, tilorone-pretreated animals exhibited no significant differences from controls in either the absence (27.1 ± 4.7 vs 27.9 ± 1.2) or presence (12.8 ± 0.5 vs 13.2 ± 1.3) of the AcCoA regenerating system. Cytosolic protein content and kidney wet weights were not affected by tilorone pretreatment.

To determine the effect of tilorone pretreatment on another non-hepatic site of acetylation, NAT activity was determined in whole blood collected

Table 1. Effect of tilorone pretreatment on apparent Michaelis–Menten parameters for NAT activity toward PA in hepatic cell cytosol in the absence or presence of an AcCoA regenerating system*

	No. of animals	K_m (PA) ($\times 10^{-4}$ M)	V_{max} (PA) (nmol NAPA formed/min/mg protein)
Standard incubation†			
Control	4	2.26 ± 0.24	1.21 ± 0.11
Tilorone	4	2.50 ± 0.73	1.30 ± 0.18
Regenerating system incubation‡			
Control	8	1.83 ± 0.70	0.57 ± 0.13
Tilorone	7	1.88 ± 0.78	0.55 ± 0.10

* Tilorone-HCl (50 mg/kg) or saline was administered p.o. and rats were killed 48 hr later. Values are means ± SD.

† Apparent Michaelis–Menten parameters were determined at an initial AcCoA incubation concentration of 0.42 mM.

‡ Apparent Michaelis–Menten parameters were determined at an initial incubation AcCoA concentration of 0.1 mM in the presence of acetylcarnitine (5 mM) and carnitine *O*-acyltransferase (1 U/mL).

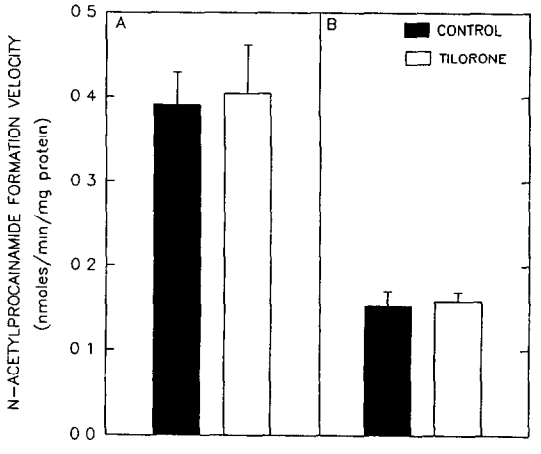

Fig. 2. NAPA formation velocity determined *in vitro* in control and tilorone-pretreated rats. Tilorone-HCl (50 mg/kg) or saline was administered p.o. and rats were killed 48 hr later. Renal cytosol, isolated as described in the text, served as the source of NAT in incubation mixtures containing (A) 0.6 mM PA and 0.42 mM AcCoA, or (B) 0.6 mM PA, 0.1 mM AcCoA and the AcCoA regenerating system. Values are means ± SD; N equals 6 for each group in (A) and 4 for each group in (B).

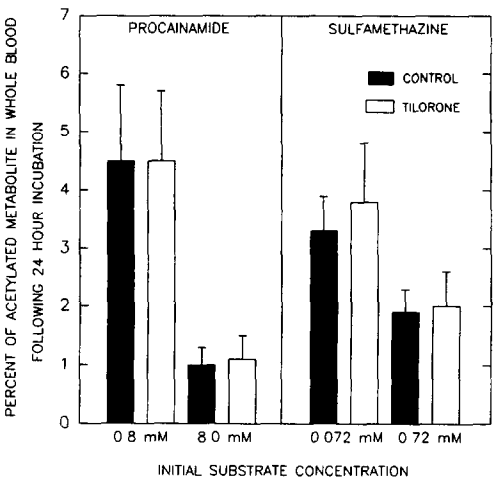

Fig. 3. Effect of tilorone pretreatment on *ex vivo* acetylation of PA and SMZ in whole blood. Tilorone-HCl (50 mg/kg) or saline was administered p.o. and rats were killed 48 hr later. Whole blood was collected immediately prior to sacrifice. Substrates were added to whole blood to give initial concentration of substrates indicated and incubated for 24 hr prior to determination of parent and acetylated metabolite content. Values are calculated as acetylated metabolite divided by total arylamine × 100. Values are means ± SD; N equals 6 for control and 5 for treated.

from treated and control animals utilizing PA and SMZ as substrates. The results, summarized in Fig. 3, indicate that immunomodulator pretreatment had no significant effect on the acetylation activity of whole blood.

Effect of tilorone pretreatment on hepatic cytosolic AcCoA content. Figure 4 illustrates the effect of tilorone pretreatment on cytosolic AcCoA content. In Exp. 1 tilorone pretreatment produced a significant ($P < 0.05$) decrease in cytosolic AcCoA content (5.10 ± 2.1 vs 11.97 ± 2.2 nmol/mg protein, treated vs control). The magnitude of change represents an over 50% decrease in cytosolic AcCoA content in treated animals.

To verify that tilorone pretreatment did indeed decrease hepatic cytosolic AcCoA content the experiment was repeated. The results of the second experiment were essentially identical to those of Exp. 1. In both experiments, tilorone-pretreated animals did not differ from control animals in regard to liver weights and hepatic cytosolic protein content.

DISCUSSION

Acetylation is a significant biotransformation pathway for the elimination of several important

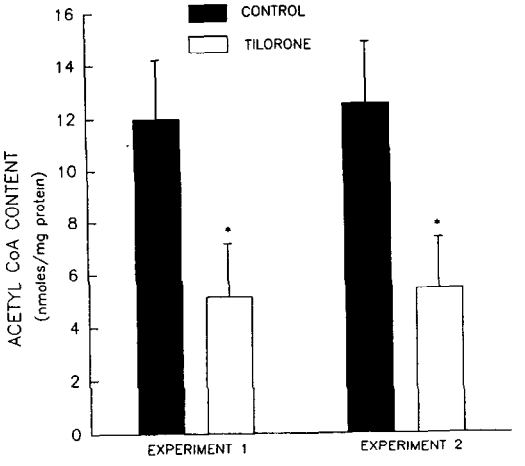

Fig. 4. Effect of tilorone pretreatment on hepatic cytosolic AcCoA content in two separate experiments. Tilorone-HCl (50 mg/kg) or saline was administered p.o. and rats were killed 48 hr later. Ultrafiltrate of hepatic cytosol was isolated and AcCoA content subsequently determined as described in the text. Values are means ± SD; N equals 5 for each group in Expt. 1 and 6 for each group in Exp. 2. Key: * $P < 0.05$ vs control.

therapeutic agents, including procainamide, isoniazid and several sulfa drugs. Differences in acetylation capacity have been shown to result in clinically significant effects [17]. The studies reported herein represent our attempts to elucidate the biochemical alteration induced by immunomodulator pretreatment which results in the observed increase in acetylation activity [6–8]. Addition of tilorone to incubation mixtures containing cytosolic NAT and the required cosubstrates PA and AcCoA decreased the acetylation rate of PA at tilorone concentrations greater than 0.25 mM, indicating that the increase in acetylation activity observed *in vivo* [8] is not due to a direct effect of tilorone on NAT.

Ex vivo determination of the apparent Michaelis–Menten parameters for hepatic cytosolic NAT was conducted to examine if tilorone pretreatment increased *in vivo* acetylation of PA by enhancing NAT activity. In the presence of an initial AcCoA concentration of 0.42 mM, we found that the apparent Michaelis–Menten parameters for NAT toward PA did not differ between control and treated animals. However, based upon our observation that tilorone could inhibit NAT activity *in vitro*, it was essential to examine if the *ex vivo* determinations of the apparent K_m and V_{max} values were affected by the presence of residual tilorone in the cytosol of treated animals. This possibility was ruled out by the failure to detect tilorone in cytosol of treated animals 48 hr after pretreatment. Tilorone, if present at concentrations below our level of detection, should have no effect on PA acetylation. If tilorone induces a change in NAT activity as a secondary effect, this change was not detected *ex vivo*. These results, however, do not rule out the possibility that the presence of residual metabolites of tilorone may inhibit NAT.

It should be noted, however, that the initial studies were conducted with AcCoA concentrations at supraphysiologic levels. The inital AcCoA concentration in these studies was 0.42 mM, while the average cytosolic AcCoA concentration in fasted male Sprague–Dawley rats is approximately 0.1 mM [18]. While the conditions utilized in this initial experiment are the same as those used by previous investigators [10], they can lead to spurious results. In particular, based upon the mechanism of N-acetylation (bi-bi ping-pong) [17, 19], the higher concentrations of AcCoA would not give physiologic estimates of NAT K_m and V_{max}. Moreover, as the reaction proceeds coenzyme A, which may be inhibitory for the reaction [20], will accumulate as a by-product and possibly alter enzyme kinetics.

To avoid these shortcomings, an AcCoA recycling system described by Andres *et al.* [13] was utilized in a subsequent experiment. The use of this recycling system prevents AcCoA depletion and coenzyme A accumulation, thereby allowing physiologic AcCoA concentrations to be utilized. Comparison of the results obtained in the presence and absence of the recycling system supports the conclusion that tilorone pretreatment did not increase acetylation via an alteration in hepatic NAT activity or content (Table 1).

NAT is distributed in various tissues throughout the body. While the results of Schneck *et al.* [10] indicate that the liver is the primary source for acetylation of PA in the rat, it is possible that enhancement of extra-hepatic NAT activity is responsible for the increased acetylation *in vivo*. However, our observation of a lack of effect of tilorone on renal and whole blood acetylation invalidates this alternative hypothesis.

As previously mentioned, the acetylation of drugs catalyzed by NAT proceeds via a bi-bi ping-pong mechanism [17, 19]. The first step in this reaction is the acetylation of NAT by AcCoA. Thus, based on this mechanism AcCoA concentration should be a rate-limiting factor for N-acetylation. This conclusion is supported by the observation that administration of compounds which serve as AcCoA precursors (e.g. ethanol and citrate) increases acetylation *in vivo* and in isolated cells [21–23]. These observations suggest that an alteration in the *available* AcCoA pool could be responsible for immunomodulator enhancement of drug acetylation.

However, in contrast to its effects on NAT activity, we found that tilorone pretreatment significantly *reduced* cytosolic AcCoA content (Fig. 4). While in conflict with our proposed hypothesis, these results are consistent with those of Kilpatrick *et al.* [24] who found that total hepatic AcCoA content decreases by approximately 50% following endotoxin pretreatment in rats. The demonstration that immunomodulator pretreatment reduced cytosolic AcCoA content suggests that (a) AcCoA is not rate limiting as presumed by previous results, or (b) some other mechanism may compensate for the expected reduction in acetylation secondary to the reduced AcCoA content. In regard to the possibility that AcCoA may not be rate-limiting, the observations

of Griffeth *et al.* [25] should be considered. These investigators found that a 22% decrease in *in vitro* isoniazid acetylation after model trauma paralleled a 23% decrease in the plasma clearance of isoniazid despite a 16% increase in hepatic AcCoA content.

One shortcoming of the described experiments is our inability to simultaneously measure acetylation, NAT and AcCoA. Such measurements are precluded with *in vivo* studies since measurement of the latter two parameters requires killing the animal. Studies in isolated hepatic cells, which are currently in progress, should allow simultaneous measurement of these three parameters and a more definitive elucidation of the biochemical alterations which produce the observed enhancement in drug acetylation *in vivo*.

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REFERENCES

1. Mannering GJ, Renton KW, El Azhary R and Deloria LB, Effects of interferon-inducing agents on hepatic cytochrome P-450 drug metabolizing systems. *Ann NY Acad Sci* **350**: 314–331, 1980.
2. Renton KW and Mannering GJ, Depression of hepatic cytochrome P-450-dependent monooxygenase systems with administration of interferon inducing agents. *Biochem Biophys Res Commun* **73**: 343–348, 1976.
3. Mannering GJ and Deloria LB, The pharmacology and toxicology of the interferons: An overview. *Annu Rev Pharmacol Toxicol* **26**: 455–516, 1986.
4. Singh G, Renton KW and Stebbing N, Homogeneous interferon from *E. coli* depresses hepatic cytochrome P-450 and drug biotransformation. *Biochem Biophys Res Commun* **106**: 1256–1261, 1982.
5. Blaschke TF, Horning SJ, Merigan TC, Gurley VF, Brown MI and Atiba JO, Recombinant βser-interferon inhibits antipyrine clearance in man. *Clin Res* **33**: 19A, 1985.
6. Zídek Z, Friebová M, Jankú I and Elis J, Influence of sex and Freund's adjuvant on liver *N*-acetyltransferase activity and elimination of sulphadimidine in urine of rats. *Biochem Pharmacol* **26**: 69–70, 1977.
7. duSouich P and Courteau H, Induction of acetylating capacity with complete Freund's adjuvant and hydrocortisone in the rabbit. *Drug Metab Dispos* **9**: 279–283, 1981.
8. Svensson CK and Knowlton PW, Effect of the immunomodulator tilorone on the *in vivo* acetylation of procainamide in the rat. *Pharmacol Res* **6**: 477–480, 1989.
9. Svensson CK, Effect of the immunomodulator tilorone on antipyrine disposition in the rat. *J Pharm Sci* **75**: 946–948, 1986.
10. Schneck DW, Sprouse JS, Hayes AH Jr and Shiroff RA, The effect of hydralazine and other drugs on the kinetics of procainamide acetylation by rat liver and kidney *N*-acetyltransferase. *J Pharmacol Exp Ther* **204**: 212–218, 1978.
11. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
12. Liu L-L, Knowlton PW and Svensson CK, Effect of amiodarone on the disposition of procainamide in the rat. *J Pharm Sci* **77**: 662–665, 1988.
13. Andres HH, Klem AJ, Szabo SM and Weber WW, New spectrophotometric and radiochemical assays for acetyl-CoA:arylamine *N*-acetyltransferase applicable to a variety of arylamines. *Anal Biochem* **145**: 367–375, 1985.
14. Decker K, UV-method for acetyl coenzyme A. In: *Methods of Enzymatic Analysis* (Eds. Bergmeyer HU, Bergmeyer J and Grabl M), 3rd Edn, Vol. 7, pp. 188–193. VCH Publishers, New York, 1985.
15. Lindsay RM and Baty JD, A novel method for the estimation of blood acetyl-CoA levels. *Biochem Soc Trans* **17**: 921–922, 1989.
16. Reeves PT, Minchin RF and Ilett KF, Induction of sulfamethazine acetylation by hydrocortisone in the rabbit. *Drug Metab Dispos* **16**: 110–115, 1988.
17. Weber WW and Hein DW, N-Acetylation pharmacogenetics. *Pharmacol Rev* **37**: 25–79, 1985.
18. Seiss EA, Brocks DG and Wieland OH, Subcellular distribution of key metabolites in isolated liver cells from fasted rats. *FEBS Lett* **69**: 265–271, 1976.
19. Cleland WW, The kinetics of enzyme-catalyzed reactions with two or more substrates or products. I. Nomenclature and rate equations. *Biochim Biophys Acta* **67**: 104–137, 1963.
20. Weber WW and Cohen SN, N-Acetylation of drugs: Isolation and properties of an N-acetyltransferase from rabbit liver. *Mol Pharmacol* **3**: 266–273, 1967.
21. Olsen H and Morland J, Ethanol-induced increase in drug acetylation in man and isolated rat liver cells. *Br Med J* **2**: 1260–1262, 1978.
22. Olsen H, Interaction between drug acetylation and ethanol, acetate, pyruvate, citrate and L-carnitine in isolated rat liver parenchymal cells. *Acta Pharmacol Toxicol* **50**: 67–74, 1982.
23. Olsen H and Morland J, Ethanol-induced increase in procainamide acetylation in man. *Br J Clin Pharmacol* **13**: 203–208, 1982.
24. Kilpatrick LE, Polin RA, Douglas SD and Corkey BE, Hepatic metabolic alterations in rats treated with low-dose endotoxin and aspirin: An animal model of Reye's syndrome. *Metabolism* **38**: 73–77, 1989.
25. Griffeth LK, Rosen GM and Rauckman EJ, Effect of model traumatic injury on hepatic drug metabolism in the rat. V. Sulfation and acetylation. *Drug Metab Dispos* **13**: 398–405, 1985.