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SELECTIVE INHIBITORY EFFECT OF ORGANOPHOSPHATES ON UDP-GLUCURONYL TRANSFERASE ACTIVITIES IN RAT LIVER MICROSOMES

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Abstract—The effects of acute and subacute administration of diisopropylfluorophosphate (DFP) and acute administration of Soman, Sarin and Tabun on UDP-glucuronyltransferase (GT) activity towards 4-nitrophenol, 4-methylumbelliferone, phenolphthalein and testosterone in rat liver microsomes were investigated. Twenty-four hours after a single injection of DFP, the activity of GT towards 4-nitrophenol and 4-methylumbelliferone was inhibited, and the inhibitory effect continued for 3 days. The activity had recovered by 7 days after injection. The activity of GT towards phenolphthalein and testosterone was not affected at any time after injection. Soman, Sarin and Tabun showed the same effect as DFP after a single injection. After daily DFP injections, the activity of GT towards 4-nitrophenol and 4-methylumbelliferone was decreased to the same level as found following acute treatment with DFP. The *in vitro* addition of DFP to liver microsomes did not affect GT activity towards 4-nitrophenol. It is suggested that these changes are not due to a direct effect of DFP. Furthermore, the effects of two enzyme inducers on GT activity in the presence and absence of DFP were investigated. In the 3-methylcholanthrene (MC) pretreatment group, DFP inhibited only the GT activity towards 4-nitrophenol and 4-methylumbelliferone. On the other hand, in the phenobarbital (PB) pretreatment group, DFP did not inhibit the GT activity towards 4-nitrophenol and 4-methylumbelliferone. It was also demonstrated that MC pretreatment increased the mortality in the DFP-treated rats but that PB pretreatment suppressed it. These results suggest that DFP and other organophosphorus agents may be useful agents for studies on the heterogeneity of GT.

Diisopropylfluorophosphate (DFP†) is one of the most extensively studied organophosphorus cholinesterase inhibitors [1]. Soman (pinacolyl methylphosphonofluoridate), Sarin (isopropyl-methylphosphonofluoridate) and Tabun (ethyl *N*-dimethylphosphoramidocyanidate), which were developed as warfare agents in Germany by Schrader's group, are also anti-cholinesterase agents [1]. Organophosphate insecticides which are anti-cholinesterases are known to alter the hydroxylation of steroids and O- and N-demethylase activities in the liver [2–4]. In contrast to the phase I reactions, e.g. hydroxylation, there have been few reports concerning the effects of organophosphates on phase II reactions, i.e. conjugation. Glucuronidation is an important pathway in the detoxication of endogenous and exogenous compounds. Hepatic microsomal UDP-glucuronyltransferase (GT) catalyzes the transfer of glucuronic acid from UDP-glucuronic acid to various compounds [5]. Various forms of GT activities have been reported in the literature. Some investigators [6, 7] classify these activities as the late-fetal group and the neonatal group on the basis of their developmental period from low to high values. Other groups [8, 9] also divide them into two groups on the basis of their inducibility by 3-methylcholanthrene (MC) and phenobarbital (PB) as GT_1 and GT_2 respectively. However, there is a remarkable similarity between late-fetal and GT_1 substrates (4-nitrophenol, 4-methylumbelliferone, 1-naphthol, etc.) and between neonatal and GT_2 substrates (morphine, testosterone, phenolphthalein, etc.). Recently, three GT isoenzymes have been separated from rat livers [10]. One isoenzyme is capable of conjugating 4-nitrophenol, 1-naphthol and 4-methylumbelliferone. A second isoenzyme is active towards testosterone, the 17-OH-position of β-estradiol and 4-nitrophenol. A third isoenzyme conjugates androsterone and etiocholanolone. Therefore, in the present study, we examined the effects of organophosphate agents on the GT activity in rat liver microsomes using five substrates (4-nitrophenol, 4-methylumbelliferone, phenolphthalein, testosterone, and androsterone).

MATERIALS AND METHODS

Chemicals. Radioactive steroids, [1α,2α-^{3}H(N)]-testosterone (53.0 Ci/mmole) and [1,2-^{3}H]androsterone (44.5 Ci/mmole), were purchased from the New England Nuclear Corp., Boston, MA. UDP-glucuronic acid (disodium salt), testosterone, androsterone, 4-methylumbelliferone, 4-methylumbelliferyl-β-D-glucuronide, 3-methylcholanthrene (MC)

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† Abbreviations: DFP, diisopropylfluorophosphate; GT, UDP-glucuronyltransferase (EC 2.4.1.17); MC, 3-methylcholanthrene; and PB, phenobarbital.

and Triton X-100 were obtained from the Sigma Chemical Co., St. Louis, MO. Phenolphthalein and 4-nitrophenol were purchased from the Aldrich Chemical Co., Milwaukee, WI. Phenobarbital sodium salt (PB) was obtained from the Mallinckrodt Chemical Works, St. Louis, MO. Diisopropylfluorophosphate (DFP) was obtained from the Calbiochem-Behring Corp., La Jolla, CA. Soman, Sarin and Tabun were supplied by the U.S. Army Medical Research and Development Command (Fort Detrick, MD).

Animals. Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 200–260 g, were used throughout the study. The animals were housed four to a cage with free access to food and water.

Administration of DFP, Soman, Sarin and Tabun. Freshly prepared solutions of DFP, Soman, Sarin, and Tabun in saline (0.9% NaCl, w/v) were administered subcutaneously in volumes of 0.1 ml/100 g body wt. Both acute and subacute treatments with DFP were carried out. In the acute treatments, a single DFP dose of 2 mg/kg was injected, and the animals were killed by decapitation 2, 6, 24, 48, 72 or 168 hr after the treatment. In subacute treatments with DFP, a dose of 1 mg/kg was administered daily for 8, 14 or 21 days. Saline-treated (0.1 ml/100 g body wt) controls and DFP-treated animals were decapitated 24 hr after the last treatment.

The doses of Soman, Sarin and Tabun used were 120, 120 and 240 μg/kg respectively. Saline-treated controls, and Soman-, Sarin- and Tabun-treated animals were killed by decapitation 6 or 24 hr after the treatment.

Combined administration of DFP and enzyme inducers. The animals were divided into six treatment groups (saline-treated control, DFP-, MC+DFP-, MC-, PB+DFP- and PB-treated). MC (40 mg/kg) in corn oil was injected intraperitoneally 72 hr before decapitation. PB (75 mg/kg) in saline was administered intraperitoneally at 72, 48 and 24 hr before decapitation. DFP (2 mg/kg) in saline was injected subcutaneously 24 hr before decapitation. Animals in the MC+DFP group were treated with MC 72 hr and with DFP 24 hr before decapitation. Animals in the PB+DFP group were treated with PB at 72, 48 and 24 hr before decapitation and with DFP 24 hr before decapitation. Control rats were administered corn oil 72 hr and saline 24 hr before decapitation.

Preparation of microsomal fractions. The animals were decapitated and a 20% (w/v) liver homogenate was prepared in ice-cold 0.25 M sucrose with a Teflon/glass homogenizer. Microsomal fractions were obtained by differential centrifugations (2,000 *g* for 10 min, 16,000 *g* for 45 min and 105,000 *g* for 60 min). Microsomes were stored at −70° until used. The microsomal protein was determined by the method of Lowry *et al.* [11] with bovine serum albumin as a standard.

Assays of GT activity. The GT activity towards 4-nitrophenol, phenolphthalein, testosterone and androsterone was assayed by a slight modification of the method described previously [12, 13]. An ethanolic solution of each substrate was evaporated under nitrogen and the residue was dissolved in one drop of propylene glycol. The standard incubation medium contained the microsomal fraction (0.5 to 0.8 mg protein), 0.1 M Tris/HCl buffer (pH 7.4) containing 40 μM EDTA, 10 mM $MgCl_2$, 2 mM UDP-glucuronic acid and 360 μM 4-nitrophenol, 120 μM phenolphthalein, 692 μM [^{3}H]testosterone (0.046 μCi) or 172 μM [^{3}H]androsterone (0.064 μCi) in a total volume of 1.0 ml. In the assays of GT activity towards 4-nitrophenol and phenolphthalein, the incubation was performed at 37° for 20 min and was stopped by addition of 7 ml of 0.2 M glycine-NaOH buffer (pH 10.4). In the assays of GT activity towards testosterone (or androsterone), the incubation was carried out at 37° for 60 min (or 40 min) and was terminated by adding 4.0 ml of methanol. Activity of GT for 4-methylumbelliferone was measured using 1.0 mM substrate as previously described [14] with the following modification: a 0.5 ml reaction volume contained the microsomal fraction (0.3 to 0.6 mg protein), 0.1 M Tris/HCl buffer (pH 7.4), 40 μM EDTA, 10 mM $MgCl_2$ and 4 mM UDP-glucuronic acid. The incubation was performed at 37° for 10 min and was stopped by addition of 10 ml of water-saturated dichloromethane. For the assay of the modified GT activity, Triton X-100 was added to the incubation medium in which UDP-glucuronic acid was omitted. For the GT activity towards 4-nitrophenol, phenolphthalein and 4-methylumbelliferone, the enzyme activities were maximally activated with 0.025% (w/v) Triton X-100. For the GT activity towards testosterone and androsterone, they were maximally activated with 0.01% (w/v) Triton X-100. The mixture was then preincubated at 4° for 30 min; the reaction was started by addition of 0.1 ml of 20 mM UDP-glucuronic acid. Blank values were obtained from control incubations in which UDP-glucuronic acid was omitted. The transferase activity towards 4-nitrophenol and phenolphthalein was assayed colorimetrically from the disappearance of the substrate [12, 13]. The activity towards testosterone and androsterone was measured radiometrically from the appearance of testosterone glucuronide or androsterone glucuronide [12, 13]. The activity towards 4-methylumbelliferone was assayed by measuring the fluorescence of 4-methylumbelliferyl-β-D-glucuronide [14]. The specific activity of GT was expressed as nmoles of glucuronide formed per min per mg of protein.

Radioactivity measurements. The radioactivity was counted in a Beckman LS 1800 scintillation spectrometer. The sample (0.5 ml) was dissolved in 10 ml of Aquasol (New England Nuclear Corp.).

Assay of cholinesterase activity. The cholinesterase activity was determined according to the method of Ellman *et al.* [15]. The activity was expressed as nmoles of acetylthiocholine hydrolyzed per min per mg of protein.

In vitro *effect of DFP on GT activity towards 4-nitrophenol and on cholinesterase activity.* To compare the inhibitory effect of DFP (10^{-4} or 10^{-3} M) on the GT activity towards 4-nitrophenol and on the cholinesterase activity, aliquots of 0.4 ml of liver microsomal fractions activated with 0.025% Triton X-100 were incubated in 0.1 ml of saline with different concentrations of DFP for 10 min at 37°. After the incubation, the GT activity towards 4-nitro-

Table 1. Time–course of the specific activity of the GT and the protein concentration in rat liver microsomes after a single injection of DFP (2 mg/kg)

Time (hr)	GT activity (nmoles/min/mg protein)				Protein conc (mg/g liver)
	4-Nitrophenol	4-Methyl-umbelliferone	Phenolphthalein	Testosterone	
0	17.0 ± 1.0	103 ± 6	2.9 ± 0.1	2.7 ± 0.3	5.4 ± 0.1
2	14.4 ± 0.8	108 ± 4	3.5 ± 0.3	2.4 ± 0.1	5.5 ± 0.3
6	14.9 ± 1.3	105 ± 8	3.0 ± 0.2	2.8 ± 0.3	5.5 ± 0.4
24	10.0 ± 1.5*	78 ± 6*	3.1 ± 0.1	2.2 ± 0.1	7.1 ± 0.3†
48	10.9 ± 1.0*	81 ± 3†	2.9 ± 0.3	2.5 ± 0.5	6.2 ± 0.2†
72	9.6 ± 1.3*	66 ± 6†	3.1 ± 0.3	2.7 ± 0.2	7.1 ± 0.4†
168	16.4 ± 0.8	112 ± 6	3.0 ± 0.2	3.0 ± 0.5	7.8 ± 0.4‡

Each value is the mean ± S.E.M. for four or five animals. Experimental details for the enzyme assay are described in Materials and Methods.

*–‡ Significantly different from 0 time level (Student's *t*-test): *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$.

Table 2. Individual difference in the androsterone GT activity

Group	No. of animals	Androsterone GT activity (nmoles/min/mg protein)	Activity ratio
High	13	1.58 ± 0.36	13.2
Intermediate	3	0.70 ± 0.17	5.8
Low	4	0.12 ± 0.05	1.0

Each value is the mean ± S.E.M. Experimental details for the enzyme assay are described in Materials and Methods.

phenol and the cholinesterase activity were measured using the methods described above.

RESULTS

Effect of DFP on GT activity and microsomal protein concentration in liver. Table 1 shows the time–course of effects of acute DFP treatment on GT activity and protein concentration in liver microsomes. There were no changes in GT activity and protein concentration in liver microsomes within 6 hr after a single DFP injection. After 24 hr, the activity of GT towards 4-nitrophenol and 4-methylumbelliferone was reduced significantly to 59 and 76% of the zero time activity, respectively, and the GT activity returned to the zero time level after 7 days. On the other hand, the activity of GT towards phenolphthalein and testosterone was not affected by DFP treatment at any time after injection. In contrast to the GT activity, the microsomal protein concentration was increased significantly 24 hr after the injection and the increase continued after 7 days.

Matsui *et al.* [12] reported that there was no remarkable individual difference of GT activity towards androsterone in Sprague–Dawley rats. Falany and Tephly [10] separated the isoenzyme which conjugated androsterone but which did not conjugate 4-nitrophenol, 4-methylumbelliferone and testosterone. Although we measured the activity of GT towards androsterone, we could not estimate the effect of DFP on the activity of GT towards androsterone, since there was a remarkable individual difference in non-treated rats as shown in Table 2.

After subacute administration of DFP, there was a substantial inhibition of the GT activity towards 4-nitrophenol and 4-methylumbelliferone (Table 3). The activity of GT towards 4-nitrophenol and 4-

Table 3. Effect of the subacute administration of DFP on the GT activity in rat liver microsomes

Treatment	GT activity (nmoles/min/mg protein)			
	4-Nitrophenol	4-Methyl-umbelliferone	Phenolphthalein	Testosterone
Control	19.4 ± 1.5	101 ± 3	3.3 ± 0.3	2.9 ± 0.4
1 mg/kg × 8 days	12.7 ± 0.8*	80 ± 4*	2.9 ± 0.2	2.2 ± 0.1
1 mg/kg × 14 days	13.4 ± 1.1†	78 ± 6*	3.7 ± 0.4	2.5 ± 0.1
1 mg/kg × 21 days	11.6 ± 1.3*	71 ± 6*	3.2 ± 0.3	2.2 ± 0.3

Each value is the mean ± S.E.M. for four or five animals. Experimental details for the enzyme assay are described in Materials and Methods.

*,† Significantly different from control level (Student's *t*-test): *$P < 0.01$ and †$P < 0.05$.

Table 4. Effects of Soman, Sarin and Tabun on the GT activity in rat liver microsomes

Treatment after injection	GT activity (nmoles/min/mg protein)			
	4-Nitrophenol	4-Methyl-umbelliferone	Phenolphthalein	Testosterone
6 hr Control	19.8 ± 1.1	92 ± 8	3.8 ± 0.1	2.1 ± 0.2
Soman	17.7 ± 0.9	80 ± 16	4.0 ± 0.2	1.8 ± 0.1
Sarin	17.6 ± 0.6	75 ± 10	3.4 ± 0.8	1.8 ± 0.2
Tabun	18.2 ± 0.7	92 ± 6	4.0 ± 0.2	1.9 ± 0.2
24 hr Control	17.5 ± 1.2	92 ± 15	3.0 ± 0.1	2.3 ± 0.6
Soman	8.5 ± 0.8*	37 ± 3*	2.5 ± 0.3	1.7 ± 0.2
Sarin	8.6 ± 0.4*	42 ± 6*	2.6 ± 0.3	1.6 ± 0.1
Tabun	9.2 ± 0.5*	44 ± 10*	2.8 ± 0.1	1.7 ± 0.4

Each value is the mean ± S.E.M. for four or five animals. Experimental details for the enzyme assay are described in Materials and Methods.

* Significantly different from control level (Student's *t*-test): $P < 0.01$.

methylumbelliferone was decreased significantly to 60–69 and 71–79% of the control activity, respectively, by daily treatment with DFP for 8–21 days.

Effects of Soman, Sarin and Tabun on GT activity in liver. The activity of GT towards 4-nitrophenol, 4-methylumbelliferone, phenolphthalein and testosterone in liver microsomes from rats treated with Soman, Sarin or Tabun is shown in Table 4. Six hours after injection, glucuronidation of the four substrates was not affected. After 24 hr, the activity of GT towards 4-nitrophenol and 4-methylumbelliferone in liver microsomes from rats treated with Soman, Sarin and Tabun was reduced significantly but the activity of GT towards phenolphthalein and testosterone was not affected. These results were similar to those obtained after the acute DFP treatment.

Effect of pretreatment with enzyme inducers on GT activity and mortality in DFP-treated rats. Table 5 shows the effects of MC and PB pretreatment on liver microsomal GT activity towards 4-nitrophenol, 4-methylumbelliferone, phenolphthalein and testosterone as well as mortality in DFP-treated rats. In microsomes from MC-treated rats, the activity of GT towards 4-nitrophenol and 4-methylumbelliferone was increased significantly (approximately 3.7- and 2.9-fold higher, respectively, than control), while the activity of GT towards phenolphthalein and testosterone was not changed. On the other hand, in microsomes from PB-treated rats, the activity of GT towards 4-nitrophenol and 4-methylumbelliferone was not affected, but activity towards phenolphthalein and testosterone was increased significantly (approximately 1.3- and 1.6-fold higher than control) DFP suppressed the induction of GT activity towards 4-nitrophenol and 4-methylumbelliferone by MC and the induction of GT activity towards phenolphthalein and testosterone by PB. PB suppressed the inhibitory effect of DFP on GT activity towards 4-nitrophenol and 4-methylumbelliferone.

A dose of 2 mg/kg DFP, which caused 33% mortality in native rats, caused 63 and 13% mortality in the MC- and PB-pretreated rats respectively.

Table 5. Effects of 3-methylcholanthrene (MC) and phenobarbital (PB) pretreatment on the liver microsomal GT activity and the mortality in rats administered DFP (2 mg/kg)

Treatment	GT activity (nmoles/min/mg protein)				Mortality*
	4-Nitrophenol	4-Methyl-umbelliferone	Phenolphthalein	Testosterone	
Control	21.3 ± 0.6	108 ± 5	3.2 ± 0.1	2.7 ± 0.1	
DFP	14.9 ± 0.6†	70 ± 7†	3.1 ± 0.2	2.6 ± 0.1	33% (10/30)
MC	79.2 ± 5.1	312 ± 11	2.9 ± 0.2	2.7 ± 0.1	
MC + DFP	52.3 ± 3.3‡	247 ± 16‡	2.9 ± 0.1	3.0 ± 0.2	63% (10/16)
PB	22.5 ± 1.4	109 ± 13	4.0 ± 0.2†	4.3 ± 0.1†	
PB + DFP	19.6 ± 0.8§	119 ± 9§	3.0 ± 0.1‖	3.2 ± 0.1	13% (2/16)

Each value is the mean ± S.E.M. for five or six animals. Experimental details for the enzyme assay are described in Materials and Methods.

* Percentage of animals dead within 24 hr after DFP injection.

† Statistically significant difference compared with control: $P < 0.01$.

‡ Statistically significant difference compared with MC treatment: $P < 0.01$.

§ Statistically significant difference compared with DFP treatment: $P < 0.01$.

‖ Statistically significant difference compared with PB treatment: $P < 0.01$.

Table 6. Effect of DFP *in vitro* on the GT activity towards 4-nitrophenol and on the cholinesterase activity in rat liver microsomes

Treatment	GT activity: 4-Nitrophenol (nmoles/min/mg protein)	Cholinesterase activity (nmoles/min/mg protein)
Control	23.8 ± 2.0	8.3 ± 0.8
DFP, 1×10^{-3} M	21.3 ± 1.0	0.3 ± 0.3*
DFP, 1×10^{-4} M	22.8 ± 1.2	0.4 ± 0.2*

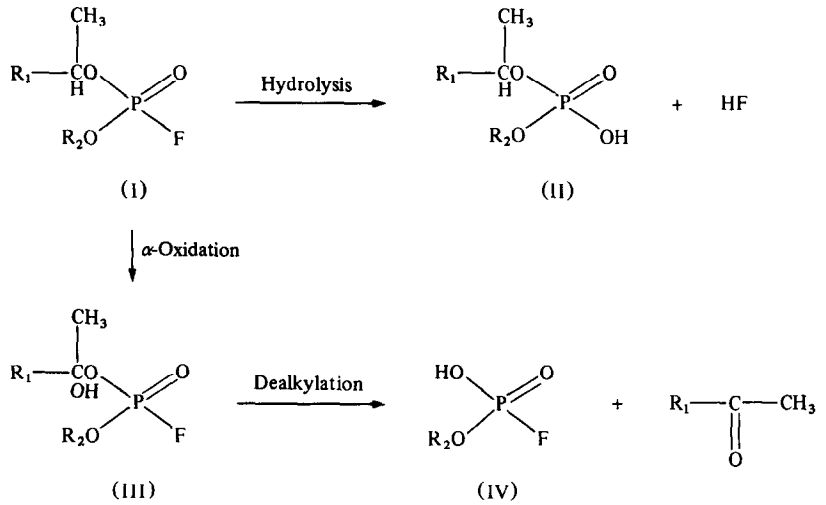
Each value is the mean ± S.E.M. for three or five animals. Experimental details for the enzyme assay are described in Materials and Methods.
* Significantly different from control level (Student's *t*-test): $P < 0.001$.

Effect of in vitro *addition of DFP on GT activity and on cholinesterase activity in liver microsomes.* The *in vitro* presence of DFP at 10^{-4} and 10^{-3} M inhibited markedly the activity of cholinesterase but failed to affect the GT activity towards 4-nitrophenol in liver microsomes (Table 6).

DISCUSSION

The organophosphorus agents, DFP, Soman, Sarin and Tabun are potent inhibitors of cholinesterase, producing a stable inactive phosphorylated enzyme [1, 16]. The cholinesterase in brains and livers of rats is inhibited within 30 min to 2 hr after a single injection of DFP (data not shown). The half-life of organophosphates *in vivo* is relatively short [17]. Although we thought DFP would affect the GT activity in liver microsomes within 2 hr after injection, the inhibitory effect by DFP on the GT activity towards 4-nitrophenol and 4-methylumbelliferone was not shown until 24 hr after the treatment. This change was not due to a direct effect of DFP on the GT activity towards 4-nitrophenol and 4-methylumbelliferone, since no inhibition of the GT activity was observed when a high concentration (10^{-3} M) of DFP was added *in vitro* to the microsomes. The activity of GT towards 4-nitrophenol and 4-methylumbelliferone was also inhibited by subacute treatment with DFP.

The GT activity towards 4-nitrophenol and 4-methylumbelliferone was inhibited 24 hr after treatment with Soman, Sarin or Tabun as much as after DFP treatment. The doses of 50% inhibition of the GT activity towards 4-nitrophenol (ID_{50}) by acute treatment with Soman, Sarin, Tabun and DFP were 0.63, 0.83, 1.57 and 13.6 μmoles/kg respectively. Sivam *et al.* [18] have demonstrated that the LD_{50} values for Soman, Sarin, Tabun and DFP are 0.88, 1.4, 1.9 and 14.5 μmoles/kg respectively. The *in vivo* assessment of the inhibition of GT activity towards 4-nitrophenol revealed that the rank order of potency



	R_1	R_2
DFP	CH_3	i-Pro
Soman	t-Butyl	CH_3
Sarin	CH_3	CH_3

Fig. 1. Structures and metabolism of DFP, Soman and Sarin.

calculated on the basis of ID_{50} values was fairly in agreement with that based on the LD_{50} values.

4-Nitrophenol and 4-methylumbelliferone are substrates for the late-fetal group enzyme (GT_1) which achieves adult activity by the 20th fetal day [6, 7] and is induced by MC [8, 9]. On the other hand, phenolphthalein and testosterone are substrates for the neonatal group enzyme (GT_2) which achieves adult activity during the first 2 postnatal days [6, 7] and is induced by PB [8, 9]. DFP demonstrated an inhibitory effect on the GT_1 activities in MC-pretreated animals as well as in non-pretreated animals. On the other hand, in the PB-pretreated animals, this compound did not alter the GT_1 activities. DFP had no inhibitory effects on GT_2 activities but it significantly reduced the inductive effect of PB on GT_2 activities as if DFP had had an inhibitory effect on GT_2 activities (Table 5). It is of considerable interest that DFP seems to selectively inhibit only one GT isoenzyme.

However, DFP did not inhibit GT_1 activities in *in vitro* study. From this point, DFP and other organophosphates seem to need metabolic activation. DFP, Soman and Sarin have similar structures, as shown in Fig. 1. These compounds can be metabolized by three pathways (hydrolysis, α-oxidation and dealkylation). The hydrolysis is regarded as an inactivation reaction. Parathion (*O*,*O*-diethyl-*O*-4-nitrophenyl phosphorothioate), which is also an anticholinesterase agent, is hydrolyzed to diethylphosphorothioic acid and 4-nitrophenol by A-esterase [16, 19]. Many investigators [19–21] have reported that PB reduces the toxicity of parathion in rats and mice. In our present study, PB also reduced the toxicity of DFP. It has been suggested that the decrease of the toxicity is due to the induction of A-esterase by PB, because the A-esterase activity in rats is increased by PB treatment [22]. Donninger *et al.* [23] reported that chorfenvinphos [2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate] is dealkylated via hydroxylation of the α-carbon atom of the alkoxy group by NADPH-dependent microsomal oxidase and that dimethyl and diisopropyl phosphate triesters also are dealkylated by this enzyme. The hydroxylation of the α-carbon atom is considered as an important pathway to activate the carcinogenic and mutagenic dialkylnitrosamines [24–26]. Hence, we think the α-carbon hydroxylated metabolite (III) (as shown in Fig. 1) which might be unstable is an active form of DFP and other organophosphates which directly inhibits the GT_1 activities.

Recently, Falany and Tephly [10] separated three GT isoenzymes from livers of Sprague–Dawley rats by using chromatofocusing and affinity chromatography. Furthermore, six to seven GT isoenzymes have been separated from livers of Gunn and Wistar rats [27]. Certainly, further study is required to elucidate which enzyme forms are selectively inhibited by organophosphorus agents.

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