Protective Effect of a High Protein Diet against the Toxicity of Some Organophosphorus Compounds in Albino Rats

Ajit K. Chatterjee and Usha Kaveeshwar

Defense Research and Development Establishment, Tansen Road, Gwalior 474002, India

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ABSTRACT—The present investigation deals with determining the efficacy of a high protein diet (HPD) in combating toxicity in albino rats of some organophosphorus compounds (OPCs) that follow dissimilar metabolic patterns in a living system. As assessed by an increase or decrease in the levels of some biochemical and nutritional parameters, the high protein diet containing 59% protein seems to have a beneficial effect in alleviating toxicity of low but prolonged doses of OPCs over the standard diet (SD) containing 19% protein. OPCs undergoing direct detoxication in a living system like diisopropyl phosphoro-fluoridate (DFP) appear to be more susceptible to HPD than those undergoing biotransformation like EPN (O-ethyl O-p-nitrophenyl phenylphosphonothioate) and malathion (S-(1,2-dicarbethoxyethyl) O,O-dimethyldithiophosphate).

Nutritional factors play an important role in the metabolism and pharmacotoxicological activities of various drugs and xenobiotics (1). A dietary reduction in either quality or quantity of protein causes a depression of hepatic microsomal mixed function oxidase activities. According to Boyd (2), these results have a direct application to the toxicity of drugs and chemical agents in persons whose dietary protein is low. Kato et al. (3) reported decreased microsomal drug metabolizing enzymes in rats on a protein-deficient diet. The functions of these enzymes are to metabolize and thereby detoxify any xenobiotic (4). The original reports of increased toxicity of drugs during protein deficiency were submitted by Drill in 1952 (5).

Chemical toxicity can be divided into two categories: parent compound-induced toxicity and metabolite mediated toxicity. The relationship of biotransformation to toxicity in the former category is rather simple as biotransformation means detoxication. In the lat-

ter case, on the other hand, the relationship is more complicated. Biotransformation may lead to biotransformation or bioactivation. The toxicities in vivo of foreign compounds administered to protein-deficient animals apparently depend on whether the principal reaction step represents intoxication or detoxication. For a chemical that gets bioactivated, the test animal is exposed to the stress of a more toxic metabolite for a longer period when on a high protein diet (HPD).

An attempt has been made in the present investigation to study the efficacy of HPD in alleviating toxic effects of some organophosphorus compounds (OPCs) that are either similar, *vis a vis* chemical structure and toxicity, to some chemical warfare agents (6) like diisopropyl phosphorofluoridate (DFP) or are insecticides of low toxicity like malathion (S-(1,2-dicarbethoxyethyl) O,O-dimethyldithiophosphate) or EPN (O-ethyl O-p-nitrophenyl phenylphosphonothioate).

DFP is a structural analogue of the nerve

gas Sarin (methyl isopropyl phosphonofluoridate), and hence expected to behave as sarin because of the close interrelationship between chemical constitution and physiological activity in certain compounds (7).

Two other pesticides of low toxicity, malathion and EPN, were included in the present study as they follow a different metabolic pattern than DFP in a living system: they become biotoxicated first, instead of undergoing direct detoxication (6).

The biochemical studies carried out on male albino rats included determination of serum cholinesterase (ChE) and aminotransferase activities (SGOT and SGPT), liver glycogen, serum albumin/globulin (A/G) ratio and liver/body wt. index. It is well-established in the literature that under conditions of toxicity, serum ChE activity, liver glycogen levels and A/G ratios are lowered and serum aminotransferase activity and liver/body wt. indices are increased with respect to their corresponding control values.

The nutritional aspect, dealing with studies on growth rates in albino rats under combined effects of low but prolonged doses of OPCs (DFP, EPN and malathion), taken separately, and respective standard (SD) and modified diets like low-protein diet (LPD) and HPD were communicated earlier (8, 9).

From our earlier studies (8, 9), elevations in growth rates under HPD alone and also in all the three OPC-treated groups under HPD could be found as compared to the SD control. In the case of DFP combined with HPD (containing 59% protein), the elevation in growth rate was 76.4%; for EPN, 6.1%; and for malathion, 51.8%. Whereas in the case of DFP combined with SD (containing 19% protein), the elevation in growth rate was 22.7%, for EPN, the depression in growth rate was 35.5% and for malathion, the depression in growth rate was 50.9%. For the HPD control, the percent elevation in growth rate was 134.3 as compared to SD. Thus the beneficial effect of HPD was obvious, and the capacity of alleviation of toxicity increased with the increase in protein content. The protective action was

most pronounced in the case of DFP and least in the case of EPN. The difference in the mode of biotransformations of the two compounds was probably the cause.

The diets on which the animals were kept were assessed as suitable, if on administration of a daily low dose (approx. 5% LD₅₀) of the toxic OPCs for 20 days, the toxic effects were not observed.

In the present study, inhibition of serum ChE activity, rise in serum aminotransferase(s) activity, depletion of liver glycogen, lowering of serum A/G ratio and rise in liver/body wt. indices compared to the controls were taken as standards for the manifestation of toxicity.

MATERIALS AND METHODS

Male albino rats of the Wistar strain (body wt. 130 ± 15 g) were divided into eight groups of eight animals each. Groups I and V served as controls for their respective dietary categories, A and B being given SD containing 19% protein and isocaloric HPD containing 59% protein, respectively. The composition of the two isocaloric diets are as described by Purshottam & Kaveeshwar (10) in Table 1.

These two groups received the same quantity of diluents as the experimental animals in various groups that were used for the dilution of OPCs.

Groups II, III and IV were kept on SD and daily s.c. doses of DFP (0.025 mg/rat/day), EPN (0.3 mg/rat/day) and malathion (10.3 mg/rat/day), respectively, for 20 days, which was equivalent to 5% of their respective LD₅₀ values.

Groups VI, VII and VIII received in addition to the HPD described above, daily doses of DFP, EPN or malathion for the same period and in the same doses.

The dilutions of OPCs were made in distilled water with propylene glycol (1 ml) being initially added in each. The quantity of food given was 17 g/rat/day, which had no left-over as studied by separate experiments.

On the 21st day, the overnight fasted rats

Table 1. Composition of different diets (dietary constituents in g/100 g diet)

Constituents	Diet	
	standard	high protein
Sucrose	60	20
Cascien	19	59
Dalda*	10	10
Cod liver oil	1	1
Yeast powder	2	2
Tetra cycline	0.01	0.01
Salt mixture**	2	2
Cellulose	6	6
Kcal/100 g	415	415
g/day/rat	17	17
Kcal intake/rat/day***	70.5	70.5

*Dalda is the commercial name of common edible hydrogenated vegetable oils mainly composed of ground nut and repressed oils. **USP 17 salt mixture. ***The caloric intake was calculated assuming that the animals consumed all the food given to them.

(for reasons of attaining a steady state of body metabolism) were separately weighed and anesthetized with ether; and samples of blood (approx. 3 ml) were collected from the orbital sinus (11) into tubes and then centrifuged to obtain the serum. The whole liver from each animal was taken out, soaked in folds of filter paper and weighed for the determination of liver/body wt. indices. A weighed portion (approx. 1 g) of each liver sample was dipped into tubes containing 30% KOH (6 ml) for the estimation of glycogen. The serum samples were used for the analyses of ChE, SGOT, SGPT and A/G ratios.

Assay of ChE based on the disappearance of acetylcholine (ACh) determined as the ferric aldoxime complex (12) was performed as reported by Augustinson (13). The aminotransferase(s) activities in serum was measured by the Reitman and Frankel method (14). Glycogen was isolated by Hawk's procedure (15) and estimated as glucose (16). A/G ratios were determined after separation of albumin from the total globulins in the serum by low voltage paper electrophoresis at 100 volts for 16 hr using barbitone buffer (pH 8.6).

RESULTS

Table 2 shows that under SD alone, there is statistically no change in the serum ChE activity from that when HPD was taken alone (c.f. group I versus group V). However, a slightly higher ChE activity is observed in the HPD-treated group.

In Table 2, category A (SD), under the combined effects of SD and DFP/EPN, taken separately, highly significant ($P < 0.001$) inhibitions in serum ChE activities could be seen (c.f. groups II and III versus group I). In contrast, in category B (HPD), under HPD either alone or in combination with DFP/EPN/malathion, the ChE activities remained unaffected (c.f. groups VI, VII and VIII versus group V).

If the levels of ChE in categories B and A in separate DFP, EPN and malathion treated groups are compared, their levels will be found to be significantly more ($P < 0.001$, $P < 0.001$ and $P < 0.05$) in category B (HPD treated group) than their corresponding values in category A (under SD).

Changes in activities of serum aminotransferase(s) are also recorded in Table 2. Compared to the SD group I in category A, SGOT and SGPT levels under HPD alone in group V in category B are significantly lower ($P < 0.001$ and $P < 0.05$).

Under the combined action of SD and DFP/EPN, there were significant decreases ($P < 0.001$ and $P < 0.05$) of SGPT levels (c.f. groups II and III versus group I). However, for malathion under similar conditions, there was a significant increase ($P < 0.001$) in SGPT level (c.f. group IV versus group I).

Under the combined effects of HPD and various OPCs, there were no observable changes in either SGOT or SGPT levels (c.f. groups VI, VII and VIII versus group V).

Liver glycogen values are also shown in Table 2. The control value of glycogen under HPD was significantly higher than that under SD (c.f. group V versus group I). Also, the values of glycogen under the combined actions of HPD and various OPCs, separately taken,

Table 2. Changes in serum AChE, aminotransferases and liver glycogen in male albino rats under combined effects of daily low doses of OPCs and isocaloric diets for 20 days

Category	Group	Treatment	Serum AChE expressed as l mole ACh hydrolysed /ml/min	SGOT l mole/min	SGPT l mole/min	Liver glycogen expressed as g% glucose/100 g of liver tissue
A (SD)	I	SD containing 19% protein	1.60 ± 0.05	70.90 ± 2.0	81.84 ± 5.26	0.64 ± 0.05
	II	SD + DFP (5% LD ₅₀) daily for 20 days	0.93 ± 0.05**	63.61 ± 3.02	45.12 ± 2.68**	0.69 ± 0.05
	III	SD + EPN	1.0 ± 0.05**	64.50 ± 3.50	59.75 ± 4.50*	0.68 ± 0.04
	IV	SD + Malathion	1.50 ± 0.10	60.40 ± 5.0	115.80 ± 2.96**	0.67 ± 0.41
B (HPD)	V	HPD containing 59% protein	1.83 ± 0.12	59.10 ± 2.0	58.80 ± 4.85*	2.64 ± 0.04**
	VI	HPD + DFP (5% LD ₅₀) daily for 20 days	1.76 ± 0.06	66.50 ± 6.04	53.70 ± 4.40	2.08 ± 0.40
	VII	HPD + EPN	1.81 ± 0.11	43.35 ± 6.05	51.15 ± 6.80	2.63 ± 0.40
	VIII	HPD + Malathion	1.81 ± 0.08	60.20 ± 1.14	61.40 ± 7.80	2.14 ± 0.40

Values are mean ± S.E., 8 animals in each group. *P < 0.05, **P < 0.001. AChE: acetylcholinesterase, OPCs: organophosphorus compounds, SD: standard diet, DFP: diisopropyl phosphorofluoridate, HPD: high-protein diet.

Table 3. Changes in A/G ratio and liver/body wt. ratio in male albino rats under combined effects of daily low doses of OPCs and isocaloric diets for 20 days

Category	Group	Treatment	A/G ratio	Liver/Body wt. ratio × 100
A (SD)	I	SD containing 19% protein	0.28 ± 0.07	4.12 ± 0.40
	II	SD + DFP (5% LD ₅₀) daily for 20 days	0.32 ± 0.05	3.91 ± 0.19
	III	SD + EPN	0.33 ± 0.08	3.81 ± 0.13
	IV	SD + Malathion	0.36 ± 0.07	3.18 ± 0.13
B (HPD)	V	HPD containing 59% protein	0.32 ± 0.06	3.82 ± 0.20
	VI	HPD + DFP (5% LD ₅₀) daily for 20 days	0.39 ± 0.08	3.90 ± 0.08
	VII	HPD + EPN	0.39 ± 0.07	3.82 ± 0.14
	VIII	HPD + Malathion	0.37 ± 0.08	3.90 ± 0.08

Values are mean ± S.E., 8 animals in each group. A/G: albumin/globulin. Other abbreviations are as in Table 2.

were significantly higher than their corresponding values under SD.

Table 3 indicated no changes in A/G ratios and liver/body wt. indices in various groups both under combined effects of SD or HPD and individual OPCs.

DISCUSSION

The primary action of OPCs are to inhibit AChE activity leading to accumulation of ACh in cholinergic nerve endings, which in turn is responsible for toxic effects (17) due to continuous stimulation of cholinergic fibers through out the central and peripheral nervous system.

Highly significant lowerings of serum ChE levels could be seen in the case of DFP/EPN, taken separately, along with SD indicating non-alleviation of toxicity under SD, of course with the exception of the SD/malathion combination. However, on changing over to HPD, ChE activities were not lowered by any of the three OPCs, probably due to rapid detoxication of OPCs by microsomal drug metabolizing enzymes formed in the liver under HPD. Another possibility could be that in the 20-day feeding experiments with a high protein diet, there may be an enhanced *de novo* synthesis of ChE, which is an enzyme protein, and which can compensate for the irreversibly phosphorylated enzyme. This shows the efficacy of HPD over SD.

Increase in the levels of serum aminotransferase(s) is an indicator of toxic stress (18). It can be hepatotoxicity, cardiotoxicity and nephrotoxicity. Assessed with respect to the increase in the levels of these enzymes, HPD seems to be a better diet than SD as there is no increase in these enzyme levels under the combined effects HPD/OPCs. Again, the cause may be due to rapid detoxication of toxic OPCs under HPD. It may be noticed under the SD/malathion combination, there is a significant increase in SGPT activity indicating non-alleviation of toxicity by SD. Also, under HPD, the control values are significantly lower compared to the SD control, indicating less

ser toxicity of HPD.

Additional synthesis of liver glycogen could be seen under HPD alone. Also, under HPD/OPCs combinations, the glycogen values remained at comparatively higher levels than their corresponding levels under SD. This shows the beneficial effect of HPD over SD as extra glycogen means extra stored energy in a living system.

Table 3 shows no changes in A/G ratios and liver/body wt. indices in various groups both under SD and HPD. A/G ratios are lowered under hepatotoxicity (19) and liver/body wt. indices increase under formation of fatty liver. Considering all the parameters together, HPD seems to be superior to SD in alleviating the toxicity of OPCs.

The mode of action of protein in the diet seems to be two fold. First, it helps in the extra synthesis of ChE in a living system which in turn counteracts the toxicity caused by inhibition of native ChE. The enzyme ChE is a protein and dietary deficiency of protein can result in lower ChE activity in liver microsomes and serum of rats (20). Casterline et al. (21) observed that Che inhibition of some OPCs like parathion and banol is more at lower dietary levels of casein than at higher levels. This proves that the toxicity of these enzyme inhibitors is greater at lower dietary protein levels. Caldara (22) observed that a protein diet protected rats from acute poisoning by 98% parathion (500 mg/kg, s.c.). The morphological changes in liver tissue and decreases in glycogen and nucleic acid content were less severe in rats given a high protein diet than in those given a diet with a normal protein content.

On the other side, protein in the diet increases the over-all concentration of cytochrome-P-450 linked monooxygenases (drug metabolizing enzymes or mixed function oxidases) that are predominantly present in liver microsomes (4). The direct role of dietary protein in increasing the concentration of cytochrome-P-450 linked monooxygenases is not known. However, Kato (3) reported decreased microsomal drug metabolizing enzymes in rats on a protein-deficient diet,

thereby proving the other role of protein.

The microsomal drug metabolising enzyme plays a very important part in chemical toxicity and can detoxify (and also activate in some cases) any xenobiotic (4). The enzymes in the liver have been shown to be susceptible to the influences of environmental factors (23, 24) out of which dietary factors are of prime importance.

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