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SHORT COMMUNICATION

Comparative Evaluation of High Protein against Normal Protein Diet in Combination with Carbamates against Organophosphorus Intoxication in Rats

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ABSTRACT

The relative efficacy of an isocaloric high protein diet (HPD) containing 59 per cent protein, in comparison to a conventional diet containing 21 per cent protein, as applied in the alleviation of toxicity of diisopropyl phosphorofluoridate (DFP) and methyl isopropyl phosphonofluoridate (sarin), has been reported. In combination with well-known prophylactics like carbamates and cholinolytics like atropine against nerve gas toxicity, HPD appears to be superior to the conventional diet as studied by survival time measurements. Apart from carbamates, atropine and mecamylamine, HPD may be treated as an additional prophylactic agent to guard against the toxicity of DFP and sarin, which are being used as war chemicals.

1. INTRODUCTION

To alleviate the toxicity of nerve gases including organophosphates by prophylactic means, two approaches are cited in literature. Some authors[1,2] have reported the use of carbamates, viz. physostigmine and pyridostigmine as pretreatment drugs to rapidly improve the incapacitating effects of nerve gases and other organophosphate(s) intoxication in various animal species. Sometimes, cholinolytics and antimuscarinic/ antinicotinic agents like atropine and mecamylamine are also used alongwith carbamates.

The carbamates reversibly bind to the active sites of the enzyme acetylcholinesterase (*AChE, E.C.*3.1.1.7) in a living system forming carbamylated derivatives thereby protecting the enzyme from subsequent irreversible binding to the organophosphorus compounds including highly toxic nerve gases. This results in nerve gases being quickly metabolised in the body or bound to non-specific binding sites.

The second approach has been the use of high protein diet (HPD)[3-5]. The dietary protein seems to have a two-pronged action[5]. On one side, it helps in the extra synthesis of *ChE*, which in turn, counteracts the toxicity caused by the inhibition of native *ChE*. On the other hand, protein in the diet increases the concentration of cyt-*P*-450-linked mono-oxygenases (drug metabolising enzymes or mixed function oxidases), which are predominantly present in liver microsomes[6]. The direct role of dietary protein in increasing the concentration of cyt-*P*-450 linked mono-oxygenases is not known. However, in protein deficient diet, decreased microsomal drug metabolising enzymes in rats have been reported[7], proving the second role of protein.

In the present study the effects of an isocaloric HPD, with 59 per cent protein content (either used alone or in combination with individual carbamates and atropine), in the alleviation of toxicity of various doses of DFP and sarin have been reported. The objective was to compare the results with those obtained earlier[2] in similar studies on rats under identical experimental conditions and kept on a conventional diet containing

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21 per cent protein. The parameter chosen for this study was survival time measurement.

It was expected that such a comparison would immediately reveal the usefulness or otherwise of HPD over that of a conventional diet having normal quantity of protein, in the alleviation of toxicity of DFP and sarin. It was intended to introduce HPD, if found beneficial, as an additional prophylactic agent, alongwith carbamates, atropine and/or mecamylamine in the alleviation of toxicity of nerve gases.

2. MATERIALS AND METHODS

Male albino rats of Wister strain (body wt. 125±10 g) were divided into five groups, consisting of six subgroups each. Each subgroup had five animals. All the animals were fed for 18 days with HPD, at the rate of a calculated average of 17 g per rat per day. The quantity of food given was found to be optimum, with no left-over as studied by separate experiments. Before the actual feeding with HPD, it was confirmed by growth rate studies on a separate group of animals, that the animals remained in a positive nitrogen balance when fed on HPD, showing steady increase in body weights. Both the conventional and HPD diets were non-toxic and growth promoting[3]. The compositions of the conventional diet and isocaloric HPD are given in Table 1.

Table 1. Composition of different diets

(dietary constituents in g/100 g diet)

Constituents	Conventional Diet (CD)	Isocaloric HPD
Protein	21.0	59.0 (as casein)
Fat	5.0	5.0 (as dalda)
Carbohydrate	53.0	15.0 (as sucrose)
Crude fibre (cellulose powder)	4.0	4.0
Ash	8.0	8.0
Calcium + phosphorus	1.6	1.6
Metabolising energy in k cal/100 g	360	360
Dose, g/rat/day	17	17
intake/rat/day, k cal (b)	61.2	61.2

(a) : USP 17 salt mixture; (b) : caloric intake was calculated assuming that the animals consumed all the food given to them.

Group I in Table 2 was taken as control group and treated with HPD. Animals of subgroups 1-3 of Group I were administered 2,4 and 8 LD_{50} doses of DFP, respectively and subgroups 4-6 with sarin. The survival

Table 2. Effect of treatment with physostigmine, pyridostigmine and atropine on survival time of 18 day HPD-fed rats, intoxicated with DFP and sarin

Groups	Treatement	Survival time (min)					
					sarin (mg/kg)		
		6.6 (a)	13.2 (b)	26.4 (c)	O.406 (a)	0.812 (b)	1.624 (c)
I.	HPD	>24 hr	>24 hr	>24 hr	17.0±0.50	14.0±0.20	4.3±0.10
II	CD (d)	(47.6±3.70)	(25.0±2.60)	(14.2±0.38)	(6.6±0.50)	(5.4±0.25)	(3.2±0.11)
III	HPD+physostigmine	>24 hr	>24 hr	>24 hr	>24 hr	(23.0±0.40	(6.0±0.12)
IV	CD+physostigmine	(>24 hr)	(>24 hr)	(25.8±0.68)	(7.8±1.20)	(5.4±0.25)	(4.6±0.13)
V	HPD+pyridostigmine	>24 hr	>24 hr	>24 hr	26.0±0.50	25.0±0.50	21.0±0.40
	CD+pyridostigmine	(16.4±0.50)	(9.0±0.45)	(8.0±0.45)	(5.4±0.25)	(4.6±0.25)	(2.8±0.22)
VII	HPD+physostigmine +atropine	>24 hr	>24 hr	>24 hr	>24 hr	26.0±0.20	22.0±0.20
VIII	CD+physostigmine +atropine	(>24 hr)	(>24 hr)	(>24 hr)	(4.3±0.19)	(4.4±0.25)	(4.4±0.12)
IX	HPD+physostigmine +atropine	>24 hr	>24 hr	>24 hr	>12 hr	21.0±0.25	18.0±0.40
	CD+physostigmine +atropine	(>24 hr)	(>24 hr)	(>24 hr)	(4.2±0.12)	(4.5±0.22)	(4.2±0.12)

Values are mean ± SE; (a): 2 LD_{50}; (b): 4 LD_{50}; (c): 8 LD_{50}; (d) CD: Conventional diet (Values taken from Ref. 2)

times were measured/observed upto 24 hr in each case. The time of administration of DFP/sarin was considered as zero time for the measurement of survival time. Fresh aqueous solutions of DFP (LD_{50} 3.3 mg/kg, sc) and sarin (LD_{50} 203.4 μg/kg, sc) were prepared each time before use. Sarin and DFP were synthesised in the Synthetic Chemistry Division of the Laboratory by two of the authors (NS and AKS) and were found to be about 98 per cent pure, as identified by gas liquid chromatographic procedure and infrared spectrophotometric analysis.

A maximum sign-free dose[1,8] of an aqueous solution of physostigmine and pyridostigmine (0.1 mg/kg, im) were administered to each animal of groups III and V 30 minutes prior to the challenge doses of DFP or sarin, in their respective subgroups and survival times recorded.

To each of the animals of groups VII and IX in Table 2, an additional dose of atropine (0.5 mg/kg, ip), prepared in distilled water, was administered within 30 seconds of organophosphate(s) treatment, with other details remaining exactly the same as described for groups III and V.

The physostigmine sulphate was obtained from E Merck A G, Darmstadt; pyridostigmine bromide from Sigma Chemical Company, St Louis, USA; and atropine sulphate, from Vikash Pharma, Bombay. Students *t* test was applied for statistical analysis.

3. RESULTS AND DISCUSSION

On the basis of studies of the protection of animals against organophosphate intoxication by carbamate pretreatment, it has been observed[8] that the dose of carbamate is not critical. The protection is essentially constant for doses ranging from half to four times the maximum sign-free dose.

In Group I animals, the survival times of 18-day HPD-treated rats, arranged in various subgroups, are compared with their corresponding control values under isocaloric conventional dietary conditions containing 21 per cent protein (as reported earlier[2] and shown in parenthesis in Group II). It can be found that the survival times are significantly more ($P < 0.001$) for HPD- treated animals, both against DFP and sarin challenge, in all the three (2,4 and 8 LD_{50}) doses tested. This proves beyond doubt the beneficial effect of HPD over the conventional one as a protecting agent against organophosphate intoxication.

In Group III, under the combined action of HPD and physostigmine, the survival times are significantly more ($P < 0.001$) under the action of 8 LD_{50} dose of DFP and all the three doses of sarin, as compared to that under conventional diet in Group IV (shown in parentheses) under various subgroups[2].

In Group V, under the combined action of HPD and pyridostigmine, the survival times in all three doses of DFP and sarin are significantly more ($P < 0.001$) when compared with the corresponding values under conventional diet and shown in parentheses in Group VI[2]. This shows that HPD has a better performance in combination with both the carbamates tested, as compared to carbamates working under normal dietary conditions.

In Groups VII and IX, under the combined action of HPD, atropine and respective carbamates, the survival times are significantly more ($P < 0.001$), atleast, in all three doses of sarin, as compared to their corresponding basal values under normal diet (shown respectively in Groups VIII and X in parentheses[2]). It seems that the combination of the anticholinergic agent, atropine and carbamate under HPD gives better performance over normal dietary control under similar conditions[2], atleast, for sarin.

The possibility of high carbohydrate diet and high fat diet being used as a protecting agent against organophosphate toxicity has been eliminated by our earlier work (unpublished) on growth rate studies in rats, where it was found that only protein diet causes elevation in growth rate against organophosphate intoxication.

The present study was sought to find out unambiguously, whether HPD makes a change in a living system unlike normal protein diet, so that, it can better withstand the toxic effects of nerve gases and provides more protection to the system.

The second objective was to see whether HPD gives same enhanced protection in the presence of conventional protective agents, like carbamates and atropine. HPD appears to serve both the objectives. With some more experimentation, HPD can be recommended for the soldiers as well as civilian personnel, atleast for a short period, before their actual exposure to war chemicals or organophosphorus

insecticides, though its long term use may not prove to be feasible, due to socio-economic and physiological reasons. In this way, one can have further guard against nerve gas toxicity.

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REFERENCES

1 Somani, S.M. & Dube, S.N. Physostigmine — an overview as pretreatment drug for organophosphate intoxication. *Int. J. Pharmacol. Ther. & Toxicol.*, 1989, **27**, 367-87.

2. Chatterjee, A.K. Comparative evaluation of carbamates as prophylactic agents against organophosphate intoxication in rats. *Def. Sci. J.*, 1992, **42**, 85-87.

3. Chatterjee, A.K. & Kaveeshwar, U. Alleviating effect of high protein diet on the toxic effect of organopnosphorus compounds on the growth of rats. *Def. Sci. J.*, 1989, **39**, 109-12.

4. Chatterjee, A.K. Comparative evaluation of the alleviating effect of standard, low and high protein diets on the toxic effects of organophosphorus compounds on the growth of rats. *Def. Sci. J.*, 1989, **39**, 201-04.

5. Chatterjee, A.K. & Kaveeshwar, U. Protective effect of a high protein diet against the toxicity of some organophosphorus compounds in albino rats. *Japan J. Pharmacol.*, 1991, **57**, 147-52.

6. Sato, A. & Nakajima, T. Dietary carbohydrate and ethanol induced alteration of the metabolism and toxicity of chemical substances. *Nutr. Cancer.*, 1984, **6**, 121-25.

7. Kato, R.; Chiesara, E. & Vassanelli, P. Factors influencing induction of hepatic microsomal drug metabolizing enzymes. *Biochemical Pharmacol.*, 1962, **11**, 211-20.

8. Gordon, J.J.; Leadbeater, L. & Maidment, M.P. The protection of animals against OP poisoning by pretreatment with a carbamate. *Toxicol. Appl. Pharmacol.*, 1978, **43**, 207-16.