Original Paper

Lipid Profiles in Cerebral and Hepatic Tissues of Wistar Rats During Chronic Phosphamidon Toxicity

Srikanth K, Sahitya Chetan P, Wisweswari G, Rajendra W*

ABSTRACT

The possibility of alterations in function of lipids in structural components of membranes such as storage, protective coating on the surface, cell recognition, and tissue immunity during phosphamidon stress, prompted the present investigation.

Specifically chosen doses of phosphamidon, viz, acute dose (AD) (1/2 LD_{50}, 6.63 mg/kg body wt/day), and subacute dose (ASD) (1/3 $LD_{50;}$ 0.10 mg/kg body wt/day) were administered to male Wistar rats orally by gavage. After the stipulated treatment period, brain and liver from these rats were examined for lipid profiles such as cholesterol triglycerides and phospholipids, using standard biochemical procedures.

An increase in lipid peroxidation in conjunction with reduced levels of lipid profiles indicated that the lipid profiles were variously affected depending on different altered chemical reactons in the tissues of animal under stress. The study shows that induced lipid peroxidation was on par with the reduced levels of lipid profiles, indicating that the lipid profiles under continued daily dosing of phosphamidon could generate free radicals, in spite of elevation in the levels of antioxidants, or antioxidant enzyme activity, or both.

Key Words: Lipid peroxidation, Antioxidant, Free radical, Phosphamidon

Introduction

The consumption of food containing environmental contaminants is a potentially significant source of human exposure to numerous metals and pesticides.[1] Organophosphorus compounds (OPs), used the world over are one set of culprits. They are known to exert their acute toxicity by inhibition of acetylcholinesterase (Ache), though the toxic symptoms of OPs cannot be explained by esterase inhibition alone.

Although quite a few studies on the effects of OPs on carbohydrate and protein metabolism have been made, investigation into the effects of these insecticides on lipid metabolism has been relatively neglected.[2-8] In the present study, the effect of chronic, oral administration of the OP compound, phosphamidon on lipid profile of Wistar rats was examined in cerebral and hepatic tissues. This was considered important in clearly elucidating the alterations in lipid levels during toxic insults. There have been reports on the altered levels of membrane lipids in several pathological conditions, and also of lipids serving as good substrates for peroxidation reaction.[9,10]

The possibility of modulation of functions of lipids in biological systems such as structural components of membranes, involving storage and transport forms of metabolic fuel, protective coating on the surface, and as cell surface components concerned in cell recognition and tissue immunity during phsphamidon stress has prompted the present investigation.[11]

Department of Zoology, Division of Molecular Biology, Sri Venkateswara University, Tirupati, Andhra Pradesh 517502
*Author for correspondence: Phones: +91-87740021, +91-9849667236
E-mail: rajendraw@yahoo.co.in

Materials & Methods

Selection of Animals

Male Wistar rats weighing 110 ± 10 g obtained from the Indian Institute of Science, Bangalore, were maintained at the following laboratory conditions: temperature 25-27°C, 70-75% relative humidity, and a photoperiod of 12 h light /12 h dark cycle. The animals were fed a standard pellet diet, with water *ad libitum*.

Technical Information

Phosphamidon (80% purity) was obtained from Ciba-Geigy, India. Acute (AD) ½ LD_{50}; 6.63 mg/kg body wt/day), sub-acute (SAD) (1/3LD_{50}: 4.43 mg/kg body wt/day) and no-effect level (NEL) (1/100 LD_{50}; 0.10 mg/kg body wt/day) doses of phosphamidon were administered to the rats orally by gavage.

Treatment Protocol

The animals were divided into 3 groups. Group I comprised 4 sub-groups, each having 5 rats. Sub-group A was used as control (vehicle-treated). Sub-groups B and C wee treated with sub-acute and no-effect level doses of phosphamidon respectively, for 4 weeks. Sub-group D received acute dose for one day.

Group II comprised 3 sub-groups, each having 5 rats. Sub-groups B and C received sub-acute and no-effect level doses of phosphamidon respectively, for 8 weeks, keeping sub-group A as respective control (vehicle-treated).

Group III comprised 3 sub-groups having 5 rats. The sub-groups B and C were administered with sub-acute no-effect level doses respectively, for 12 weeks, keeping sub-group A as respective control (vehicle-treated). All control groups received corn oil vehicle by gavage. The corn oil was compatible with both test material and animal. After the stipulated treatment duration, the animals were sacrificed by cervical dislocation, and tissues such as liver and brain were isolated immediately, and frozen in liquid nitrogen (170^{0}C). The isolated tissues were stored at –40^{0}C until analyses.

Assays

Total cholesterol and triglyceride contents in the isolated tissues were estimated by the method of Natelson, 1971,[11] while the phospholipid content was estimated by the method of Zilversmith and Davis, 1950,[12] and lipid peroxidation (LP) was measured by determining malondialdehyde (MDA) content using thiobarbituric acid (TBA) as suggested by Ohakawa et al, 1979.[13]

Statistical Analyses

All the data obtained were subjected to Analysis of Variance and Student-Newman-Keul's test. The statistical tool of Steele and Torrie, 1960[14] was used to determine the significance of differences among means.

Results

Phosphamidon treatment was found to reduce cholesterol, triglyceride, and phospholipid levels in the liver of rats. However, no-effect level dosing for 4 weeks period did not significantly alter lipid levels (**Tables 1 & 2**). On the other hand, elevation in lipid peroxidation was observed in the liver of rats on no-effect level, sub-acute, and acute treatments, but the extent of elevation in the liver of 4 week-treated rats was only 6.4, and therefore not significant (**Table 1**). The brain samples of phosphamidon-treated rats were also found to show elevated lipid peroxidation, except in the brain of 4 week-treatment with no-effect level dose, where MDA content was lowered significantly by 21.1% (**Table 2**). Further treatment of rats with no-effect level and sub-acute doses of phosphamidon for 8 and 12 weeks caused progressive enhancement in lipid peroxidation.

The results show that phosphamidon intoxication caused reduction in lipid profiled in liver and brain of rats, but only a slight change during no-effect level dosing for 4 weeks period (**Tables 1 & 2**).

Discussion

The decreased cholesterol level during phosphamidon stress may suggest the following possibilities: (i) membrane architectural damage, (ii) reduced de novo synthesis of cholesterol from acetyl Co-A and (iii) increased input of cholesterol, or mitigating free radical effects during toxic stress.[15] The drop in cholesterol content in the present study can also be attributed to its involvement in the synthesis of steroid hormones under stressfull conditions.

The decrease in triglyceride levels during phosphamidon stress is presumably due to enhanced lipase activity. Some earlier studies are in agreement with this presumption.[16,17] The energy crisis induced during toxic stress may be taken as the nearest possibility for stimulation of lipase activity to step up triglyceride hydrolysis, and its mobilization to meet energy demands, and the concomitant decreased triglyceride levels may also thus be expected.

Table 1 Alterations in cholesterol, triglycerides, phospholipids, and lipid peroxidation in the liver of wistar rats

Assay	4 weeks treatment		8 weeks treatment			12 weeks treatment				1 day
	CON	NEL	SAD	CON	NEL	SAD	CON	NEL	SAD	AD
Cholesterol	60.57 ±0.28	58.79 ±0.28 (-2.94)*	52.08 ±0.28 (-14.01)	70.3 ±0.29	63.28 ±0.29 (-9.98)	54.29 ±0.21 (-22.77)	74.16 ±0.23	60.08 ±0.07 (-18.99)	52.3 ±0.23 (-29.47)	47.31 ±0.35 (-21.89)
TG	0.41 ±0.01	0.4 ±0.01 (-1.68)*	0.32 ±0.01 (-21.44)	0.48 ±0.0008	0.4 ±0.007 (-16.39)	0.37 ±0.008 (-21.37)	0.5 ±0.0009	0.38 ±0.008 (-25.00)	0.34 ±0.006 (-31.29)	0.29 ±0.007 (-28.43)
PL	13.48 ±0.33	13.44 ±0.3 (-0.26)*	10.08 ±0.1 (-25.23)	11.35 ±0.19	8.49 ±0.13 (-25.16)	5.4 ±0.23 (-52.41)	10.31 ±0.22	5.96 ±0.06 (-42.18)	4.77 ±0.06 (-53.74)	7.78 ±0.08 (-42.30)
LP	41 ±0.74	43.64 ±0.46 (6.4)*	52.24 ±0.55 (27.4)	43.96 ±0.55	49.85 ±0.48 (13.4)	58.52 ±0.76 (42.2)	47.19 ±0.36	55.04 ±0.55 (16.6)	67.52 ±0.73 (43.07)	55.67 ±0.54 (35.78)

TG = Triglycerides PL = Phospholipids LP = Lipid peroxidation
All the values are mean ± standard deviation of five individual observations; ± value indicates standard deviation; values in parentheses indicate percent changes over respective controls; *signifies non-significant change; all the values are significant at 5 per cent level in SNK test

Table 2 Alterations in cholesterol, triglycerides, phospholipids, and lipid peroxidation in the liver of wistar rats

Assay	4 weeks treatment		8 weeks treatment			12 weeks treatment				1 day
	CON	NEL	SAD	CON	NEL	SAD	CON	NEL	SAD	AD
Cholesterol	214.2 6.87	221.0 11.06 (3.17)*	159.0 6.52 (-25.77)	252.2 11.96	165.4 5.94 (-34.42)	154.4 4.03 (-38.77)	310.2 7.98	151.2 5.97 (-51.25)	142.4 3.97 (-54.09)	145.2 5.54 (-32.21)
TG	0.461 0.009	0.462 0.01 (0.217)*	0.399 0.007 (-13.45)	0.486 0.005	0.399 0.008 (-17.9)	0.366 0.008 (-24.69)	0.503 0.006	0.372 0.009 (-26.04)	0.331 0.008 (34.19)	0.382 0.008 (-17.14)
PL	15.14 0.135	15.18 0.236 (0.264)*	12.19 0.113 (-19.44)	13.15 0.122	10.070. 106 (-23.39)	8.71 0.113 (-33.78)	12.27 0.167	8.10 0.081 (-33.94)	7.39 0.185 (-39.76)	11.12 0.119 (-26.52)
LP	30.90 0.747	24.37 0.56 (-21.1)	38.88 0.602 (25.8)	33.12 0.96	38.59 0.598 (16.5)	45.33 0.271 (36.8)	36.5 0.444	47.61 0.402 (30.4)	51.11 0.719 (40.2)	41.96 0.752 (35.8)

TG = Triglycerides PL = Phospholipids LP = Lipid peroxidation
All the values are mean ± standard deviation of five individual observations; ± value indicates standard deviation; values in parentheses indicate percent changes over respective controls; *signifies non-significant change; all the values are significant at 5 per cent level in SNK test

The decrease in phospholipd content seen in the study, probably serves as a metabolic alarm to the animal, in the sense that the membrane integrity is lost. The decrease in phospholipid content could also be due to its immediate utilization for energy purposes, as it is relatively actively degradable among the lipids.[17,18] Reddy et al[15] also reported a drop in phospholipid content in brain and liver when exposed to cypermethrin toxicity, and an increase in lipid peroxidation observed in the present study concurs with the reports of Porumb[19] who stated that increased phospholipase A2 activity was responsible for depleted membrane phospholipids and stress conditions. Induction of lipid peroxidation may alter the permeability of biological membranes, thereby affecting metabolic activity of cells leading eventually to cell death. It is also obvious from the present investigation that the increment of lipid peroxidation was progressive with the treatment period in both liver and brain, without showing any sign of recovery towards normalcy.

The present study clearly shows that induced lipid peroxidation is on par with the reduced levels of lipid profiles, and continued daily dosing of phosphamidon could generate free radicals, inspite of elevation in the levels of antioxidants or antioxidant enzyme activity, or both, to lessen the free radical mediated toxicity.[20]

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