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Membrane Disordering Effect of Thiram as Assessed by Brain Synaptosomal and Erythrocyte Membrane Constituents

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Biological membranes are complex structures that deal with a multiplicity of functions including secretion, transport, endocytosis and signal transduction (Muriel and Mourelle 1990). Lipids of the bilayer provide the basic structure and subserve functions of the membranes, while proteins are responsible for receiving and transducting chemical messangers from neighbouring cells or the intracellular fluid (Alberts et al. 1983). The activity and functioning of these proteins in, or associated with the membrane are largely determined by the fluidity of their lipid micro-environment (Shinitzky and Henkart 1979) notable determinants of which are phospholipids (PL) and cholesterol. Alteration in cholesterol/phospholipid molar ratio could induce neurotoxicity (Taranova 1976). Among the thiocarbamate fungicides, thiram effect on the membrane structure has not been studied in detail although dithiocarbamates are reported to cause CNS malfunction (Lyon 1976). Acute poisoning of thiram has been shown to raise the PL levels above normal (Boguszewski et al. 1984).

Thus in the present investigation the effect of different concentrations of thiram for different durations on protein, phospholipids, cholesterol, cholesterol/phospholipid molar ratio and lipid peroxidation in mice was studied.

MATERIALS AND METHODS

Adult LACA mice of either sex weighing 28 ± 5g were maintained at 25°C and fed on the normal rat diet.

Thiram of 99% purity was obtained from Fluka AG, Switzerland and was used as such. It was given intraperitoneally, in 2% propanol-2 to 16h fasted mice.

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The doses of thiram and time of sacrifice of animals by decapitation, after the administration of the toxicant is given below:

Dose (uM Kg^{-1} body wt.) and schedule of administration	Time of sacrifice after the administration of thiram (h)
1/4 LD_{50} (670.0), single i.p.	0.75, 4 and 168
1/4 LD_{50} (670.0), single i.p. once daily for three consecutive days	72
1/2 LD_{50} (1330.0), single i.p.	0.75
LD_{50} (2660.0), single i.p.	0.75

Just before sacrifice, animals were weighed, blood was drawn from the eye of mice into heparinized tubes and the animals were decapitated. All proceedings thereafter were conducted at $4^{o}C$. Brains were removed rapidly and washed with ice cold 10% (w/v) sucrose. They were weighed after draining between folds of filter paper and homogenate was prepared in 19 volumes of 10% (w/v) ice cold sucrose solution.

Crude brain synaptosomes (Westcott and Weiner 1983) and erythrocyte membranes (Hanahen et al. 1974) were prepared by the methods described elsewhere. **Protein** content of tissue preparation was determined by the method of Lowry et al. (1951). Bovine serum albumin was used as a standard. **Lipid peroxidation** measured by the method of Placer et al. (1966) was expressed as nmoles of malonaldehyde formed. **Lipid extraction** was done by the method of Folch et al(1975). Chloroform: methanol:water (3:48:47) extract was used for estimatiion of **Cholesterol** (Zlatibis et al 1953) and **Phospholipids** (Bartlett, 1959).

Data shown in the study were statistically evaluated by student's t-test. A difference between two means was considered significant with $p < 0.05$, $p < 0.01$ and $p<0.001$, and comparison to a control are indicated in tables by 'a_1','a_2', 'a_3'.

RESULTS AND DISCUSSION

The results showed that thiram had a marked effect on membrane constituents.

Increase in dosage of thiram (1/4 LD_{50}, 1/2 LD_{50} and LD_{50}) and duration of observation from 0.75h to 4h and

Table 1. Effect of different doses of thiram (i.p. injection) after 0.75h of its administration on mice brain synaptosomes.

Constituents[a]	Control	Dose (uM Kg^{-1} body wt.) 670 ($1/4LD_{50}$)	1330 ($1/2LD_{50}$)	2600 (LD_{50})
Protein[b]	12.43 ±3.04	25.07 ±4.39[a3]	40.75 ±4.48[a3]	49.07 ±1.97[a3]
Phospholipids[b]	2.21 ±0.47	1.70 ±0.20[a2]	1.55 ±0.13[a3]	0.63 ±0.05[a3]
Cholesterol[b]	0.78 ±0.15	2.07 ±1.17[a3]	1.50 ±0.20[a3]	0.41 ±0.08[a3]
Cholesterol/PL	0.35 ±0.03	1.22 ±0.03[a3]	0.97 ±0.06[a3]	0.66 ±0.03[a3]
Lipid - peroxidation[c]	65.00 ±2.11	248.77 ±130.00[a3]	299.04 ±53.23[a3]	469.07 ±66.47[a3]

[a]Each value is the mean ± S.D. of the observations from six independent experiments
[b]Contents are expressed as mg per g protein; [c]nmol malonaldohyde formed per mg protein
[a1]$p < 0.05$ vs control; [a2] $p < 0.01$ vs control;
[a3]$p < 0.001$ vs control

168h increased the protein content of synaptosomes but the same doses and durations suppressed the protein content of erythrocyte membranes. Three consecutive days of injection of thiram produced a rise in protein content in synaptosomes but a fall in erythrocyte membranes (Table 1-4).

Phospholipids were decreased with all the doses in brain synaptosomes (Table 1) and were increased with LD_{50} dose of thiram in erythrocyte membranes (Table 2). After 4h and 168h of administration of thirmam, there was no significant change in PL content as compared with the control in both the tissues (Table 3-4). Cummulative dose of thiram produced a rise in PL level in erythrocyte membranes (Table 4) while in brain synaptosomes (Table 3), there was no significant change.

$1/4$ LD_{50} dose of thiram increased the cholesterol content which decreased with increase in dose in both the tissues studied (Table 1-2). Cholesterol content rose in brain synaptosomes with increase in duration of observation to 4h and decreased after 168h (Table 3) as compared with control. On the other hand, cholestrerol content was increased after 4h and 168h of administration of thiram in erythrocyte membranes (Table 4) as compared with control. Cummulative dose of

thiram raised the cholesterol level above the control in brain synaptosomes (Table 3) and produced a fall in erythrocyte membranes (Table 4).

Cholesterol/PL molar ratio was found to increase with the increase in dose in synaptosomes (Table 1) while a reverse pattern was seen in erythrocyte membranes (Table 2). The ratio was also increased in synaptosomes after 4h of administration of thiram (Table 3), which decreased after 168h. There was no significant change in molar ratio in erythrocyte membranes at both the durations (Table 4). Three consecutive days of injection of thiram produced a suppression of cholesterol/PL ratio in erythrocyte membranes (Table 4) but there was no significant change in synaptosomes (Table 3) as compared with the control.

Table 2. Effect of different doses of thiram (i.p. injection) after 0.75h of its administration on mice erythrocyte membranes.

Constituents[a]	Control	Dose (uM Kg^{-1} body wt) 670 ($1/4LD_{50}$)	1330 ($1/2Ld_{50}$)	2600 (LD_{50})
Protein[b]	3.00 ±0.70	1.72 ±0.20[a1]	1.56 ±0.21[a2]	1.26 ±0.24[a2]
Phospholipids[b]	0.39 ±0.03	0.47 ±0.09	0.50 ±0.09	0.73 ±0.10[a2]
Cholesterol[b]	0.56 ±0.10	0.74 ±0.2[a1]	0.64 ±0.02	0.10 ±0.00[a3]
Cholesterol/PL	1.43 ±0.30	1.56 ±0.05	1.21 ±0.04	0.14 ±0.01[a3]
Lipid - Peroxidation[c]	14.40 ±4.10	23.24 ±0.98[a1]	34.02 ±6.10[a2]	37.10 ±5.10

[a]Each value is the mean ± S.D. of the observations from three independent experiments
[b]Contents are expressed as mg per g protein; [c]nmol malonaldohyde formed per mg protein
[a1]$p < 0.05$ vs control; [a2] $p < 0.01$ vs control;
[a3]$p < 0.001$ vs control

Lipid peroxidation was found to increase in both the tissues at all the dosages and duration of the dithiocarbamate into the cellular membranes. It has been shown that lipophilic thiram complexes can be formed and it is well known that lipid solubility is a physical property of the major importance for the passage of molecule accross cellular membranes (Jasim et al. 1984). The decrease in phospholipids, due to thiram intoxication might be because of the impairment of phospholipid biosynthesis which might affect the membrane/function especially the

"viscotropic properties" and neurotransmission. Similarly, alteration in cholesterol content also affects the general metabolism and erythrocyte membranes. Therefore, the depletion of cholesterol in present study might lead to a decrease in surface exposure, an increase in osmotic fragility and glycerol permeability.

Table 3. Effect of 1/4 LD_{50} dose of thiram (i.p. injection) on constituents of mice brain synaptosomes after different durations of observations.

Constituents[a]	Control	Time of Sacrifice (h) 4.0	168.0	72.0
Protein[b]	12.43 ±3.04*	23.11 ±1.16[a3]	27.23 ±1.39[a3]	23.57 ±1.36[a3]
	15.60 ±3.14**			
Phospholipids	2.21 ±0.47*	2.11 ±0.27	1.94 ±0.41	2.48 ±0.20
	2.00 ±0.40*			
Cholesterol[b]	0.78 ±0.15*	0.97 ±0.13[a3]	0.51 ±0.15[a3]	0.92 ±0.09[a2]
	0.80 ±0.10**			
Cholesterol/PL molar ratio	0.35 ±0.03*	0.46 ±0.02[a3]	0.26 ±0.03[a3]	0.37 ±0.12
	0.40 ±0.04**			
Lipid per oxidation[c]	65.00 ±2.11*	231.17 ±6.37[a3]	249.48 ±32.22[a3]	259.65 ±27.91[a3]

*control for the animals sacrificed after 4h and 168h of administration of thiram.
**control for animals sacrificed after 72h of administration of thiram.
[a]each value is mean ± $S.D_b$. of the observation form six independent experiments; contents are expressed as mg per g protein; malonalodohyde formed per mg protein.
[a1]p <0.05 vs control; [a2]p <0.01 vs control;
[a3]p < 0.01 vs control

A change in cholesterol/PL ratio can also be seen in the present study, which also reflects the change in

membrane fluidity. Both in vitro and in vivo studies have demonstrated that increase in cholesterol/PL increase the microviscosity of the membranes (synaptosomal plasma membrane/erythrocytes). Many drugs (like tranquilizers, anaesthetics and toxicants etc.) which can cross the blood brain barrier have been shown to affect the membrane founctions by altering the vital membrane constituents (Aberlin and Litman 1979).

Table 4. Effect of 1/4 LD_{50} dose of thiram (i.p. injection) on constituents of mice erythrocyte membranes after different durations of observations.

Constituents[a]	Control	Time of Sacrifice (h) 4.0	168.0	72.0
Protein[b]	3.00 ±0.70*	2.00 ±0.80	2.00 ±1.00	1.28 ±0.60[a1]
	2.86 ±0.50**			
Phospholipids	0.39 ±0.03*	0.44 ±0.07	0.44 ±0.06	0.67 ±0.70[a2]
	0.40 ±0.40*			
Cholesterol[b]	0.56 ±0.15*	0.90 ±0.13[a3]	0.80 ±0.15[a3]	0,33 ±0.09[a2]
	0.50 ±0.05**			
Cholesterol/PL molar ratio	1.43 ±0.30*	2.04 ±0.07	1.82 ±0.08	0.49 ±0.07[a1]
	1.25 ±0.20**			
Lipid per oxidation[c]	14.40 ±4.10*	43.28 ±7.75[a3]	49.48 ±14.23[a2]	259.65 ±6.25

*control for the animals sacrificed after 4h and 168h of administration of thiram.
**control for animals sacrificed after 72h of administration of thiram.
[a]each value is mean ± $S.D_b$. of the observation from three independent experiments; contents are expressed as mg per g protein; milanalodohyde formed per mg protein.
[a1]$p < 0.05$ vs control; [a2]$p < 0.01$ vs control;
[a3]$p < 0.01$ vs control

Organochlorine insecticide DDT, which is claimed to be a neurotoxicant has been shown to disturb the bilayer order in fluid native membranes of mitochondria and sarocoplasmic reticulum, but moderate disordering effects are noticed in brain microsomes and erythrocyte membranes enriched in cholesterol (Madeira and Madeira 1990). Similarly, fluorescence polarizing studies have shown that lindane disorders the upper regions and the inner core of the bilayer, in the gel phase of dimyristoyl phosphatidyl choline (Maderia et al. 1990) showing that the membrane fluidity is the main parameter affecting lindane incorporation and probably, its toxicity. Thus, in the light of the above reported literature, it can be said that thiram might be toxic due to its ability to cause disorder in the lipid bilayer system. A uniform increase in lipid peroxidation in brain synaptosomes and erythrocyte membranes in the presetn study might be due to a deficit in the supply of energy which may be due to a depletion of ATP due to thiram intoxication which might lead to a alteration of membrane integrity, permeability and deformability (Lub and Chiu 1982).

On the basis of foregoing results, on the effects of thiram on synaptosomes and erthrocytes membranes, it could be suggested that it could affect the membrane viscosity and thus it is a potential neurotoxicant and could also affect the functions and metabolism of erythrocytes.

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