

Age related effects of organochlorine insecticide lindane on intestinal brush border membrane in rats

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The effect of oral administration of lindane (γ -HCH) has been studied on the intestine in 10-day, 20-day and 100-day old rats. In 10 day-old suckling pups exposed to lindane, there was a significant decrease in the activities of sucrase (29%), lactase (20%) and that of alkaline phosphatase (24%) compared to control. Sialic acid content of the brush borders was significantly decreased (29%) in 10-day old as well as in 20- and 100-day old rats (20 and 25% respectively), while fucose content of the membranes was significantly enhanced in all the age groups upon pesticide treatment. Among the brush border lipids, cholesterol content was significantly increased in all the age groups studied, the maximum increase of 35% being observed in 10-day-old rats. Membrane phospholipids were also increased in 20- and 100-day old animals (22% each) on lindane exposure. The present studies indicated that brush border membranes of suckling rat intestine were more susceptible to pesticide induced changes compared to older animals.

Lindane (γ -hexachlorocyclohexane, HCH), a persistent organochlorine pesticide, is widely used for crop protection and as an ectoparasiticide to treat lice and scabies infestation. The increasing usage of HCH in recent years in India is reflected by the fact that its concentration in some of the food materials has been detected above the recommended tolerance limits, particularly in the dairy products¹⁻³. Significant levels of HCH and DDT have also been found in cord blood collected 48 hr after childbirth from nursing mothers, indicating the passage of organochlorines across the placenta to the neonates⁴. However, not much information is available about the age related toxic effects of the pesticides. Ingestion of pesticide contaminated foods is the main source of exposure to these compounds and intestinal tissue constitutes the primary target being exposed to the pesticide. Following oral administration of HCH, residues of γ -HCH, have been shown to be present in enterocytes lining the rat intestine. In mice, hydrolytic enzymes of small intestine, such as disaccharidases, dipeptidase, lipase, amylase and phosphatases have been found to be affected by dietary HCH and γ -HCH⁶. The present study was undertaken to examine the age related effects of lindane on intestinal brush border membrane (BBM) in rats.

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Abbreviations: γ -GTP, γ -glutamyl transpeptidase; LAP, leucine aminopeptidase

Materials and Methods

Treatment of animals

Male albino rats (Sprague Dawley strain) of three different ages, namely (a), 10 days old suckling (both sexes) (b), 20 days old post weaning rats and (c), 100 days old adult rats were used. The number of animals used in each group was 15, 12 and 6 respectively. Older rats were maintained on standard rat pellet diet with water *ad libitum*, while the suckling pups were maintained with their dams. The 20 and 100 day old rats were orally administered lindane (γ -HCH 99.9%, Sigma Chemical Company, St. Louis, USA) at a dose of 20 mg/kg body weight daily, dissolved in 0.2 ml corn oil, for 7 days. Suckling rats were given 4.4 mg lindane/kg body weight in 0.1 ml oil. At a higher pesticide dose, there was a high mortality rate among the pups. Control animals received equal amounts of the vehicle alone. At the end of the experimental period, overnight fasted animals were sacrificed under light ether anesthesia. Starting from the ligament of Treitz, the small intestine was removed, flushed with ice-cold saline and used for various biochemical studies.

BBM preparation

Brush border membranes were isolated following the method of Kessler *et al.*⁷. The final membrane preparation was suspended in 50 mM sodium maleate buffer (pH 6.8) and exhibited 15-20 fold enrichment of brush border membrane marker enzymes over the crude homogenate. To prepare membranes from 10

day and 20 day old rats, tissues from 3 animals were generally pooled.

Enzyme assays

Sucrase and lactase activities were assayed by the method of Dahlquist⁸. Alkaline phosphatase activity was determined by the method of Bergmeyer⁹. γ -glutamyl transpeptidase activity was determined as described by Naftalin *et al.*¹⁰ and leucine aminopeptidase activity was assayed by the method of Goldbarg and Rutenberg¹¹. Protein was determined by the method of Lowry *et al.*¹², using bovine serum albumin as the standard.

Chemical composition of BBM

For lipid analysis, BBM prepared from intestines of 2-3 animals were pooled and 3 such preparations were used. Lipids were extracted from BBM following the method of Folch *et al.*¹³. Phospholipid phosphorous was determined by the method of Marinetti *et al.*¹⁴. Total cholesterol was estimated as described by Zlatkis *et al.*¹⁵, while free cholesterol was determined by the method of Courchain *et al.*¹⁶. Triglycerides were determined by the method of Van Handel and Zilversmit¹⁷.

Membrane sialic acid was determined by the method of Skoza and Mohos¹⁸. Fucose was estimated by the method of Dische and Shettles¹⁹. The amount of total hexoses in BBM was determined by the method of Gatt and Berman²⁰ and hexosamines were determined by the method of Roe²¹.

All enzyme activities were expressed as units/mg protein. One enzyme unit is equal to the amount of enzyme that transforms 1 μ mole of the substrate per minute into product under standard assay conditions.

Statistical analysis

Statistical analysis of the data was done using the student's t test.

Results

Table 1 shows the effect of lindane administration on brush border enzymes in different age groups of rats. Sucrase and lactase activities were significantly decreased by 29% and 20% respectively by lindane treatment in 10-day-old rats compared to the controls. In contrast, sucrase activity was significantly elevated in 20 day (15%, $p < 0.05$) and 100 day (32%, $p < 0.01$) old rats upon lindane exposure. Alkaline phosphatase activity was reduced ($p < 0.001$) by 24% in pups, while it was enhanced by 20% in 100-day-old rats ($p < 0.05$) upon lindane exposure. The activities of leucine aminopeptidase and γ -glutamyl transpeptidase were essentially unaltered in pesticide treated 10-day, 20-day and 100-day old animals.

The data on the sugar contents of the brush borders in rats exposed to lindane are shown in Table 2. There was a significant decrease in the sialic acid content of the membranes in 10-day (29%, $p < 0.01$), 20-day (20%, $p < 0.05$) and 100-day (25%, $p < 0.05$) old rats after lindane treatment of the animals compared to the controls. However, the membrane fucose content was significantly enhanced in all the age groups under these conditions. Hexosamine content of the brush borders was augmented (35%) in 100-day-old rats but remained unaltered in other age groups of rats upon pesticide administration, compared to the controls. Membrane hexoses were also elevated in 10- and 100-day-old rats (16 and 27% respectively) exposed to lindane.

Analysis of membrane lipids revealed that the total cholesterol content increased by 35% and 20% in 10- and 20-day-old rats respectively, upon pesticide exposure (Table 3). In 100-day-old rats, both the total and free cholesterol contents of brush borders were enhanced by 18% and 16% respectively, albeit significantly ($p < 0.05$) upon lindane administration. Membrane phospholipids were enhanced by 22% in 20- and 100-day old animals exposed to the pesticide, but there was no change in membrane phospholipids

Table 1—Effect of lindane on intestinal brush border enzymes of different age groups of rats

[Values are mean $\pm$ SD of 5 observations]

Enzyme (units/mg protein)	10 days		20 days		100 days	
	Control	Treated	Control	Treated	Control	Treated
Sucrase	0.080 $\pm$ 0.001	0.057 $\pm$ 0.01**	0.502 $\pm$ 0.07	0.576 $\pm$ 0.01*	0.586 $\pm$ 0.04	0.776 $\pm$ 0.09**
Lactase	0.220 $\pm$ 0.02	0.176 $\pm$ 0.02**	0.018 $\pm$ 0.002	0.018 $\pm$ 0.002	0.012 $\pm$ 0.002	0.010 $\pm$ 0.01
Alkaline phosphatase	0.758 $\pm$ 0.04	0.576 $\pm$ 0.05***	0.822 $\pm$ 0.09	0.894 $\pm$ 0.09	1.297 $\pm$ 0.5	0 1.558 $\pm$ 0.09*
γ -GTP	0.312 $\pm$ 0.05	0.328 $\pm$ 0.03	0.313 $\pm$ 0.03	0.315 $\pm$ 0.02	0.329 $\pm$ 0.04	0.334 $\pm$ 0.04
LAP	0.295 $\pm$ 0.04	0.275 $\pm$ 0.03	0.316 $\pm$ 0.02	0.342 $\pm$ 0.03	0.336 $\pm$ 0.07	0.382 $\pm$ 0.04

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs control

Table 2—Effect of lindane on intestinal brush border saccharides of different age groups of rats

[Values are mean $\pm$ SD of 5 observations]

Membrane saccharides	10 days		20 days		100 days	
	Control	Treated	Control	Treated	Control	Treated
Sialic acid (nmole/mg protein)	109.2 $\pm$ 13.8	77.7 $\pm$ 12.4**	68.8 $\pm$ 8.6	55.1 $\pm$ 7.2*	37.6 $\pm$ 6.1	28.1 $\pm$ 3.8*
Fucose (nmole/mg protein)	80.8 $\pm$ 6.7	96.1 $\pm$ 6.1**	105.4 $\pm$ 12.3	124.5 $\pm$ 12.0*	228.5 $\pm$ 11.3	287.3 $\pm$ 28.2**
Total hexoses (μ g/mg protein)	79.4 $\pm$ 6.0	92.1 $\pm$ 5.5*	75.8 $\pm$ 8.0	81.2 $\pm$ 11.5	63.8 $\pm$ 8.6	80.9 $\pm$ 8.6*
Hexosamines (μ g/mg protein)	84.5 $\pm$ 9.2	81.7 $\pm$ 9.6	79.4 $\pm$ 7.8	81.8 $\pm$ 10.2	75.5 $\pm$ 9.8	102.0 $\pm$ 15.8*

* $p < 0.05$, ** $p < 0.01$ vs control

Table 3—Effect of lindane on intestinal brush border lipids of different age groups of rats

[Values are mean $\pm$ SD of 3 observations]

Lipid fraction μ g/mg protein	10 days		20 days		100 days	
	Control	Treated	Control	Treated	Control	Treated
Phospholipids (PL)	350.0 $\pm$ 24.9	369.2 $\pm$ 30.6	329.1 $\pm$ 32.5	401.7 $\pm$ 28.8*	212.6 $\pm$ 19.6	259.0 $\pm$ 9.3*
Total cholesterol	198.6 $\pm$ 19.5	267.7 $\pm$ 22.3*	188.2 $\pm$ 19.0	225.7 $\pm$ 13.2*	147.8 $\pm$ 11.9	174.9 $\pm$ 10.1*
Free cholesterol	94.8 $\pm$ 10.1	90.7 $\pm$ 12.1	90.4 $\pm$ 17.0	86.8 $\pm$ 13.6	84.0 $\pm$ 3.3	97.5 $\pm$ 7.0*
Triglycerides	85.5 $\pm$ 6.3	84.8 $\pm$ 9.2	76.4 $\pm$ 11.6	73.6 $\pm$ 8.1	65.5 $\pm$ 9.0	66.1 $\pm$ 7.3
PL/cholesterol mole/mole	0.852	0.666	0.844	0.861	0.694	0.714

* $p < 0.01$ vs control

of the pups. Membrane triglycerides (65.5-85.5 μ g/mg protein) were essentially unaltered under these conditions.

Discussion

The results presented herein indicate generalized impairment in the digestive functions of the intestine of 10-day-old rats exposed to lindane. The observed decrease in lactase activity is particularly significant, since it would influence the digestion of lactose present in milk which is the only diet of suckling animals. In contrast, pesticide administration stimulated the activities of some of the brush border enzymes in the older rats. These findings suggest the differential effects of lindane exposure in the suckling and the mature rats. Chlorinated hydrocarbon pesticides have been shown to exhibit steroidal hormone actions²². The observed decrease in sucrase and lactase activities in suckling rats exposed to lindane suggests a specific effect of the pesticide, which is unrelated to maturational developments of the tissue as observed in response to cortisone treatment. It may suggest interplay of complex regulatory and adaptive mechanisms in response to pesticide treatment in the developing rat intestine. Interestingly, the activities of peptidases were not affected by pesticide treatment. Changes observed in

disaccharidases and alkaline phosphatase reflect the alteration in membrane structure whereas the peptidases are not only membrane bound but also exist in the cytosol. It is likely that only the membrane bound enzymes are modulated in response to pesticide treatment. It is also known that several peptidases with different specificities²³ are present in intestine which may be differently affected under these conditions. No reports are available on the effects of pesticides on intestinal functions in suckling rats, but in adult animals, chlorinated hydrocarbon pesticides endosulfan, DDT, dieldrin and lindane have been reported to modify the activities of various brush border enzymes, though the effects are variable²⁴⁻²⁷.

The glycosylation changes that normally take place at the time of weaning are characterized by a shift from sialylation to fucosylation in rat intestine²⁸. Decrease in sialic acid and an increase in fucose content of brush borders in suckling rats in response to lindane administration observed in the present studies indicates that it influences the normal development of the glycosylation pattern.

Analysis of membrane lipids revealed an increase in phospholipid and cholesterol levels of treated animals. Increase in lipid content of microvillus membrane upon pesticide treatment may result in reduced membrane fluidity²⁹ thus modifying the

functional activities of the membrane. Similar results have been described in enterocytes and renal proximal tubules in lindane treated rats^{30,31}. Microvillus membrane in suckling rat intestine contains relatively high amounts of lipids compared to that in adult animals³². This may facilitate the absorption and accumulation of lipophilic xenobiotics such as lindane in these animals, thus making them more susceptible to the toxic effects of the drug. However, the mole/mole ratio between phospholipids and cholesterol was reduced from 0.852 in 10 day old control pups to 0.666 in pesticide treated animals. There was essentially no change in the phospholipid/cholesterol molar ratio in 20-and 100-day-old rats upon pesticide treatment. An increase in intestinal phospholipids in chicks treated with lindane has been reported by Moreno *et al.*³³. Similar results in rats exposed to DDT have been described by Dudeja and Mahmood²⁴. Hepatic fat content was increased in HCH fed rats of 5 and 10 weeks of age but was not affected in 16 or 32 weeks or 16 month-old rats³⁴. The observed increase in levels of phospholipids and cholesterol may be the result of enhanced biosynthesis of these compounds, since lindane exposure has been reported to modify phospholipid and cholesterol biosynthesis in various tissues^{30,35,36}.

In conclusion, the present findings demonstrate that the effects of lindane administration on brush border enzymes and on chemical composition of the membranes in rat intestine are different in different age groups. The suckling rats are more susceptible to the toxic effects of the pesticide, compared to adult animals.

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