J. Physiol. Biochem., 52 (1), 37-44, 1996
Revista española de Fisiología

Effects of different subchronic treatments with lindane on some brush border enzymes in rat jejunum

M. J. Moreno, S. Pellicer and M. P. Fernández-Otero

Departamento de Fisiología y Nutrición,
Universidad de Navarra, 31008 Pamplona (Spain)

(Received on November 6, 1995)

M. J. MORENO, S. PELLICER and M. P. FERNÁNDEZ-OTERO. *Effects of different subchronic treatments with lindane on some brush border enzymes in rat jejunum.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (1), 37-44, 1996.

Subchronic lindane (γ-HCH) intoxication by oral or s.c. injection over 7 and 15 days, induced a significant inhibition in rat jejunum maltase activity when the pesticide was administered at doses of 20 mg/kg b. wt. However, maltase levels remained unaffected in those animals injected with 10 mg/kg of lindane. A longer period of s.c. lindane exposure (30 days) at doses of 10 mg/kg induced a significant decrease in maltase activity, although the injection of 20 mg/kg over the same period did not alter this enzyme activity. When this lindane dose was s.c. injected over 20 days a significant inhibition of maltase activity was observed. However no changes in this enzyme were found in rats injected over 25 days. This fact seems to suggest that between 20-25 days of pesticide exposure the organism develops possible regulatory mechanisms to counteract the alterations induced by this dose of lindane on maltase activity. Lactase and alkaline phosphatase activities were not altered by lindane action in different treatments performed. Sucrase activity was only altered in oral injected rats at doses of 20 mg/kg over 15 days. In conclusion, maltase activity seems to be more sensitive to lindane action than other brush border enzymatic proteins; lindane effects on this enzyme depend on the injected dose and the pesticide administration period duration.

Key words: Lindane, γ-Hexachlorocyclohexane, Pesticide, Disaccharidases, Alkaline phosphatase, Intestinal membrane.

*Correspondence to M. P. Fernández-Otero. (Tel.: 3448-105600 - Ext. 6325; FAX: 3448-105649).
e-mail: pfernandez@farma.unav.es

γ-Hexachlorocyclohexane (γ-HCH), commonly known as lindane, is an organochlorine pesticide widely used at present in veterinary, agricultural, and medical products in many countries where the application of lindane is limited to certain crops, and its use is not permitted in processing or during storage (25). Because of lindane previous abundance, its persistence and subsequent accumulation in the environment, may reach animal organisms, including man, by inhalation into lungs, by diffusion through skin, and above all, through the food chain (11, 20, 32). Therefore, the environmental and biological persistence of organochlorinated pesticides, such as lindane, presents a problem of chronic toxicity, resulting from repeated low-level exposure to these compounds (19).

The adverse health effects of lindane have been studied extensively. The principal effects of lindane exposure seem to be neurological and behavioural (31, 17). Biochemical and histological alterations in liver, kidney and reproductive organs have been described (8, 33, 35). However, the exact mechanism(s) of lindane toxic action is not well known.

Lindane is a small lipophilic molecule likely to accumulate in lipid membrane, and consequently, it has been suggested that its toxic effects may be related to its accumulation in cell membranes (1, 15).

Intestinal epithelium constitutes one of the most important cellular membranes due to its role in digestive and absorptive functions (12) and it is primarily exposed to low but persistent quantities of lindane when this toxicant enters animal organisms after oral uptake of contaminated food. Little is known about the effect of lindane on the gastrointestinal tract. It is of relevance to find out the effects of lindane on the intestinal epithelium since any alteration in digestive and absorptive functions of the small intestine could lead to an inadequate nutrient bioavailability in the organism. Previous studies have described some alterations in structure and functions of intestinal membranes related to lindane accumulation (7, 23, 26). In this sense, earlier studies carried out by our group have shown that lindane treatment altered intestinal mucosa composition (22), as well as the intestinal absorption of galactose and leucine in chicken enterocytes (21).

The aim of the present work was to study the effect of the exposure to repeated low lindane doses (10 and 20 mg/kg b. wt.) over different periods of time and by different pathways, on some brush border digestive enzyme activities in the rat intestine.

Materials and Methods

Animals and treatment.– Male albino Wistar rats (*Rattus norvegicus*), weighing 180-270 g at the end of the different treatments were used. Rats were housed in a temperature-controlled room and maintained on a standard breeding diet; food and water were given *ad libitum*. Rats were divided into different experimental groups of eight animals each, according to the lindane administered dose: 10 or 20 mg/kg b.w. Lindane was dissolved in sesame oil. Control rats were injected with the oil only. Injections were given at 9:00 a.m. Different subchronic lindane treatments were carried out. Thus, the pesticide was injected via oral or s.c., and over different periods: 7 and 15 days (oral and s.c. treatments) and 20, 25 and 30 days (s.c. treatments alone).

Once the different experimental periods were over, and after 24 h of starvation, the animals were killed by decapitation, minimizing stress conditions, between 9-11 a.m. A jejunum segment of 20 cm from the Treitz's ligament was quickly

removed and flushed with 10 ml of ice-cold 150 mM NaCl to remove luminal content. It was longitudinally opened, the mucosa was scraped off completely with a glass slide and the mucosa was stored (–20 °C) for enzymatic assays.

Preparation of brush border membranes.– Brush border membranes were isolated from jejunal mucosa following the method of SCHMITZ *et al.* (27). Briefly, a jejunal mucosa fragment was homogenized in 2 mM Tris-HCl (pH 7.1) solution with 50 mM mannitol (Heidolph Type RZR1, 30 sec at full speed). 10 mM $CaCl_2$ was added and left for 10 min. After centrifugation at 2000 *g* for 10 min, the supernatant was centrifuged at 20,000 *g* for 15 min. Finally, the pellet obtained was resuspended in 150 mM NaCl.

Measurement of disaccharidases and alkaline phosphatase activities.– Aliquots of mucosa homogenate (obtained by mucosa homogenization in 150 mM NaCl at 300 rpm for 3 min) or of brush border membrane were used for determination of enzymatic activities. Lactase (EC 3.2.1. 23), sucrase (EC 3.2.1. 48) and maltase (EC 3.2.1. 20) were assayed by the method of DAHLQVIST (9) using 0.056 mM lactose, sucrose and maltose (Merck) as substrates in 0.1 M sodium maleate buffer, pH 6.0. Alkaline phosphatase (EC 3.1.3.1) was determined by a Sigma diagnostic kit, using as substrate 7.6 mM *p*-nitrophenyl phosphate (104 Sigma) in 50 % (v/v) alkaline buffer solution (221 Sigma). Protein was assayed by the method of LOWRY *et al.* (16).

Statistical treatment of the results.– Results were expressed as mean ± SEM. Data were evaluated statistically by one-way analysis of variance. When significant differences were found, comparisons between control and treated groups were made applying the Fisher PLSD test. Comparisons of 2 means were made using Student's t-test. In all cases $p < 0.05$ was considered to be the statistically significant level.

Results and Discussion

Different subchronic lindane treatments were carried out to study their effects on some intestinal brush border enzymatic proteins.

Similar doses of lindane have been used previously to study the effects of several subchronic lindane treatments on different biochemical and physiological processes. Thus, CAMON *et al.* (5) studied cerebral glucose uptake in rats injected with 10 mg/kg of lindane over 7 days; this same dose administered over 12 days was used by PÉREZ-ALBARSANZ *et al.* (24) to study the effects of lindane on fluidity and lipid composition in rat renal cortex membrane; COOPER *et al.* (8) administered 10 and 20 mg/kg of lindane over 120 days to evaluate pesticide effect on hormonal control of reproductive function in female rats.

Although in many studies lindane is administered mixed with diet (10, 28, 29), the quantity taken by each animal is difficult to ascertain. Thus we administered the pesticide, like many authors (6, 30) by oral gavage, since this guarantees that each animal receives an established dose.

Table I shows the effect of two different oral lindane treatments on intestinal disaccharidase activities. Lindane oral treatment over 7 days can be observed to induce a significant decrease ($p < 0.05$) in maltase activity in rats injected with 20 mg/kg of the pesticide, but no changes were found in those rats treated at the low dose of 10 mg/kg. Lactase activity was not modified by oral lindane injection of either dose over 7 days. There were no

Table I. *Effect of different oral lindane treatment periods on intestinal lactase, sucrase and maltase activities in rat*

Enzymatic assays were performed in jejunal mucosa homogenate from rats injected with two different oral doses 10 (L 10) and 20 (L 20) mg/kg of lindane dissolved in sesame oil over 7 and 15 days and from control rats (injected with sesame oil alone). Results are expressed as mean ± SE. In parentheses the number of animals. Values are given as μmoles of lactose, sucrose or maltose hydrolyzed per min per g protein. $^{*}p < 0.05$, $^{**}p < 0.01$.

	7 days			15 days		
	Control (6)	L 10 (6)	L 20 (6)	Control (8)	L 10 (8)	L 20 (8)
Lactase	25.45±2.30	27.02±4.07	18.68±2.07	23.14±0.97	23.42±3.75	21.05±2.24
Sucrase	76.27±8.93	79.06±6.90	57.30±7.53	152.91±9.93	141.61±13.76	106.39±4.57*
Maltase	721.26±60.82	723.78±27.58	565.25±49.37*	645.27±24.58	630.51±70.92	448.96±47.4**

changes in sucrase activity in rats treated with 10 mg/kg of lindane, while a non significant decrease (24.87 %) can be observed in this enzyme activity in lindane–treated rats at a 20 mg/kg dose. When oral lindane treatment was performed over 15 days, a similar decrease ($p < 0.01$) in maltase activity in intestinal mucosa homogenate from rats injected at 20 mg/kg doses was observed. Lactase levels remained unaffected, but a significant decrease (30.50 %, $p < 0.05$) in sucrase activity in jejunum from 20 mg/kg lindane-treated rats was found.

After oral administration lindane reaches the enterocyte brush border membrane directly. Therefore, the lindane inhibitory effect on maltase might be caused by a local direct action of the pesticide on this membrane protein during its resorption in the small intestine. To find out if the lindane effects on intestinal enzymes are similar when the pesticide does not reach the brush border membrane directly, the pesticide was injected by another pathway, reaching the intestine from systemic circulation. One of the routes most frequently used to administer liposoluble toxicants, like lindane, is subcutaneous injection (13, 14). In addition, the studies of CARRERO *et al.* (7) showed that epithelial cells from rat intestinal mucosa accumulated lindane when animals were injected subcutaneously with 10 mg/kg of this pesticide for 12 days.

For this purpose, disaccharidase and alkaline phosphatase activities in jejunal brush border vesicles from rats s.c. injected with 10 and 20 mg/kg of lindane over 7 and 15 days were determined. Results showed that s.c. lindane administration induced similar effects to oral treatment. Thus, pesticide injection at 10 mg/kg doses over 7 or 15 days did not induce changes in any enzyme activity. Lactase and alkaline phosphatase activities remained unaffected in those animals injected with 20 mg/kg over both treatment periods. Neither was there a change in sucrase activity in rats treated with this higher dose (data not shown), which contrasts with the decrease observed in this enzymatic activity after oral lindane treatment. However, a significant decrease ($p < 0.05$) in maltase activity of lindane-treated rats at a 20 mg/kg dose over both treatment periods was observed (table II).

When lindane s.c. treatment was performed at two same doses over a longer period (30 days) no changes were found in lactase, sucrase and alkaline phosphatase activities (data not shown). A significant inhibition ($p < 0.05$) in maltase activity by lindane treatment was found in rats injected with 10 mg/kg of lindane. However daily administration of 20 mg/kg of lin-

Table II. *Effect of different subcutaneous lindane treatment periods on intestinal maltase, activity in rat*
Enzymatic assays were performed in jejunal brush border vesicles from rats injected s.c. with lindane dissolved in sesame oil at doses of 10 (L10) and 20 (L20) mg./kg over 7, 15 and 30 days and from control rats (injected with sesame oil alone). Results are expressed as mean ± SE. In parentheses the number of animals. Values are given as μmoles of maltose hydrolyzed per min per g protein. *$p < 0.05$.

	7 days (8)	15 days (7)	30 days (7)
Control	3897.64 ± 250.19	3947.43 ± 304.02	3104.72 ± 235.67
L 10	3681.06 ± 256.99	3269.05 ± 252.24	2109.71 ± 312.02*
L 20	3141.36 ± 202.52*	3129.16 ± 248.61*	2576.04 ± 191.35

dane did not modify this enzyme activity (table II).

The present results seem to suggest that maltase activity is more sensitive to lindane action than other disaccharidases, such as lactase and sucrase.

Previous studies with lindane have shown the ability of this toxicant to alter the activity of some intestinal brush border membrane enzymes. NEDKOVA-BRATANOVA *et al.* (23) found an inhibition of lactase, sucrase and maltase activities after 90 days of lindane treatment at a 14.8 μmol/kg dose. Such lengthy duration of the treatment could explain the differences with respect to our results. RAVINDER *et al.* (26) observed that hydrolytic enzymes in mice small intestine, such as disaccharidases, amylase, dipeptidases and phosphatases were affected after dietary intoxication with 200 and 400 ppm of γ-HCH for two weeks. These authors, however, contrary to what was observed in the present study, found a significant decrease in lactase and sucrase activities without changes in maltase levels. Earlier studies performed by our group on chickens injected with 10 and 20 mg/kg of lindane over 7 days showed this, as in this study, maltase activity decreased in chickens injected with 20 mg/kg of lindane without changes in sucrase levels. However, a marked inhibition of alkaline phosphatase activity was observed in two groups of chickens injected with lindane (22) unlike what was observed in rats. Similar alterations in intestinal enzyme activities have been described after treatment with other organochlorinated pesticides such as endosulfan (34) and DDT (12).

Lindane has been shown to induce alterations in lipid composition and membrane fluidity in rat isolated enterocytes (7). In this sense, previous studies carried out by our group have shown that 30 mg/kg lindane administration to chickens during 7 days leads to changes in intestinal mucosa lipid composition (22).

Intestinal brush border enzymes are membrane-bound proteins. The activities of some of these enzymatic proteins have been observed to appear to be influenced by changes in the lipid composition and/or fluidity of the rat intestinal microvillus membrane (2, 3). In this sense, the studies of BRASITUS *et al.* (4) showed that the activities of lactase, sucrase and maltase –which are largely external to the membrane– do not seem to be influenced by either lipid or fluidity alterations in rat intestinal microvillus membrane. However, the activity of alkaline phosphatase –a smaller molecular weight protein that appears more deeply embedded within the hydrophobic core of the membrane (18)– is more dependent upon the lipid environment of this membrane. Therefore, we suggest that changes in the maltase activity observed in lindane-treated rats might

be due to a direct action of the pesticide on hydrophobic domains of this enzymatic protein rather than on a secondary effect as a result of modifications induced by lindane treatment in intestinal mucosa lipid composition.

Lindane inhibitory effect on maltase activity was related to the injected dose and the duration of the pesticide exposure period, but it seems to be independent of the route of lindane administration. Both oral or s.c. lindane administration at dose of 20 mg/kg over 7 and 15 days can be observed to decrease maltase activity, but a longer period of lindane exposure (30 days) was necessary to observe alterations in this enzyme activity when lindane is administered at a lower dose (10 mg/kg). These facts seem to suggest the necessity of a certain level of pesticide accumulation in intestinal epithelium to induce significant alterations on maltase activity. The higher dose administration and a longer period of pesticide exposure would facilitate lindane accumulation. However, the inhibitory effect in maltase activity observed in rats injected with 20 mg/kg of lindane over 7 and 15 days did not appear in the small intestine of over 30 days treated rats. This fact seems to suggest that the organism develops possible regulatory mechanisms to oppose the alterations induced by the lindane higher dose. To determine when these possible mechanisms were able to counteract lindane effects on intestinal maltase during the pesticide exposure period, s.c. treatments at a 20 mg/kg dose over 20 and 25 days were carried out. Results show that lindane administration (20 mg/kg) over 20 days induced a significant decrease ($p < 0.05$) in intestinal maltase activity, but that there were no significant changes in this enzyme activity in brush border vesicles from over 25 days lindane-treated rats (fig. 1). This suggests that the accommodation effect to the toxic lindane action took place between 20 and 25 days of pesticide exposure. A similar effect has been described by NEDKOVA-BRATANOVA *et al.* (23). These authors observed that lindane treatment at a 14.8 µmol/kg dose over 15 days induces a significant decrease in the activity of some brush border dipeptidases. However, these enzyme activities remained unaffected in rats injected with the pesticide over 60 days, whereas the inhibitory effect was again observed in animals treated during 90 days. Therefore, their results, similar to ours, seem to suggest the development of possible regulatory mechanisms to oppose the alterations induced by lindane presence, but these mechanisms would not be able to counteract lindane toxic action, if pesticide exposure were prolonged over a longer period.

In conclusion, the present results suggest that lindane treatment induces alterations in intestinal maltase activity, which are related to the administered dose and

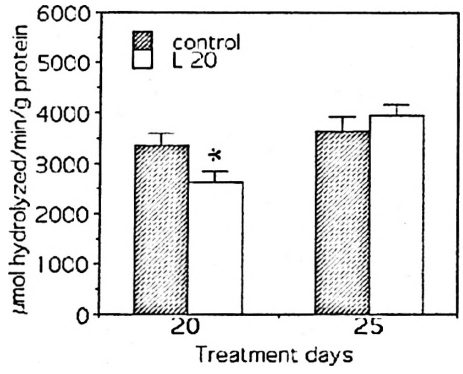

Fig. 1. *Maltase activity in jejunal brush border vesicles of rats injected s.c. with 20 mg/kg of lindane dissolved in sesame oil during 20 and 25 days in comparison with the control groups (injected with sesame oil alone).*

Results are expressed as mean ± SE. Values are given as µmoles of maltose hydrolyzed per min per g protein. The number of animals per groups was eight. *$p < 0.05$.

the duration of the lindane exposure period. In addition, present data seem to suggest the development of adaptative processes to oppose lindane toxic action. Further studies are needed to elucidate the mechanism(s) involved in intestinal alterations induced by lindane treatment.

Acknowledgements

This work was supported by the Spanish "Ministerio de Educación y Ciencia" CICYT nº SAL 90-0627. M. J. Moreno has a fellowship from Consejo Asesor de Investigación de la Diputación General de Aragón (Spain).

M. J. MORENO, S. PELLICER y M. P. FERNÁNDEZ-OTERO. *Efecto de diferentes tratamientos subcrónicos con lindano sobre algunas enzimas del borde en cepillo de yeyuno de rata.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (1), 37-44, 1996.

La administración subcrónica de lindano (20 mg/kg, 7 y 15 días) por vía oral o s.c., inhibe significativamente la actividad maltasa de yeyuno de rata, sin que se observen alteraciones en los animales inyectados con 10 mg/kg de lindano. Cuando la administración s.c. de lindano (10 mg/kg) se prolonga hasta 30 días se observa una disminución significativa en la actividad maltasa, sin que la inyección de una dosis de 20 mg/kg altere la actividad de dicha enzima. Cuando esta dosis de lindano se inyecta durante 20 días se observa una disminución significativa de la actividad maltasa, sin observarse cambios si el tratamiento se prolonga a 25 días. Este hecho parece sugerir que entre los días 20 y 25 de exposición al pesticida, el organismo desarrolla posibles mecanismos de defensa para contrarrestar las alteraciones inducidas por la dosis de 20 mg/kg de lindano sobre la actividad maltasa. Las actividades lactasa y fosfatasa alcalina no se ven modificadas por acción del lindano en ninguno de los diferentes tratamientos llevados a cabo, y la actividad sacarasa sólo se modifica tras la administración oral de 20 mg/kg durante 15 días. En conclusión, la actividad maltasa parece ser más sensible a la acción del lindano que la actividad de otras enzimas del borde en cepillo y, los efectos del lindano sobre esta proteína enzimática dependen de la dosis administrada y de la duración del período de exposición a la acción del pesticida.

Palabras clave: Lindano, Pesticidas, Disacaridasas, Fosfatasa alcalina, Membrana apical intestinal.

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