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Induction of Lipid Peroxidation and Alteration of Glutathione Redox Status by Endosulfan

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ABSTRACT

The oxidant stress-inducing effects of endosulfan, a chlorinated hydrocarbon insecticide of the cyclodiene group, have been examined following ig administration of single and repeated doses. A single dose of 30 mg/kg (~30% LD_{50}) endosulfan significantly ($p < 0.001$) increased the TBARS and, hence, the lipid peroxidation in cerebral and hepatic tissues of rats. Administration of endosulfan with doses of 10 or 15 mg/kg/d for 5 d has also induced lipid peroxidation significantly ($p < 0.05$). The same doses caused a significant alteration in glutathione redox status of cerebral and hepatic tissues, where total glutathione and oxidized glutathione were measured by an enzymatic cycling procedure. Selenium levels were also determined and compared with controls. With repeated doses, oxidant stress was more pronounced in cerebral tissue, where endosulfan shows a GABA-antagonistic activity. The possible relationship between the neurotoxicity of endosulfan and its oxidant stress-inducing effect was discussed.

Index Entries: Cyclodiene insecticide endosulfan; induction of lipid peroxidation; alteration of glutathione; tissue selenium levels in rats; GABA antagonistic effect.

INTRODUCTION

Endosulfan (CAS 115-29-7) is a chlorinated hydrocarbon insecticide developed and introduced in the early 1950s. With its chlorinated cyclodiene structure (Fig. 1), endosulfan belongs to a group of insecticides of which various members, such as endrin, dieldrin, or aldrin, have been banned owing to their longer persistence and higher toxicity to mam-

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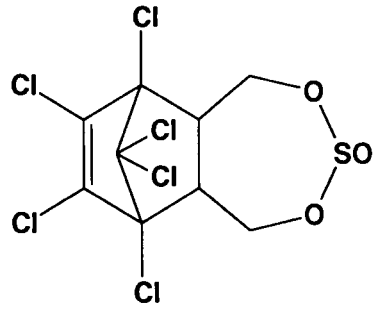

Fig. 1. Chemical structure of endosulfan.

mals. However, endosulfan is rapidly metabolized and still in use worldwide. It is not only one of the top ten insecticides consumed in large amounts, but also the only organochlorine insecticide used in agriculture in Turkey. Its widespread use has caused our concern about the effects of persistent exposure of human and animal systems to it either directly or as an environmental pollutant. Perturbation of cell membranes and lipid peroxidation were reported as a consequence of various organochlorine insecticides *(1,2)*. We have, therefore, been interested in the oxidant stress-inducing effects of endosulfan, and examined the glutathione redox status, lipid peroxidation, and selenium levels in cerebral and hepatic tissues of rats exposed to endosulfan.

MATERIALS AND METHODS

Male albino Wistar rats, weighing 180 ± 20 g, were used. The animals were maintained in standard laboratory chow, and allowed free access to food and water. Technical-grade endosulfan was a gift from Bayer, İstanbul. Rats received ig endosulfan for 1 or 5 d in corn oil or the vehicle, and were decapitated 24 h after the last dose.

Total glutathione and oxidized glutathione (GSSG) content of tissues was determined by an enzymatic cycling assay as described by Tietze *(3)*, and modified by Hazelton and Lang *(4)*. Glutathione redox ratio was calculated as [(GSSG/(GSH + GSSG)] × 100. The concentration of thiobarbituric acid reactive substances (TBARS) of whole homogenized liver and cerebral tissue was determined as a measure of lipid peroxidation according to the procedure of Ohkawa et al *(5)*.

Tissue selenium levels were determined by a spectrofluorimetric method *(6)*. Verification of precision, accuracy, and sensitivity was accomplished by the direct use of Standard Reference Material (SRM) (Seronorm TM Trace S by Nycomed, Oslo, Norway). Protein concentrations were determined by the standard method of Lowry *(7)*. The data

Table 1
Glutathione Redox Status in Cerebral and Hepatic Tissues of Rats Treated with Endosulfan

Tissue	Parameter	Mean ± SEM			
		Control, $n = 6$	Endosulfan 30 mg/kg, ig, $n = 4$	Endosulfan* 10 mg/kg/d, ig $n = 6$	Endosulfan* 15 mg/kg/d, ig $n = 6$
Cerebral	GSSG**	8.4 ± 0.70	9.9 ± 0.15[a]	12.5 ± 1.00[c]	12.6 ± 0.80[e]
	Total GSH***	1.88 ± 0.07	1.77 ± 0.02	1.75 ± 0.12	1.80 ± 0.08
	Glutathione redox ratio (GSSG%)	0.44 ± 0.03	0.56 ± 0.01[c]	0.72 ± 0.04[e]	0.71 ± 0.05[e]
Hepatic	GSSG**	132.6 ± 9.30	138.8 ± 4.70	186.2 ± 21.2[a]	170.7 ± 3.10[d]
	Total GSH***	7.38 ± 0.33	6.32 ± 0.68	7.64 ± 0.39	7.94 ± 0.48
	Glutathione redox ratio (GSSG%)	1.78 ± 0.08	2.25 ± 0.17[b]	2.42 ± 0.20[c]	2.20 ± 0.16[a]

*Repeated doses for 5 d.
**nmol GSH Eq/g wet wt.
***µmol GSH Eq/g wet wt.
[a]$p < 0.05$, [b]$p < 0.02$, [c]$p < 0.01$, [d]$p < 0.002$, [e]$p < 0.001$ v control.

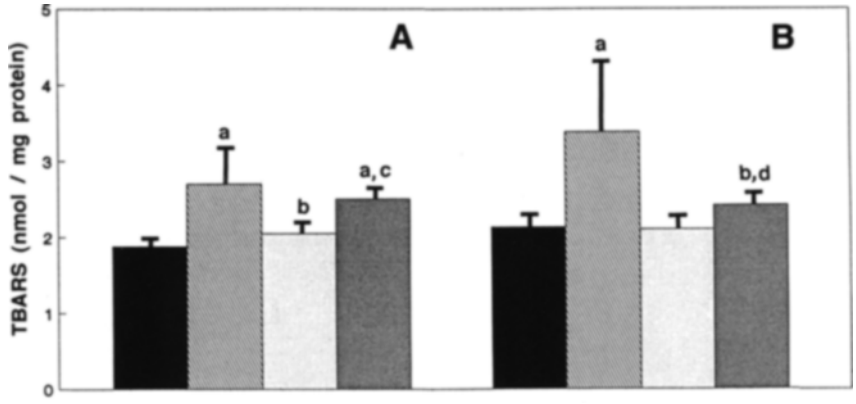

Fig. 2. TBARS levels (measured 24 h after the last dose and given as mean ± SEM) in cerebral (A) and hepatic (B) tissues of rats treated with ig endosulfan. ■ Control group (n = 15); ▨ 30 mg/kg (single dose), ■ 10 mg/kg/d and ▨ 15 mg/kg/d (repeatedly dosed for 5 d) endosulfan groups (n = 6, each). $^{a}p < 0.001$, $^{b}p < 0.02$ vs controls; $^{c}p < 0.002$, $^{d}p < 0.02$ vs 10 mg/kg/d endosulfan group.

Table 2
Selenium Levels in Cerebral and Hepatic Tissues of Rats Treated with Endosulfan

	Selenium, ng/g tissue, mean ± SEM, n = 6	
Tissue	Controls	15 mg/kg/d, ig, endosulfan, 5 d
Cerebral	254 ± 23	221 ± 25
Hepatic	920 ± 56	803 ± 58

were analyzed for significant differences by one-way Anova and Student's t-test using the Microsta statistical package.

RESULTS

As shown in Table 1, ig endosulfan treatment of rats for 5 d did not cause any significant alteration in brain and liver GSH content. However, GSSG levels and, hence, the glutathione redox ratio increased significantly. At different doses, the alterations of the glutathione redox status were not significantly different, possibly because of the small difference between the two doses used (~10–15% of LD_{50} value).

With a single dose of endosulfan (30 mg/kg, ~30% of LD_{50}), significantly high levels of TBARS were measured in both tissues. The same was observed with repeated doses and the response was dose-dependent

(Fig. 2). Selenium levels were measured only in cerebral and hepatic tissues of rats, exposed to 15 mg/kg/d endosulfan for 5 d. Although a tendency toward decrease was observed, as seen in Table 2, the differences were not significant.

DISCUSSION

"Oxidant stress" is associated with a disturbance in the pro-oxidant-antioxidant balance in favor of the pro-oxidant. Alteration in this balance is a key feature of many physiological and pathophysiological phenomena, and processes as diverse as inflammation, aging, carcinogenesis, drug action, and drug toxicity *(8)*. Glutathione-dependent mechanisms in the cell constitute important defenses against oxidant stress. During the destruction of H_2O_2 and lipid hydroperoxides by glutathione peroxidase (GSH-Px), GSH is oxidized to GSSG, which is then reduced back to GSH by glutathione reductase-utilizing NADPH. By measuring the glutathione redox status in the target organs, brain and liver, we have, hence, directly assessed the magnitude of oxidant stress. Our results indicate that endosulfan causes an oxidant stress in both tissues, being more pronounced in cerebral tissue. Lipid peroxidation, which can lead to cell dysfunction or death, is often caused by oxidant stress. Our findings regarding TBARS induction are parallel to glutathione redox status alterations and support the occurrence of an oxidant stress with exposure to endosulfan. On the other hand, selenium is involved, in concert with vitamin E, in protecting polyunsaturated membrane lipids from oxidative degradation that would result in membrane dysfunction. Three forms of selenium-containing GSH-Px have been identified. Both the soluble cytosolic and the plasma forms metabolize H_2O_2 and lipid hydroperoxides *(9)*. The third form, thought to be associated with cell membranes, can also metabolize phospholipid hydroperoxides, which are not metabolized by intracellular and extracellular forms of the enzyme *(10)*. Therefore, instead of GSH-Px measurement, we examined the tissue selenium status. A decrease of 11% was observed in both whole brain and liver selenium content. However, possibly because of short-term (5 d) exposure, alterations were not significant.

Like other members of cyclodiene insecticides, the acute toxic effects of endosulfan are primarily the result of hyperexcitation in the nervous system. The mechanism of action of these compounds has been only recently elucidated and purported to involve binding at the picrotoxinin site in δ-aminobutyric acid (GABA) chloride ionophore complex *(11)*. Thus, cyclodienes mimic the action of picrotoxin, the nerve excitant and antagonist of the neurotransmitter, GABA, which induces the uptake of chloride ion by neurons. The blockage of this activity results in a state of uncontrolled excitation. Cyclodienes are also potent inhibitors of Na^+, K^+-ATPase, and more importantly the enzyme Ca^{2+}, Mg^{2+}-ATPase that is essential for transport of calcium across membranes. These inhibitions

result in an accumulation of intracellular free calcium ions with the promotion of calcium-induced release of neurotransmitters and, hence, the propagation of stimuli throughout the CNS *(12)*.

On the other hand, dysfunctions of GABA system have been associated with certain neurological disorders and in the etiology of degenerative diseases, including ischemia and seizure-related brain damages. In addition to several other factors, free radical processes may play an important role *(13)*. Furthermore, the synthesis and the effects of GABA are in a sensitive balance with the excitatory neurotransmitter L-glutamate. It has been reported that the release of endogenous glutamate and aspartate from rat hippocampal slices was significantly increased when the slices were incubated with xanthine oxidase plus xanthine to produce superoxide and hydroxyl free radicals locally *(14)*. These releases and neuronal damages observed with neuroexitatoxin kainate have been reported to be prevented by free radical scavengers, such as allopurinol, SOD, and mannitol *(14,15)*. Therefore, in the neurotoxicity of endosulfan, free radicals and, hence, the induction of oxidant stress, as shown in this study, might be involved. However, further studies are certainly needed for such a conclusion.

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