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# Effect of Intratracheal Administration of DDT and Endosulfan on Cytochrome P-450 and Glutathione-s-transferase in Lung and Liver of Rats

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The lungs are a prominent target organ for numerous types of chemically induced pathological changes (Witschi and Cote 1977). They are situated in a primary site for exposure to xenobiotics both externally by inhalation and internally by circulation (Remmer 1974; Gram 1980). Lungs contain enzymatic systems capable of metabolizing drugs and other chemicals, and metabolic activation of certain compounds is critical for the onset of cellular damage (Gillett et al. 1974; Boyd 1980). Lung also contains defence mechanisms against reactive electrophiles and nucleophiles generated during metabolism of chemicals (Dunbar et al. 1981; Boyd et al. 1982). There are several studies which show that DDT and endosulfan enhance the activities of hepatic drug metabolizing enzymes in animals (Casida 1978; Agarwal et al. 1978; Dorough et al. 1978; Down and Chasseaud 1978, 1981; Tyagi et al. 1983). However very little information is available on the effect of these insecticides when given through pulmonary route, on the drug metabolizing enzymes of rats. Reduced glutathione has been shown to provide protection to lung and other organs against reactive intermediates. Using in vitro studies it has been shown that styrene oxide, benzo(a)pyrene affect the activity of glutathione-s-transferase and the ratio of reduced:oxidized glutathione (Smith and Bend 1981; Steel et al. 1981). No such information is available on the effect of DDT and endosulfan given through pulmonary route. In view of these, studies were undertaken to assess the effect of intratracheally administered DDT and endosulfan on pulmonary and hepatic drug metabolic activity, glutathione-s-transferase activity and the levels of reduced and oxidized glutathione in rats, and the results are reported.

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## MATERIALS AND METHODS

Male rats (wistar strain, weighing 180g to 200g) were fed Hind Lever Diet (Hindustan Lever Ltd., Bombay, India) ad-libitum and had free access to water all the time. The rats were divided into three groups and given the treatments shown below:

Rats of group A were given DDT intratracheally (5mg/100g body weight) for three consecutive days. Rats of group B were given endosulfan intratracheally (1mg/100g body weight) for three consecutive days. Rats of group C were given only the vehicle solution intratracheally for the same period and were the controls. The insecticides were dissolved in a minimum volume of ground nut oil and were administered intratracheally through mouth with a plastic cannula. Twenty four hours after the administration of the last dose of respective insecticides, rats were fasted over night and anaesthetized with sodium pentobarbital (Abbott laboratories, Bombay, India; 6 mg/100 g body weight, given i.p.) and their thoracic cavities opened. The lungs were perfused through right ventricle with ice cold physiological saline. After perfusion the lungs and livers were removed, immersed in ice cold saline, cleaned and weighed. Microsomes were isolated from lung and liver for P-450 estimation by the method of Ritter and Malejka-Giganti (1982) and for other enzymatic assays by the procedure of Zannoni et al. (1972). The protein was estimated by the method of Lowry et al. (1951).

The cytochrome P-450 in lung and liver microsomal fraction was determined as described by Omura and Sato (1964). However for the lung, only the 450 nm peak was used for calculation. The activities of NADPH-cytochrome c reductase (Christensen and Wissing 1972), N-demethylase (Kato and Gillette 1965), and aniline hydroxylase (Imai et al. 1966) were also determined. Glutathione-s-transferase activity was assayed by the method of Habig et al. (1974). The levels of reduced and oxidized glutathione in lung and liver tissues were determined according to Keizomaita et al. (1983).

## RESULTS AND DISCUSSION

Intratracheal administration of DDT and endosulfan did not significantly affect the body weights, lung and liver weights, except that DDT significantly increased the liver wet weight as compared to the control (Table 1,2). Because of differences in chemical structure between DDT and endosulfan, the latter is more polar (Peterson and Robinson 1964; Gupta and Gupta 1979). DDT administration significantly increased both the microsomal protein and cytochrome P-450 content of lung and liver whereas no such effects were seen in endosulfan treated

Table 1. Effect of intratracheal administration of DDT and endosulfan on rat lung microsomal mixed function oxidases.

| | Control | DDT | Endosulfan |
|---|---|---|---|
| Organ Weight (g) | 1.93±0.11 | 2.16±0.82 | 1.78±0.21 |
| Microsomal protein (mg/g tissue) | 3.61±0.11 | 4.41±0.29* | 4.11±0.31 |
| Cytochrome P-450 (nmol/mg protein) | 0.077±0.008 | 0.097±0.005* | 0.071±0.005 |
| NADPH-cyt c reductase (nmol cyt c red/ min/mg protein. | 35.95±2.39 | 39.58±2.75 | 39.50±2.62 |
| Aminopyrine N-demethylase (nmol HCHO formed/ hr/mg protein) | 29.72±1.11 | 34.34±1.86 | 31.00±1.31 |
| Aniline hydroxylase (nmol p-aminophenol formed/hr/mg protein) | 11.49±0.35 | 13.58±1.61 | 15.75±3.05 |

Values are presented in mean±SE from at least six to eight animals in each group.

* Significantly different from controls ($p \leq 0.05$).

rats (Table 1,2). Similar effect of DDT on cytochrome P-450 have been reported by others in studies where DDT was given orally (Bunyan et al. 1970; Bunyan and Page 1973; Bickers et al. 1974). Endosulfan has been reported to be a weak inducer of cytochrome P-450 (Dorough et al. 1978; Tyagi et al. 1983). In the present investigation insecticides were given intratracheally, therefore their effects in liver would be due to either untransformed or metabolites of DDT and endosulfan, which got into the blood circulation and reached to other extrapulmonary tissues. Another interesting observation was that the induction of cytochrome P-450 by DDT was tissue specific. In liver DDT brought about a 52% increase in cytochrome P-450 level whereas in lung it was only about 25% (Table 1,2). DDT administration did not affect the activity of NADPH-cytochrome c reductase, aminopyrine N-demethylase and aniline hydroxylase in lung, whereas in liver DDT

Table 2. Effect of intratracheal administration of DDT and endosulfan on rat liver microsomal mixed function oxidases.

| | Control | DDT | Endosulfan |
|---|---|---|---|
| Organ weight(g) | 5.31±0.16 | 6.67±0.26* | 5.31±0.31 |
| Microsomal protein (mg/g tissue) | 22.64±0.94 | 29.40±0.79* | 20.25±0.97 |
| Cytochrome P-450 (nmol/mg protein) | 0.882±0.108 | 1.343±0.084* | 0.699±0.085 |
| NADPH-cyt c reductase (nmol cyt c red/ min/mg protein. | 73.96±2.99 | 116.08±4.29* | 69.49±4.90 |
| Aminopyrine N-demethylase (nmol HCHO formed/ hr/mg protein) | 94.07±3.93 | 120.81±6.40* | 117.09±4.21* |
| Aniline hydroxy-lase (nmol p-amino phenol formed/hr/ mg protein) | 25.49±0.35 | 27.43±0.54 | 23.40±0.43 |

Values are expressed as mean±SE from six to eight animals in each group.

* Significantly different from controls($p \leq 0.05$).

significantly increased activities of NADPH-cytochrome c reductase and aminopyrine N-demethylase. The reason for this could be that the induction of cytochrome P-450 level in lung was not enough to effect the activity of other components of mixed function oxidase system (Copeland and Cranmer 1974; Sell et al. 1972). Endosulfan administration did not affect the activities of NADPH-cytochrome c reductase and aniline hydroxylase in lung and liver of rats except that of aminopyrine N-demethylase in liver which was increased significantly as compared to the control (Table 1,2). These results are similar to those reported by others where endosulfan was given orally (Agarwal et al, 1978; Tyagi et al. 1983).

Table 3. Glutathione-s-transferase activity, GSH and GSSG levels in rat lung and liver by intratracheal administration of DDT and endosulfan.

| Tissue | Control | DDT | Endosulfan |
|---|---|---|---|
| | | Cytosolic Protein(mg/g tissue) | |
| Lung | 41.15±3.85 | 42.25±1.02 | 38.99±1.95 |
| Liver | 52.59±2.61 | 49.71±0.53 | 54.07±0.82 |
| | | Glutathione-s-transferase (nmol. 2:4-dinitrochlorobenzene conjugated/min/mg protein) | |
| Lung | 739.00±33.00 | 805.00±70.00 | 809.00±41.51 |
| Liver | 1735.17±105.00 | 1836.11±133.62 | 1683.89±41.51 |
| | | Reduced Glutathione(GSH) | |
| Lung | 0.04±0.001 | 0.03±0.002 | 0.03±0.01 |
| Liver | 2.34±0.22 | 3.95±0.23* | 2.98±0.39 |
| | | Oxidized Glutathione(GSSG) (mg/organ) | |
| Lung | 0.21±0.02 | 0.47±0.03* | 0.31±0.02* |
| Liver | 1.43±0.05 | 1.40±0.09 | 0.44±0.08* |
| | | GSH:GSSG Ratio | |
| Lung | 0.19 | 0.07 | 0.10 |
| Liver | 1.64 | 2.82 | 6.77 |

Each value represents the mean±SE of the results from six to eight animals in each group.

* Significantly different from controls ($p < 0.05$).

Reduced glutathione levels (GSH) have been shown to provide protection to lung and other organs against reactive intermediates. Glutathione-s-transferase catalyzes the conjugation of reduced glutathione with various xenobiotics. In *in vitro* studies it has been shown that benzo(a)pyrene and styrene oxide affect the activity of glutathione-s-transferase and the ratio of GSH:GSSG(Smith and Bend 1981; Steel et al. 1981). It has been suggested that the amounts of GSH *in vivo* could be the limiting factor for induced glutathione-s-transferase activity. We have estimated the levels

of GSH and GSSG and glutathione-s-transferase activity (Table 3) to assess the protection afforded by this system against the toxicity of intratracheally administered DDT and endosulfan. The results showed that neither of these insecticides affected the activity of glutathione-s-transferase either in lung or in liver. DDT significantly increased the levels of GSH in liver and of GSSG in lung whereas endosulfan did not affect the levels of GSH neither in lung nor in liver.However endosulfan significantly raised the levels of GSSG in both lung and liver. The ratio of GSH:GSSG in the cells are influenced by the activity of glutathione synthetase, glutathione peroxidase, glutathione reductase and glutathione-s-transferase. The increase in ratio of GSH:GSSG in liver of DDT and endosulfan treated rats could be either at the glutathione peroxidase step or at glutathione synthetase step. The ratio of GSH:GSSG was profoundly reduced in lung suggesting an enhanced scavanging of peroxides by GSH, under these experimental conditions. Normally GSH would be regenerated through the action of glutathione reductase which utilizes NADPH. It is likely that the increased level of GSSG may be either due to the reduced activity of glutathione reductase or due to the limitations on the availability of the reducing equivalents. Further work is in progress along these lines.

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