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Dimethoate Induced Lipid Peroxidation and Inhibition of Type-I Iodothyronine 5'-Monodeiodinase Activity in Young Cockerel

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Dimethoate (O,O-dimethyl S-methyl-carbamoyl-methyl phosphorodithioate), a moderately toxic organophosphorus systemic pesticide (WHO 1989), is primarily known as a neurotoxin (Jeyaratnam and Maroni 1994). It is also known to inhibit metabolic enzymes (Awasthi et al. 1984). However, not a single report is available at present on dimethoate toxicity in relation to thyroid function. In general, literature on dimethoate toxicity in birds is meagre (Francis et al. 1985), despite the fact that birds are considered as the main non-target animals to be infected by the pesticide. Therefore, the present study was primarily aimed to investigate the possible interference by dimethoate in thyroid function of cockerels with a special emphasis on 5'-monodeiodination of thyroxine (T_4) to triiodothyronine (T_3), the principal pathway of T_3 generation (Kuhn 1990).

Free radical induced lipid peroxidation (LPO) of biomembranes leading to the loss of membrane integrity is well established (Halliwell and Gutteridge 1985). Since the cellular toxicity is correlated with the generation of free radicals (Bagchi and Stohs 1993), an attempt has also been made to correlate the pesticide induced alterations in type-I iodothyronine 5'-monodeiodinase (5'-D) activity, if any, with the possible involvement of free radicals as evidenced by changes in lipid peroxidative process.

MATERIALS AND METHODS

Dimethoate (Rogor 30% EC) was obtained from Rallis India Ltd., Bombay. L-thyroxine and dithiothreitol (DTT) were purchased from Sigma Chemical Co., USA; thiobarbituric acid (TBA), sodium dodecyl sulphate (SDS), ethylenediamine

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tetra acetic acid (EDTA) and all other chemicals were of reagent grade and were supplied by Loba Chemie, India. Radioimmunoassay (RIA) kits for the estimation of T_4 and T_3 were obtained from Bhabha Atomic Research Centre, Bombay, India.

Day old male broiler chicks were supplied by local commercial producer and were divided into four groups of eight each. They were housed in temperature (27±1°C) and light (14 hr light : 10 hr dark) controlled room with the provision of grower feed and water *ad libitum.* After one week of acclimatization; groups 2, 3 and 4 were administered orally with dimethoate (dissolved in corn oil) at the doses of 2, 4 and 8 mg/kg body weight, respectively, daily for four weeks. These doses correspond to 5, 10 and 20% of the oral LD_{50} (Sanderson and Edson 1964). The duration of exposure was according to the earlier study made in broiler chicks (Majumder et al. 1994). Group 1, receiving equivalent amount of vehicle only, served as control.

On the last day, the birds were sacrificed, blood of each was collected by cardiac puncture method, centrifuged and serum samples were stored at -20°C for the estimation of T_3 and T_4

For the assay of 5'-D, the liver was homogenized in 3 volume (wt/volume) ice cold 0. 1*M* phosphate buffer (pH 6.5) containing 0.25 *M* sucrose and 5 m*M* EDTA using a Potter-Elvehjem teflon-glass homogenizer. The homogenate was then centrifuged at 2000 g at 4°C for 30 min and the supernatant was used for the assay of 5'-D according to the method of Kahl et al. (1987) with some modifications. In brief, the supernatant was incubated with T_4 (1.3 µ*M*) for 1 hr at 37°C. The incubation mixture contained 100 µL supernatant, 200 µL assay buffer, 200 µL DTT (2m*M* final concentration) and 10 µL T_4. The incubation was terminated with the addition of 1 mL 100% ethanol and kept at 4°C overnight. Samples were centrifuged and the resulting supernatants were kept at -20°C until the *in vitro* generation of T_3 was determined. Each incubation assay included a blank tube which was devoid of T_4. *In vitro* generation of T_3, due to the incubation of liver homogenate in the presence of exogenous T_4 was expressed as ng T_3 produced per hr per mg protein.

Serum concentrations of T_4 and T_3 were estimated by RIA following the method of Brown et al (1970) with little modification (Kar and Chandola-Saklani 1985). RIA for the specific measurement of *in vitro* generation of T_3 (for the assay of 5'-D) was done according to the method described earlier (Visser et al. 1975).

The assay of LPO was done by the thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to the peroxidation of lipids, according to the method of Ohkawa et al. (1979). The liver and kidney were

homogenized in ice cold 0.15 *M* phosphate buffer (pH 7.4, 10% wt/volume) and the homogenates were centrifuged at 2000 g at 4°C for 30 min. The amount of MDA formed was measured by taking the absorbance at 532 nm (extinction coefficient, $\varepsilon = 1.52 \times 10^5$) using a Shimadzu UV-160A spectrophotometer (Japan).

Hepatic and renal protein contents were estimated following the method of Lowry et al. (1951).

For statistical evaluation of the data analysis of variance (ANOVA), and student's t-test were followed.

RESULTS AND DISCUSSION

While serum T_4 decreased significantly in all the treated groups ($P < 0.001$ for all three doses; 33.7%, 33.3% and 26.6% in low, medium and high dose treated groups, respectively), T_3 concentration decreased significantly only by the two higher doses of pesticide, *i.e.*, 4 and 8 mg/kg body weight ($P < 0.001$ for both; 60.5% and 68.8% decrease, respectively; figure 1). The significant decrease in hepatic 5'-D activity was observed only in the medium (46.7%, $P < 0.001$) and highest (59.1%, $p<0.001$) dosed groups compared to the control value (figure 1). The decreases in T_3 concentration and 5'-D activity in lowest dose treated group were only 4.6% and 6.6%, respectively. A dose dependent increase in LPO (figure 2) was observed in both liver and kidney ($p<0.001$ for all the three doses when compared to respective control values for both the tissues) of dimethoate treated birds. The percentage increases in LPO were 68.1%, 87.5% and 125% in liver; and 55.1%, 74.4% and 96.2% in kidney for the doses 2, 4 and 8 mg/kg body weight, respectively.

Serum T_4 concentration decreased at all the doses of dimethoate indicating that a dose as low as 2 mg/kg body weight of this insecticide is thyroid inhibitory in nature. Since thyroid gland is the only organ that is capable of synthesizing T_4, it appears that dimethoate inhibits T_4 synthesis and/or release at the glandular level. Similar observations have been made in fish exposed to another organophosphate pesticide (cythion) in which thyroidal ^{131}I uptake and the conversion ratio of protein bound ^{131}I in relation to total ^{131}I uptake in serum were reduced following pesticide treatment (Singh and Singh 1980). Decreases in serum T_3 concentration and hepatic 5'-D activity indicate that dimethoate inhibits the peripheral conversion of T_4 to T_3. In fact, T_3 is primarily generated through the 5'-monodeiodination of phenolic ring of T_4 in the peripheral tissues including liver and kidney (Kuhn 1990). Since no scientific investigation has been made so far on the dimethoate induced alterations

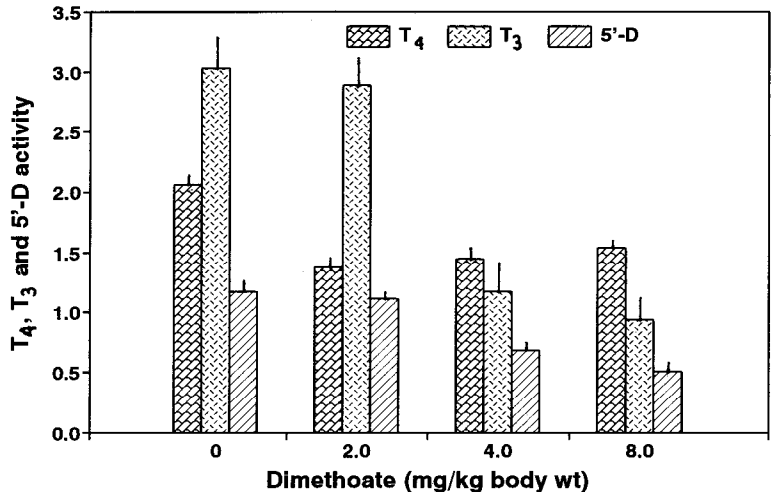

figure 1. Alterations in serum concentrations of T_4 (µg/100 mL) and T_3 (ng/mL); and hepatic 5'-D activity (ng T_3/hr/mg protein) following dimethoate administrations (mg/kg body weight) in young cockerels. Vertical lines indicate standard errors of the means.

in thyroid function in birds, our results can be compared to that of another organophosphate pesticide, *i.e.*, malathion, in fish, *Heteropneustes fossilis* (Yadav and Singh 1986). In this fish, malathion decreased T_4 concentration, as observed in the present study. However, it increased T_3 concentration , which is contradictory to the effect of dimethoate in cockerel. This discrepancy in the results may be a species specific or pesticide specific variation.

Increased LPO both in liver and kidney indicates the hepatotoxic and nephrotoxic effects of the pesticide. Since generation of free radicals has been observed earlier due to insecticide poisoning (Bagchi and Stohs 1993), in the present investigation, it appears that the toxic effects of dimethoate could be the result of pesticide induced free radical generation. Free radical generating system stimulates LPO in the biomembranes and continuous fragmentation of fatty acid side chains produces aldehydes and hydrocarbons eventually leading to the loss of membrane integrity (Halliwell and Gutteridge 1985). As dimethoate decreased the activity of 5'-D, a membrane bound enzyme (Sakame and Chopra 1990), the pesticide induced increase in LPO may be correlated with the decreased activity of 5'-D. This indicates

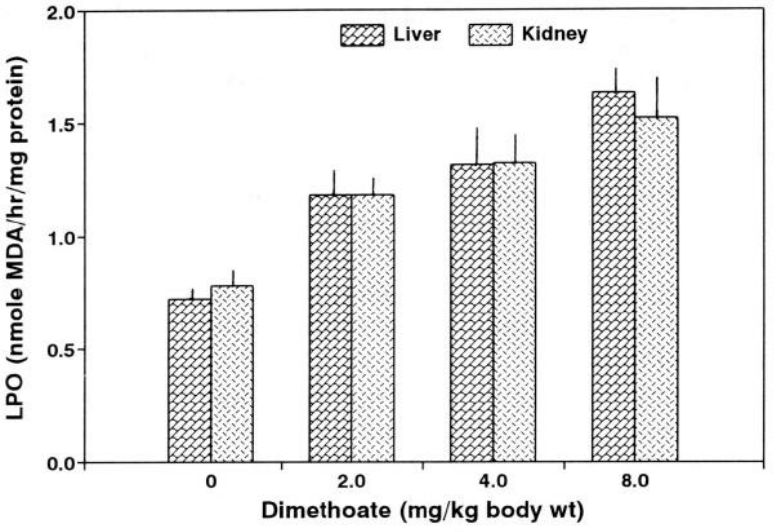

Figure 2. Induction of lipid peroxidation (nmole of MDA formed/hr/mg protein) in liver and kidney by different doses of dimethoate (mg/kg body weight) in cockerels. Vertical lines indicate standard errors of the means.

the possible impact of LPO on the activity of the said enzyme, as has been suggested earlier in rat (Huang et al. 1987) and in mice (Maiti et al. 1995).

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