Acute Effects of Organotins on Brain, Liver and Kidney in Rats

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Abstract: Effects of dioctyltin oxide (DOTO) tricyclohexyltin hydroxide (TCHTOH) and tributyltin oxide (TBTO) were examined on some enzymic activities in liver and kidney and biogenic amines level in brain of rats at 24 hours after single subcutaneous administration (25 μmole/100 g B. Wt.). All the organotin compounds produced a significant increase in the activity of alkaline phosphatase and adenosine triphosphatase and decrease in monoamine oxidase in both liver and kidney. DOTO and TCHTOH were more effective in impairing the activity of succinate dehydrogenase in liver. Concentrations of γ-aminobutyric acid (GABA) and dopamine were found to be significantly decreased in brain however, acetylcholine concentration remained unaltered. These results suggest that organotin compounds DOTO and TCHTOH are more toxic to rats than TBTO.

Key words: Organotin Compounds—Toxicity—Sulfhydryl—Oxidative Phosphorylation—GABA—Dopamine—Acetylcholine

INTRODUCTION

In continuation to the studies on inorganic tin\(^1\)\(^2\) we have extended our investigation towards the effect of organotin compounds on biological system. Organotin compounds have become of major interest in recent years due to their widespread use as heat stabilizer in the prevention of thermal degradation of many chlorinated compounds such as transformer oils, PVC (polyvinylidene chloride), chlorinated rubbers and modified plastics in our country. The dioctyltin derivatives were specifically developed for PVC items. Trisubstituted organotin derivative like TBTO and TCHTOH are extremely effective in controlling bacteria and fungus in hospitals. Due to the effective biocidal properties TBTO is extensively used in marine lumber preservatation. These compounds are also used in

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both agriculture and industry. The potential values of these biocidal organo compounds have stimulated an examination of their toxicity. These compounds are known to produce a range of neurological symptoms/disorders such as behavioral abnormalities, muscle weakness, cerebral edema and convulsions, and lowering in the level of biogenic amines in brain of experimental animals. It is also reported that organotin compounds produce significant alteration in delta aminolevulinic acid dehydratase and heme oxygenase (sulfhydryl dependent enzymes of heme metabolism), together with the depletion in cytochrome P-450 in liver; thus resulting to heavy loss into the drug oxidative mechanism of the system. Cyclic-AMP metabolism has also been found to be disturbed as a result of the exposure of organotin compounds.

On account of these facts, an attempt has, therefore, been made to examine the toxic effects of organotin compounds viz. dioctyltin oxide (DOTO), tricyclohexyltin hydroxide (TCHTOH) and tributyltin oxide (TBTO) on sulfhydryl dependent metabolic functions and energy metabolism of the liver and kidney and the concentration of biogenic amines in brain of rats to get an insight of their actions. The enzymes, lactate dehydrogenase, succinate dehydrogenase, monoamine oxidase, adenosine triphosphatase and alkaline phosphatase and biogenic amines \( \gamma \)-aminobutyric acid (GABA) dopamine and acetylcholine which are linked to each other have been considered for the present investigation.

**MATERIALS AND METHODS**

**Chemical:** Dioctyltin oxide and tributyltin oxide were purchased from Alpha Chemical Company, Danvers, Massachusetts, USA. Tricyclohexyltin hydroxide was synthesized as reported by Ingham et al. All other chemicals used were either BDH (AR) or E. Merck extrapure grade.

**Animals and Treatment:** Male albino-Wistar rats inbred in Industrial Toxicology Research Centre Colony (approximately 200 gm body weight), maintained on pellet diet (Hindustan Levers Ltd., Bombay, India) and tap water ad libitum were used throughout the experiment. Animals of six month old were divided into four groups of six animals each. Organotin compounds dissolved in ground nut oil (used as vehicle) were administered subcutaneously (SC) (25 \( \mu \)mole tin/0.5 ml/100 gm body wt.) in the first-three groups while fourth group received ground nut oil alone and served as control. Animals of each group were starved overnight and sacrificed by decapitation 24 hrs after the administration. Dose of the compounds and duration of the exposure to rats were chosen in view of our previous observation that tin at a dose (25 \( \mu \)mole tin/100 gm body weight) produced maximum biochemical alterations at 24 hours after its administration. Furthermore, the dose dependent studies of organotin compounds are in progress to explore out the severity of the effects at multiple dose treatment. Liver, kidney
and brain were removed immediately and processed for biochemical estimations.

**Biochemical Estimations:** Enzymic activities and the concentration of biogenic amines were determined respectively in whole homogenate (5% w/v) of liver, kidney and brain as reported.

1. Lactate dehydrogenase (LDH—EC 1.1.1.27).[^14]
2. Succinate dehydrogenase (SDH—SC 1.3.99.1).[^15]
3. Monoamine oxidase (MAO—EC 1.4.3.4).[^16]
4. Adenosine triphosphatase (ATPase—EC 3.6.1.3).[^17]
5. Alkaline phosphatase (EC—3.1.3.1).[^18]
6. γ-aminobutyric acid (GABA).[^19]
7. Dopamine.[^20]
8. Acetylcholine.[^21]

Total protein content was determined according to the procedure of Lowry *et al.*[^22] using bovine serum albumin as a reference standard.

**Statistical analysis:**

Data were presented as the mean plus or minus the standard error of the mean. Significance of difference between control and experimental values were calculated by use of Student’s t-test as described by Fisher.[^23]

### RESULTS

1. **Effects of organotin compounds on liver enzymes:**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Control</th>
<th>Dioctyltin oxide (DOTO)</th>
<th>Tricyclohexyltin hydroxide (TCHTOH)</th>
<th>Tributyltin oxide (TBTO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lactate dehydrogenase (LHD) n mole of NADH oxidised ×10/min/mg protein</td>
<td>77.19±3.22</td>
<td>50.75±6.42[^a]</td>
<td>44.75±3.50[^a]</td>
<td>73.98±3.95</td>
</tr>
<tr>
<td>2. Succinate dehydrogenase (SDH) n mole of K₅ Fe (CN) reduced/min/mg protein</td>
<td>31.10±0.22</td>
<td>21.00±0.24[^a]</td>
<td>24.80±0.70[^a]</td>
<td>32.38±1.56</td>
</tr>
<tr>
<td>3. Monoamine oxidase (MAO) n mole of benzaldehyde/min/mg protein</td>
<td>5.46±0.12</td>
<td>2.46±2.17[^a]</td>
<td>2.67±0.24[^a]</td>
<td>3.25±0.15[^a]</td>
</tr>
<tr>
<td>4. Adenosine triphosphatase (ATPase) n mole of Pi liberated/min/mg protein</td>
<td>54.94±1.81</td>
<td>93.15±3.59[^a]</td>
<td>137.94±4.44[^a]</td>
<td>117.75±4.16[^a]</td>
</tr>
<tr>
<td>5. Alkaline phosphatase (n mole of phenol liberated/min/mg protein)</td>
<td>5.75±1.40</td>
<td>12.88±1.28[^a]</td>
<td>14.99±1.58[^a]</td>
<td>10.46±1.21[^a]</td>
</tr>
</tbody>
</table>

Each value represents the mean±SEM for six rats.

[^a]: Statistically significant (p<0.05) from control.
Alterations in the activity of various enzymes in the liver of rats by organotin compounds are shown in Table 1. Results indicate that the extent of enzymatic alterations produced by organotin compounds were different from one another. A consistent increase in the activity of alkaline phosphatase and ATPase and decrease in MAO was reflected by all the three compounds. Activity of LDH and SDH decreased significantly in case of DOTO and TCHTOH while both the enzymes remained practically unaltered by TBTO.

2. Effect of organotin compounds on kidney enzymes:
The effects of various organotin compounds on the enzymic activities of kidney are shown in Table 2. Results indicate that the enzymatic alterations produced by all the three organotin compounds in alkaline phosphatase, ATPase and MAO were same as in liver. A significant inhibition in the activity of SDH was produced only by DOTO while none of the compounds altered the activity of LDH.

3. Effects of organotin compounds on the level of biogenic amines: in brain:
Concentration of biogenic amines in brain of rats treated with organotin compounds is given in Table 3. A significant lowering was observed in GABA and dopamine while concentration of acetylcholine remained unaffected. A significant decrease in MAO activity was also observed in brain homogenates of organotin treated rats. DOTO and TCHTOH were more effective in reducing the concentration of GABA and dopamine than TBTO.

Table 2. Alterations in the activity of various enzymes in kidney of rats exposed with organotin compounds (25 micromole Sn++/100 gm body weight)

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Control</th>
<th>Diocetyl tin oxide (DOTO)</th>
<th>Tricyclohexyl tin hydroxide (TCHTOH)</th>
<th>Tributyl tin oxide (TBTO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lactate dehydrogenase (LDH) (n mole NADH oxidised × 10 min/mg protein)</td>
<td>25.72 ± 1.44</td>
<td>25.09 ± 0.87</td>
<td>24.47 ± 0.60</td>
<td>23.28 ± 0.56</td>
</tr>
<tr>
<td>2. Succinate dehydrogenase (SDH) (n mole K₂Fe(CN) reduced/min/mg protein)</td>
<td>46.65 ± 1.34</td>
<td>32.34 ± 1.86 a)</td>
<td>45.81 ± 2.07</td>
<td>43.41 ± 1.98</td>
</tr>
<tr>
<td>3. Monoamine oxidase (MAO) (n mole benzaldehyde/min/mg protein)</td>
<td>2.55 ± 0.14</td>
<td>1.28 ± 0.13 a)</td>
<td>1.75 ± 0.16 a)</td>
<td>2.10 ± 0.15 a)</td>
</tr>
<tr>
<td>4. Adenosin triphosphatase (ATPase) (n mole Pi liberated/min/mg protein)</td>
<td>91.95 ± 2.90</td>
<td>104.75 ± 4.51 a)</td>
<td>143.10 ± 10.75 a)</td>
<td>120.60 ± 5.15 a)</td>
</tr>
<tr>
<td>5. Alkaline phosphatase (n mole phenol liberated min/mg protein)</td>
<td>660.80 ± 46.50</td>
<td>880.00 ± 22.00 a)</td>
<td>947.13 ± 30.00 a)</td>
<td>778.50 ± 11.40 a)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM for six rats.

a) Statistically significant (p<0.05) from control.
DISCUSSION

It has been suggested that in biological media, organotin compounds get ionised into organotin ions which in turn impair the functioning of various intracellular biochemical processes. A significant increase in alkaline phosphatase activity is due to a general response of the liver and kidney to overcome an insult produced by organotin compounds. The elevation in alkaline phosphatase activity might also be due to an accelerated membrane transport function related to anion hydroxide exchange across the lipid biomembranes mediated by DOTO, TCHTOH and TBTO. Increased activity of ATPase, in whole homogenate of our experiment is in good correlation with an increase in membrane bound ATPase after the treatment of organotin compounds. Increase in membrane bound ATPase might also be correlated with an acceleration in membrane transport function by organotin ions. Involvement of the organotin ions with Zn\(^{++}\)/Mg\(^{++}\) component of alkaline phosphatase and Na\(^{+}\)/K\(^{+}\)/Ca\(^{++}\)/Mg\(^{++}\) of ATPase could be of some significance in modulating their enzymatic behaviour. However, Leaw et al. demonstrated a decrease in adenylate cyclase activity by organotin compounds, which is directly related to ATPase activity. Further investigation is needed to explore out this possibility.

Since tin is known to bind with the -SH containing moiety a significant decrease in LDH, SDH and MAO activity in our experiment may be the result of an interaction of organotin ions with the sulphydryl component of the enzymes. Impairment in MAO activity of liver as well as of brain is an indication of abnormalities of the central nervous systems. A significant depletion in concentration of GABA and dopamine in brain (substances of inhibitory neurons) enhances the neurotoxic effects of organotin compounds. Mailman et al. have also reported recently a deficit in the concentration of GABA and glutamic acid by organotin compounds in rats. An alteration in monoamine oxidase activity in brain and consecutive decrease in biogenic amine concentration in brain of rats

<table>
<thead>
<tr>
<th>Table 3. Alteration in the level of biogenic amines in the brain of control and organotin treated rats (25 micromole Sn(^{++})/100 gm body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>(\gamma)-aminobutyric acid (GABA) ((\mu) mole/gm)</td>
</tr>
<tr>
<td>Dopamine ((\mu) gm/gm)</td>
</tr>
<tr>
<td>Acetylcholine ((\mu) gm/gm)</td>
</tr>
<tr>
<td>Monoamine oxidase (MAO) (n mole benzaldehyde/min/mg protein)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM for six rats.
* Statistically significant (p<0.05) from control.
during investigation could be of significance in part at least, for the altered
behavioural changes observed in this experiment, due to hyperactivity and thus
reflecting an aberration in central nervous system functioning.

Variations in the enzymic alterations produced by different organotin compounds
could be considered of the different structural and functional activity of the organic
moiety attached to tin and the degree of their ionization.

It may, thus be concluded that DOTO, TCHTOH and TBTO exert their
principal toxic action on oxidative phosphorylation mechanism and central nervous
system by impairing the various enzymic activities and the concentration of biogenic
amines. Compounds DOTO and TCHTOH are more toxic as judged by the
biochemical alterations produced in liver, kidney and brain in comparison to
TBTO. Further studies are in progress to elucidate the biochemical basis of
neurotoxic actions of organotin compounds.

ACKNOWLEDGEMENTS

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REFERENCES

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