Vitamin E prevents deleterious effects of di (2-ethyl hexyl) phthalate, a plasticizer used in PVC blood storage bags

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Vitamin E administration prevented DEHP induced deleterious effects like (i) degenerative changes in the brain and thyroid, (ii) decrease in the activity of neuronal membrane Na⁺ - K⁺ ATPase, (iii) decrease in the concentration of insulin, cortisol and TSH, and (iv) the increase in T₃ and T₄ in female Albino rats. The results suggest use of vitamin E to prevent harmful effects of repeated transfusion of DEHP containing blood as in thalassemia patient. The possibility of using vitamin E to prevent the harmful effects of repeated transfusion of DEHP containing blood, as in thalassemia patients, is discussed.

Keywords: DEHP, vitamin E, Na⁺ - K⁺ ATPase, Insulin, Glucose, T₃, T₄, TSH, Cortisol.

Appreciable amounts of di (2 ethyl hexyl) phthalate (DEHP) leaches out into blood (about 10 - 15 mg/100 ml) stored in DEHP plasticized PVC bags¹ ², resulting in the exposure of DEHP to recipients of blood transfusion³ ⁴. Administration of DEHP to rats at low levels caused significant inhibition of membrane Na⁺ - K⁺ ATPase in brain, liver and RBCs⁵, an important observation in view of the reports of a decrease in the enzyme activity in a number of disorders like neurological disorders, hypertension, diabetes, coronary artery disease and stroke, tumours etc⁶-¹⁴.

Further, a decrease in serum insulin, increase in blood glucose, decrease in liver glycogen, increase in T₃ and T₄ and decrease in cortisol were observed in rats administered DEHP at low doses¹⁵. Similar changes were also observed in blood stored in DEHP plasticized bags. Decrease in insulin and increase in blood glucose are involved in diabetes, increase in T₃ and T₄ in hyperthyroidism and decrease in cortisol in adrenocortical dysfunction.

These observations suggest the possibility of predisposing recipients of transfusion of blood stored in DEHP plasticized bags to various disorders mentioned above. However, since these effects of DEHP were reversed in experimental animals when administration of DEHP was stopped⁶,¹⁵, it may not be a problem in the case of a few transfusions, but in patients receiving repeated blood transfusion like thalassemia patients, the possibility of this risk has to be considered. In fact neurological, cardiological and other complications have been reported in thalassemia patients receiving repeated transfusion of blood stored in DEHP plasticized bags¹⁶.

A decrease in vitamin E concentration in blood and tissues in rats administered DEHP at low doses (150 to 750µg/100g body weight) and also in human blood stored in DEHP plasticized bags has been reported¹⁷. Administration of vitamin E (at a dose of 450µg/100g body weight) to rats given DEHP or incorporation of vitamin E in the additive solution in the blood stored in DEHP plasticized bags, (20mg/100ml blood) prevented this decrease¹⁷.

Vitamin E (200µg/ml blood) minimized the increase in lipid peroxidation in RBCs in blood stored in DEHP plasticized PVC bags¹⁸. RBCs in blood stored in DEHP plasticized bags take up DEHP and most of it is present in the RBC membrane¹⁹. A decrease in vitamin E in the RBC membrane was observed under these conditions¹⁷. When RBCs were incubated with DEHP in vitro in the presence of vitamin E, there was progressive decrease in the membrane bound DEHP²⁰. These observations suggest that vitamin E may displace DEHP bound to the cell membrane.

In view of these observations, it was considered necessary to investigate whether vitamin E can prevent the deleterious effects of DEHP. The effects...
of administration of vitamin E along with DEHP in rats on histopathology of brain and thyroid, activity of Na⁺-K⁺ ATPase in the brain, level of serum insulin, T₃, T₄ and TSH, cortisol and blood glucose are reported in this communication.

Ethical clearance for this study was obtained from the Institutional Animals Ethics Committee.

Materials and Methods
Female albino rats (Wistar strain, body weight 120-180g) were grouped randomly into following three groups of 12 rats each. Group 1: control rats, Group 2: rats given DEHP (750µg/100g body weight) and Group 3: rats given DEHP + Vitamin E (2mg/100g body weight).

Emulsion of DEHP and vitamin E was prepared in 2.2% glycerol containing 1.2% egg yolk lecithin by sonication under sterile conditions and was administered intraperitoneally as described earlier. Control rats received the same volume of vehicle. The animals were caged individually in polypropylene cages and maintained on normal laboratory feed in rooms maintained at 28 ± 1°C. Food and water were available to the rats ad libitum.

Administration of DEHP / vitamin E was made on alternate days and a total of 7 injections were given. At the end of 14 days, the animals were deprived of food overnight and blood was collected from ocular vein. They were then sacrificed by decapitation. Brain was collected to cooled containers for estimation of Na⁺-K⁺ ATPase. Brain and thyroid tissues were collected in 10% buffered formalin for histopathological examination.

Analytical Procedures

Estimation of membrane Na⁺-K⁺ ATPase activity
(EC 3.6.1.37) in membrane of brain cells was carried out as described earlier.

For histopathological examination of the brain and thyroid, the tissues transferred to buffered 10% formalin were sectioned (1-2 microns) and stained with haematoxylin and eosin. Commercial kits were used for estimation of serum insulin (Merckodia Insulin ELISA, Merckodia AB, Sweden), T₃, T₄ and TSH (Monobind Inc. CA., Sweden), cortisol (Equiafar) and blood glucose (Randox, U.K).

Statistical analysis—The results are presented as the mean ± SD. Statistical analysis of the results was performed by ‘ANOVA’. Differences among the means of the groups were assessed using the Duncan Procedure to determine which mean values were significantly different at P< 0.01 and P between 0.01 and 0.05.

Results

Histopathological examination

Brain—Histopathological examination of the brain (Fig. 1) of rats administered DEHP alone (group 2) revealed areas of moderate focal degenerative changes in the cerebrum and cerebellum. Neurons had become shrunken, eosinophilic and contained pyknotic nuclei. Brain of control rats (group 1) and of those administered vitamin E along with DEHP (group 3) appeared normal.

Thyroid—Thyroid gland in the rats receiving DEHP alone (group 2) showed areas of functional hyperplasia of the epithelial cells, which were enlarged in size and increased in number (Fig. 2). However, in control rats (group 1) and those administered vitamin E along with DEHP (group 3), the thyroid gland appeared normal.

Activity of Na⁺-K⁺ ATPase in the membrane of brain cells—There was significant inhibition of the enzyme activity in the membrane of brain cells in rats administered DEHP, when compared to that in control rats. This effect was prevented and near normal value of enzyme activity was observed in rats administered vitamin E along with DEHP (Table 1).

Serum levels of T₃, T₄ and TSH—Concentration of serum T₃ and T₄ showed significant increase in the rats administered DEHP when compared to control rats, while that of TSH showed significant decrease. But the concentration of T₃, T₄ and TSH in rats administered vitamin E along with DEHP showed values similar to that in control animals (Table 1).

Concentration of blood glucose and serum insulin—There was significant increase in blood glucose and decrease in insulin in rats administrated DEHP when compared to control rats. These changes were however prevented in rats administered vitamin E along with DEHP and very near normal values were obtained (Table 1).

Concentration of serum cortisol—Serum cortisol showed significant decrease in the rats administrated DEHP when compared to control rats. This decrease was also prevented in rats receiving vitamin E along with DEHP (Table 1).
Fig. 1—Micrograph of brain from rats receiving 750μg/100g body weight by successive administration (A) Control rats (normal); (B) rats receiving DEHP (at a dose of 750μg/100g body weight). Note the early vulnerable response of degeneration of neurons (N), which has become shrunken, eosinophilic and with pyknotic nuclei; (C) rats receiving vitamin E (at a dose of 2mg/100g body weight) along with DEHP. No much cellular changes as evident in B. x 100; H & E.

Fig. 2—Micrograph of thyroid from rats receiving 750μg/100g body weight by successive administration. (A) Control rats (normal); (B) rats receiving DEHP (at a dose of 750μg/100g body weight) Note the hyperplastic acinar cells (AC) with large nuclei and with scanty colloid. Acinar cells are seen crowded at few areas; (C) rats receiving vitamin E (at a dose of 2mg/100g body weight) along with DEHP. No evidence of any acinar hyperplasia. x 100; H & E.
Table 1—Effect of administration of DEHP and Vitamin E + DEHP to rats on the activity of membrane Na⁺K⁺ ATPase in the brain, levels of thyroid hormones, glucose, insulin and cortisol in the experimental animals

[Values are mean ± SD from 6 rats in each group]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Membrane Na⁺K⁺ ATPase activity (μmoles of ATP hydrolyzed/hr/mg protein)</th>
<th>T3 (ng/dl)</th>
<th>T4 (μg/dl)</th>
<th>TSH (mIU/ml)</th>
<th>Blood glucose (mg/dl)</th>
<th>Serum insulin (mU/l)</th>
<th>Serum cortisol (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>7.44 ± 1.11</td>
<td>51.67 ± 6.8</td>
<td>2.45 ± 0.51</td>
<td>0.15 ± 0.06</td>
<td>52.2 ± 7.36</td>
<td>0.37 ± 0.05</td>
<td>12.4 ± 3.02</td>
</tr>
<tr>
<td>DEHP (Group 2)</td>
<td>3.98 ± 1.81*</td>
<td>62.5 ± 6.83</td>
<td>3.23 ± 0.47</td>
<td>0.07 ± 0.016</td>
<td>64.4 ± 6.02</td>
<td>0.28 ± 0.042</td>
<td>7.07 ± 2.59</td>
</tr>
<tr>
<td>Vitamin E + DEHP (Group 3)</td>
<td>7.56 ± 1.16*</td>
<td>48.33 ± 6.82</td>
<td>2.13 ± 0.61</td>
<td>0.14 ± 0.058</td>
<td>54.16 ± 6.95</td>
<td>0.55 ± 0.11*</td>
<td>11.95 ± 2.94*</td>
</tr>
</tbody>
</table>

Group 2 is compared with group 1 and group 3 with group 2. *P values: < 0.01, b between 0.01 and 0.05
Group 3 is also compared with group 1. *P values: < 0.01.

Discussion

The observation that administration of vitamin E can prevent the degenerative changes in the brain caused by DEHP is very significant. The dose of DEHP administered on alternate days corresponded to the successive transfusion of 10 units of blood in a recipient of blood transfusion. The results also indicate that administration of vitamin E prevents the inhibition of membrane Na⁺K⁺ ATPase in the brain caused by DEHP. An inhibition of this enzyme has been observed in many neurological and psychiatric disorders (epilepsy, Parkinson’s disease, schizophrenia, manic depressive psychosis etc.) (12, 19). The inhibition of this enzyme activity in the brain has also been observed in quinolinic acid induced neurodegeneration in the heart in isoprenaline-induced myocardial infarction, both in rats (20). The observation that vitamin E can also prevent the decrease in insulin and increase in blood glucose in diabetes is also significant. Decrease in insulin and hypoglycemia are manifestations of diabetes caused by DEHP and is also significant. Decrease in insulin and hypoglycemia are manifestations of diabetes caused by DEHP. An inhibition of this enzyme activity in the brain has also been observed in quinolinic acid induced neurodegeneration in the heart in isoprenaline-induced myocardial infarction, both in rats (20). The observation that vitamin E can also prevent the decrease in insulin and increase in blood glucose in diabetes caused by DEHP and is also significant. Decrease in insulin and hypoglycemia are manifestations of diabetes caused by DEHP. An inhibition of this enzyme activity in the brain has also been observed in quinolinic acid induced neurodegeneration in the heart in isoprenaline-induced myocardial infarction, both in rats (20). The observation that vitamin E can also prevent the decrease in insulin and increase in blood glucose in diabetes caused by DEHP and is also significant. Decrease in insulin and hypoglycemia are manifestations of diabetes caused by DEHP. An inhibition of this enzyme activity in the brain has also been observed in quinolinic acid induced neurodegeneration in the heart in isoprenaline-induced myocardial infarction, both in rats (20).
minimizes the increased lipid peroxidation and free radical damage caused by DEHP\textsuperscript{18}.

References


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