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ADMINISTRATION OF THE PLASTICIZER DI (2-ETHYLHEXYL) PHTHALATE ALTERS GLYCOCONJUGATE PROFILE

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Abstract: This study was undertaken to evaluate the level of glycoproteins and sialic acid in rats fed di (2-ethylhexyl)-phthalate (DEHP) in the diet for 24 weeks. Protein-bound hexose, hexosamine and sialic acid were increased in plasma and liver of rats treated with DEHP, whereas the erythrocyte membrane showed a reduction following DEHP administration. Evaluation of glycoproteins is a useful indicator of the carcinogenic process. It is suggested that profound alterations in membrane components observed in the present study may be related to the carcinogenic potential of DEHP.

Key words: Di(2-ethylhexyl) phthalate, membrane, glycoproteins, sialic acid

INTRODUCTION

Many properties of mamalian cells are mediated through the cell surface. Glycoproteins and sialic acid, major constituents of cell membrane which play an important role in maintaining the integrity of cell membrane show variations during neoplastic transformation (1). Membrane glycoproteins are responsible for cell recognition and various immunological phenomena like autoimmune diseases and cancer. Sialic acid, a membrane constituent involved in cell contact phenomena, growth control and cellular invasiveness has been demonstrated to be increased at the surface of cancer cells in humans (2).

Phthalate esters such as di (2-ethylhexyl) phthalate (DEHP) are extensively used as plasticizers in the manufacture of polyvinylchloride. DEHP has been demonstrated to induce peroxisome proliferation and hepatocellular carcinomas in rodents (3). Reddy and Lalwani (4) proposed an increase in the synthesis of H_2O_2 generating peroxisomal β-oxidation enzyme systems, causing profound intrahepatic oxidative stress and initiation of hepatic neoplasia.

Xenobiotics are known to cause profound alterations in the cell membrane. Although hepatic effects of DEHP have been extensively documented, studies on DEHP-induced changes in cell surface components in general and glycoproteins in particular are limited. Previous studies from this laboratory have shown preneoplastic changes and alterations in membrane associated enzymes and components in rats following chronic DEHP administration (5, 6). This preliminary report evaluates levels of protein bound hexose, hexosamine and sialic acid in liver, plasma and erythrocyte membrane of rats following DEHP administration.

METHODS

Wistar male rats weighing 100-130g were housed in polycarbonate cages in a temperature and humidity controlled environment, with a 12-h light/dark cycle. Standard pellets were obtained from Mysore Feed Ltd. Mysore. The animals had free access to food and water. Rats were fed 2% DEHP in the diet for 24 weeks. Age and sex matched animals were used as controls.

Biochemical assays: Blood was collected in heparinised tubes. The plasma was separated

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after centrifugation at 1000xg for 15 min. The buffy coat was removed and the packed cells were washed thrice with physiological saline. The erythrocyte membrane was isolated (7) with a change in buffer according to Quist (8). Protein-bound hexose (9), protein-bound hexosamine (10) and sialic acid were estimated (11). Statistical significance was calculated using student's 't' test.

RESULTS

Table I shows the levels of protein-bound hexose, hexosamine and sialic acid in liver, plasma and erythrocyte membrane of rats exposed to DEHP. Liver and plasma glycoproteins and sialic acid were found to be increased in rats treated with DEHP, whereas erythrocyte membrane showed a reduction.

TABLE I : Effect of DEHP administration on protein-bound hexose, protein-bound hexosamine and sialic acid in rats.

Parameters	*Liver (mg/g defatted tissue)*		*Plasma (mg/dl)*		*Erythrocyte Membrane (mg/mg protein)*	
	Control	*DEHP*	*Control*	*DEHP*	*Control*	*DEHP*
Protein-bound hexose	26.0 ± 0.70	36.75 ± 0.82*	210 ± 8.16	313 ± 12.47*	0.473 ± 0.03	0.297 ± 0.004*
Protein-bound hexosamine	5.7 ± 0.20	9.26 ± 0.32*	49.99 ± 1.16	59.04 ± 1.57*	0.17 ± 0.01	0.106 ± 0.02*
Sialic acid	6.46 ± 0.11	7.88 ± 0.11*	87.37 ± 0.97	100.94 ± 1.34*	0.293 ± 0.01	0.150 ± 0.01*

Values represent mean ± SD from 6 animals in each group; *P < 0.001 than control animals.

DISCUSSION

Increased glycoprotein levels have been documented in several neoplams (12). Alterations in the levels of plasma glycoproteins observed in this study can be explained in relation to changes observed in hepatic cells as well as the erythrocyte membrane. Various animal tissues are composed of neutral glycoproteins with the same carbohydrate constituents as that of plasma. Hence, an increase in hepatic level of these glycoproteins may increase the level of glycoproteins in circulation. Macbeth et al (12) postulated that presence of a tumor induces hepatic cells to synthesize glycoproteins which subsequently appear in circulation. The depletion in erythrocyte membrane glycoproteins observed in this study may be due to increased shedding of glycoproteins into circulation. Thus, enhanced levels of plasma glycoproteins in DEHP treated rats could be as a result of release from liver as well as erythrocyte membrane.

Cell surface conjugates, major constituents of cell membrane are important in malignancy. Neoplasms often have an increased level of sialic acid on the tumor cell surface. Kloppel et al (13) observed an increase in the concentration of sialic acid on tumor cell surface and suggested that sialoglycoproteins are shed or secreted by these cells which in turn can bring about an increase in circulation. Changes in sialic acid content observed in this study is probably a reflection of cell surface changes.

Sialic acid residues are responsible for the net negative charge on the surface of erythrocyte membrane. Alterations in the content of sialic acid may alter the rigidity of the cell membrane. A reduction or deficiency of glycosyl transferases may be responsible for the decreased amount of erythrocyte membrane sialic acid, observed in this study. We have undertaken this study as part of an investigation to evaluate the carcinogenic potential of DEHP. The present study suggests that DEHP-induced alterations in membrane components, may affect the functions and rigidity of biomembrane. Efforts are underway to correlate changes in cell surface glycoprotein profile with the carcinogenic potential of DEHP.

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