**In vivo** effect of vitamin E on serum and tissue glycoprotein levels in perchloroethylene induced cytotoxicity

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Received 21 June 1994; accepted 14 October 1994

Abstract

The antioxidant efficacy of vitamin E on Perchloroethylene (PER) induced cytotoxicity has been studied in rats. Feeding PER to rats for 42 days using sesame oil as vehicle alters total protein and protein bound carbohydrate components in liver and kidney of experimental animals. Supplementation of vitamin E prevented the changes observed in total protein and protein bound carbohydrate components of PER administered rats. Histopathological studies also show the effectiveness of vitamin E on PER administered rats in protecting the cellular architecture of liver and kidney from PER induced cytotoxicity. (Mol Cell Biochem 144: 13–18, 1995)

Key words: perchloroethylene, vitamin E, total protein, glycoproteins

Introduction

Many chemical substances such as organic solvents, mycotoxins, plant toxins and drugs are capable of producing acute liver and kidney damage characterized primarily by necrosis or cell death [1].

Perchloroethylene (PER) is a chlorinated hydrocarbon which has important industrial applications in dry cleaning and in degreasing of metal parts [2]. The ability of PER to produce acute hepatotoxicity and nephrotoxicity to a number of laboratory animals was reported [3]. Subsequently, administration of PER was shown to produce high incidence of hepatocellular carcinoma and renal adenoma in mammalian species [4]. The carcinogenic effect of PER has been documented in various animal models, and its mechanism to produce the mutagenic effect has been investigated in detail in the past two decades. It is generally accepted that the mutagenic action of PER is mediated via its immediate metabolic products [5]. Specifically, the formation of metabolic products which are able to alkylate the genetic material (or) the protein associated carbohydrate materials [6].

Glycoproteins are common components of animal cell surfaces, and are also commonly found as constituents of lysosomes and among the products exported by the cell [7]. The cell surfaces glycoproteins have been shown to play important roles in pinocytosis, tumorigenesis and as mediators of immunological specificity [8]. Carbohydrate moieties of glycoproteins have also implicated in the transport of metabolites across cell membranes and also observed a direct relationship between glycoprotein and tumorigenesis [9]. Sialic acid is an important constituent of the renal glomerular poly anion present in glycoproteins of the glomerular basement membrane [10].

Vitamin E is one of the most abundant, naturally occurring, biologically active antioxidant in the system. There is evidence to suggest that vitamin E plays a major role in reducing the incidence of cancer, by acting as free radical scavenger that inhibits the lipid peroxidation [11]. It has been suggested...
that the structure of their side chain allows the tocopherols to interact specifically with the poly unsaturated fatty acids of membrane lipids and to act as a membrane stabilizer [12]. Cook has reported that vitamin E supplementation reduced the number and incidence of chemically induced tumors in animals [13].

Considering the cytotoxic and tumorigenic nature of PER and the antioxidant effect of vitamin E, the present study was proposed to investigate the protective efficacy of vitamin E against PER induced cytotoxicity in liver and kidney with reference to protein and protein bound carbohydrate levels as they are one of the indicators of tumorigenesis.

Materials and methods

Materials

1,1,2,2- Perchloroethylene (Analar grade 96%) was obtained from BDH Chemicals Limited (Poole, Dorset, UK). Vitamin E was obtained from Sigma Chemicals, St. Louis, USA. Or-cinol, p-dimethyl benzaldehyde and thiobarbituric acid (all from E. Merck analytical grade) were used.

Animal model

Adult male Wistar strain rats weighing 160–190 g were maintained with free access to food and water. The rats were divided into three groups of six each. Group I served as control animals, fed with normal commercial rat feed along with sesame oil (10 ml/kg body wt/day), while Group II animals were fed with PER (3000 mg/kg body wt/day) dissolved in 10 ml of sesame oil for 42 days, and the Group III animals were fed with PER along with vitamin E (400 mg/kg body wt/day) for 42 days by oral gavage.

After the experimental period the animals were killed by cervical decapitation. Blood was collected and was allowed to clot before centrifugation and serum was collected and stored at 4°C. Liver and kidney were removed immediately, washed well with ice cold saline and homogenised in Tris-HCl (pH 7.5). A part of the tissue of each of the organs was processed in 10% formalin for histopathological studies.

Analysis of total protein and protein bound carbohydrate components in serum and tissue homogenates were carried out. Total protein in serum and tissue homogenates were estimated by the method of Lowry et al. [14].

Glycoprotein analysis

Hydrolysis of glycoprotein for hexose and hexosamine determination was carried out. A known amount of defatted tissue was taken in test tube to which 1 ml of 2N HCl was added, and the tubes were sealed. Hydrolysis was completed by keeping the sealed tubes in 100°C for 16–18 h. After hydrolysis the contents were neutralised with sodium hydroxide and made up to known volume, and aliquots were used for hexose and hexosamine determination. Hexose and Hexosamine in the aliquots were determined by the method of Niebes [15] and Wagner [16] respectively.

Analysis of sialic acid

A known amount of defatted tissue was hydrolysed with 0.1N H₂SO₄ at 90°C and neutralised. The hydrolysed extract was used for the determination of sialic acid by the method of Warren [17].

Statistical analysis

In order to minimise interassay variation, analyses were carried out in groups with equal number of samples from PER administered, PER administered along with vitamin E and control each group. Statistical significance between groups were compared, using student’s t-test.

Results

Effect of vitamin E on total protein levels in PER administered animals

In order to assess the effect of vitamin E administration, serum, liver and kidney protein contents in PER administered animals were determined. As shown in Table 1, the total protein levels were found to be significantly elevated (p < 0.001) in PER administered animals, when compared with controls.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Group I Control</th>
<th>Group II PER administered</th>
<th>Group III PER + vitamin E treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>5.44 ± 0.24</td>
<td>6.63 ± 0.43***</td>
<td>6.11 ± 0.58*</td>
</tr>
<tr>
<td>Liver</td>
<td>225.20 ± 16.90</td>
<td>277.40 ± 20.90***</td>
<td>244.50 ± 24.30*</td>
</tr>
<tr>
<td>Kidney</td>
<td>193.40 ± 13.0</td>
<td>244.50 ± 24.30**</td>
<td>202.0 ± 21.60 NS</td>
</tr>
</tbody>
</table>

Values are expressed as mg/g in tissues and g/dl in serum ± standard deviation. Values are taken as a mean of six individual experiments. For statistical analysis Group II was compared with Group I and Group III was compared with Group II. p* < 0.05, p** < 0.01, p*** < 0.001, NS – not significant.