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Orjinal Article

The effects of inhaled formaldehyde on the activities of some metabolic enzymes in the liver of male rats: subchronic (13-weeks) effects

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Abstract. We aimed to investigate the effects of different formaldehyde (FA) concentrations on some enzyme activities that take part in metabolic pathways in the liver. The enzymes studied were hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) which are included in the three main metabolic pathways; glycolysis, citric acid cycle, and pentose phosphate pathway. Thirty male Wistar albino rats were raudemly divided into 3 separate groups (10 per group). The first ten rats were used as control (grup I). Rats were exposed to atmosphere containing 10 and 20 ppm FA continuously (8 hours per day, 5 days per week) in groups II and III. HK, G6PD, 6PGD, LDH, and MDH activities were measured in liver tissues. The results showed a remarkable reduction in G6PD and 6PGD activities, significant increase in MDH activity in liver tissues from rats exposed to 10 ppm FA for 13-weeks. There were no differences in the activities of HK and LDH between test and control groups. Conversely G6PD activity was decreased and MDH activity was increased in liver tissues from rats exposed to 20 ppm FA for 13-weeks. These results may suggest the possible changing effect of FA inhalation on metabolic enzymes in liver.

Key words: formaldehyde, hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, liver.

1. Introduction

Formaldehyde (FA), which is known to be genotoxic and irritant, is present in side-stream tobacco smoke, gasoline and diesel exhaust, consumer products, and many occupational settings (1,2). Exposure to FA has been implicated in many diseases because it is an irritant giving rise to dermatitis, eye irritation, respiratory irritation, asthma, and pulmonary edema (3). Exposure to FA occurs especially among anatomists, embalmers, and pathologists (1). The studies conducted among anatomists, pathologist, and morticians suggested that there was an increase in the number of deaths due to brain cancer, leukemia, and colon cancer (4). On the other hand, cells exposed to FA developed both DNA protein cross-links and single strand breaks (2). The mechanisms underlying cancer etiopathogenesis related to FA has not yet been elucidated. We hypothesized that the possible relationship between cancer and FA may indirectly depend on the changing effect of FA on metabolic enzymes. The effect of FA on liver HK, G6PD, 6PGD, LDH and MDH metabolic enzymes has not been studied up to now. In the present report, the effect of subchronic FA on rat liver was studied by assaying the activities of

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some selected 'marker' enzymes functioning in energy generation and in reducing equivalents biosynthesis.

2.Materials and methods

The FA gas was generated from paraformaldehyde (Merck KGaA, 64271 Darmstadt, Germany) by thermal depolymerization according to the method described by Chang et al. (5). The FA level of the test atmosphere was monitored by gas chromatography.

Three groups of adult male albino Wistar rats consisting of each 10 (weighing 250 ± 5 gm) were obtained from the Biomedical Research Unit of the Institute of Health Sciences, Firat University, Elazig. After an acclimatization period of 1 week, the rats were assigned by computer randomization to 3 equal groups. The first 10 rats were used as control group (group I). Other two groups were exposed (whole body exposure) to atmospheres containing 10 (Group II) and 20 (Group III) ppm FA continuously (8 h a day, 5 days per week) during 13 weeks. The exposure was performed in a glass chamber (5). Animals were housed (5 rats per cage) in a chamber ventilated with 10 lt./min, maintained at 25 ± 2 °C, with a relative humidity of 45-55% and a 12-h light/dark cycle during the observation periods. Neither food nor drinking water was present in the inhalation chambers during the exposures. During the non-exposure periods, the animals were provided with bottled tap water and the Institute's stock diet for rats *ad libitum*. The rats were checked daily and body weights were recorded weekly. At the end of the 13-week exposure period, the rats were killed by decapitation under ether anesthesia, autopsied and examined grossly for pathological changes. After liver tissues were obtained for biochemical analysis, the specimens were washed out from contaminating blood with ice-cold buffered saline. They were weighted and cut very thinly with a clean scalpel blade and then homogenized in 0.15 M ice-cold KCI for three minutes at 16000 rpm with an homogenizer (Ultra Turrax Type T-25-B; Labortechnic, Staufen, Germany). All parameters were done on the homogenate.

2.1.Enzyme activity determinations

The activities of HK, G6PD, 6PGD, LDH, and MDH enzymes were determined spectrophotometrically (6), from the oxidation of NADH (for MDH and LDH) to NAD^+ or the reduction of $NADP^+$ (for HK and G6PD) to NADPH by recording the absorbance (A_{340}) decrease and increase, respectively, of reaction mixtures at 25 ºC for a minute period. Enzymatic assay of 6PGD was carried out according to the method of Rudack et al. (7).

2.2.Protein assay

Tissue protein levels were determined by the Lowry procedure using bovine serum albumin as a standard (8).

2.3.Statistical analysis

Data were analyzed by using SPSS® for Windows computing program. Non-parametric statistical methods were used to analyze the data. Mann-Whitney U tests were used to examine between the groups' comparison. Bivariate comparisons were examined using Pearson rank correlation coefficients and values were corrected for ties. Two-tailed significance values were used. A p value less than 0.05 was considered significant.

3.Results

Liver tissue HK, G6PD, 6PGD, LDH, and MDH activities were presented in Table 1. Glucose-6-phosphate dehydrogenase activity was decreased significantly in both Group II (mean ± SD; 5.22 ± 2.39 mU/mg protein) and Group III (6.99 ± 2.14 mU/mg protein) when compared to group I (22.88 ± 3.83 mU/mg protein) ($p<0.01$). Tissue 6PGD activity was significantly decreased in Group II (5.29 ± 1.21 mU/mg protein) when compared to group I (7.81 ± 2.23 mU/mg protein) ($p<0.05$). Tissue MDH activity was increased significantly in both Group II (mean ± SD; 2382 ± 393 mU/mg protein) and Group III (2827 ± 455 mU/mg protein) when compared to group I (1905 ± 229 mU/mg protein)($p<0.05$ and $p<0.01$, respectively). There were no statistical differences in HK and LDH activities in FA-exposed rats when compared to control rats. It has been noticed a statistically significant positive correlation between HK and G6PD ($r= 0.710$, $p<0.048$) and between G6PD and 6PGD ($r= 0.711$, $p<0.05$) in Group III.

4.Discussion

In this study, a significant decrease in G6PD activity was seen in both Group II and Group III, and 6PGD activity in Group II (Table 1). In addition, we found a positive linear correlation between G6PD and 6PGD ($r=0.711$, $p<0.05$) in Group III. However, Cassee and Feron (9) found

Table 1.

Hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) activities in liver tissue of rats exposed to formaldehyde (FA) in different concentrations. The results are expressed as mean ± SD specific activity (mU / mg protein).

Groups	HK	G6PD	6PGD	LDH	MDH
Grup I (n=10)	0.80 ± 0.39	22.88 ± 3.83	7.81 ± 2.23	1744 ± 413	1905 ± 229
Group II (10 ppm) (n=10)	1.08 ± 0.63	5.22 ± 2.39**	5.29 ± 1.21*	1535 ± 380	2382 ± 393*
Group III (20 ppm) (n=10)	0.83 ± 0.34	6.99 ± 2.14**	7.16 ± 1.71	1433 ± 282	2827 ± 455**

*p<0.05, **p<0.01 when compared with group I
*p<0.05 when compared with Group I I

that G6PD, glutathione reductase, glutathione s-transferase, FA dehydrogenase activities in nasal epithelium of rats were not affected by FA. Cassee and Feron found no changes in the activities of these enzymes when they used FA in low dose and at short time interval using 3-day intermittent exposure to FA 3.6 ppm (9). However, they found that FA induced rhinitis, degeneration, frank necrosis, hyperplasia and squamous metaplasia of the ciliated and non-ciliated nasal respiratory epithelium (9). G6PD and 6PGD play a key role in the hexose monophosphate pathway and through these reactions, generates NADPH, which is required as electron donor in various biosynthetic pathways and for the regeneration of reduced glutathione, which helps protect the cells against oxidative damage (10). G6PD and 6PGD enzymes were inhibited by FA. There are two possibilities here: First, reduced glutathione level was decreased after FA exposure resulting in the decreased activity of G6PD. Second, decreased activity of G6PD can be caused by extracellular and intracellular lipid peroxidation (11). Hepatocyte susceptibility to FA was also increased, if hepatocyte GSH was depleted beforehand so as to inhibit the GSH-dependent aldehyde dehydrogenase, which catalyses HCHO oxidation to formate (12). Various inhibitors of aldehyde dehydrogenase were shown to inhibit FA metabolism in GSH depleted hepatocytes, which also markedly increased FA-induced cytotoxicity.

Individual risk of toxicity or cancer can be affected by one's exposure to sufficiently high doses of particular environmental agents (or mixtures), combined with each person's underlying genetic predisposition. Oxidative stress elicits an adaptive antioxidant response, which varies with tissue type. Both G6PD and 6PGD can be accepted as antioxidant enzymes because of their support to the reducing equivalent amount against ROS.

Formaldehyde is widely used in various manufacturing processes including the production of wood products, textiles, rubber, and cement and is also commonly used as an intermediate in the synthesis of other industrial chemicals (4). Worldwide production of formaldehyde is in the millions of tons each year. It is water-soluble and readily reacts with cells of the upper respiratory tract and the conjunctiva (1). Several experiments have shown that inhaled FA is rapidly oxidized to formate and carbon dioxide or is incorporated into macromolecules by one-carbon metabolic pathways (13). In a study carried out by Grafstrom et al. (2), the number of DNA protein cross-links induced by 100 μM FA in epithelial cells and fibroblasts was found similar (0.65 and 0.83 unit, respectively), and the frequency of these cross-links was found proportional to the concentration of FA (2). Cheng et al (14) found in their experimental study that low G6PD activity in patients with nasopharyngeal carcinoma was associated with poor prognosis.

We found a significantl increase in MDH activity in both Group II and III (Table 1). Metanol is oxidized to FA and then to formate by the enzymes of liver (15). Skrzydlewska and Farbiszewski found that the activities of Cu, Zn-superoxide dismutase and catalase were significantly increased in rat liver homogenates after 6 h following methanol ingestion in doses of 3.0 and 6.0 g/kg b.w. and persisted up to 2-5 days (15).

We did not find differences in HK and LDH activities between the groups. Similarly, Schlink et al. found that O^6-methylguanine DNA methyltransferase activity (MGMT) was not changed among medical students; before FA exposure and after 50 days exposure (131.1 ± 15.8 fmol MGMT.10^6 cells; p=0.56). Indeed no significant difference was observed between thestudents before and after 111 days exposure (128.2 ± 19.0 fmol MGMT.10^6 cells; p=0.87) (1).

Liver has a central role in the body for intermediary metabolism. Carbohydrates, proteins, lipids, and amino acids were processed in this metabolically active tissue for different objectives. In addition, liver put forward a lot of processed metabolites to the bioavailibity of the extra hepatic tissues. Consequently; FA inhalation in different doses and time intervals affect the metabolic pathway of the liver. At least, the catabolism of FA in the liver clearly seems to alter pentose phosphate pathway because 10 ppm FA exposure decreased the G6PD and 6PGD enzymes. The prevention of the pentose phosphate pathway may affect the oxidant/antioxidant balance of the liver and results in lipid peroxidation, consequently cellular damage. In conclusion, we report here that there is a decrease in G6PD and 6PGD activities and an increase in the activities of MDH in FA groups when compared with control group. This suggests that subchronic FA exposure causes enzyme changes which partly arise as a result of enormous production of free radicals in the system.

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