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Histopathologic Changes of Rat Liver Following Formaldehyde Exposure

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Abstract: Formaldehyde is a chemical, which is used traditionally for fixing the cadaver. It is vaporized during dissection and practical study on the cadaver. Studies show that this vapor can cause some clinical symptoms such as throat, eye, skin and nasal irritation. This study was designed to determine the histopathologic changes of rat liver while all of the experiments were exposed to formaldehyde vapor for 18 weeks. The study was performed on 28, 6-7 weeks postnatal albino Wistar rats. The rats were divided into 3 case groups and 1 control group. The group E1 was exposed 4 h/day and 4 day/week. The exposure protocol for group E2 was 2 h/day, 4 day/week. The group E3 was exposed 2 h/day and 2 day/week. There was no exposure for control group. After 18 weeks of formaldehyde exposure, the rats of all groups were sacrificed by chloroform anesthesia and the liver specimens were extracted, fixed in formaldehyde buffer solution and stained with H & E technique for histopathologic study. By microscopic study in cases of group E3, eosinophilic cytoplasm and oncocytic view were seen in some cells, while other cells showed focal ground glass view as well as sinusoidal and portal artery dilation. In group E2, sinusoidal dilation and congestion were seen. Mononuclear cell infiltration and regeneration of hepatocytes in periportal space were seen in cases of group E1. No histological changes were seen in control group. The findings of this study showed that formaldehyde vapor in the concentrations similar to our study, can make histopathologic changes which could be detectable by light microscope. But there is no direct relationship between the duration of exposure to formaldehyde vapor and the intensity of histopathologic changes in the liver.

Key words: Formaldehyde, liver, rat, histopathology

INTRODUCTION

Formaldehyde (CH₂O) is a flammable, colorless reactive, readily polymerized gas at normal room temperature and pressure, with a relative molecular mass of 30.03 and a pungent odor. Formaldehyde is soluble in water, ethanol and diethyl ether. Also, it is used in polymerized form (Paraformaldehyde) (WHO, 1998).

Under atmospheric conditions, formaldehyde is readily photo-oxidized by sunlight to carbon dioxide. In the absence of nitrogen dioxide, the half-life of formaldehyde is approximately 50 min during the daytime; while in the presence of nitrogen dioxide, it drops to 35 min (WHO, 1998).

There are various sources of formaldehyde, but the major anthropogenic sources which affect humans are in the indoor environments. Other anthropogenic sources include direct emissions; especially from the production and use of formaldehyde (WHO, 1998).

Its potential to act as an electrophile and act with macromolecules such as DNA, RNA and protein to form reversible adducts or irreversible cross-links (IARC, 1995) makes it as a conventional tissue fixative (particularly in cadaver's fixation).

Acute formaldehyde exposure produces mainly mucosal irritation of the eye and upper respiratory tract in humans (Zwart *et al.*, 1988) and a long-term exposure leads to histopathologic changes of trachea (Davarian *et al.*, 2005), even nasal tumors in rodents (Monticello *et al.*, 1996). Formaldehyde also causes pulmonary function impairment (Berbstein *et al.*, 1984) and asthmatic reactions in sensitized individuals (Burge *et al.*, 1985; Gorski and Krokowiak, 1991).

In the dissection lab and during cadaver's dissection, instructors of anatomy and medical students are exposed to formaldehyde vapor derived from cadaver's fixative.

In order to study the histopathologic changes in the liver tissue due to formaldehyde exposure in the dissection lab and determining its relationship with the duration of exposure, this study was performed on Albino Wistar rats.

MATERIALS AND METHODS

The present study was conducted in Department of Anatomy, Gorgan Faculty of Medicine, Gorgan, Iran in 2005. This study was performed on 28, 6-7 weeks

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postnatal Albino Wistar rats (bought from Iranian Pasteur Institute). The animals were divided randomly into three equal case groups based on the differences between exposure periods: E1 (4 h/d, 4 d/w), E2 (2 h/d, 4 h/w), E3 (2 h/d, 2 d/w) and a control group without any exposure.

Using a digital scale, the mean weights for each group were 252 g (E1), 209 g (E2) and 222 g (E3) and 195 g (control group). The concentration of formaldehyde vapor was measured at the beginning, during and at the end of the study by means of Detector Tube and Dragger Pump (model 31, made in Germany) after the covers of the cadavers were removed. The mean vapor concentration of dissection room was 1.5 ppm. The temperature of dissection room was 20-26°C and the air pressure was 760-763 atm.

At non-exposure times, all groups were kept in laboratory animal house, which was far from the place of exposure with no formaldehyde detection. The animal house was ventilated and its temperature was kept around 21°C with air conditioner system and adequate light was prepared. All groups were fed with a standard similar diet (bought from Iranian Pastor Institute) two times a day (morning and afternoon); but water was available all the time (*ad libitum*).

The cages of the case groups were placed at a height the same as cadaver's height with a distance of 15 cm apart from them for 18 weeks, corresponding time protocols mentioned above. During each period of exposure, the control group was kept in the animal house.

When the exposure period was expired, each of the rats of both experiments and control groups were anaesthetized with chloroform. After cervical dislocation, the abdomen was dissected and whole of the liver was extracted. Then specimens with sizes of 2 × 4 × 4 cm were taken from each specimen. These specimens were fixed in Formaldehyde buffer solution for 48 h.

After tissue processing and paraffin embedding, 10-12 sections from each specimen were cut at 5 µm and stained with Hematoxylin and Eosin (H & E). All of the sections were studied by OLYMPUS light microscope with multiple magnifications (40 x, 100 x, 400 x).

RESULTS

In group E1, mild infiltration of mononuclear cells was seen in the portal space. In addition, some lymphocytes infiltrated into intra-acinar area. These findings were suggestive for lobular hepatitis. Some hepatocytes, particularly in periportal area, showed evidences of regeneration (Fig. 1).

In group E2, Dilatation and congestion of sinusoids and centrallobular veins were found. The dilatation was more severe in some cases and appeared as cyst like formations rarely (Fig. 2).

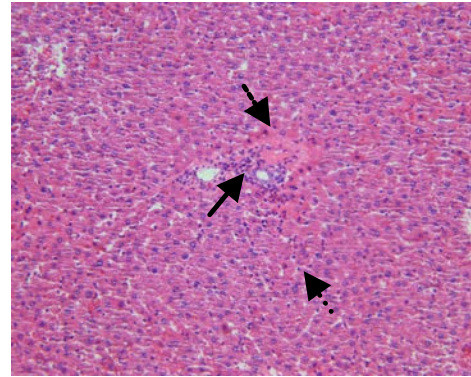


Fig. 1: The histologic section of rat liver in group E1; showing mild mononuclear infiltration in portal space (→), lymphocytic infiltration in intra-acinar area (•••→) and evidences of hepatocyte regeneration (•••→). (H & E, 100X)

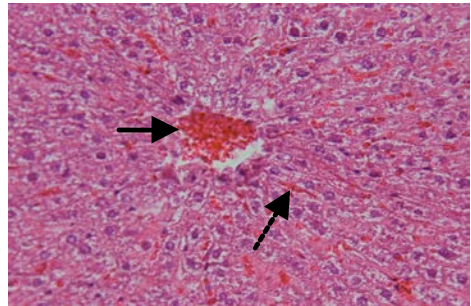


Fig. 2: The histologic section of rat liver in group E2; showing dilatation and congestion in centrallobular vein (→) and sinusoids (•••→). (H & E, 400X)

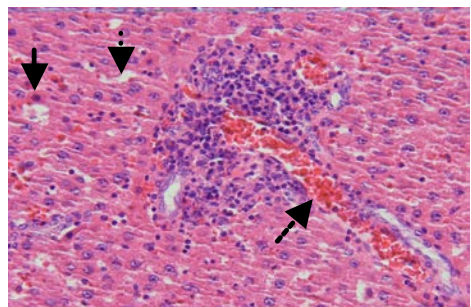


Fig. 3: The histologic section of rat liver in group E3; showing sinusoidal dilatation (→), centrallobular congestion (•••→) and ground glass appearance (•••→). (H & E, 400X)

In group E3, Some cells showed ground glass appearance focally. The sinusoids appeared slight

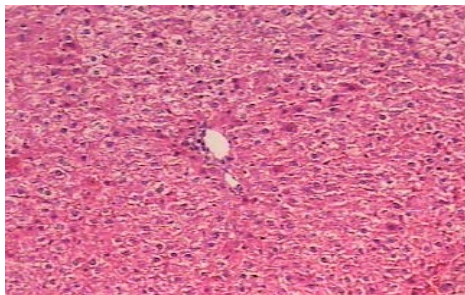


Fig. 4: The histologic section showing normal appearance of rat liver in control group. (H & E, 400X)

dilatation and congestion (Fig. 3). Cytoplasm of some cells was eosinophilic with oncocytic appearance. No evidences of parenchymal necrosis were found in all case groups.

No histopathologic changes were seen in control specimens (Fig. 4). It is important to remember that the mentioned findings were found in all sections of each group.

DISCUSSION

This study showed that formaldehyde exposure can cause mononuclear cell infiltration in portal space, hepatocyte regeneration and sinusoidal dilatation and congestion accompanied with arterial dilatation in portal space. In addition, some cells showed oncocytic appearance.

According to Berstein *et al.* (1984) study, formaldehyde exposure can cause edema in the liver. The edema can occur following sinusoidal dilatation which also was seen in our study.

Teng *et al.* (2001) found hepatotoxicity and decreasing in the level of anti-oxidant enzymes in formaldehyde-fed rodents. These effects can confirm our observations about hepatocyte regeneration.

Bandman (1994) exposed the experiments to formaldehyde (200 mg/m³ during 122 days) and observed inflammatory changes in the liver specimens. The mononuclear cell infiltration found in our study can be supported by these findings.

Kuchmeister *et al.* (1998) study showed that formaldehyde exposure can induce oncogenesis in the rodents. Kerns *et al.* (1983) also showed evidences for carcinogenicity of formaldehyde. In addition, it was reported that all aldehydes can cause DNA-Protein cross-link. The aldehydes are very active and can interact with cytoplasmic macromolecules, such as protein and

mRNA, in many cells. These evidences can highlight the role of formaldehyde in developing oncocytic appearance of some hepatocytes seen in our study. The hepatic cells with oncocytic appearance were the cells with granular eosinophilic cytoplasm and round central nucleus.

In contrast to these findings, Woutersen *et al.* (1987) found no microscopic lesions in liver specimens of rats exposed to 20 ppm (6 h/d; 5 d/w for 13 weeks).

Considering to kinetics of formaldehyde in human body and the rapid metabolism of absorbed formaldehyde within blood (<1.5 min) (Dart, 2004); the cytotoxic effects of formaldehyde can attributed to its main metabolite, formic acid. Formic acid is excreted through hepatic metabolism and urinary excretion. Saturation of formaldehyde-metabolizing enzymes of liver, primary and systemic metabolic acidosis as well as lactic acidosis in kidney due to inhibition of cytochrome oxidase (Dart, 2004) can reduce the hepatic and urinary excretion of formic acid. As the excretion decreases, the concentration of formic acid can reach to cytotoxic levels and cause the histologic changes were seen.

According to present study, the formaldehyde exposure with mentioned concentration and duration can cause histopathologic changes in rat liver. However, it seems that there is no relationship between severity of exposure-induced changes and the duration of exposure.

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