Effect of formaldehyde inhalation on rat livers: A light and electron microscopic study

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Abstract
It is well known that formaldehyde (FA) is cytotoxic and potentially carcinogenic. Although the individual effects of this reactant on cells has been investigated, the cytotoxicity exerted by the coexistence of FA is poorly understood. The aim of this study was to investigate the effects of FA on the liver in rats, by light and electron microscopic level. We used 18 Wistar albino rats divided into three groups, exposed to 0 (control), 19.7 ppm FA gas for a total of 4 weeks, 8 h/day, 5 days a week (subacute) and 20.3 parts per million (ppm) FA gas for a total of 13 weeks, 8 h/day, 5 days a week (subchronic). After the completion of the exposure period, they were sacrificed by decapitation and their liver tissue samples were taken in order to be processed for light and electron microscopic studies. Light microscopic evaluation of liver tissue samples of FA-exposed rats revealed enlarged sinusoids filled with blood and mononuclear cell infiltration in the portal areas and around the central veins. In addition, some of the hepatocytes showed loss of cytoplasm, and some had a hyperchromatic nucleus. The cells of FA-exposed livers, on the other hand, showed an electron-lucent ground-cytoplasm and a hypertrophy of the smooth-surfaced endoplasmic reticulum. In conclusion, we observed that exposure FA caused diverse histopathological changes indicating the destruction in the liver tissue and this destruction has direct relationship with the length of the exposure period.

Keywords
formaldehyde, liver, light microscope, electron microscope, rat

Introduction
Formaldehyde (FA) is a water soluble, colorless gas whose pure form is irritant and fetor. Its solid state is called paraformaldehyde and it can turn into its gas state, FA, in room temperature. FA concentration is generally explained as parts per million (ppm; 1 ppm = 1.25 mg/m³), and 37%–50% of its aqueous solution is called formalin (WHO, 2001). FA is the member of the aldehyde group in which some compounds such as acetaldehyde, malondialdehyde, acrolein, benzaldehyde, citral and vanillin are present and is an organic compound with a very reactive composition. FA is consumed via foods in different ratios and it is estimated that it is taken on an average of 1.5–14 mg/day in a day (Johannsen et al., 1986). It is also possible to take FA via respiratory and derm. FA metabolized by the liver is transferred into blood as format. The discharge of FA can be through urine as format salts or through lungs by turning into CO₂. Some of them enter into the carbon structure of protein and nucleic acids and are kept in the body (Bolt, 1987; Köppel et al., 1990; WHO, 2001; William, 2003).

FA, which has a wide area of usage was first used in industrial field in 1868. Formalin and urea-FA are the most commonly used forms. It is frequently used in medicine because it is both fixative and antimicrobial agent (Köppel et al., 1990). WHO declared the

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products that contain FA in a detailed way in 1985. According to this declaration, the products containing FA are as follows: adhesives and glues, cosmetic goods, deodorants, detergents, soaps, paints and especially white papers, explosives, fertilizers, filters and chemicals, protective covering used in keeping foods, and in the production and tanning of leather and fur, parquets, plywood, formica, rubber, latex, eraser, polish, varnish, textile goods and water softeners (WHO, 2001).

Many studies on the negative effects of FA have been conducted, which have a wide area of usage in human health. It is possible to categorize these negative effects in two headings such as irritant (acute and chronic) and carcinogenic (Bolt, 1987). The negative effects of FA have been published as a table by International Aldehyde Research Staff. According to this table, it is stated that there are no symptoms in 0–0.05 ppm, neurophysiological effect in 0.05–1.5 ppm, a change in scent threshold in 0.05–1 ppm, eye irritation in 0.01–2 ppm, upper airway irritation in 0.10–25 ppm, lower airway and pulmonary effects in 5–30 ppm, pulmonary edema and inflammation in 50–100 ppm and mortalities in 100 and over ppm (Bernstein et al., 1984).

In epidemiological and experimental animal studies, FA induced a variety of toxic effects, especially in liver tissue, after inhalation. These effects included dose-related focal hepatic necrosis, hepatic enlargement, decreased weight, and hepatocellular fatty degeneration (Beall and Ulsamer, 1984; Heck et al., 2004; Kamata et al., 1997; Rusch et al., 1983). In addition, FA is cytotoxic (IARC, 1995; Monticello et al., 1989; Swenberg et al., 1980), a potent upper respiratory tract irritant (Kriebel et al., 2001; Monticello et al., 1989; Sari et al., 2004), and induced squamous cell carcinoma in the nasal cavity of rats (Feron, et al., 2001; Kamata et al., 1997; Swenberg et al., 1980). Prolonged exposure to FA may cause degeneration in the proximal tubules and necrosis in the kidney (Greenberg, 1982).

However, studies on the toxic effects at the liver are limited. Considering this point, we have aimed to present the toxic effects of FA gas on the liver, on the levels of light and electron microscopic.

Materials and methods

Animals

The experimental protocol was approved by the Ethical Committee of Trakya University Medical Faculty, Turkey. In this study, 18 healthy male Wistar albino rats weighing 250–300 g and averaging at 12 weeks old were utilized. The animals were produced in Laboratory Animals Research, Department of Trakya University, and housed in individual cages (360 × 200 × 190 mm) 1 month before the start of the experiments. Food and tap water were available ad libitum. In the windowless animal quarter, automatic temperature (22°C ± 2°C) and lighting controls (12 h light/12 h dark cycle) was performed. Humidity ranged from 50% to 55%. All animals received human care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health.

FA generation and monitoring

The FA gas was generated from paraformaldehyde (Merck KgaA, 64271 Darmstadt, Germany) by thermal depolymerization. This method was originally described by Chang et al. (1983). FA level of the test atmosphere was monitored continually by an FA monitor (Environmental Sensors Co., Boca Raton, FL, USA) maintained within the exposure chamber. The FA monitor was calibrated with an FA permeation tube (Vici Metronics, Santa Clara, CA, USA), at a permeation rate of about 60 ng/min per centimeter. The actual permeation rates were confirmed periodically by using the FA Meter ES300 (advised by OSHA).

Formaldehyde exposure

Rats were exposed to FA, 0 (control), 19.7 ppm FA gas, for a total of 4 weeks, 8 h/day, 5 days a week (subacute) and 20.3 ppm FA gas for a total of 13 weeks, 8 h/day, 5 days a week (subchronic), in order that their whole body would be affected. Animals were not given water or food during the inhalation period, but were allowed to eat and drink at all other times. In glass chambers (90 cm breadth, 50 cm height and 60 cm depth), the temperature was maintained at 25°C ± 5°C, humidity at 47%–55% and airflow was adjusted to 10 L/min.

Light microscopic examination

After treatment, the animals were sacrificed and liver tissues were removed for histological investigation. Liver tissues were harvested from the sacrificed animals, and the tissues were fixed in 10% neutral buffered formaline, embedded in paraffin, sectioned at
5 mm thickness and then stained with hematoxyline-eosine. Histological specimens were examined in light microscopy (Nikon Optiphot II, Japan).

**Transmission electron microscopic examination**

For electron microscopical observation, liver specimens in 1 × 2 mm size were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer and after primary fixations tissues were washed in 0.1 M phosphate buffer overnight. The tissues were postfixed with 1% osmium tetroxide in phosphate buffer for 1 h at 4°C. Then, the postfixed tissues were washed in 0.1 M phosphate buffer and dehydrated by graded ethyl alcohol and finally with propyleneoxide. Dehydrated tissues were processed for making araldite blocks. Ultrathin section were obtained by ultramicrotome (RMC-MTX Ultramicrotome-USA) and collected on copper grids for double staining (uranyl acetate and Reynold’s lead citrate). Stained sections were finally observed under a Jeol-JEM 1010 transmission electron microscope.

**Results**

**Light microscope**

The sections of control group were stained with hematoxylin-eosin and examined under the light microscope. After that, liver tissue was observed to be in normal structure (Figure 1A). In the liver of the rats exposed to FA, vena centralis and sinusoids were observed to be slightly enlarged. Partial loss of cytoplasm was detected in some of the hepatocytes. In addition, mononuclear cell infiltration was distinguished in portal tract (Figure 1B). In the liver of the rats exposed to subchronic FA, on the other hand, vena centralis and sinusoids were observed to enlarge much more compared to the group exposed to subacute FA. Loss of cytoplasm in hepatocytes was ascertained to rise noticeably. Mononuclear cell infiltration in portal tract was not distinguished in the subjects of this group (Figure 1C and D).

**Electron microscope**

The liver tissue of the control group was found to be normal ultrastructurally under EM examination (Figure 2A). It was observed that most of the mitochondria in the subacute group were smaller than the ones in the control group, granular endoplasmic reticulum was irregular, dilatation took place in smooth endoplasmic reticulum and villi in the space of disse, on the other hand, were disrupted. Moreover, the beginning of pycnosis as a result of chromatine coarsening was seen as well as slight loss of cytoplasm (Figure 2B). It was detected that mitochondria in subchronic group were small like the ones in subacute group, but the numbers of them showed increase and the nucleolus was divided into two parts; on the other hand, chromatine coarsening rose. Besides, it was observed that granular endoplasmic reticulum was irregular, there was dilatation of smooth endoplasmic reticulum and the loss of a major part of cytoplasm as well as resolutions. Villi in the space of disse of hepatocytes, on the other hand, disappeared completely and focal postnecrotic cirrhotic changes were determined as a result of the development of collagen fibrils in this region (Figure 2C and D).

**Discussion**

The most remarkable effect of FA (beside the irritant effects mentioned above) is the mutagenic and carcinogenic effect. After the exposure of test animals to the acute, sub-acute, sub-chronic and chronic FA, focal-nonneoplastic and neoplastic changes in the respiratory epithelium of nasal mucous membrane had been observed.

In the epidemiologic studies carried out on humans, it cannot be proved to lead to nasal and lung cancer even if there is an increase in neoplasm of the upper respiratory tracts. It is stated in literature that FA had various effects on many more systems in addition to the respiratory system, eye, allergic, mutagenic and carcinogenic effects (Bernstein et al., 1984; Vargova et al., 1993; William, 2003).

The great majority of these studies, in both our country and other countries, are in the interpretation form of clinical complaints and tissue samples’ assessments, which is done on the people, working in a place using FA very often. When looking at foreign articles, if they are the majority of the experimental studies on respiratory system, there are studies about the other systems. When looking at these experimental studies especially about liver: Harrington exposed rabbits to 40% formalin solution from 4–6 mL, which was gained from FA gas, in the study in 1898. Eventually, it was declared that there was degeneration on some liver’s cell in central lobules and dilatation on hepatic veins (Harrington, 1898). Fischer, in his study in 1905, found that the solution, which was diluted in rates 1:1000 and 1:2000 formalin, was injected by intraperitoneal way in a period...
which was distributed from the 4th day the 38th day. In the end, he found inflammation in liver, cloudy swelling and focal hepatic necrosis (Fischer, 1905). Skog gave high doses with the way of inhalation (500–1400) and FA subcutan (0.15–0.46 and 0.30–0.64 g/kg) to rats in 1950. In the end, he declared that hyperemia in liver caused perivascular edema and necrosis (Beall and Ulsamer, 1984). Gofmekler applied 0.83 and 0.01 ppm FA gas to rats 22 days/hour for 6 weeks, in his study in 1968. He found slight hypertrophy in kupffer cells, multisegmented configuration in sinusoids and lack of glicogen in the lobules’ peripheric pieces in liver (Gofmekler, 1968). Feld’man and Bonashevskaya (1971), found histopathologic changes in the livers of the groups, who were applied only 0.83 and 2.45 ppm doses, as a result of that rats were exposed to 0.001,0.03,0.83 and 2.45 ppm doses of FA gas in 3 months (nuclear polimorfism, focal hyperplasia, moderate declination in the glycogen amount of the liver cells, declination and coarsening in density of RNA granuls (Feld’man and Bonashevskaya, 1971). In the study, which was carried out in 1978 at the laboratory of Battle Columbus, rats and mice were exposed to 2, 5, 6 and 14.1 ppm doses FA gas for 6, 12, 18 and 24 months. At the end, in the group that was exposed to 14.1 ppm for 18 months, degeneration in hepatocellular, centrilobular and

Figure 1. (A-D) Light microscopic images in different groups belonging to the liver tissue. A: The normal structure of liver tissue belonging to the control group. B: In the liver of the rats belonging to the subacute group exposed to formaldehyde, slight dilatation of vena centralis and sinusoids and as well as partial loss of some hepatocyte cytoplasm, mononuclear cell infiltration in portal tract have been discriminated. C: In the liver of the rats belonging to the subchronic group exposed to formaldehyde, moderate dilatation of vena centralis and sinusoids and the loss of a major part of cytoplasm in most hepatocytes compared to acute group are striking. D: In the liver of the rats belonging to the subchronic group exposed to formaldehyde, the disappearance of mononuclear cell infiltration in portal tract as well as loss of cytoplasm in most of the hepatocytes are conspicuous. CV: vena centralis; S: sinusoid; the arrow head: the loss of cytoplasm; Star: mononuclear cell infiltration; PT: portal tract; H&E ×200.
cytoplasmic vacuoles and in the other group who was exposed to the same amount dose for 6 months, centrilobular cytoplasmic vacuolization were ascertained. The research carried out in 1981, at Battelle Norwest Laboratory, exposed 40 ppm FA gas to mice during 6 hours a day, 5 days a week and for 13 weeks. In the end, focal necrosis in liver cells were asserted (Beall and Ulsamer, 1984). Strubelt et al. gave 10 mmol/1 FA as an initial dose in the study of liver perfusion, and then attended electronmicroscopic analyzing by taking incisions. After all, damage was retained at the FA in mitochondrias (membrana ruptures, loss in crystals) and some endoplasmic reticulums with granule (Strubelt, 1989).

In our study, 18 rats were separated into 3 groups of 6. One of them was control group, the first of other two groups was exposed to 19.7 ppm FA gas during 4 weeks (5 days in a week and 8 hours in a day) and the last group was exposed to 20.3 ppm FA gas during 13 weeks (5 days in a week and 8 hours in a day). Then in our light microscopic analyses, we have observed vena centralis and slight widening at sinusoids on livers of the rats that were applied subacute FA. We have assigned partial cytoplasmic loss on some hepatocytes. Also, we have differentiated mononuclear cell infiltration in the portal area. We have observed more widening on vena centralis and sinusoids at the rats’ livers that were exposed to

Figure 2. (A-D) Electron microscopic (EM) images in different groups belonging to liver tissue. A: The appearance of normal hepatocyte under EM examination of the control group. B: In thin section belonging to the subacute group exposed to formaldehyde; distinct dystrophic and primary necrotic changes are displayed. C: In thin section belonging to the subchronic group exposed to formaldehyde; as well as dystrophic and regenerative changes, primary fibrosis are shown. D: In thin section belonging to the subchronic group exposed to formaldehyde; necrotic changes are striking in addition to the disruption and fragmentation. N: nucleus, M: mitochondrion, the arrow head: granular endoplasmic reticulum, O: glycogen; L: lysosome; arrow: lipid droplet; asterisk: smooth endoplasmic reticulum; S: gall canaliculus; D: the space of disse; C: collagen. A, B ×8000, C ×5000, D ×6000.
subchronic FA than the other group that was exposed to subacute FA. We have assigned cytoplasmic loss on hepatocytes markedly. We have not differentiated mononuclear cell infiltration at the portal area in this group.

In our microscopic analysis, we have observed that many of subacute group mitochondrias have been smaller than the control group’s, lack of order on endoplasmic reticulum with granule, dilatation on the smooth endoplasmic reticulum and deformation on villi at the space of disse. Furthermore, we have seen that there has been a beginning of pyknosis according to rare degree on the cytoplasmic loss with the cause of coarsening chromatin. In the subchronic group, mitochondrias have been smaller than similar mitochondrias that have been like in subacute group’s, but their amounts have been getting more and the nucleolus has been divided in two parts and the chromatin has coarsened.

Moreover; lack of order on endoplasmic reticulum with granule has been getting more, there has been dilatation on the smooth endoplasmic reticulum and there has been big loss in cytoplasm with dissociations. We have ascertained that hepatocytes and villi in the space of disse have disappeared and there have been focal postnecrotic cirrhotic changes as a result of development of collagen fibrils in this area.

These findings have been such as to support the histopathologic changes that have been declared at the literature. In addition to this, although applied doses have been very closer, changes in subchronic group have been more advanced than subacute’s, this has showed that there has been direct proportion with tissue damages, which have been declared with duration of exposure to FA.

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Corrigendum

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Corrigendum to the above article as follows:

The authors omitted to include the following sentence in the published article:

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