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Histologic and histometric assessments of the potential formaldehyde immunotoxicity in the mice

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ABSTRACT

Formaldehyde (FA) as a major source of occupational pollutant is commonly used in the industrial and medical settings. It has been demonstrated that FA exposure in industry causes health impairment especially immune profile. Therefore, the aim of the present work was to characterize its potential immunotoxicity in mice. Forty male Balb/C mice were exposed to FA gas as levels of 0 (control), 7, 14 and 28 ppm, during 8 hours/day over a period of 30 consecutive days. In the end of experiments, all of mice were anesthetized; the blood samples were taken and total as well as differential leucocytes counting were carried out. In addition, tissue specimens were taken and histological alterations of the spleens, thymuses, adrenal glands and sub iliac lymph nodes were examined using optical microscope. The diameters of splenic as well as lymph nodes follicles were lower than the control, whereas the splenic megakaryocyte counts were found to be higher in the FA exposed animals. Also, thymus tissue in the experimental animals has been affected and its lymphoid cells population shows a significant decreasing. Moreover, histometric assessment revealed the diameters of tymic cortex and medulla was significantly lower than those of the controls. Finally, the total leucocytes count significantly declined in the FA exposed animals. Other examined immunological parameters as well as adrenal glands's structure was not different from those of the controls. Histological study, histometrical analysis and peripheral blood cells counting indicate that inhalation of FA gas has a harmful impact on the immunocompetent organs in the mice.

Keywords: Histological, formaldehyde, mice, histometrical, immunotoxicity

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INTRODUCTION

Formaldehyde (FA), a member of the aldehyde family and one of the simplest organic molecules, is a bactericidal agent and tissue preservative [1, 2, 3]. It is widely used in industrial and medical settings and is a major source of occupational pollution compound [4] which can rapidly diffuse into many tissues [5]. Recently, there has been increased awareness of the possible toxic effects of FA on the human health [6, 7] and as well as laboratory animal [8, 9]. In the previous work we have been demonstrated that inhalation of FA in 7, 14 and 28 ppm induced toxic morphological and structural alterations in the placental tissue [8].

Lymphoid tissues alterations recently have received considerable attention as a target organ for the achieving the chemical materials toxicity [10]. In this regards, it is reported that FA and/or xylene may affect the systemic cellular immunity, as well as local immunity in bronchus (BALT) particularly in young and adult rats [11]. A study also demonstrated that FA exposure in the worker induces significant increasing in the TNF-ά amount, and also statistically decreasing in the IgG and IgM levels [6].

Since the understanding of the toxic actions of FA on the lymphoid organs is very important for occupational and public health; therefore, the aim of this study was to determine overall its effect on immune system by histologic and histometric methods.

MATERIALS AND METHODS

Animals

Forty male Balb/C mice aged 7 to 8 weeks were purchased from Razi Institute (Karaj, Iran). The animals were maintained in a controlled environment at a temperature of 23±1° C; a humidity of 45±5% and natural 12:12 h light-dark cycle and had ad-lib access to drinking water and food. Mice were allowed to be acclimatized to the laboratory environment at least 6 days before commencement of testing.

Experimental design and FA exposing

FA (CH2O) was obtained from Biochem Chemical Company (Tehran, Iran). The mice were randomly alloted into one control group and three experimental groups. The mice of each group were placed in a glass chamber that has two holes for the air to come in or out. In experimental groups, the FA in gas phase was pumped for the period and at the amount desired into glass containers from where it was expelled with an air pump. 10 lt/min airs was given to the mice in the control group. The experimental animals were exposed to 10 lt/min airs mixed with FA gas as levels of 7, 14 and 28 ppm, during 8 hours/day over a period of 30 consecutive days. The concentrations were determined on the basis of a primary study. Glass covered containers were cleaned every 3–4 days, and a thick layer of sawdust was placed under them.

Histological and histometrical assessment

At the end of the administration period; the animals were anaesthetized with chloroform vapor, quickly brought out of the jar and sacrificed. Heparinized blood samples were drawn by cardiac puncture. For tissue assessment; the specimens from spleens, thymuses, adrenal glands and sub iliac lymph nodes were immersion imprisoned overnight in 10% neutral buffered formalin to be fixed. Then the specimens were mounted to allow 5-µm sections. Sections were stained via hematoxylin and eosin method and photographed directly using a stereo microscope with Microsoft system.

A histometrical analyze was carried out to exact determination of the lymphatic organ's structural alterations. For this purpose, splenic megakaryocytes in unit area of (1.44×10^4 μm^2 tissue area) were determined by counting in 10 randomly selected areas in subcapsular white pulp regions [12] using Image Tool® 3.0 software (UTHSCSA, San Antonio, TX, USA). Also, in each animal from all of the groups 10 tissue sections (7µm) were taken at 21 µm intervals, and splenic capsule thickness, and also splenic follicular diameter were recorded. Furthermore, thymic capsule thickness, thymic cortex diameter as well as thymic medulla diameters were recorded. In addition, the thickness of the glomerular, fascicular, reticular and medullary layers of adrenal glands has been determined. Finally, the thickness of the lymph node´s capsule and the diameter of the lymph nodes follicles were determined.

Statistical analysis

All statistical analyses were carried out using Statistical Package for Social Scientist (SPSS version 13, Chicago, IL, USA). Results were tested for normal distribution (Shapiro-Wilks) and homogeneity of variances (Bartlett test) and then expressed as standard error of the mean (SEM). The analysis of variance (ANOVA) was used to test the overall significance of differences among the means. Tukey-Kramer´s Multiple Comparison Test was applied for post hoc comparison. A probability level of less than 5% ($P < 0.05$) was considered as significant.

RESULTS

Spleen

The diameters of splenic follicles were lower than the control, whereas the splenic megakaryocyte counts as well as the thickness of the spleen´s capsule were found to be higher in the FA exposed animals ($p<0.05$) (Table1) (Figure 1-a). It was not found any outstanding differences in the histological results between three experimental groups.

Adrenal glands

In the control group the adrenal glands have been displayed normal histology with a large cortical and medullar compartments. Similarly, the adrenal gland's structure was not different from those of the controls (Figure 1-b) and (Table 1).

Thymus

Thymus tissue in the experimental animals has been affected and its lymphoid cells population shows a significant decreasing. Also, histometric assessment revealed the diameters of tymic cortex and medulla was significantly lower than those of the controls. Furthermore, thymus tissue of the control group had normal cellular population in its cortical and medullar compartments ($P<0.01$) ($p<0.05$) (Table 1) (Figure 1-c).

Lymph node

The diameter of the lymph nodes follicles was lower than the control, whereas the thickness of lymph nodes capsule was found to be higher in the FA exposed animals (Table 1) (Figure 1-d).

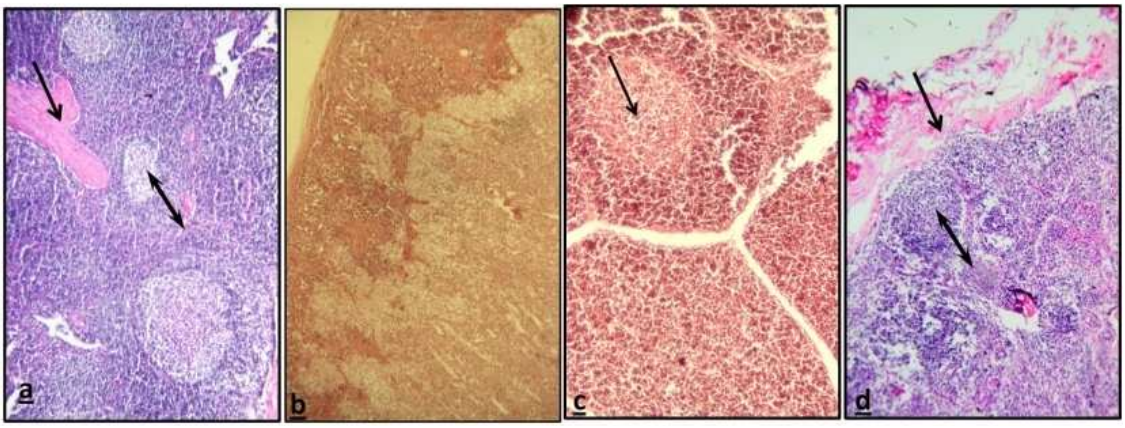

Figure1: a: Spleen transverse sections of the 7 ppm formaldehyde treated animals; shows decreasing of diameters of splenic follicles (double head arrow) and also elevation in the thickness of spleen´s capsule(arrow). b: Adrenal transverse sections of the 14 ppm formaldehyde treated animals; displayed normal histology with a large cortical and medullar compartments. c: Thymus transverse sections of the 28 ppm formaldehyde treated animals; shows decreasing in diameters of the diameter of the tymic cortex and medulla (arrow). d: Lymph node transverse sections of the 14 ppm formaldehyde treated animals; shows decreasing of diameters of lymphatics follicles (double head arrow) and also elevation in the thickness of lymph node´s capsule (arrow). (Haematoxylin and Eosine stain) (a, b,c,d: × 200)

Peripheral blood cells

Total leucocytes count significantly declined in the FA treated animals ($P<0.01$) (Table 2) compared with control samples. The number of lymphocytes significant decreased were also observed in the peripheral white blood cells ratio of the FA treatment groups ($p<0.05$). Other examined peripheral blood cell was not different from those of the controls (Table 2).

DISCUSSION

Although preventive measures aimed at reducing FA contaminant have been implemented, exposure to FA remains one of the most prominent environmental health problems [13, 14, 15]. In the present work, histological study, histometrical analysis and peripheral blood cells count indicates the potential immunotoxic properties of FA exposure at different concentration in the mice. In line with these findings, it has been claimed that functions of both the immune and hematopoietic systems could be affected by chronic exposure to FA, phenol and organic chlorohydrocarbons chemical substances [16].

It had been suggested that chronic exposure to FA might be associated with immunological hypersensitivity, as measured by elevated circulating IgG and IgE auto-antibodies to human serum albumin [17, 18]. In contrast, exposure to low concentration of FA in the workers was related to significant decreasing in the IgG and IgM levels [6].

In the current study cellularity of spleen and its structural integrity was affected by FA administration. In other words, morphometric results have indicated that FA caused profound suppression in the splenocytes population in the treated mice. Splenic immune-suppression may be attributing to the decreased different lymphatic cells numbers in the spleen as well as other immune organ. In line with these results, similarly, previous studies have demonstrated the FA administered at 20, 40, 80 mg/kg for 4 weeks by gavage method causes narrowed thymus-dependent zones of PALS in the spleen tissue [19]. Furthermore, a study of the bone marrow and spleen of animals injected with 6.25–25 mg/kg FA, intraperitoneally, showed no induction of chromosomal aberration [19]. Similarly, a research has shown that FA vapor can cause morphometric changes in the white pulp of the spleen in rats [20].

Our results show the significant decrease in the diameter of the lymph node´s follicles, whereas an elevation in the thickness of lymph nodes capsule in FA given animals. Other studies have shown that FA can decrease human body weight and increase lymph node weight during oral exposure, although there is no effect on the cellularity of lymphoid tissue [21].

In this study, FA administration caused significant alterations in the thymus tissue and its lymphoid cells population. In line with this fingings, FA at the concentration of 10 mg/m^3 exerted the negative effect on the thymus [22].

In the FA treated animals, total leucocytes count was significantly declined; also, lymphocytes number showed a significantly reduction. This finding may be suggesting an immune suppression. In fact, the regressive histological alterations of lymphatic organs could further interpret the decreases of total leucocytes count. Although exact causative mechanism(s) and factors for FA induced immunological toxicity is not clear, but chromosomal damage by FA exposure in human peripheral blood cells was been claimed to occur [23, 24]. Also, chromosomal damage leading to micronucleated lymphocytes was found to be more frequent in highly exposed pathology and anatomy laboratory workers than in controls. The difference was suggested to be due to a higher frequency of chromosome loss, suggesting FA-induced defects in the mitotic apparatus [25].

Upon to this study, it is concluded that FA has toxic effects on immune cell populations in the immune-competent organs of the mice. On the other hand, our study also suggests the immune-suppressive and immune-toxic properties of FA exposure. Finally, further studies will be needed to explore exact causative mechanism(s) and factors for FA induced immunological toxicity.

Table 1. Summarized histometric changes in spleens, thymuses, adrenal glands and sub iliac lymph nodes of the mice exposed to different concentrations of formaldehyde.

Experimental groups/Parameters		Control	7ppm, 8hours/day FA	14ppm, 8hours/day FA	28ppm, 8hours/day FA	Significant
Spleen	**SCT(µm)**	3.3 ±0.45	4.99 ±0.4	4.7 ±0.86	5.2±0.7	*
	GCD (µm)	78.9 ±7.5	26.4 ±3.3	45.8 ±9.3	26.4 ±5.4	*
	MC/UA	1.4 ±0.08	3.7 ±0.01	6.5 ±0.09	4.7 ±0.07	*
Adrenal gland	**GLT(µm)**	67.3 ±7.5	66.6 ±13.1	64.8 ±07.2	67.2 ±7.8	-
	FLT(µm)	84.7 ±8.4	85.3 ±6.3	88.3 ±7.5	84.7 ±4.4	-
	RLT(µm)	63.1 ±4.6	63.4 ±7.0	62.9 ±9.3	64.4 ±7.1	-
	MT(µm)	74.6 ±5.7	74.3 ±2.5	77.2 ±7.2	76.7 ±6.4	-
Thymus	**TCT (µm)**	5.5 ±0.4	5.2 ±0.9	5.3 ±0.6	5.4 ±0.4	-
	TMD(µm)	94.9 ±6.9	47.2 ±2.5	36.9 ±4.8	34.1 ±4.7	*
	TCD(µm)	57.6 ±2.8	34.7 ±3.7	25.3 ±4.9	24.6 ±5.9	**
Lymph node	**LCT(µm)**	6.3 ±0.07	9.6 ±0.08	8.9 ±0.05	11.3 ±0.6	*
	LFD(µm)	73.8 ±4.9	24.9 ±0.6	33.5 ±3.7	43.4 ±3.6	**

*-: not significant, * : P<0.05, ** : P<0.01*

SCT: Splenic capsule-thickness, GCD: The diameter of germinal center of the lymphoid follicles, MC/UA: Megakaryocyte count/unit (1.44×104µm2) tissue area, GLT: Glomerular layer thickness of adrenal, FLT: Fascicular layer thickness of adrenal, RLT: Reticular layer thickness of adrenal, MT: Medullary layer thickness of adrenal, TCT: Thymic capsule thickness, TMD: Thymic medulla diameter, TCD: Thymic cortex diameter, LCT: Lymph node capsule thickness, LFD: Lymph node follicular diameter.

Table 2. Mean± standard deviation and range of the leukocyte differential count values of the mice exposed to different concentrations of formaldehyde.

Groups/ Parameters		Control	7ppm, 8hours/day FA	14ppm, 8hours/day FA	28ppm, 8hours/day FA	Significance Significant
Total leucocytes count ($\times 10^3$ /ul)		18.2 ±1.76	10.4 ±1.7	11.3 ±0.8	10.4 ±0.4	**
Neutrophils	**(%)**	21.3 ±0.5	2.6 ±0.5	18.4 ±0.5	22.5 ±0.6	*
	Range	20.3 -23.6	1.1 -2.4	16.4 -20.3	20.8 -22.9	-
Eosinophils	**(%)**	0.4 ±0.08	2.3 ±0.04	2.5 ±0.05	3.5 ±0.05	*
	Range	0.1 -2.4	0.09 -1.9	2.1 -2.8	2.4 -4.1	-
Basophils	**(%)**	0.1 ±0.04	2.8 ±0.04	4.8 ±0.02	3.5 ±0.02	*
	Range	0.06 -0.8	1.3 -4.1	2.3 -6.7	2.8 -3.9	-
Lymphocytes	**(%)**	76.8±0.4	56.6 ±5.7	38.9 ±2.4	36.4 ±3.9	**
	Range	45.7 -78.9	48.3 -66.2	35.3 -43.5	31.2 -54.1	-
Monocytes	**(%)**	1.4 ±0.05	35.7 ±0.09	35.4 ±0.6	34.1 ±0.5	**
	Range	1.1 -2.4	31.5 -37.8	28.9 -39.4	27.5-46.4	-

*-: not significant, * : P<0.05, ** : P<0.01*

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