

Evaluation of Bleach Vapors on Mouse Lower Respiratory System

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Abstract: The aim of this study was to examine the pathological effects of inhalation of bleach vapors, both short and long term, on the lower respiratory system of mice. This laboratory experimental study was done on 28 male mice which divided into four groups; Control: no contact with the toxic vapors and then they autopsied after 6 weeks. Acute: exposed to the toxic vapor (300ppm) for 2 hours and then autopsied immediately. Sub-acute: exposed to the vapors for 2 hours, had no contact with the toxic materials for 6 weeks and then autopsied. Chronic: exposed to the toxic materials 2 hours per day for 6 weeks and then were autopsied. The results were compared according to the time and the amount of the exposure. In the control group the score for involvement was zero and no pathological changes were observed. In the acute group, infiltration of acute and chronic inflammatory cells, bleeding and edema (with different scores) were observed in 100% of the test subjects, while the score of fibroblastic reaction was zero. In the sub-acute group, the score for the acute inflammatory cells was zero. As a result of the infiltration of the chronic inflammatory cells in this group, bleeding, edema and fibroblastic reaction with the score of one were detected in 40%, leaving the rest (60%) unchanged. In the chronic group, there were no acute inflammatory cells, bleeding or edema, while in 100%, chronic inflammatory cells infiltration and fibroblastic reaction were observed. The inhalation of bleach vapors can stimulate immune and fibroblastic systems which can lead to fibrosing disease in people who have come to contact with these materials.

Key words: Bleach vapor • Pneumonitis • Mice • Fibroblastic reaction • Exposure

INTRODUCTION

Restrictive lung diseases (RLD) are characterized by reduced compliance. Since predominant changes are localized in the interstitium, RLD is usually known as interstitial lung diseases [1]. The chronic restrictive diseases of the lung are a heterogeneous group with an unknown cause and pathogenesis. The more common examples are occupational exposure, drug related and immunologic lung disease. Hypersensitivity pneumonitis is an immunologically mediated inflammatory lung disease that primarily affects the alveoli and is therefore often called allergic alveolitis. Generally it is an occupational disease that results from heightened sensitivity to inhaled antigens [2]. Among the numerous causes is chloramine gas.

Sodium hypochlorite and chloramine gases are produced when the bleaching solutions are mixed together. These gases are the strong stimulant materials that damage the lung tissue. Inhalation of these gases which are produced by using the household products at home and other places, causes signs and symptoms such as asthma, temporary mucosal inflammation, lung edema, transient bronchospasm, toxic pneumonitis and RADS (Reactive Airways Dysfunction Syndrome) and even death [3, 4].

Based on these facts we decided to develop a new method to evaluate the histopathological effects of these gases on the lower respiratory tract of mice based on temporary and long term effects with the duration of contact. It was evident that if we got the special figure of variations in the lung based on our questions and

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hypothesis, we could teach the general public to avoid the inhalation of these vapors or to use the necessary protections, as well as informing the physicians of their effects.

MATERIALS AND METHODS

In this experimental -laboratorial study some male and female mice were selected from the animal's house in Shahid Sadoughi Medical Collage and put them in the same separate cages to mature (fertile) females. Then we separated 28 new born male mice and put them in the same physiological and environmental conditions to grow and reach to our favorite size (30 gr). The mice were randomly divided into four groups, one control group (were put in a box without vapors of bleaching solution for six weeks each day for two hours) and then were autopsied after six weeks. Second group (acute) which received 300 ppm vapors (i.e vapors ascendant from 150 cc Golrang solution containing Na hypochlorite, Na hydroxide, chlorine and chloramines in a closed aluminum box with 0.5 m³ volume) for 2 hours and immediately were autopsied. Third group (sub acute) received 300 ppm of vapors of the above mentioned bleaching solution for 2 hours, were kept for six weeks unaffected and then were autopsied. Fourth group (chronic) received 300 ppm for six weeks, two hours per day and were autopsied at the end of the sixth week. Our selection of 300 ppm was based on the initial failure of 400 ppm that we had chosen by the advice of a veterinarian. Since the mice died with 400 ppm, we had to titrate the dose to the endurable amount of 300ppm.

The results from each group were compared and noted in terms of the duration of the contact and the inhalation of vapors with pathological changes on the lungs of mice.

The decision of the scores was made based on the pathology reference books and studies done in USA in 2005 as below: [5,6].

"Score 0: *No macrophages no hemorrhage, no fibroblastic proliferation.*

Score 1: *A few macrophages with a few fibroblastic proliferations.*

Score 2: *Mild macrophages infiltration, lymphocytic infiltration and fibroblastic proliferation, mild interstitial thickening.*

Score 3: *Moderate macrophages infiltration lymphocytic infiltration and fibroblastic proliferation, moderate alveolar collapse.*

Score 4: *Severe fibroblastic proliferation, collapsed alveolar spaces.*

First assessment was done on slides stained by H&E method for hemorrhage, edema and infiltration of the inflammatory cells. For the detection of fibrosis, all of the slides were stained with reticulin and trichrome staining method.

Finally the collected data were analyzed by the kroskal- wallis test and using minitab soft ware. P value less than 0.05 was considered statistically significant.

RESULTS

In the control group, all the subjects had a score of zero and no pathological changes were seen in their lungs. In the acute group there was an infiltration of acute and chronic inflammatory cells and edema with different scores in 100% of the cases. However the score of fibroblastic reaction was zero in all of them. In the sub acute group, we detected chronic inflammatory cells infiltration in 100% of the cases, which 60% did not have fibroblastic reaction, hemorrhage and edema, while 40% of the cases the score of one was observed. In the chronic group, no infiltration of acute inflammatory cells, edema and hemorrhage were detected. However, chronic inflammatory cells infiltration and fibroblastic reaction were seen in 100% of the cases. No sign of acute inflammatory cells infiltration in chronic, control and sub acute groups were observed, instead, all mice in the acute group had this infiltration. 60% of them had score 3, 20% had score 2 and 20% had score 4. According to p-value =0.000 which was obtained by using the Kruskal-Wallis test, we concluded that acute inflammatory cells infiltration in control, chronic and sub acute groups are the same. Meanwhile the acute group has a meaningful difference from the remainder groups (Table 1). Chronic inflammatory cells infiltration was present in all the tested mice, regardless of the group. This infiltration in sub acute group was less than the chronic and acute groups. Since the p-value =0.000 was reached from the Kruskal-Wallis test, we concluded that chronic inflammatory cells infiltration, were the same in the chronic and acute groups, but more than the sub acute group. Also the chronic inflammatory cells infiltration in

Table 1: Frequency of acute inflammatory cells infiltration based on groups

Score group	0		1		2		3		4		Total	
	#	Percent	#	Percent	#	Percent	#	Percent	#	Percent	#	Percent
Control	7	100%	0	0%	0	0%	0	0%	0	0%	7	100%
Acute	0	0%	0	0%	1	20%	3	60%	1	20%	5	100%
Subacute	5	100%	0	0%	0	0%	0	0%	0	0%	5	100%
Chronic	7	100%	0	0%	0	0%	0	0%	0	0%	7	100%

Table 2: Frequency of chronic inflammatory cells infiltration based on groups

Score group	0		1		2		3		4		Total	
	#	Percent	#	Percent	#	Percent	#	Percent	#	Percent	#	Percent
Control	7	100%	0	0	0	0%	0	0%	0	0%	7	100%
Acute	0	0%	0	0	1	20%	2	40%	2	40%	5	100%
Subacute	0	0%	3	60	2	40%	0	0%	0	0%	5	100%
Chronic	0	0%	1	14	2	29%	4	57%	0	0%	7	100%

Table 3: Frequency of hemorrhage based on the test groups

Score group	0		1		2		3		4		Total	
	#	Percent	#	Percent	#	Percent	#	Percent	#	Percent	#	Percent
Control	7	100%	0	0%	0	0%	0	0%	0	0%	7	100%
Acute	0	0%	1	20%	1	20%	2	40%	1	20%	5	100%
Subacute	3	60%	2	40%	0	0%	0	0%	0	0%	5	100%
Chronic	7	100%	0	0%	0	0%	0	0%	0	0%	7	100%

the acute, sub acute and chronic groups was more than the control group (Table 2). There was no hemorrhage in the chronic group. In the sub acute group, 40% of the cases had score 4. In the acute group, 40% of the cases had score 3, 20 % had score 2 and 20 % had score 4. Based on the P-value =0.007 from the Kruskal-Wallis test, we concluded that hemorrhage was the same in the control, chronic and sub acute groups with a meaningful difference for the acute group (Table 3). The cases with edema were '0' in the chronic group and 40% with score 1 in the sub acute group. In the acute group, edema was found in 60 % of the cases with score 3, 20 % with score 2 and 20 % with score 4. So according to the p-value =0.006 from the Kruskal-Wallis test, we concluded that edema is the same in the chronic and sub acute groups with a meaningful difference of the control and acute groups. There was no fibroblastic reaction in the control and acute groups. In the sub acute group, only in 40 % of the cases fibroblastic reaction with score 1 was seen and in the chronic group 57 % of cases had score 4. According to the p-value= 0.002 from the Kroskal-Wallis test, we decided that the score of fibroblastic reaction in the chronic group has a meaningful difference with the control, acute and sub acute groups.

DISCUSSION

Bleach is used in several applications in our lives. Some of the more applications are in disinfecting, sanitation, odor control, chlorination of drinking water & swimming pools, cleaning clothes & bacterial control. Bleach is used across the world and praised for its antibacterial & antiviral properties. Infrequent, short exposure to bleach fumes usually does not pose any problems. However since sodium hypochlorite is an oxidizing agents and contains chlorine, it can cause pulmonary symptoms. According to this we decided to perform a modern method obtained by various searches to evaluate the effects of the inhalation of these vapors on the lower respiratory tract of mice.

One of the limitations of this research was its impossibility to perform ethically on human beings. Therefore we were forced to do this on laboratory animals and make this a unique research. In the past similar studies have performed based on the clinical findings on human beings. Earlier literature reported that exposure to a concentration of about 5 ppm caused respiratory complaints, corrosion of the teeth, inflammation of the mucous membranes of the nose and susceptibility to

tuberculosis among chronically exposed workers. However many of these effects are not confirmed in recent studies and are of very dubious significans [7]. Since the clinical sings on animals, are not detectable, we compared autopsy findings of animals' lungs with the clinical signs established on the human beings. It is necessary to mention that we did not have any mean for preparing the exact dose of 300 ppm and we were compelled to spray 150 cc of the material manually and experimentally. In spite of all the necessary precautions contraption and using various reviews for choosing the box for mice, some died due to hypoxia. In many of the previous articles and studies, the acute effects of the bleaching agents were tested. However, our research evaluated the chronic effects of these materials as well as the acute effects; therefore it has an especial value. In one study animals surviving sublethal inhalation exposures for 15-193 days showed marked emphysema, which has associated with bronchiolitis and pneumonia [8]. Chlorine injected into the anterior chamber of rabbit's eyes resulted in severe damage with inflammation, opacification of the cornea, atrophy of the iris and injury to the lens [9]. Severe acute effects of chlorine exposure on human have been well documented since World War I when chlorine gas was used as a chemical warfare agent. Other severe exposures have resulted from the accidental rupture of chlorine tanks. These exposures have caused death, lung congestion, pulmonary edema, pneumonia, pleurisy and bronchitis [10]. In a study done in Japan in 1992, it was concluded that housewives who clean lavatories with bleaching agents, were afflicted with acute respiratory syndrome [11]. Also in a study in USA in 1998, performed on 79 soldiers, the effects of these vapors caused acute respiratory syndrome [12]. We can generalize the obtained results in our research with the previous studies that the infiltration of acute inflammatory cells in the control, chronic and sub acute groups, are similar, but had a meaningful difference with acute group. Also scores of hemorrhage in control, chronic and sub acute groups were nearly similar and had a meaningful difference with the acute group. Our study shows that the similarity of edema degree in chronic, sub acute and control groups ($P_v = 0.006$) has a meaningful difference with the acute group.

According to these results and because the exudative phase of ARDS, characterized by capillary congestion, alveolar epithelial cells necrosis, edema, interstitial and intra alveolar hemorrhage and neutrophilic aggregation in capillaries [1], we can conclude that obtained results can show RDS in mice. This confirms the results of other

studies. In studies done in Japan and USA, one case of death was reported [11, 12]. Death had occurred to two mice afflicted with acute inhalation of the bleaching materials and two mice with encountering sub acute inhalation of these materials, (which is possible by inhaling rather than absorption through the skin). Since the number of deaths were not large enough to affect the statistical measurement, it was not taken in account. In addition, the above explanations can answer the questions and hypothesis such as the existence of death after given the dose. One study that was done in Turkey in 2004 showed the inhalation of these vapors could lead to airway dysfunction (ARDS). Also this study reported respiratory insufficiency in one person and showed the resulted complications are longer and more fatal in older people with less free space, or when these materials mix together sooner [3]. There was not any fibroblastic reaction in the control and acute groups and in sub acute group, we found this reaction only in 40 % of the cases with score 1. Nevertheless, in the chronic group, fibroblastic reaction was seen in the majority of mice with different scores, which shows meaningful difference in comparison to the other three groups ($p_v = 0.002$). So we can conclude that once the time of the inhalation is longer, the probability of irreversible and fatal effect is more and it can also answer this question and hypothesis that the long term contact can increase the incidence of complications. In one study done in 1986, Reiszuger reported that three patients were afflicted with threatening pneumonitis as a result of mixing the bleaching solutions over the span of one year [13]. One of the common causes of hypersensitivity pneumonitis is inhalation of the chemical agents and this disease presents as a predominantly restrictive lung disease with decreased diffusion capacity, lung compliance and total lung volume [1]. Because the density and frequency of the contact as well as the quality and the size of antigen particle are effective in disease process [2], therefore we can say the longer the time of the contact with bleaching agents, the probability of decreased diffusion capacity and irreversible changes such as fibroblastic reaction are increased. This in turn can cause a life-threatening pneumonitis Also one research in 1993, studied a group of about 216 prisoners and followed them up in 12 months after the gas exposure. The conclusion was: 200 cases improved during 6 hours, 16 cases were symptomatic after six hours, 14 cases cured at home, 71 cases were hospitalized and received oxygen and steroid therapy and 70 cases had only chest X-Ray signs. They concluded that although all cases were symptomatic,

most of them cured at home [14]. This study suggests that prevention of further contact with bleaching agents, even after one acute contact can be effective in decreasing the signs and improving the healing process.

Suggestions: Finally according to all the results, the public education is recommended. We suggest that the service workers, whenever possible, should not come to any contact with these materials. These materials should not be used in a closed environment because the vapors arising from them can cause life-threatening diseases such as ARDS. Surely when the usage of these agents is unavoidable, there must be enough ventilation. Educating physicians about the quality of vapors' effects, is also recommended. Also, we do recommend a further research on humans to determine the minimum concentration of the vapors to cause any damaging effects and accordingly, to take necessary precautions to avoid such concentrations. Furthermore, we suggest that with more research on people with IPF (Idiopathic Pulmonary Fibrosis), both genders included, the history of contact with these harmful vapors, would be recognized as one of the causes of IPF.

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