2008-5435/11/32-76-80
INTERNATIONAL JOURNAL OF OCCUPATIONAL HYGIENE

IJOH 3: 76-80, 2011

ORIGINAL ARTICLE

Effect of Vitamin C on Styrene Induced Respiratory Toxicity

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Received January 12, 2011; Revised June 3, 2011; Accepted July 27, 2011

This paper is available on-line at *http://ijoh.tums.ac.ir*

ABSTRACT

Styrene (ethylbenzene) is widely used as a solvent in many industrial setting. Occupational exposure to ST can result in pulmonary toxicity. For better understanding of the mechanism by which styrene caused lung injury, this study was undertaken to investigate the effect of styrene on rat respiratory epithelial cells. The role of vitamin C (Vit C) on styrene induced toxicity was also investigated. Adult male rats were given ST (ip) at doses of 0, 200, 400 or 600 mg/kg. Another series of rats were pretreated with Vit C (300 mg/kg, ip) 30 min prior administration of various doses of ST. 24 h later, animals were killed with overdose of sodium pentobarbital. Lung and trachea tissues were removed, fixed and processed for light microscopy. Results demonstrated that styrene induced dose-dependant injury in respiratory epithelial cells. The antioxidant, Vit C protected cells against styrene toxicity. The results support the view that generation of oxidative stress is responsible for ST-induced damage in respiratory airway. The finding that Vit C has potential to protect respiratory epithelial cells against ST toxicity further support this hypothesis.

Keywords: *Styrene vitamin C, Lung, Trachea, Rat*

INTRODUCTION

Styrene (ST) is widely used organic solvent. This chemical is used in the production of many products including polymers which are incorporated into products such as plastic, rubber, fiberglass, carpet backing and food containers. ST exposure has been associated with numerous health effects in human and laboratory animals. Occupational exposure to this chemical can cause fatigue, memory lost, and lung plus liver damage [1]. ST may be absorbed into blood stream by all routes of administrations [2-6].

Numbers of studies have indentified ST induced toxicity in humans [7-11]. Exposure to ST in humans results in effect on respiratory system with symptom such as chest tightness, wheeze and respiratory mucus membrane irritation [8, 9]. Occupational exposure to ST has been reported to cause asthma and induced hypersensitivity response [8, 9]. Roder-Stolinski et al. found ST–induced pulmonary inflammatory response in the workers occupational exposed to ST. These authors also reported that generation of oxidative stress is responsible for lung injury [12]. Similarly, Mogel et al. demonstrated that ST produced lung hypersensitivity in the workers exposed to ST, N-acetyl cysteine as antioxidant was able to prevent inflammatory reactions in lung epithelial cells [11]. Occupational exposure to ST diminished lung functions and induced oxidative stress [13].

The pneumotoxicity of ST in experimental animals were reported by several investigators [4-6,14]. Chronic exposure of mice to ST produced pulmonary injury [6].

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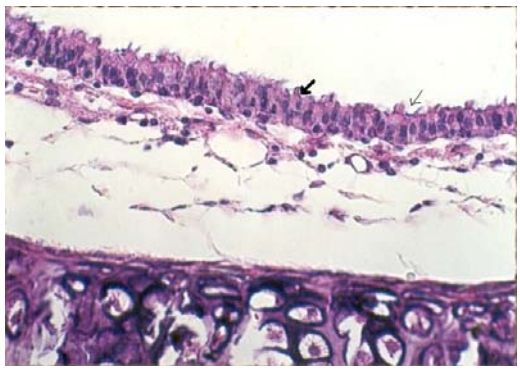

Fig 1. Light micrograph of tracheal respiratory epithelial cells of control rat. The ciliated cells (thick arrow) and nonciliated cells (thin arrow) are Intact. H&E x 200

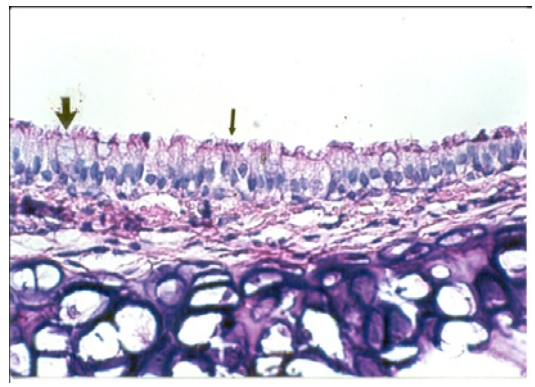

Fig 2. Light micrograph of rat trachea treated with 600 mg/kg ST. Note damage in ciliated (small arrow) and non ciliated (large arrow) respiratory epithelial cells. H&E stain x 200

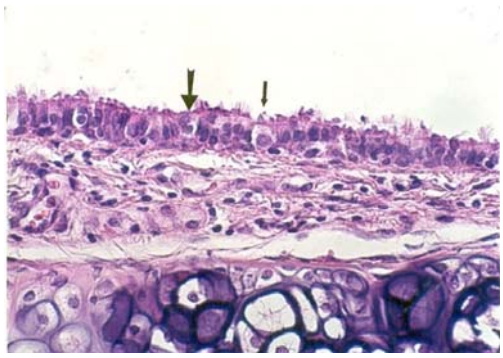

Fig 3. Light micrograph of rat trachea pretreated with 300 mg/kg vitamin C and received 600 mg/kg ST. Showing ST-induced cell injury diminished in compare with non-pretreated animals which received the same dose of this agent (Fig. 2). H&E x200

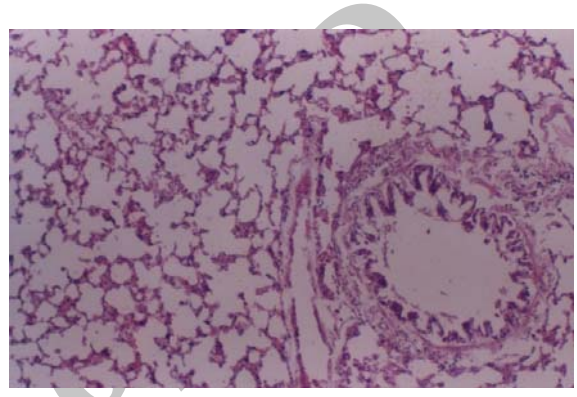

Fig 4. Light micrograph of control rat lung. There was no obvious injury in the lung cells. H&E X 200

Degenerative lesion in mice Clara cells were reported after exposure to ST [5, 14]. Hialcrvhuck and Carlson reported that ST induced histopathological injury in mice lung [14]. It is generally accepted that oxidative stress plays an important role in ST-induced toxicity. Carlson found that the level of GSH significantly decreased in mice after treated with ST [15]. Styrene produced toxicity may be related to oxidative stress [16].

Vitamin C plays an important role as an antioxidant to prevent cellular damage from free radical. This agent acts directly to scavenge free radicals and also protecting cell membrane by regenerating the antioxidant [17-19]. The large body of evidence indicated that styrene caused toxicity in experimental animals is similar to that reported in human exposure to styrene vapor [12-16, 20-22].

As clinical symptoms were noted following exposure to ST in human and experimental animals, thus the antioxidant chemicals may have the potential to diminish ST toxicity.

The study of the effect of ST on experimental animals may be useful for better understanding of the clinical pictures following ST exposure in humans.

This experimental in vivo study was conducted to investigate the effect of styrene on rat respiratory epithelial cells. Further, the role of vitamin C on ST toxicity was investigated. Having in mind that ST is an organic solvent with wide industrial applications and the significant potential of occupational exposure, this study may lead to a better understanding of mechanisms by which ST may induce pulmonary toxicity.

MATERIALS AND METHODS

Adult male Wistar rats (250-300 g) were housed in groups of 3 in clear polypropylene cages in a light cycle (12 h light and 12 h dark) and temperature-controlled room. The animals were allowed food and tap water ad libitum. The animals were pretreated with vitamin C (ip) at doses of 300 mg/kg [19]. Control rats received vehicle only (distilled water, D H_2O). Thirty minutes later animals were given styrene (ST) at doses 0, 200, 400, or 600 mg/kg, ip [4]. Twenty four h later, all animals were killed with over dose of sodium pentobarbital. The lung and tracheal tissues were removed, fixed and processed for light microscopy. The tissue was fixed in 10% buffered formalin for 24 hours, routinely processed and paraffin embedded. Five histological sections each at least 15 μm apart were taken from each tissue block and stained with Hematoxylin and Eosin, H&E. The criteria for cell injury included: nuclear dilation, loss of staining capacity and obvious cellular swelling. Ten animals

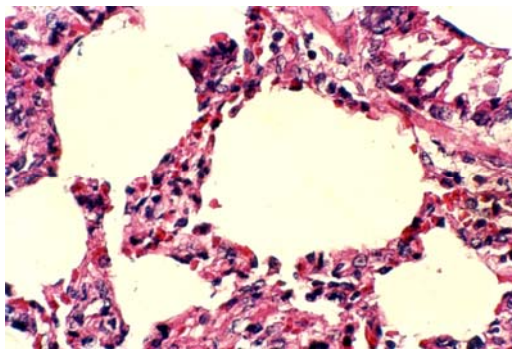

Fig 5. Light micrograph of rat lung treated with 600 mg/kg ST. Showing extensive injury including loss of staining capacity, dilatation of the nucleus and cellular swelling in lung, marked infiltration of inflammatory cells in to the alveolar space and septal thickening. Dilatation and vacuolization of type II pneumocytes (arrow). H&E x400

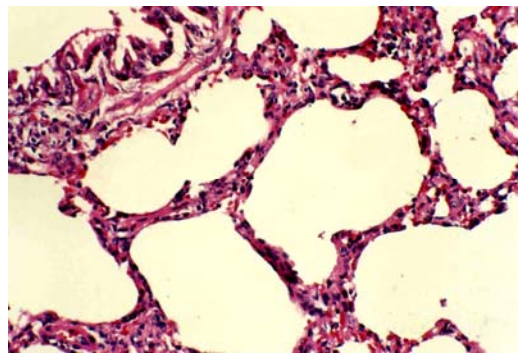

Fig 6. Light micrograph of rat lung pretreated with 300 mg/kg vitamin C and received 600 mg/kg ST. Showing ST-induced lung injury diminished in compare with non-pretreated animals which received the same dose of this agent (Fig. 5). H&E x200

were used for each treated group. The protocol was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences.

RESULTS

Administration of normal saline (vehicle) alone did not produce detectable injury in rat tracheal respiratory epithelial cells (Fig 1). However, cell injury was observed in the various morphological levels and regions of the trachea following treated with ST. Light microscopy revealed that both ciliated and nonciliated tracheal epithelial cells were swollen, had loss of staining capacity, and nuclei appeared to be dilated (Fig 2). However, the degree of injury varied in different levels and regions of tracheal epithelium. The extent of injury was related to higher dose of the ST exposure. Vitamin C had no effect on tracheal respiratory epithelial cells. However, the number of damaged cells significantly decreased in ST treated- animals pretreated with Vitamin C when compared to the ST treated rats pretreated with normal saline (Fig 3).

In control rats the lung was intact and there was no detectable injury (Fig 4). However, ST-induced damage in the lung tissue marked infiltration of inflammatory cells in to the alveolar space and septal thickening. Dilatation and vacuolization of type II pneumocytes and nonciliated Clara cells were observed in ST treated rats (Fig 5). The extent of damage was increased in dose dependant manner. Vitamin C had no detectable injury in rat lung and the lung tissue was similar to control animals. However, this agent markedly decreased pulmonary damage cells in ST treated rats (Fig 6).

DISCUSSION

The respiratory epithelium is the first line to contact inhaled of toxicants. ST is widely used with significant human exposure, particularly in the reinforced plastic industry. Although exposure of ST–induced adverse effects on respiratory system, little effort has been made to characteriz the effect of ST on respiratory airway epithelial cells. We observed dose dependant morphological changes occurring in the respiratory epithelial cells after systemic (interapertoneal, ip) administration of ST into rats. To determine the toxic effect of ST on various organs, many investigators were used systemic route (ip) of administration [4, 20, 23].

Coccini et al . observed histopathological alterations of rat respiratory tract after either inhalation of ST vapor or systemic (ip) treatment. However, these authors reported that pneumotoxic effect of ip administration of ST tend to be more severe than those seen in rats exposed to ST by inhalation for longer period of time [4].

The present study showed that ST induced dose – dependant toxicity in rat lung. Injury was mostly observed in type II and nonciliated Clara cells. Histopathological damage in lung was noted in experimental animals following exposure to ST [4-6]. These data lead to conclude that biotransformation of ST in situ at least in part is responsible for ST–induced pulmonary toxicity. As another possibility for ST induced lung injury is that translocation of ST metabolites from the liver to lung via general circulation produced respiratory toxicity.

The large body of evidence support the view that styrene caused pulmonary toxicity in experimental animals is similar to that reported in human exposure to styrene vapor [12-16, 20-22].

Although the mode of action ST-induced pulmonary injury is not completely understood, but sufficient evidence demonstrated that it may be related to oxidative stress including reduction of the level of GSH [11, 13, 16, 22].

Sati et al. studied the effect of ST on lung function and oxidative stress in occupationally exposed workers in plastic factory. These authors reported that inhalation of ST by workers significantly reduced lung functions and enhanced the level of oxidative stress. They concluded that generation of oxidative stress is responsible for ST-induced lung damage [13]. Oxidative stress acts as a primary molecular response mechanism of human lung epithelial cells to ST exposure [22].

Administration of ST caused depletion of GSH and induced toxicity in mice lung [23]. In vitro study has been shown that Clara cells are the main target cells for ST-induced pulmonary toxicity in human [22]. Antioxidant agents such as N-acetylcysteine (NAC) and glutathione protected liver cells against ST metabolite induced toxicity in mice [16]. Cruzan et al. observed that inhalation of ST by mice results cytotoxicity in terminal bronchioles [24]. Thus, it appears that ST produced toxicity in respiratory epithelial cells following metabolic activation and generation of reactive toxic metabolites. Acute exposure to ST caused an increased in lipid peroxidation and decreased glutathione level in mice. These authors suggested that enhancement of lipid peroxidation in lung is a consequence of depletion of glutathione on certain critical levels [20**].** Occupational exposure to ST-induced inflammatory response in respiratory system [11, 12]. We observed that vitamin C protected lung and respiratory epithelial cells against ST induced toxicity. On the basis of these results, we conclude that vitamin C may prevent the occurrence of ST induced adverse effect in humans respiratory system. The mechanism by which vitamin C protected cells against ST toxicity may be related to vitamin C is able to reduce reactive metabolites and/or supporting glutathione biosynthesis that serves directly as an antioxidant. Asthma associated with occupational exposure to ST was reported by several investigators [7, 8, 25]. Hays et al. described occupational asthma in ST exposure workers [8]. Roder-Stolinski et al. reported that ST-induced release of the inflammatory mediators by the human airway epithelial cells. These authors found that NAC inhibited the release of mediators [12]. Morbet et al. concluded that oxidative stress act as a primary molecular response mechanism of human lung epithelial cells to ST exposure [22]. Mogel et al. observed ST induced inflammatory reactions in human lung epithelial cells and NAC was capable to prevent the cells against ST toxicity. These authors suggested that generation of oxidative stress was responsible for ST–produced lung injury [11]. Result of our study along with others support the view that generation of oxidative stress is likely involved in ST–induced toxicity in humans and experimental animals. These data also support the use of antioxidants in order to ameliorate the adverse effects of ST in respiratory system.

In conclusion, ST produced dose dependant injury in lung and respiratory airway epithelial cells. This finding supports the view that these cells may have the potential to bioactivate ST. The observation that vitamin C had potential to ameliorating ST toxicity further support this hypothesis.

ACKNOWLEDGEMENTS

This study was supported by Physiology Research Center and the Research Deputy of Ahvaz Jundishapur University of Medical Sciences (Grant NO. PRC-76). Our special thanks go to Dr. A. Malaeke for reviewing histopathological samples. The authors declare that there is no conflict of interests.

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