The Probable Protective Role of Vitamin C against Cyclosporine an Induced Pulmonary Changes in Mice

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Abstract—Cyclosporine A (Cs-A) is a frequently used immunosuppressive agent in transplant medicine to prevent rejection and in the treatment of autoimmune diseases. Vitamin c is a potent antioxidant that has the ability to scavenge factors causing free radical formation in animals receiving Cs-A. It plays a major role in disease prevention. Cs-A induced histological and ultrastructural alterations in the pulmonary tissue. These included congestion and hemorrhage of most blood capillaries, increased number of lymphocytes within the thickened interalveolar septa and destruction of the inner border of the respiratory bronchioles and increase of collagen fibers. Changes were seen in type I & II alveolar cells in the form of vacuolation, increase in the number of lysosomes, shortening of the apical microvilli and irregularity and swollen of the nuclei. Also, an increase in number of macrophages and fragmentation of the rough endoplasmic reticulum. Most of the main cell types in the alveolar tissue had restored their normal histological and ultrastructural appearance by VC. Conclusion: Antioxidant nutrients (VC) can improve lung histological and ultrastructural damage produced by Cs-A administration.

Index Terms—Antioxidant capacity, cyclosporine, pulmonary damage, vitamin c

I. INTRODUCTION

Cs-A is a frequently used immunosuppressive agent in transplant medicine to prevent rejection and in the treatment of autoimmune diseases [1], [2], [3]. Its molecular mechanism of action has been well defined in Tcells and involved inhibition of critical signalling pathways that regulated T-cell activation [4]. In fact, CsA inhibited calcineurin phosphatase activity and thereby activation of the transcription factor, nuclear factor of activated T cells [5]. Unfortunately, its full clinical utility is limited due to some adverse effects, including nephrotoxicity, hepatotoxicity, cardiotoxicity, vascular toxic effects and increased blood pressure [2], [3], [5]. These negative effects have been identified through morphological alterations and/or clinical parameters. Moreover, the side effects are usually dose-dependent and are reversed following dose reduction. It has been demonstrated that Cs-A increases the synthesis of reactive oxygen species (ROS) and lipid peroxidation products in vitro and in vivo studied by Ergüder et al. [6], but its possible toxic effects in lung tissue have not been defined yet.

On the other hand, VC is the most common nontoxic essential dietary antioxidant. It is the main water-soluble antioxidant in human plasma where it consumes oxygen free radicals [7] & [8]. High concentration are naturally found in the fluid of the lung to protect against free radicals generated by toxic chemicals in air [9]. It plays an important role in the regulation of intracellular redox state [10]. Moreover, VC is useful agents for attenuating the lung injury caused by increased oxidative stress and neutrophil accumulation [11]. In addition, administration of a moderately large dose of vitamin C almost completely prevents protein damage, apoptosis and the lung injury [12].

The present study was designed to determine the effect of cyclosporine A on lung tissue and to investigate whether VC has a possible protective effect on Cs-A-induced lung toxicities, through light and ultrastructure microscopic evaluation in mice.

II. MATERIALS AND METHODS

Healthy, male albino mice obtained from Theodore Bilhars research Institute, Imbaba Giza (3 months old) weighing 25 ± 5 g were housed in stainless steel cages, maintained on a photoperiod of 12 h light/12 h dark and fed a standard laboratory Pelleted food and water ad libitum.

The animals divided to three groups 10 each as following: Group I (control group) received distilled water. Group II (Cs-A-treated group) were daily administrated the therapeutic dose of Cs-A (0.06 mg/kg b.w.) Group III (VC group) received a daily oral the therapeutic dose of VC (1.25 mg/kg b.w.) simultaneously with Cs-A (0.06 mg/kg b.w.) All groups received the doses daily for 30 days.

Mice were sacrificed 24 hours after the last administration and carefully dissected, the lungs were rapidly removed and processed to histological and ultrastructure studies.
III. RESULTS

The lungs of control group of mice (group I), showed normal spongy histological structure (Fig. 1, Fig. 2 and Fig. 7). Type I pneumocytes are highly attenuated with flattened nuclei (Fig. 8). Type II pneumocytes are cuboidal in shape and possess vacuolated cytoplasm and rounded nuclei (Fig. 9). Macrophages are found in the alveolar wall or free in the alveolar space (Fig. 7). Examination of lung by light microscopy revealed characteristic Cs-A-induce pulmonary lesions. The most prominent feature in this group is the vascular changes. Such changes were represented by the congestion of the blood capillaries that were engorged with erythrocytes and extravasations of red blood cells (RBCs) within the alveolar lumen. Most of the alveolar septa were thickened and heavy infiltration of inflammatory cells, mainly lymphocytes associated with thickened wall pulmonary blood vessels. Destruction of the inner border of the respiratory bronchioles and intrabronchial cellular debris associated with RBCs and alveolar macrophages (Fig. 3 and Fig. 4). Electron microscopic observations showed conspicuous degenerative features were encountered in the interalveolar septum and interstitium (Fig. 10-Fig. 14). Type I pneumocytes displayed certain degenerative changes in the form of fragmented ER and vacuolated cytoplasm (Fig. 11), while pneumocytes type II depicted atypical vacuolation with degenerative changes of their lamellar bodies leaving irregular empty spaces and fragmented their apical microvilli. The endoplasmic reticulum has been severely fragmented into small vesicles and devastated mitochondria (Fig. 12 and Fig. 13). In addition, occasional degenerative areas were detected in the interstitium accompanied by obvious increase of collagen fibers (Fig. 11, Fig. 13 and Fig. 14). Macrophages with large, indented nuclei and many lysosomes were frequently encountered within the alveolar lumen (Fig. 10 and Fig. 12). The interalveolar septa were thickened with cellular infiltration (Fig. 10 and Fig. 14). Concomitant administration of Cs-A with VC (group III) showed evident reduction of all alveolar changes except for mild thickening of interalveolar septa with mild inflammatory cellular infiltration (Fig. 5, Fig. 6, Fig. 15 and Fig. 16).
Figure 6. Protected mice lung with vitamin C (group III) showing mild inflammatory cellular infiltration (IC) of interalveolar septa and around a respiratory bronchiole (RB). H&E ×400.

Figure 7. Control mice lung (group I) showing the tissue component of the alveolar wall between two adjacent alveoli (Al) with pneumocytes type I (P1), pneumocytes type II (P II), fibroblast (F) with irregular nucleus and dense chromatin masses, macrophage with its pseudopodia (↑) and their cytoplasm contains numerous lysosomes (Ly) and blood vessel (BV) are also detected. Transmission electron microscopy ×3000.

Figure 8. Control mice lung (group I) showing pneumocytes type I having oval nucleus (N). Its cytoplasm with rough endoplasmic reticulum (RER) and pinocytotic vesicles (PV). Transmission electron microscopy ×10000.

Figure 9. Control mice lung (group I) showing pneumocytes type II having large rounded euchromatic nucleus (N) and short microvilli on the cell surface (MV). Its cytoplasm shows lamellar bodies (LB) and mitochondria (M). Transmission electron microscopy ×6000.

Figure 10. Cyclosporine A-treated mice lung (group II) showing a pneumocyte type I (P I) lining the wall of an alveolus with oval nucleus and vacuolated cytoplasm. Notice the presence of interstitial septal cells (N), pneumocytes type II (P II) with degenerative changes of their cytoplasmic organelles and devastating mitochondria (M). An intra-alveolar macrophage with irregular nucleus (↑). Transmission electron microscopy ×4000.

Figure 11. Cyclosporine A-treated mice lung (group II) showing pneumocyte type I lining the wall of an alveolus fragmented endoplasmic reticulum (RER) and vacuolated cytoplasm and interstitial collagen fibers (CF) are increased. Transmission electron microscopy ×10000.

Figure 12. Cyclosporine A-treated mice lung (group II) showing an alveoli lined by many electron-dense pneumocytes type II with vacuolated cytoplasm (v), degenerative microvilli (MV), vesiculated endoplasmic reticulum (ER) and devastated mitochondria (M). Also, intra-alveolar macrophage with large indented nucleus (N) and many cytoplasmic lysosomes (Ly). Transmission electron microscopy ×6000.

Figure 13. Cyclosporine A-treated mice lung (group II) showing a pneumocyte type II with electron-dense nucleus (N) and degenerative changes of their lamellar bodies leaving irregular empty vacuoles (v), fragmented endoplasmic reticulum (RER) and extraordinary increase of interstitial collagen fibers (CF) are also observed. Transmission electron microscopy ×75000.
IV. DISCUSSION

Histopathological studies revealed that some type II pneumocytes are precursor (stem) cells for type I pneumocytes and their proliferation in the lungs is a common response to damage of the alveolar walls [13]. Thus, in the present study, proliferation of type II pneumocytes might be considered as a sign of repair of the destroyed epithelium. Proliferation of type II pneumocytes occurs under certain conditions such as pulmonary tumors induced by diethyl nitrosamine in mice [14], acute primary blast injury by Brown et al. [15], and lung emphysema [16]. The extent of tissue damage is the result of the balance between the free radicals generated and the antioxidant protective defense system [17]. Thickening some of interalveolar septa, could be explained by the increased interstitial collagen fiber deposition and marked cellular infiltration with lymphocytes, neutrophils, eosinophils, and macrophages [18]. Vascular congestion and cellular infiltration of the lung tissue observed in this study could be referred to changes of the vascular integrity of the lung vessels causing disruption of the endothelial barrier and increased capillary permeability evoking an inflammatory response through activation of oxidative stress-sensitive signaling pathways [19]. The current changes were found in pulmonary tissue following different toxicological agents such as treated with nicotine [20], after Pulmonary blast injury in mice using laser-induced stress waves [21], effects of blast waves [15], long-term administration of amiodarone [18], amiodarone lung disease [22] and also due to 6 months ovariectomized albino rats [23]. Regardless of the source of injury, the progression of drug-induced lung toxicity is often quite similar, involving (1) parenchymal damage, (2) recruitment of inflammatory cells, and (3) progression of the inflammatory process. If the inflammatory response is sufficiently severe and disperse, increased collagen can be deposited in interstitial and intra-alveolar areas [24]. There are several hypotheses to explain the mechanism of Cs-A-induced adverse effects, including the formation of free oxygen radicals and lipid peroxidation [6], [25]. Oxidative stress can be done indirectly by assaying products of oxidative damage as malondialdehyde (MDA) that indicates membrane lipid peroxidation and cellular injury [26]. The inflammatory cells, detected in the present study following Cs-A treatment produce ROS [27] and [28]. These activated oxygen species could cause DNA strand breaks and lipid peroxidation and induce cell injury. Cotran et al. [27] reported that the destruction of lung parenchyma is due to the action of proteolytic enzymes (Proteases, mainly elastase). One important source of these proteases is leukocytes associated with pulmonary inflammation. Furthermore, they added that the inflammatory cells produce reactive oxygen that could cause lipid peroxidation and induces cell injury. Thus, ROS may be important contributory agents to the pathogenesis of Cs-A associated lung injury. In contrast, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are endogenous antioxidants that play a role in prevention of oxidative injury [29]. Therefore, the imbalance between production of free oxygen radicals and antioxidant defense systems, due to Cs-A administration, is a mechanism responsible for oxidative stress [2]. Moreover, Some authors have reported a direct correlation between Cs-A-induced cytotoxicity and changes in mitochondrial enzyme activity [30] and [31]. Cs-A has also the ability to interact
with receptors within the nucleus to inhibit genetic transcription of proteins secreted by fibroblasts, endothelial cells, macrophages, and monocytes [32]. Cs-A stimulates fibroblast proliferation through mediators produced by airway epithelial cells, raising the possibility that Cs-A may contribute to the development of Obliterative bronchiolitis [OB] after lung transplantation [33]. Finally, Cs-A affects cell types differently and that the disruption of organ architecture is the result of multiple effects at the cell level [34]. Simultaneous treatment of mice with VC revealed that most of the main cell types of pulmonary tissue have restored their normal histological and ultrastructural appearance. Our results were compatible with Baltalarli et al. [11]. In addition, Banerjee et al. [12] concluded that the pathophysiological events in lung are prevented by a moderately large dose of VC. The antioxidant species help the lungs ward off the deleterious consequences of a wide variety of oxidants/reactive oxygen species [35]. VC is useful agents for attenuating the lung injury caused by increased oxidative stress and neutrophil accumulation [11]. VC, a water-soluble vitamin, is effective in scavenging free radicals and act as a two-electron reducing agent and confers protection by contributing an electron to reduce free radicals. Thus neutralizing these compounds in the extracellular aqueous environment prior to their reaction with biological molecules antioxidant [36]. Reduction of the ascorbate free radical (AFR) at the plasma membrane provides an efficient mechanism to preserve the vitamin in a location where it can recycle alpha-tocopherol and thus prevent lipid peroxidation [37]. Moreover, the antioxidant potential of VC is not only attributed to its ability to quench ROS, but also to its ability to regenerate other small molecules antioxidants [36].

V. CONCLUSION

The Cs-A-therapy results in pulmonary hemorrhage and congestion, as well as rupture and/or thickening of the alveolar walls. The current results demonstrate that VC had a significant antioxidant activity thereby protecting the lung from free radical damage produced by the Cs-A-therapy.

REFERENCES


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