Journal of Animal and Veterinary Advances 10 (8): 1080-1088, 2011
ISSN: 1680-5593

Efficacy of L-Carnitine Administration on Lungs of Neonatal Rats Exposed to Hyperoxia

[1]Ibrahim Caner, [1]Ayhan Tastekin, [2]Fatih Saruhan, [2]Ahmet Hacimuftuoglu,
[1]Atilla Cayir, [3]Deniz Unal, [3]Jale Selli and [4]Nesrin Gursan
[1]Department of Pediatrics, [2]Department of Pharmacology,
[3]Department of Histology and Embryology,
[4]Department of Pathology, School of Medicine, Ataturk University, Erzurum, Turkey

Abstract: Oxygen toxicity is believed to play a prominent role in the lung injury that leads to the development of bronchopulmonary dysplasia. L-carnitine (LCAR) is an antioxidant and prevents the accumulation of end products of lipid peroxidation, acts as a free radical scavenger and protects cells from reactive oxygen species. The aim of the present study was to evaluate the effects of LCAR on the histopathologic characteristics of oxygen-induced lung injury. Thirty one rat pups were divided into 4 groups: Healthy control group (group 1, n = 8), hyperoxia-exposed group (group 2, n = 7), hyperoxia-exposed and 100 mg kg^{-1} LCAR-treated group (goup 3, n = 10), hyperoxia-exposed and 200 mg kg^{-1} LCAR-treated group (goup 4, n = 6). Although in group given 100 mg kg^{-1} LCAR together with hyperoxia-exposure, it was observed some improvement, histopathologic findings obtained from animals treated with 200 mg kg^{-1} LCAR were similar to normal surprisingly. In conclusion, it should be focused on more the possible protective effect mechanism of LCAR and it should be made more effort to be able to use it in routine.

Key words: Bronchopulmonary dysplasia, prematurity, L-carnitine, oxygen toxicity, rats, Turkey

INTRODUCTION

Although, oxidative stress has long been linked with the development of Bronchopulmonary Dysplasia (BPD) in lung, the pathogenesis of this condition is still unclear and a number of risk factors have been identified. Preterm delivery, respiratory failure, oxygen and mechanical ventilation constitute the major risk factors for BPD development (Kinsella *et al*., 2006; Saugstad, 2001; Schroeder and Gortner, 1999). BPD was originally linked to oxygen toxicity because of increased production of cytotoxic oxygen free radicals which can overwhelm the host antioxidant defense mechanisms and cause lung injury. Histopathologic characteristics of lung injury in BPD include a decrease in alveolarization, an abnormal capillary morphology and an interstitium with variable cellularity and fibroproliferation (Kinsella *et al*., 2006; Bancalari *et al*., 2003).

Because L-carnitine (LCAR) is an integral component of the membrane phospholipid, it is possible that carnitine (B-hydroxy-N-trimethyl aminobutyric acid) causes lung maturation via membrane phospholipid repair activity. L-carnitine is also an antioxidant and prevents the accumulation of end products of lipid peroxidation. Several mechanisms by which LCAR acts have been proposed. Carnitine plays a major role as a cofactor in the transportation of Free Fatty Acids (FFAs) from the cytosol to the mitochondria. FFAs degrade to acyl-CoA by β-oxidation and these substances enter the Tricarboxylic Acid (TCA) cycle.

A large amount of oxygen is consumed in this reaction and Adenosine Triphosphate (ATP) is synthesized in the steps of the electron transport chain and oxidative phosphorylation. Oxygen is reduced to water (H_2O) at the end of the TCA cycle and the oxygen concentration decreases. Thus, toxic products such as Reactive Oxygen Species (ROS) and products of FFAs are cleared from the cell (Gomez-Amores *et al*., 2007; Gulcin, 2006).

The BPD treatment is aimed to minimize ongoing injury, reduce inflammation, maintain adequate oxygenation and facilitate lung growth. Although, it istempting to try preventing BPD by supplementing infants with antioxidants, a wide variety of these agents were used without any significant benefit. Prophylactic supplementation of antioxidant agents is a promising

Corresponding Author: Ibrahim Caner, Department of Pediatrics, School of Medicine, Ataturk University, Erzurum, Turkey

method for preventing the development of BPD (Laughon *et al*., 2009). The aim of the present study was to evaluate the effects of LCAR treatment on the histopathologic characteristics of oxygen-induced lung injury and to address the potential role for LCAR treatment in neonates with BPD.

MATERIALS AND METHODS

Animals were housed in facilities accredited by international guidelines and studies were approved by and conducted in accordance with the Institutional Animal Care and Use committee of Ataturk University. In this study, it was used adult (12 weeks old) female Sprague dawley rats with dated pregnancies from Ataturk University Experimental Animal Laboratory. The animals were housed in groups of 5 cage^{-1} until delivery under controlled conditions of constant temperature/humidity and exposed to a 12 h light/dark cycle. The rat pups were delivered spontaneously and reared with their dams along with experimentation.

Forty new born rats were allocated randomly into 4 groups which were healthy control group (group 1, n = 8), hyperoxia-exposed group (group 2, n = 7), hyperoxia-exposed and 100 mg kg^{-1} LCAR-treated group (group 3, n = 10) and hyperoxia-exposed and 200 mg $^{-1}$ kg LCAR-treated groups (group 4, n = 6). The experiment was begun on the 3rd day and ended 14th day after birth in a total 31 rats. At the end of experiments, all animals were killed by intraperitoneal injections of pentobarbital (200 mg kg^{-1}).

Experimental model: Animals in group 1 accepted as control were kept in room air. In group 2, there were animals with hyperoxic exposure. These animals were maintained in Plexiglas chambers (Natus oxydome 2, Seatle, USA) throughout 23 and then 1 h kept in room air for providing relative hypoxia per day between 3rd and 14th days after birth.

Oxygen concentration of Plexiglas chambers was ≥80%. As so long as experimental period, oxygen concentration of the chamber was monitored terdiurnal. Humidity was maintained at >80% and CO_2 was removed by soda lime absorption.

Pups in groups 3 and 4 were injected intraperitoneally with 100 and 200 mg kg^{-1} L-carnitine (Sigma chemical Co., St. Louis, MO), respectively between 3rd and 14th day after birth. The 1st dose of LCAR to these 2 groups was given 6 h before the hyperoxia exposure. Other procedures were same with group 2.

Tissue preparation: The lungs were resected by thoracotomy and rapidly fixed in 10% buffered formalin for 24-48 h for histological examination. After fixation and routine preparation of samples according to conventional light microscopical technique respectively, it was embedded in paraffin. About 5 μm thick sections were cut with a microtome (Leica RM2125RT) and stained with hematoxylin-eosin for routine histological examination. All sections were studied and photographed by a light photomicroscope (Olympus BH 40) (Altunkaynak *et al*., 2008; Halici *et al*., 2008).

RESULTS AND DISCUSSION

Healthy control group (group 1): In the lungs, we evaluated the last segments of the conductive portion of the respiratory system. The respiratory portion of the respiratory system which composed of respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli observed normally (Fig. 1A and D). Terminal bronchioles lined by ciliated simple cuboidal epithelium were normal and have regular counters (Fig. 1A and H). Bronchi were characterized by the presence of glands and supporting cartilage (Fig. 1D and N). The cartilages supporting the bronchi were typically found in several small pieces (Fig. 1N). Bronchi were surrounded by a layer of smooth muscle which is located between the cartilage and epithelium (Fig. 1H).

Alveoli were separated from one another by thin alveolar walls; each alveolus was lined by simple squamous epithelium (Fig. 1A and G). The walls of the capillaries were in direct contact with the epithelial lining of the alveoli. Type 1 pneumocytes were extremely flattened (as thin as 0.05 μm) and form the bulk of the surface of the alveolar walls (Fig. 1Q). Type 2 pneumocytes were normal shaped (sometimes cuboidal) (Fig. 1P).

Alveolar ducts had no walls and (Fig. 1B) separated from one another by a thin connective tissue, the Interalveolar septum (Fig. 1G) containing capillaries (Fig. 1F). There were alveolar macrophages being appeared sometime in interalveolar septum, sometime in alveolar air space (Fig. 1P).

Hyperoxia-exposed group (group 2): In this group, alveolar ducts were enlarged and a great number of erythrocytes appeared in the alveolar ducts (Fig. 2A and B). Significant destructions were seen in the alveolar structures (Fig. 2F and 3J, M). Although, morphological

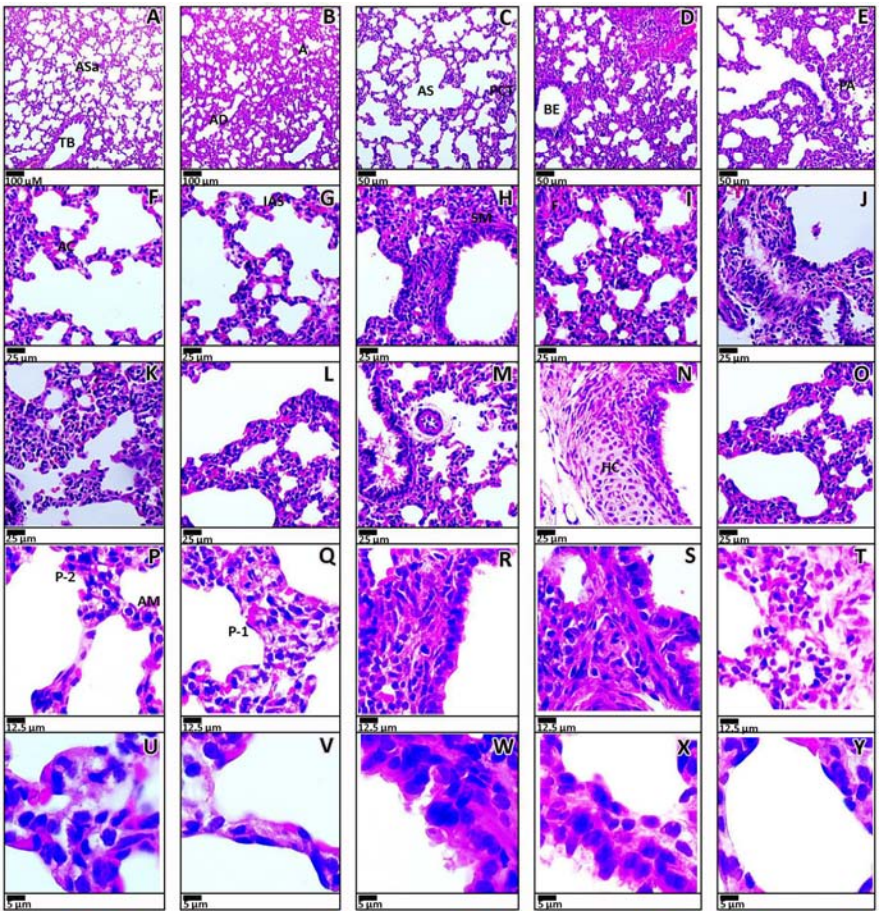

Fig. 1: Light microscopic photomicrograph of control group. The section was stained with hematoxylin-eosin. A: Alveolus, AC: Alveolar Capillary, AD: Alveolar Duct, AM: Alveolar Macrophages (Dust cells), AS: Air Space, ASa: Alveolar Sac, BE: Bronchiole Epithelium, F: Fibroblast, IAS: Interalveolar Septa, P-1: Type 1 Pneumocytes, P-2: Type 2 Pneumocytes, PA: Pulmonary Artery, PCT: Pulmoner Connective Tissue, SM: Smoot Muscle, TB: Terminal Bronchioles, HC: Hyaline Cartilage

changes of the endothelium in lung of oxygen-exposed group were uncommon in some field of view, prominent and swollen endothelial cells were encountered in endothelium (Fig. 2G and 3C, G, L and H). It was determined either edema or irregularities in thickness of interalveolar septa which may be indicate an increase in vascular permeability (Fig. 3L, Q and T). In addition to these findings in vascular endothelial cells of interalveolar septa, it was observed some pathological changes such as hyperchromasia, nuclear atypia (Fig. 2C and 3B) and vacuolization (Fig. 2C and 3B).

In majority of alveolar sacks at higher magnification, it was shown mild pathological changes characterized by the combination of fibrin and cellular debris called as hyaline membrane (Fig. 3Q and R) which were the result of leakage of blood components into the alveolar sacks. Type 2 pneumocytes had normal structure (Fig. 2K). It was seen erythrocyte extravasations in the lung parenchyma (Fig. 2C, D and 3A and K). Terminal bronchioles were destructed (Fig. 3C, G and K) and intraluminal erythrocytes were observed in bronchi (Fig. 2K and L). Structural irregularities were noted in smooth muscle (Fig. 3F), in vessel walls (Fig. 3S).

Hyperoxia-exposed and 100 mg kg^{-1} LCAR-treated group (group 3): In this group, alveolar destruction was more pronounced (Fig. 4D, O and 5H). Alveolar ducts were enlarged (Fig. 4C). Significant intralveolar bleeding was found (Fig. 4I, J and 5G). It was determined in some areas of pulmonary connective tissue by increasing number of cells (Fig. 4G). At higher magnification, typical moderate laminated hyaline membranes in vascular lumen (Fig. 4L and 5D) were observed. Significant increase in thickness of interalveolar

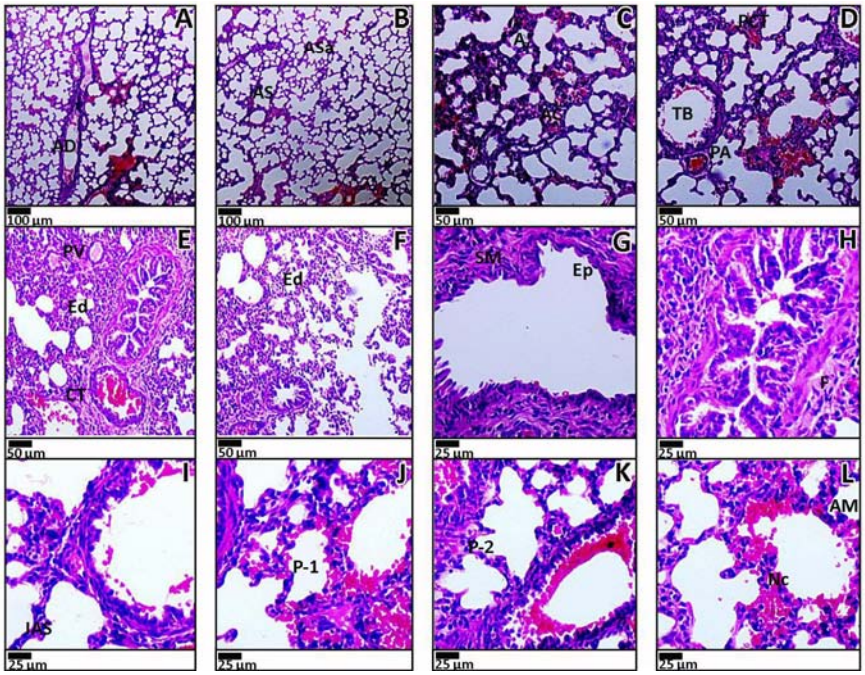

Fig. 2: Light microscopic photomicrograph of oxygen-exposed group. The section was stained with hematoxylin-eosin. AC: Alveolar Capillary, AD: Alveolar Duct, AM: Alveolar Macrophages (Dust cells), ASa: Alveolar Sac, Ep: Epithelium, Ed: Edema, F: Fibroblast, IAS: Interalveolar Septa, Nc: Necrotic cell, P-1: type 1 Pneumocytes, P-2: type 2 Pneumocytes, PA: Pulmonary Artery, PCT: Pulmoner Connective Tissue, PV: Pulmonary Vein, SM: Smoot Muscle, TB: Terminal Bronchioles

septa was examined (Fig. 5C, F and I). Interstial edema was clear in some areas (Fig. 5B and T). Another remarkable finding was proliferation of alveolar macrophages in interalveolar septum (Fig. 4O and 5G) indicating preceding alveolar damage. In this group, there were also some abnormalities in the terminal bronchi such as not only structural changes but also large amount of erythrocytes and inflammatory cells (Fig. 4E, F, M and 5E). It was encountered with same findings in respiratory bronchi (Fig. 4K, N and 5A). Another important finding was an increase in number of type 2 pneumocytes (Fig. 4L) and destruction of capillary walls (Fig. 5K and L).

Hyperoxia-exposed and 200 mg kg^{-1} LCAR-treated group (group 4): In this group, lung tissue did not have any abnormal appearance to near normal (Fig. 6A and B). There were no erythrocytes within alveolar structure (Fig. 7C and D). Appearances of alveolar ducts (Fig. 6C-F), interalveolar septa (Fig. 7I and J), terminal bronchi (Fig. 6A, E, H and 7F) and type 2 pneumocytes (Fig. 7A and B) were closely similar to normal group although, it was determined some unimportant changes such as a small amount of epithelial desquamation in terminal bronchi (Fig. 6B) or a little destruction of vascular endothelium (Fig. 6J). Bronchopulmonary dysplasia, chronic lung disease of infancy is a chronic lung disorder that is commonly occurred in children who have got some risk factors such as prematurity, low birth weight and receiving prolonged mechanical ventilation to treat Respiratory Distress Syndrome (RDS).

Mature alveoli are not present before birth. A more open expression, development and remodeling of the acinus occurs during fetal life and childhood. Multiplication of the acinar air spaces and the structural changes, they undergo have been described and represented schematically by summarizing previous studies, particularly those of the last two decades (Hislop and Reid, 1974). During the last 2 months of prenatal life, the number of terminal sacs increases steadily and cells lining the sacs known as type 1 alveolar epithelial cells become thinner so that surrounding capillaries protrude into the alveolar sacs.

Until the end of the 6th month, endothelial cells and flat alveolar epithelial cells, another cell type known as type 2 alveolar epithelial cells which produce surfactant, a phospholipid-rich fluid capable of lowering surface tension at the air-alveolar interface keep on going its own development (Sadler and Langman, 2000; Moore and Persaud, 2007). Surfactant is particularly important for survival of the premature infant. When surfactant is

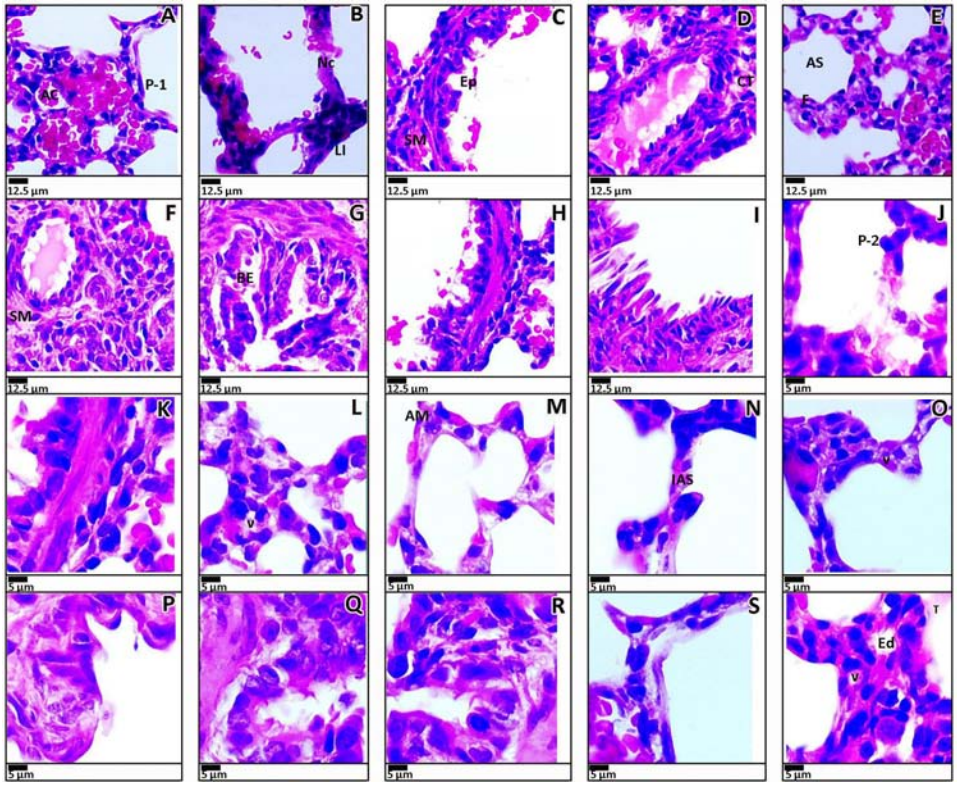

Fig. 3: Light microscopic photomicrograph of oxygen-exposed group. The section was stained with hematoxylin-eosin. AC: Alveolar Capillary, AM: Alveolar Macrophages (Dust cells), AS: Air Space, BE: Bronchiole Epithelium, CT: Connective Tissue, Ep: Epithelium, Ed: Edema, F: Fibroblast, IAS: Interalveolar Septa, Nc: Necrotic Cell, P-1: type I Pneumocytes, P-2: type 2 Pneumocytes, SM: Smoot Muscle, TB: Terminal Bronchioles, v: Vacuolization, LI: Lymphocyte infiltration

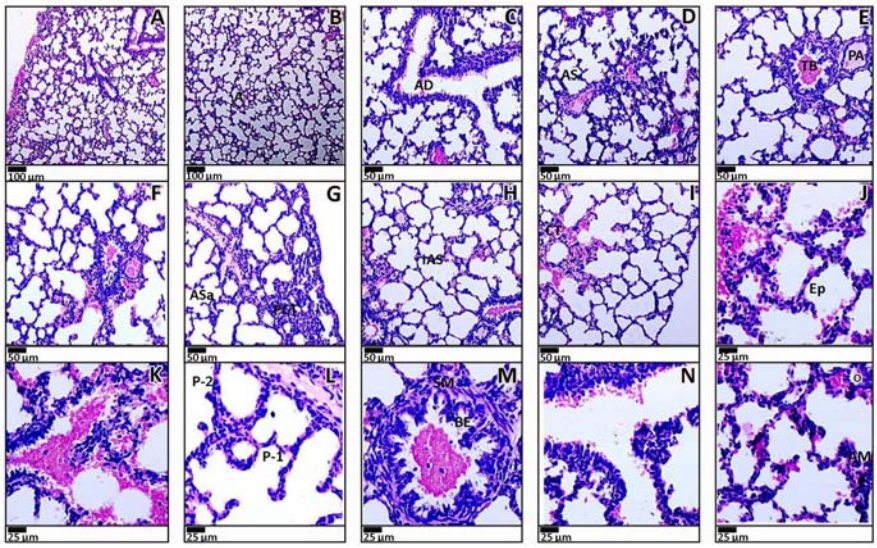

Fig. 4: Light microscopic photomicrograph of 100 mg kg^{-1}, LCAR-treated and oxygen-exposed group. The section was stained with hematoxylin-eosin. A: Alveolus, AD: Alveolar Duct, AM: Alveolar Macrophages (Dust cells), AS: Air Space, ASa: Alveolar Sac, CT: Connective Tissue, Ep: Epithelium, IAS: Interalveolar Septa, P-1: type 1 Pneumocytes, P-2: type 2 Pneumocytes, PA: Pulmonary Artery

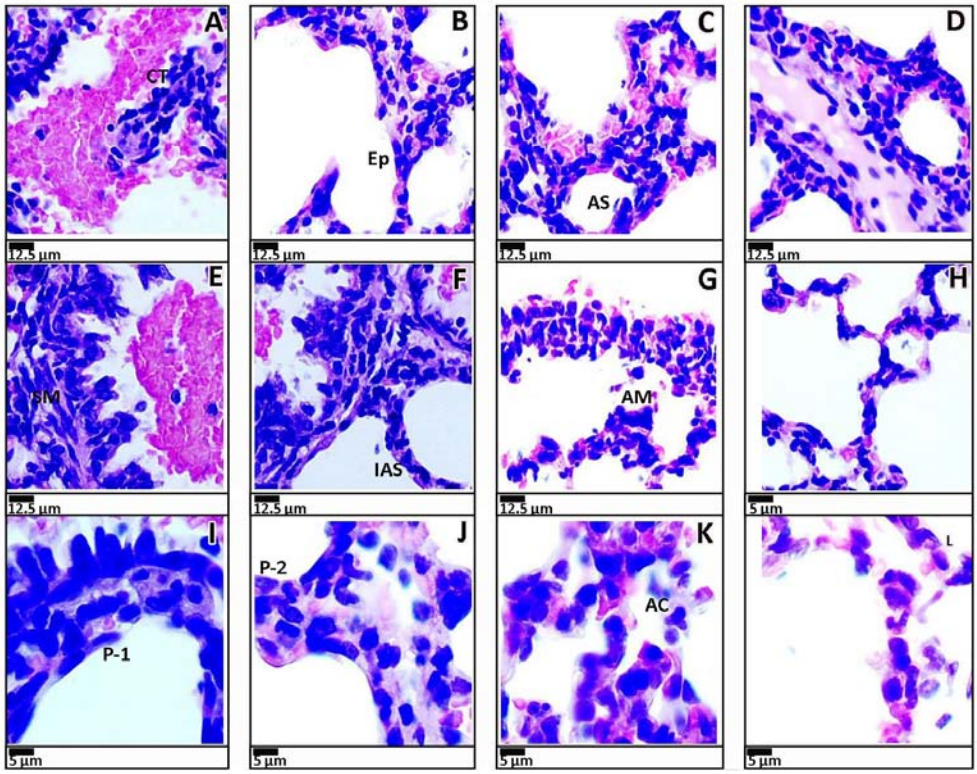

Fig. 5: Light microscopic photomicrograph of 100 mg kg^{-1}, LCAR-treated and oxygen-exposed group. The section was stained with hematoxylin-eosin. AC: Alveolar Capillary, AM: Alveolar Macrophages (Dust cells), AS: Air Space, CT: Connective Tissue, Ep; Epithelium, IAS: Interalveolar Septa, P-1: type 1 Pneumocytes, P-2: type 2 Pneumocytes, SM: Smoot Muscle

insufficient, the air-water (blood) surface membrane tension becomes high, bringing great risk that alveoli will collapse during expiration. As a result, RDS develops. This is a common cause of death in the premature infant (Sadler and Langman, 2000; Moore and Persaud, 2007). That is to say if oxygen support is necessary in premature infants, we have to apply it according to defined procedures and doses. Otherwise, it may be inevitable to enter asphyxia of premature infants. It is known that asphyxia represents a serious problem worldwide, resulting in ~1 million deaths and an equal number of serious sequelae annually (Saugstad, 2001).

Again, it is well known that this treatment causes other problems as BPD including inflammation, fibrosis and developmental pause of lung tissue (Speer and Groneck, 1998). Last one triggers the start-up of several mechanisms including a rise in the level of tumor necrosis factor-alpha, generation of ROS such superoxide anion and hydroxyl radicals (Sayed-Ahmed *et al*., 2004). Under normal conditions, there are magnificent balance between prooxidant and antioxidant systems to be able to cope with oxidative stress (Calabrese *et al*., 2005). When the rate of free radical generation exceeds, the capacity of antioxidant defenses, it is not easy to prevent possible devastative effects of oxidative stress. Under pathological conditions as both premature infant with RDS and applying oxygen therapy as a result of this, ROS are over-produced and result in oxidative stress again (Gulcin, 2006).

This is a terrible dilemma for clinician. Today in a lot of new born clinic and also in experimental studies, it has been tried to find a satisfying solution. When looked at these studies, it will be seen that is related to applying of agents with the antioxidant activity of most studies. It has been received successful results from giving of erythropoietin that is an antioxidant due to decreasing the plasma iron concentration and increasing the ability of plasma to inhibit lipid peroxidation (Ozer *et al*., 2005a; Bany-Mohammed *et al*., 1996), retinoic acid that has got antioxidant activity via activating gene transcription by specific nuclear receptors (Hong and Lee-Kim, 2009; Ozer *et al*., 2005b).

Today, it has been shown via many studies that LCAR is a vital component in lipid metabolism for the

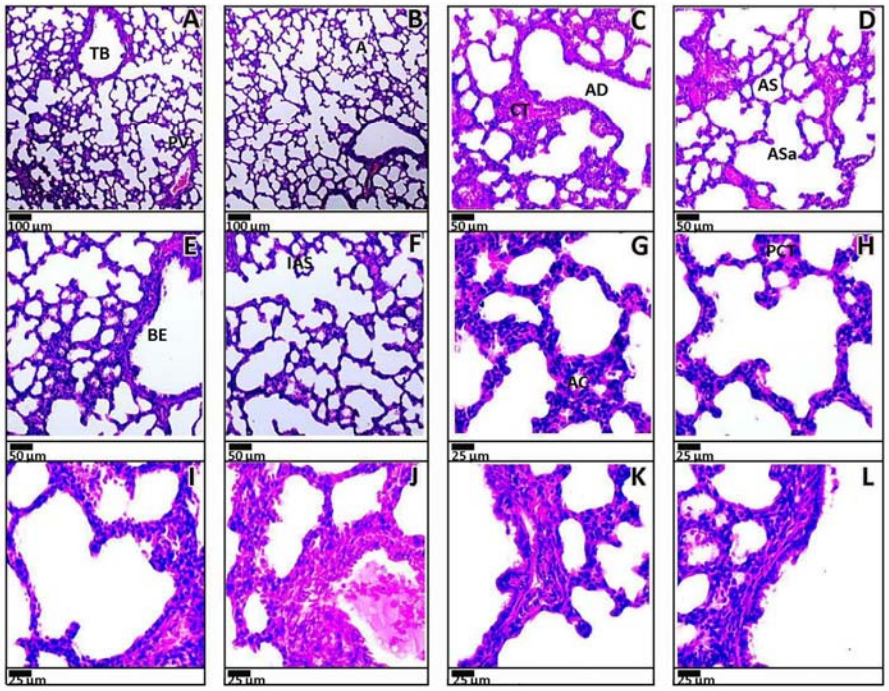

Fig. 6: Light microscopic photomicrograph of 200 mg kg^{-1}, LCAR-treated and oxygen-exposed group. The section was stained with hematoxylin-eosin. A: Alveolus, AC; Alveolar Capillary, AD: Alveolar Duct, AS: Air Space, ASa: Alveolar Sac, BE; Bronchiole Epithelium, CT: Connective Tissue, IAS: Interalveolar Septa, PCT: Pulmoner Connective Tissue, PV: Pulmonary Vein, TB: Terminal Bronchioles

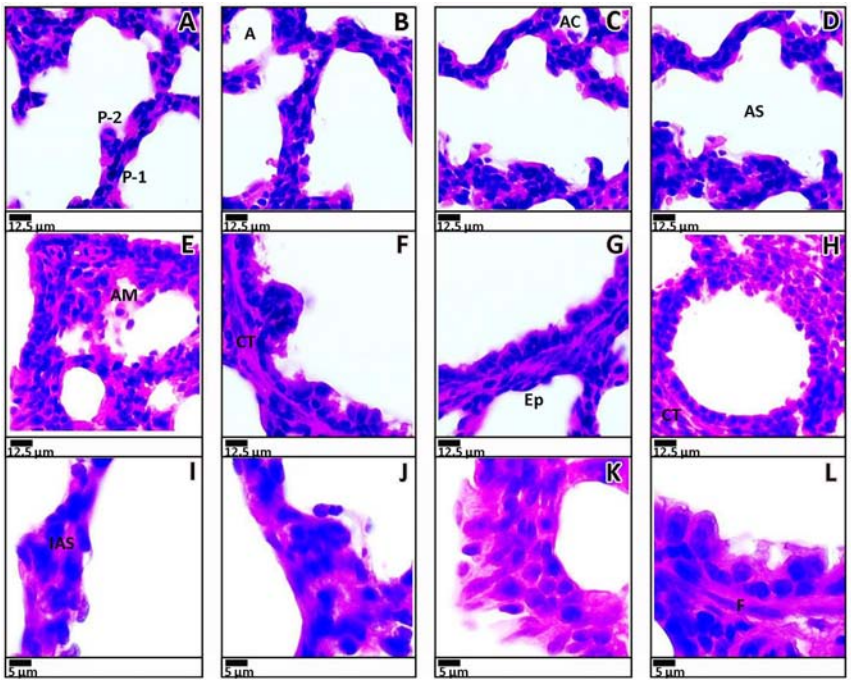

Fig. 7: Light microscopic photomicrograph of 200 mg kg^{-1}, LCAR-treated and oxygen-exposed group. The section was stained with hematoxylin-eosin. A: Alveolus, AC: Alveolar Capillary, AM: Alveolar Macrophages (Dust cells), AS: Air Space, CT: Connective Tissue, Ep: Epithelium, F: Fibroblast, IAS: Interalveolar Septa, P-1: type 1 Pneumocytes, P-2: type 2 Pneumocytes

production of ATP through the β-oxidation of long-chain fatty acids (Gomez-Amores *et al*., 2007). An antioxidant promoting action has been suggested for this compound. Thus, LCAR acts as a free radical scavenger (Kalaiselvi and Panneerselvam, 1998; Arockia Rani and Panneerselvam, 2001) and protects cells from ROS (Sushamakumari *et al*., 1989).

We have not found any document focused on the adverse effects of LCAR. Although, various aspects of LCAR have been dealt with whether there is a relationship between carnitine level obtained from premature infant formula and lipid metabolism and growth (Seong *et al*., 2010) that is investigated serum free carnitine levels in preterm infants with RDS and controls during the first week of postnatal life (Ozturk *et al*., 2006), it has not yet dwelled on direct therapeutic effect of LCAR in premature children with RDS to whom oxygen must be given in terms of histopathology of lung.

CONCLUSION

Protective effects of LCAR depending on dose on lung injury of hyperoxia-exposed premature rats at the histopathologic level were investigated. Briefly, this study revealed that there were a lot of dramatic pathological changes in lung of oxygen-exposed group as shown elsewhere (26-28), LCAR exhibited a strong protective effect in especially treated group with 200 mg kg^{-1} rather than 100 mg kg^{-1} treated animals and histopathological findings of treated group with 200 mg kg^{-1} LCAR were generally close to normal and sometime almost same with healthy control group surprisingly.

In this study, it should be focused on more the possible protective effect mechanism of LCAR and it should be made more effort to be able to use it in routine.

REFERENCES

Altunkaynak, M.E., E. Ozbek, B.Z. Altunkaynak, I. Can, D. Unal and B. Unal, 2008. The effects of high-fat diet on the renal structure and morphometric parametric of kidneys in rats. J. Anat., 212: 845-852.

Arockia Rani, P.J. and C. Panneerselvam, 2001. Carnitine as a free radical scavenger in aging. Exp. Gerontol., 36: 1713-1726.

Bancalari, E., N. Claure, I.R.S. Sosenko, 2003. Bronchopulmonary dysplasia: Changes in pathogenesis, epidemiology and definition. Semin. Fetal Neonatal, 8: 63-71.

Bany-Mohammed, F.M., S. Slivka and M. Hallman, 1996. Recombinant human erythropoietin: Possible role as an antioxidant in premature rabbits. Pediatr. Res., 40: 381-387.

Calabrese, V., A. Ravagna, C. Colombrita, G. Scapagnini and E. Guagliano *et al*., 2005. Acetylcarnitine induces heme oxygenase in rat astrocytes and protects against oxidative stress: Involvement of the transcription factor Nrf2. J. Neurosci. Res., 79: 509-521.

Gomez-Amores, L., A. Mate, J.L. Miguel-Carrasco, L. Jimenez and A. Jos *et al*., 2007. L-carnitine attenuates oxidative stress in hypertensive rats. J. Nutr. Biochem., 18: 533-540.

Gulcin, I., 2006. Antioxidant and antiradical activities of L-carnitine. Life Sci., 78: 803-811.

Halici, Z., O.N. Keles, D. Unal, M. Albayrak and H. Suleyman *et al*., 2008. Chronically administered risperidone did not change the number of hepatocytes in rats: A stereological and histopathological study. Basic Clin. Pharmacol. Toxicol., 102: 426-432.

Hislop, A. and L. Reid, 1974. Development of the acinus in the human lung. Thorax, 29: 90-94.

Hong, T.K. and Y.C. Lee-Kim, 2009. Effects of retinoic acid isomers on apoptosis and enzymatic antioxidant system in human breast cancer cells. Nutr. Res. Pract., 3: 77-83.

Kalaiselvi, T. and C. Panneerselvam, 1998. Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. J. Nutr. Biochem., 9: 575-581.

Kinsella, J.P., A. Greenough and S.H. Abman, 2006. Bronchopulmonary dysplasia. Lancet, 367: 1421-1431.

Laughon, M.M., P.B. Smith and C. Bose, 2009. Prevention of bronchopulmonary dysplasia. Semin. Fetal Neonatal Med., 14: 374-382.

Moore, K.L. and T.V.N. Persaud, 2007. Before We Are Born: Essentials of Embryology and Birth Defects. 7th Edn., Saunders Elsevier, Philadelphia.

Ozer, E.A., A. Kumral, E. Ozer, N. Duman, O. Yilmaz, S. Ozkal and H. Ozkan, 2005a. Effect of retinoic acid on oxygen-induced lung injury in the newborn rat. Pediatr. Pulmonol., 39: 35-40.

Ozer, E.A., A. Kumral, E. Ozer, O. Yilmaz and N. Duman *et al*., 2005b. Effects of erythropoietin on hyperoxic lung injury in neonatal rats. Pediatr. Res., 58: 38-41.

Ozturk, M.A., T. Gunes, E. Koklu and A. Erciyes, 2006. Free carnitine levels in respiratory distress syndrome during the first week of life. Am. J. Perinatol., 23: 445-449.

Sadler, T.W. and J. Langman, 2000. Langman's Medical Embryology. 8th Edn., Lippincott Williams and Wilkins, Philadelphia, ISBN-13: 9780683306507, pp: 504.

Saugstad, O.D., 2001. Chronic lung disease: Oxygen dogma revisited. Acta Paediatr., 90: 113-115.

Sayed-Ahmed, M.M., H.H. Mansour, O.A. Gharib and H.F.Hafez, 2004. Acetyl-L-carnitine modulates bleomycin-induced oxidative stress and energy depletion in lung tissues. J. Egypt Natl. Canc. Inst., 16: 237-243.

Schroeder, P. and L. Gortner, 1999. Predicting bronchopulmonary dysplasia. Intensive Care Med., 25: 241-242.

Seong, S.H., S.C. Cho, Y. Park and Y.S. Cha, 2010. L-Carnitine-supplemented parenteral nutrition improves fat metabolism but fails to support compensatory growth in premature Korean infants. Nutr. Res., 30: 233-239.

Speer, C.P. and P. Groneck, 1998. Oxygen radicals, cytokines, adhesion molecules and lung injury in neonates. Semin. Fetal Neonatal, 3: 219-228.

Sushamakumari, S., A. Jayadeep, J.S. Kumar and V.P. Menon, 1989. Effect of carnitine on malondialdehyde, taurine and glutathione levels in heart of rats subjected to myocardial stress by isoproterenol. Indian J. Exp. Biol., 27: 134-137.