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The role of taurine added to pulmonary reperfusion solutions in isolated guinea pig lungs

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Summary. An experimental comparative study on isolated guinea pig-lungs has been undertaken to determine the probable beneficial effects of adding taurine to pulmonary reperfusion solutions in lung ischemia-reperfusion. 20 guinea pigs were used. The isolated lungs (n = 10 in each group) previously being perfused by oxygenated Krebs-Henseleit solution were put in normothermic ischemic conditions. After 3 hours of normothermic ischemia the lungs were reperfused (with Krebs-Henseleit solution in the control group, Krebs-Henseleit solution plus taurine 10^{-2}M in the experiment group) for 20 minutes. Pulmonary artery pressures, tissue malondialdehyde (MDA) and glutathione (GSH) levels were measured before and after the ischemic period and also at the end of reperfusion. Malondialdehyde and glutathione levels of the pefusate were measured before ischemic period and at the end of reperfusion. An electron microscopic analysis was performed on the lung tissues before and after the ischemic period and also at the end of reperfusion. Decreased pulmonary artery pressure, tissue perfusate MDA levels and increased perfusate GSH levels were observed in taurine added group. Electron microscopic evaluation supported our findings indicating preservation of lamellar bodies of type II pneumocytes. It is concluded that taurine may play an important role in protecting tissue against ischemia-reperfusion injury by functioning as an antioxidant.

Keywords: Amino acids – Lung – Ischemia-reperfusion injury – Taurine

Introduction

The lung has a unique position in the body due to its direct contact with the external environment unlike other organ systems. The lung may be damaged with free radical mediate reactions. The reason for this is that the lungs are exposed not only by 02 tensions but also by a lot of toxic agents which are

oxidants or free radicals associated with inspired air (ozone, nitrogen dioxide) or cigarette smoke, inorganic dust inhalation (asbestosis, silicosis) (Smith, 1986; Sylvester, 1996).

Reactive oxygen species are suspected to play a role in lung injury caused by ischemia – reperfusion (lung transplantation, cardiopulmonary bypass, reexpansion pulmonary edema, and pulmonary thromboembolism) (Sylvester, 1996).

The clinical success of lung transplantation depends on effective organ preservation and protection of the lung from reperfusion injury. Oxygen-derived free radicals can be generated in the transplanted lung either by reintroduction of molecular oxygen on reperfusion or by activated neutrophils (Sylvester, 1996).

Taurine is the most abundant free aminoacid in many tissues. Various researchers have shown that taurine has protective properties in vivo and in vitro in several different organs, particularly the lungs, heart and liver (Öz et al., 1999). There are various known and proposed roles of taurine. Proposed functions include removal of hypochlorous acid in tissues where oxidants are generated such as neutrophils and the retina, modulation of calcium levels, maintenance of osmolarity and stabilization of membranes (Timbrell et al., 1995).

The greatest significance observed is that taurine is protective when it is only present during the reperfusion period i.e., after the membran damage has occured. Therefore it does not prevent the degree of damage, but it ameliorates the consequences of this damage. (Huxtable, 1992)

With this study we aimed to investigate the protective role of taurine against reperfusion damage by adding taurine to pulmonary reperfusion solution.

Methods

Animals

Lungs were obtained from male guinea pigs (n = 20) weighing 300 to 400 g. All animals received human care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No.80-23, revised 1978).

The animals were anesthetized with urethane and given 200 units of heparin into the femoral vein. After insertion of a No:16 cannula into the trachea by an open tracheostomy, a sternotomy was performed. After cannulation of the pulmonary artery via the right ventricle, the lungs and the heart were rapidly harvested.

Perfusion techniques and solutions

The lungs were mounted on a Modified Langendorf Perfusion Apparatus. The lungs were inflated with room air and then began perfusion with a gassed (oxygen 95%, carbondioxide 5%) Krebs-Henseleit solution at a rate of 10 ml/min at 37°C. The composition of the solution was as follows: $NaHCO_3$: 25 mM, NaCl: 112 mM, NaH_2PO_4: 1 mM, KCl: 5 mM, $MgCl_2$: 0.5 mM, $CaCl_2$: 2,5 mM and glucose: 11,5 mM. Krebs-Henseleit solu-

tion was used as the pulmonary solution in the control group (Soncul et al., 1999). 10^{-2} M taurine was added to the Krebs-Henseleit solution in the experimental group (Kaplan et al., 1993).

Protocol

At the twentieth minute of perfusion, perfusate samples from the left atrium were collected to determine malondialdehyde (MDA) and glutathione (GSH) levels and one of the lung segments (left lower lobe) was excised to determine tissue malondialdehyde (Kurtel et al., 1992) and glutathione levels (Casini et al., 1986) and pulmonary artery mean pressures were recorded then perfusion was stopped. During the ischemic period (3 hours) the lungs were kept at 37°C in an isotonic saline bath.

After 3 hours of ischemia, the reperfusion with the Krebs-Henseleit solution in the control group was started and in the experiment group, taurine was added to Krebs-Henseleit solution. Pulmonary artery mean pressures were recorded during perfusion and reperfusion time. Tissue pieces were excised again at the beginning of the reperfusion and also at the twentieth minute of reperfusion (Fig. 1).

Before and after ischemic period and also at the end of the reperfusion the lungs were prepared for electron microscopic studies. Lung samples were cut into approximately 1 mm^3 fragments. This fragments were fixed in 2% gluteraldehyde + cocodilate solution for 24 hours in 4°C. After fixation they were washed with cocodilate solution (0.1 M, pH 7.4). Seconder fixation was done with 2% 0_s0_4 for 2 hours in room temparature. Then the tissues were dehydrated by inserting in an increasing series of alcohol. The tissues were stained with uranyl acetate and phosphothungustic acid solution for 2 hours in 4°C. Finally tissues samples were embedded into araldite combination. Thin sections were examined with electron microscop Carl Zeiss 900.

Data analysis and statistics

Pulmonary artery pressure (mm Hg) were measured with a patient monitor (Datascope 2001A monitor; Datascope Corp. Montvale, NJ) and a pressure monitoring kit (Viggo-Spectramed Inc; Oxnard, CA). The levels of tissue MDA (nmol/g tissue), perfusate MDA (nmol/mL), tissue GSH (μmol/g tissue) and perfusate GSH (mmol/mL) were measured.

The results are presented as mean (± SEM). The overall significance of differences between the groups was determined by the Mann-Whitney U Test using data analysis software (Mikrosoft Excel; Mikrosoft Corp: Redmond, WA).

Results

Because of the changes in lung sizes in different animals and our standard perfusion volume (10 ml/min), the alterations of pulmonary artery pressures were calculated as the percentage change of the preischemic value in each experiment. The mean pulmonary artery pressures increased in the both of the groups after the ischemic period according to preischemic period ($p < 0.05$). Pulmonary artery pressures which were recorded at the end of reperfusion were found statistically significant among two groups ($p < 0.05$) (Table 1) (Fig. 2).

In the control group, the mean preischemic tissue and perfusate MDA levels increased noticeably at the end of reperfusion ($p < 0.05$) (Table 1). In the experimental group, the mean preischemic perfusate MDA level de-

Table 1. Effects of taurine on ischemia-reperfusion injury*

	Pre-ischemic	Post-ischemic	Post-reperfusion
PA pressure, % change			
Control group (n = 10)	100	207 ± 69	143 ± 35
Experimental group (n = 10)	100	202 ± 41	117 ± 19
Tissue MDA levels			
Control group (n = 10)	37.13 ± 1.98	40.69 ± 2.92	50.95 ± 10
Experimental group (n = 10)	39.90 ± 4.40	38.60 ± 2.73	37.61 ± 4.71
Perfusate MDA levels			
Control group (n = 10)	4.80 ± 0.59	–	5.19 ± 0.51
Experimental group (n = 10)	5.07 ± 0.11	–	4.62 ± 0.27
Tissue GSH levels			
Control group (n = 10)	2.11 ± 0.24	2.29 ± 0.24	2.16 ± 0.14
Experimental group (n = 10)	2.26 ± 0.13	2.28 ± 0.11	2.28 ± 0.15
Perfusate GSH levels			
Control group (n = 10)	81.61 ± 17.61	–	62.39 ± 2.66
Experimental group (n = 10)	81.12 ± 3.75	–	103.06 ± 9.97

*Values are mean ± SEM. *PA*, Pulmonary artery.

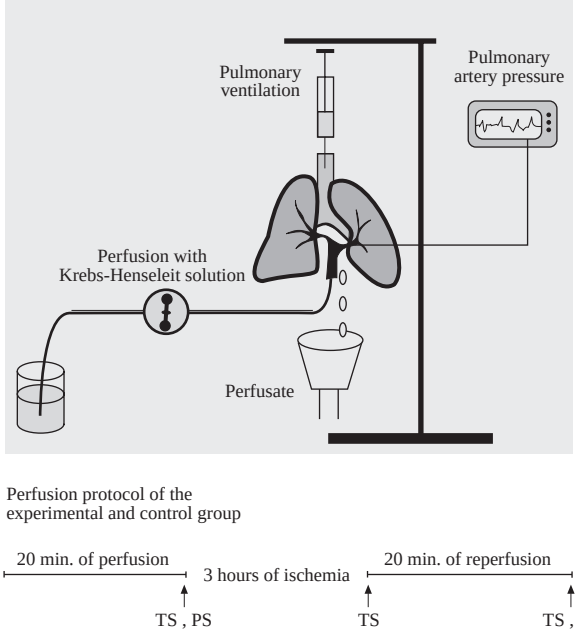

Fig. 1. Research system and protocol. *TS*, Collection of tissue samples; *PS*, collection of perfusate samples

creased noticeably at the end of reperfusion ($p < 0.05$) (Table 1). Either tissue and perfusate MDA levels following reperfusion were also statistically significant among two groups ($p < 0.05$) (Table 1) (Figs. 3–4).

In the control group, the mean preischemic perfusate GSH level decreased significantly at the end of reperfusion ($p < 0.05$) (Table 1). In the experimental group, the mean preischemic perfusate GSH level increased

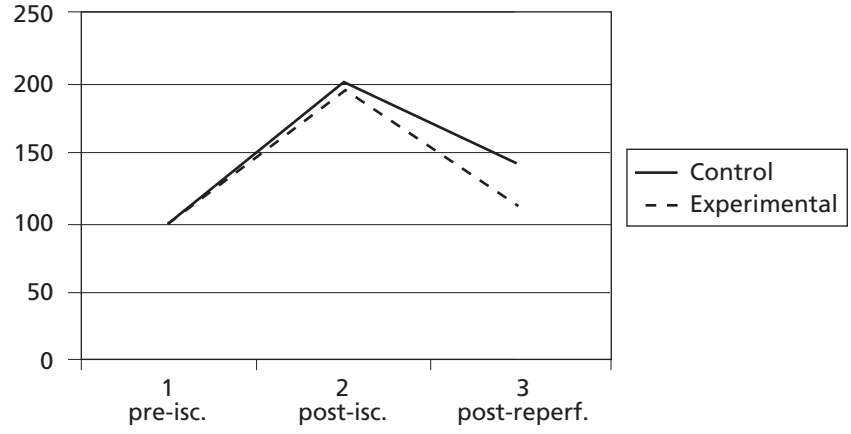

Fig. 2. Pulmonary artery pressure

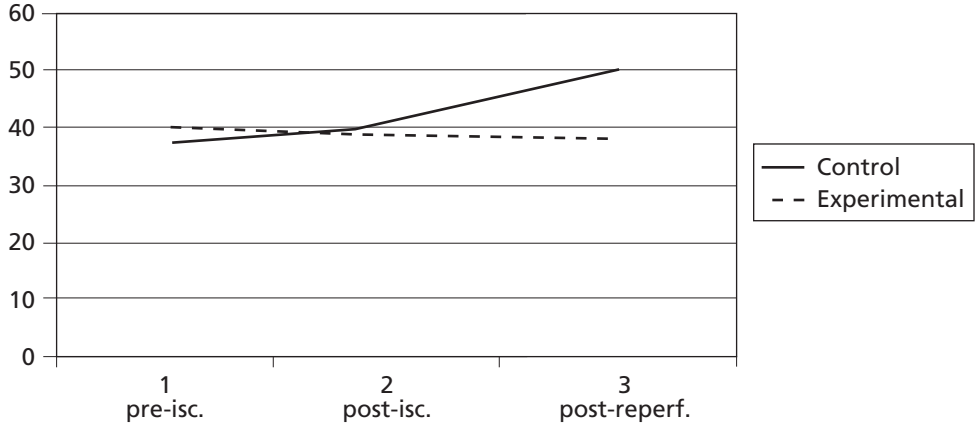

Fig. 3. Tissue MDA levels

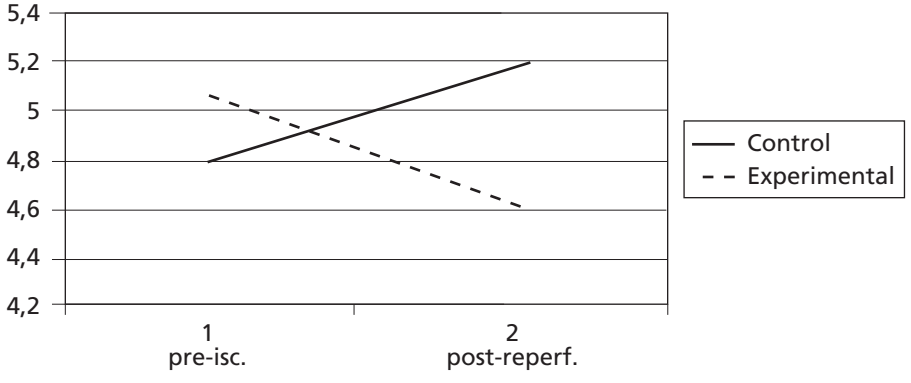

Fig. 4. Perfusate MDA levels

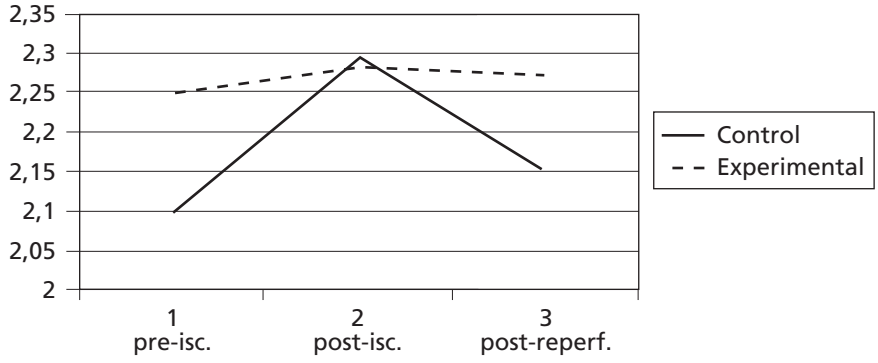

Fig. 5. Tissue GSH levels

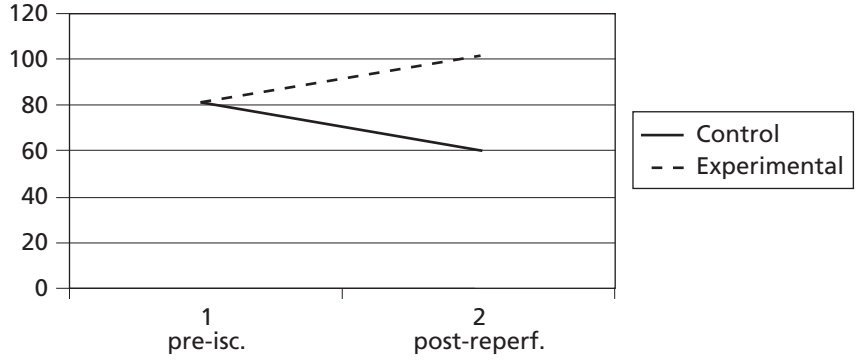

Fig. 6. Perfusate GSH levels

significantly at the end of reperfusion ($p < 0.05$) (Table 1). So far, perfusate glutathione levels were found significantly increased in the experimental group with respect to control group at the end of reperfusion ($p < 0.01$) (Fig. 6).

The ultrastructural analysis of the lung tissues in the preischemic control and taurine groups showed normal cell elements (Figs. 7–10 respectively).

In the postischemic control and taurine groups the ultrastructural analysis of the lung tissues showed degenerative changes of the type II cells (Figs. 8–11 respectively).

The ultrastructural analysis of the lung tissues in the postreperfusion control group showed that some deterioration had occured in lamellar bodies (Fig. 9).

The ultrastructural analysis of the lung tissues in the postreperfusion taurine group showed that cellular structures were nearly normal (Fig. 12).

Discussion

Ischemia-reperfusion lung injury occurs after various clinical procedurs, including cardiopulmonary bypass and lung transplantation. The lung was resistant to ischemic injury because of its dual pulmonary and bronchial

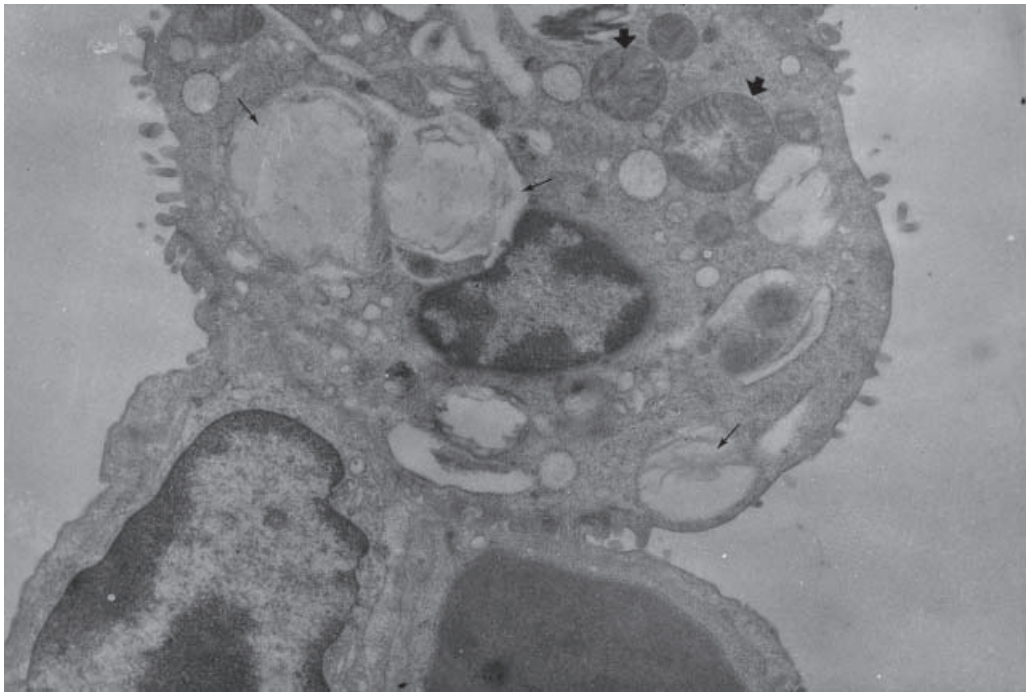

Fig. 7. Preischemic control group, ↑: Mitochondrion, ↑: Lamellar bodies (Uranyl acetate, Lead citrate ×21,000)

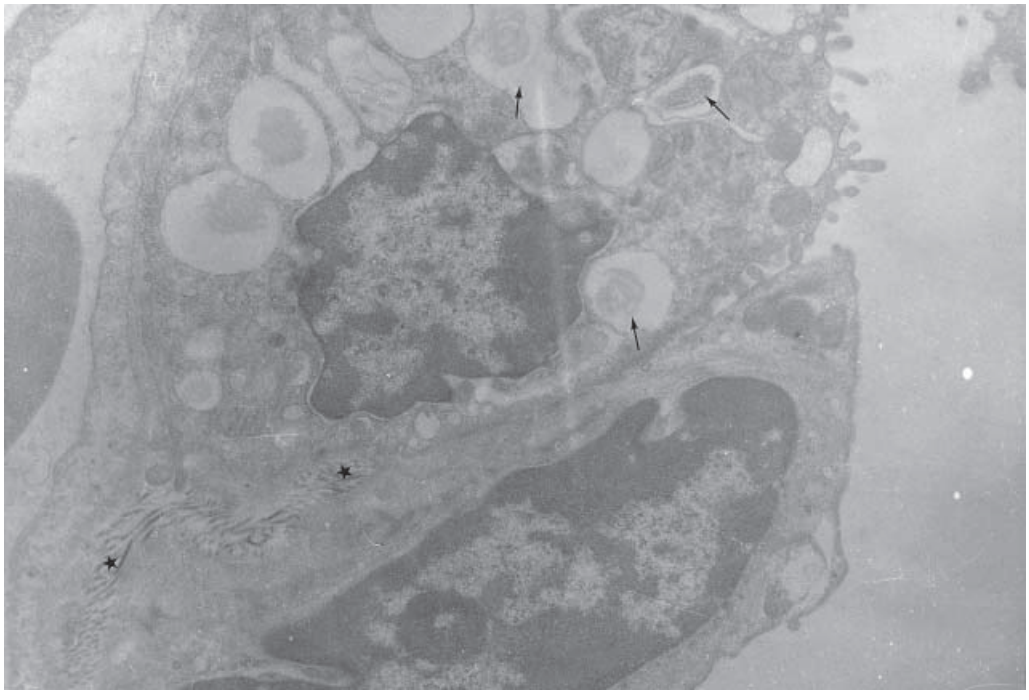

Fig. 8. Postischemic control group, ↑: Lamellar bodies, *Collagen fibers in interstitiel tissues. (Uranyl acetate ×21,000)

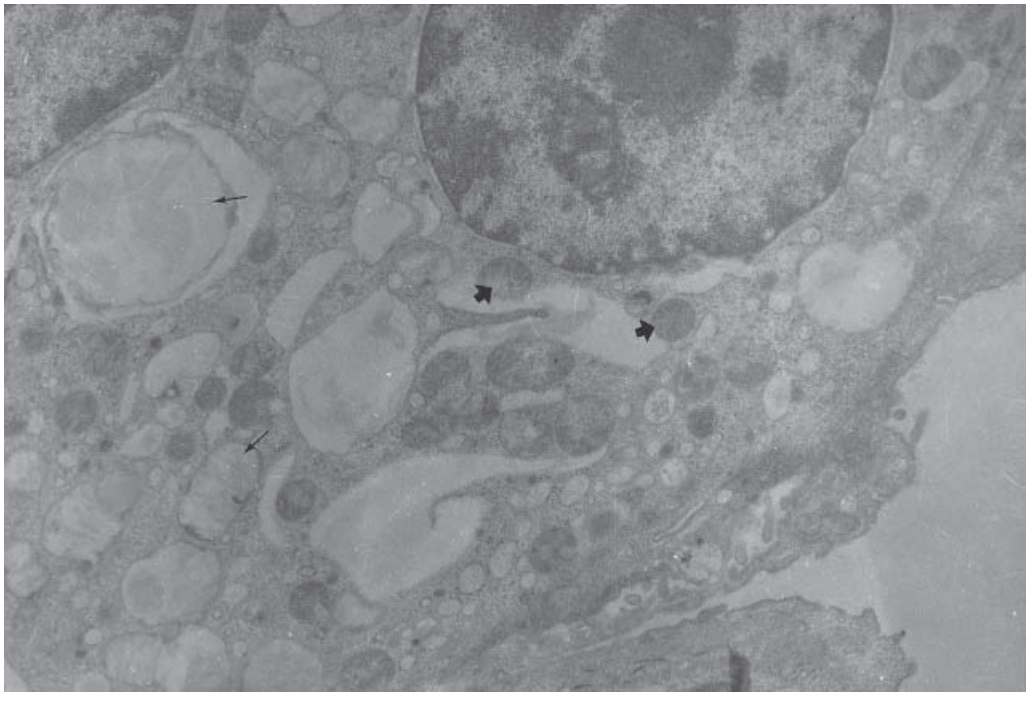

Fig. 9. Post-reperfusion control group ↑: Mitochondrion, ↑: Lamellar bodies, (Uranyl acetate, Lead citrate ×21,000)

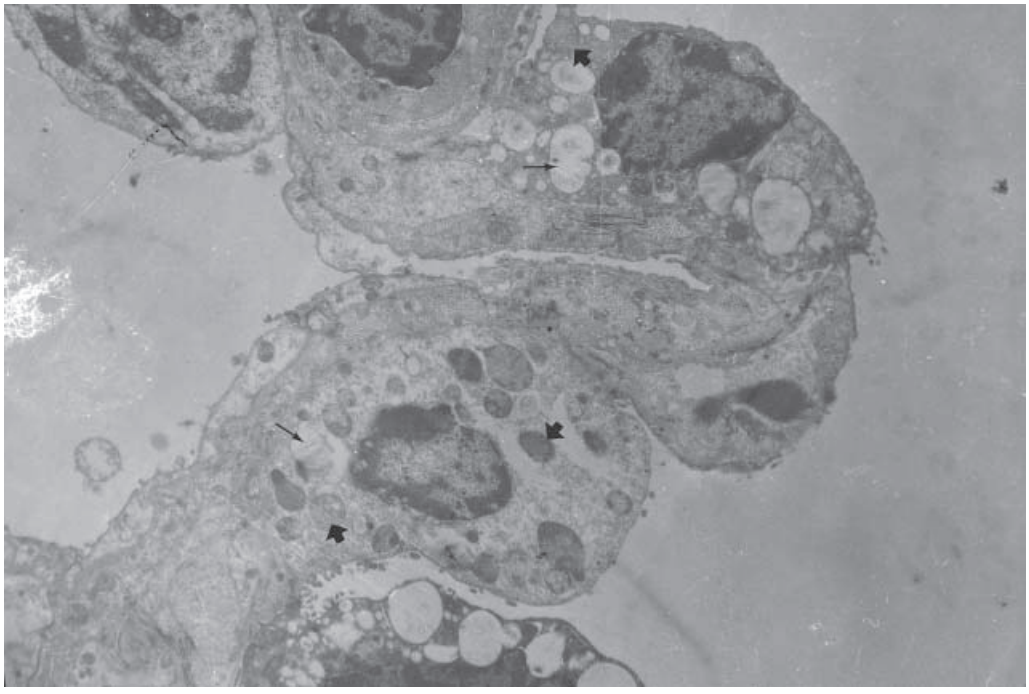

Fig. 10. Preischemic taurine group, ↑: Mitochondrion, ↑: Lamellar bodies (Uranyl acetate, Lead citrate ×9,000)

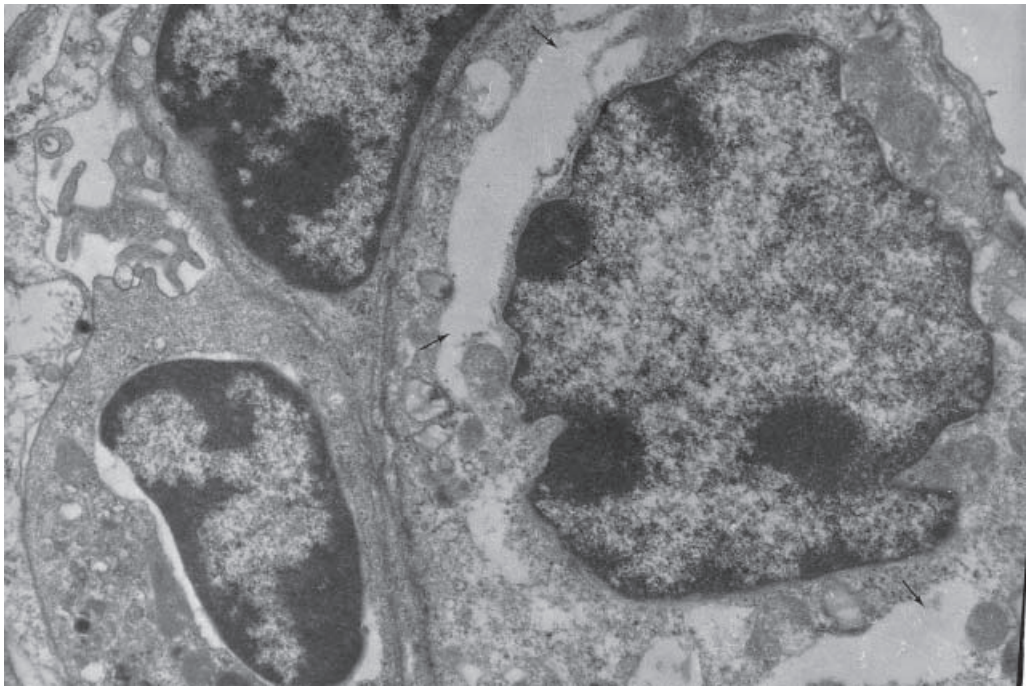

Fig. 11. Postischemic taurine group, ↑: Degenerative orifies (Uranyl acetate, Lead citrate ×21,000)

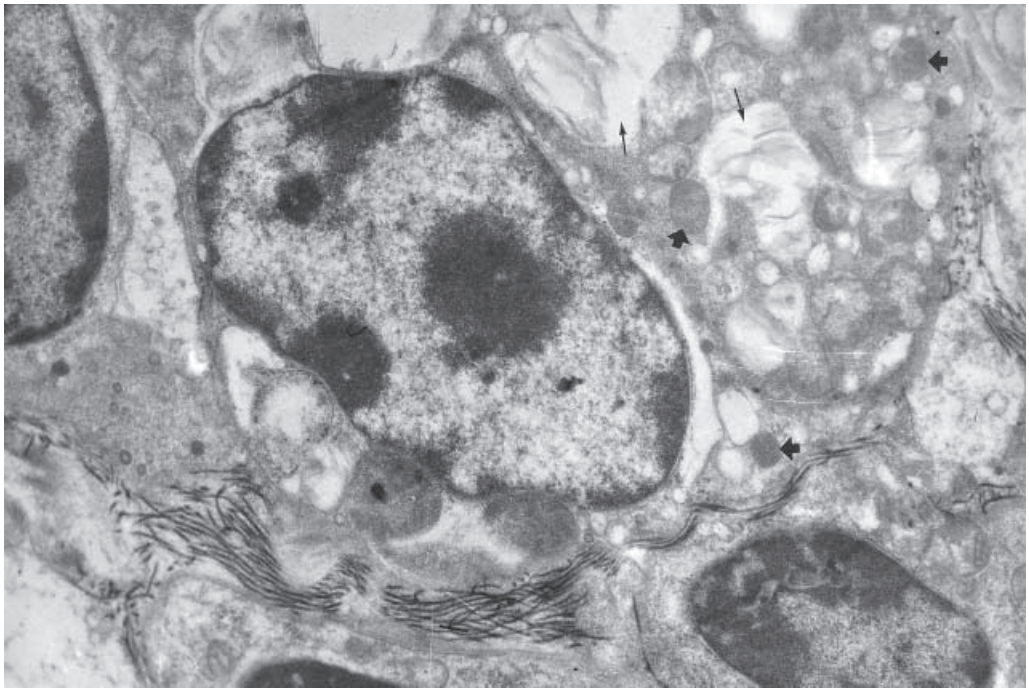

Fig. 12. Post-reperfusion taurine group, ↑: Mitochondrion, ↑: Lamellar bodies, (Uranyl acetate, Lead citrate ×21,000)

arterial blood supply and its independent source of oxygen available from the alveolar space (Heffner and Fracica, 1996). In contrast, much more difficulty has been associated with the protection of the lung than with the protection of solid organs in vitro because of the lung's unique structure, especially the close apposition of blood and air compartments in the alveoli (Date et al., 1993).

Although lung transplantation has become an effective therapy for a variety of patients with end-stage lung disease, severe ischemia-reperfusion injury occurs in about 10–20% of transplantated patients. Several studies have suggested that oxygen-derived free radicals produced by various mechanisms play a significant role in the injury sustained by a transplanted organ (Hernandez and Granger, 1988; Adkins and Taylor, 1990).

Previous experiments have demonstrated that lung functions began to deteriorate when preservation time was extended to 30 hours under hypothermic conditions (Miyoshi et al., 1992). Due to difficulties in keeping standardized experimental environment for such long periods in our laboratory conditions, in the current experiment, it was preferred to use 3 hours of normothermic ischemia.

Taurine has protective functions against free radical injury, for example; Gordon et al. (Gordon et al., 1986) had prophilactically administrated taurine to hamsters drinking water. As a result the hamsters were completely protected against inflammation and morphological changes in the lungs caused by exposure to nitrogen dioxide were discovered. The same group also observed that taurine administered in the drinking water prior to dosing reduced the pathological changes in the lungs of hamsters caused by administration of paraquat and bleomycin. Other workers have shown that taurine gives partial protection against pulmonary fibrosis caused by bleomycin in hamsters (Wang et al., 1991).

The role of ROS (Reactive oxygen species) in ischemia-reperfusion lung injury has been examined by detecting by-products of target molecule oxidation (lipid peroxidation and protein oxidation) and by determining the consumption of tissue antioxidants, such as glutathione. There have been numerous studies indicating a positive correlation between increased antioxidant enzymes in lung and/or decreased antioxidant defenses (Heffner and Fracica, 1996).

Taurine may play an important role in preventing ischemia-reperfusion injury by acting as an antioxidant. Taurine, an amino acid found in large quantities in neutrophils, is a powerful endogeneous anti-oxidant. Taurine was effective in preventing neutrophil-mediated pulmonary microvascular injury. (Barry et al., 1997)

Using isolated rat alveolar macrophages, Banks et al. (Banks et al., 1990; Banks et al., 1992) showed that preincubation in taurine significantly decreased the toxicity and ameliorated the biochemical effects of ozone exposure including lipid peroxidation, loss of ATP ase and leakage of glutathione. Taurine was protective in adriamycin induced cardiotoxicity and indicators of oxidative stress such as malondialdehyde and depletion of glutathione were normalized by taurine treatment (Azuma et al., 1987). Alveolar

macrophages isolated by pulmonary lavage from partially taurine-depleted rats demonstrated increased chemiluminescence due to the extracellular reaction between exogenous zymosan and various reactive forms of oxygen compared to macrophages isolated from control animals. These data suggest that taurine has antioxidant properties and that taurine depletion is potentially deleterious to alveolar macrophages and pulmonary tissues (Zhang and Lombardini, 1998). Dietary taurine has been shown to protect rat and hamster lung epithelia from acute oxidant injury induced by ozone exposure (Gordon et al., 1998). Administration of taurine can significantly decrease the mRNA transcription of procollagen and the synthesis of type I collagen in the interalveolar septa and can decrease the hydroxyproline content of the pulmonary tissue. These data suggest that taurine is protective against pulmonary injury (Song et al., 1998).

Malondyaldehyde is relatively stable end product of lipid peroxidation. In our study, in taurine added group, both tissue and perfusate MDA levels decreased significantly compared to control group ($p < 0.05$).

Glutathione has been shown to be an important cellular antioxidant, protecting cells from the damaging effects of oxidation products (e.g., hydrogen peroxide, superoxide, hydroxyl radicals) that are normally produced and destroyed by the cell during metabolism. The supply of glutathione may play a critical role in antioxidant defense. Under oxidant stress the redox state of the cell could become oxidized and then the cell would have in adequate antioxidant defenses to prevent irreversible damage such as lipid peroxidation (Meister, 1991).

Glutathione cannot efficiently be taken up by the cell because glutathione is continually being used up by the cell for that reason it was observed that glutathione has been depleted by the tissue and passed to perfusate (Timbrell et al., 1995; Meister, 1991).

Pulmonary artery pressure is a relative parameter for pulmonary vascular resistance. Ischemia is known to cause intense pulmonary vasospasm. Thus a remarkable increases of the pulmonary artery pressures were observed in both groups during the post ischemic period. On the other hand remarkable decrease of the pulmonary artery pressure was observed in experimental group at the end of reperfusion.

Constitutive levels of several antioxidant enzymes are relatively higher in type II cells than in the rest of the lung (Forman and Fisher, 1981) This may also partially account for the relative resistance of experimental group because of the preservation of the lamellar bodies of type II pneumocytes in this group under electron microscopic evaluations.

As a result, our study suggests that addition of taurine to pulmonary reperfusion solutions may reduce ischemic lung injury normothermic conditions.

Conclusion

In conclusion, it can be argued that the addition of taurine to pulmonary reperfusion solutions significantly improves lung recovery from ischemic cellular injury.

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