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Albumin resuscitation protects against traumatic/hemorrhagic shock-induced lung apoptosis in rats*

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Abstract: Objective: To determine the effects of albumin administration on lung injury and apoptosis in traumatic/hemorrhagic shock (T/HS) rats. Methods: Studies were performed on an in vivo model of spontaneously breathing rats with induced T/HS; the rats were subjected to femur fracture, ischemia for 30 min, and reperfusion for 20 min with Ringer's lactate solution (RS) or 5% (w/v) albumin (ALB), and the left lower lobes of the lungs were resected. Results: Albumin administered during reperfusion markedly attenuated injury of the lung and decreased the concentration of lactic acid and the number of in situ TdT-mediated dUTP nick-end labelling (TUNEL)-positive cells. Moreover, immunohistochemistry performed 24 h after reperfusion revealed increases in the level of nuclear factor κB (NF-κB), and phosphorylated p38 mitogen-activated protein kinase (MAPK) in the albumin-untreated group was down-regulated by albumin treatment when compared with the sham rats. Conclusion: Resuscitation with albumin attenuates tissue injury and inhibits T/HS-induced apoptosis in the lung via the p38 MAPK signal transduction pathway that functions to stimulate the activation of NF-κB.

Key words: Traumatic/hemorrhagic shock (T/HS), Mitogen-activated protein kinase (MAPK), Nuclear factor κB (NF-κB), Albumin, Apoptosis

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INTRODUCTION

Fluid resuscitation is vital in the treatment of traumatic/hemorrhagic shock (T/HS). Shifts in paradigm have suggested that the majority of cellular injuries occur during resuscitation but not ischemic period (Waxman, 1996). Adverse sequelae after shock treatment are the result of systemic inflammation, leading to the generation of reactive oxygen species, arachidonic acid metabolites, and inflammatory cytokines which perpetuate a pathologic state, even after the restoration of intravascular volume (Nuytinck *et al*., 1988). Clinically, this can contribute to the development of adult respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS), which are important determinants of outcome in trauma (Peitzman *et al*., 1995). Recent data have suggested that the type of resuscitation fluid used to treat hemorrhagic shock can affect the physiologic response (Sun *et al*., 1999), and that colloid versus crystalloid resuscitation in the acute trauma setting remains a controversial subject of debate. Despite the negative perception for resuscitation, recent studies showed that human albumin, as a broadly binding protein, has been characterized as a scavenger in addition to being an anti-apoptotic agent or antioxidant (Esposito *et al*., 1989; Emerson, 1989; Zoellner *et al*., 1996; Kentner *et al*., 2002; Osband *et al*., 2004b), and

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no increased mortality has been documented with albumin administration in the trauma population (Cochrane Injuries Group Albumin Reviewers, 1998; Vincent *et al*., 2004; Dubois *et al*., 2006).

Recently, the mechanisms involved in pulmonary apoptosis following lung ischaemia/reperfusion (I/R) have begun to be understood. Cell death that occurs following ischemic insult has long been associated with necrosis. However, recent reports have established apoptosis as a significant contributor to cell death after I/R (Genescà *et al*., 2002; Wang X. *et al*., 2004). A wide variety of stimuli such as oxidative stress can induce endothelial cell apoptosis and endothelial dysfunction, which may be regulated by different signaling pathways (de Nigris *et al*., 2003; Jiang *et al*., 2006). Mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinase 1/2 (ERK1/2) and p38, are a family of central signaling molecules that respond to numerous stimuli and are known to participate in decisions regarding cell survival and death. ERK1/2 phosphorylation promotes cell survival, whereas p38 MAPK activation induces apoptotic responses in endothelial cells (Hoefen and Berk, 2002). Recent studies have suggested that p38 MAPK may be an effective contributor to the development of acute organ injury, via the regulation of the nuclear factor κB (NF-κB)-activation pathway (Kim *et al*., 2006; Wang H. *et al*., 2007). Novel therapeutic interventions for the attenuation of T/HS-induced endothelial cell apoptosis and organ dysfunction remain a major focus area of research.

All these led to our hypothesis that albumin would also protect lung epithelia from injury and apoptosis induced by T/HS.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (8~10 weeks old, weighing 200~250 g) were purchased from the Animal Resource Center, School of Medicine, Zhejiang University, China. This study was approved by the Institutional Laboratory Review Board for the Care and Use of Animals, and based on the principles stated in the Guide for the Care and Use of Laboratory Animals by National Institutes of Health Publication in 1985.

Establishment of T/HS in rats

The rats were anesthetized intraperitoneally with 200 mg/kg chloral hydrate, and were allowed to waken. Under aseptic conditions, catheters were inserted into the right jugular vein for fluid resuscitation, the right carotid artery for measurement of mean arterial pressure (MAP), and the left femoral artery for the induction of hemorrhage. After an open, mid-diaphyseal transverse fracture was performed in the left femur, the animals were bled through the femoral artery catheter to an MAP of (30±5) mmHg and this was maintained for 30 min. After this period, rats were resuscitated with two times the shed blood volume in the form of Ringer's lactate solution (RS) or 5% (w/v) albumin (ALB). Finally, the catheters were removed, the vessels were ligated, and the incisions were closed.

Animal grouping and sample collection

The rats (n=36) were randomly divided into three groups: (1) sham group (n=12) rats underwent the same anesthetic and surgical procedures, but neither trauma/hemorrhage nor fluid resuscitation was performed; (2) T/HS+RS group (n=12) rats were resuscitated with RS; and (3) T/HS+ALB group (n=12) rats with 5% (w/v) albumin. For each of the T/HS groups and the sham group, blood was collected from the rats for analysis at 1, 3, and 24 h postresuscitation, and the left lower lobes of the lungs were resected at 24 h postresuscitation.

Blood analysis

The PaO_2/FiO_2 ratio levels in heparinized arterial blood were measured using a Radiometer ABL 700 blood gas analyzer (Copenhagen, Denmark), and the lactate measurements were performed using a lactic acid kit (Jianchen, Nanjing, China). The plasma samples were stored at −70 °C until analysis.

In situ TdT-mediated dUTP nick-end labelling (TUNEL)

Lung tissue samples were washed by perfusion with ice-cold phosphate buffered solution (PBS) for 1 min and then fixed using ice-cold 4% (w/v) paraformaldehyde in PBS. Following treatment with fixation solution for 24 h, tissue samples were cryosectioned at a thickness of 10 μm, and the serial sections obtained were stained with in situ TUNEL reagents (In

Situ Cell Death Detection Kit, POD; Roche, Mannheim, Germany) according to the manufacturer's instructions. The cell types were identified by hematoxylin staining (Vector Laboratories, Burlingame, CA, USA). The number of TUNEL-positive cells was counted against the total number of hematoxylin-stained cells.

The nuclei were counted under a light microscope in 8~10 microscopic fields per slide; 500 cells were counted. The percentage of apoptosis (apoptotic index) was calculated by dividing the number of cells that took up the stain by the total cell counts in an open field.

Immunohistochemistry

The paraformaldeyde-fixed tissues were cryosectioned at a thickness of 10 μm and were prepared for immunoperoxidase staining that was performed using the Envision System (Dako, CA, USA). In brief, endogenous peroxidase was quenched by treatment with 0.3% (v/v) H_2O_2 in methanol for 30 min. The nonspecific immunoglobulin-binding sites were blocked with normal goat serum for 1 h, and the sections were then incubated with anti-phospho-p38 MAPK or anti-phospho-p65 NF-κB as the primary antibody (Cell Signaling Technology, Beverly, MA, USA) for 1 h at room temperature. Further, the sections were incubated for 30 min with a biotinylated secondary antibody solution, followed by incubation for 30 min with the Envision System (Dako). Immunoglobulin complexes were visualized following incubation with 3,3′-diaminobenzidine (Sigma, St Louis, MO, USA); the sections were then washed, counterstained with hematoxylin, cleared, mounted, and examined by light microscopy. In total, 10 fields were observed for each slide. Tissue sections that were treated with normal serum instead of the primary antibodies were used as negative controls for the immunohistochemical staining. Routine histological examination was conducted by hematoxylin and eosin staining. The images were analyzed by using a computer-assisted image analyzer system that comprised a microscope (Olympus IPP6.0; Tokyo, Japan) equipped with a high-resolution video camera (JVC TK-890E; Japan). This analysis was performed for at least 10 fields per section and 2 sections per animal at a magnification of 400×. The staining intensity (on a scale of 0~3 for the lowest to the highest intensity) and the proportion of stained cells (on a scale of 0~4 for 0% to more than 70% cells stained) were semi-quantitatively determined as described previously (Zhou *et al*., 2003). A combined score of ≥6 was considered as overexpression. All the slides were scored by 2 observers who were blinded to the pathology and experiment features of T/HS in the study.

Statistical analysis

Differences were considered significant at $P<0.05$ for data comparison. The data were compared among groups by analysis of variance (ANOVA) and Tukey's test. The data were represented as the mean±*SD*.

RESULTS

Establishment of the T/HS model in rats

In our T/HS model, a bone fracture and continuous bleeding were induced. The MAPs of T/HS-affected rats that had been resuscitated with 5% (w/v) albumin or RS, together with the sham control rats, were longitudinally measured in Fig.1.

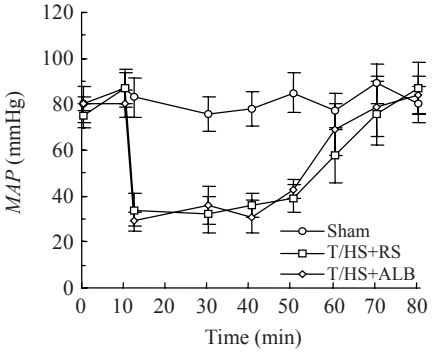

Fig.1 The mean blood pressure was monitored throughout the experimental period. The MAP at baseline was (81±11.9) mmHg. All animals exhibited a sharp drop in their MAP immediately after hemorrhage induction (10 min), and no significant differences in the end-resuscitation blood pressure were observed between the T/HS+RS and T/HS+ALB groups when compared with the sham group. The data shown are the mean±*SEM* recorded for each rat group

In the sham control rats, the MAP recorded remained constant at a value of 85 mmHg, while in the experimental rat groups, it increased slightly immediately after trauma induction and then decreased rapidly to a value of about 30 mmHg after bleeding. Following a compensatory increase in the MAP

during the shock phase, the MAP increased gradually toward the value recorded at baseline postresuscitation in the experimental rats, regardless of the type of fluid administered. The rapid drop in the MAP in the experimental rats following T/HS induction and the gradual recovery of MAP postresuscitation reflected the establishment of a T/HS model. Interestingly, no significant difference was observed among the rat groups with regard to the MAP recorded at the end of resuscitation, indicating that the resuscitation with albumin was not responsible for the observed MAP fluctuations in this rat model.

Effects of albumin treatment on the plasma lactic acid and the PaO_2/FiO_2 ratio in the T/HS-affected rats

T/HS induction significantly increased the plasma concentrations of lactic acid from the baseline values by 3~4 times immediately after the hemorrhage phase (Fig.2a). These increases were transient, and 3 h after complete resuscitation with RS, these concentrations dropped and attained the average levels. The PaO_2/FiO_2 ratio decreased significantly in the T/HS-affected rats 1 h after resuscitation (Fig.2b). In contrast, the changes in lactic acid (T/HS+RS group (8.79±1.23) mmol/L vs T/HS+ALB group (4.95±1.65) mmol/L, $P<0.01$) and the PaO_2/FiO_2 ratio (T/HS+RS group (160±32) mmHg vs T/HS+ALB group (258±57) mmHg, $P<0.01$) were significantly reversed 1 and 3 h after resuscitation in albumin-treated rats respectively. Thus, albumin treatment promoted the recovery of lung function following T/HS.

Comparative effects of albumin treatment on T/HS-induced lung injury and apoptosis

The effects of albumin treatment on T/HS-induced lung injury and apoptosis were evaluated and compared. The administration of albumin during reperfusion period attenuated injury and apoptosis (Fig.3). Injuries that developed in the T/HS rats, such as marked lung edema and inflammation, were improved in the albumin-treated group. Meantime, quantitative analysis revealed a significant decrease in the proportion of TUNEL-positive cells in the lungs of the T/HS+ALB group when compared with the T/HS+RS group (proportion of TUNEL-positive cells: (10.8±2.4)% and (19.9±6.1)% for the albumin-treated and untreated groups respectively, $P<0.05$).

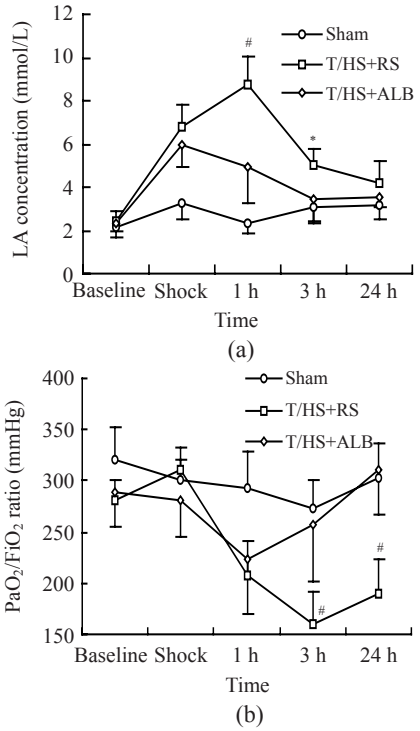

Fig.2 Effects of albumin treatment on (a) the plasma lactic acid (LA) and (b) the PaO_2/FiO_2 ratio in T/HS-affected rats. Blood samples were obtained before hemorrhage induction (baseline), after hemorrhage induction (shock), and at 1, 3, and 24 h postresuscitation. The data are expressed as the mean±*SD*. $^*P<0.05$ and $^\#P<0.01$, T/HS+RS group vs T/HS+ALB group

p38 MAPK activation following T/HS induction and albumin treatment

To determine the role played by MAPK, if any, in the lungs following T/HS induction, we performed immunohistochemical analyses using specific phospho-antibodies against p38 MAPK. Fig.4a shows that the levels of phosphorylated p38 increased following T/HS induction and resuscitation, and that albumin treatment attenuated the activity of phosphorylated p38 MAPK in the lungs. The immunohistochemical staining results were scored to determine the inter-group differences in the distribution patterns and the intensity of p38 MAPK immunolabelling in the lung tissue. The staining intensity (scored from 0 to 3 for the lowest to highest intensity) and the proportion of stained cells (scored from 0 to 4 for 0% to more than 70% cells stained) were semi-quantitatively determined as described previously. Based on the scores, the immunolabelling intensity of p38 MAPK was considered to have increased in the T/HS+RS group when compared with the sham group.

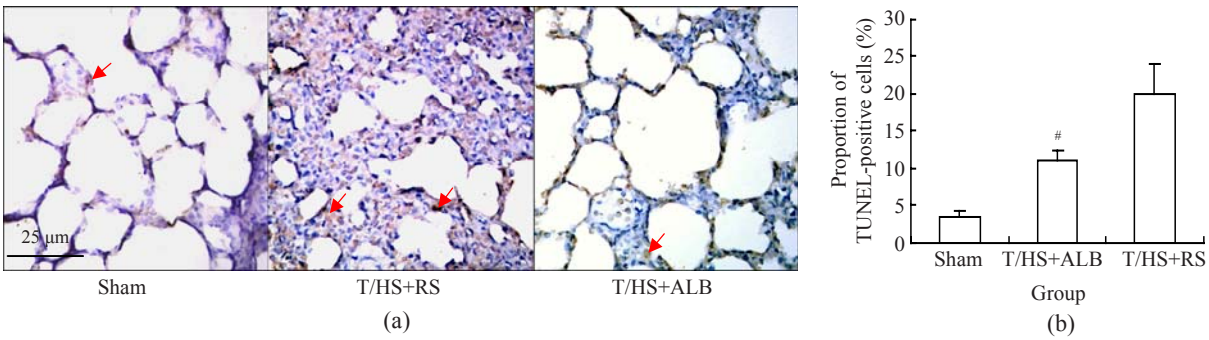

Fig.3 Effects of albumin treatment on T/HS-induced cellular apoptosis in the lungs. (a) DNA-strand fragmentation, as demonstrated by TUNEL technique. The positive (apoptotic, red arrow) nuclei were stained brown during the TUNEL reaction, and the negative ones were stained blue; (b) Percentage of TUNEL-positive cells in lungs obtained from all the rat groups. $^{\#}P$<0.01, T/HS+RS group vs T/HS+ALB group

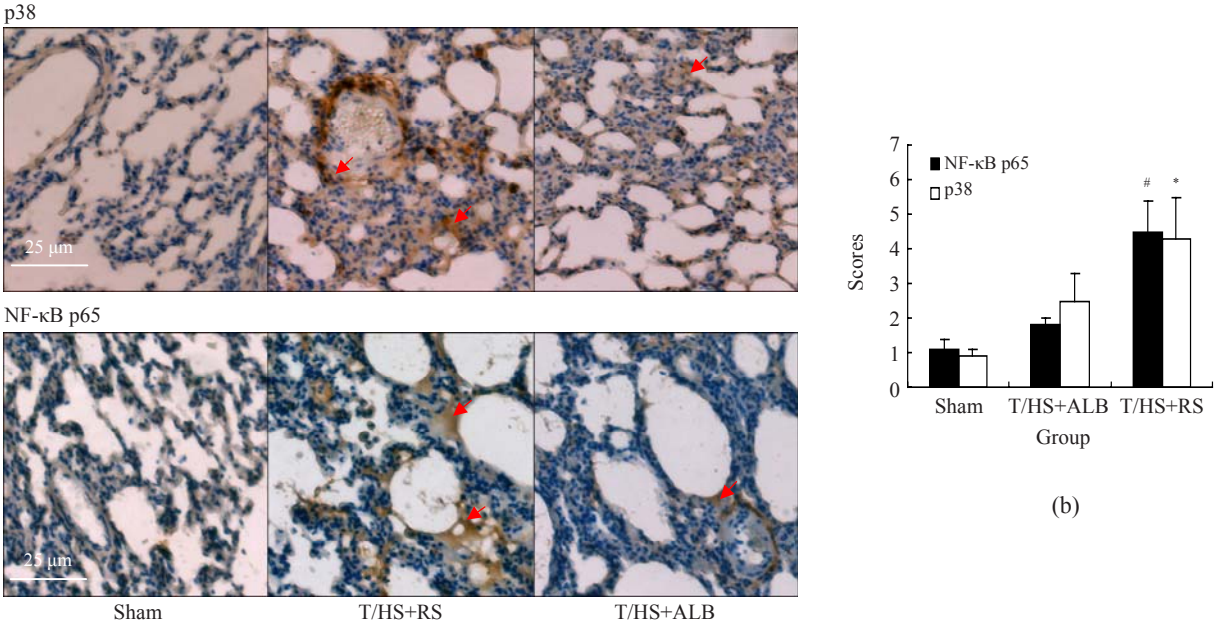

Fig.4 (a) Immunolabelling of phospho-p38 and phospho-NF-κB p65 in the lungs obtained from the albumin-treated and untreated rats and the sham group rats; (b) Intensities of phospho-p38 and phospho-NF-κB p65 immunolabelling. A semiquantitative scoring system was used to determine the staining intensity (scored 0~3 for the lowest to highest intensity) and the proportion of stained cells (scored 0~4 for 0% to more than 70% cells stained). $^{*}P$<0.05 and $^{\#}P$<0.01, T/HS+RS group vs T/HS+ALB group for the lung tissue samples

Further, a significant reduction in the p38 MAPK expression was noted in the albumin-treated rats when compared with the untreated rats (T/HS+ALB group (2.5±0.8)% vs T/HS+RS group (4.3±1.2)%, P<0.05).

NF-κB p65 activation following T/HS induction and albumin treatment

We performed immunohistochemical staining to assess the localization of phosphorylated NF-κB p65 expression. The results were scored to determine the inter-group differences in the distribution patterns and the immunolabelling intensity of phosphorylated NF-κB p65 in the lung tissue (Fig.4a). The staining intensity and the proportion of stained cells were scored according to the same semiquantitative system as described above. The expression patterns of phosphorylated NF-κB p65 were observed to be similar to those exhibited by phosphorylated p38 MAPK. Thus, albumin may attenuate the increased expression of phosphorylated NF-κB p65 in the lungs induced by T/HS (T/HS+ALB group (1.8±0.2)% vs T/HS+RS group (4.5±0.9)%, P<0.01).

DISCUSSION

T/HS patients run the risk of developing ARDS and subsequent MODS. T/HS has been shown to induce cellular apoptosis (Lu *et al*., 2005), which is an important contributor to cell dysfunction, organ failure, and mortality (Deb *et al*., 1999; 2000). Blood volume therapy can help to maintain hemodynamic stability and diminish the risk of developing hypovolemia and tissue malperfusion; however, the manner in which T/HS-induced cell apoptosis can be directly attenuated remains largely unclear. Hence, the discovery that resuscitation with albumin protects lung epithelia from apoptosis in T/HS rats significantly, as well as binds fatty acid, scavenges free radicals, and improves microvascular permeability (Horstick *et al*., 2002; Osband *et al*., 2004a), is highly significant.

We examined the effects of albumin treatment on the hemodynamics, lung functions, and cellular apoptosis in the lung tissue by using a rat model, wherein T/HS that mimicked the clinical conditions was induced and the animals were subsequently resuscitated with RS or 5% (w/v) albumin.

First, we observed that immediate resuscitation following T/HS can rapidly restore hemodynamic stability, with or without albumin. However, albumin treatment increased the PaO_2/FiO_2 ratio and decreased the plasma lactic acid concentrations when compared with those in RS resuscitated rats. These three clinically available parameters were used to test the effectiveness of volume expansion and tissue perfusion with different resuscitation fluids, and the difference in gas exchange or in lung edema formation. The results are consistent with several reports on the protective effects of albumin on I/R-induced organ dysfunction (Boura *et al*., 2003; Zhang *et al*., 2003; Su *et al*., 2007).

Second, two distinct modes of cell death, apoptosis and necrosis, have been reported to be involved in the destruction of small intestinal epithelial cells (Ikeda *et al*., 1998), lung epithelial cells (Ayala *et al*., 2001), and renal tubular cells (Engel *et al*., 2001) during I/R; however, apoptosis is noted to be the primary mode of cell death that greatly contributes to organ dysfunction, and alveolar apoptosis after hemorrhagic shock and associated toxic byproducts have been suggested to contribute to lung injury (Buckley *et al*., 1998; Compton *et al*., 1998; Magnotti *et al*., 1998). RS resuscitation has been demonstrated to activate neutrophils and raise apoptosis in the liver and small intestine of hemorrhaged rats (Deb *et al*., 1999). On the contrary, albumin has been well characterized as a general scavenger in addition to being an anti-apoptotic agent and an antioxidant. Our laboratory has also shown that the type of fluid used affects the extent of apoptosis in rat lungs after T/HS and resuscitation. In this study, it was observed that 5% (w/v) albumin resuscitation resulted in significantly less apoptosis in the lung than RS resuscitation by the TUNEL staining. Based on this finding, we set out to determine which signaling pathways were responsible for regulating apoptosis in the lungs of T/HS rats.

Considering the antiapoptotic effects of alumin in patients with I/R injury and the important role played by p38 MAPK in pulmonary injury by reperfusion (Khan *et al*., 2003; 2004; Lai *et al*., 2004), we aimed to determine whether albumin-induced organ protection is mediated by regulation of the activities of MAPK family members. Our study demonstrates, for the first time, that the expression level of phosphorylated p38 in the lung is higher in animals with T/HS injury and reperfusion with RS, when compared with normal animals; this is consistent with previous findings regarding the pathologic sequelae of cardiac, hepatic, and renal reperfusion injuries (Toledo-Pereyra *et al*., 2004; Matot *et al*., 2006). In this study, albumin administration attenuated both p38 MAPK expression and apoptosis.

Furthermore, we examined the expression of NF-κB p65 in tissue samples obtained from the T/HS-affected hosts, and found that T/HS increased this expression, while albumin treatment attenuated it. These data indicate the unfavorable role played by p38 MAPK in T/HS-induced apoptosis associated with the development of acute organ injury via its upstream action on NF-κB.

In summary, our study on a rat model demonstrates that T/HS can induce cellular apoptosis in the lungs. Albumin inhibits the apoptosis of the lungs induced by T/HS, and exhibits organ protective activity. Many factors are known to modulate the programmed cell death, and our previous study suggested that the anti-apoptotic effect of albumin against T/HS-induced injury may be mediated by

down-regulation of p38 MAPK and NF-κB p65 activation. Further studies are required to precisely explore the signaling pathways of T/HS-induced apoptosis by specific inhibition of one or more signaling molecules (phosphatidylinositol 3 (PI3), protein kinase B (AKT), C-Jun, Smads, and the above mentioned) to delineate the benefits of albumin. Whether albumin treatment can improve organ function in T/HS patients also remains to be investigated.

References

Ayala, A., Chung, C.S., Song, G.Y., Chaudry, I.H., 2001. IL-10 mediation of activation-induced TH1 cell apoptosis and lymphoid dysfunction in polymicrobial sepsis. *Cytokine*, **14**(1):37-48. [doi:10.1006/cyto.2001.0848]

Boura, C., Caron, A., Longrois, D., Mertes, P.M., Labrude, P., Menu, P., 2003. Volume expansion with modified hemoglobin solution, colloids, or crystalloid after hemorrhagic shock in rabbits: effects in skeletal muscle oxygen pressure and use versus arterial blood velocity and resistance. *Shock*, **19**(2):176-182. [doi:10.1097/00024382-200302000-00015]

Buckley, S., Barsky, L., Driscoll, B., Weinberg, K., Anderson, K.D., Warburton, D., 1998. Apoptosis and DNA damage in type 2 alveolar epithelial cells cultured from hyperoxic rats. *Am. J. Physiol.*, **274**(5 Pt 1):714-720.

Cochrane Injuries Group Albumin Reviewers, 1998. Human albumin administration in critically ill patients: systematic review of randomised controlled trials. Cochrane Injuries Group Albumin Reviewers. *BMJ*, **317**(7153): 235-240.

Compton, C.N., Franko, A.P., Murray, M.T., Diebel, L.N., Dulchavsky, S.A., 1998. Signaling of apoptotic lung injury by lipid hydroperoxides. *J. Trauma*, **44**(5):783-788.

de Nigris, F., Lerman, A., Ignarro, L.J., Williams-Ignarro, S., Sica, V., Baker, A.H., Lerman, L.O., Geng, Y.J., Napoli, C., 2003. Oxidation-sensitive mechanisms, vascular apoptosis and atherosclerosis. *Trends Mol. Med.*, **9**(8): 351-359. [doi:10.1016/S1471-4914(03)00139-4]

Deb, S., Martin, B., Sun, L., Ruff, P., Burris, D., Rich, N., DeBreux, S., Austin, B., Rhee, P., 1999. Resuscitation with lactated Ringer's solution in rats with hemorrhagic shock induces immediate apoptosis. *J. Trauma*, **46**(4): 582-588.

Deb, S., Sun, L., Martin, B., Talens, E., Burris, D., Kaufmann, C., Rich, N., Rhee, P., 2000. Lactated Ringer's solution and hetastarch but not plasma resuscitation after rat hemorrhagic shock is associated with immediate lung apoptosis by the up-regulation of the Bax protein. *J. Trauma*, **49**(1):47-53.

Dubois, M.J., Orellana-Jimenez, C., Melot, C., De Backer, D., Berre, J., Leeman, M., Brimioulle, S., Appoloni, O., Creteur, J., Vincent, J.L., 2006. Albumin administration improves organ function in critically ill hypoalbuminemic patients: a prospective, randomized, controlled, pilot study. *Crit. Care Med.*, **34**(10):2536-2540. [doi:10.1097/01.CCM.0000239119.57544.0C]

Emerson, T.E.Jr., 1989. Unique features of albumin: a brief review. *Crit. Care Med.*, **17**(7):690-694. [doi:10.1097/00003246-198907000-00020]

Engel, J.M., Welters, I., Rupp, M., Langefeld, T., Ruwoldt, R., Menges, T., Hempelmann, G., 2001. Influence of colloid fluids on polymorphonuclear granulocyte function in vivo. *Acta Anaesthesiol. Scand.*, **45**(3):385-389. [doi:10.1034/j.1399-6576.2001.045003385.x]

Esposito, C., Gerlach, H., Brett, J., Stern, D., Vlassara, H., 1989. Endothelial receptor-mediated binding of glucose-modified albumin is associated with increased monolayer permeability and modulation of cell surface coagulant properties. *J. Exp. Med.*, **170**(4):1387-1407. [doi:10.1084/jem.170.4.1387]

Genescà, M., Sola, A., Miquel, R., Pi, F., Xaus, C., Alfaro, V., Hotter, G., 2002. Role of changes in tissular nucleotides on the development of apoptosis during ischemia/reperfusion in rat small bowel. *Am. J. Pathol.*, **161**(5): 1839-1847.

Hoefen, R.J., Berk, B.C., 2002. The role of MAP kinases in endothelial activation. *Vascul. Pharmacol.*, **38**(5): 271-273. [doi:10.1016/S1537-1891(02)00251-3]

Horstick, G., Lauterbach, M., Kempf, T., Bhakdi, S., Heimann, A., Horstick, M., Meyer, J., Kempski, O., 2002. Early albumin infusion improves global and local hemodynamics and reduces inflammatory response in hemorrhagic shock. *Crit. Care Med.*, **30**(4):851-855. [doi:10.1097/00003246-200204000-00023]

Ikeda, H., Suzuki, Y., Suzuki, M., Koike, M., Tamura, J., Tong, J., Nomura, M., Itoh, G., 1998. Apoptosis is a major mode of cell death caused by ischaemia and ischaemia/reperfusion injury to the rat intestinal epithelium. *Gut*, **42**(4):530-537.

Jiang, D.J., Jia, S.J., Dai, Z., Li, Y.J., 2006. Asymmetric dimethylarginine induces apoptosis via p38 MAPK/caspase-3-dependent signaling pathway in endothelial cells. *J. Mol. Cell. Cardiol.*, **40**(4):529-539. [doi:10.1016/j.yjmcc.2006.01.021]

Kentner, R., Safar, P., Behringer, W., Wu, X., Kagan, V.E., Tyurina, Y.Y., Henchir, J., Ma, L., Hsia, C.J., Tisherman, S.A., 2002. Early antioxidant therapy with Tempol during hemorrhagic shock increases survival in rats. *J. Trauma*, **53**(5):968-977.

Khan, T.A., Bianchi, C., Araujo, E.G., Ruel, M., Voisine, P., Sellke, F.W., 2003. Activation of pulmonary mitogen-activated protein kinases during cardiopulmonary bypass. *J. Surg. Res.*, **115**(1):56-62. [doi:10.1016/S0022-4804(03)00236-1]

Khan, T.A., Bianchi, C., Ruel, M., Voisine, P., Sellke, F.W., 2004. Mitogen-activated protein kinase pathways and cardiac surgery. *J. Thorac. Cardiovasc. Surg.*, **127**(3): 806-811. [doi:10.1016/j.jtcvs.2003.04.001]

Kim, H.J., Lee, H.S., Chong, Y.H., Kang, J.L., 2006. p38 mitogen-activated protein kinase up-regulates LPS-induced NF-kappaB activation in the development of

lung injury and RAW 264.7 macrophages. *Toxicology*, **225**(1):36-47. [doi:10.1016/j.tox.2006.04.053]

Lai, E.W., Toledo-Pereyra, L.H., Walsh, J., Lopez-Neblina, F., Anaya-Prado, R., 2004. The role of MAP kinases in trauma and ischemia-reperfusion. *J. Invest. Surg.*, **17**(1): 45-53. [doi:10.1080/08941930490269646]

Lu, Y.Q., Cai, X.J., Gu, L.H., Fan, Y.J., Wang, Q., Bao, D.G., 2005. Effects of three fluid resuscitation methods on apoptosis of visceral organs in rats with hemorrhagic shock. *J. Zhejiang Univ. Sci. B*, **6**(9):907-912. [doi:10.1631/jzus.2005.B0907]

Magnotti, L.J., Upperman, J.S., Xu, D.Z., Lu, Q., Deitch, E.A., 1998. Gut-derived mesenteric lymph but not portal blood increases endothelial cell permeability and promotes lung injury after hemorrhagic shock. *Ann. Surg.*, **228**(4): 518-527. [doi:10.1097/00000658-199810000-00008]

Matot, I., Zeira, E., Weiniger, C.F., Galun, E., Joshi, B.V., Jacobson, K.A., 2006. A3 adenosine receptors and mitogen-activated protein kinases in lung injury following in vivo reperfusion. *Crit. Care*, **10**(2):R65. [doi:10.1186/cc4893]

Nuytinck, H.K., Offermans, X.J., Kubat, K., Goris, J.A., 1988. Whole-body inflammation in trauma patients. An autopsy study. *Arch. Surg.*, **123**(12):1519-1524.

Osband, A.J., Deitch, E.A., Hauser, C.J., Lu, Q., Zaets, S., Berezina, T., Machiedo, G.W., Rajwani, K.K., Xu, D.Z., 2004a. Albumin protects against gut-induced lung injury in vitro and in vivo. *Ann. Surg.*, **24**(2):331-339. [doi:10.1097/01.sla.0000133359.12284.6b]

Osband, A.J., Sifri, Z.C., Wang, L., Cohen, D., Hauser, C.J., Mohr, A.M., Deitch, E.A., Livingston, D.H., 2004b. Small volume albumin administration protects against hemorrhagic shock-induced bone marrow dysfunction. *J. Trauma*, **56**(2):279-283.

Peitzman, A.B., Billiar, T.R., Harbrecht, B.G., Kelly, E., Udekwu, A.O., Simmons, R.L., 1995. Hemorrhagic shock. *Curr. Probl. Surg.*, **32**(11):925-1002. [doi:10.1016/S0011-3840(05)80008-5]

Su, F., Cai, Y., Wang, Z., Rogiers, P., Vincent, J.L., 2007. Fluid resuscitation in severe sepsis and septic shock: albumin, hydroxyethyl starch, gelatin or Ringer's lactate: does it really make a difference? *Shock*, **27**(5):520-526. [doi:10.1097/01.shk.0000248583.33270.12]

Sun, L.L., Ruff, P., Austin, B., Deb, S., Martin, B., Burris, D., Rhee, P., 1999. Early up-regulation of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 expression in rats with hemorrhagic shock and resuscitation. *Shock*, **11**(6):416-422.

Toledo-Pereyra, L.H., Toledo, A.H., Walsh, J., Lopez-Neblina, F., 2004. Molecular signaling pathways in ischemia/reperfusion. *Exp. Clin. Transplant*, **2**(1):174-177.

Vincent, J.L., Navickis, R.J., Wilkes, M.M., 2004. Morbidity in hospitalized patients receiving human albumin: a meta-analysis of randomized, controlled trials. *Crit. Care Med.*, **32**(10):2029-2038. [doi:10.1097/01.CCM.0000142574.00425.E9]

Wang, H., Wang, Z., Chen, J., Wu, J., 2007. Apoptosis induced by NO via phosphorylation of p38 MAPK that stimulates NF-kappaB, p53 and caspase-3 activation in rabbit articular chondrocytes. *Cell Biol. Int.*, **31**(9):1027-1035. [doi:10.1016/j.cellbi.2007.03.017]

Wang, X., Zhou, Y., Kim, H.P., Song, R., Zarnegar, R., Ryter, S.W., Choi, A.M., 2004. Hepatocyte growth factor protects against hypoxia/reoxygenation-induced apoptosis in endothelial cells. *J. Biol. Chem.*, **279**(7):5237-5243. [doi:10.1074/jbc.M309271200]

Waxman, K., 1996. Shock: ischemia, reperfusion, and inflammation. *New Horiz.*, **4**(2):153-160.

Zhang, H., Voglis, S., Kim, C.H., Slutsky, A.S., 2003. Effects of albumin and Ringer's lactate on production of lung cytokines and hydrogen peroxide after resuscitated hemorrhage and endotoxemia in rats. *Crit. Care Med.*, **31**(5): 1515-1522. [doi:10.1097/01.CCM.0000065271.23556.FF]

Zhou, Y.Q., He, C., Chen, Y.Q., Wang, D., Wang, M.H., 2003. Altered expression of the RON receptor tyrosine kinase in primary human colorectal adenocarcinomas: generation of different splicing variants and their oncogenic potential. *Oncogene*, **22**(2):186-197. [doi:10.1038/sj.onc.1206075]

Zoellner, H., Höfler, M., Beckmann, R., Hufnagl, P., Vanyek, E., Bielek, E., Wojta, J., Fabry, A., Lockie, S., Binder, B.R., 1996. Serum albumin is a specific inhibitor of apoptosis in human endothelial cells. *J. Cell Sci.*, **109**(Pt 10): 2571-2580.