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Taurine attenuates ischemia-reperfusion injury and prevents magnesium efflux from isolated rat liver

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ABSTRACT

Ischemia reperfusion injury (IRI) and severe disruption of inorganic ion homeostasis lead to massive cell death. Taurine is a sulfur-containing amino acid that maintains cell homeostasis and functions with ion flux regulating properties. We hypothesize that pre- and post-ischemic treatment with taurine reduces ischemia-reperfusion injury and inhibits magnesium (Mg^{2+}) efflux, which promotes tissue survival from oxidative stress. Isolated rat livers were flushed with Krebs-Henseleit Buffer (KHB) for 5-10 min followed by Mg^{2+} free KHB for 10 min and whole organ ischemia was induced by stopping perfusion for 30 min. Livers were treated with taurine (20 mmol/L) 5 min before (BT-isc) and after (AT-isc) ischemia. The livers were then reperfused with oxygenated Mg^{2+} free KHB for 90 min and the necessary effluents were collected for estimation of Mg^{2+} efflux by AAS. Lactate dehydrogenase (LDH) levels and superoxide dismutase (SOD) activity were measured by colorimetric assay. Total ERK (tERK1/2); Phospho ERK (pERK1/2); both are the members of p44/42 MAPK, Bcl and Bax proteins were measured in liver homogenate by western blotting method. Mg^{2+} efflux, LDH, SOD, tERK1/2, pERK1/2, Bax and Bcl significantly ($p<0.05$) increased during reperfusion in the non-treated control (NT-isc) livers. Taurine treatment before and after ischemia significantly reduced Mg^{2+} flux into the effluent. LDH and SOD activity in liver tissue during the reoxygenation period were found to be significantly decreased compared to the untreated group. Taurine treatment significantly decreased total and phospho ERK1/2, Bax and Bcl proteins in perfused livers compared to untreated controls. Comparison between pre- and post-ischemia taurine treatment indicated that taurine pre-treatment provided better protection against reperfusion injury than post-ischemia treatment. Taurine supplementation treatment before and after ischemic liver injury may be beneficial for better recovery of Mg^{2+} in the isolated liver and may be effective in protecting the transplanted liver from ischemia-reperfusion injury in the recipient.

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Introduction

Ischemia-reperfusion injury (IRI) to the hepatic tissue occurs during a variety of medical conditions, including liver trauma, hepatectomy, and transplantation (Papadopoulos *et al*., 2013). The oxidative stress and inflammatory response followed by I/R leads to hepatocyte necrosis and apoptosis (programmed cell death) leading to organ dysfunction (Guan *et al*., 2008). Taurine is an inclusive sulfur-containing amino acid in most tissues with many important functions, such as antioxidant (Baliou *et al*., 2021; Surai *et al*., 2021). Previous investigations have shown that taurine prevents gut mucosal damage and inhibits intestinal epithelial cell apoptosis following IRI in rats (Sukhotnik *et al*., 2016). It also may protect against hepatic IRI after liver transplantation (Sun *et al*., 2012). Similarly, Mg^{2+} is an inorganic anion that plays an important role in cellular function by modulating hundreds of enzymatic reactions in the cell. In the present investigation, we hypothesized that taurine has an inhibitory effect on Mg^{2+} efflux from ischemic liver tissue. Several previous studies have described protection against IRI using drugs that employ different strategies, such as modulating cell-survival pathways, avoiding oxidative damage, physically protecting cell membrane integrity, and increasing cell strength (Guo *et al*., 2011; Soares *et al*., 2019). Necrosis has classically been considered the only mode of cell death induced by IRI, however, increasing evidence indicates that apoptosis also has an important role (Cursio *et al*., 1999). The swelling process is followed by inflammation with collateral damage to cells and tissues leading to cell rupture and release of contents. Theories of the 1950s proposed that disruption of ion homeostasis was central to the pathogenic process of apoptosis (Barros *et al*., 2002; Trapani *et al*., 2011). Because, with the development of anoxic or oxidative tissue destruction, ion and water balance are important, especially Ca^{2+} and Mg^{2+} were known to influence protein activity. Currently, the ion theory of cell necrosis is well established and rapid advances are being made in our understanding of the molecular mechanisms involved. It is also reported that necrosis or apoptosis of lethally injured mammalian cells can be confirmed by the initial activation of specific ion channels on the cell surface (Barros *et al*., 2002). A previous study by Kim *et al*., (2011) showed that administration of Mg^{2+} before reperfusion in liver transplantation is protective against IRI by reducing blood lactate levels. It has important implications for hepatocytes under cellular conditions where the intramitochondrial ATP pool size is depleted, such as in hypoxia or ischemia, or during reperfusion when the mitochondria are exposed to increased oxidative stress (Hagen *et al.,* 2003). Evidence indicates that extracellular Mg^{2+} deficiency induces apoptosis, mainly through increased oxidative stress, while intracellular Mg^{2+} mobilization from intracellular stores and consequent increase of cytosolic free Mg^{2+} seem to act in the effector phase (Trapani *et al*., 2011). Previous studies in isolated rat hearts have shown that leakage of Mg^{2+} is due to the release of intracellular Mg^{2+} into the extracellular space, as high Mg^{2+} prior to ischemia or during reperfusion may be protective by affecting Ca^{2+}, K^{+} and Na^{+} distribution and flux (Kirkels *et al*., 1989). In this context, taurine has been identified as an organic osmolyte in various liver cells that is accumulated or released by cells in response to hyperosmotic cell shrinkage or hyposmotic cell swelling, respectively, to maintain cell volume homeostasis (Warskulat *et al*., 1997; Peters-Regehr *et al*., 1999) and thus protective effect of taurine has been found in warm ischemia and reoxygenation in perfused rat livers (Wettstein *et al*., 1997). The membrane-stabilizing effects, inhibiting effects on ROS-producing enzymes, as well as the indirect antioxidant effects of taurine via redox balance maintenance associated with the modulation of various transcription factors like Nrf2 and NF-κB have been stated elsewhere (Surai *et al.,* 2021). The use of taurine to protect the liver from endotoxin-induced injury, particularly after IRI, through its anti-inflammatory, anti-oxidative and anti-apoptotic activities is approved by the study of Zhang *et al.,* (2010). Considering the above multifaceted recognized role of taurine, the present study was designed to investigate whether inhibition of Mg^{2+} efflux from isolated-ischemic rat liver by taurine could reduce ischemia-reperfusion injury and thus be effective in liver transplantation.

Materials and methods

Animal preparation

Male Sprague-Dawley rats, 6 weeks of age and weighing 240-260 g, were purchased from the Korean Research Institute of Bioscience and Biotechnology. Rats were housed in a room with a temperature of 23 ± 5 °C and a 12 h dark-light cycle. Animals were fed standard rat chow (crude protein 20%, crude fat 4.5%, crude fiber 6.0% crude ash 7%, calcium 0.5% and phosphorus 1%) without regard to taurine. The study was approved by the Ethical Committee of Chonbuk National University for the use of laboratory animals. The investigation was under the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Liver isolation and ischemia-reperfusion procedure

Animals were anesthetized with pentobarbital, and livers were isolated and attached to the perfusion system as the procedure described previously (Sugano *et al*., 1978). Briefly, the liver was first perfused in vivo with KHB by inserting a catheter into the portal vein (inflow) and another into the hepatic vein (outflow), then removed and reconnected to a non-circulating Langendorff perfusion system (AD Instruments Pty Ltd, Bella Vista, NSW, Australia) and perfusion continued with KHB (NaCl-120 mM, KCl-3.5 mM, Pyruvate-2 mM, Glucose-10 mM, Tris-base-10 mM, Tris-acid-10 mM, KH_2PO_4-1.2 mM, NaH_2CO_3-25 mM and KH_2CO_3-12 mM) during the test period. The working KHB solution was slightly modified by omitting 1.2 mM of $MgCl_2$ present in the regular KHB solution. The inflow portal vein, outflow hepatic vein, and bile duct were ligated to prevent bile flow. After catheter placement, the in vivo liver was flushed with 15 mL of 4°C chilled KHB. During the transfer period, the inflow and outflow catheters were closed by clips to avoid air bubbles. The livers were then isolated and transferred and reattached to the Langendorff perfusion system via an inflow catheter. The mean isolation and transfer time was 10±2 min. Organs were perfused with oxygenated KHB (37 °C) for 80-90 min at a flow rate of 15 ml/min. Whole organ ischemia was induced by stopping perfusion for 30 min.

Livers were perfused according to the following schedule.

10 min Normal Perfusion	5 min Pre-Isc Treat	30 min No flow Ischemia	Reperfusion			
			5 min Post-Isc Treat	Early Reperfusion (10 min)	30 min (Continuous Reperfusion)	Late Reperfusion (10 min)

Treatment and sample collection

Livers were first perfused with magnesium-free 37°C oxygenated KHB (O_2/CO_2; 95/5) for 10 min, and then 30 min of no-flow whole-organ ischemia was induced by stopping perfusion and keeping the liver fully immersed in the tissue bath during this period. Following the hypoxic period, organs were resuscitated with oxygenated KHB. Treatment was applied to divided groups, referred to as treatment before ischemia (BT-Isc) and treatment after ischemia (AT-Isc) and the group that did not received any treatment is considered as Control. Both treated groups received taurine (20 mmol) with KHB for 5 min. Effluent perfusate samples were collected for Mg^{2+} efflux measurement. After completion of perfusion, 1-2 gm liver samples were collected and aliquots are stored at -80°C for evaluation of LDH, SOD activity and protein expression by western blot in tissues.

Measurement of total Mg^{2+} efflux

Effluent perfusate was collected and used to measure total Mg^{2+}. The first set of samples was taken 10 min before ischemia. The 2nd set of samples was collected immediately after 5 min of reperfusion and the 3rd set was taken 60 min after reperfusion. After 10 min of washing (0~10 min), the effluent was sampled at l-min intervals. The first 10 minutes (10~20 minutes) of sampling provided a baseline condition for all livers, and then it was used to initiate ischemia by blocking perfusion. The 2nd set of samples was considered as early or immediate after reoxygenation (50~60 minutes)

and the 3rd set of samples was acquired during the last 10 min of reperfusion during the 80~90 minutes. All samples (~2 mL) were collected in borosilicate glass tubes and Mg^{2+} content was measured by atomic absorption spectrophotometer at a wavelength of 285.2 nm. (Analab 9200, Seoul, Korea).

LDH and SOD Assays procedure

LDH and SOD were measured in liver samples stored at -80°C by their respective colorimetric assay methods. Briefly, liver tissue was homogenized in 1 part of the tissue in 9 parts of saline and centrifuged at 4000 g in a 4°C centrifuge machine and the supernatant was collected in an EP tube and placed in an ice box until the assay was prepared. The LDH kit was used to measure LDH activity by a colorimetric method (Sigma Aldrich, St. Louis, MO) by measuring the level of pyruvic acid in the samples. Absorbance was recorded in a microplate spectrophotometer at the wavelength of 490 nm. SOD activity was measured in the same samples using the SOD Assay Kit-WST (Dojindo Molecular Technologies Inc. Kumamoto, Japan). The assay was prepared following the company's protocol and the absorbance was measured in a microplate spectrophotometer (SpectraMax-M2 with SoftMax program, Molecular Devices, USA) at the wavelength of 490 nm.

Western blot analysis for the ERK 1/2 (MAPK), Bcl and Bax

The aliquot of liver samples, 40 mg, was homogenized in 1 ml RIPA buffer by ultrasonication for five pulses at 40% duty cycle (Sonics & Materials, Ultrasonic Processor, USA) and microfuged for 30 min at 14000 rpm. Protein concentration was determined in the supernatants with a bicinchoninic acid assay kit (Pierce Biotechnology, Inc., Rockford, Illinois) according to the manufacturer's instructions. Supernatant samples were diluted with RIPA buffer so that all samples contained equal amounts of protein, and 15 µl of sample from each group was loaded onto a 7.5% SDS-PAGE gel for electrophoresis. After conducting electrophoresis, the protein was electrotransferred to a Hybond-ECL nitrocellulose membrane. The membrane was blocked with Tris-buffered saline (20 mM Tris and 140 mM NaCl, pH 7.6) containing 0.1% Tween20 (TBST) and 5% skimmed milk for 2 h at room temperature. The membrane was then incubated overnight with monoclonal phosphospecific p44/42 MAPK antibody (Cell Signaling Technology, Inc. Danvers, MA), which was the primary antibody, at 1:1000 dilution in TBST with 5% skimmed-milk. During this time temperature was maintained at 4°C. The blot was then washed for 5 min with three changes of TBST containing horseradish peroxide-conjugated anti-rabbit antibody according to the manufacturer's instructions, and bands were detected using ECL. The blots were quantified via laser scanning densitometry (Molecular Imager, ChemiDoc XRS+ System) with Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

Data analysis

Statistical analysis was performed using the one-way ANOVA method followed by Duncan's multiple-range test. Prism statistical software version 9 was used for data analysis. A p value <0.05 was taken as statistically significant. Data are presented as mean ± SEM.

Results

Effect of pre and post ischemia taurine treatment on Mg^{2+} efflux in ischemia-reperfused rat liver

Fig. 1 shows the changes in Mg^{2+} content in the effluent of control livers (Con) and livers following treatment with 20 mmol taurine for 5 min before or after induction of whole organ ischemia. A persistent elevation of Mg^{2+} flux was found in the non-treated ischemic group. Both pre- and post-treatment with taurine alleviated Mg^{2+} efflux in early and late samples. No statistically significant differences in effluent Mg^{2+}

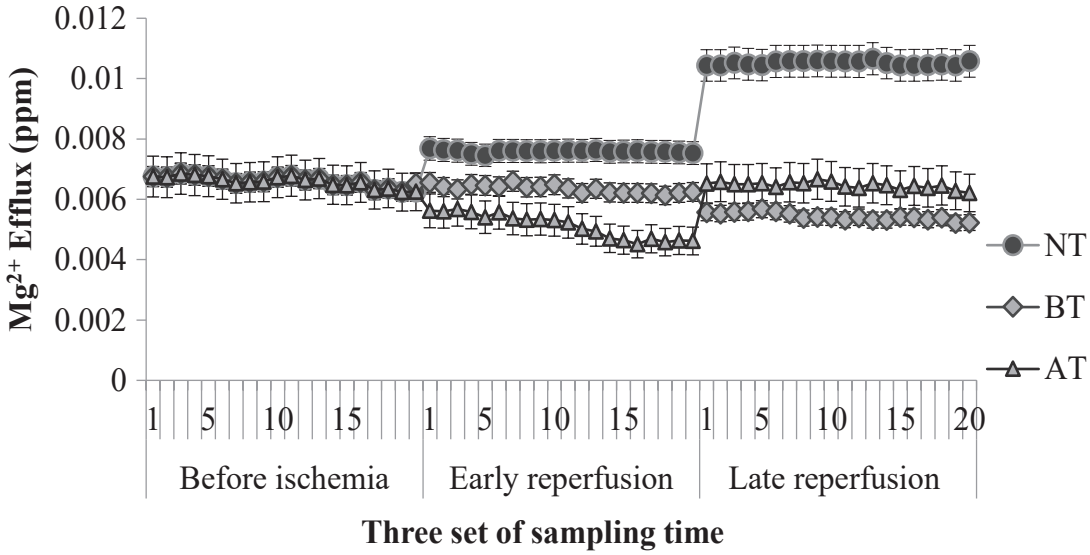

Fig. 1. Effect pre and post ischemia treatment of taurine on Mg^{2+} efflux from isolated rat liver in effluent perfusate, three phage effluent samples were collected during normal perfusion before ischemia, early sample-immediate after reperfusion and late sample-after 80 min of reperfusion. Taurine 20 mmol treatment in KHB was given for 5 min pre ischemia treatment (BT-isc) and post ischemia treatment (AT-isc) was given for 5 min. One group was without treatment and considered as ischemic control (NT-isc). Data plotted are Mean±SEM, $p<0.05$ considered statistically significant, representative of six independent experiments for each condition.

levels were observed between groups during the first 10 min (0 to 10 min) of sampling before ischemia induction. The results indicated that post-ischemic taurine treatment effectively reduced Mg^{2+} efflux more than the pretreatment group but was not sustained in the long term. This shows a slight elevation of Mg^{2+} flux in the late perfusate and a slight change in Mg^{2+} efflux over time. The treatment before ischemia completely inhibited early Mg^{2+} efflux from the liver and also found a little decrease in effluent Mg^{2+} in the late effluent samples. Data indicate that pre-ischemia treatment is more effective than post-ischemic treatment with taurine in maintaining Mg^{2+} retention in the liver under ischemic injury.

LDH Measurement

LDH activity was measured as an indicator of cell injury. LDH activity peaked in non-treated liver tissue and decreased significantly in both pre- and post-treatment groups in the early reperfution sample. A similar effect also observed in the late samples. LDH activity was statistically significant for both time points compared to normal tissue. Both pre- and post-treatment effects significantly reduced LDH activity in the ischemic liver compared to the untreated liver. A slight difference in LDH activity between pre- and post-treatment groups was observed in early and late reperfused samples but was statistically insignificant (Fig. 2). When livers were treated with taurine before and after ischemia, tolerance to reoxygenation injury was greater than in untreated livers.

SOD Measurement

Three phase measurements of SOD activity in 3 different groups show the SOD activity was significantly higher in the untreated group immediately after and at the end of reperfusion. Whereas taurine treatment reduced SOD activity in liver tissue both before and after ischemia compared to tissue during normal perfusion (n=5, $p<0.01$).Pretreatment showed almost similar SOD levels during prolonged perfusion but post-treatment resulted in more SOD activity in liver tissue (Fig. 3).

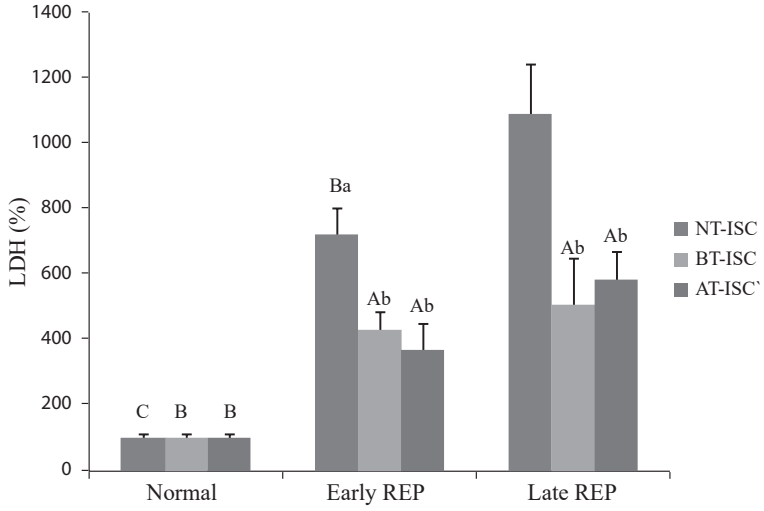

Fig. 2. LDH activity rate was measured as an index of injury and lysis of hepatocyte. LDH was measured in the liver tissue collected from Pre-ischemia period (Normal) liver, early reperfused liver (immediate of reperfusion) and late reperfused liver (at the end of reperfusion). Data are presented as Mean±SEM (five independent experiments for each). $p<0.05$ is considered statistically significant, a small letter represents the difference at the same time between groups, large letter represents the difference of the same group between times, which means the same letters for specific condition are not significantly different. BT-isc- pre ischemia treatment; AT-isc- post ischemia treatment; NT-isc- Un-treated control.

Effects of pre and post ischemia treatment of taurine on MAPK and Bcl-2 family proteins

To determine whether ERK pathways are involved in ischemic injury and their phenomena in response to the pre and post ischemia taurine treatment on an isolated perfused rat liver was used. We examined the activation of ERKs by monitoring the changes in their total and phosphorylation (activated) forms ok ERKs at the end of reperfusion (Fig. 4). Data shows the protein expression levels of tERK1/2, pERK1/2, BCL and Bax. All those protein were measured by Western blot in samples collected from different group in different time points. Immunoblot and densitometry analysis showed that ischemia reoxygenation injury significantly increased tERK and pERK in untreated liver but taurine treatment significantly inhibited the activation of tERK before and after ischemia due to reoxygenation injury. The expression of both Bcl and Bax was significantly up-regulated by reoxygenation injury in non-treated livers, which was significantly down-regulated in pre- and post-ischemia taurine treated livers.

Discussion

Taurine is an amino acid with many biological functions, including membrane stabilization, mediation of homeostasis, and protection against oxidative stress (Baliou *et al*., 2021). Cell apoptosis is thought to be responsible for liver damage during hepatic IRI (Cannistra *et al*., 2016). Data from the present investigation show that taurine appears to be a potent protective compound of Mg^{2+} flush in rat liver under ischemia-reoxygenation injury (Fig. 1). This may be due to membrane stabilizing effects or positive effects on cell viability during IRI because taurine plays an important role in restoring mineral and trace element homeostasis (Jafri *et al*., 2017) and protect organs from IRI. Evidence from various cell, tissue, and organ experiments suggests that disruption of ion and molecule homeostasis is a major factor in determining tissue death. Since only 6% of cytosolic Mg^{2+} is free, most of it exists in complexes with proteins and ATP (Corkey *et al*., 1986), although it is present in the mM range. Its role in cell death is not well understood but after cell death the integrity of the cytosolic complex does not exist so Mg^{2+} can be free and efflux. For

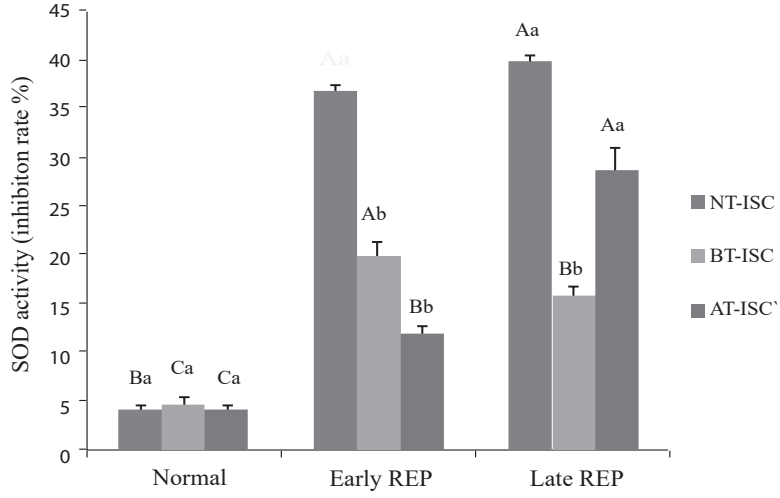

Fig. 3. SOD activity or inhibition rate: SOD activity was measured as an index of antioxidative activity. SOD activity was measured in the perfusate during Pre-ischemia (Normal), and during Early (beginning) and Late (end) of reperfusion. 20 min continuous effluent perfusate was collected in each phase. Liver ischemia for 30 min was induced by stopping the perfusion flow. 20 mmol taurine pre and post treatment was given in KHB for 5 min. Data are presented as Mean±SEM (five independent experiments for each). $p<0.05$ is considered statistically significant, a small letter represents the difference at the same time between groups, large letter represents the difference of the same group between times, which means the same letters for specific condition are not significantly different. BT-isc- pre ischemia treatment; AT-isc- post ischemia treatment; NT-isc- Un-treated control.

example, ATP is hydrolyzed in acutely stressed cells and Mg^{2+} is released due to its low affinity for ADP and AMP (Harman *et al*., 1990). An investigation by Maulik *et al*., (2001) showed that apoptosis induced by ligation of FAS, the resting level of free Mg^{2+} increased prior to DNA fragmentation and phosphatidylserine externalization. The molecular mechanism for Mg^{2+}-dependent modulation of apoptosis was shown by Eskes *et al*., (1998) where Bax-induced cytochrome C was found to be potentiated in mitochondria by mM concentrations of this divalent ion. Taurine improves graft function in transplanted kidneys and protects against apoptosis and necrosis, possibly through an antioxidation mechanism (Guan *et al*., 2008). Another study showed that taurine treatment prior to intestinal IRI elicited significantly lower apoptotic indices in jejunum and ileum that had a higher Bcl-2/Bax ratio than IR animals (Sukhotnik *et al*., 2016). The protective effect of taurine against hepatic IRI after liver transplantation is the down-regulation of IRAK-4 and downstream nuclear factor **κ**B and TNF-**α** expression in Kupffer cells (Sun *et al*., 2012). The protective effect of Mg^{2+} on IRI in liver transplant patients was studied by Chung *et al*., (2013), which showed that Mg^{2+} pretreatment alleviated post-reperfusion syndrome and Th2 cell activity, potentiating the Th1-to-Th2 cytokine balance in favor of Th2. Another study showed that improvement of liver function by ATP-$MgCl_2$ infusion after ischemia may be due to improvement of energy metabolism during hepatic IRI (Jeong and Lee, 2000).

Measurement of the glycolytic enzyme LDH in tissue homogenates during IRI gave an elevated value in this study suggesting further clinical significance. Its high levels can be a sign of tissue damage. This effect is attenuated by both pre- and post-ischemic treatment with taurine (Fig. 2). The results indicate that taurine likely protects tissue from acute death from IRI in the present study. Previous study shows that taurine improves graft survival and reduces injury to liver by reducing LDH activity (Schemmer *et al*., 2005). Taurine-induced inhibition of LDH leakage in contrast to the positive effect on cell viability in perfused rat livers has already been described by others (Brass *et al*., 1993) may be due to taurine-induced antioxidant activity. Serum LDH is reported to be markedly elevated in ischemic hepatitis

(Cassidy and Reynolds, 1994). SOD constitutes a very important antioxidant defense against oxidative stress. The present study showed that taurine effectively alleviated oxidative stress before and after ischemia as determined by SOD activity in I/R liver tissue (Fig. 3). Combining these findings, several previous comparative studies have demonstrated the beneficial effects of taurine in this regard. Superoxide radicals are produced in the cytoplasm and play a definitive role in tissue injury after reperfusion of the liver and at the same time, antioxidant enzymes expressions are observed so that oxygen radicals are scavenged to attenuate the I/R-induced liver injury (Chen *et al*., 2007; Guo *et al*., 2011). Studies on testicular ischemia-reperfusion injury show that taurine reduces ROS generation and neutrophil recruitment to the testis. (Wei *et al*., 2007). Utilization of taurine in protecting livers against endotoxin-induced injury especially after HIT, by its anti-inflammatory, anti-oxidative and anti-apoptotic activities (Zhang *et al*., 2010). Taurine

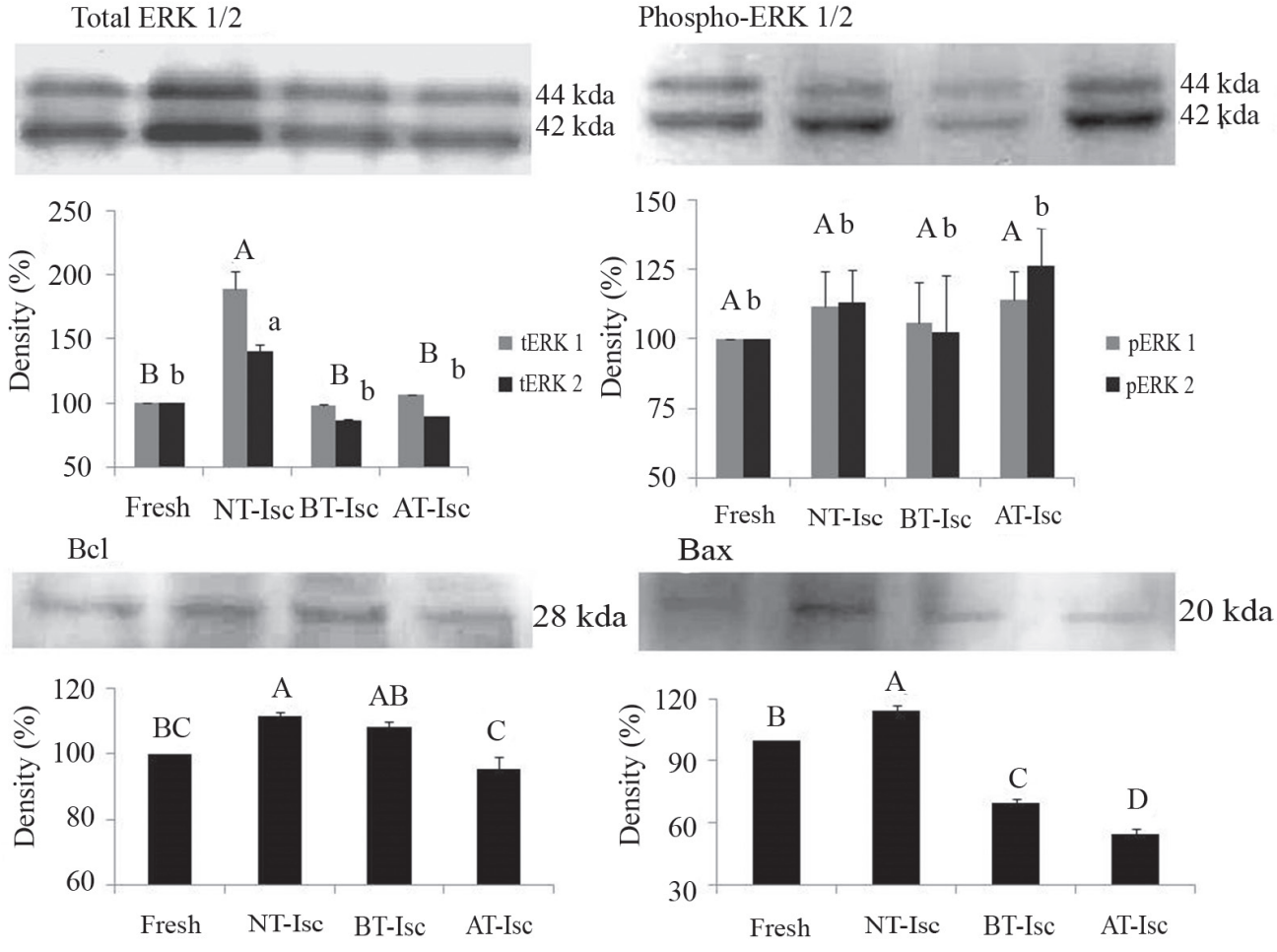

Fig. 4. Effects of pre and post taurine treatment in ischemia-reoxygenation injury in isolated perfused rat liver. Rat livers were perfused with KHB equilibrated with O_2/CO_2 (95/5 by volume). During the 30 min no flow ischemic period the liver was sunk under the perfusate is tissue bathe at 37 ^{0}C and was equilibrated with N2/CO2 (95/5 by volume), and then oxygen was reinstituted again. Data are presented as Mean±SEM (five independent experiments for each). $p<0.05$ is considered statistically significant, a small letter represents the difference at the same time between groups, large letter represents the difference of the same group between times, which means the same letters for specific condition are not significantly different. BT-isc- pre ischemia treatment; AT-isc- post ischemia treatment; NT-isc- Un-treated control. Fresh - fresh tissue.

alleviates lipopolysaccharide-induced liver injury by anti-inflammation and antioxidants in rats (Surai *et al*., 2021). In the present study, ischemic liver reperfusion induced higher SOD activity to scavenge oxygen radicals in the untreated group whereas taurine itself has antioxidant activity so lower SOD activity was observed in the treated group which could further reduce I/R-induced liver injury in the treated group. SOD activity was decreased both in before and after treatment with taurine compared to control but slightly increased during late reperfusion samples collected from post-treated groups compared to pre-treated livers, which could not be explained. However, taurine pre-treatment is thought to increase cellular osmolarity and integrity before ischemia induction, which was lacking in post-treatment livers.

ERKs are important components of the intracellular network of proteins involved in intracellular reactions. The results of the present study showed increased activation of ERK, which was inhibited by taurine treatment before and after IRI (Fig. 4). This result is supported by a study by Yang *et al*., (2004) who reported that from 5 min before reperfusion, infusion of PD98059, an inhibitor of ERK activation, abolished postconditioning-mediated protection and addition of anisomycin, a JNK/p38MAPK activator, alleviated inhibition of apoptosis by postconditioning and Mg^{2+} deficiency promotes PI3K/MAPK. Taurine protects against cerebral ischemia and apoptotic cell death pathways mediated by m-calpain and caspase-3 by inhibiting caspase-3 expression (Sun and Xu, 2008). Bcl abd Bax are both Bcl-2 family proteins where Bcl acts as an anti- or pro-apoptotic regulator and is involved in a variety of cellular activities. Bax is also pro-apoptotic and is believed to interact with and induce mitochondrial voltage-dependent anion channels. Alternatively, growing evidence suggest that activated Bax and/or Bak results in the release of cytochrome c and other pro-apoptotic factors from the mitochondria, leading to the activation of caspases (Cursio *et al*., 1999).

Conclusion

In summary, the first few minutes before or after taurine administration in perfusion buffer may protect the liver from injury due to IRI. It prevents Mg^{2+} flush from tissues and thus protects against pro-necrotic, as well as pro-apoptotic threats. Thus, Taurine improves the protection of isolated liver against oxidative stress by its antioxidant properties. The present study indicates that taurine supplementation for both donors and recipients may have beneficial effects in protecting the transplanted liver from ischemic injury. Although taurine confers protection against IRI further investigation needs to explore research gaps involving unexplained cellular mechanisms.

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