International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2019.872.879

Research Article

Protective Effect of Probiotic Potentiate with Thiopental in Intestinal Ischemia-Reperfusion Induced Lung Injury

Wenxia Jia, Zhijia Guo, Xiang Yu, Jieping Lv, Baozhong Yang and Shouyuan Tian

Department of Anesthesiology, First Hospital of Shanxi Medical University, 030001 Taiyuan, Shanxi, China

Abstract

Background and Objective: Intestinal ischemia/reperfusion leads to development of multiple organ dysfunctions including lung. The aim of present investigation was to determine the effect of thiopental alone and in combination with probiotic on I/R induced lung injured rats. **Materials and Methods:** Animals were treated with probiotics (1×10^9 CFU) and thiopental (20 mg kg^{-1}, i.p.) alone and in combination. Probiotics was administered with 3 days prior to the surgery and thiopental was administered 15 min after the induction of reperfusion. The ELISA method was used to determine the level of mediators of inflammation and oxidative stress in the lung tissue. Moreover Western blot, RT-PCR assay were performed by the estimation of expression of several proteins in the lung tissue homogenates. **Results:** Data of the investigation revealed that thiopental alone and in combination with probiotic reduced the inflammatory cytokines oxidative stress in the lung tissues compared to I/R group. Moreover expression of TLR-4, iNOS and NF-κB proteins were reduced in the lung tissue of thiopental alone and in combination with probiotic treated group compared to I/R group of rats. Treatment with thiopental alone and in combination with probiotic attenuated the altered histopathology of intestinal I/R induced lung injured rats. **Conclusion:** In conclusion, data of investigation suggested that thiopental ameliorated the lung injury by regulating iNOS/TLR-4/NF-κB pathway in intestinal I/R induced lung injured rats model. Moreover study also revealed that thiopental showed synergistic effect against intestinal I/R induced lung injury when treated with probiotics.

Key words: Ischemia/reperfusion, lung tissue, thiopental, probiotic, inflammatory cytokines, lung injury

Citation: Wenxia Jia, Zhijia Guo, Xiang Yu, Jieping Lv, Baozhong Yang and Shouyuan Tian, 2019. Protective effect of probiotic potentiate with thiopental in intestinal ischemia-reperfusion induced lung injury. Int. J. Pharmacol., 15: 872-879.

Corresponding Author: Shouyuan Tian, Department of Anesthesiology, First Hospital of Shanxi Medical University, 030001 Taiyuan, Shanxi, China
Tel/Fax: 0086-0351-4639114

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Patients going through many surgeries such as liver transplantation, small bowel transplantation and cardiac surgery suffer from a challenging complication intestinal ischemia/reperfusion[1]. There are many pathogenic pathways like inflammatory mediator's activation, immune cell and oxidative stress due to Intestinal I/R[2]. This causes injury to many organs including lung injury, which is having high mortality rate. It is well documented that production of reactive oxygen species (ROS) was induced by hemorrhagic resuscitation and shock which leads to lung injury[3]. Mast cells widely available in lung which regulated its function and degranulation of mast cells were observed by ROS causes the development of inflammatory diseases[4]. Literature revealed that degranulation of mast cell contributed in the development of lung injury[5]. Conventional drug used for the management of lung injury has several limitations. Thus, there is a need to develop a new drug and therapy for the treatment of lung injury. The I/R of the gut cause inflammatory response by enhancing the release of bacteria-derived endotoxins and pro inflammatory cytokines which leads to organ dysfunction[6]. Probiotic administration reported to has protective role for the management of lung injury[7]. Moreover thiopental which is used as anesthetic belongs to barbiturate category. Thiopental reported to inhibit the lipid peroxidation by which it demonstrated the strong antioxidant property[8]. Literature revealed that function of neutrophil was attenuated by reducing the production of ROS[9]. Thiopental also reported to posse's protective effect against lung edema induced by alpha-naphthyl thiourea and renoprotective effect against ischemia reperfusion induced renal injury[10]. Thiopental has shown protective effect against ischemia/reperfusion induced injury and probiotic administration attenuated the inflammation and oxidative stress against I/R of the gut. Thus the present investigation determined the protective effect of thiopental alone and in combination with probiotic in intestinal ischemia/reperfusion induced lung injury rat model.

MATERIALS AND METHODS

Animals: The SD rats of either sex with 180-250 g weight were kept under the 12 h of light and dark cycle in the standard condition such as 60±5% of humidity, 24±3°C of temperature. Present work was conducted in the lab of First hospital of Shanxi Medical University, China from March, 2017-October, 2017. All the protocols of the study were approved by The Institutional Animal Care and Use Committee of First hospital of Shanxi Medical University, China (IACUC/FH-SMU/2017/06).

Experiment: All the animals were separated into 5 groups such as; Sham group, I/R group, I/R+probiotic which received probiotics (1×10^9 CFU) for 3 days prior to the surgery, I/R+thiopental which received thiopental 20 mg kg^{-1}, i.p., 15 min after the induction of reperfusion, I/R+probiotic+ thiopental receives pretreatment of probiotic (1×10^9 CFU) 3 days before the induction of ischemia and reperfusion and thiopental (20 mg kg^{-1}, i.p.) 15 min after the induction of reperfusion.

Body temperature was maintained at 36°C for all the rats after anesthetizing them with 50 mg kg^{-1} of thiopental (i.p.). Rats were kept on supine position at operation table and after cleaning with antiseptic abdomen were shaved. Midline laparotomy (1 c.m.) was done to open the abdomen and ischemia was induced for 1 h by applying a traumatic micro bulldog clamp on the superior mesenteric artery (SMA). Further reperfusion of was applied onto the SMA by releasing the clamp after 1 h. Reperfusion was done for 2 h till the pink color of tissue re established. Survival rate was observed after the reperfusion.

Preparation of tissue sample: Rats were sacrificed at the end of protocol by cervical dislocation method and lungs were exposed by performing sternotomy. Lungs lobes were isolated from the rats for determining the biochemical parameters and examination of histopathology of lung tissue was estimated in the isolated lung tissues.

Determination of inflammatory cytokines: Inflammatory cytokines such as MCP-1, MIP-2, TNF-α, NF-κB, IL-6 and IL-18 concentration in the lung tissue of all the animals was estimated by using ELISA kit as per the directions given by the manufacturer of kit.

Determination of oxidative stress: Level of MDA and reduced glutathione (GSH) and activity of xanthine oxidase (XO) were estimated in the lung tissues as the instruction given by the kits and level of iNOS was determined by using ELISA kit in the lung tissues.

Western blot assay: Extraction of total protein from the isolated lung tissue was done after treating it with NP40

protein lysis buffer and DC protein assay was performed for the estimation of concentration of total protein. Sodium dodecyl sulfate (SDS)-polyacrylamide gel (10%) was used for the separation of isolated protein and further polyvinylidene difluoride membrane used for the filtration of it and 5% fresh non-fat dry milk was treated with membrane to block the reaction. Membrane was incubated at 4°C for the duration of overnight with primary anti bodies such as TLR-4, IκB-α, NF-κB, iNOS and β-actin and thereafter incubate it with secondary antibodies for 60 min at room temperature. Chemiluminescence was used to enhance the blot and ImageLab software was used to perform the densitometric analysis of blots.

RT-PCR: Separated lung tissue was used to isolate the RNA by Trizol Reagent. RevertAid First Strand cDNA Synthesis Kit was used to reversely transcribe the RNA. Primers mention below was mixed with RT 2 SYBR Green Master to determine the gene expression by Quantitative SYBR Green PCR assays. The program used in all samples was 98°C for 2 min and then 25-40 cycles of 98°C for 10 sec, 55°C for 5 sec and 72°C for 20 sec. The mRNA expression levels were calculated according to relative standard curves. The curves were generated by plotting the quantification cycle (Cq) against the log amount of total cDNA added to the reaction. The relative target gene expression levels were determined using the $2^{-\Delta\Delta Cq}$ method (Table 1).

Histopathology: Isolated lung tissues were stained by using hematoxylin and eosin (HE) staining as per previously reported method[11]. Lung tissue was seeded in the molten liquid paraffin and microtome was used to section the tissue of 4 μm thickness. Hematoxylin was used to stain the lung tissue for the period of 1 min at 60°C and wash it under running water. Further 1% hydrochloric acid alcohol was poured on the tissue and incubated for the period of 2 sec. thereafter lung tissue were stained with eosin (0.5%) for 1 min.

Injury score was determined by estimating the level of lung injury from the scale 0-3. Several histopathological observations such as cellular hyperplasia, intra-alveolar hemorrhage/debris, infiltration of neutrophil and hyperemia/congestion were determined. 0: Absent of exudates, 1: Slight exudates, 2: Alveolar walls thickening and moderate edematous, 3: Extensive occurrence of alveolar exudates.

Statistical analysis: All data were expressed as Mean±SEM (n = 10). The statistical analysis was performed using one way ANOVA. *Post-hoc* comparison of means was carried out by Dunnett's *post hoc* test (Gradpad prism 6.1., CA, USA). The level of statistical significance was set at $p<0.05$.

RESULTS

Effect of thiopental and probiotic on mediators of inflammation: Effect of thiopental and probiotic on the inflammatory mediators was shown in Fig. 1. Mediators of inflammation such as MIP-2, NF-κB, IL-6, IL-1β, TNF-α and MCP-1 were enhanced in the tissue homogenate of lung in I/R group than sham group of rats. Thiopental alone and in combination with probiotic treatment reduces the level of these mediators of inflammation than I/R group. Moreover Thiopental treated with probiotic reduces the level of inflammatory cytokines in lung tissue compared to I/R+probiotic and I/R+thiopental treated groups.

Effect of thiopental and probiotic on the parameters of oxidative stress: The data in Table 2 shows the effect of thiopental and probiotic on the markers of oxidative stress in I/R induced lung injured rats. Level of GSH was reduced and MDA level was enhanced in the lung tissue of I/R group than sham group. Moreover activity of iNOS and XO was enhanced in the lung tissue of I/R group than sham group. It was observed that treatment with thiopental alone and in combination with probiotic attenuates the altered level of parameters of oxidative stress in the lung tissue of I/R induced lung injured rats.

Table 1: Primer sequence

Protein	Forward	Reverse
TLR7	5′-GGTGGCAAAATTGGAAGATCC-3′	5′-AGCTGTATGCTCTGGGAAAGGTT-3′
iNOS	5′-GTTCTCAAGGCACAGGTCTC-3′	5′-GCAGGTCACTTATGTCACTTATC-3′
GAPDH	5′-ACCACCATGGAGAAGGCTGG-3′	5′-CTCAGTGTAGCCCAGGATGC-3′

NOS: Nitric oxide synthase, TLR7: Toll-like receptor 7, GAPDH: Glyceraldehyde phosphate dehydrogenase

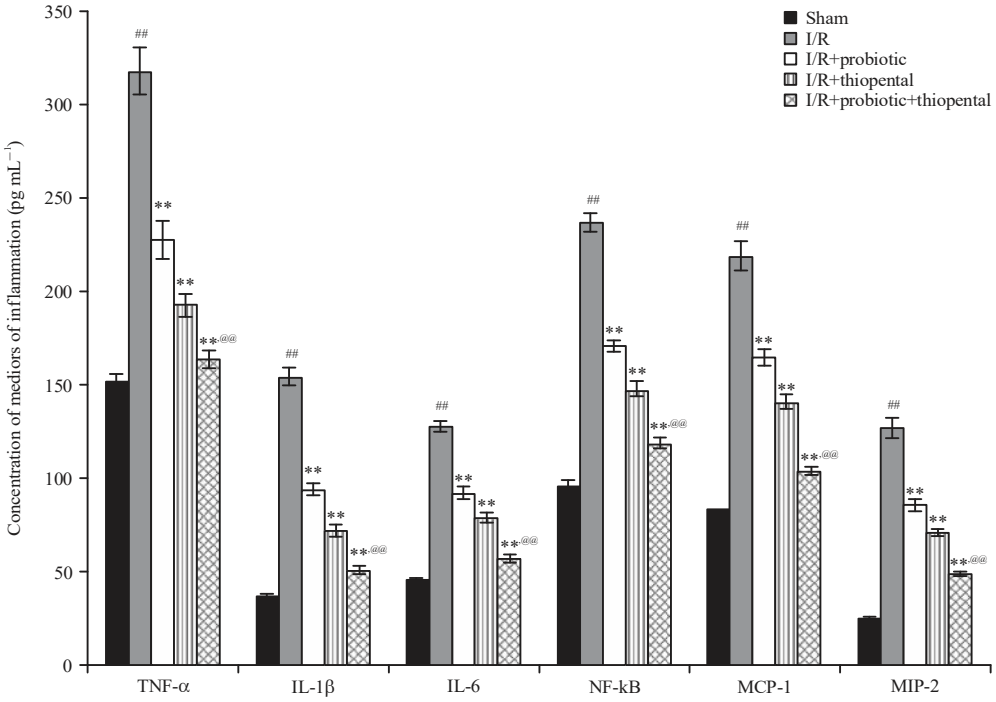

Fig. 1: Effect of thiopental alone and in combination with probiotic on the inflammation mediators in I/R induced lung injured tissue

Mean±SEM (n = 10), ##p<0.01 compared to sham group, **p<0.01 compared to I/R group, @@p<0.01 compared to I/R+probiotic and I/R+thiopental group, TNF-α: Tumor necrosis factor alpha, IL-1β: Interleukin 1 beta, IL-6: Interleukin 6, NF-κB: Nuclear factor Kappa b cell, MCP-1: Monocyte chemoattractant protein 1, MIP-2: Macrophage inhibitor protein 2

Effect of thiopental and probiotic on the expression of TLR-4, I κB-α, NF-κB and iNOS protein: Effect of thiopental and probiotic on the expression of TLR-4, I κB-α, NF-κB and iNOS protein in the I/R induced lung injured rats (Fig. 2). There was increase in the expression of TLR-4, NF-κB and iNOS and decrease in IκB-α protein in the I/R group than sham group. Expression of TLR-4, NF-κB and iNOS were significantly ($p<0.01$) reduced and the expression of IkB-α was enhanced in the lung tissues of thiopental alone and in combination with probiotic treated group than I/R group.

Effect of thiopental and probiotic on the mRNA expression of TLR-4 and iNOS protein: Effect of thiopental and probiotic on the mRNA expression of TLR-4 and iNOS in the I/R induced lung injured rats (Fig. 3). mRNA expression of TL-4 and iNOS proteins were enhanced in the lung tissue of I/R group than sham group of rats. It was observed that thiopental alone and in combination with probiotic treated group mRNA expression of TLR-4 and iNOS reduced compared to I/R group.

Effect of thiopental and probiotic on the histopathology of lung: The results in Fig. 4 Showed the effect of thiopental and probiotic on the histopathology of lung tissue of I/R induced lung injured rats. Sham operated group shows the normal structure of lung tissues. Whereas histopathology of lung tissues of I/R group shows lung injury associated with infiltration of neutrophil, haemorrhage to mesenchyme and alveoli, edema and alveolar collapse. However treatment with thiopental alone and in combination with probiotic reverses the lung injury (Fig. 4a). Lug injury score was found to be higher in the lung tissue of I/R group than sham group of rats. However treatment with thiopental alone and in combination with probiotic ameliorates the lung injury in the intestinal I/R induced lung injured rats (Fig. 4b).

DISCUSSION

Intestinal ischemia/reperfusion leads to development of multiple organ dysfunctions including lung[12]. In present investigation the level of mediators of inflammation and oxidative stress were estimated in the lung tissue by ELISA test. Moreover Western blot, RT-PCR assay were performed by the estimation of expression of several proteins in the lung tissue homogenates. Moreover histopathology study was done for determining the lung injury score.

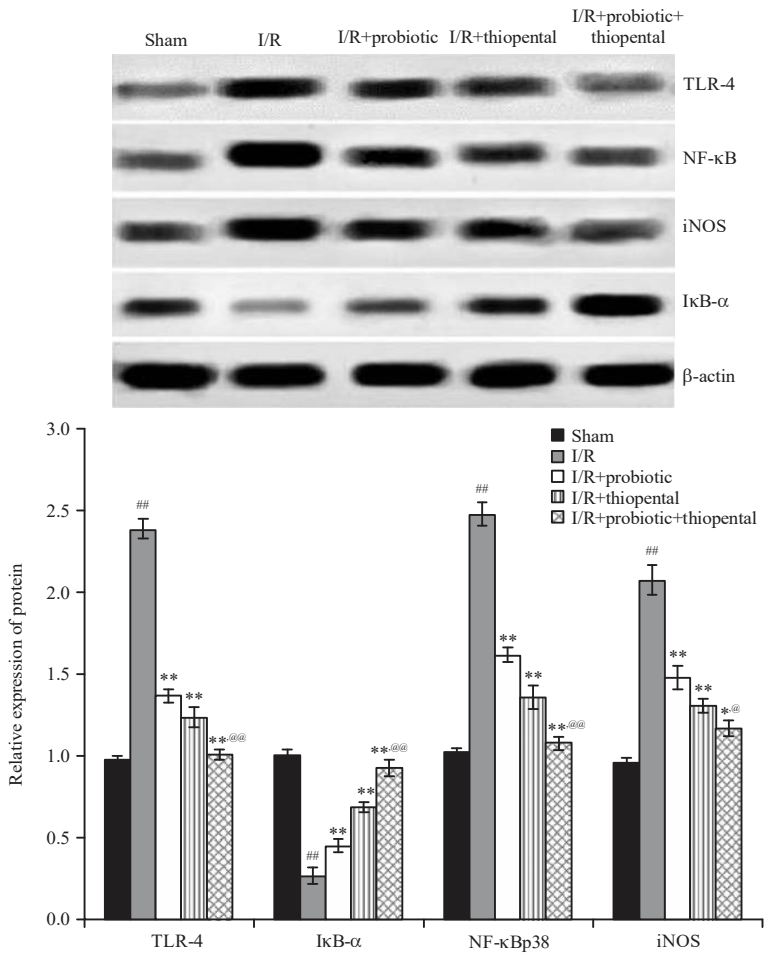

Fig. 2: Effect of thiopental alone and in combination on the protein expression of TLR-4, IκB-α, NF-κB and iNOS protein in I/R induced lung injured tissue

Mean±SEM (n = 10), ##p<0.01 compared to sham group, **p<0.01 compared to I/R group, @@p<0.01 compared to I/R+probiotic and I/R+thiopental group, TLR-4: Toll-like receptor 4, IκB: Inhibitor κB protein, NF-κB: Nuclear factor Kappa B cell, iNOS: Inducible nitric oxide synthase

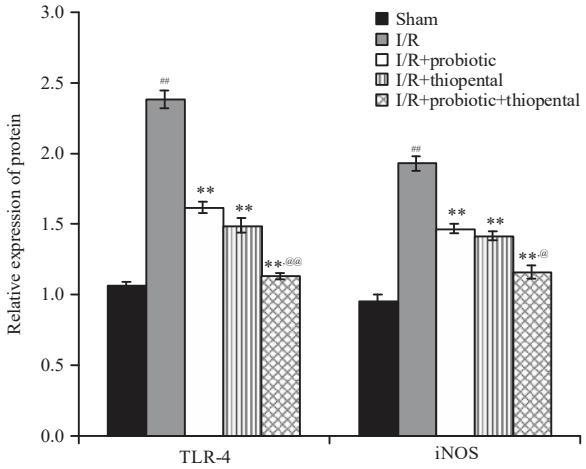

Fig. 3: Effect of thiopental alone and in combination with probiotic on mRNA expression of TLR-4 and iNOS protein in I/R induced lung injured tissue

Mean±SEM (n = 10), ##p<0.01 compared to sham group, **p<0.01 compared to I/R group, @@p<0.01 compared to I/R+probiotic and I/R+thiopental group, TLR-4: Toll-like receptor 4, iNOS: Inducible nitric oxide synthase

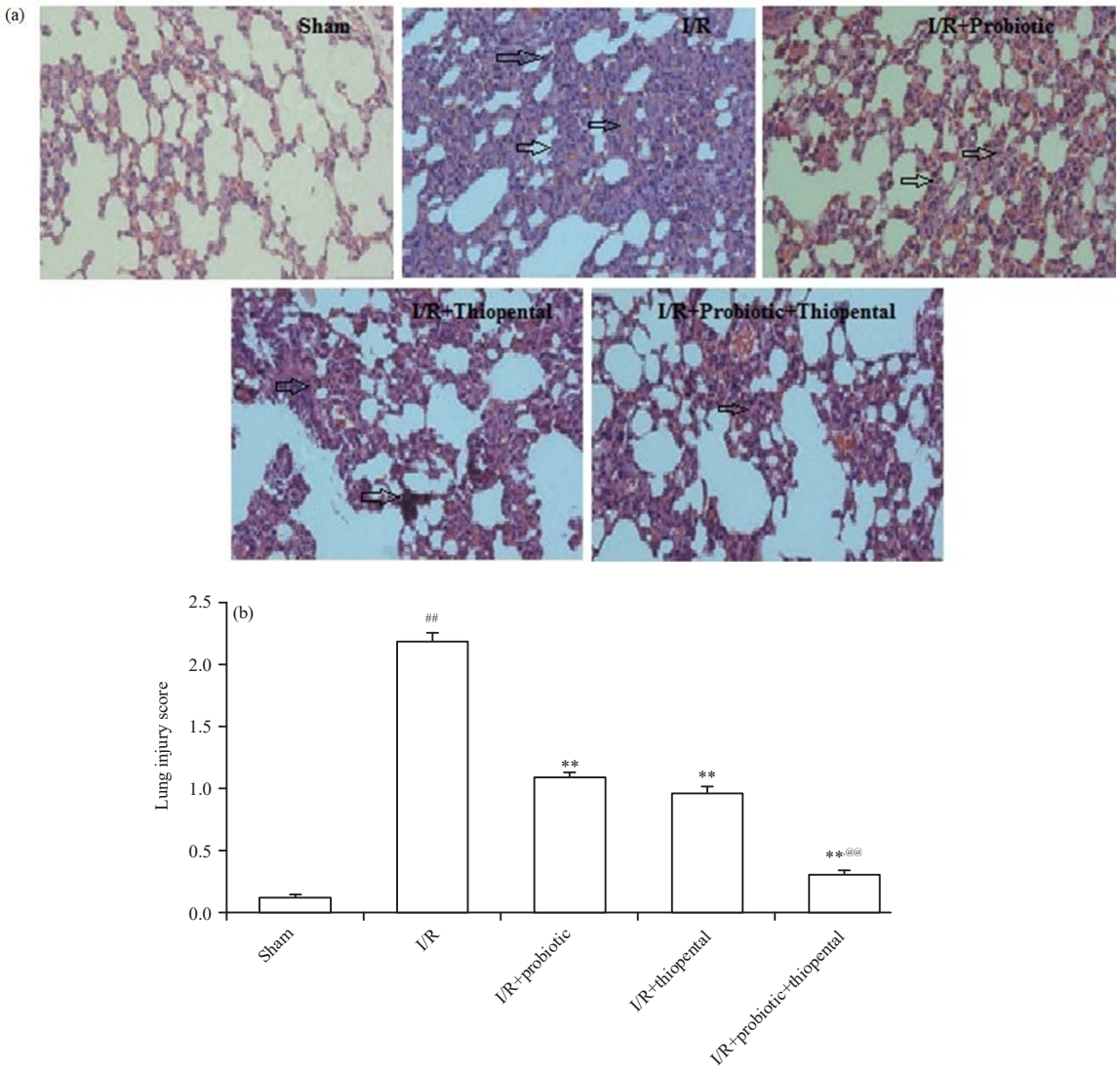

Fig. 4(a-b): Effect of thiopental alone and in combination with probiotic on pathophysiology of lung tissue in I/R induced lung injured tissue, (a) Histopathology of lung tissue and (b) Lung injury score

Arrows are showing the lung injury (hemorrhage and edema) in the tissue section of lungs of I/R induced lung injured rats, Mean±SEM (n = 10), ##p<0.01 compared to sham group, **p<0.01 compared to I/R group, @@p<0.01 compared to I/R+probiotic and I/R+thiopental group

Table 2: Effect of thiopental alone and in combination on the markers of oxidative stress in I/R induced lung injured rats

Groups	MDA (nmol mg^{-1} protein)	GSH (nmol mg^{-1} protein)	XO (mU mg^{-1} protein)	iNOS (U mg^{-1} protein)
Sham	1.16±0.02	9.62±0.26	1.26±0.05	1.28±0.07
I/R	7.41±0.06##	3.74±0.09##	2.13±0.07##	2.24±0.16##
I/R+probiotic	4.82±0.03**	6.49±0.14**	1.82±0.09**	1.86±0.09**
I/R+thiopental	3.47±0.04**	5.13±0.12**	1.68±0.04**	1.61±0.07**
I/R+probiotic+thiopental	1.79±0.02**'@@	8.16±0.18**'@@	1.43±0.02**'@@	1.42±0.04**'@@

Mean±SEM (n = 10), ##p<0.01 compared to sham group, **p<0.01 compared to I/R group, @@p<0.01 compared to I/R+probiotic and I/R+thiopental group, MDA: Melanodialdehyde, GSH: Glutathione, XO: Xanthine oxidase, iNOS: Inducible nitric oxide synthase

Reported data revealed that in intestinal I/R several factors such as enhanced level of mediators of inflammation and oxidative stress induced the lung injury[13]. Probiotic administration reported to reduces the oxidative stress and inflammation[14]. Moreover thiopental also reduces the production of ROS and inflammatory cytokines[15]. Literature revealed that reduction in the inflammatory cytokines and oxidative stress showed the protective effect against

intestinal ischemia/reperfusion induced lung injury[16]. Present investigation revealed that thiopental attenuated the altered level of oxidative stress and cytokines in the lung tissues and thiopental treated with probiotic showed the synergistic effect against oxidative stress and inflammation. Data of the study revealed that the expression of TLR-4, NF-κB and iNOS also been reduced in the thiopental treated group compared to I/R group. Literature suggested that iNOS reduced the recruitment of neutophils in the lung tissue and thereby reduced the production of inflammatory cytokines and causes lung injury[17]. iNOS inhibitor has reported for their protective role against lung injury[18]. TLR-4 expression was reported to be enhanced in the intestinal epithelial lining and lung tissues in intestinal I/R induced injury[19]. Moreover inhibition of expression of TLR-4 attenuated the lung injury in intestinal I/R induced injured rats [20]. Moreover thiopental administered with probiotic showed protective role against intestinal I/R induced lung injury rats.

CONCLUSION

In conclusion, data of investigation concluded that thiopental ameliorated the lung injury by regulating iNOS/TLR-4/NF-κB pathway in intestinal I/R induced lung injured rats model. Moreover study also revealed that thiopental showed synergistic effect against intestinal I/R induced lung injury when treated with probiotics.

SIGNIFICANCE STATEMENT

This study discovered the protective effect of thiopental and probiotic administration on the intestinal ischemia/reperfusion induced lung injury rats that can be beneficial for the management of lung injury. This study will help the researchers to uncover the thiopental alone and in combination with probiotic for the treatment of intestinal ischemia/reperfusion induced lung injury by regulating iNOS/TLR-4/NF-κB pathway that many researchers were not able to explore.

ACKNOWLEDGMENT

All the author of this manuscript are thankful to Innovation fund of the first Hospital of Shanxi Medical University (YC1431), China for providing the necessary facility to conduct the presented work.

REFERENCES

1. Bharadwaj, S., P. Tandon, T.D. Gohel, J. Brown and E. Steiger *et al*., 2017. Current status of intestinal and multivisceral transplantation. Gastroenterol. Rep., 5: 20-28.
2. Bhattacharyya, A., R. Chattopadhyay, S. Mitra and S.E. Crowe, 2014. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol. Rev., 94: 329-354.
3. Kao, R.L.C., X. Xu, A. Xenocostas, N. Parry, T. Mele, C.M. Martin and T. Rui, 2014. Induction of acute lung inflammation in mice with hemorrhagic shock and resuscitation: Role of HMGB1. J. Inflamm., Vol. 11, No. 1. 10.1186/s12950-014-0030-7.
4. Beghdadi, W., L.C. Madjene, M. Benhamou, N. Charles, G. Gautier, P. Launay and U. Blank, 2011. Mast cells as cellular sensors in inflammation and immunity. Front. Immunol., Vol. 2. 10.3389/fimmu.2011.00037.
5. Cruse, G. and P. Bradding, 2016. Mast cells in airway diseases and interstitial lung disease. Eur. J. Pharmacol., 778: 125-138.
6. Chen, L., H. Deng, H. Cui, J. Fang and Z. Zuo *et al*., 2017. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 9: 7204-7218.
7. Mortaz, E., I.M. Adcock, G. Folkerts, P.J. Barnes, A. Paul Vos and J. Garssen, 2013. Probiotics in the management of lung diseases. Mediat. Inflamm., Vol. 2013. 10.1155/2013/751068.
8. Murphy, P.G., J.R. Bennett, D.S. Myers, M.J. Davies and J.G. Jones, 1993. The effect of propofol anaesthesia on free radical-induced lipid peroxidation in rat liver microsomes. Eur. J. Anaesthesiol., 10: 261-266.
9. Wu, S.Y., J.H. Huang, W.Y. Chen, Y.C. Chan and C.H. Lin *et al*., 2017. Cell intrinsic galectin-3 attenuates neutrophil ROS-dependent killing of Candida by modulating CR3 downstream Syk activation. Front. Immunol., Vol. 8. 10.3389/fimmu.2017.00048.
10. Sipahi, E., H. Ustun and F.N. Ayoglu, 2002. Acute effects of pentobarbital, thiopental and urethane on lung oedema induced by alpha-naphthythiourea (ANTU). Pharmacol. Res., 45: 235-239.
11. Martina, J.D., C. Simmons and D.M. Jukic, 2011. High-definition hematoxylin and eosin staining in a transition to digital pathology. J. Pathol. Inform., Vol. 2. 10.4103/2153-3539.86284.
12. Pierro, A. and S. Eaton, 2004. Intestinal ischemia reperfusion injury and multisystem organ failure. Semin. Pediatric Surg., 13: 11-17.
13. Sun, Q., Y. Wu, F. Zhao and J. Wang, 2017. Maresin 1 ameliorates lung ischemia/reperfusion injury by suppressing oxidative stress via activation of the Nrf-2-mediated HO-1 signaling pathway. Oxidat. Med. Cell. Longevity, Vol. 2017. 10.1155/2017/9634803.
14. Mohammadi, A.A., S. Jazayeri, K. Khosravi-Darani, Z. Solati and N. Mohammadpour *et al*., 2015. Effects of probiotics on biomarkers of oxidative stress and inflammatory factors in petrochemical workers: A randomized, double-blind, placebo-controlled trial. Int. J. Prev. Med., Vol. 6. 10.4103/2008-7802.164146.

15. Cruz, F.F., P.R.M. Rocco and P. Pelosi, 2017. Anti-inflammatory properties of anesthetic agents. Crit. Care, Vol. 21, No. 1. 10.1186/s13054-017-1645-x.
16. Wang, J., L. Qiao, S. Li and G. Yang, 2013. Protective effect of ginsenoside Rb1 against lung injury induced by intestinal ischemia-reperfusion in rats. Molecules, 18: 1214-1226.
17. Speyer, C.L., T.A. Neff, R.L. Warner, R.F. Guo and J.V. Sarma *et al*., 2003. Regulatory effects of iNOS on acute lung inflammatory responses in mice. Am. J. Pathol., 163: 2319-2328.
18. Dugo, L., S. Marzocco, E. Mazzon, R.D. Paola, T. Genovese, A.P. Caputi and S. Cuzzocrea, 2004. Effects of GW274150, a novel and selective inhibitor of iNOS activity, in acute lung inflammation. Br. J. Pharmacol., 141: 979-987.
19. Sodhi, C.P., M.D. Neal, R. Siggers, S. Sho and C. Ma *et al*., 2012. Intestinal epithelial Toll-like receptor 4 regulates goblet cell development and is required for necrotizing enterocolitis in mice. Gastroenterology, 143: 708-718.
20. Chi, X., W. Yao, A. Zhang, M. Ge and J. Cai *et al*., 2015. Downregulation of lung toll-like receptor 4 could effectively attenuate liver transplantation-induced pulmonary damage at the early stage of reperfusion. Mediat. Inflamm., Vol. 2015. 10.1155/2015/383907.