Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 09, pp.57385-57389, September, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EFFECT OF METOPROLOL ON HEPATIC ISCHEMIA-REPERFUSION INJURY

[*,1]Silvio Marcio Pegoraro Balzan, [2]AlexandreRieger, [1]VinículusGrandoGava, [2]Daniel Pra, [3]Pedro Lúcio de Souza, [3]Rafael AntoniazziAbaid, [4]DanieliRosaneDallemole and [5]Caio Fernando de Oliveira

[1]Department of Biology and Pharmacy, School of Medicine, University of Santa Cruz do Sul (UNISC) and Department of Surgery, Moinhos de Vento Hospital, Porto Alegre, Brazil
[2]Department of Biology and Pharmacy, School of Medicine, University of Santa Cruz do Sul (UNISC); Postgraduation Course of Health Promotion, UNISC; Laboratory of Biotechnology and Genetics, UNISC; Brazil
[3]Department of Biology and Pharmacy, School of Medicine, University of Santa Cruz doSul (UNISC), Brazil
[4]Postgraduation Course on Pharmaceutic Science, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil
[5]Department of Biology and Pharmacy, School of Medicine, University of Santa Cruz do Sul (UNISC); Postgraduation Course of Health Promotion, UNISC, Brazil

ARTICLE INFO

Article History:
Received 08th June, 2017
Received in revised form
05th July, 2017
Accepted 19th August, 2017
Published online 29th September, 2017

Key words:

Liver, Metoprolol, Reperfusion injury.

ABSTRACT

Purpose: Beta-blockers show promise in the attenuation of ischemia reperfusion during cardiac surgery, however its role on injury during liver surgery is not known.
Methods: This preclinical study evaluated the use of beta-blockers during induction of anesthesia in a swine model of liver ischemia-reperfusion injury (metoprolol and control groups).
Results: A total of 28 animals were studied and no difference was observed between groups in biochemical markers sampled before and after liver ischemia and reperfusion.
Conclusion: The use of beta-blockers does not have a clinical impact on attenuating liver ischemia reperfusion injury.

Citation: Silvio Marcio Pegoraro Balzan, AlexandreRieger, VinículusGrandoGava *et al*. 2017. "Effect of metoprolol on hepatic ischemia-reperfusion injury", *International Journal of Current Research*, 9, (09), 57385-57389.

INTRODUCTION

Temporary interruption of hepatic blood inflow, with consequent hepatic ischemia, is anessential step in liver transplantation and many liver resections (Balzan *et al*., 2005; Farges *et al*., 1999; Abdalla *et al*., 2004). The initial detrimental effects due to ischemia can be worsenby a number of lesions that occur after reperfusion, the so-called ischemia-reperfusion syndrome (Zimmerman *et al*., 2017; Hu and Li, 2017). The complex pathophysiology of ischemia-reperfusion is not entirely understood. Production of reactive oxygen radicals during reperfusion leads to tissue damage associated to infiltration by activated polymorphonuclear leukocytes and platelets. There is also cytokine production, complement activation, local imbalance in nitric oxid levels, accumulation of platelet activating factors and endothelial cell adhesion molecules, and formation of free radicals. These metabolic processes can eventually lead to cell apoptosis and tissue necrosis. Several approaches to attenuate hepatic ischemia-reperfusion injury were developed including ischemia preconditioning and postconditioning, and the use of pharmacological agents (Gurusamy *et al*., 2010; Koti *et al*., 2003; Santos *et al*., 2010; Song *et al*., 2012). The protective effects of ischemic and pharmacological preconditioning have been reported in experimental and clinical studies, despite the mechanism through which protection occurs is not clear yet (Hu and Li, 2017; Balzan *et al*., 2014; Rodríguez-Lara *et al*., 2016). Benefits of ischemic postconditioning (brief intermittent cycles of ischemia-reperfusion after the prolonged period of ischemia but prior to permanent reperfusion) were shown in experimental studies (Santos *et al*., 2010; Song *et al*., 2012; Rodríguez-Lara *et al*., 2016). Various pharmacological interventions have been attempted to decrease ischemia-reperfusion injury, such as the use of methyl prednisolone, amino acids, N-acetylcysteine, among others (Bogetti *et al*., 2005; Junnarkar *et al*., 2009; Abu-Amara *et al*., 2010; Robinson *et al*., 2013 and Grendar *et al*., 2016). However, none of these pharmacological agents are recommended for routine use in hepatic surgery. More recently some studies evaluated

******Corresponding author:* **Silvio Marcio Pegoraro Balzan,**
Department of Biology and Pharmacy, School of Medicine, University of Santa Cruz do Sul (UNISC) and Department of Surgery, Moinhos de Vento Hospital, Porto Alegre, Brazil.

the protective effect of beta blockers on cardiac ischemia-reperfusion injury (Ulger *et al*., 2015). Beta blockers could prevent ischemia-reperfusion lesions by its effect on reducing lipid peroxidation, reducing circulating levels of inflammatory cytokines and attenuating oxidative stress (Kalaycioglu *et al*., 1999; Ohtsuka *et al*., 2001; Wang *et al*., 2015). This experimental study was designed to evaluate the effects of metoprolol on biochemical markers of hepatic injury using a pig model of ischemia-reperfusion.

MATERIALS AND METHODS

Animals and study design

28 male swine weighing 20-25 kg, aged 3-4 mo, were fed with standard chow until 12h before the surgical procedure. They were randomized in two groups (Figure 1): metoprolol group and control group. Animals in both groups were submitted to a hepatic ischemia period of 30 min followed by a reperfusion period (30 min). In the metoprolol group, animals received 2.5mg of *in bolus* intravenous metoprolol during induction of general anesthesia. Blood samples from the jugular vein were taken during anesthetic induction, immediately after the ischemia period and after the reperfusion period. Following the reperfusion period animals were sacrificed. Ischemia-reperfusion injury was evaluated through serum analysis of hepatocellular and systemic inflammatory markers. Animals were housed and sacrificed according to institutional animal care policies. The protocol was approved by the Ethics Committee for the use of animals at our institution and it is in accord with Brazilian law the Council for International Organization of Medical Sciences.

Standard surgical procedure

After sedation with intramuscular ketamine at 5 mg/Kgand midazolam at 0.3 mg/Kg, each animal was placed on a proper operating table and electrocardiographic monitoring was initiated. Peripheral venous access was obtained and tracheostomy performed under local anesthesia with lidocaine. Immediately after a definitive airway was obtained, endovenous anesthesia was performed using fentanyl at 0.05 mg/Kg, midazolam at 0.3 mg/Kg, and pancuronium at 0.1 mg/Kg (with reinfusion as needed). All animals were mechanically ventilated. A median laparotomy was performed and access to the upper abdomen aided by a Balfour retractor (Edlo, Canoas, Brazil). The lesser omentum was open and an umbilical tape used to encircle the hepatic pedicle. Pringle maneuver was used to perform hepatic ischemia. Blood samples were obtained by punctuation of the right or left jugular vein.

Biochemical tests

All tests were performed using serum; blood was collected by needle from a vein and then incubated for 20 min at 37 °C. Blood was then centrifuged for 5 min at 3,500 RPM and the supernatant separated for biochemical tests. Biochemical analysis of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP), total bilirubin (TBIL), and C-reactive protein (CRP) was performed by enzymatic photometric methodology using proper reagents (Kovalent) and the automated system Miura 200 (I.S.E., Rome, Italy) according to standard methodology of medical laboratories.

Statistical analysis

Data were entered in spreadsheets and statistical analysis was performed using SPSS for Windows 11.5 (SPSS Inc., Chicago, IL, USA). Data are presented as mean (± standard deviation). Results were compared using two-way ANOVA with Bonferroni's multiple comparisons test. A value of $P<0.05$ was considered significant.

RESULTS

All animals survived until the end of reperfusion period. There was no significant difference on heart rate between the groups, despite a tendency to reduction in the metoprolol group (mean of 110 ±13 on metoprolol group vs. 122 ±21 on control group, data not shown).Three swines in the control group were excluded from analysis due to the presence of abnormal biochemical measures on initial samples. Serum level of biochemical markers are shown in Table 1. The serum levels of aspartate transaminases increased after the ischemia and reperfusion periods. This was observed in both control (from 47±4 U/L to 79±12 U/L after ischemia and 89±11 U/L after reperfusion, $P<0.05$) and metoprolol (from 49±4 U/L to 91±16 U/L after ischemia and 92±9 U/L after reperfusion, $P<0.05$) groups. Alanine aminotransferase and alkaline phosphatase also increased after ischemia on metoprolol group ($P<0.05$) and total bilirubin increased after reperfusion in this group ($P<0.05$). Comparison of the variation of serum level of biochemical markers between the control e metoprolol groups showed very similar values, with no statistical difference (Figure 2).

Table 1. Biochemical markers compared between moments M1, M2 and M3 and the control and metropolol groups

	M1		M2		M3	
	Control (n=11)	Metoprolol (n=14)	Control (n=11)	Metoprolol (n=11)	Control (n=14)	Metoprolol (n=14)
ALT (U/L)	32.45±1.94[d]	30.07±2.55	35.00±1.67	34.45±1.69[d,f]	36.86±2.81	32.36±2.84[f]
AST (U/L)	46.73±4.18[a,b]	49.21±3.92[d,e]	79.45±11.57[a]	88.55±11.24[d]	91.57±15.63[b]	91.64±9.15[e]
ALT/AST	1.47±0.14[b]	1.70±0.14[d,e]	2.38±0.39	2.71±0.44[d]	2.79±0.56[b]	3.15±0.48[e]
GGT (U/L)	28.45±2.36	25.36±1.93	25.82±2.34	27.18±2.37	28.50±2.58	26.00±2.12
ALP (U/L)	222.24±12.58	195.95±19.14[d,e]	229.45±15.74	233.38±17.05[d]	222.16±23.08	214.96±20.57[e]
TBL (mg/dL)	0.11±0.01	0.10±0.01	0.09±0.01	0.13±0.02	0.09±0.01	0.14±0.02
CRP (mg/dL)	0.61±0.10	0.72±0.13	0.64±0.12	0.66±0.11	0.70±0.12	0.80±0.13

Two way ANOVA with Bonferroni's multiple comparisons test (α = 0.05);
Differences significant for Control groups: [a]M1 vs M2; [b]M1 vs M3; [c]M2 vs M3.
Differences significant for Metoprolol groups: [d]M1 vs M2; [e]M1 vs M3; [f]M2 vs M3;
No significant differences found between control and metoprolol groups

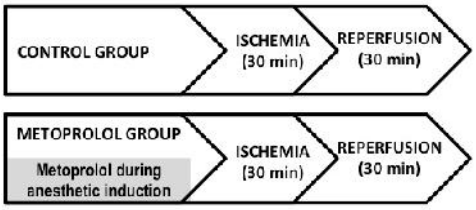

Figure 1. Experimental protocol. Animals were randomized into two gropus (n=14 per group). They underwent 30 min of ischemia followed by 30 min of reperfusion. Control group did not received any intercention and metoprolol group received 2.5mg of intravenous metoprolol before ischemia

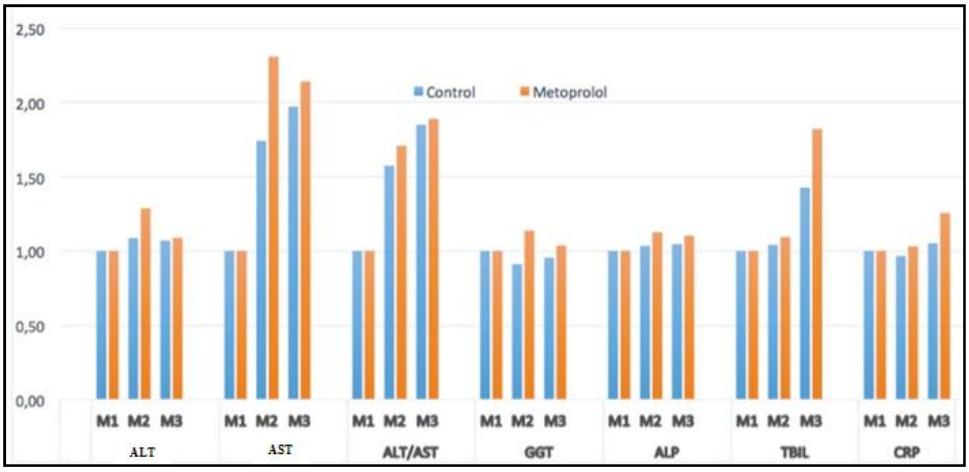

Figure 2. Biochemical results standardized considering mean value at M1 as references. M1: before ischemia, M2: after 30 min ischemia, M3: after 30min reperfusion

DISCUSSION

Vascular clamping is often used during hepatic surgery in order to reduce blood loss with the Pringle maneuver being the most frequently used. Unfortunately, interruption of blood flow followed by its reestablishment causes ischemia-reperfusion injury in the liver. Despite various mechanisms have been described to explain the development of the so-called hepaticischemia-reperfusion syndrome, its precise pathophysiology is not entire understood. During ischemia period occurs depletion of cellular energy, accumulation of intracellular sodium, calcium, and reactive oxygen species, and activation of multiple enzyme systems, leading to cell damage (Serracino-Inglott *et al*., 2001; Zhang *et al*., 2017). After reperfusion tissue damage is potentiated by the infiltration of activated polymorphonuclear leukocytes and platelets, production of cytokines (including tumor necrosis factor-alpha, platelet activating factors, and interleukins IL-1 and IL-10), complement activation, Kupffer cell activation, local imbalance in nitric oxid levels, and formation of free radicals (Arab *et al*., 2009; Jaeschke, 2011). All these inflammatory mechanisms lead to microcirculatory failure, metabolic acidosis, and changes in mitochondrial membrane permeability, resulting ultimately in increased level of cell apoptosis (Fondevila *et al*., 2003; Menger *et al*., 1999; Rudiger *et al*., 2003; Kohli *et al*., 1999). In clinical practice, hepatic ischemia-reperfusion injury can compromise liver functioning thereby increasing risk of postoperative morbidity and mortality in hepatic surgery. A number of methods aimed to prevent the injury secondary to ischemia-reperfusion and to reduce its consequences have been used. Among the surgical methods applied in hepatic surgery, the use of intermittent vascular clamping, ischemic preconditioning or postconditioning, and parenchymal hypothermia have been applied in clinical practice. Also, pharmacological interventions have been tested to prevent ischemia-reperfusion injury, such as the use of prednisolone, amino acids, N-acetylcysteine, and others (Bogetti *et al*., 2005; Junnarkar *et al*., 2009; Abu-Amara *et al*., 2010; Robinson *et al*., 2013; Grendar *et al*., 2016). However, despite some of these strategies show promise in pre-clinical models to reduce hepatic ischemia-reperfusion injury, the lack of clinical trials has became their routine use in clinical practice a controversial issue. Some authors have proposed that beta-blockers reduce cardiac ischemia-reperfusion injury in patients with acute coronary syndrome and this might result in clinical benefits (Ibanez *et al*., 2013; Ndrepepa *et al*., 2013). Due to reperfusion lesions could be prevented by beta-blockers due its effect on reducing lipid peroxidation, reducing circulating levels of inflammatory cytokines and attenuating oxidative stress (Kalaycioglu *et al*., 1999; Ohtsuka *et al*., 2001; Wang *et al*., 2015). To our knowledge, this pharmacological strategy hadnot been tested to attenuate hepatic ischemia-reperfusion. Our study intended to evaluate the effect of intravenous metoprolol, a β1 receptor blocker, on serum level of biochemical markers of hepatic injury using a pig model of ischemia-reperfusion (Balzan *et al*., 2014). Our results suggest that the use of metoprolol before hepatic ischemia and reperfusion does not affect the mostcommon serum markers of hepatocellular injury routinely used on clinical practice. Thus, this approach should not be suggested for human studies until others experimental researchesproof otherwise.

Some criticisms should be considered in the present study. First, only serum biochemical markers routinely used in clinical practice were measured. The use of more sensitive serum markers of inflammation or cellular damage, such as some cytokines (interleukines IL-6 and IL-10, tumor necrosis factor-alfa), total oxidant and antioxidant status, thiobarbituric acid reactive substances (TBARS), among others, or histopathological analysis of hepatic tissue could provide

different results. However, our results with biomarkers routinely used in clinical practice could suggest that, if present, the benefit of metoprolol before hepatic ischemia-reperfusion would have a non-significant clinical impact. Another point to consider is the dosage of beta-blockers. The 2.5 mg of metoprolol infused intravenously during induction of general anesthesia was chosen after a pilot study where the heart rate did not increased during surgical procedure in animals that received metoprolol.

Conclusion

In conclusion, our results suggest that using a beta-blocker before a procedure including hepatic ischemia-reperfusion does not have clinical impact on attenuating the ischemia-reperfusion injury.

REFERENCES

Abdalla, E.K., Noun, R., Belghiti, J. 2004. Hepatic vascular occlusion: which technique? *Surg Clin North Am*., 84(2): 563–85. DOI 10.1016/S0039-6109(03)00231-7

Abu-Amara, M., Gurusamy, K., Hori, S., Glantzounis, G., Fuller, B., Davidson, B.R. 2010. Systematic review of randomized controlled trials of pharmacological interventions to reduce ischaemia-reperfusion injury in elective liver resection with vascular occlusion. HPB, 12(1):4–14. PMID 20495639

Arab, H.A., Sasani, F., Rafiee, M.H., Fatemi, A., Javaheri, A. 2009. Histological and biochemical alterations in early-stage lobar ischemia-reperfusion in rat liver. *World J Gastroenterol*., 15(16):1951–7. PMID 19399926

Balzan, S., Belghiti, J., Farges, O., Ogata, S., Sauvanet, A., Delefosse, D., Durand, F. 2005. The “50-50 criteria” on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg*., 242(6):824–8. PMID 16327492

Balzan, S.M.P., Gava, V.G., Rieger, A., Pra, D., Trombini, L., Zenkner, F.F., Horta, J.A., Azambuja, G., Schopf, L., de Souza, P.L. 2014. Ischemic versus pharmacologic hepatic preconditioning. *J Surg Res*., 191(1):134–9. PMID 24853611

Bogetti, D., Jarzembowski, T.M., Sankary, H.N., Manzelli, A., Knight, P.S., Chejfec, G., Cotler, S., Oberholzer, J., Testa, G., Benedetti, E. 2005. Hepatic ischemia/reperfusion injury can be modulated with thymoglobulin induction therapy. *Transplant Proc*., 37(1):404–6. PMID 15808659

Farges, O., Malassagne, B., Flejou, J.F., Balzan, S., Sauvanet, A., Belghiti, J. 1999. Risk of major liver resection in patients with underlying chronic liver disease: A reappraisal. *Ann Surg*., 229(2):210–5. PMID 10024102

Fondevila, C., Busuttil, R.W., Kupiec-Weglinski, J.W. 2003. Hepatic ischemia/reperfusion injury—a fresh look. *Exp Mol Pathol*., 74(2):86–93. PMID 12710939

Grendar, J., Ouellet, J.F., McKay, A., Sutherland, F.R., Bathe, O.F., Ball, C.G., Dixon, E. 2016. Effect of N-acetylcysteine on liver recovery after resection: A randomized clinical trial. *J Surg Oncol*., 114(4):446–50. PMID 27302646

Gurusamy, K.S., Gonzalez, H.D., Davidson, B.R. 2010. Current protective strategies in liver surgery. *World J Gastroenterol*., 16(48):6098–103. PMID 21182224

Hu, C., Li, L. 2017. Pre-conditions for eliminating mitochondrial dysfunction and maintaining liver function after hepatic ischaemia reperfusion. *J Cell Mol Med*., Mar 16. PMID 28301072

Ibanez, B., Macaya, C., Sánchez-Brunete, V., Pizarro, G., Fernández-Friera, L., Mateos, A., Fernandez-Ortiz, A., Garcia-Ruiz, J.M., Garcia-Alvarez, A., Iniguez, A., Jimenez-Borreguero, J., Lopez-Romero, P., Fernandez-Jeminez, R., Goicolea, J., Ruiz-Mateos, B., Bastante, T., Arias, M., Iglesias-Vazquez, J.A., Rodriguez, M.D., Escalera, N., Acebal, C., Cabrera, J.A., Valenciano, J., Perez de Prado, A., Fernandez-Campos, M.J., Casado, I., Garcia-Rubira, J.C., Garcia-Prieto, J., Sanz-Rosa, D., Cuellas, C., Hernandez-Antolin, R., Albarran, A., Fernandez-Vazquez, F., de la Torre-Hernandez, J.M., pocock, S., Sanz, G., Fuster, V. 2013. Effect of Early Metoprolol on Infarct Size in ST-Segment Elevation Myocardial Infarction Patients Undergoing Primary PCI: The METOCARD-CNIC Trial. Circulation. 128(14):1495-503. PMID 24002794

Jaeschke, H. 2011. Reactive oxygen and mechanisms of inflammatory liver injury: Present concepts. *J Gastroenterol Hepatol*., Jan 26; p. 173–9. DOI: 101111/j. 1440-1746.2010.06592.x

Junnarkar, S.P., Tapuria, N., Dutt, N., Fuller, B., Seifalian, A.M., Davidson, B.R. 2009. Bucillamine improves hepatic microcirculation and reduces hepatocellular injury after liver warm ischaemia-reperfusion injury. *HPB*, 11(3):264–73. PMID 19590658

Kalaycioglu, S., Sinci, V., Imren, Y., Oz, E. 1999. Metoprolol prevents ischemia-reperfusion injury by reducing lipid peroxidation. *Jpn Circ J*., 63(9):718–21. PIMD 10496488

Kohli, V., Selzner, M., Madden, J.F., Bentley, R.C., Clavien, P.A. 1999. Endothelial cell and hepatocyte deaths occur by apoptosis after ischemia-reperfusion injury in the rat liver. Transplantation, 67(8):1099–105. PMID 10232558

Koti, R.S., Seifalian, A.M., Davidson, B.R. 2003. Protection of the liver by ischemic preconditioning: a review of mechanisms and clinical applications. *Dig Surg*., 20(5):383–96. PMID 12840597

Menger, M.D., Richter, S., Yamauchi, J., Vollmar, B. 1999. Role of microcirculation in hepatic ischemia/reperfusion injury. Vol. 46, *Hepatogastroenterology*, p. 1452–7. PMID 1043 1706

Ndrepepa, G. and Kastrati, A. 2013. Intravenous Beta Blockers in Primary PCI: New Hope for an Old Therapy. Circulation. 128(14):1487-9. PMID 24002793

Ohtsuka, T., Hamada, M., Hiasa, G., Sasaki, O., Suzuki, M., Hara, Y., Shigematsu, Y., HIwada, K. 2001. Effect of beta-blockers on circulating levels of inflammatory and anti-inflammatory cytokines in patients with dilated cardiomyopathy. *J Am Coll Cardiol*., 37(2):412–7. PMID 11216955

Robinson, S.M., Saif, R., Sen, G., French, J.J., Jaques, BC., Charnley, R.M., Manas, D.M., White, S.A. 2013. N-acetylcysteine administration does not improve patient outcome after liver resection. HPB, 15(6):457–62.PMID 23458723

Rodríguez-Lara, S.Q., Cardona-Muñoz, E.G., Ramírez-Lizardo, E.J., Totsuka-Sutto, S.E., Castillo-Romero, A., García-Cobián, T.A., Garcia-Benavides, L. 2016. Alternative Interventions to Prevent Oxidative Damage following Ischemia/ Reperfusion. *Oxid Med Cell Longev*., 7190943. PMID 28116037

Rudiger, H.A., Graf, R., Clavien, P.A. 2003. Liver ischemia: Apoptosis as a central mechanism of injury. *J Investig Surg*., 16(3):149–59. PMID 12775431

Santos, C.H.M. dos, Pontes, J.C.D.V., Miiji, L.N.O., Nakamura DI, Galhardo CAV, Aguena SM. 2010.

Postconditioning effect in the hepatic ischemia and reperfusion in rats. *Acta Cir Bras*., 25(2):163–8. PMID: 20305883

Serracino-Inglott, F., Habib, N.A., Mathie RT. 2001. Hepatic ischemia-reperfusion injury. *Am J Surg*., 181(2):160–6. PMID 11425059

Song, X., Zhang, N., XU H, Cao, L, Zhang, H. 2012. Combined Preconditioning and Postconditioning Provides Synergistic Protection against Liver Ischemic Reperfusion Injury. *Int J Biol Sci*., 8(5):707–18. PMID 22701341

Ulger, B.V., Erbis, H., Turkcu, G., Ekinci, A., Turkoglu, M.A, Ekinci, C,. Yilmaz, V.T., Bac, B. 2015. Nebivolol Ameliorates Hepatic Ischemia/Reperfusion Injury on Liver But Not on Distant Organs. *J Investig Surg*., 28(5):245–52. DOI 10. 3109/08941939.2015.1031923

Wang, X., Cheng, Y., Xue, H., Yue, Y., Zhang, W., Li, X. 2015. Fargesin as a potential β1 adrenergic receptor antagonist protects the hearts against ischemia/reperfusion injury in rats via attenuating oxidative stress and apoptosis. Fitoterapia. 105:16–25. PMID 26025856

Zhang, Y.Q., Ding, N., Zeng, Y.F., Xiang, Y.Y., Yang, M.W., Hong FF, Yang SL. 2017. New progress in roles of nitric oxide during hepatic ischemia reperfusion injury. *World J Gastroenterol*., 23(14):2505–10. PMID 28465634

Zimmerman, M., Martin, A., Yee, J., Schiller, J., Hong, J.. 2017. Natural Killer T Cells in Liver Ischemia–Reperfusion Injury. *J Clin Med*., 6(4):41. PMID28368299

****