Impact of three methods of ischemic preconditioning on ischemia-reperfusion injury in a pig model of liver transplantation

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Abstract

Background. Ischemic preconditioning (IPC), either direct (DIPC) or remote (RIPC), is a procedure aimed at reducing the harmful effects of ischemia-reperfusion injury. Aims. To assess the local and systemic effects of DIPC, RIPC, and both combined, in the pig liver transplant model.

Methods. Twenty-four pigs underwent orthotopic liver transplantation and were divided into 4 groups according to the procedures applied: direct donor preconditioning; indirect preconditioning at the recipient and a group with direct donor and indirect recipient preconditioning. The following parameters were recorded: donor and recipient weight, graft-to-recipient weight ratio (GRWR), surgery time, hot and cold ischemia time, and intraoperative hemodynamic values. Blood samples were collected before native liver removal (BL) and at 0h, 1h, 3h, 6h, 12h, 18h, and 24h post-reperfusion and the following biochemical tests were performed: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), creatinine, BUN (blood urea nitrogen), lactate, total and direct bilirubin. Histopathological examination of liver, gut, kidney, and lung fragments were performed, as well as molecular analyses for expression of the apoptosis-related BAX (pro-apoptotic) and Bcl-XL (anti-apoptotic) genes, eNOS (endothelial nitric oxide synthase) gene, and IL-6 gene related to inflammatory ischemia-reperfusion injury, using real-time polymerase chain reaction (RT-PCR).

Results. There were no differences between the groups regarding biochemical and histopathological parameters. We found a reduced ratio between the expression of the pro-apoptotic BAX gene and the expression of the anti-apoptotic Bcl gene in the livers of animals with IPC versus the control group.

Conclusions. DIPC, RIPC or a combination of both produce local beneficial effects only at the molecular level but do not translate into biochemical or histological changes.

Background

Primary graft dysfunction due to ischemia-reperfusion (I/R) injury is one of the most serious complications of liver transplantation.[1,2] Ischemic preconditioning (IPC) is an important approach to reduce the harmful effects of this process. It consists of producing brief periods of ischemia followed by brief periods of reperfusion prior to the prolonged ischemic insult, which should induce greater organ tolerance to prolonged ischemia and subsequent reperfusion.[3,4] Its beneficial effects are thought to result from the release of adenosine by the ischemic tissue promoting vasodilation, inhibition of platelet aggregation and neutrophil adherence, inhibition of endothelin synthesis and reactive oxygen species, in addition to increased production of nitric oxide.[4]

IPC can be direct at the target organ or indirect (remote). Direct ischemic preconditioning (DIPC) has the disadvantage of causing mechanical stress to the main vascular structures of the organ.[5] In remote ischemic preconditioning (RIPC), the procedure is applied to another organ, with the protective effect on the target organ being exerted by biochemical mediators activated at a distance and carried by the blood

stream, without direct stress or trauma to the organ.[6] The effect of RIPC was first demonstrated on the myocardium of rats submitted to renal IPC.[5] So far, there is no consensus about the best method of RIPC, i.e., the number of I/R cycles, the effective I/R time required to trigger the protective stimulus, and the choice of the I/R site to maximize the beneficial effects of IPC with the least possible damage to the body. However, notwithstanding these unanswered questions, the short-term occlusion of the mesenteric artery has been proven of great importance, with positive effects on several organs.[5]

Several studies demonstrate the beneficial effects of DIPC and RIPC. A recent study also showed that the combination of both types of IPC enhanced the protective effects of this procedure in a mouse liver transplant model.[7]

The pig model has clear anatomical, morphological, and physiological similarities with humans. It also allows for easy and extensive monitoring and ensures the feasibility of the orthotopic liver transplantation, thus being useful to investigate issues that have direct clinical relevance.[7,8] The potential benefits of RIPC in transplants performed on medium-sized animals, the association of direct and remote ischemic preconditioning, and the comparative effects of these two methods have been reported in very few studies. Thus, the objectives of the present study were to evaluate the local and systemic effects of DIPC, RIPC and their combination in a liver transplant model in pigs of similar weights. The groups were evaluated by biochemical, histopathological and molecular analyses.

Methods

The experimental study protocol was approved by the Institutional Animal Use Ethics Committee. The funding body had no participation in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript. All procedures performed in the animals were in accordance with the ethical standards of our Institution. Twenty-four hybrid pigs of both sexes weighing on average 28.7±2.4 kg were randomly divided into the following 4 groups (n=6 each) and submitted to orthotopic liver transplantation. The number of animals in each group was based in previous similar studies.[9,10]

1. control group (C)
2. direct donor preconditioning (D)
3. indirect recipient preconditioning (R)
4. direct donor and indirect recipient preconditioning (D+R).

The animals (donors and recipients) were fasted for 12 hours, then at 7 a.m. injected with intra muscular xylazine (2.0 mg/Kg) and ketamine (10.0mg/Kg) as pre-anesthetic 15 minutes before anesthesia, which was induced with propofol (5.0-10.0 mg/Kg) and maintained with endotracheal intubation, 40% oxygen supply, and isoflurane (1.3 to 2.0%) in inspired air, along with a continuous intravenous infusion of fentanyl (0.05 µg/Kg/min). Catheters were introduced into the jugular vein for fluid infusion and central venous pressure (CVP) measurement, and into the carotid artery for invasive mean arterial pressure (iMAP) measurement and blood sampling for biochemical analyses. Recipient animals were continuously

monitored until the end of the surgery and post-surgery recovery with electrocardiogram, oximetry, end tidal carbon dioxide monitoring (EtCO2), respiratory rate, and pressure measurements - CVP and iMAP.

According to the groups where they were allocated, the animals underwent one of two types of ischemic preconditioning (groups D and R) or both combined (group D+R). Direct organ preconditioning was performed by clamping the donor whole hepatic pedicle (portal vein, hepatic artery, and bile duct); indirect preconditioning was applied to the recipient gut, by clamping the superior mesenteric artery. Both types of preconditioning consisted of three cycles with 5 minutes' ischemia followed by 5 minutes' reperfusion.

Surgical procedures on donor and recipient animals were performed according to previously published technique by our laboratory. After a large experience, we standardized the porcine liver transplantation without the use of venovenous bypass. [9,10]

After surgery, recipient animals remained extubated and conscious in private stalls in our laboratory for 24 hours, with catheters in the jugular vein and carotid artery for medication infusion and blood sampling. Blood samples were collected at the following time points: before native liver removal (BL) and periods after graft reperfusion: 0h, 1h, 3h, 6h, 12h, 18h, and 24h. At the end of this period, the animals were anesthetized, intubated and connected to the mechanical ventilator for gut, kidney, lung, and liver biopsies. After that, the animals were euthanized with an overdose of inhaled anesthetic 5% isoflurane and intravenous administration of 10 mL/kg of 19.1% potassium chloride.

The following parameters were recorded: donor and recipient weight, surgery time, hot and cold ischemia time, and intraoperative hemodynamic values. Additionally, the following biochemical tests were performed: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), creatinine, BUN, lactate, total and direct bilirubin.

Histopathological analyses were performed on 4 liver fragments obtained at the following time points: before removal from the donor (BL), immediately after release of the hepatic artery flow of the recipient (0h), after 1 hour of reperfusion (1h) and after 24 hours of reperfusion (24h). For the other organs (gut, kidney, and lung), only one biopsy was performed after 24 hours of observation. Samples were fixed in neutral formalin, dehydrated and embedded in paraffin blocks, then sectioned (4 µm-sections) and stained with hematoxylin-eosin.

The liver injury was assessed based on endpoints analyzed and quantified according to the criteria described by Scheuer et al11. This score was used for its easy reproducibility and for describing the inflammatory response. Lung histology assessment was based on the modified VILI score, with 4 parameters evaluated and quantified to measure the degree of tissue damage[12]. Kidney injury was assessed using the Banff score with 4 parameters evaluated and quantified[13]. To assess histological damage to the gut, the Chiu score was used.[14]

All the tissue sections were examined under light microscopy by 3 different blind readers who assigned scores to the identified injuries. For the statistical analysis, each section was assigned the average of

these 3 scores.

For the molecular analyses, a fragment from each biopsy specimen was examined for expression of the apoptosis-related genes, i.e., BAX (pro-apoptotic) and Bcl-XL (anti-apoptotic), the eNOS (endothelial nitric oxide synthase) gene, and the IL-6 gene related to the inflammatory ischemia-reperfusion injury, using RT-PCR. To study the balance between pro- and anti-apoptotic gene expression, the BAX/Bcl-XL ratio for all organs was calculated at different time points.

Statistical Analysis

Data were recorded and stored on a spreadsheet of the Stata statistical package. For the qualitative variables, the absolute and relative frequencies were calculated. For quantitative variables, the mean, standard deviation, median, minimum and maximum values were calculated and displayed in graphic format with the values.

Data with normal distribution regarding quantitative variables and gene expression were assessed using analysis of variance (ANOVA); differences between groups were identified using the Bonferroni test; and differences between timepoints in the groups were identified using the t-test. Histomorphometric (qualitative) data were compared using the nonparametric Kruskall-Wallis method.

The null hypothesis of equality of means was rejected when $p<0.05$.

Results

The recipient weights and ischemia times in different groups were similar ($p>0.05$, Fig. 1). The lengths of warm ischemia, cold ischemia, and recipient surgery are shown in Table 1. We can verify that there were no differences among the groups, regarding these parameters.

Table 1. Lengths in minutes of cold ischemia, warm ischemia and recipient surgery (median, with maximum and minimum values) for the experimental groups.

	Cold Ischemia*			Warm Ischemia**			Recipient Surgery***		
Group	Median	Max	Min	Median	Max	Min	Median	Max	Min
C	225	255	160	60	118	54	138	246	135
D	222	325	180	66	125	39	172	245	146
R	239	317	208	68	80	61	191	220	175
D+R	229	288	180	56	71	45	178	188	141

*comparison among groups p = 0.78

**comparison among groups p = 0.39

***comparison among groups p = 0.34

Biochemical Analysis

The behavior of the different serum biochemical markers studied in the different groups throughout the experimental period is shown in Fig. 2.

Regarding serum AST levels, all groups showed similarly disperse patterns, with values increasing over the experimental time points and stabilizing between 12 and 24 hours. There was no difference between groups.

For gamma-GT, some groups showed great variation within the group at the experimental time points, but no difference was observed between groups. Also, alkaline phosphatase levels showed no differences between groups over the experimental time points.

All groups showed an increase in creatinine values compared to baseline. At 3h and 6h, values in the R group were lower than in controls (p=0.003 and p=0.042, respectively).

There was no difference in arterial lactate levels between groups during the experimental period. All groups showed a consistent increase in the immediate post-transplant period, followed by a return, by the end of the experiment, to baseline values.

There was wide variation in total bilirubin levels in the R and D+R groups at 6h, 12h, 18h, and 24h, but the only significant difference observed in this variable was an increase in the D+R group compared to the controls at 24h (p=0.046). The direct bilirubin levels showed the same behavior as total bilirubin, i.e. wide variation within the groups, but with higher levels in the D+R group compared to the controls at 6h (p=0.033).

Histopathological Analysis

The histopathological analysis of the liver revealed typical ischemia-reperfusion lesions found in major surgeries: lobular and inflammatory cells infiltrate, hepatic sinusoid hyperemia and hepatocellular necrosis. In the small intestine, cell lysis, Grünhagen spaces formation, enlargement of the distance between villi, dilated capillaries, presence of inflammatory cells, and destruction of the villi with intraluminal hemorrhage were observed. In the kidney, the typical lesions were capillary tuft retraction plus interstitial, tubule and vessel congestion. In the lung, alveolar congestion was observed and in some cases hemorrhage and leukocyte infiltrates inside the alveoli and bronchi (Fig. 3). Histopathological scores in the different tissues and groups are summarized in Table 2. Despite individual observations, no statistically significant differences were found in tissue scores between the groups.

Table 2. Median scores, with maximum and minimum values, of histopathological analyses of different tissues (liver, gut, lung, and kidney), by group and time points (BL, 0h, 1h, and

24h).

up	Time	Liver			Gut			Kidney			Lung		
		Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min
	BL	1	2	1									
	T0h	2	2	1									
	T1h	3	3	1									
	T24h	3	3	2	0.5	3	0	2	2	2	3	4	1
	BL	1	2	0									
	T0h	1.5	2	1									
	T1h	2	5	2									
	T24h	4	7	4	2.5	3	0	2	3	0	2	5	0
	BL	0.5	2	0									
	T0h	2	2	1									
	T1h	2	2	1									
	T24h	3	4	2	0	1	0	1.5	4	0	2	3	1
R													
	BL	0.5	2	0									
	T0h	2	3	1									
	T1h	2	2	2									
	T24h	2.5	4	2	0.5	3	0	2	3	1.0	3.5	6	2

Molecular Analysis

The gene expressions in the liver, gut, kidney, and lung of the study animals are shown in Fig. 4. In the liver, concerning the BAX gene expression, the D+R group showed lower values at 24h when compared to the controls (p=0.039). The expression of the IL-6 gene in D group animals was higher at 24h when compared to the R group (p=0.001) and the D+R group (p=0.02). The Bcl-XL values did not increase during the experimental period in the controls, while in the groups that received any preconditioning there was an increase in the gene expression towards the end of the experimental period; the D group and the D+R group showed higher values at 24h (p=0.034 and p=0.006, respectively) when compared to the controls. Regarding the e-NOS gene, by 24h the values of the D+R were higher when compared to the controls (p=0.031), the D group (p=0.003), and the R group (p=0.021).

In the gut, no differences in the expression of the BAX and IL-6 genes were observed in the groups at 24h. However, the Bcl-XL gene expression was higher in the R group than in the controls (p=0.004). In addition, the eNOS gene expression was higher in the D group animals when compared to the controls (p=0.001) and the R group (p=0.001). In the kidney, it was observed that there was no difference in the expression of

the BAX and Bcl-XL genes in the various groups at 24h. IL-6 in the R group showed higher values than in the controls (p=0.004). Also, no difference between groups in the expression of the BAX, IL-6 and Bcl-XL genes in the lung tissue, although the eNOS gene expression was higher in the D group than in the R group (p=0.007). Finally, the ratio of pro-apoptotic BAX gene expression to anti-apoptotic Bcl-XL gene expression was lower in the liver by 24 h in all groups receiving IPC (D, R, and D+R groups) when compared to the controls (Fig. 5).

Discussion

After the advent of the experimental evidences of positive effects of IPC in I/R injury, several clinical studies started to show conflicting results about the real benefits of this procedure in medical practice. Recently, studies with a high level of evidence, as clinical trials, systematic reviews, and meta-analyses have demonstrated beneficial effects of direct or remote IPC in clinical situations involving myocardial[15,16], renal[17], and neuronal ischemia[18]. However, it is known that different tissue and organ sensitivities to ischemia are diverse so that such benefits cannot be generalized.

In the current literature context, the real effects of IPC in hepatic I/R injury, namely in liver transplantation, are not clear. Besides, different modalities of IPC are impractical procedures that may further increase the already high complexity of transplantation surgery. So, although several experimental investigations have shown promising results of IPC in liver IRI of small animals,[19-22] there are few studies with models of cold and hot ischemia in liver transplantation performed on medium-sized animals[23,24] and this motivated us to evaluate such effects utilizing a model that simulates the human condition.

We have been utilizing the porcine model in our laboratory because it mimics the clinical situation of liver transplantation.[9,10,25,26] In a previous study, utilizing a model similar to the present one, we observed that IPC resulted in partial attenuation of the harmful effects of I/R injury.[10] In the current study, we also aimed at identifying if the positive effects of IPC remained after a longer observation time, thus providing a rationale for its use in clinical practice.

While studies of IPC are not new, still there is no consensus about technical issues such as ischemia time and number of I/R cycles needed to effectively achieve protective effects. Therefore, in our model, we used 3 alternating 5-minute cycles of ischemia followed by the same reperfusion time, to prevent severe hemodynamic repercussions in the animals, while avoiding a too short IPC period that might have less evident effects. Another objective of this experimental study was to assess RIPC as a means to protect the target organ without causing the direct stress of ischemia-reperfusion. By using the two techniques in separate groups as well as combined, in a specific group that received the two types of IPC, our goal was to ascertain if there would be a difference between preconditioning the graft or the recipient, and to check potential cumulative effects when both procedures are used concomitantly.

For RIPC, the most widely used technique is clamping one limb of a patient[27] or animal.[28] However, for our project we chose a different target territory, i.e. the gut, by clamping the superior mesenteric artery.

This organ was chosen because it has one of the highest levels of metabolic activity and is very susceptible to oxygen pressure variations, and therefore is quickly responsive to short periods of ischemia, which should maximize the protective effect of the RIPC. Finally, it is important to stress that the gut is a territory that markedly suffers from blood stasis during the anehepatic phase of the transplant procedure.

We performed the current experiments to clarify local and systemic effects of IPC in liver transplantation and assess the potential influence of our conditioning models on common problems caused by I/R injury in organ transplants, such as acute kidney injury.[29-32] Unlike previous publications, our biochemical results fail to show any benefit of DIPC or RIPC in liver transplantation. Serum AST and ALT levels are consistent with the degree of hepatocellular injury and are used as indicators of graft distress, bearing correlation with different levels of primary graft dysfunction. All groups in our study were comparable in terms of enzymatic profile. In the D+R group, AST showed high variability, with a trend towards higher median values at 12h, 18h, and 24h, suggesting a possible harmful effect of the addition of the two IPC procedures, even though the difference was not significant.

Our results differ from those of studies of liver transplantation in small animals (rats), which showed lower transaminase values and improvement of histopathological aspects in animals submitted to RIPC compared to controls after 24h of reperfusion.[26] On the other hand, our results are consistent with those from human studies in which direct and remote IPC increased AST and ALT values 24h after reperfusion.[25,30]

We made a refinement in this current investigation, by adding some molecular analyses that could show some beneficial effects of IPC. In the liver tissue, the combined IPC approach resulted in marked positive changes in gene expression. The eNOS gene expression in the liver tissue was higher in the D+R group at 24h, and such expression is usually related to improvement in ischemia. In addition, lower expression of pro-inflammatory genes (BAX) and higher expression of anti-inflammatory genes (Bcl-XL) were observed in this same group. DIPC also had positive effects, leading to increased expression of the IL-6 and Bcl-XL genes.

The BAX/Bcl-XL ratio showed lower values for all treated groups when compared with controls at 24h. This finding may suggest an IPC-driven potential decrease in cell apoptosis secondary to I/R injury. Furthermore, in the gut, kidney, and lung tissues, some molecular changes were detected demonstrating beneficial effects of each IPC separately.

Although gene expression suggests positive effects, with results showing broad ranges probably due to the small sample size, we cannot infer that in the complex situation of liver transplantation these results would be sufficient to indicate an IPC procedure. In addition, the results of biochemical and histopathological analyses, consistent with human studies, did not confirm any benefits from IPC, which raises questions about the feasibility of extrapolating results obtained in small animals to medium-sized animals and humans. The first hypothesis to explain this difference would be a higher susceptibility of

certain species, e.g. rodents, to the IPC procedure, either in situ (direct) or remote, with this propensity diminishing as we advance phylogenetically towards humans, as in the case of pigs.

Also, worth mentioning are the physiological, anatomical, and surgical variations involved in organ transplantation in different species. Despite the highly ingenious technical solutions found to overcome difficulties and enable organ transplantation in rats and mice, the surgical procedure in these animals cannot match what happens in human surgery, including technical hurdles, hemodynamic instability, surgical time, and postoperative follow-up. The human context is optimally mimicked by transplantation performed in medium-sized animals, which yields similar results.

Finally, it remains an important question: although considered a good idea, why the ischemic preconditioning in the pig model did not work out? Probably the ischemia-reperfusion represents intense stress to liver graft, that all benefits of ischemic preconditioning are covered by the real ischemic impact.

Considering the great complexity of the transplant surgery and based on the results of the current investigation, we may conclude that the three methods of IPC herein utilized may not be utilized in clinical practice.

Conclusions

In the pig model of orthotopic liver transplantation, direct or remote IPC, or a combination of both, produces local beneficial effects only at the molecular level but do not translate into biochemical or histological changes.

Abbreviations

IPC: Ischemic preconditioning

DIPC: Direct ischemic preconditioning

RIPC: Remote ischemic preconditioning

GRWR: Graft-to-recipient weight ratio

BL: Before native liver removal

AST: aspartate aminotransferase

ALT: alanine aminotransferase

ALP: alkaline phosphatase

GGT: gamma-glutamyl transferase

BUN: blood urea nitrogen

RT-PCR: real-time polymerase chain reaction BAX

CVP: central venous pressure

iMAP: invasive mean arterial pressure

Declarations

Availability of data and materials

The datasets used and analyzed during the current study will be available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The experimental study protocol number 034/14 was approved by the Institutional Animal Use Ethics Committee (University of Sao Paulo Medical School, Sao Paulo, Brazil). All applicable international, national, and institutional guidelines for the care and use of animals were followed. The landrace white pigs were commercially obtained from the Company Granja RG (Suzano – São Paulo, Brazil). A written informed consent to use the animals in the study was obtained from the Company.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files]

Competing interests

The authors declare that they have no conflict of interest.

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Author's contribution

ARB, ACAT, RRG, and JLF performed the animal experiments; AMS, SS, JOG, CSF, ASA, VRP performed all laboratory studies; DARM performed the statistical analyses and UT was the responsible for the manuscript.

All authors have read and approved the manuscript.

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References

1. Liu Q, Bruns H, Schultze D, Xue Y, Zorn M, Flechtenmacher C, Straub BK, Rauen U, Schemmer P. HTK-N, a modified HTK solution, decreases preservation injury in a model of microsteatotic rat liver transplantation. Langenbecks Arch Surg 2012;397:1323-1331.
2. Kupiec-Weglinski JW, Busuttil RW. Ischemia and reperfusion injury in liver transplantation. Transplant Proc. 2005;37:1653-1656.
3. Theodoraki K, Tympa A, Karmaniolou I, Tsaroucha A, Arkadopoulos N, Smyrniotis V. Ischemia/reperfusion injury in liver resection: a review of preconditioning methods. Surg Today 2011;41:620-629.
4. Miranda LEC, Viaro F, Ceneviva R, Evora PRB. As bases experimentais da lesão por isquemia e reperfusão do fígado: revisão. Acta Cirurgica Bras 2004;19:1-12.
5. Tapuria N, Kumar Y, Habib MM, Abu Amara M, Seifalian AM, Davidson BR. Remote ischemic preconditioning: a novel protective method from ischemia reperfusion injury–a review. J Surg Res 2008;150:304-330.
6. Song X, Zhang N, Xu H, Cao L, Zhang H. Combined preconditioning and postconditioning provides synergistic protection against liver ischemic reperfusion injury. Int J Biol Sci 2012;8:707-718.
7. Mendes-Braz M, Elias-Miro M, Jimenez-Castro MB, Casillas-Ramirez A, Ramalho FS, Peralta C. The current state of knowledge of hepatic ischemia-reperfusion injury based on its study in experimental models. J Biomed Biotechnol 2012;2012:298657.
8. Yandza T, Tauc M, Saint-Paul MC, Ouaissi M, Gugenheim J, Hebuterne X. The pig as a preclinical model for intestinal ischemia-reperfusion and transplantation studies. J Surg Res 2012;178:807-819.
9. Rangel Moreira D de A, Aoun Tannuri AC, Belon AR, Mendonça Coelho MC, Oliveira Gonçalves J, Serafini S, Roberto Lima F, Agostini LO, Guimarães RR, Tannuri U. Large-for-size liver transplantation: a flowmetry study in pigs. J Surg Res. 2014;189:313-320.
10. Leal AJ, Tannuri AC, Belon AR, Guimarães RR, Coelho MC, Gonçalves J de O, Serafini S, Melo ES, Tannuri U. Effects of ischemic preconditioning in a pig model of large-for-size liver transplantation. Clinics (Sao Paulo) 2015;70(2):126-135.
11. Scheuer PJ, Standish RA, Dhillon AP. Scoring of chronic hepatitis. Clin Liver Dis 2002;6:335-347.
12. Belperio JA, Keane MP, Burdick MD, Londhe V, Xue YY, Li K, Phillips RJ, Strieter RM. Critical role for CXCR2 and CXCR2 ligands during the pathogenesis of ventilator-induced lung injury. J Clin Invest 2002;110:1703-1716.

13. Racusen LC, Halloran PF, Solez K. Banff 2003 meeting report: new diagnostic insights and standards. Am J Transplant 2004;4:1562-1566.
14. Chiu CJ, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. Arch Surg 1970;101:478-483.
15. Candilio L, Hausenloy DJ, Yellon DM. Remote ischemic conditioning: a clinical trial's update. J Cardiovasc Pharmacol Ther 2011;16:304-312.
16. McLeod SL, Iansavichene A, Cheskes S. Remote ischemic preconditioning to reduce reperfusion injury during acute ST-segment-elevation myocardial infarction: A systematic review and meta-Analysis. J Am Heart Assoc 2017;6:e005522..
17. Bei WJ, Duan CY, Chen JY, Wang K, Liu YH, Liu Y, Tan N. Remote ischemic conditioning for preventing contrast-induced acute kidney injury in patients undergoing percutaneous coronary interventions/coronary angiography: a meta-analysis of randomized controlled trials.. J Cardiovasc Pharmacol Ther 2016;21:53-63.
18. Landman TRJ, Schoon Y, Warlé MC, de Leeuw FE, Thijssen DHJ. Remote ischemic conditioning as an additional treatment for acute ischemic stroke. Stroke 2019;50:1934-1939.
19. Gomes PFM, Tannuri ACA, Nogueira TM, Iuamoto LR, Paes VR, Coelho MCM, Gonçalves JO, Serafini S, Tannuri U. Remote ischemic preconditioning is efficient in reducing hepatic ischemia-reperfusion injury in a growing rat model and does not promote histologic lesions in distant organs. Transplant Proc 2018;50:3840-3844.
20. Koti RS, Seifalian AM, McBride AG, Yang W, Davidson BR. The relationship of hepatic tissue oxygenation with nitric oxide metabolism in ischemic preconditioning of the liver. FASEB J 2002;16:1654–1656.
21. Koti RS, YangW, DashwoodMR, DavidsonBR, Seifalian AM. Effect of ischemic preconditioning on hepatic microcirculation and function in a rat model of ischemia reperfusion injury. Liver Transpl 2002; 8:1182–1191.
22. Gustafsson BI, Friman S, Wallin M, Heiman J, Delbro DS. Effect of remote preconditioning on mild or severe ischemia-reperfusion injury to rat liver. Transplant Proc 2006;38:2708-2709.
23. Shimoda M, Iwasaki Y, Sawada T, Kubota K. Protective effect of ischemic preconditioning against liver injury after major hepatectomy using the intermittent Pringle maneuver in swine. Pathobiology 2007;74:42-49.
24. Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. Circulation 1993;87:893-9.
25. Leal AJG Tannuri AC, Belon AR, Guimarães RR, Coelho MC, Oliveira Gonçalves Jd, Sokol SS, De Melo ES, Otoch JP, Tannuri U. A simplified experimental model of large-for-size liver transplantation in pigs. Clinics (Sao Paulo) 2013;68:1152-1156.
26. Tannuri ACA, de Albuquerque Rangel Moreira D, Belon A, Coelho MCM, Gonçalves JO, Serafini S, Tannuri U. Does a meso-caval shunt have positive effects in a pig large-for-size liver transplantation

model? Pediatr Transplant 2017;21(5).

27. Robertson FP, Goswami R, Wright GP, Imber C, Sharma D, Malago M, Fuller BJ, Davidson BR. Remote ischaemic preconditioning in orthotopic liver transplantation (RIPCOLT trial): a pilot randomized controlled feasibility study. HPB (Oxford) 2017;19:757-767.
28. Czigany Z, Bleilevens C, Beckers C, Stoppe C, Möhring M, Fülöp A, Szijarto A, Lurje G, Neumann UP, Tolba RH. Limb remote ischemic conditioning of the recipient protects the liver in a rat model of arterialized orthotopic liver transplantation. PLoS One 2018;13:e0195507.
29. Liu X, Cao L, Zhang T, Guo R, Lin W. Effect of Remote Ischemic preconditioning in patients undergoing hepatectomy with portal triad clamping: A randomized controlled trial. Anesth Analg 2019;129:1742-1748.
30. Barri YM, Sanchez EQ, Jennings LW, Melton LB, Hays S, Levy MF, Klintmalm GB. Acute kidney injury following liver transplantation: definition and outcome. Liver Transpl 2009;15:475–483.
31. Yalavarthy R, Edelstein CL, Teitelbaum I. (2007) Acute renal failure and chronic kidney disease following liver transplantation. Hemodial Int 11:S7–S12.
32. Koneru B, Shareef A, Dikdan G, Desai K, Klein KM, Peng B, Wachsberg RH, de la Torre AN, Debroy M, Fisher A, Wilson DJ, Samanta AK. The ischemic preconditioning paradox in deceased donor liver transplantation-evidence from a prospective randomized single blind clinical trial. Am J Transplant 2007;7:2788-2796.

Figures

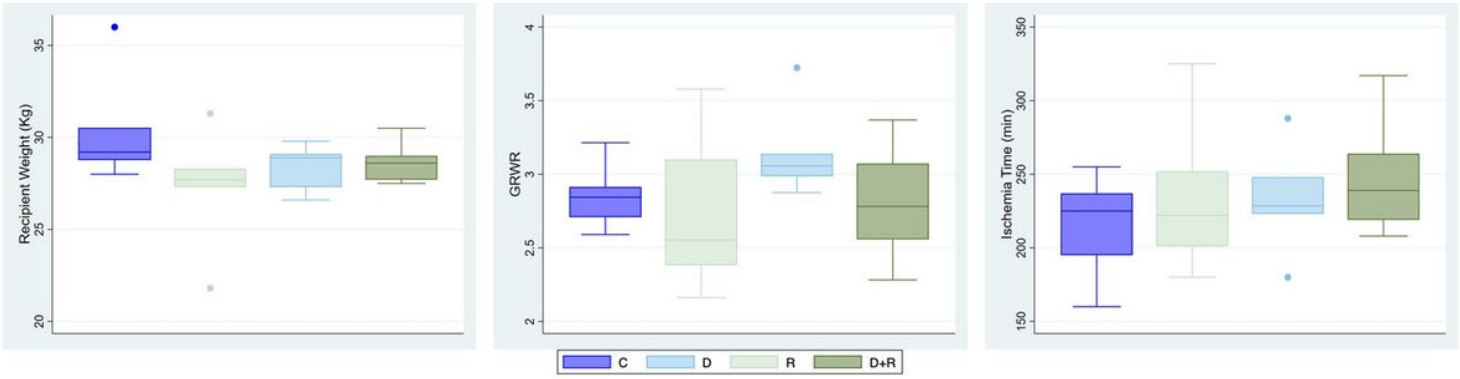

Figure 1

Recipient weight, GRWR and ischemia time in different groups. C: control group; D: direct donor preconditioning group; R: indirect recipient preconditioning group; and D+R: direct donor and indirect recipient preconditioning group. p>0.05 for all comparisons. Note the outliers.

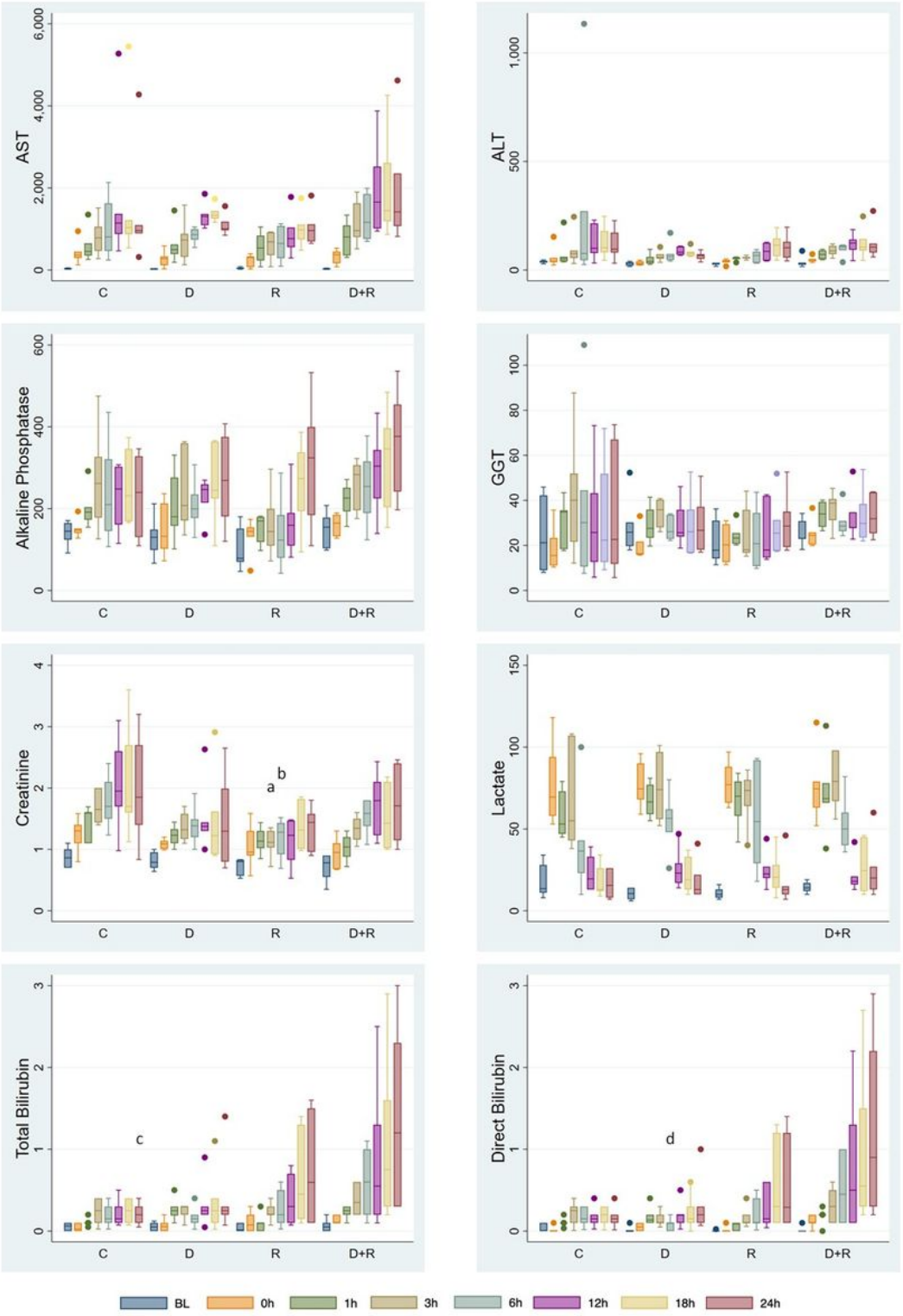

Figure 2

Measurements of blood biochemical parameters, in different groups by time point. Creatinine: C group vs. R group, p=0.003; b: C group vs. D+R group, p=0.040. Total bilirubin: D group vs. D+R group, p=0.046. Direct bilirubin: D group vs. D+R group, p=0.033.

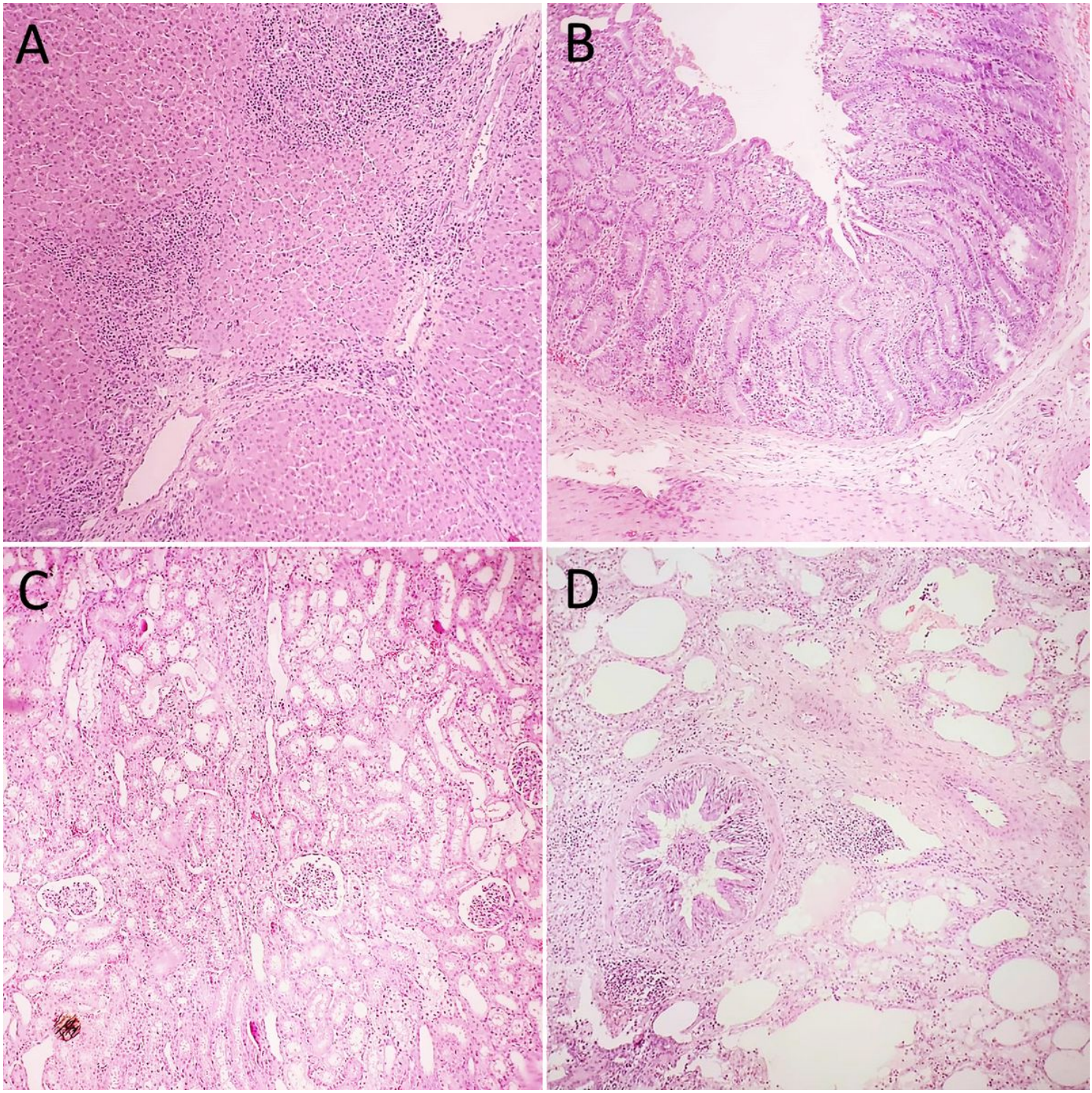

Figure 3

Histopathological analysis: A: Liver: observe the lobular and inflammatory cells infiltrate; B: Gut: note the inflammatory cells and destruction of the villi; C: Kidney: an intense capillary tuft retraction is observed; D: Lung: observe the inflammatory cell infiltrate in the alveolar septa and inside the bronchus.

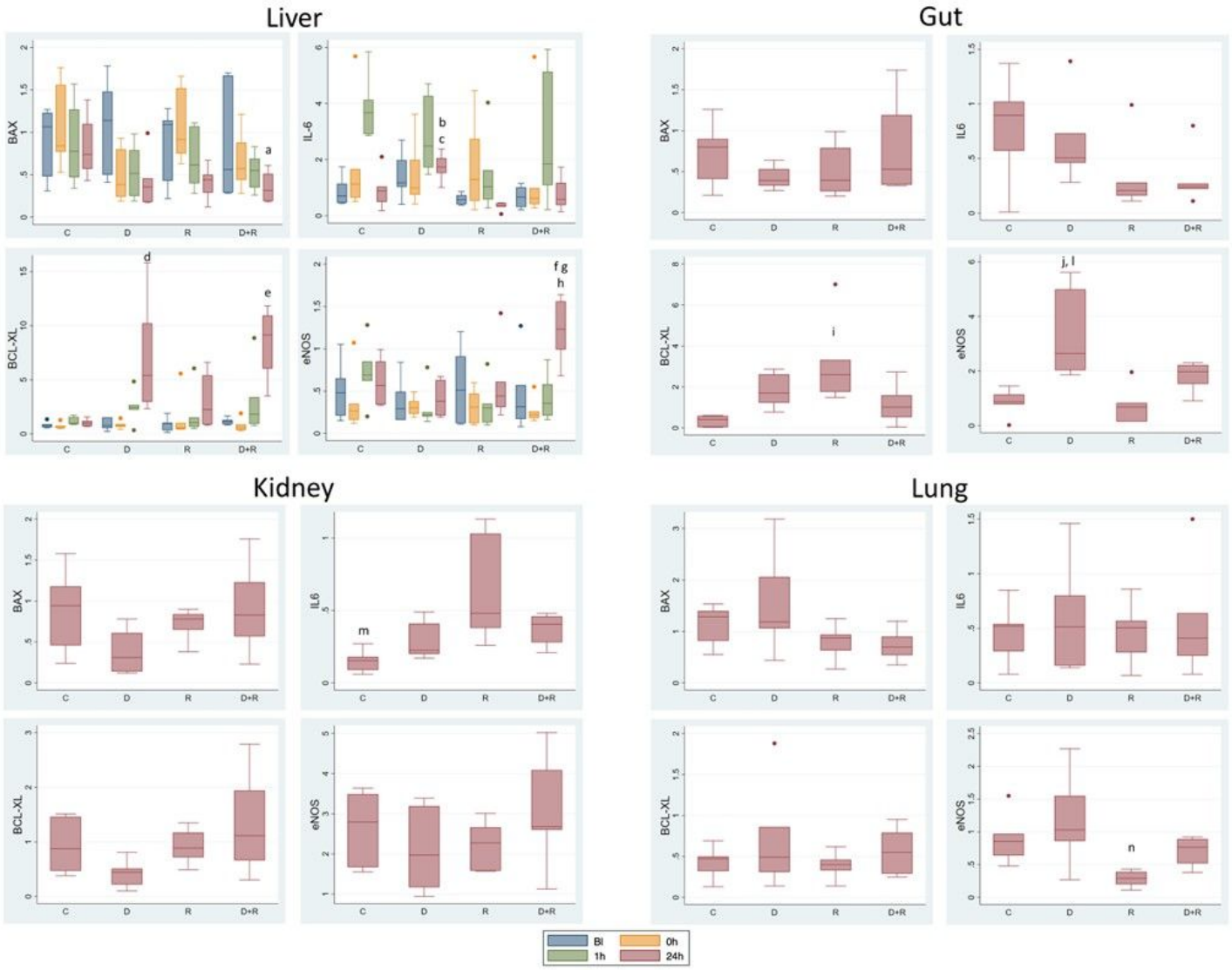

Figure 4

Expression of BAX, IL-6, Bcl, and e-NOS genes in the liver, gut, kidney, and lung tissues, in different groups by time point. Liver - BAX gene: C group vs. D+R group, p=0.039; IL-6 gene: D group vs. R group, p=0.001; D group vs. D+R group, p=0.020; gene BCL-XL: C group vs. D group, p=0.034; C group vs. D+R group, p=0.006; eNOS gene: C group vs. D+R group, p=0.031; D group vs. D+R group, p=0.003; R group vs. D+R group, p=0,021. Gut - BCL-XL gene: C group vs. R group, p=0.004; eNOS gene: C group vs. D group, p=0,001; D group vs. R group, p=0.001; Kidney - IL6 gene: C group vs. R group, p=0.004. Lung - eNOS gene, D group vs. R group, p=0.007.

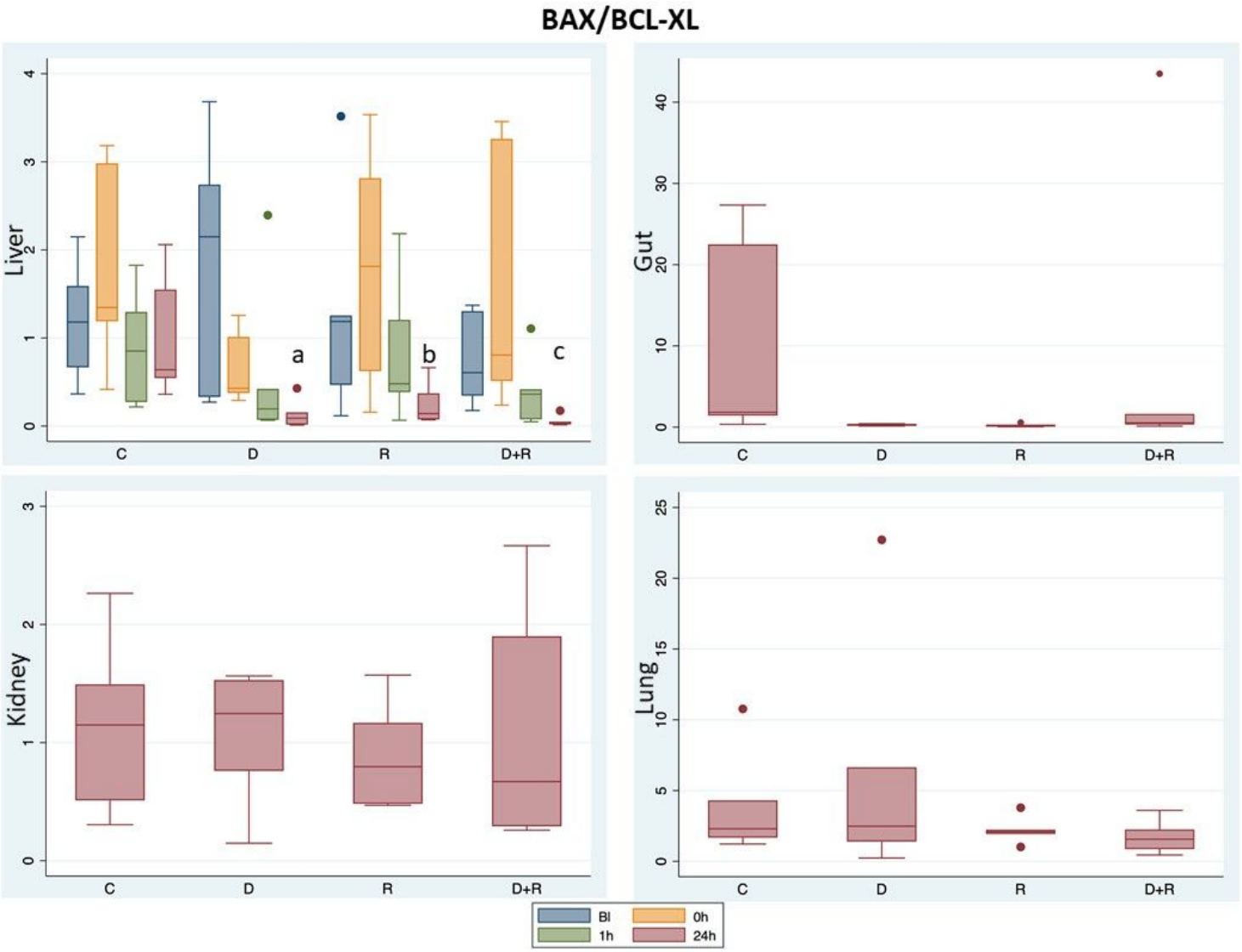

Figure 5

BAX/Bcl ratio in the liver, gut, kidney, and lung tissues, in different groups by time point. C group vs. D group, p=0.005; C group vs. R group, p=0.017; C group vs. D+R group, p=0.002. Note the outliers.

Supplementary Files

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