King's Research Portal

DOI:

[10.1016/j.jss.2017.07.038](https://doi.org/10.1016/j.jss.2017.07.038)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Fantozzi, E. T., Breithaupt-Faloppa, A. C., Ricardo-da-Silva, F. Y., Rodrigues-Garbin, S., Romero, D. C., da Silva Rodrigues, A., Riffo-Vasquez, Y., & Tavares-de-Lima, W. (2018). Estradiol mediates the long-lasting lung inflammation induced by intestinal ischemia and reperfusion. *Journal of Surgical Research*, 221, 1-7. <https://doi.org/10.1016/j.jss.2017.07.038>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Estradiol mediates the long-lasting lung inflammation induced by intestinal ischemia and reperfusion

Short Title: Intestinal IR alters lung homeostasis

Evelyn Thais Fantozzi¹, Ana Cristina Breithaupt-Faloppa², Fernanda Yamamoto Ricardo-da-Silva², Sara Rodrigues-Garbin¹, Daniel Cancelli Romero¹, Adriana da Silva Rodrigues¹, Yanira Riffio-Vasquez,³ Wothan Tavares-de-Lima¹.

¹Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil, ²Laboratory of Cardiovascular Surgery and Physiopathology of Circulation (LIM-11), Heart Institute (InCor), Medicine School of University of São Paulo; ³Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Sciences, King's College London

Words: 2,237

Corresponding Author: Wothan Tavares-de-Lima, PhD
Laboratório de Fisiopatologia da Inflamação Experimental
Instituto de Ciências Biomédicas
University of São Paulo
Av. Prof. Lineu Prestes, 1524
05508-900 São Paulo, Brazil.
e-mail: wtavares@usp.br

Contribution of the authors:

ET Fantozzi: Contributed to the experimental design, performed experiments, analyzed data and contributed to the writing of the manuscript. AC Breithaupt-Faloppa: performed experiments, analyzed data and contributed to the discussion of the results. FY Ricardo-Silva: performed experiments. S Rodrigues-Garbin: performed experiments. DC Romero: performed experiments. AS Rodrigues: performed experiments. Y Riffio-Vasquez: Contributed to the discussion of the results and writing of the manuscript. W Tavares-de-Lima: Contributed to the experimental design, discussion of the results and writing of the manuscript.

Key words: intestinal ischemia and reperfusion, 17 β -estradiol, lung, inflammation, cytokines, uric acid.

Abstract

Background: Lung inflammation is one of the main consequences of intestinal ischemia reperfusion (intestinal IR) and, in severe cases, can lead to acute respiratory distress syndrome (ARDS) and death. We have previously demonstrated that estradiol exerts a protective effect on lung edema and cytokine release caused by intestinal IR in male rats.

Materials and Methods: We investigated the role of estradiol on the generation of IL-1 β , IL-10, VEGF, and CINC-1 in a female rat model of intestinal IR. Blood and bone marrow leukocytes were also quantified. Seven-days-ovariectomized (OVx) rats were subjected to intestinal IR by occlusion of the superior mesenteric artery for 45 min. After re-perfusion of the tissue for 2 hours, the rats were sacrificed. Lung tissue was collected, cultured for 24 hours and assayed.

Results: We observed a significant increase in serum levels of IL-10, CINC-1, uric acid and circulating, but not bone marrow, leukocyte numbers. In addition, intestinal IR induced a significant increase in the *ex-vivo* lung levels of IL-1 β , IL-10 and VEGF. Treatment with 17 β -estradiol before the induction of intestinal IR prevented the systemic release of IL-10, CINC-1 and uric acid, but it did not affect the leukocytosis. In addition, 17 β -estradiol significantly prevented the *ex-vivo* release of IL-1 β and VEGF from lung tissue.

Conclusions: We demonstrated that intestinal IR interferes with lung homeostasis, priming the tissue to generate pro-inflammatory mediators for at least 24 hours post-ischemia. Furthermore, our data confirm that the inflammatory responses caused by intestinal IR are estradiol mediated.

Introduction

Intestinal ischemia and reperfusion (IR) is a life-threatening medical disorder characterized by the systemic release of a wide spectrum of inflammatory mediators and oxygen radicals, thus activating the innate immune response and resulting in local and remote organ dysfunction ^{1, 2, 3}. During ischemia, low tissue oxygenation leads to an energy imbalance whereby the Adenosine Triphosphate/Adenosine Monophosphate (ATP/AMP) ratio is shifted to increased levels of AMP that, in turn, are catabolized to hypoxanthine and uric acid ⁴. The release of uric acid is elevated after an ischemic event and hence, is considered an indicator of low oxygenation of the gut tissue ⁵. Uric acid is also a well-recognized stimulus of the innate and adaptive immune response ^{6, 7}.

Acute lung inflammation is a major consequence of intestinal IR and is characterized by leukocyte migration, increased microvascular permeability and release of pro-inflammatory mediators. In severe cases, this inflammation can lead to acute respiratory distress syndrome (ARDS), multiple organ failure and death. Despite advances in pharmacological therapies, the mortality rate of ARDS is still high (around 40%), indicating a need for a better understanding of its underlying mechanisms ^{8, 9}. We have previously demonstrated that ovariectomized rats develop a significantly higher lung microvascular permeability and neutrophil influx after intestinal IR in comparison to female rats with intact ovaries. Interestingly, the replenishment of these ovariectomized rats with 17 β -estradiol prevented the increase of lung microvascular permeability by a nitric oxide-mediated mechanism, but it did not affect neutrophil migration into the lung ¹⁰. These results suggest that endogenous estrogens protect female rats against some of the inflammatory repercussions of intestinal IR. Moreover, we have also shown that treatment with 17 β -estradiol protects male rats against lung injury induced by intestinal IR, confirming the important role of female sex hormones in this process ¹¹. Estradiol has also been shown to be protective in experimental models of ischemia-reperfusion in other organs such as heart ^{12, 13}, brain ^{14, 15}, kidney ¹⁶, and liver ^{17, 18}.

1 In this study, we sought to investigate the role of estradiol on the systemic release of inflammatory
2 mediators after two hours of intestinal reperfusion in female rats, and on the *ex-vivo* release of
3 mediators by lung tissue after 24 hours of culture. To this purpose, we measured estradiol
4 modulation of the release of serum and tissue cytokines, the number of leukocytes in the bone
5 marrow and blood compartment, and the generation of uric acid in an ovariectomized rat model.
6

Materials and Methods

Animals and study groups

Seven days after ovariectomy, female Wistar rats (180–220 g) from our Department's animal facilities were assigned to three groups: (1) Sham IR: rats surgically manipulated but not submitted to intestinal IR; (2) IR: rats submitted to intestinal IR and (3) IR+E: rats treated with estradiol and 24 hours later submitted to intestinal IR. These studies were approved by the Animal Care Committee of the Institute of Biomedical Sciences, University of Sao Paulo, following the guidelines of the National Council of Animal Experimentation that regulates animal research according to Brazilian Federal Law (Report no. 111/10/03 - 2013).

Ovariectomy (OVx)

Rats were anesthetized with isoflurane (2%) and an incision was made on the lower part of the abdomen; the ovaries were identified and removed free from the adherent tissue. After OVx, the animals received a single dose of Pentantibiotic® (570 mg/kg, i.m.), Tramadol® (5 mg/kg, i.p.) and Paracetamol® (530 µg/ml, in the drinking water *ad libitum* for three days). The effectiveness of the ovariectomy was verified 7 days later by cell patterns in vaginal smears and only rats with smears compatible with diestrus phase (elevated number of leukocytes) were used in these experiments. In addition, at the end of each experiment the weight of the uterus was measured and compared to uteri from rats with intact ovaries. Animals with significant loss in weight of their uterus in comparison to controls were considered successfully ovariectomized.

Intestinal IR model

After 7 days of OVx, the rats were anesthetized with ketamine-xylazine (100 and 20 mg/kg, respectively, intraperitoneally) and the superior mesentery artery was occluded for 45 min., after which the clip was removed and sutures performed to close the abdomen. After two hours of reperfusion all animals were euthanized by an over dose of ketamine-xylazine.

Ex vivo culture of lung tissue

Lungs were perfused through the pulmonary artery with 20 mL of phosphate buffer salt solution (PBS, 30ml/min). Samples of lung parenchyma were cut into small pieces and incubated in 24-well plastic microplates (four pieces per well) containing Dulbecco's Modified Eagle Medium (DMEM) at 37° C in a humidified atmosphere with 5% CO₂. Aliquots of the supernatants were collected after 24 hours of incubation for cytokine quantification.

Quantification of IL-1 β , IL-10, VEGF and CINC-1

The levels of interleukin (IL) 1 β , 10, vascular endothelial growth factor (VEGF) and of cytokine-induced neutrophil chemoattractant 1 (CINC-1) were determined in serum by Milliplex[®] commercial ELISA kit (Merck Millipore, EUA). The concentration of IL-1 β , IL-10 and VEGF were quantified using Duo Set[®] commercial ELISA kit (R&D System, USA) in aliquots of DMEM collected from lung tissue cultures. All assays were conducted following the specifications of the manufacturer.

Circulating blood leukocytes and bone marrow cell numbers

Peripheral blood leukocytes were collected from the tail vein before and after induction of intestinal IR and mixed in Turk's solution (1:20). Total cells from bone marrow were obtained by flushing the femur with a syringe containing 10 mL of PBS. The suspension was then placed in a solution of violet crystal 0.2% (1:10). Cells were quantified using optical light microscopy with a Neubauer chamber.

Quantification of serum levels of uric acid

Uric acid concentrations in serum were determined colorimetrically, using a commercial kit according to the specification of the manufacturer (Bioclin/Quibasa, Minas Gerais, Brazil).

Pharmacological treatment

Ovariectomized rats (OVx) were treated with a single subcutaneous injection of 17 β -estradiol (280 μ g/kg – Sigma-Aldrich, Darmstadt, Germany) diluted in vehicle (Sunflower Oil, Liza, Brazil) 24 hours prior to the induction of intestinal IR.

1 *Statistical analysis*

2 Data are expressed as mean $\pm$ SEM. Comparisons between groups were made by one way, ANOVA
3 followed by Tukey post-test using the GraphPad Prism (version 6.0). Values of $P < 0.05$ were
4 considered significant.

5

1 **Results**

2 *Cytokines levels in serum*

3 To analyze the systemic effects of intestinal IR we determined the levels of cytokines in serum from
 4 blood samples obtained from ovariectomized rats following 2 hours of intestinal reperfusion. As
 5 shown in figure 1, there was a significant increase in IL-10 and CINC-1 levels for IR group of rats
 6 in comparison to Sham IR rats (Figure 1, panels B and D). Pre-treatment with 17 β -estradiol
 7 significantly prevented the increase of these mediators in the serum. Systemic release of IL-1 β and
 8 VEGF was not significantly altered by intestinal IR or 17 β -estradiol (Figure 1, panels A and C).

9 *Cytokines levels in ex-vivo culture of lung tissue*

10 Following 2 hours of intestinal reperfusion, lung samples were collected from the euthanized,
 11 ovariectomized rats. Figure 2 shows the levels of cytokines released by lung tissue after 24 hours of
 12 culture in DMEM. Panels A, B and C demonstrate that lung collected from IR rats produced
 13 significantly higher levels of IL-1 β , IL-10 and VEGF in comparison to samples from Sham IR
 14 animals. *In vivo* treatment of ovariectomized rats with 17 β -estradiol prior to induction of intestinal
 15 IR significantly prevented the release of IL1- β and VEGF but did not affect the release of IL-10 by
 16 lung tissue 24 hours later.

17 *Effect of 17 β -estradiol treatment on leukocytes count following intestinal IR*

18 As shown in Figure 3 (panel A), intestinal IR induced a significant increase in circulating
 19 leukocytes in rats that was not affected by pre-treatment with 17 β -estradiol. When compared to
 20 Sham IR rats, the number of leukocytes in the bone marrow was not significantly affected by
 21 intestinal IR or pre-treatment with 17 β -estradiol (Figure 3, panel B).

22 *Effect of 17 β -estradiol treatment on uric acid levels following intestinal IR*

23 Figure 4 shows that intestinal IR significantly increased the levels of uric acid in the IR group
 24 compared to Sham IR rats. Pre-treatment with 17 β -estradiol significantly prevented the increase of

systemic uric acid after intestinal IR, with uric acid levels for IR+E rats similar to values found in Sham IR animals.

Discussion

In previous studies, we have shown that the lungs of male rats generate inflammatory mediators in response to intestinal IR and that this response can be modulated by treatment with 17 β -estradiol after to the induction of ischemia ¹¹. In this study, we have investigated the effects exerted by estradiol on the lung and systemic inflammation caused by intestinal IR in female ovariectomized rats. We show that intestinal IR induced a significant increase in both the levels of circulating IL-10 and CINC-1 as well as lung tissue levels of IL-1 β , IL-10 and VEGF, measured after 24 hours of culture.

Experimental and clinical evidence indicates that IL-1 β is an important mediator in several models of systemic inflammation ¹⁹, sepsis ^{20, 21}, trauma hemorrhagic shock ²², and intestinal IR ²³. In fact, IL-1 β induces mobilization of leukocytes between compartments and is a marker of inflammasome activation ^{24, 25}. In our OVx rat model, intestinal IR resulted in a significant increase in IL-1 β levels in cultured lung tissue, but not in the systemic circulation. Furthermore, this increase was inhibited by pre-treatment with 17 β -estradiol, indicating that this response is modified by the female sex hormone.

Similar to IL-1 β , when compared to sham IR animals, intestinal IR caused an increase in VEGF levels measured in cultured lung tissue, but not in the circulation. Again, this effect is modified by 17 β -estradiol. Mura et al. (2006) ²⁶, using an intestinal IR model in mice found high levels of VEGF in the lung of both sham and intestinal IR groups after 4 hours of intestinal reperfusion. VEGF has been shown to be important in leukocyte recruitment into the lung during inflammation ^{27, 28, 29} but its role in microvascular permeability is also well recognized. Interestingly, VEGF increases vascular permeability by a nitric oxide mediated mechanism ³⁰. We have previously reported that

estradiol downregulates lung vascular permeability induced by intestinal IR in OVx rats by a mechanism dependent on nitric oxide activity¹⁰.

Taken together, these data demonstrate that intestinal IR causes changes in lung tissue homeostasis that persists for at least 24 hours after intestinal reperfusion. More importantly, our *ex-vivo* lung cultures strongly suggest that the ability of the lung tissue to continuously produce pro-inflammatory mediators is not dependent on further stimulation from the system or the gut. Lung tissue culture is a well-established method for measuring *ex-vivo* ongoing release of mediators from the tissue. However, there are limitations with this method which should be taken into consideration. Among these are the use of a single time-point and the exclusion of other endogenous factors that could influence the response *in vivo*.

Moreover, this study suggests that estradiol differentially regulates the systemic and lung release of cytokines induced by intestinal IR, confirming previous observations of the protective role of estrogen in lung injury in male rats. Thus, we hypothesized that the low levels of estradiol induced by the removal of the ovaries increases the generation/release of inflammatory mediators such as IL-1 β and VEGF, worsening the lung injury induced by intestinal IR. Supporting our hypothesis, estradiol did not affect the release of the anti-inflammatory cytokine IL-10 by the lung tissue, possibly released as an attempt to control the inflammatory response induced in OVx rats³¹.

In this study, we have also found that at reduced levels of estrogen, intestinal IR caused a robust circulating leukocytosis that was not accompanied by significant changes in bone marrow cell numbers. We have also found an increased level of CINC-1 in serum, a potent neutrophil chemotactic agent³² that was significantly inhibited by pre-treatment with estradiol. We have previously reported that estradiol fails to down regulate the migration of neutrophils into the lung of female rats. However, this study suggests that an effect of estradiol on circulating chemotactic factors may occur¹¹. It is important to highlight that even though estrogen does not regulate leukocyte migration into the lung, CINC 1 in the circulation may be having its effects elsewhere³³.

In our model, we found a significant increase in the levels of uric acid in the circulation after 2 hours of reperfusion, indicating a decrease in tissue oxygenation⁵. Furthermore, we found that estradiol had an inhibitory effect on the intestinal IR-induced increase in uric acid, suggesting an interaction between this hormone and uric acid generation. These data allow us to speculate that downregulation of the expression of danger signals may be one of the mechanisms by which estrogen protects females against lung damage induced by intestinal IR. Supporting our findings, Mumford et al. (2013)³⁴ demonstrated that in pre-menopausal women, estradiol reduces uric acid levels, which is associated with the protective effects of estradiol on cardiovascular diseases in this cohort. Noteworthy, Gasse et al. (2009)³⁵ also established a correlation among increased levels of uric acid and activation of inflammasome NLRP3 in a model of acute lung injury in mice.

In conclusion, our study indicates that intestinal IR induces an inflammatory status in the lung that remains for a long period after the mesenteric reperfusion has been re-established. This status is characterized by the release of pro-inflammatory mediators and danger signals. Our study also presents strong evidence that this persistent inflammatory status in the lung, observed after intestinal IR, is downregulated by estrogen in female OVx rats.

Acknowledgments: This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP: 2009-54823-2; 2013-15291-0) and Conselho Nacional de Pesquisa (CNPq). Wothan Tavares de Lima is a fellow researcher of CNPq. The authors are grateful to Helori Vanni Domingos by her technical assistance and Dr. Ricardo Martins Oliveira-Filho by his contribution in the discussion of the results. We acknowledge the support given by Prof. B. Boris Vargaftig, especially regarding the lungs explant method.

References

1. Turnage RH, Guice KS, Oldham KT. Endotoxemia and Remote Organ Injury Following Intestinal Reperfusion. *J Surg Res.* 1994;56(6):571-578. doi:10.1006/jsre.1994.1091.
2. James IAO, Chen C-L, Huang G, Zhang H-Y, Velten M, Besner GE. HB-EGF protects the lungs after intestinal ischemia/reperfusion injury. *J Surg Res.* 2010;163(1):86-95. doi:10.1016/j.jss.2010.03.062.
3. Hsieh Y-H, McCartney K, Moore TA, et al. Intestinal ischemia-reperfusion injury leads to inflammatory changes in the brain. *Shock.* 2011;36(4):424-430. doi:10.1097/SHK.0b013e3182295f91.
4. Oka H. [Xanthine oxidoreductase activity in rat brain tissue: the changes after decapitation]. *No To Shinkei.* 1989;41(6):575-581. <http://www.ncbi.nlm.nih.gov/pubmed/2803824>. Accessed March 30, 2017.
5. Schmidt H, Weigand MA, Li C, Schmidt W, Martin E, Bardenheuer HJ. Intestinal Formation of Hypoxanthine and Uric Acid during Endotoxemia. *J Surg Res.* 1997;71(1):61-66. doi:10.1006/jsre.1997.5098.
6. Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature.* 2003;425(6957):516-521. doi:10.1038/nature01991.
7. Amaro S, Llull L, Renú A, et al. Uric acid improves glucose-driven oxidative stress in human ischemic stroke. *Ann Neurol.* 2015;77(5):775-783. doi:10.1002/ana.24378.
8. Villar J, Blanco J, Añón JM, et al. The ALIEN study: incidence and outcome of acute respiratory distress syndrome in the era of lung protective ventilation. *Intensive Care Med.* 2011;37(12):1932-1941. doi:10.1007/s00134-011-2380-4.
9. Gregová K, Číkoš Š, Bilecová-Rabajdová M, et al. Intestinal ischemia-reperfusion injury mediates expression of inflammatory cytokines in rats. *Gen Physiol Biophys.* 2015;34(1):95-99. doi:10.4149/gpb_2014030.

10. Breithaupt-Faloppa AC, Breithaupt-Faloppa AC, Fantozzi ET, et al. Protective effect of estradiol on acute lung inflammation induced by an intestinal ischemic insult is dependent on nitric oxide. *Shock*. 2013;40(3):203-209. doi:10.1097/SHK.0b013e3182a01e24.
11. Breithaupt-Faloppa AC, Thais Fantozzi E, Romero DC, et al. Acute Effects of Estradiol on Lung Inflammation Due to Intestinal Ischemic Insult in Male Rats. *Shock*. 2014;41(3):208-213. doi:10.1097/SHK.0000000000000092.
12. Wang L, Gu H, Turrentine M, Wang M. Estradiol treatment promotes cardiac stem cell (CSC)-derived growth factors, thus improving CSC-mediated cardioprotection after acute ischemia/reperfusion. *Surgery*. 2014;156(2):243-252. doi:10.1016/j.surg.2014.04.002.
13. Hale SL, Birnbaum Y, Kloner RA. beta-Estradiol, but not alpha-estradiol, reduced myocardial necrosis in rabbits after ischemia and reperfusion. *Am Heart J*. 1996;132(2 Pt 1):258-262. <http://www.ncbi.nlm.nih.gov/pubmed/8701884>. Accessed March 30, 2017.
14. Koh P-O. Estradiol alleviates the ischemic brain injury-induced decrease of neuronal calcium sensor protein hippocalcin. *Neurosci Lett*. 2014;582:32-37. doi:10.1016/j.neulet.2014.08.045.
15. Toung TJ, Traystman RJ, Hurn PD. Estrogen-mediated neuroprotection after experimental stroke in male rats. *Stroke*. 1998;29(8):1666-1670. <http://www.ncbi.nlm.nih.gov/pubmed/9707210>. Accessed March 30, 2017.
16. Tanaka R, Tsutsui H, Kobuchi S, et al. Protective effect of 17 β -estradiol on ischemic acute kidney injury through the renal sympathetic nervous system. *Eur J Pharmacol*. 2012;683(1-3):270-275. doi:10.1016/j.ejphar.2012.02.044.
17. Lin FS, Shen SQ, Chen ZB, Yan RC. 17 β -estradiol attenuates reduced-size hepatic ischemia/reperfusion injury by inhibition apoptosis via mitochondrial pathway in rats. *Shock*. 2012;37(2):183-190. doi:10.1097/SHK.0b013e31823f1918.
18. Burkhardt M, Slotta JE, Garcia P, Seekamp A, Menger MD, Pohlemann T. The effect of estrogen on hepatic microcirculation after ischemia/reperfusion. *Int J Colorectal Dis*. 2008;23(1):113-119. doi:10.1007/s00384-007-0360-5.

19. Clark BD, Bedrosian I, Schindler R, et al. Detection of interleukin 1 alpha and 1 beta in rabbit tissues during endotoxemia using sensitive radioimmunoassays. *J Appl Physiol.* 1991;71(6):2412-2418. <http://www.ncbi.nlm.nih.gov/pubmed/1778940>. Accessed March 30, 2017.
20. Damas P, Reuter A, Gysen P, Demonty J, Lamy M, Franchimont P. Tumor necrosis factor and interleukin-1 serum levels during severe sepsis in humans. *Crit Care Med.* 1989;17(10):975-978. <http://www.ncbi.nlm.nih.gov/pubmed/2791581>. Accessed March 30, 2017.
21. Jacobs RF, Tabor DR. The immunology of sepsis and meningitis--cytokine biology. *Scand J Infect Dis Suppl.* 1990;73:7-15. <http://www.ncbi.nlm.nih.gov/pubmed/2091257>. Accessed March 30, 2017.
22. Roumen RM, Hendriks T, van der Ven-Jongekrijg J, et al. Cytokine patterns in patients after major vascular surgery, hemorrhagic shock, and severe blunt trauma. Relation with subsequent adult respiratory distress syndrome and multiple organ failure. *Ann Surg.* 1993;218(6):769-776. <http://www.ncbi.nlm.nih.gov/pubmed/8257227>. Accessed March 30, 2017.
23. Chang DM, Hsu K, Ding YA, Chiang CH. Interleukin-1 in ischemia-reperfusion acute lung injury. *Am J Respir Crit Care Med.* 1997;156(4 Pt 1):1230-1234. doi:10.1164/ajrccm.156.4.9702095.
24. Kwak A, Lee Y, Kim H, Kim S. Intracellular interleukin (IL)-1 family cytokine processing enzyme. *Arch Pharm Res.* 2016;39(11):1556-1564. doi:10.1007/s12272-016-0855-0.
25. Harris J, Lang T, Thomas JPW, Sukkar MB, Nabar NR, Kehrl JH. Autophagy and inflammasomes. *Mol Immunol.* February 2017. doi:10.1016/j.molimm.2017.02.013.
26. Mura M, Han B, Andrade CF, et al. The early responses of VEGF and its receptors during acute lung injury: implication of VEGF in alveolar epithelial cell survival. *Crit Care.* 2006;10(5):R130. doi:10.1186/cc5042.

- 1 27. Karpaliotis D, Kosmidou I, Ingenito EP, et al. Angiogenic growth factors in the
2 pathophysiology of a murine model of acute lung injury. *Am J Physiol Lung Cell Mol*
3 *Physiol.* 2002;283(3):L585-95. doi:10.1152/ajplung.00048.2002.
- 4 28. Hamada N, Kuwano K, Yamada M, et al. Anti-vascular endothelial growth factor gene
5 therapy attenuates lung injury and fibrosis in mice. *J Immunol.* 2005;175(2):1224-1231.
6 <http://www.ncbi.nlm.nih.gov/pubmed/16002726>. Accessed March 30, 2017.
- 7 29. Hua F, Wang X, Zhu L. Terlipressin decreases vascular endothelial growth factor expression
8 and improves oxygenation in patients with acute respiratory distress syndrome and shock. *J*
9 *Emerg Med.* 2013;44(2):434-439. doi:10.1016/j.jemermed.2012.02.073.
- 10 30. Murohara T, Horowitz JR, Silver M, et al. Vascular endothelial growth factor/vascular
11 permeability factor enhances vascular permeability via nitric oxide and prostacyclin.
12 *Circulation.* 1998;97(1):99-107. doi:10.1161/01.cir.97.1.99.
- 13 31. Lane JS, Todd KE, Lewis MP, et al. Interleukin-10 reduces the systemic inflammatory
14 response in a murine model of intestinal ischemia/reperfusion. *Surgery.* 1997;122(2):288-
15 294. <http://www.ncbi.nlm.nih.gov/pubmed/9288134>. Accessed March 30, 2017.
- 16 32. Lojek A, Milancíz, Slavíková H, et al. Leukocyte mobilization, chemiluminescence response,
17 and antioxidative capacity of the blood in intestinal ischemia and reperfusion. *Free Radic*
18 *Res.* 1997;27(4):359-367. <http://www.ncbi.nlm.nih.gov/pubmed/9416464>. Accessed March
19 30, 2017.
- 20 33. Stringa P, Lausada N, Romanin D, et al. Pretreatment Combination Reduces Remote Organ
21 Damage Secondary to Intestinal Reperfusion Injury in Mice: Follow-up Study. *Transplant*
22 *Proc.* 2016;48(1):210-216. doi:10.1016/j.transproceed.2015.12.002.
- 23 34. Mumford SL, Dasharathy SS, Pollack AZ, et al. Serum uric acid in relation to endogenous
24 reproductive hormones during the menstrual cycle: findings from the BioCycle study. *Hum*
25 *Reprod.* 2013;28(7):1853-1862. doi:10.1093/humrep/det085.
- 26 35. Gasse P, Riteau N, Charron S, et al. Uric acid is a danger signal activating NALP3

- 1 inflammasome in lung injury inflammation and fibrosis. *Am J Respir Crit Care Med*.
- 2 2009;179(10):903-913. doi:10.1164/rccm.200808-1274OC.

3

4

Legends of the figures

Figure 1. Cytokine quantification in serum collected 2h after intestinal IR: IL-1 β (A), IL-10 (B), VEGF (C) and CINC-1 (D). Sham IR were rats manipulated but not subjected to intestinal IR (open bar), IR were subjected to intestinal IR (grey bar) and IR+E were subjected to intestinal IR and treated with estradiol (280 μ g/kg, s.c) 24 hours before ischemia induction (black bar). Data was analyzed by one-way ANOVA and expressed as mean $\pm$ SEM. In panel B: * $p < 0.05$ vs Sham IR; $^{\circ}p < 0.05$ vs IR. In panel D: * $p < 0.05$ vs Sham IR; $^{\circ}p < 0.05$ vs IR.

Figure 2. Cytokine quantification in supernatants of lung tissue collected at 24h of incubation in DMEM. IL-1 β (A), IL-10 (B) and VEGF (C) Lung samples were collected 2h after intestinal IR. Sham IR were rats manipulated but not subjected to intestinal IR (open bar), IR were subjected to intestinal IR (grey bar) and IR+E were subjected to intestinal IR and treated with estradiol (280 μ g/kg, s.c.) 24 hours before ischemia induction (black bar). Data was analyzed by one-way ANOVA and expressed as mean $\pm$ SEM. In panel A: * $p < 0.05$ vs Sham IR; $^{\circ}p < 0.05$ vs IR. In panel B: * $p < 0.05$ vs Sham IR. In panel C: * $p < 0.0001$ vs Sham IR; $^{\circ}p < 0.05$ vs IR.

Figure 3. Cell count determined in peripheral blood (A) and bone marrow (B) collected 2h after intestinal IR. Sham IR were rats manipulated but not subjected to intestinal IR (open bar), IR were subjected to intestinal IR (grey bar) and IR+E were subjected to intestinal IR and treated with estradiol (280 μ g/kg, s.c) 24 hours before ischemia induction (black bar). Data was analyzed by one-way ANOVA and expressed Data expressed as mean $\pm$ SEM from 6 to 8 animals. In A: * $p < 0.05$ vs Sham IR.

Figure 4. Quantification of Uric Acid in serum collected 2h after intestinal IR. Sham IR were rats manipulated but not subjected to intestinal IR (open bar), IR were subjected to intestinal IR (grey bar) and IR+E were subjected to intestinal IR and treated with estradiol (280 μ g/kg, s.c.) 24 hours before ischemia induction (black bar). Data was analyzed by one-way ANOVA and expressed as mean $\pm$ SEM from 6 to 8 animals. * $p < 0.05$ vs Sham IR; $^{\circ}p < 0.05$ vs IR.

Figure 1. Fantozzi et al. 2017

Figure 3 Fantozzi et al., 2017

Figure 4. Fantozzi et al. 2017

Figure 2 Fantozzi et al., 2017