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Evaluation of Distant Organ Effect of Renal Ischemia and Reperfusion with Claudin-5

Claudin-5 ile Renal İskemi ve Reperfüzyonun Uzak Organ Etkisinin Değerlendirilmesi

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ABSTRACT

Purpose: Claudins are the barrier in the cells. They can regulate intracellular permeability, form pores or increase water permeability. They have important effects in determining the permeability of epithelial cells. Mammals have 27 claudins grouped in eight subgroups. The distribution patterns are tissue-specific. They are located in contact areas between the epithelium.

The aim of this study was to determine the results of claudin-5 level of experimental ischemia reperfusion injury in rats kidney and indirect effects of liver, lung and heart.

Materials and Methods: Rats (average 250-300 grams) were divided into two equal groups, 7 rats in each group. The ischemia / reperfusion (IR) group (= experimental group) was administered ischemia to the kidney vessels of rats for 60 min. Sham group did not have ischemia. The rats in both groups were sacrified 24 hours after the occlusion of the rats. The liver, heart and lungs were removed and placed in containers containing 10% formalin.

Slayts were evaluated with light microscope. Cytoplasmic membrane staining was considered positive. Four grades were evaluated.

Results: Statistically, when sham and experimental group were compared in terms of staining intensity, moderate staining was found to be significantly lower in the experimental group (P = 0.031).

It was noted that the stain of the sham group in the lungs and heart was greater than experimental group.

Conclusion: In experimental renal IR injury, claudin-5 level in the lung and heart were more affected than in the liver.

Keywords: Ischemia, Liver, Lung, Heart, Claudin Proteins

ÖZ

Amaç: Claudinler, hücrelerdeki bariyerlerdir. Hücre içi geçirgenliği düzenleyebilir, gözenekler oluşturabilir veya su geçirgenliğini artırabilirler. Epitel hücrelerinin geçirgenliğinin belirlenmesinde önemli etkileri vardır. Memeliler, sekiz alt grupta toplanmış 27 claudin'e sahiptir. Dağılım paternleri dokuya özgüdür. Epitel arasındaki temas alanlarında bulunurlar.

Bu çalışmanın amacı, rat böbreklerinde deneysel iskemi reperfüzyon hasarının karaciğer, akciğer ve kalp üzerine indirekt etkilerinin Claudin-5 düzeyindeki sonuçlarını tespit etmektir.

Gereç ve Yöntem: Ratlar (ortalama 250-300 gram) her grupta 7 rat olmak üzere iki eşit gruba ayrıldı. İskemi / reperfüzyon (IR) grubuna (= deney grubu) 60 dakika boyunca böbrek damarlarına iskemi uygulandı. Sham grubuna deney uygulanmadı. Her iki gruptaki ratlar, ratların oklüzyonundan 24 saat sonra sakrifiye edildi. Karaciğer, kalp ve akciğerler çıkarıldı ve % 10 formalin içeren kaplara yerleştirildi.

Hazırlanan lamlar ışık mikroskobu ile değerlendirildi. Sitoplazmik membran boyanma pozitif olarak kabul edildi. Dört derecede değerlendirildi.

Bulgular: İstatistiksel olarak, sham ve deney grubu boyanma yoğunluğu açısından karşılaştırıldığında, deney grubundaki orta derecede boyanmanın anlamlı derecede düşük olduğu bulundu (P = 0,031).

Sham grubunun akciğer ve kalpteki boyanma oranının deney grubundan daha yüksek olduğu belirtildi.

Sonuç: Deneysel böbrek IR yaralanmasında akciğerde ve kalpte claudin-5 düzeyi karaciğerden daha fazla etkilenmiştir.

Anahtar Sözcükler: claudin-5, karaciğer, akciğer, kalp, iskemi

Introduction

Ischemia can be defined as inadequate blood flow to tissues due. to obstruction of the arterial system. It started to attract attention at the beginning of the 19th century. In the last 30 years, very effective findings have been detected. During ischemia, the knowledge about molecular, cellular, tissue-specific and systemic events has greatly increased. Evidence has been discovered that reperfusion induces necrosis and also increases the severity of damage (1).

Research on reperfusion has provided great acceleration. Because this compound of tissue damage is suitable for therapeutic intervention (1,2).

The degree of dysfunction in the cell varies according to the duration and severity of ischemia. Thus, revascularization and restoration of blood flow as soon as possible is the basis of all current therapeutic approaches to ischemia. However, since the organs of the organs are different, their response to ischemia varies. In addition, oxygen and nutrients need to reach tissues for the continuation of cell metabolism. However, it is clear that reperfusion may result in pathogenic processes that exacerbate injuries caused by ischemia and can lead to tissue damage. Reperfusion causes the formation of mediators in the bloodstream causing damage to distant organs (2). Despite years of intensive research, the underlying mechanisms of ischemia and reperfusion injury have not been fully explained (1,2).

Contact points between cells are required for many functions (3). These contact points are important in the formation of epithelial layers (4). Tight junctions (TJs), adhesion points, gap connections and desmosomes are cell membrane structures involved in cell-cell contact (4,5).

TJs are one of the special intracellular binding complexes that mediate adhesion between epithelial cells (6).

TJ is a specific membrane site located in the apical region of epithelial and endothelial cells. It is also necessary to achieve the paracellular transport of the soluble substances, as well as the diffusion of lipids and proteins, and cell polarization (7).

The claudin protein family, which is located in the transmembrane region, has a very important role in TJ formation. These proteins consist of approximately 27 members (8,9).

Claudin-5 is a four-transmembrane protein of 23 kilodaltons, known to form TJs between endothelial cells (10).

Claudin-5 is often expressed in tight junctions of pancreatic cells, alveolar cells in lung, epithelial cells in colon and endothelial cells forming the blood-brain barrier and endoneural blood-nerve barrier. In colonic regions, the expression is mainly related to the paracellular sealing of TJs (11). Claudin-5 and its redistribution and downregulation may alter the structure of TJs leading to barrier dysfunction in active inflammatory disease (11).

Hassoun et al. reported that distant organ injury induced by renal ischemia-reperfusion (IR) injury is strongly associated with activated leukocyte activation and systemic inflammatory responses during the reperfusion phase (12).

In this study, we tried to demonstrate the differences in claudin-5 expression due to renal ischemia and reperfusion injury in the lung, liver and heart.

Material and method

This study was evaluated and accepted by the Animal Ethics Committee of the Faculty of Medicine (Date: 30.01.2018, Issue: 02).

Rats (250-300 g) were divided into two equal groups, 7 rats in each group. The IR group (= experiemental group) was subjected to renal ischemia by obstruction of kidney vessels of rats for 60 min. Sham group was not subjected to ischemia. The rats in both groups were sacrified 24 hours after the occlusion of the vessels. The liver, heart and lungs were removed and placed in containers containing 10% formalin.

Samples were taken from the tissues and then sections with a thickness of 5µ were taken on the poly- laminated slide. Prepared for immunohistochemical study. A Leica Bond-Max IHK staining device (Vision Biosystems, Melbourne, Australia) was used for the immunohistochemical study. It was stained with Claudin-5 (Epitomics (AC-0212A), 0.1ml (1:100).

Slides were evaluated with light microscope. Cytoplasmic membrane staining was considered positive. Four grades were evaluated. 0 staining (no staining), 1 + (mild) (1% to 10%) staining, 2 + (moderate) staining (11% to 50%), 3+ (severe) staining (over 50%) were evaluated (Figures 1-2).

Statistical analysis

The techniques used to evaluate the differences between sham and experimental groups were Mann-Whitney U-shaped with the Shapiro Wilk test. P value of 0.05 and above was considered statistically significant. SPSS 21.0 package program was used for statistical analysis.

Results

Staining intensity in the heart was mild (14.3%, 71.4%) and moderate (85.7%, 28.6%) in sham and experimental groups, respectively. Intense staining was not observed.

When sham and experimental group were compared in terms of staining intensity, moderate staining was found to be significantly lower in the experimental group (P = 0.031).

Staining intensity in the lung was moderate (14.3%, 42.9%) and severe (85.7%, 57.1%) in the sham and experimental groups, respectively.

There was no significant difference in sham and experimental group when compared with the intensity of staining (P = 0.237).

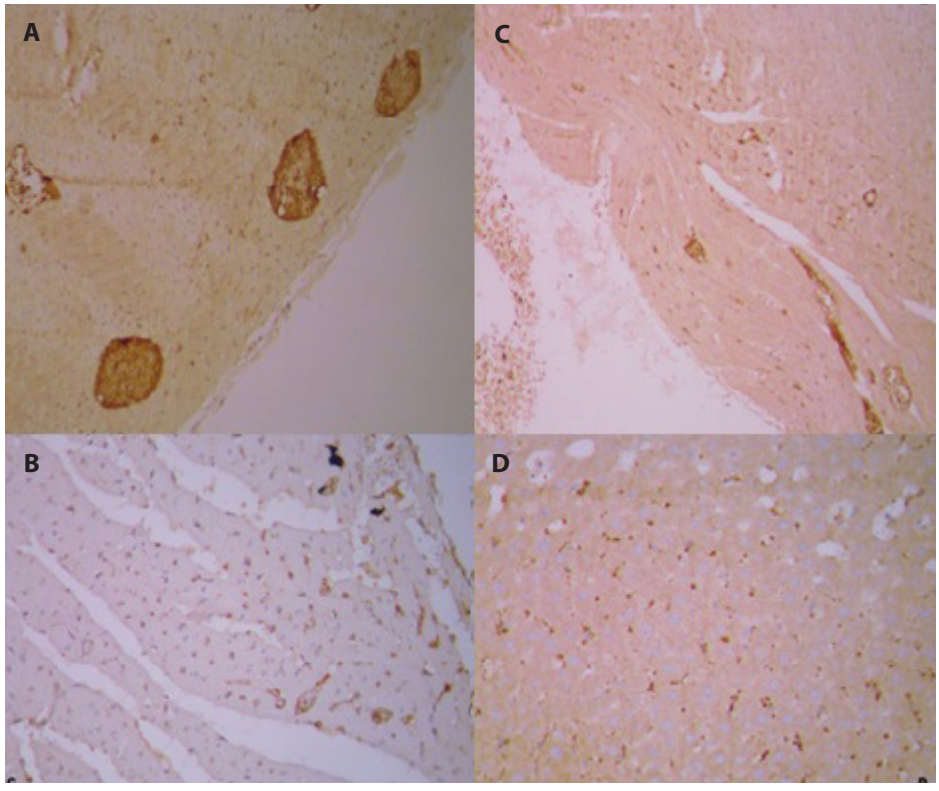

Figure 1. **A.** In the heart, moderate grade claudin 5 expression, sham group (claudin-5 x 200), **B.** In the heart , mild grade claudin 5 expression, experimental group (claudin-5 x 200), **C.** In the liver, moderate grade sinusoidal claudin 5 expression, experimental group (claudin-5 x 400), **D.** In the liver, severe grade claudin 5 expression, sham group (claudin-5 x 200).

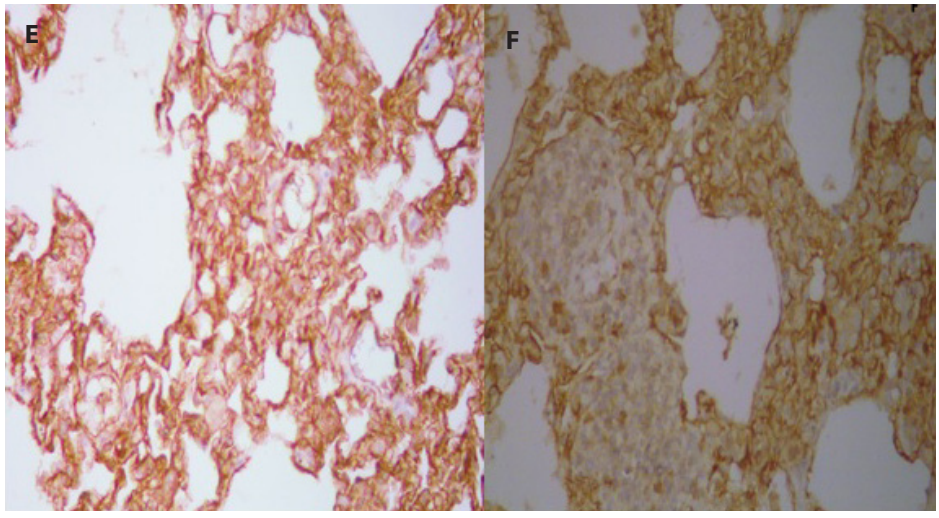

Figure 2. **E.** In the lung, Severe grade, claudin 5 expression, sham group (claudin-5 x 400), **F.** In the lung, moderate grade claudin 5 expression, experimental group (claudin-5 x 400).

Staining intensity in liver was moderate (14.3%, 0%) and severe (85.7%, 100%) in the sham and experimental groups, respectively. The degree of staining in the liver was found to be moderate or severe.

No significant difference was determined in the sham and experimental group in terms of intensity of staining grade (P = 0.299) (Table 1).

Discussion

Renal IR injury produces tissue damage in many distant organs such as the lung, liver and heart (13,14). In many studies, it has been shown that reactive oxygen radicals (ROS) are produced during IR. Exposure to oxygen may also damage biomembranes

Table 1. Sham and experimental group in terms of intensity of staining grade

		Sham		experimental		
		n	%	n	%	P
Heart	1	1	14,3	5	71,4	0.031
	2	6	85,7	2	28,6	
Lung	2	1	14,3	3	42,9	0.237
	3	6	85,7	4	57,1	
Liver	2	1	14,3	0	0,0	0.299
	3	6	85,7	7	100,0	

and enzyme proteins as well as cell apoptosis and may support leukocyte-endothelial cell adherence (15). ROS and nitric oxide (NO) mediate cell damage during IR injury. Inflammation is an important contributor to the pathogenesis of IR for certain cells, adhesion molecules and cytokines (16).

One of the distant organ damage caused by IR is liver injury. Acute renal failure due to liver disease is a common clinical etiology (17).

It is believed that IR injury plays an important role in reactive oxygen and nitrogen species in the pathophysiology of renal injury, leading to inflammatory response resulting in tissue damage in many organs (18).

Liver redox status appears to be impaired by reperfusion for at least 8 hours, as in the kidney. Prolonged reperfusion time has been reported to be the main injury factors (15).

In a study with transmission electron microscopy, first group (1 hour reperfusion after 1 hour ischemia) showed incompatibility of intercellular conjunctions. Second group (4-hour reperfusion), intracellular connections disappeared and hepatocytes fused to basal cytoplasmic membrane structures (15). Third group (8-hour reperfusion), when the membrane structure was present, the binders were almost lost (15).

In this study, renal ischemia by obstruction of kidney vessels of rats were exposed to ischemia for 1 hour and sacrified after 24 hours. No comparison was made between ischemic groups since longer ischemic groups were not established.

In this study, it was noticed that the expression of claudin-5 on the liver sham group and experimental group did not differ.

In studies with immunoblot analysis or immunofluorescence microscopy, studies have shown that claudin 1, 2, 3 and 5 are excreted in the liver but no cell-specific differences are reported. In these studies, Claudin 5 was shown only in the combination of endothelium in portal veins and hepatic artery. Claudin 5 was not detected in sinusoidal endothelial cells, but it was not found to be absent (10,19). In this study, it was noticed that the expression of claudin-5 did not change among the groups, and sinusoidal staining was associated with it. This finding seems different from the results of previous studies (10,19).

Claudin 1, 3, 4, 5, 7, 8 and 18 have been reported to be expressed in human bronchi and bronchioles (20).

In this study, the prominence of claudin-5 expression in the lungs was noted in alveoli. Different staining intensities were observed between the experimental group and the sham group. These differences were reduced in the experimental group.

Claudin 5 is reported to cause epithelial leakage when overexpressed in bronchial transplant cells. Claudin 3 and 5 in rat alveolar cells have been associated with leakier phenotypes. Paradoxically, claudin 5 has been claimed to cause barrier loosening of both bronchial and alveolar cells (21-23).

In rat alveolar cells claudins 3 and 5 have been associated with a leakier phenotype. Paradoxically, claudin 5 causes both the bronchial and alveolar cells to loosen the barrier (21-23). Oxidative stress may be a factor that causes relaxation of mesothelial barrier due to many diseases such as inflammation. In a recent report on this subject, it was shown that claudin 1, 3, 5 and 7 levels decreased due to pleural inflammation and claudin 2 increased in mesothelial cells (24).

We can say that the barrier weakness in the lung alveolar increases as a result of renal ischemia and reperfusion.

In this study, the effect of renal ischemia and reperfusion on the distribution of claudin-5 on the heart was evaluated. It was observed that pericardial staining was more intense. Different staining intensities were observed between the sham group and the experimental group. It was observed that the claudin-5 level decreased in the study group.

Cardiomyocyte loss is known to be important in the pathogenesis of congestive heart failure. There may be a decrease in cardiac function due to an increase in cardiac apoptosis after renal ischemia (25).

Studies have shown that claudin-5 is localized in normal cardiomyocytes and endothelial cells, and it has also been shown that the protein of claudin-5 is dramatically reduced in heart failure. In recent publications it has been claimed that the reduction of claudin-5 level occurs before the onset of cardiac damage and the presence of claudin-5 in a mouse model prevents the onset of cardiomyopathy. It has been described that in the light of this spectrum, with the prevalence of claudin-5 reduction in human heart failure, claudin-5 may be useful in preventing cardiomyopathy early in its progression (26-28).

Claudin-5 is only one of the four genders that have been found hypermethylated and have had a failure in failure against unsuccessful human hearts. It is stated that future epigenetic studies of Claudin-5 gene regulation may provide a basis for the development of novel therapeutics for heart failure (28).

TJ proteins are known to contain redox-sensitive proteins. With the increase of oxidative stress, the expression of claudine decreased and consequently the membrane localization was decreased and the joint barriers were loosened (29).

Conclusion

The reduction in expression of claudin-5 in distant organs may be attributed to the systemic effects of ROS.

The effect of claudins on permeability is an important factor in many systemic diseases. Lung, liver and heart disease have many pathogenic effects. The effect of these pathogens on the expression of claudin or its effect on cell distribution is still unclear and more research is needed. The change in the expression of Claudin plays an important role in most cases. Claudins affecting through manipulation of alveolar, cardiomyocyte or endothelial permeability may be a future target in the treatment of distant organ injury.

AUTHOR CONTRIBUTIONS:

Concept: HE; **Design:** EC, HE; **Supervision:** AK, MAÇ; Fundings: AK; **Materials:** HE, EÇ; **Data Collection and/or Processing:** AK; **Analysis and/or Interpretation**: HE; **Writing Manuscript**: AK; **Critical Review:** HE.

Ethics Committee Approval: Animal Ethics Committee of theFaculty of Medicine (Date: 30.01.2018, Issue: 02).

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References

1. O'Donnell CJ, Nabel EG. Genomics of cardiovascular disease. N Engl J Med. 2011;365(22):2098-109.
2. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury.Int Rev Cell Mol Biol, 298(2012) 229.
3. Gumbiner BM. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell. 1996;84(3):345-57.
4. Shin K, Fogg VC, Margolis B. Tight junctions and cell polarity. Annu Rev Cell Dev Biol 2006; 22: 207-235.
5. Dejana E, Del Maschio A. Molecular organization and functional regulation of cell to cell junctions in the endothelium. Thromb Haemost. 1995;74(1):309-12.
6. Gumbiner B, Stevenson B, Grimaldi A. The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. J Cell Biol. 1988;107(4):1575-87.
7. Elkouby-Naor L, Ben-Yosef T. Functions of claudin tight junction proteins and their complex interactions in various physiological systems. Int Rev Cell Mol Biol. 2010;279:1-32
8. Lal-Nag M, Morin PJ. The claudins. Genome Biol. 2009;10(8):235.
9. Krause G, Winkler L, Piehl C, Blasig I, Piontek J, Müller SL. Structure and function of extracellular claudin domains. Ann N Y Acad Sci. 2009;1165:34-43.
10. Morita K, Sasaki H, Furuse M, Tsukita S. Endothelial claudin: claudin-5/TMVCF constitutes tight junction strands in endothelial cells. J Cell Biol. 1999 4;147(1):185-94.
11. Zeissig S, Bürgel N, Günzel D, Richter J, Mankertz J, Wahnschaffe U et al. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. Gut, 56 (2007) 61.
12. Hassoun HT, Grigoryev DN, Lie ML, Liu M, Cheadle C, Tuder RM et al. Ischemic acute kidney injury induces a distant organ functional and genomic response distinguishable from bilateral nephrectomy. Am J Physiol Renal Physiol. 2007;293(1):F30-40.
13. Kelly KJ: Distant effects of experimental renal ischemia/ reperfusion injury. J Am Soc Nephrol. 2003;14(6):1549-58.
14. Gulec B, Coskun K, Yigitler C, Yigit T, Aydin A, Oner K .Ischemia-reperfusion injury in the liver during renal transplantation: does perfusion solution play any role? Transplant Proc. 2008;40(1):59-62.
15. B. Wang, M. Bai, Y. Bai, Q. Li. Liver Injury Following Renal Ischemia Reperfusion in Rats. Transplant Proc. 2010;42(9):3422-6.
16. Ysebaert DK, De Greef KE, De Beuf A, Van Rompay AR, Vercauteren S, Persy VP et al. T cells as mediators in renal ischemia/reperfusion injury. Kidney Int. 2004;66(2):491-6.
17. Melin J, Hellberg O, Akyürek LM, Källskog O, Larsson E, Fellström BC. Ischemia causes rapidly progssive nephropathy in the diabetic rat. Kidney Int. 1997;52(4):985-91.
18. Erdogan H, Fadillioglu E, Yagmurca M, Uçar M, Irmak MK. Protein oxidation and lipid peroxidation after renal ischemia-reperfusion injury: protective effects of erdosteine and N-acetylcysteine. Urol Res. 2006;34(1):41-6.
19. Furuse M, Fujita K, Hiiragi T, Fujimoto K,Tsukita S. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol. 1998;141(7):1539-50.
20. Ylermi Soini. Claudins in lung diseases. Respir Res. 2011 27;12:70.
21. Coyne CB, Gambling TM, Boucher RC, Carson JL, Johnson LG. Role of claudin interactions in airway tight junctional permeability. Am J Physiol Lung Cell Mol Physiol. 2003;285(5):L1166-78
22. Nitta T, Hata M, Gotoh S, Seo Y, Sasaki H, Hashimoto N et al. Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. J Cell Biol. 2003;161(3):653-60.
23. Chen SP, Zhou B, Willis BC, Sandoval AJ, Liebler JM, Kim KJ et al. Effects of trans differentiation and EGF on claudin isoform expression in alveolar epithelial cells. J Appl Physiol (1985). 2005;98(1):322-8.
24. Markov AG, Voronkova MA, Volgin GN, Yablonsky PK, Fromm M , Amasheh S. Tight junction proteins contribute to barrier properties in human pleura. Respir Physiol Neurobiol. 2011;175(3):331-5.
25. Wencker D, Nguyen KT, Khine CC, Chandra M, Garantziotis S, Ng K et al. Myocyte apoptosis is sufficient to cause dilated cardiomyopathy. Circulation. 1999;100(suppl I):I-17.
26. Delfín DA, Xu Y, Schill KE, Mays TA, Canan BD, Zang KE et al. Sustaining cardiac claudin-5 levels prevents functional hallmarks of cardiomyopathy in a muscular dystrophy mouse model. Mol Ther. 2012;20(7):1378-83.
27. Koczor CA, Lee EK, Torres RA, Boyd A, Vega JD, Uppal K et al. Detection of differentially methylated gene promoters in failing and nonfailing human left ventricle myocardium using computation analysis. Physiol Genomics. 2013;45(14):597-605.
28. Swager SA, Delfín DA, Rastogi N, Wang H, Canan BD, Fedorov VV et al. Claudin-5 levels are reduced from multiple cell types in human failing hearts and are associated with mislocalization of ephrin-B1. Cardiovasc Pathol. 2015 May-Jun;24(3):160-167.
29. Manish Mittal, Mohammad Rizwan Siddiqui, Khiem Tran, Sekhar P. Reddy, Asrar B. Malik. Reactive Oxygen Species in Inflammation and Tissue Injury. Antioxid Redox Signal. 2014;20(7):1126-67.