10.5152/turkjnephrol.2020.4015

Original Article

Turkish Journal of Nephrology

Comparison of the Preventive Effects of Carvedilol and Nebivolol on Kidney Ischemia Reperfusion Injury

Murat Küçük[1], Özer Ural Çakıcı[2], İpek Işık Gönül[3], Mustafa Kavutçu[4], Selim Turgay Arınsoy[5]

[1]Unit of Intensive Care, Dokuz Eylül University School of Medicine, İzmir, Turkey
[2]Department of Urology, Medical Park Ankara Hospital, Ankara, Turkey
[3]Department of Pathology, Gazi University School of Medicine, Ankara, Turkey
[4]Department of Biochemistry, Gazi University School of Medicine, Ankara, Turkey
[5]Department of Nephrology, Gazi University School of Medicine, Ankara, Turkey

Abstract

Objective: To compare the ameliorating effects of carvedilol and nebivolol on kidney Ischemia/Reperfusion Injury (IRI).
Materials and Methods: A total of 24 rats were separated into 4 groups. The experimental model group and 2 treatment groups underwent right nephrectomy and left ischemia/reperfusion. The sham group underwent laparotomy. Carvedilol or nebivolol was administered between the first and sixth days to the treatment groups. On the sixth day, an experimental IRI model was created by right nephrectomy and left renal arterial clamping, followed by reperfusion. After reperfusion, kidney function markers and kidney injury molecule levels were determined, histological examinations were carried out, a tissue oxidative product level, and the antioxidant enzyme activities were measured.
Results: All groups showed similar basal results. The nebivolol and carvedilol groups did not show differences in the kidney functions and the injury molecules vs. the experimental model group. Only the carvedilol group's Kidney-Injury-Molecule-1 (KIM-1) levels showed statistically significant amelioration. The carvedilol and nebivolol groups only showed significant effect on the tissue with Glutathione S-Transferase activity. The carvedilol group showed attenuated tubular congestion compared with the experimental model group. Other histological findings were similar between all groups.
Conclusion: In our study, both carvedilol and nebivolol failed to show any benefits against IRI on a single-kidney experimental model in terms of routine kidney function tests, Malondialdehyde levels, Superoxide Dismutase and Catalase activities, and histological findings. Both molecules had favorable effects on the tissue with Glutathione S-Transferase activity. Carvedilol showed a better ameliorating effect on urinary KIM-1 levels and histological tubular congestion scores.
Keywords: Antihypertensive agents, carvedilol, ischemia/reperfusion, kidney, nebivolol, single kidney

Corresponding Author: Özer Ural Çakıcı ✉ ozerural@hotmail.com **Received:** 25.06.2019 **Accepted:** 22.07.202 **Available Online Date:** 05.11.2020

Cite this article as: Küçük M, Çakıcı ÖU, Işık Gönül İ, Kavutçu M, Arınsoy ST. Comparison of the Preventive Effects of Carvedilol and Nebivolol on Kidney Ischemia Reperfusion Injury. Turk J Nephrol 2021; 30(1): 9-16.

INTRODUCTION

Ischemia is defined as compromised or totally lost tissue perfusion, which leads to a lack of oxygen in the tissues. The inevitable result of ischemia is cellular damage, either reversible or irreversible (1, 2). Reiteration of the blood flow to the tissue may induce further damage via reactive oxygen species (ROS) that are derived from polymorphonuclear leukocytes. This phenomenon is defined as ischemia/reperfusion injury (IRI) (3).

Systemic hypotensive states and hypovolemic shock, low-output cardiac disorders, and surgical procedures involving partial clamping of the renal vasculature (e.g., partial nephrectomy or renal revascularization) or renal transplantation are the main factors that lead to renal ischemia and further IRI if the blood flow can be re-provided (4). The injury may be seen anywhere in a wide spectrum, from a mild and transient rise of kidney function tests to a total loss of the functional glomeruli (4, 5).

After discerning the roles of ROS in the ischemia/reperfusion (I/R) process, preventive roles of antioxidants were extensively hypothesized. Several different molecules have been evaluated for distinct clinical or experimental

situations to overcome the disruptive effects of ROS on the cellular metabolism. Carvedilol, 1-[carbazolyl-(4)-oxy]-3-[(2-methoxyphenoxy-ethyl) amino]-2 propanol, is a beta-adrenergic blocker and has been used in the treatment of heart failure and hypertension. It has an additional antioxidant effect through inhibiting the release of ROS from activated leucocytes and inhibiting lipid peroxidation (6). Nebivolol, 1-(6-fluorochroman-2-yl)-2-[[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino] ethanol, is also a selective beta-blocker with antioxidant and kidney-protective effects (7, 8). Both are widely used in clinical practice. This study aimed to compare the preventive effects of these two molecules in a single-kidney I/R experimental model and to provide evidence of beta-blockers in patients with single kidneys and vulnerability to further kidney damage, with the results providing new information to assist in clinical choices.

Several molecules, tests, and calculation methods have been used in the diagnosis and grading of a kidney injury. In the contemporary literature, neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) are used as early indicators of early kidney injury and excess of kidney damage, respectively (9-12).

In our study, we evaluated and compared the efficacy of nebivolol and carvedilol on preventing an IRI in terms of urinary NGAL and KIM-1 levels, a tissue oxidative product, and tissue antioxidant enzymes.

MATERIALS AND METHODS

Experimental Model and Animals

A total of 24 Wistar-Albino male rats (6 weeks of age and 260±13 g weight) were separated into 4 groups, with each group including 6 rats. All the rats were weighed just before caging, were fed ad libitum during the whole study, and had a light and dark cycle of 12/12 h. On the first day, before the experimental protocol, and on the sixth day, after completion of the experimental interventions, 24 h urine samples were obtained using metabolic cages, and the blood samples were drawn from tail veins to calculate basal creatinine clearance (CrCl) rates. All the blood samples were drawn between 09:00 and 11:00 h to avoid circadian variations. After the first day, all the rats were taken into regular individual cages.

Main Points

- In our study, both carvedilol and nebivolol failed to ameliorate global kidney functions in a single-kidney ischemia/reperfusion injury experimental model.
- Carvedilol showed amelioration in terms of Kidney Injury Molecule-1 levels, which shows early kidney injury, and tissue Malonaldehyde levels, which shows tissue oxidative stress.
- In patients with a solitary kidney, the choice of antihypertensive agents should be individualized, and we need further clinical surveys to study the effects of beta-blockers on kidney functions on patients who are vulnerable to ischemic kidney damage.

On the sixth day of the study, all surgical procedures were carried out under general anesthesia using a dosage of 40 mg/kg ketamine (Ketalar, Eczacibasi Pharmaceuticals; İstanbul, Turkey).

The first group formed the sham group, where only a laparotomy was carried out with a general anesthesia protocol. The second group formed the experimental group and underwent right nephrectomy (RN) with consequent left I/R using temporary left renal artery clamping for 45 min (RN+I/R, the experimental model group). The third and fourth groups had undergone RN with temporary left arterial clamping with treatments with either carvedilol or nebivolol, respectively.

Starting from the first through to the sixth day of the study, carvedilol (Vasoxen 5 mg, Berlin-Chemie AG; Berlin, Germany) and nebivolol (Arlec 6.25 mg, Ali Raif Pharmaceuticals; İstanbul, Turkey) were administered daily at dosages of 2 mg/kg/day dissolved into 2 mL of drinking water, via nasogastric catheters to the third (RN+I/R+C, the carvedilol group) and fourth (RN+I/R+N, the nebivolol) groups. The same volume of drinking water was also administered via nasal gavage to the sham and experimental model groups. Additional doses of carvedilol and nebivolol were administered through nasogastric catheters just before clamping the left renal artery in the third and fourth groups, and additional vehicle drinking water was administered via a nasal gavage in the sham and control groups at the same time with the treatment groups. Thus, a total of 6 doses of treatment were used for all groups. In the dosing of beta-blockers, the preconditioning and ischemia timeline for I/R injury was based on previous experimental models by Singh et al. (13) and Gandhi et al. (14). After completion of the procedures, all the rats were taken back into metabolic cages. In addition, 24 h urine samples were obtained, and simultaneous venous blood samples were drawn. All the rats were sacrificed with cardiac blood withdrawal under Xylazine (Rompun, Bayer; İstanbul, Turkey) anesthesia at a dosage of 5 mg/kg. Left nephrectomy procedures were carried out, and the kidney tissues were obtained by slicing them into two identical parts. One of the parts was fixed in 10% formalin solution for histological examination, while the matching part was kept under an environment of -80° C to undergo tissue preparation and measurement of enzyme activities. The study protocol is summarized in Figure 1.

Determination of Urinary Marker Levels, Oxidative Product Levels and Antioxidant Enzyme Activities

NGAL and KIM-1 levels were determined with enzyme-linked immunosorbent assay tests purchased commercially from Boster Immunoleader (EK0882 and EK0855). Tissue superoxide dismutase (SOD) activity was evaluated using the methods described by Durak et al. (15). Tissue catalase activity was determined using Aebi's (16) method. Glutathione-S-transferase

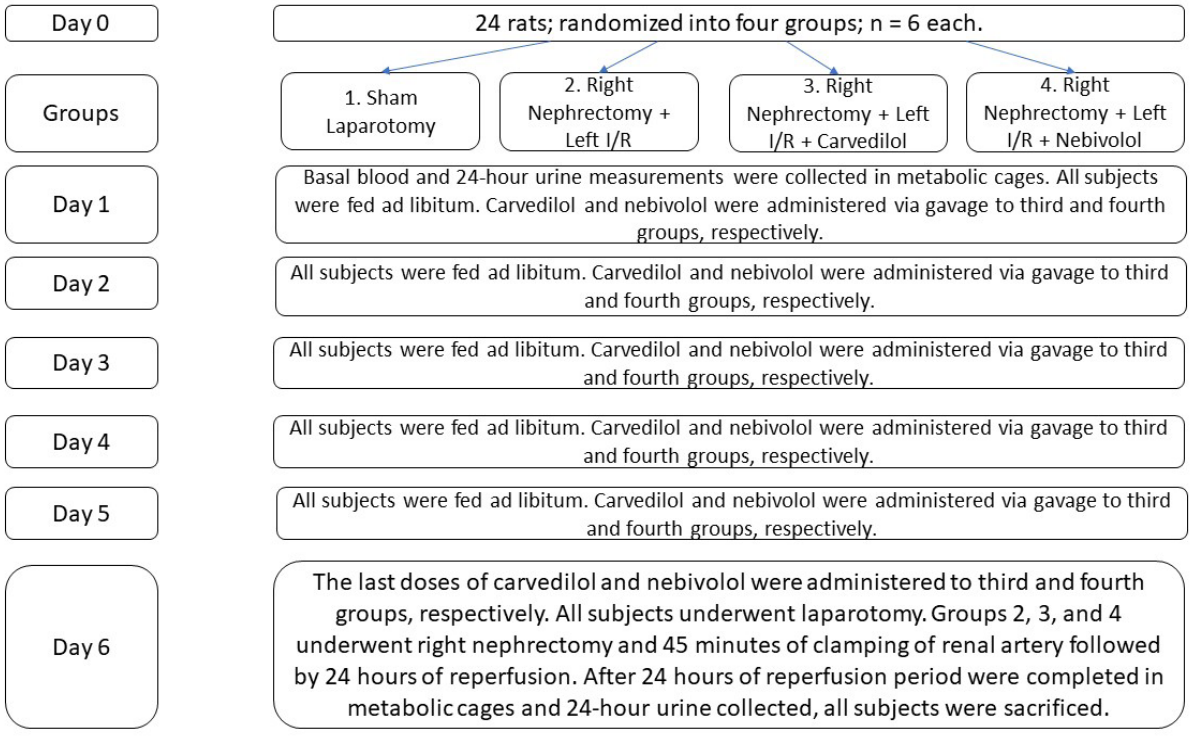

Figure 1. The diagram summarizes the study protocol

(GST) enzyme activities in the tissues were measured using the method described by Habig et al. (17). Malondialdehyde (MDA) levels in the tissues were determined using the method by Van Ye et al. (18).

Preparation of the Histological Specimens

Histological preparation of the resected kidney tissues was carried out after 24 h of fixation in a solution of 10% formalin. The resected tissues were embedded in paraffin blocks, and the blocks were sliced with a thickness of 4 μm. Obtained slices were stained using the hematoxylin and eosin protocol and examined under light microscopy. Medullar congestion was graded using a semiquantitative method and was classified as follows: insignificant (score null, congestion can be noticed only under ×400 optical enhancement), mild congestion (score 1, congestion is visible under ×200 optical enhancement), moderate congestion (score 2, congestion can be observed under ×100 optical enhancement), and severe congestion (score 3, apparent congestion under ×40 optical enhancement). Tubular necrosis was graded calculating the necrosis area to the total parenchyma (0: no necrosis, 1: mild necrosis involving less than 25% of total parenchyma, 2: moderate necrosis involving between 25% and 50% of total parenchyma, and 3: severe necrosis involving more than 75% of the total parenchyma).

Expression of the Results and Ethical Approval

Serum creatinine (Cr) and blood urea nitrogen (BUN) levels were expressed in mg/dL; CrCl rates were expressed in mL/min, and 24 h urinary NGAL and KIM-1 levels were expressed in ng/mL and pg/mL, respectively.

MDA levels were expressed as micromoles per gram tissue. SOD and GST activities were expressed as IU in gram protein, and catalase activity was determined using the velocity of the products and expressed in k per milligram protein.

Ethics committee approval was received for this study from the Animal Care Ethical Committee of Gazi University (Approval Date: November 3, 2020; Approval Number: GUET 14.067).

Statistical Analysis

All the biochemical levels and enzyme activities were expressed together with their standard deviations. Statistical tests were performed using the IBM Statistical Package for Social Sciences version 22.0 for Windows (IBM SPSS Corp.; Armonk, NY, USA).

For comparison of the kidney function tests, oxidative product, and enzyme activities between the groups, the Kruskal-Wallis H test was performed, and the multiple comparison test was conducted to show which group holds difference over other groups if a significant difference was found by the Kruskal-Wallis H test. The Wilcoxon signed-rank test was used to evaluate whether any significant difference existed in each group's own results before and after the experiments.

To compare the histological scores, an analysis of variance (ANOVA) test was used to evaluate the difference between the

groups. The Tukey's honestly significant difference test was carried out in case the ANOVA test revealed a significant difference for the sub-scores.

RESULTS

Renal Function Tests and Urinary Markers

Basal serum Cr and BUN levels, 24 h urine volumes and NGAL levels, as well as CrCl rates were statistically similar in all the groups. In contrast, basal KIM-1 levels showed a significant difference between the groups, being higher in the treatment groups than the sham and experimental model groups. Significant differences were observed between the basal and sixth-day results in all groups, except the sham group. Changes in the CrCl rates, serum Cr and BUN levels, 24 h urine volumes, and 24 h urine NGAL and KIM-1 levels during the study period are presented in Table 1. None of the rats were lost during the study.

All three intervention groups showed a significant increase in serum Cr and serum BUN levels and a decrease in CrCl rates compared with the sham group. Both carvedilol and nebivolol groups showed a similar increase in the serum Cr and BUN levels and reduction in the CrCl rates when compared with the experimental model group.

All three intervention groups showed higher 24 h urine NGAL and KIM-1 levels than the sham group. The carvedilol and nebiv-

Table 1. Comparison of the kidney function tests, their derivatives and kidney injury markers between the groups

Parameter	RN+I/R	S	RN+I/R+C	RN+I/R+N
Serum creatinine (mg/dL)				
Basal	0.64±0.15	0.53±0.07	0.58±0.10	0.55±0.09
Sixth day	1.2±0.23	0.40±0.04	1.10±0.14	1.15±0.15
p*	0.028	0.046	0.029	0.030
Creatinine clearance (mL/min)				
Basal	1.32±0.23	1.26±0.67	1.19±0.84	1.28±0.74
Sixth day	0.31±0.11	1.02±0.26	0.41±0.11	0.40±0.19
p*	0.028	0.116	0.028	0.028
Serum urea nitrogen (mg/dL)				
Basal	30.5±7.6	23.2±2.7	25.4±3.57	25.16±2.85
Sixth day	84.6±30.6	20.06±2.6	60.25±21.6	75.28±27.5
p*	0.026	0.075	0.027	0.027
Urine NGAL (ng/mL)				
Basal	73.97±53.43	104.44±30.6	71.71±31.11	130.9±33.6
Sixth day	132.44±61.8	71.13±48.9	117.35±43.92	208.98±96.9
p*	0.022	0.075	0.028	0.028
Urine KIM-1 (pg/mL)				
Basal	59.22±14.24	62.65±9.36	84.25±23.67	88.26±27.55
Sixth day	158.6±21.01	60.54±12.41	133.2±33.90	176.9±47.97
p*	0.025	0.60	0.028	0.028

*p indicates comparisons of the basal values and the sixth-day values of the presented groups using the Wilcoxon signed-rank tests
RN+I/R: right radical nephrectomy and consequent left renal ischemia/reperfusion group; S: sham group that has undergone laparotomy; RN+I/R+C: right radical nephrectomy and consequent left renal ischemia/reperfusion group that is preconditioned with carvedilol; RN+I/R+N: right radical nephrectomy and consequent left renal ischemia/reperfusion group that is preconditioned with nebivolol; NGAL: neutrophil gelatinase-associated lipocalin; KIM-1: kidney injury molecule-1

olol groups showed similar increment in the 24 h urine NGAL levels compared with the experimental model group (p=0.06 for both comparisons).

Basal 24 h urinary KIM-1 levels showed a significant difference between the groups. Basal 24 h urinary KIM-1 levels were significantly higher in the carvedilol and nebivolol groups. Significant increases in the 24 h urine KIM-1 levels were observed on the sixth day in the experimental model group, the carvedilol group and the nebivolol group (p<0.01, p<0.01, and p=0.01, respectively). The sham group showed no change in KIM-1 levels during the study. Compared with the RN+I/R group, the carvedilol group showed a significantly lower increase in the 24 h urine KIM-1 levels (p=0.02). The nebivolol group showed a similar increase in the 24 h urinary KIM-1 levels compared with the experimental model group (p=0.08). Changes in the 24 h urine NGAL and KIM-1 levels during the study are presented in Table 2.

Tissue Oxidative Marker Levels and Antioxidant Enzyme Activities

Tissue SOD and catalase activities showed no differences among the sham, experimental model, and treatment groups (p=0.21 and p=0.12, respectively). Tissue MDA levels were significantly higher in the experimental model group than the sham group (p=0.006), whereas no difference was observed between the experimental model group and the treatment groups, or between the 2 treatment groups (p=0.53 and p=0.71, respectively).

GST enzyme activity showed a significant decrease in the experimental model group compared with the sham group (p=0.005). The carvedilol and nebivolol groups showed a significantly less decrease in GST enzyme activity compared with the experimental model group (p=0.005 and p=0.009, respectively). No significant differences were observed in the GST enzyme activity between the carvedilol- and nebivolol-administered groups.

The tissue oxidative stress product levels and antioxidant enzyme activities are presented in Table 2.

Histological Findings

Comparative scores, number of experimental animals, and histological findings between the groups are presented in Table 3.

The experimental group showed significant deterioration in the histological findings compared with the sham group. The treatment groups did not show any significant amelioration, with

Table 2. Comparison of the renal tissue oxidative stress markers between the groups*

Parameter	RN+I/R	S	RN+K+I/R	RN+N+I/R	p#
SOD (IU/mg protein)	108.9±41.8	126.4±48.8	119.7±40.9	113.7±32.6	0.213
Catalase (k/mg protein)	547.7±228.4	752.08±118.04	573.1±85.2	604.07±122.6	0.126
GST (IU/mg protein)	0.042±0.007[a,b]	0.072±0.015	0.063±0.015[a]	0.058±0.007[b]	0.004
MDA (µmol/g tissue)	0.62±0.078[a]	0.049±0.034[a,b,c]	0.24±0.11[b]	0.23±0.086[c]	0.02

*The superscripted letters indicate statistically significant difference between the groups marked by the same letters
#p indicates the comparisons of all groups using the Kruskal-Wallis H test. The multiple comparison test was carried out to show which groups show statistical differences in case a significant difference was found in the Kruskal-Wallis H test
RN+I/R: right radical nephrectomy and consequent left renal ischemia/reperfusion group; S: sham group that has undergone laparotomy; RN+I/R+C: right radical nephrectomy and consequent left renal ischemia/reperfusion group that is preconditioned with carvedilol; RN+I/R+N: right radical nephrectomy and consequent left renal ischemia/reperfusion group that is preconditioned with nebivolol; SOD: superoxide dismutase; GST: glutathion S-transferase; MDA: malondialdehyde; NOS: nitric oxide synthase

Table 3. Distribution of number of the experimental animals owing to the histological findings

Groups	Congestion scores				Inflammation scores				Tubular necrosis scores				Tubular dilation scores			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Sham	-	6	-	-	6	-	-	-	6	-	-	-	6	-	-	-
RN+I/R	-	1	3	2	6	-	-	-	1	2	3	-	5	1	-	-
RN+I/R+N	-	3	3	-	5	1	-	-	1	2	3	-	3	3	-	-
RN+I/R+C	1	4	1	-	5	1	-	-	4	2	-	-	2	3	1	-

RN+I/R: right radical nephrectomy and consequent left renal ischemia/reperfusion group; RN+I/R+C: right radical nephrectomy and consequent left renal ischemia/reperfusion group that is preconditioned with carvedilol; RN+I/R+N: right radical nephrectomy and consequent left renal ischemia/reperfusion group that is preconditioned with nebivolol

the only exception being significantly better tubular congestion scores in the carvedilol group (p=0.03).

Figure 2 shows the normal histology of the sham group. Figure 3 presents the congestive and necrotic findings in the RN+I/R group. Figure 4 illustrates the findings in the carvedilol- and nebivolol-administered groups.

DISCUSSION

Ischemic acute renal failure (ARF) induced by hypoxia constitutes 70% of community-acquired ARF and 40% of hospital-acquired ARF cases (19). Because of its significant consequences and high incidence, several studies have been carried out on the devastating effects of ischemia. After characterizing the further damage of reperfusion on tissues, academic interest has tended towards defining the pathways of an IRI as well as finding candidate molecules in avoiding the injury. Free oxygen radicals have been proposed as the main culprit of IRI, and it has been shown that the radicals are induce significant cell damage via lipid peroxidation and degradation of cross-links in protein molecules and nucleic acids (20).

In our study, we evaluated and compared the preventive roles of carvedilol and nebivolol, which are shown to have antioxi-

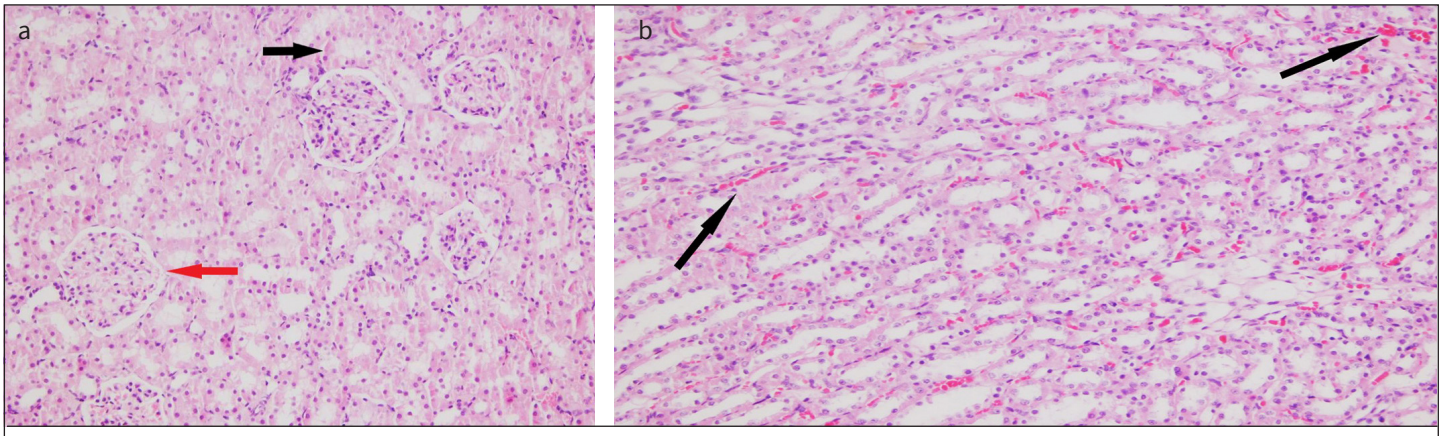

Figure 2. a, b. Histological image a) from one of the sham group rats showing normal kidney glomerulus (red arrow), tubular epithelium (black arrow), and parenchyma. Histological image b) from one of the sham group rats showing widespread minimal medullar congestion (arrows)

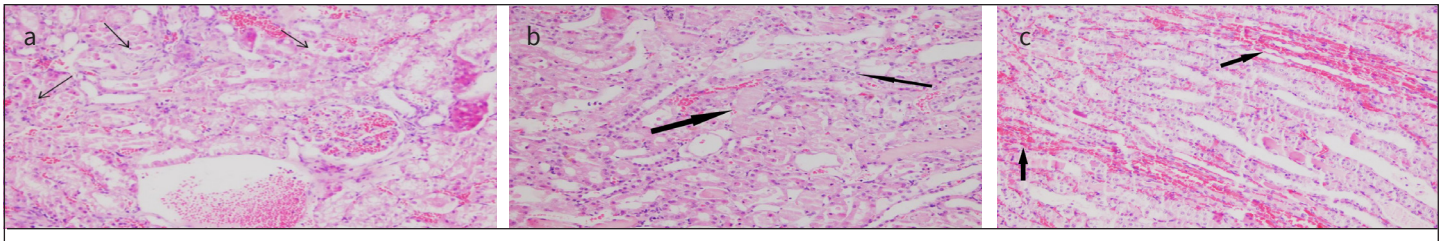

Figure 3. a-c. Histological image a) from right radical nephrectomy and consequent left renal ischemia/reperfusion group indicating necrosis (arrows) and swelling of the tubular epithelium with glomerular and capillary congestion. Wide necrosis areas b) that are ubiquitously observed (arrows) in the right radical nephrectomy and consequent left renal ischemia/reperfusion group. Severe vascular congestion c) that is commonly noted (arrows) in the right radical nephrectomy and consequent left renal ischemia/reperfusion group

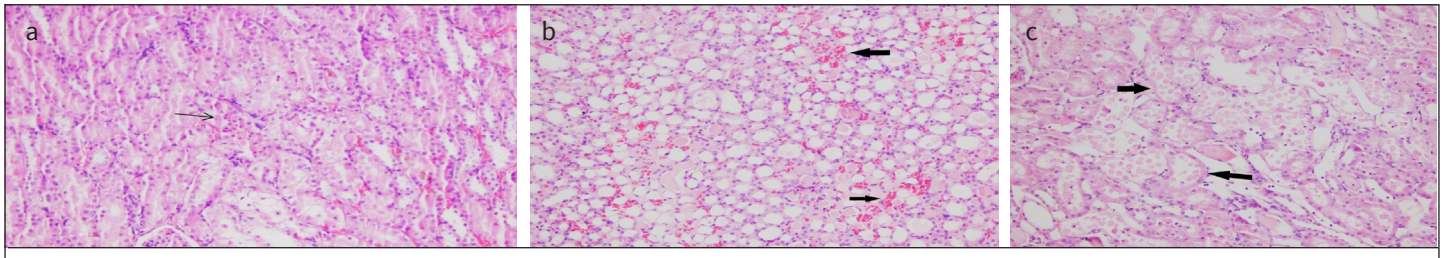

Figure 4. a-c. Histological image a) demonstrates amelioration of tubular necrosis that is characterized by mild and discrete necrotic areas (arrows) in right radical nephrectomy and consequent left renal ischemia/reperfusion group. Histological image b) demonstrates amelioration of tubular necrosis, which is characterized by discrete and intermediate necrotic areas (arrow) in right radical nephrectomy and consequent left renal ischemia/reperfusion preconditioned with the nebivolol group. Histological image c) demonstrates amelioration of medullary congestion that is characterized by mild to intermediate congestion (arrow) in the right radical nephrectomy and consequent left renal ischemia/reperfusion groups

dant effects, in an I/R injury. We used conventional tests (serum BUN, Cr levels and CrCl rates), contemporary molecular indicators (24 h urine NGAL and KIM-1), a tissue-damage indicator, antioxidative enzymes, and histological findings. To the best of our knowledge, our study provides the first evidence on the comparison of carvedilol and nebivolol on ameliorating IRI. Our I/R model and the dosages used were shown to be reliable from previous studies (13, 14, 21).

Its electron-stabilizing and SOD-clearing effects, ameliorating effects on endothelium dysfunction, and preventive effects against the renal fibrosis of nebivolol have been shown in previous studies (7, 22, 23). Carvedilol is shown to be preventive against lipid peroxidation in cell membrane via intracellular pathways (24). Several reports are available on the effects of carvedilol to decrease the migration of neutrophils to ischemic areas while inhibiting the myeloperoxidase and oxidase activity in them, thus decreasing the ischemia-related injury in the tissue (25, 26).

Singh et al. (13) reported significant amelioration in kidney function tests with increased antioxidant enzymes and decreased peroxidation in tissues using carvedilol in an I/R injury model. Hayashi et al. (6) reported better histological findings and improved kidney functions in carvedilol-administered rats compared with the isolated I/R model. Hayashi et al. (6) further compared the preventive effects of carvedilol and metoprolol in the I/R model and reported better kidney functions in the carvedilol group.

We observed significantly deteriorated kidney function from the tests in the experimental group. Therefore, we can understand that our experimental model was successful. However, we can only report a partial beneficial effect of carvedilol and nebivolol.

We did not observe any significant benefit of both carvedilol and nebivolol on the serum Cr and BUN levels, as well as CrCl rates against IRI in a single-kidney experimental model. Thus, we can report that, at least owing to the results we obtained, none of the molecules seems preventive against deterioration in basic kidney function tests.

We found amelioration in the 24 h urinary KIM-1 levels in the carvedilol group, whereas such an effect was not apparent in the nebivolol group. Furthermore, both groups did not show any changes in the 24 h urinary NGAL levels compared with the experimental model group. Thus, we can indicate that there is only a partially beneficial effect on early kidney injury in favor of carvedilol as observed in our study.

The tissue activities of SOD and catalase did not show a significant difference between the groups (p=0.21). SOD and catalase are major antioxidative enzyme systems that react to oxidative stress. Thus, our hypothesis was in favor of observing the increased activities of enzymes in the experimental model group respective to the sham group, as well as a diminished increase of the activities in the treatment groups respective to the experimental model group. However, we observed a similar activity in the sham, experimental model, and treatment groups.

The GST activity was found to be significantly decreased in the experimental model group compared with the sham group (p=0.005), whereas the GST activities were found to be significantly higher in the carvedilol and nebivolol groups than in the experimental model group (p=0.005 and p=0.009, respectively). The difference between the carvedilol and nebivolol groups was insignificant. I/R significantly increased the tissue MDA levels in the experimental model group compared with the sham group (p=0.006), and treatment with nebivolol or carvedilol decreased this alteration compared with the experimental model group (p=0.006 and p=0.003, respectively). The effects of both molecules were statistically similar to each other. GST is a well-known antioxidant enzyme system, whereas MDA is the end product of lipid peroxidation. Thus, we can conclude that both the beta-blockers used in our study were equally capable of preventing oxidative injury induced by tissue I/R. Our results are compatible with the previously shown effects of both molecules.

Evaluating the histological findings, it was found that tubular necrosis and corticomedullary congestion scores were significantly higher in the experimental model group than the sham group (p<0.01 for all subdomains). Both the carvedilol and nebivolol groups showed similar results with the experimental model group, on most of the subdomains. Only the cortical congestion scores showed a significant difference in favor of the carvedilol group compared with the experimental model group (p=0.03). Thus, we can summarize that only carvedilol merely showed some partial beneficial effect histologically.

In our study, we used the previously accepted single-kidney I/R experimental model. We evaluated and compared the protective roles of carvedilol and nebivolol in terms of kidney functions tests, contemporary kidney injury markers, tissue antioxidant enzymes, and histological findings. Our study brings the first one-to-one comparison of the ameliorating effects of carvedilol and nebivolol in a kidney I/R injury. Our trials showed that carvedilol has better antioxidant activity as well as an ameliorating effect on acute kidney injury markers after an IRI in a single-kidney experimental model. We cannot report any ameliorating effect in countenance of both molecules on routinely used basic kidney function tests.

CONCLUSION

In conclusion, the choice between these molecules in the clinical management of patients vulnerable to an IRI should be based on the physicians' preference. However, owing to our experimental results, we can suggest that carvedilol seems a more rational choice in patients with single-kidney units and

who are vulnerable to kidney ischemia. We think that further research and comparative studies can lead to a more rational usage of beta-blockers in patients with a single kidney or who have a compromised global renal function.

Ethics Committee Approval: Ethics committee approval was received for this study from the Animal Care Ethical Committee of Gazi University (Approval Date: November 3, 2020; Approval Number: GUET 14.067).

Informed Consent: Informed consent was not obtained due to the nature of this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.T.A.; Design - S.T.A., M.K.; Supervision - S.T.A.; Resources - S.T.A., M.K., Ö.U.Ç.; Materials - M.K.; Data Collection and/or Processing - M.K., Ö.U.Ç., M.K., İ.I.G.; Analysis and/or Interpretation - M.K., Ö.U.Ç., M.K., İ.I.G.; Literature Search - M.K., Ö.U.Ç.; Writing - Ö.U.Ç., M.K.; Critical Review - S.T.A.

Acknowledgements: The manuscript is reproduced from the residency thesis of Dr. Küçük, under the supervision of Prof. Arınsoy.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Welbourn CR, Goldman G, Paterson IS, Valeri CR, Shepro D, Hechtman HB. Pathophysiology of ischaemia reperfusion injury: Central role of the neutrophil. Br J Surg 1991; 78: 651-5. [Crossref]
2. Atilla K, Coker A, Sagol O, Coker I. Protective effects of carnitine in an experimental ischemia-reperfusion injury. Clinical Nutrition 2002; 4: 309-13. [Crossref]
3. Eltzschig HK, Eckle T. Ischemia and reperfusion--from mechanism to translation. Nat Med 2011; 17: 1391-401. [Crossref]
4. Woolfson RG, Millar CG, Neild GH. Ischaemia and reperfusion injury in the kidney: Current status and future direction. Nephrol Dial Transplant 1994; 9: 1529-31.
5. Sivarajah A, Chatterjee PK, Patel NS, Todorovic Z, Hattori Y, Brown PA, et al. Agonists of peroxisome-proliferator activated receptor-gamma reduce renal ischemia-reperfusion injury. Am J Nephrol 2003; 23: 267-76. [Crossref]
6. Hayashi T, De Velasco MA, Saitou Y, Nose K, Nishioka T, Ishii T, et al. Carvedilol protects tubular epithelial cells from ischemia-reperfusion injury by inhibiting oxidative stress. Int J Urol 2010; 17: 989-95. [Crossref]
7. Pires MJ, Rodríguez-Peña AB, Arévalo M, Cenador B, Evangelista S, Esteller A, et al. Long-term nebivolol administration reduces renal fibrosis and prevents endothelial dysfunction in rats with hypertension induced by renal mass reduction. J Hypertens 2007; 25: 2486-496. [Crossref]
8. Groot AA, Mathy MJ, Zwieten PA, Peters SL. Antioxidant activity of nebivolol in the rat aorta. J Cardiovasc Pharmacol 2004; 43: 148-53.[Crossref]
9. Gonzalez F, Vincent F. Biomarkers for acute kidney injury in critically ill patients. Minerva Anestesiol 2012; 78: 1394-403.
10. Bagshaw SM, Bellomo R. Early diagnosis of acute kidney injury. Curr Opin Crit Care 2007; 13: 638-44. [Crossref]
11. Liang XL, Liu SX, Chen YH, Yan LJ, Li H, Xuan HJ, et al. Combination of urinary kidney injury molecule-1 and interleukin-18 as early biomarker for the diagnosis andprogressive assessment of acute kidney injury following cardiopulmonary bypasssurgery: A prospectivenested case-controlstudy. Biomarkers 2010; 15: 332-9. [Crossref]
12. Song L, Xue L, Yu J, Zhao J, Zhang W, Fu Y. Kidney injury molecule-1 expression is closely associated with renal allograft damage. Bosn J Basic Med Sci 2013; 13: 1704. [Crossref]
13. Singh D, Chander V, Chopra K. Carvedilol attenuates ischemia-reperfusion-induced oxidative renal injury in rats. Fundam Clin Pharmacol 2004; 18: 627-34. [Crossref]
14. Gandhi C, Zalawadia R, Balaraman R. Nebivolol reduces experimentally induced warm renal ischemia reperfusion injury in rats. Ren Fail 2008; 30: 921-30. [Crossref]
15. Durak I, Canbolat O, Kavutcu M, Ozturk HS, Yurtarslani Z. Activities of total, cytoplasmic, and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. J Clin Lab Anal 1996; 10: 17-20. [Crossref]
16. Aebi H. Catalase. Bergmeyer HU (ed). Methods of Enzymatic Analysis. New York and London: Academic Press, 1974; p.673-677. [Crossref]
17. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-9.
18. Van Ye TM, Roza AM, Pieper GM, Henderson J, Johnson JP, Adams MB, et al. Inhibition of intestinal lipid peroxidation does not minimize morphological damage. J Surg Res 1993; 55: 553-8. [Crossref]
19. Thadhani R, Pascual M, Bonventre JV. Acute renal failure. N Engl J Med 1996; 334: 1448-60. [Crossref]
20. Malek M, Nematbaksh M. Renal ischemia-reperfusion injury; from pathophysiology to treatment. J Renal Inj Prev 2015; 4: 20-7.
21. Toprak O, Cirit M, Tanrisev M, Yazici C, Canoz O, Sipahioglu M, et al. Preventive effect of nebivolol on contrast-induced nephropathy in rats. Nephrol Dial Transplant 2008; 23: 853-9. [Crossref]
22. Groot AA, Mathy MJ, Zwieten PA, Peters SL. Antioxidant activity of nebivolol in the rat aorta. J Cardiovasc Pharmacol 2004; 43: 148-53. [Crossref]
23. Mason RP, Kubant R, Jacob RF, Walter MF, Boychuk B, Malinski T. Effect of nebivolol on endothelial nitric oxide and peroxynitrite release in hypertensive animals: Role of antioxidant activity. J Cardiovasc Pharmacol 2006; 48: 862-9. [Crossref]
24. Yue TL, Cheng HY, Lysko PG, McKenna PJ, Feuerstein R, Gu JL, et al. Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger. J Pharmacol Exp Ther 1992; 263: 92-8.
25. Feuerstein G, Liu GL, Yue TL, Cheng HY, Hieble JP, Arch JR, et al. Comparison of metoprolol and carvedilol pharmacology and cardioprotection in rabbit ischemia and reperfusion model. Eur J Pharmacol 1998; 351: 341-50. [Crossref]
26. Mollnau H, Schulz E, Daiber A, Baldus S, Oelze M, August M, et al. Nebivolol prevents vascular NOS III uncoupling in experimentalhyperlipidemia and inhibits NADPH oxidase activity in inflammatory cells. Arterioscler Thromb Vasc Biol 2003; 23: 615-21. [Crossref]