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An evaluation of rivaroxaban and clopidogrel in a rat lower extremity ischemia-reperfusion model: An experimental study

Sıçan alt ekstremite iskemi-reperfüzyon modelinde rivaroksaban ve klopidogrelin değerlendirilmesi: Deneysel bir çalışma

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ABSTRACT

Background: This study aims to compare clopidogrel and rivaroxaban against ischemia-reperfusion injury after a long reperfusion time and to investigate its effects on various tissues.

Methods: A total of 40 Wistar rats were included in the study and were randomly divided into four groups (n=10 per group). Groups were defined as follows: control (Group 1), sham (Group 2), clopidogrel pre-treatment (Group 3), and rivaroxaban pre-treatment (Group 4). Ischemia (6 h) and reperfusion (8 h) were induced at the lower hind limb in Groups 2, 3, and 4. The ischemic muscle, heart, kidney, liver, and plasma tissues of the subjects were obtained to test for the oxidant (malondialdehyde) and antioxidants (glutathione, superoxide dismutase, and nitric oxide).

Results: Malondialdehyde levels were significantly higher in the sham group, compared to the controls in all tissues. Clopidogrel and rivaroxaban pre-treatment significantly decreased malondialdehyde levels, compared to the heart, ischemic muscle, liver, and blood tissues of the sham group. Kidney malondialdehyde levels were reduced only by rivaroxaban. Group 4 had significantly decreased malondialdehyde levels, compared to Group 3 in ischemic muscle ($p<0.010$). The glutathione reduction, compared to sham group, in the kidney was only significant for Group 4 ($p<0.050$). With clopidogrel and rivaroxaban pre-treatment, nitric oxide levels significantly decreased only in the heart tissue, compared to sham group ($p<0.001$ and $p<0.050$, respectively).

Conclusion: The study results suggest that rivaroxaban and clopidogrel are effective in reducing ischemia-reperfusion injury in the heart, ischemic muscle, liver, and blood. Rivaroxaban also protects the kidneys and is superior to clopidogrel in ischemic muscle protection.

Keywords: Animal experiment, clopidogrel, ischemia, reperfusion injury, rivaroxaban.

ÖZ

Amaç: Bu çalışmada, uzun bir reperfüzyon süresinden sonra iskemi-reperfüzyon hasarına karşı klopidogrel ve rivaroksaban karşılaştırıldı ve çeşitli dokular üzerindeki etkileri araştırıldı.

Çalışma planı: Toplamda 40 Wistar sıçan çalışmaya dahil edildi ve rastgele dört gruba ayrıldı (grup başına n=10). Gruplar şu şekilde belirlendi: kontrol (Grup 1), sham (Grup 2), klopidogrel ön tedavisi (Grup 3) ve rivaroksaban ön tedavisi (Grup 4). Grup 2, 3 ve 4'te sıçanların arka bacaklarında iskemi (6 saat) ve reperfüzyon (8 saat) indüklendi. Oksidan (malondialdehit) ve antioksidanları (glutatyon, süperoksit dismutaz ve nitrik oksit) çalışmak için, deneklerin iskemik kas, kalp, böbrek, karaciğer ve plazma dokuları incelendi.

Bulgular: Malondialdehit sham grubunda tüm dokularda kontrollere kıyasla anlamlı düzeyde yüksekti. Klopidogrel ve rivaroksaban ön tedavisi sham grubun kalp, iskemik kas, karaciğer ve kan dokularında malondialdehit düzeylerini anlamlı düzeyde düşürdü. Böbrek malondialdehit düzeyleri, yalnızca rivaroksaban ile azaldı. Grup 4'te, Grup 3'e kıyasla, iskemik kasta malondialdehit anlamlı düzeyde düşük bulundu ($p<0.010$). Böbrekte glutatyon düşüşü, sham grubuna kıyasla, yalnızca Grup 4 için anlamlıydı ($p<0.050$). Klopidogrel ve rivaroksaban ön tedavisi ile nitrik oksit düzeyleri, yalnızca kalp dokusunda sham grubuna kıyasla anlamlı düzeyde azaldı (sırasıyla $p<0.001$ ve $p<0.050$).

Sonuç: Çalışma sonuçları, rivaroksaban ve klopidogrelin kalp, iskemik kas, karaciğer ve kandaki iskemi-reperfüzyon hasarını azaltmada etkili olduğunu göstermektedir. Ayrıca rivaroksaban böbrekleri korur ve iskemik kas korumasında klopidogrelden üstündür.

Anahtar sözcükler: Hayvan deneyi, klopidogrel, iskemi, reperfüzyon hasarı, rivaroksaban.

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Reperfusion of ischemic tissue is known as ischemia-reperfusion (I/R).[1] Inflammatory mediators and toxic oxygen products generated during I/R may cause damage both locally and in remote organs, and I/R conditions are usually seen in vascular diseases. Rivaroxaban is used to prevent arterial embolism and has a debated place in peripheral vascular disease treatment, while clopidogrel is used in peripheral vascular disease, and both drugs are prescribed to prevent acute limb ischemia.[2-4] However, acute arterial occlusion can occur, even if these drugs are administered for thromboprophylaxis, and reperfusion is essential for tissue survival.[5] It is well-known that systemic inflammation after reperfusion becomes more severe over time, and a nine-fold increase of interleukin (IL)-6 has been demonstrated after three h of reperfusion, compared to a one h reperfusion.[1,6] Clinically, multiorgan dysfunction manifests after 24 h of I/R.[7]

There are limited reports of rivaroxaban or clopidogrel treatments preventing I/R injury induced by an ischemic extremity in rats with a reperfusion time varying between 30 min and four h.[5,8-10] To the best of our knowledge, only a study by Demirtas et al.[5] compared the effects of rivaroxaban and clopidogrel on I/R injury and the blood, heart, and kidney tissues after six h of ischemia and one h of reperfusion were examined. However, no comparison including ischemic muscle and the liver has yet been made.

In the present study, we aimed to compare the effect of pre-treatment with rivaroxaban or clopidogrel on ischemic muscle, heart, kidneys, liver, and plasma by measuring levels of the oxidant malondialdehyde (MDA) and the antioxidants glutathione (GSH), superoxide dismutase (SOD), and nitric oxide (NO) after ischemia for a longer reperfusion time (8 h).

MATERIALS AND METHODS

This study was carried out after approval was obtained from the Kocaeli University Experimental Animals Ethical Committee (KOÜ HADYEK 3/6-2013). A total of 40 male Wistar rats, 8 to 12 weeks old with a mean weight of 350±37 g were included in the study. All rats were kept in an appropriate humidity (50±5%) and temperature condition (22±2°C) under a 12:12 hour light/dark cycle. The rats were randomly divided into four equal groups of 10 rats each. All rats were given distilled water and standard rat chow. The rats in the control group (Group 1) were used to collect baseline values without I/R. In the sham group (Group 2), I/R was initiated to obtain the results of I/R without pre-treatment. The rats in the clopidogrel pre-treatment group (Group 3) were administered 0.2 mg/kg/day clopidogrel, and those in the rivaroxaban pre-treatment group (Group 4) were administered 3 mg/kg/day rivaroxaban, all via oral gavage for 10 days before I/R induction.[8,10] At the end of 10 days, the rats were anesthetized with ether, and ischemia was initiated by blockade of circulation of the right lower extremity at the trochanter major in Groups 2, 3, and 4 as described in previous studies.[8,9] Ischemia was confirmed by Doppler ultrasonography (MD2, Huntleigh Diagnostics Ltd., South Glamorgan, UK). Following six h of ischemia, reperfusion was allowed by means of tourniquet removal in the right lower extremity. Reperfusion was also confirmed by Doppler ultrasound scan. After reperfusion for eight h, all rats were sacrificed for investigation.

Blood samples were collected intracardially. Tissues were washed by administration of 100 mL 0.9% NaCl per rat. The liver, heart, kidney, and ischemic muscle tissues were extracted and homogenized in cold Tris-HCl buffer (pH 7.4). The blood, liver, heart, kidneys, and ischemic muscles were, then, used to collect measurements of the oxidant parameter MDA and the antioxidant parameters GSH, SOD, and NO. Plasma and tissue lipid peroxide levels, expressed in terms of MDA, were determined according to the method described by Buege and Aust[11] and expressed in nmoL/100 mg protein. Serum and tissue GSH levels were measured with 5,5'-dithiobis (2-nitrobenzoate) at 412 nm, according to the method described by Ellman, and expressed in nmoL/mg protein.[12] Total NO metabolites were calculated by summing nitrate (reduced to nitrite on exposure to cadmium granules) and nitrite levels, detected calorimetrically using the Griess reaction, and expressed in nmoL/100 mg protein.[13] Serum and tissue SOD levels were measured using an SOD ELISA assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). Protein concentrations of tissue homogenates were determined using the method described by Lowry et al.[14]

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 3.0 (GraphPad Inc., CA, USA) and IBM SPSS version 19 software (IBM Corp., Armonk, NY, USA). The data were expressed in mean ± standard deviation (SD). All data were tested for normal distribution. The analysis of variance (ANOVA) (post-test Tukey's multiple comparison test) was used to evaluate the data. A p value of <0.05 was considered statistically significant.

Table 1. Malondialdehyde results

	Group 1	Group 2	Group 3	Group 4	Group 1 vs. 3§	Group 1 vs. 4§
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Heart	46.9±16.4*	99.8±46.9	61.7±15.9†	62.6±23.8‡	<0.01	<0.05
Ischemic muscle	20.2±16.1**	58.1±16.4	40.4±10.1†	23.6±7.0‡‡‡	<0.01	>0.05
Kidney	30.5±12.2**	66.4±22.4	56.2±17.6	40.8±7.6‡‡	<0.01	>0.05
Liver	22.9±14.8**	63.4±33.8	43.1±17.3†	44.9±14.4‡	<0.05	<0.05
Blood	0.5±0.1*	0.6±0.0	0.5±0.0 †††	0.54±0.0‡‡‡	>0.05	>0.05

SD: Standard deviation; Passed the normality tests conducted using ANOVA and Tukey's multiple comparison tests; Heart, ischemic muscle, kidney, liver (nmoL/100 mg protein), and blood (µmol/L) MDA levels.
For paired comparison of sham with Groups 1, 3, and 4, p values are:
Group 1 vs. Group 2: * <0.010; ** <0.001;
Group 2 vs. Group 3: † <0.050; †† <0.010; ††† <0.001;
Group 2 vs. Group 4: ‡ <0.050; ‡‡ <0.010; ‡‡‡ <0.001; (comparisons without notations are statistically not significant [p>0.05]).
§ P values.
Group 1: Control group; Group 2: Sham group; Group 3: Clopidogrel pre-treatment group; Group 4: Rivaroxaban pre-treatment group.

RESULTS

Malondialdehyde

The MDA levels in all tissues and the paired comparisons among the groups are shown in Table 1. Statistical analysis revealed a significant increase in the MDA levels in all tissues in the sham group, compared to the control group. In the clopidogrel pre-treatment group, the increase in the MDA in blood was not significant compared to baseline values (p>0.050). In the rivaroxaban pre-treatment group, the increase in the MDA in the ischemic muscle, kidneys, and blood was not significant compared to baseline values. However, in the paired analysis among the control group and the treatment groups for the remaining tissues, a significant increase in the MDA was observed.

Paired analysis of the MDA levels in the treatment groups and the sham group were made. In the clopidogrel pre-treatment group, MDA was lower in kidney tissue, but not significant compared to sham (p>0.050). On the other hand, the rivaroxaban pre-treatment group had significantly decreased kidney MDA levels, compared to the sham group (p<0.010). In the clopidogrel and rivaroxaban pre-treatment, MDA levels significantly decreased in all the remaining tissues. The rivaroxaban group had significantly lower MDA levels, compared to the clopidogrel group (p<0.010) in ischemic muscle,

Table 2. Superoxide dismutase results

	Group 1	Group 2	Group 3	Group 4	Group 1 vs. 3§	Group 1 vs. 4§
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Heart	129.6±21.8**	90.8±40.4	36.8±8.9†††	52.9±9.6‡‡	<0.001	<0.001
Ischemic muscle	214.4±33.0*	170.1±40.6	154.5±23.5	126.4±18.4‡	<0.001	<0.001
Kidney	142.6±39.2***	43.0±11.5	33.9±9.5	30.9±7.7‡	<0.001	<0.001
Liver	44.6±11.2***	26.3±11.0	24.1±5.7††	28.6±9.3‡	<0.001	<0.01
Blood	63.4±5.5*	52.1±9.0	39.2±8.2††	40.2±8.0‡	<0.001	<0.001

SD: Standard deviation; Passed normality test. The ANOVA test and Tukey's multiple comparison test were used; Heart, ischemic muscle, kidney, liver (U/100 mg protein) and blood (U/mL) SOD levels.
For paired comparison of sham with Groups 1, 3 and 4 p values are :
Group 1 vs. Group 2: * <0.05; ** <0.01; *** <0.001;
Group 2 vs. Group 3: †† <0.01; ††† <0.001;
Group 2 vs. Group 4: ‡ <0.05; ‡‡ <0.01; (comparisons without notations and comparisons between group 3 and 4 are statistically not significant [p>0.05]).
§ P values.
Group 1: Control group; Group 2: Sham group; Group 3: Clopidogrel pre-treatment group; Group 4: Rivaroxaban pre-treatment group.

Table 3. Glutathione results

	Group 1	Group 2	Group 3	Group 4	Group 1 vs. 3§	Group 1 vs. 4§
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Heart	77.4±12.6**	54.1±24.0	26.8±6.1†††	33.4±7.6‡	<0.001	<0.001
Ischemic muscle	78.3±18.3***	53.3±5.9	48.9±5.5	43.5±6.8	<0.001	<0.001
Kidney	80.5±12.5***	35.6±9.2	28.5±6.8	26.7±3.6‡	<0.001	<0.001
Liver	31.5±5.5***	19.7±3.6	21.8±3.4	21.7±3.0	<0.001	<0.001
Blood	27.3±6.6*	21.4±4.6	15.8±3.0††	16.9±2.2‡	<0.001	<0.001

SD: Standard deviation; Passed normality test. The ANOVA test and Tukey's multiple comparison test were used; Heart, Ischemic muscle, Kidney, liver (nmoL/mg protein) and blood (mg/dL) GSH levels.
For paired comparison of sham with Groups 1, 3 and 4 *p* values are:
Group 1 vs. Group 2: * <0.05; ** <0.01; *** <0.001
Group 2 vs. Group 3: † <0.05; †† <0.01; ††† <0.001
Group 2 vs. Group 4: ‡ <0.05; (comparisons without notations and comparisons between group 3 and 4 are statistically not significant [p>0.05]).
§ P values.
Group 1: Control group; Group 2: Sham group; Group 3: Clopidogrel pre-treatment group; Group 4: Rivaroxaban pre-treatment group.

and no significant difference was observed in the remaining tissues (p>0.05).

Superoxide dismutase

The SOD levels in all tissues and the paired comparisons among the groups are shown in Table 2. Paired analysis showed that the SOD levels were significantly lower than control in all groups. In the clopidogrel group, the decreased SOD levels in the ischemic muscle and kidney tissues were not significant, compared to the sham group (p>0.050). However, a significant decrease of SOD in the ischemic muscle and kidney was present in the rivaroxaban pre-treatment group, compared to the sham group (p<0.050). The decrease of SOD compared to sham was significant in both treatment groups for the heart, liver, and blood.

Glutathione

The GSH levels in all tissues and the paired comparisons among the groups are shown in Table 3. Paired analysis showed that the GSH levels were significantly lower than control in all groups. In both treatment groups, the differences in GSH levels were not significant compared to the sham group for ischemic muscle and liver. In the clopidogrel pre-treatment group, the decrease of kidney GSH levels was not significantly different from the sham group (p>0.050); however, this measurement was significant for the rivaroxaban group (p<0.050). Both

Table 4. Nitric oxide results

	Group 1	Group 2	Group 3	Group 4	Group 1 vs. 3§	Group 1 vs. 4§
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Heart	32.4±12.3***	91.6±40.8	43.0±9.6†††	56.8±23.8‡	>0.05	>0.05
Ischemic muscle	38.7±15.1***	80.7±9.6	80.9±1.6	68.7±14.1	<0.001	<0.001
Kidney	23.7±9.4***	53.9±19.3	49.0±16.7	38.1±5.8	<0.01	>0.05
Liver	30.3±8.3***	54.4±15.8	52.9±7.0	54.9±8.7	<0.001	<0.001
Blood	15.3±3.5**	20.8±2.5	21.2±4.0	20.7±3.3	<0.01	<0.01

SD: Standard deviation; Passed normality test. The ANOVA test and Tukey's multiple comparison test were used; Heart, ischemic muscle, kidney, liver (µmoL/1000 mg protein) and blood (µmol /L) NO levels.
For paired comparison of sham with Groups 1,3 and 4 *p* values are:
Group 1 vs. Group 2: ** <0.01; *** <0.001
Group 2 vs. Group 3: ††† <0.001
Group 2 vs. Group 4: ‡ <0.05; (comparisons without notations and comparisons between group 3 and 4 are statistically not significant [p>0.05]).
§: P values
Group 1: Control group; Group 2: Sham group; Group 3: Clopidogrel pre-treatment group; Group 4: Rivaroxaban pre-treatment group.

pre-treatment groups had a significant reduction in heart and blood GSH levels.

Nitric oxide

The NO levels in all tissues and the paired comparison among the groups are shown in Table 4. NO levels in the sham group were significantly higher compared to the control group for all tissues. The NO levels in the heart tissue of the clopidogrel and rivaroxaban pre-treatment groups were significantly lower than in the sham group ($p<0.001$ and $p<0.050$, respectively), but there was no significant difference from baseline in the control group. There were no significant differences among the sham and treatment groups in ischemic muscle, kidney, liver, or blood. In addition, NO levels in the kidney tissue of the rivaroxaban pre-treatment group showed no significant difference from baseline values.

DISCUSSION

Oxygen free radicals are generated in tissues throughout the ischemic period. During reperfusion, these oxygen free radicals and superoxide radicals lead to endothelial damage, increased microvascular permeability, and tissue edema. Furthermore, activated adhesion molecules and cytokines can initiate a systemic inflammatory response which can lead to multiorgan dysfunction or death. This chain of events is termed an I/R injury.[15] Biochemically and histologically, a growing number of evidence shows that the systemic inflammatory response following I/R is more severe than with ischemia alone.[6] Antioxidants are defense mechanisms against free radical-induced oxidative damage, and I/R injury consists of a balance between oxidant production and oxidant removal.[16]

The I/R injury is an important risk with cardiac, vascular, and transplantation surgery, and acute lower extremity ischemia is associated with a high rate of compartment syndrome, fasciotomy, amputation, and mortality, despite reperfusion.[17-19] Further, the effects on ischemic tissue leading to reperfusion compartment syndrome and remote organ injury, come over time rather than immediately after reperfusion.[7,20] We believe that our study on the effects of clopidogrel and rivaroxaban on an I/R injury after a prolonged duration of reperfusion is more compatible with clinical practice.

Theoretically, an elevation of oxidant products and consumption of antioxidants could be expected in the sham group. Lipid peroxidation of the cell membrane is an important factor in I/R injury, and MDA is considered the final product of this reaction, as well as a sensitive indicator of lipid peroxidation. The MDA is commonly used as a biomarker to measure the levels of oxidative stress in reperfused organs.[10,21] In our study, a significant increase in the MDA levels was observed in all tissues in the sham group, compared to baseline levels. This finding demonstrates a successful I/R model. Also, the significant reduction of MDA in the rivaroxaban pre-treatment group demonstrated its beneficial effects on the ischemic tissue, heart, kidney, liver, and blood ($p<0.001$, <0.010, <0.050, and <0.001, respectively). Similarly, significant decreases in the MDA levels after clopidogrel pre-treatment in ischemic muscle, heart, liver, and blood tissue ($p<0.001$, <0.050, <0.050, and <0.001, respectively) indicate that clopidogrel confers beneficial protection against I/R injury. Furthermore, significant decreases of MDA in the rivaroxaban group, compared to the clopidogrel group, suggest that rivaroxaban provides superior protection against I/R injury in ischemic tissue ($p<0.050$). The significant reduction of blood MDA levels from both drugs is considered an evidence of the drugs, providing remote organ protection. However, there was no protection provided by clopidogrel for kidney tissues, compared to the MDA levels of the sham group ($p>0.050$).

In the present study, the differences in the MDA levels were non-significant, compared to baseline levels in ischemic muscle and kidney tissues in the rivaroxaban pre-treatment group. Additionally, the MDA difference from baseline was non-significant in the clopidogrel and rivaroxaban pre-treatment groups for blood levels. The protection shown by the near-baseline values of MDA in the pre-treatment groups indicates a significant protection against I/R injury. Our results where low plasma MDA levels show the protective effects of rivaroxaban are also consistent with the results from Caliskan et al.[10] after ischemia for six h and a short reperfusion time of 30 min. The reduction of the oxidant MDA in tissues might be related to a direct action of the drugs. We also believe that clopidogrel and rivaroxaban exert their effects via increased utilization of antioxidants, thereby, leading to decreased SOD, GSH, and NO levels.

The SOD is an enzyme which catalyzes the dismutation of the superoxide radical to hydrogen peroxide and oxygen. Superoxide, a byproduct of oxygen metabolism, can cause many types of cell damage,[22] and SOD, known to be a part of antioxidant defenses against superoxides, has been demonstrated to reduce plasma MDA levels.[21] The GSH is another endogenous antioxidant which plays a role in the elimination of hydrogen peroxide generated by SOD.[22]

In our study, the SOD and GSH levels decreased after I/R in the sham group, compared to the baseline levels of the control group. In the pre-treatment groups, a shift toward augmented consumption of SOD and GSH was evident. In particular, rivaroxaban use was more effective than clopidogrel against I/R injury due to higher SOD and GSH consumption in ischemic muscle and kidney.

The NO synthase is stimulated by cytokines, endotoxins, and lipopolysaccharides. It is a free radical which protects the cell through its inhibition of alkoxyl and peroxyl radicals.[9] The NO causes vasodilatation, prevents thrombocyte adhesion and aggregation, and modulates leukocyte-endothelial cell interactions and microvascular permeability.[15] The protective effect of NO in I/R is well-documented.[23,24] However, after ischemic endothelial damage, the production of NO decreases, and peroxynitrite molecules are formed, which are extremely toxic in the presence of cellular superoxide anions.[9,25] Yavuz et al.[26] found that NO levels had a peak concentration at two h of ischemia and, then, decreases over time, although the changes over time after reperfusion have not been studied, yet.

In our study, NO levels significantly increased after I/R in the sham group, compared to baseline values. This is similar to the results of Kanko et al.,[9] for ischemia (six h) and reperfusion (four h) in the liver, lungs, muscles, and plasma. In the study by Demirtas et al.,[5] plasma, heart, and kidney NO alterations after ischemia (six h) and reperfusion (one h), compared to the control and sham groups were not significant. On the other hand, Caliskan et al.[10] found decreased NO levels in the sham group, compared to baseline (six h) and reperfusion (30 min). The changes of NO levels might depend on reperfusion time. In our study, with the pre-treatment with clopidogrel or rivaroxaban, the only significant decreases of NO levels, compared to the sham group were found in the heart tissue ($p<0.001$ and $p<0.050$ for clopidogrel and rivaroxaban, respectively).

Demirtas et al.[5] compared the effects of clopidogrel and rivaroxaban, along with other drugs, against I/R injury in plasma, the heart, and kidneys after ischemia (6 h) and reperfusion (1 h). Elevation of MDA in the sham group and lower blood and heart levels in the groups on therapy with rivaroxaban and clopidogrel are similar to our results. In our study, decreases of kidney MDA levels in the clopidogrel pre-treatment group had no statistical significance, compared to the sham group, after reperfusion for eight h, indicating that no significant protection was conferred. Contrarily, kidney MDA levels were significantly lower after the rivaroxaban treatment, compared to sham. Thus, it can be concluded that clopidogrel loses its protective action in the kidney over time, while rivaroxaban is still protective. Kanko et al.[8] evaluated I/R injury induced by hind limb ischemia for six h and reperfusion for four h, comparing the effects of clopidogrel to placebo by measuring MDA, GSH, and SOD in liver, lung, kidney, and plasma tissues. The clopidogrel pre-treatment group had significantly lower MDA levels, compared to the sham group in the liver, kidneys, and plasma. It can be concluded that the significant protective effects of clopidogrel in the kidney, compared to the sham group, is lost between four and six h of reperfusion.

The variation of ischemia and reperfusion times between the aforementioned studies may be the cause of the different outcomes due to the broad range of chemical reactions, the mechanism of tissue injury, the interactions between reactive products and, time-dependent changes.

Clopidogrel is an antiaggregant agent used in ischemic cardiovascular diseases to inhibit the platelet activation and aggregation induced by adenosine diphosphate.[8] In our study, in the clopidogrel pre-treatment group, the decreased levels, compared to the sham group, were more significant than with the rivaroxaban pre-treatment group for SOD ($p<0.001$ vs. <0.010, respectively), GSH ($p<0.001$ vs. <0.050, respectively), and NO ($p< 0.001$ vs. <0.050, respectively) levels in the heart tissue. A relationship between clopidogrel and higher antioxidant consumption in the heart after an I/R injury is apparently present, although the balance of synthesis and consumption of antioxidants is still unknown. As no differences in heart MDA levels were present in the pre-treatment groups, a benefit of clopidogrel over rivaroxaban cannot be claimed in the heart tissue after an eight h reperfusion.

Rivaroxaban is an oral anticoagulant which rapidly induces Factor Xa inhibition in a reversible manner.[27] Additionally, rivaroxaban has direct anti-inflammatory effects by inhibiting pro-inflammatory protease activation receptors.[28] This can be a factor affecting the favorable results for rivaroxaban.

Our results show that clopidogrel and rivaroxaban agents seem to have protective efficacy against I/R damage. Clopidogrel and rivaroxaban are effective against I/R injury in the heart, ischemic muscle, the liver, and blood. Rivaroxaban is more protective on kidney and ischemic muscle, compared to clopidogrel

after eight h of reperfusion. Longer reperfusion times affect the results of the drugs on I/R.

One of the limitations of this study is the lack of pathological examinations. The main reason for this is that many parameters in multiple organs were biochemically studied simultaneously. However, the main strength of the study is that a wide spectrum of organs was studied and the ischemic muscle itself was also examined. This study contributes to the literature, as the timing of reperfusion shows the late results of I/R, compared to the early results examined in previous studies.

In conclusion, thromboprophylaxis with clopidogrel or rivaroxaban provide protection following I/R injury. Superior ischemic muscle and better remote organ protection is achieved with rivaroxaban in our ischemia-reperfusion model.

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