Acta Cardiol Sin 2024;40:77–86
Original Article
doi: 10.6515/ACS.202401_40(1).20230814C

Cardiovascular Surgery

The Anti-Apoptotic Effect of Ranolazine on Cerebral Protection during Cardiopulmonary Bypass and Carotid Artery Surgery

Engin Akgul,[1] Meliha Koldemir Gunduz,[2] Ali İhsan Parlar,[1] Yesim Guner,[1] Murat Eroglu,[1] Abdulkerim Ozhan,[1] Gulen Sezer Alptekin[1] and Ahmet Cekirdekci[1]

Background: We aimed to determine the usability of ranolazine (Rn) as a neuroprotective during cardiac surgeries and carotid artery interventions where cerebral blood flow is interrupted.

Methods: Female Wistar albino rats were used. The rats were divided into 4 groups of 8 rats each. The first group (Group 1) was the control group. Group 2 underwent ischemia induction but was not treated with Rn. Group 3 received 25 mg/kg/day and Group 4 50 mg/kg/day Rn intraperitoneally, starting 3 days before ischemia induction. Bilateral carotid arteries were explored and clamped simultaneously. Ischemia was induced for 15 minutes. After 72 hours, the experimental animals were sacrificed.

Results: Superoxide dismutase, alkaline phosphatase, and interleukin 6 levels were similar among the 4 groups. Acetylcholine esterase (Group 3: p = 0.007, Group 4: p = 0.002), tumor necrosis factor-alpha (Group 4: p = 0.01), and annexin V (Group 3: p = 0.001) levels were statistically significantly lower in the Rn-treated groups. Malondialdehyde (Group 3: p = 0.003, Group 4: p = 0.009), reduced glutathione (Group 4: p = 0.04), acid phosphatase (Group 3: p = 0.04), noradrenaline (Group 3: p = 0.01), and Bcl-2 (Group 4: p = 0.004) levels were significantly higher in the Rn-treated groups.

Conclusion: The results of this study demonstrated the antiapoptotic effect of Rn in a brain ischemia-reperfusion model of rats receiving Rn before the procedure.

Key Words: Carotid occlusion • Cerebral ischemia • Coronary bypass • Heart surgery • Ranolazine

INTRODUCTION

Ranolazine (Rn) was approved by the U.S. Food and Drug Administration in 2006 as a 'cardiac metabolic modulator' drug.[1] The term cardiac metabolic modulator refers to the change induced by Rn in the pathways used by the myocardium during adenosine triphosphate (ATP) production.[2] In conditions of an abundance of oxygen (aerobic), that is, the absence of ischemia, the primary energy production pathway of the myocardium is the 'fatty acid oxidation' pathway. In hypoxic conditions such as coronary artery disease, ATP is provided primarily by 'glucose oxidation'.[2] Although its mechanism of action is not fully known, Rn enables the myocardium to use the fatty acid oxidation pathway, which allows higher ATP production, instead of the glucose oxidation pathway in hypoxic conditions.[2] This feature paved the way for Rn to be used as an antianginal agent in stable coronary artery patients.[3,4]

Rn, which was referred to as a 'metabolic modulator' as a result of the first studies, was later defined as a 'selective inhibitor of late sodium current' after its mechanism of action had been determined.[1] Pain and epi-

Received: April 25, 2023 Accepted: August 14, 2023
[1]Department of Cardiovascular Surgery; [2]Department of Biology, Kutahya Health Science University, Turkey.
Corresponding author: Dr. Engin Akgul, Department of Cardiovascular Surgery, Kutahya Health Science University, Turkey. E-mail: engin_akgul@hotmail.com

Abbreviations	
AChE	Acetylcholine esterase
ACP	Acid phosphatase
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
Bcl-2	B-cell lymphoma 2
CA	Cerebral autoregulation
CABG	Coronary artery bypass grafting
ELISA	Enzyme-linked immunosorbent assay
GSH	Reduced glutathione
IL-6	Interleukin 6
I/R	Ischemia/reperfusion
MDA	Malondialdehyde
NA	Noradrenaline
Rn	Ranolazine
SE	Standard error
SOD	Superoxide dismutase
TNF-α	Tumor necrosis factor-alpha

leptic diseases have also been an area of study.[5]

Aldosoro et al. reported the anti-apoptotic effect of Rn on the central nervous system in vitro.[6] Therefore, the present study aimed to determine whether Rn is effective in protecting the central nervous system from ischemia-reperfusion injury and reducing oxidative stress in vivo during and after cardiovascular surgical procedures that cause hemodynamic and cerebral autoregulation variations.

METHODS

Approval for the study was obtained from the ethics committee (13/06/2019, 12650661-604.01.02). A cerebral ischemia-reperfusion injury model was created using female Wistar albino rats weighing 250-300 g. In accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication, 8th edition, 2011), the rats were maintained on a 12-h light/dark cycle at 22 ± 4 °C room temperature and were allowed free access to food and water (20 ± 1 °C). A total of 32 rats were used and divided into 4 groups of 8 animals each. The first group (Group 1) was the control group. Group 2 underwent ischemia induction but was not treated with Rn. Group 3 received 25 mg/kg/day Rn intraperitoneally, starting 3 days before ischemia induction. Group 4 consisted of rats that were given 50 mg/kg/day Rn intraperitoneally, starting 3 days before ischemia induction. It is known that the concentration of drugs in the blood can vary during cardiopulmonary bypass surgeries, including situations such as the use of a heart-lung machine and blood/fluid transfusion during the operation. Therefore, in order to effectively protect the brain against ischemia in potential emergency cases such as acute aortic dissection, we planned to work with high doses of Rn.

Before the surgical procedure, the rats were anesthetized by intraperitoneal administration of 90 mg/kg of ketamine-hydrochloride and 10 mg/kg of xylazine. The experimental animals were placed in the supine position, and a cervical midline incision was made after cleaning the surgical area. The right and left common carotid arteries were accessed by performing superficial microdissection. Two YASARGIL aneurysm clips were placed on the common carotid artery 1 cm and 3 cm proximal to the carotid bifurcation, and ischemia was achieved by keeping the clips closed for 15 minutes. Afterward, the clips were removed and the procedure was terminated. The experimental animals were sacrificed by administering high-dose anesthesia 72 hours after ischemia induction to collect blood-brain tissues for the measurement of biochemical parameters.

Cerebral tissues collected from the experimental animals after sacrifice were stored at -80 °C. After keeping the blood samples at room temperature for 2 hours, they were centrifuged for 20 minutes at 1,000 × g for serum isolation, and then stored at -80 °C until analysis.

Superoxide dismutase (SOD), acetylcholine esterase (AChE), malondialdehyde (MDA), reduced glutathione (GSH), alkaline phosphatase (ALP), acid phosphatase (ACP), interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α), and noradrenaline were measured in brain tissue samples, while annexin V and B-cell lymphoma 2 (Bcl-2) levels were measured in serum samples. These measurements were used to determine the degree of ischemia-reperfusion injury in the brain tissue.

Preparation of 10% tissue homogenate

Brain tissue samples were separately washed with physiological saline solution to clean off the blood, and then dried with a filter paper and weighed. Tissue was lysed in a homogenizer with glass beads and saline. Tissue homogenates were individually placed in Eppendorf

tubes, labeled, and stored at +4 °C until analysis.

SOD assay

Brain tissue levels of SOD after Rn administration were determined using an Uscn enzyme-linked immunosorbent assay (ELISA) kit (Uscn Life Science Inc., Wuhan).

AChE assay

Brain tissue levels of AChE after Rn administration were determined using an Uscn ELISA kit (Uscn Life Science Inc. Wuhan).

MDA assay

Levels of MDA were determined using the method of Ledwozyw (1986).[7] First, 1,250 μL of trichloroacetic acid solution (1.22 M in 0.6 M HCl) was added to 250 μL of brain tissue homogenate. After incubation at room temperature for 15 minutes, the sample was incubated with 750 μL of thiobarbituric acid solution (0.047 M) for 30 minutes in a boiling water bath. Then, 2,000 μL of commercial n-Butanol was added, and the resultant solution was centrifuged at 1,560 × g for 10 minutes. After collecting the butanol phase, absorbance was recorded at 532 nm, and MDA was calculated as nmol MDA/g protein.

GSH assay

GSH assay was carried out according to the method of Beutler (1975).[8] First, 0.3 mL of deproteinization solution containing metaphosphoric acid, NaCl, and EDTA-Na was added to 0.2 mL of brain tissue homogenate. Then, 0.2 mL of supernatant was collected from the homogenate centrifuged at 2,028 × g for 10 minutes and mixed with 0.8 mL of Na2HPO4 solution (0.3 M), and 0.1 mL of 40 mg% DTNB (5-5-dithiobis 1-2 nitrobenzoic acid) were added. The colored solution formed as a result of the reaction was analyzed spectrophotometrically at 412 nm and evaluated in nmol GSH/g protein.

ALP enzyme activity assay

ALP enzyme activity was determined according to the method of Walter.[9] The therapeutic effects of Rn on brain tissue were determined by hydrolyzing p-nitrophenyl phosphate, which is used as a substrate for the ALP enzyme, to p-nitrophenol depending on the pH of the medium. The absorbance of the resultant product was determined spectrophotometrically at 405 nm, and the ALP activity was estimated in U/g protein using the following formula: Abs × 434 (U/L).

ACP enzyme activity assay

ACP enzyme activity was determined according to the method of Walter.[9] Depending on the pH of the medium, the ACP enzyme hydrolyzes the p-nitrophenyl phosphate, which is used as a substrate, to p-nitrophenol. The absorbance of the resultant product was evaluated spectrophotometrically at 405 nm and estimated in U/g protein using the following formula: Abs × 28.8 (U/L), taking into account the supernatant dilutions.

Estimation of total protein

Protein assay was performed using the method of Bradford (1976).[10] A standard curve plot was created with stock albumin solution. Absorbances against the blank were recorded at 595 nm 15 minutes after mixing 25 μL of tissue homogenate with 775 μL of distilled water and 200 μL of commercial Bradford reagent, and the protein amounts were determined in μg/μL.

Cytokine analysis

In order to evaluate the inflammatory response after Rn administration, brain tissue samples collected from the rats were homogenized, and IL-6 and TNF-α levels were measured using an Uscn ELISA kit (Uscn Life Science Inc., Wuhan).

Plasma norepinephrine analysis

Plasma norepinephrine levels were determined using an Uscn ELISA kit (Uscn Life Science Inc., Wuhan) to determine the level of stimulation of the brain tissue after Rn administration in the acute ischemic rat model.

Determination of apoptosis levels

Annexin V and Bcl-2 levels in the serum samples were determined using an Uscn ELISA kit (Uscn Life Science Inc., Wuhan) as a result of Rn administration in carotid occlusion.

Statistical analysis

The results were analyzed using the SPSS software package. The study results were expressed as mean ± standard error (SE). The Student's t-test was used for two

group comparisons. To analyze the result of the data obtained after Rn administrationwith the control and ischemia control groups, a one-way analysis of variance (ANOVA) was performed between more than two groups. Results of the data obtained after Rn administration with the control and ischemia control groups. A p value < 0.05 was considered statistically significant.

RESULTS

Antioxidant component findings in a cerebral ischemia reperfusion model are shown at Table 1 and Figure 1. Elisa component findings in a cerebral ischemia reperfusion model are shown at Table 2 and Figure 2.

Post-ischemia brain tissue levels of SOD after Rn administration

Comparisons of the ischemia-induced groups with the control group revealed no statistically significant differences in brain tissue levels of SOD (Table 1). Comparisons of the Rn-treated groups with the ischemia control group showed statistically lower brain tissue levels of SOD after 50 mg/kg Rn administration (p = 0.03).

Post-ischemia brain tissue levels of AChE after Rn administration

Comparisons of the ischemia-induced groups with the control group showed significantly lower brain tissue levels of AChE in the Rn-treated groups (p = 0.007, p = 0.05, and p = 0.002, respectively) (Table 1). Comparisons

Table 1. Antioxidant component findings in a cerebral ischemia reperfusion model after carotid artery occlusion

Group	Group 1 (n = 8)	Group 2 (n = 8)	Group 3 (n = 7)	Group 4 (n = 6)
SOD (pg/ml)	3.89 ± 0.25	4.18 ± 0.24	4.15 ± 0.25	3.46 ± 0.18*
AChE (pg/ml)	6.48 ± 0.47	4.49 ± 0.52$^{\#}$	4.06 ± 0.75*	3.21 ± 0.37$^{\#}$
MDA	0.018 ± 0.004	0.071 ± 0.017*	0.174 ± 0.018†	0.156 ± 0.183$^{\#}$
GSH	0.006 ± 0.003	0.024 ± 0.004	0.028 ± 0.007*	0.038 ± 0.004$^{\#}$
ALP	0.00010 ± 7.81E-06	0.00016 ± 2.69E-05	0.00017 ± 1.03E-05†	0.00018 ± 2.83E-05$^{\#}$
ACP	0.094 ± 0.002	0.076 ± 0.009*	0.092 ± 0.002	0.082 ± 0.005*

AChE, acetylcholine esterase; ACP, acid phosphatase; ALP, alkaline phosphatase; GSH, reduced glutathione; MDA, malondialdehyde; SOD, superoxide dismutase. * p < 0.05, $^{\#}$ p < 0.01, † p < 0.001.

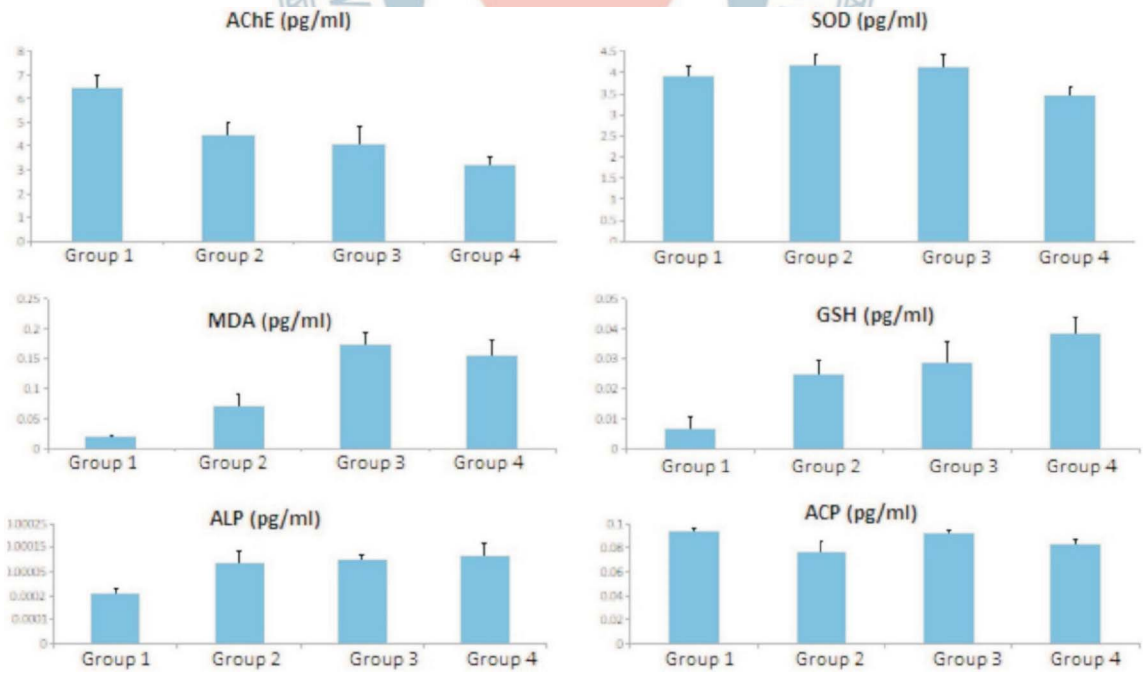

Figure 1. *Antioxidant component findings in a cerebral ischemia reperfusion model. AChE, acetylcholine esterase; ACP, acid phosphatase; ALP, alkaline phosphatase; GSH, reduced glutathione; MDA, malondialdehyde; SOD, superoxide dismutase.*

Table 2. Elisa component findings in a cerebral ischemia reperfusion model after carotid artery occlusion

Group	Group 1 (n = 8)	Group 2 (n = 8)	Group 3 (n = 7)	Group 4 (n = 6)
IL-6 (pg/ml)	76.9 ± 4.42	124.01 ± 13.51*	144.96 ± 13.09#	133.40 ± 4.3*
TNF-α (pg/ml)	1376.16 ± 127.8	1283.32 ± 55.9	1284.16 ± 146.8	1082.11 ± 87.8*
Nöradrenalin (pg/ml)	689.35 ± 113.29	959.56 ± 135.62	1121.41 ± 218.47	1795.16 ± 176.84*
Anneksin V (pg/ml)	10.76 ± 1.58	11.34 ± 1.34	7.04 ± 0.07*	4.85 ± 0.46#
Bcl2 (pg/ml)	126.89 ± 2.14	148.39 ± 6.04*	169.166 ± 11.52*	251.97 ± 23.85*

Bcl-2, B-cell lymphoma 2; IL-6, interleukin 6; TNF-α, tumor necrosis factor-alpha. * p < 0.05, # p < 0.001.

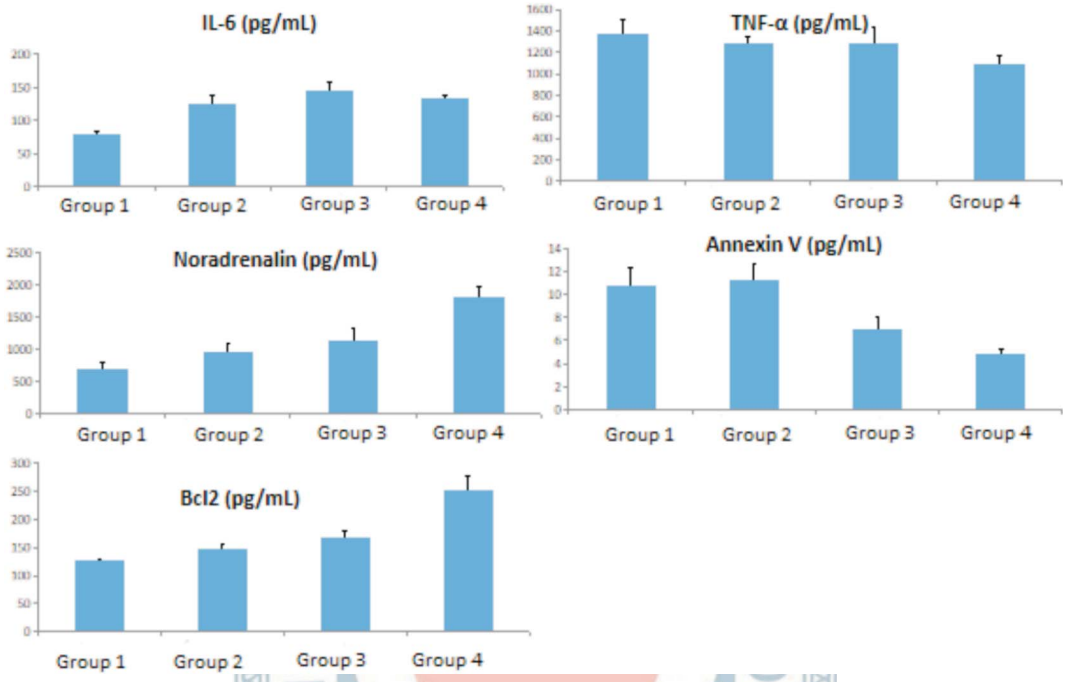

Figure 2. *Elisa component findings in a cerebral ischemia reperfusion model. Bcl-2, B-cell lymphoma 2; IL-6, interleukin 6; TNF, tumor necrosis factor-alpha.*

of the Rn-treated groups revealed lower AChE levels both in the 25 mg/kg (4.06 ± 0.75) and 50 mg/kg groups compared to the ischemia control group. This decrease at a dose of 50 mg/kg was also statistically significant (p = 0.04).

Post-ischemia brain tissue levels of MDA after Rn administration

Comparisons of the ischemia-induced groups with the control group revealed significantly higher brain tissue levels of MDA in all ischemia groups (p = 0.04, p = 0.0005, and p = 0.002 for Group 2, Group 3 and Group 4, respectively) (Table 1). The Rn-treated groups had significantly higher brain levels of MDA compared to the ischemia control group (Group 3: p = 0.003, Group 4: p = 0.009).

Post-ischemia brain tissue levels of GSH after Rn administration

Comparisons of the ischemia-induced groups with the control group showed statistically higher brain tissue levels of GSH in all ischemia groups (p = 0.05 for Group 2, p = 0.03 for Group 3, and p = 0.001 for Group 4) (Table 1). The Rn-treated groups had higher brain tissue levels of GSH compared to the ischemia control group. The group treated with 50 mg/kg Rn had a significantly higher GSH level compared to the ischemia control group (p = 0.04).

Post-ischemia brain tissue levels of ALP after Rn administration

Comparisons of the ischemia-induced groups with the control group revealed higher brain tissue levels of

ALP in all ischemia groups (p = 0.06 for Group 2, p = 0.0001 for Group 3, and p = 0.01 for Group 4) (Table 1). The Rn-treated groups had higher brain tissue levels of ALP compared to the ischemia control group, with no statistically significant differences.

Post-ischemia brain tissue levels of ACP after Rn administration

Comparisons of the ischemia-induced groups with the control group showed lower brain tissue levels of ACP in all ischemia groups (p = 0.04 for Group 2, p = 0.34 for Group 3, and p = 0.04 for Group 4) (Table 1). Comparisons of the Rn-treated groups with the ischemia control group revealed higher brain tissue levels of ACP in Group 3 and Group 4. The group treated with 25 mg/kg Rn had a significantly higher brain tissue level of ACP compared to the ischemia control group (p = 0.04).

Post-ischemia brain tissue levels of IL-6 after Rn administration

Comparisons of the ischemia-induced groups with the control group showed significantly higher IL-6 levels in the ischemia groups (p = 0.01 for Group 1, p = 0.0006 for Group 2, and p = 0.02 for Group 3) (Table 2). The Rn-treated groups had higher brain tissue levels of IL-6 compared to the ischemia control group, with no statistically significant differences.

Post-ischemia brain tissue levels of TNF-α after Rn administration

Comparisons of the ischemia-induced groups with the control group revealed lower brain tissue levels of TNF-α in the ischemia groups (Table 2). However, this decrease was not statistically significant. The Rn-treated groups had lower brain tissue levels of TNF-α compared to the ischemia control group. The group treated with 50 mg/kg Rn had a significantly lower TNF-α level compared to the ischemia control group (p = 0.01).

Post-ischemia brain tissue levels of noradrenaline (NA) after Rn administration

Comparisons of the ischemia-induced groups with the control group showed a significantly higher brain tissue level of NA in the 50 mg/kg Rn group (p = 0.0007) (Table 2). The Rn-treated groups had higher brain tissue levels of NA compared to the ischemia control group, with a statistically significant difference for Group 3 (p = 0.01).

Post-ischemia annexin V levels in the serum samples and control serum samples after Rn administration

Comparisons of Group 2 (ischemia-induced but non-Rn-treated group), Group 3 (treated with 25 mg/kg Rn), and Group 4 (treated with 50 mg/kg Rn) with the control group revealed significantly lower serum levels of annexin V in the Rn-treated groups (25 mg/kg Rn group: p = 0.03, 50 mg/kg Rn group: p = 0.008) (Table 2). The groups treated with 25 mg/kg Rn and 50 mg/kg Rn had significantly lower serum levels of annexin V compared to the ischemia control group (p = 0.01, p = 0.003).

Post-ischemia Bcl-2 levels of serum samples and control serum samples after Rn administration

Comparisons of Group 2 (post-acute carotid occlusion ischemia-induced but non-Rn-treated group), Group 3 (treated with 25 mg/kg Rn), and Group 4 (treated with 50 mg/kg Rn) with the control group showed significantly higher serum levels of Bcl-2 in all ischemia groups (ischemia-induced control group: p = 0.006, 25 mg/kg Rn group: p = 0.004, 50 mg/kg Rn group: p = 0.001) (Table 2). The Rn-treated groups had higher serum levels of Bcl-2 compared to the ischemia control group. The group treated with 50 mg/kg Rn group had a significantly higher serum level of Bcl-2 compared to the ischemia control group (p = 0.004).

DISCUSSION

Cardiopulmonary bypass surgeries affect blood circulation, so it is essential to take precautions against end organ damage. Sufficient flow and reasonable operative duration should be ensured to preserve the kidneys, liver, and other internal organs. However, the situation is somewhat more complex when it comes to the brain. In brain tissue, which has very low resistance to ischemia, irreversible damage can occur in possible hypoxic conditions. Therefore, protection of the brain against ischemia is high on the “to-do list” in such surgeries. Regardless of how effective the surgical procedure may be, if a patient develops neurological deficits, the operation can be considered to have failed. Therefore, it would be beneficial for surgeons to continuously monitor the level

of brain oxygenation during surgery, if possible, using cerebral oximetry.

The brain has the ability to maintain constant blood flow despite changes in cerebral blood pressure and perfusion values.[11] This is referred to as cerebral autoregulation (CA).[11] CA protects against cerebral oligemia/hyperemia with mean arterial pressure variations.[11] A systematic review of neurological events after coronary artery bypass grafting (CABG) reported disrupted CA after CABG surgery, thus opening the door to neurological complications.[11] These complications can be seen in a wide spectrum from simple cognitive disorders to delirium and severe neurological deficits.[12] In the literature, neurocognitive impairment has been reported in 15-66% of patients after CABG surgery until discharge, with a rate of 40% within 5 years.[12] Among these complications, the incidence of symptomatic stroke, which causes sequelae, has been reported to be approximately 6%.[13] There have been studies showing pulsatile or nonpulsatile flow,[14] low or high perfusion pressure of the cardiopulmonary bypass circuit,[14] and alterations in body temperature[14] as causes of disruption of CA and neurological events, with the most severe complications developing due to cerebral ischemia.[15] On the other hand, there are also studies demonstrating no decrease in neurological events after off-pump operations performed without the use of a cardiopulmonary bypass machine.[16,17] In addition, other studies have argued that neurological events which develop regardless of whether the operation is performed on-pump or off-pump are caused by micro/macro embolism.[18] Taken together, the likelihood of experiencing neurological events after CABG is quite high, and the exact cause is not yet known. What is known is that CA is disrupted by CABG operations, and that this is closely associated with prolonged length of hospital stay, readmission, delirium, stroke, and mortality.[11] The reason for conducting this study was to discover an agent that would help protect the cerebral tissue from CA disruption after CABG surgery, as well as from secondary neurological events. Rn was chosen because it has been demonstrated to protect neurological tissues against apoptosis in vitro.[6]

End organ damage, which may occur in cases of disrupted blood supply due to problems in the circulatory system, can sometimes result in irreversible complications. Especially in the event of a stroke, it is necessary to urgently restore blood supply to the brain tissue as early as possible.[19] However, studies have shown that cellular damage also develops as a consequence of reperfusion following ischemia, and brain edema and infarction occur after cerebral ischemia/reperfusion.[20]

SOD catalyzes the dismutation (or partitioning) of some superoxide radicals into the O_2 molecule and the degradation of some to hydrogen peroxide (H_2O_2), a less reactive molecule. These properties establish a powerful antioxidant defense system against superoxide radicals.[21,22] SOD enzyme deficiency results in mitochondrial DNA damage, which forms a firm basis for the onset of atherosclerosis,[22] and studies have shown an association between the deficiency of the SOD 3 form and atherosclerosis.[23] Our study showed no significant differences in SOD levels between the groups with and without ischemia. This result suggests that Rn does not affect the physiology of SOD and does not contribute to SOD-mediated antioxidation. However, comparisons of the ischemia groups revealed statistically low levels of SOD in Group 4. Therefore, it is necessary to investigate whether Rn has a suppressive effect on SOD in a larger study.

Higher levels of free oxygen radicals in the environment as a result of reperfusion can damage lipid structures of the cell membrane. Eventually, cell membrane lipids are oxidized, forming a toxic lipid breakdown product, MDA.[24] Therefore, the measured MDA level is directly proportional to the number of damaged cells. MDA levels measured in our study were found to be higher in all ischemia groups compared to the control group. Comparisons of the ischemia groups showed statistically higher levels of MDA in the subjects treated with Rn. Based on these results, it can be concluded that Rn has no effect on protecting the cell wall against free oxygen radicals.

GSH is a non-protein sulfhydryl compound found in mammalian cells that protects cells from oxidative stress. It removes free oxygen radicals from the environment and prevents cell lipid peroxidation.[25] A decrease in GSH has been associated with an increased incidence of diseases and adverse conditions such as neurodegenerative diseases, cardiovascular diseases, cellular aging, and cystic fibrosis.[26] Our study showed significantly higher levels of GSH in the ischemia-induced groups. Moreover, comparisons of the ischemia groups revealed statistically higher levels of GSH in the Rn-treated groups, with the highest level in Group 4. These results suggest that

Rn has a protective effect against reperfusion-related cellular damage by increasing GSH release.

Acetylcholine is one of the most well-known neurotransmitters. It is produced from 'choline', which plays a role in lipid metabolism, and 'acetyl coenzyme A', which is formed as a result of cellular respiration. It is hydrolyzed by AChE as a result of the transmission of nerve impulses in the intersynaptic space and eliminated.[27] AChE is also involved in processes such as nerve cell adhesion, migration, differentiation, apoptosis and synaptogenesis.[27] High levels of AChE have been shown to drive cells to apoptosis by activating 'glycogen synthesis kinase 3' in the nervous system.[28] In addition, increased levels of AChE have been observed in neural inflammatory conditions and after events such as stroke.[29] Therefore, high levels of AChE indicate increased neural damage.[12] The results of our study showed statistically lower levels of AChE in the Rn-treated groups. The suppression of AChE by Rn, which is expected to increase in ischemic conditions according to the literature, may therefore prevent cellular apoptosis.

Apoptosis can be of intrinsic and extrinsic origin. The Bcl-2 family is involved in the intrinsic pathway of apoptosis.[30] Depending on being a heterodimer or a homodimer, the Bcl-2 family proteins show proapoptotic or antiapoptotic activity.[31] Molecules such as BclXs, Bax and Bad are proapoptotic, while Bcl-2, Mcl-1 and Bcl-xL are antiapoptotic.[31] Studies have shown that the Bax/Bcl-2 ratio increases after ischemia/reperfusion (I/R), resulting in increased mitochondrial permeability, DNA fragmentation, and eventually cell apoptosis.[32,33] We did not measure Bax in this study, but the Bcl-2 ratio was statistically higher in the Rn-treated groups. An increase in Bcl-2 value would result in a lower Bax/Bcl-2 ratio, and consequently that antiapoptotic properties would become dominant.

TNF-α is an inflammatory cytokine secreted from tissues and macrophages,[34] and it is involved in the extrinsic pathway of the apoptotic system.[35] Previous studies have shown its substantial role in neurodegenerative diseases.[36] Cerebral I/R studies have found statistically higher levels of TNF-α in ischemia-induced groups.[37,38] Our study showed statistically lower levels of TNF-α in the Rn-treated groups. A low level of TNF-α means limited regional inflammation and apoptotic processes. This further indicates the antiapoptotic properties of Rn.

ALP is a cell surface protein widely distributed throughout the body. It is also found in soluble form in body fluids and blood. Neuronal ALP plays a vital in cortical development and aminobutyric acid metabolism.[39] Cerebrospinal fluid and plasma levels of ALP have been shown to increase in patients with brain damage and neurodegenerative events.[40] Our study revealed increased brain tissue levels of ALP after the induction of ischemic injury; however, this increase was not statistically significant in the Rn-treated groups compared to the ischemia control group. In other words, Rn did not show any pathophysiological activity to change the serum level of ALP.

ACP is responsible for the hydrolysis of phosphate monoesters, and therefore it is involved in cellular destruction.[41] As with ALP, the level of ACP increases during periods of cellular destruction, and high ACP levels have been reported to be a predictor of poor prognosis.[42] Our study showed statistically lower levels of ACP in the Rn-treated subjects compared to the control group.

IL-6, a multifunctional cytokine, can be secreted from glial cells, microglia, or neurons in brain tissue.[43] It is responsible for initiating immune reactions, stimulation of acute-phase reactants, and providing hematopoiesis in conditions such as tissue injuries and infections.[44] Although early control of its secretion can provide hematopoiesis, its long-term secretion causes chronic inflammation.[44] Increased brain tissue levels of IL-6 have been reported during ischemic events,[45,46] and microglia, phagocytic cells, and other glial cells have been shown to physiologically release IL-6.[43] Our study demonstrated higher levels of IL-6 in all ischemic groups compared to the control group, showing that Rn did not suppress IL-6 physiology in brain tissue.

NA and dopamine are neuromodulators that control many vital functions such as vigilance, memory, and learning.[47] The main source of NA in the brain is the 'locus coeruleus', which is the center of dissociative functions.[47] Increased levels of NA after brain injury and improved cognitive function in subjects medically treated with exogenous NA after injury have been reported.[48,49] NA release has been shown to increase the activation of the locus coeruleus via the nervous vagus, resulting in enhanced brain plasticity after injury.[50] Our study showed higher levels of NA in all I/R groups, and comparisons of the I/R groups revealed statistically significantly higher levels of NA in Group 4. This indicates that brain func-

tion is more likely to return to normal in subjects receiving Rn.

Annexins are a group of cellular proteins responsible for the formation of the cytoskeleton and cell shape. They also have effects on functions such as endo/exocytosis and Ca channel formation.[50] Extracellular annexins have been shown to be involved in inflammation, fibrinolysis, coagulation, and apoptosis.[50] Phosphatidylserine, which is normally found on the cytoplasmic surface of the cell membrane, translocates to the outer surface of the cell membrane if the cell is to undergo apoptosis for any reason. Annexin V in the extracellular space binds to phosphatidylserine translocated to the outer surface of the cell membrane, initiating apoptosis. Our study demonstrated statistically lower levels of annexin V in the Rn-treated groups, further indicating that Rn prevents cell apoptosis.

CONCLUSION

In conclusion, this study demonstrated that Rn was partially beneficial for cell integrity by increasing the GSH level in response to free oxygen radicals. It also had an antiapoptotic effect by increasing Bcl-2 and decreasing AChE, TNF-α and ACP levels, and also helped cerebral recovery by increasing NA levels. In light of these results, we suggest that the use of Rn in cases requiring cerebral protection may have positive results.

DECLARATION

There is not any funding to report for this submission.

The manuscript has been submitted solely to this journal and is not published, in press, or submitted elsewhere.

All the research meets the ethical guidelines, including adherence to the legal requirements of the study country.

DECLARATION OF CONFLICT OF INTEREST

All the authors declare no conflict of interest.

REFERENCES

1. Özdemir M. Ranolazin: mechanism of antianginal effects. *Turk Kardiyol Dern Ars* 2016;44:8-12.
2. Anderson JR, Nawarskas JJ. Ranolazine. A metabolic modulator for the treatment of chronic stable angina. *Cardiol Rev* 2005; 13:202-10.
3. Fihn SD, Gardin JM, Abrams J, et al. 2012 ACCF/AHA/ACP/AATS/ PCNA/SCAI/STS Guideline for the diagnosis and management of patients with stable ischemic heart disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, and the American College of Physicians, American Association for Thoracic Surgery, Preventive Cardiovascular Nurses Association, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *J Am Coll Cardiol* 2012;60:e44-164.
4. Montalescot G, Sechtem U, Achenbach S, et al. 2013 ESC guidelines on the management of stable coronary artery disease: the Task Force on the management of stable coronary artery disease of the European Society of Cardiology. *Eur Heart J* 2013;34:2949-3003.
5. Kahlig K, Lepist I, Leung K, et al. Ranolazine selectively blocks persistent current evoked by epilepsy-associated Nav1.1mutations. *Br J Pharmacol* 2010;161:1414-26.
6. Aldasoro M, Guerra-Ojeda S, Aguirre-Rueda D, et al. Effects of ranolazine on astrocytes and neurons in primary culture. *PLOS One* 2016;11:e0150619.
7. Ledwozyw A, Michalak J, Stepień A, Kadziołka A. The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. *Clin Chim Acta* 1986;155:275-83.
8. Beutler E. Glutathione in red cell metabolism: a manual of biochemical methods, 2nd edn. Grune and Stratton, New York 1975: 112-4.
9. WalterK, Schult C. Acid and alkaline phosphatase in serum (two point method). In: Bergmeyer HU (ed) 2nd ed, FLMethods of Enzymatic Analysis, pp 856-86.
10. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1977;72:248-54.
11. Caldas JR, Haunton VJ, Panerai RB, et al. Cerebral autoregulation in cardiopulmonary bypass surgery: a systematic review. *Interact Cardiovasc Thorac Surg* 2018;26:494-503.
12. Raffa G, Agnello F, Occhipinti G, et al. Neurological complications after cardiac surgery: a retrospective case-control study of risk factors and outcome. *J Cardiothorac Surg* 2019;14:23.
13. Bucerius J, Gummert JF, Borger MA, et al. Stroke after cardiac surgery: a risk factor analysis of 16,184 consecutive adult patients. *Ann Thorac Surg* 2003;75:472-8.
14. Ozhan A, Akgul E, Parlar Ai, et al. The association of blood transfusion and acute kidney injury in diabetic coronary artery bypass grafting patients. *Cardiovasc Surg Int* 2021;8:13-9.

15. Qu JZ, Kao LW, Smith JE, et al. Brain protection in aortic arch surgery: an evolving field. *J Cardiothorac Vasc Anesth* 2021;35: 1176-88.
16. Hueb W, Lopes NH, Pereira AC, et al. Five-year follow-up of a randomized comparison between off-pump and on-pump stable multivessel coronary artery bypass grafting. The MASS III Trial. *Circulation* 2010;122:48.
17. Rodriguez RA, Rubens FD, Wozny D, Nathan HJ. Cerebral emboli detected by transcranial Doppler during cardiopulmonary bypass are not correlated with postoperative cognitive deficits. *Stroke* 2010;41:2229-35.
18. Akgul E, Parlar AI, Erkul GSA, et al. Investigation of the effect of preoperative hypoalbuminemia, blood urea nitrogen and creatinine levels on postoperative atrial fibrillation on off-pump coronary bypass surgery patients. *Heart Surg Forum* 2020;23:E641-6.
19. Wang M, Wang J, Liu Z, et al. Effects of intermedin on autophagy in cerebral ischemia/reperfusion injury. *Neuropeptides* 2018;68: 15-21.
20. Aslankoç R, Demirci D, İnan Ü, et al. The role of antioxidant enzymes in oxidative stress - superoxide dismutase (Sod), catalase (Cat) and glutathione peroxidase (Gpx). *Med J SDU* 2019;26:362-9.
21. Khosravi M, Poursaleh A, Ghasempour G, et al. The effects of oxidative stress on the development of atherosclerosis. *Biological Chemistry* 2019;400:711-32.
22. Fukai T, Siegfried M, R Ushio-Fukai M, et al. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. *J Clin Invest* 2000;105:1631-9.
23. Mammadov R, Süleyman B, Bilgin AÖ. Nizatidinin bobrek iskemi reperfuzyon hasarina etkisi. *Van Tıp Dergisi* 2019;26:303-8.
24. Ahmadvand H, Babaeenezhad E, Nasri M, et al. Glutathione ameliorates liver markers, oxidative stress and inflammatory indices in rats with renal ischemia reperfusion injury. *J Renal Inj Prev* 2018;8:91-7.
25. Ballatori N, Krance SM, Notenboom S, et al. Glutathione dysregulation and the etiology and progression of human diseases. *Biol Chem* 2009;390:191-214.
26. Aoyama K. Glutathione in the brain. *Int J Mol Sci* 2021;22:5010.
27. Toiber D, Berson A, Greenberg D, et al. N-acetylcholinesterase-induced apoptosis in Alzheimer's disease. *PLOS One* 2008;3:e3108.
28. Gibson CJ, Davids MS. BCL-2 antagonism to target the intrinsic mitochondrial pathway of apoptosis. *Clin Cancer Res* 2015;21: 5021-9.
29. Coşkun G, Özgür H. Apoptoz ve nekrozun moleküler mekanizması. *aktd Eylül* 2011;20:145-58.
30. Shabanzadeh AP, D'Onofrio PM, Monnier PP, Koeberle PD. Targeting caspase-6 and caspase-8 to promote neuronal survival following ischemic stroke. *Cell Death Dis* 2015;6:e19672015.
31. Broughton BR, Reutens DC, Sobey CG. Apoptotic mechanisms after cerebral ischemia. *Stroke* 2009;40:e331-9.
32. Zhang C, Xu X, Potter BJ, et al. TNF-α contributes to endothelial dysfunction in ischemia/reperfusion injury. *Arterioscler Thromb Vasc Biol* 2006;26:475-80.
33. Montgomery SL, Bowers WJ. Tumor necrosis factor-alpha and the roles it plays in homeostatic and degenerative processes within the central nervous system. *J NeuroImmune Pharmacol* 2012;7:42-59.
34. Watters O, O'Connor JJ. A role for tumor necrosis factor-α in ischemia and ischemic preconditioning. *J Neuroinflammation* 2011;8:87.
35. Xing B, Chen H, Zhang M, et al. Ischemic post-conditioning protects brain and reduces inflammation in a rat model of focal cerebral ischemia/reperfusion. *J Neurochem* 2008;105:1737-45.
36. Li T, F Ma J, Han X, et al. Chrysin ameliorates cerebral ischemia/reperfusion (I/R) injury in rats by regulating the PI3K/Akt/mTOR pathway. *Neurochem Int* 2019;129:104496.
37. Vardy Emma RLC, Kellett Katherine AB, Cocklin Sarah L, Hooper Nigel M. Alkaline phosphatase is increased in both brain and plasma in Alzheimer's disease. *Neurodegener Dis* 2012;9:31-7.
38. Lampl Y, Paniri Y, Eshel Y, Sarova-Pinchas I. Alkaline phosphatase level in CSF in various brain tumors and pulmonary carcinomatous meningitis. *J Neurooncol* 1990;9:35-40.
39. Çelik S, Demir N, Demir Y. Serum alkalen fosfataz ve asit fosfataz enzimlerinin aktivileri üzerine lizinoprilin in vitro etkisi/the in vitro effect of lisonopril on serum alkaline phosphatase and acid phosphatase enzymes activity. *CBU J of Sci* 2017;13:233-7.
40. Henneberry MO, Engel G, Grayhack JT. Acid phosphatase. *Urol Clin North Am* 1979;6:629-41.
41. Sanchez RN, Chan CK, Garg S, et al. Interleukin-6 in retinal ischemia reperfusion injury in rats. *Invest Ophthalmol Vis Sci* 2003; 44:4006-11.
42. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* 2014;6:a016295.
43. Peters F, Nolden-Koch M. Expression of IL-6 in the ischemic penumbra. *Neuroreport* 2000;11:963-7.
44. Decharatchakul N, Settasatian C, Settasatian N, et al. Association of genetic polymorphisms in SOD2, SOD3, GPX3, and GSTT1 with hypertriglyceridemia and low HDL-C level in subjects with high risk of coronary artery disease. *PeerJ* 2019;7:e7407.
45. Ranjbar-Slamloo Y, Fazlali Z. Dopamine and noradrenaline in the brain; overlapping or dissociate functions? *Front Mol Neurosci* 2020;12:334.
46. Liu Af, Zhao Fb, Wang J, et al. Effects of vagus nerve stimulation on cognitive functioning in rats with cerebral ischemia reperfusion. *J Transl Med* 2016;14:101.
47. Bodner KE, Beversdorf DQ, Saklayen SS, Christ SE. Noradrenergic moderation of working memory impairments in adults with autism spectrum disorder. *J Int Neuropsychol Soc* 2012;18:556-64.
48. Sara SJ. The locus coeruleus and noradrenergic modulation of cognition. *Nat Rev Neurosci* 2009;10:211-23.
49. Gerke V, Creutz CE, Moss SE. Annexins: linking Ca2+ signalling to membrane dynamics. *Nat Rev Mol Cell Biol* 2005;6:449-61.
50. Lan YL, Li S, Lou JC, et al. The potential roles of dopamine in traumatic brain injury: a preclinical and clinical update. *Am J Transl Res* 2019;11:2616-31.