Original Investigation

Secukinumab Ameliorates Oxidative Damage Induced by Cerebral Ischemia-Reperfusion in Rats

Mustafa Namik OZTANIR[1], Muhammed Fatih DOGAN[2], Nese BASAK TURKMEN[3], Asli TASLIDERE[4],
Yasemin SAHIN[2], Osman CIFTCI[2]

[1]Bezmi Alem University, Faculty of Medicine, Department of Neurosurgery, Istanbul, Turkey
[2]Pamukkale University, Faculty of Medicine, Department of Pharmacology, Denizli, Turkey
[3]Inonu University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Malatya, Turkey
[4]Inonu University, Faculty of Medicine, Department of Histology and Embryology, Malatya, Turkey

Corresponding author: Osman CIFTCI ✉ osmciftci@gmail.com

ABSTRACT

AIM: To investigate the histological and biochemical neuroprotective effects of secukinumab (SEC) on cerebral ischemia–reperfusion (IR) injury in Sprague–Dawley male rats.

MATERIAL and METHODS: A total of 28 Sprague–Dawley male rats were randomly and equally divided into the following four groups: Sham, SEC, IR, and IR+SEC groups. Bilateral common carotid arteries were simultaneously separated and blocked for 15 minutes using two vascular mini clips in the IR and IR+SEC groups. The surgical procedure was similarly repeated in the Sham and SEC groups, but the carotid arteries were not clipped. Secukinumab was administered intraperitoneally to the SEC and IR+SEC groups once a week after the surgical procedure. Rat brain tissues were collected for biochemical analysis and histopathological examination 14 days after surgery.

RESULTS: Cerebral IR caused abnormal changes in oxidative stress parameters by increasing the malondialdehyde (MDA) level and by decreasing the glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) levels. IR also induced histopathological alterations, such as vascular congestion, hemorrhage, and cell infiltration in the rat brain tissues. Secukinumab treatment significantly decreased the MDA levels and increased the GPx, GSH, CAT, and SOD levels. In addition, secukinumab partially prevented histopathological alterations in the brain tissues. The percentage of immunohistochemically Caspase-3-positive cells was high in the IR group; however, SEC decreased the density of cells stained with Caspase-3.

CONCLUSION: IR injury was found to cause oxidative and histopathological changes in rat brain tissues, and secukinumab treatment ameliorated these pathological effects. Therefore, secukinumab may be useful to prevent and treat oxidative stress-induced brain damage in patients with ischemic stroke.

KEYWORDS: Secukinumab, Neuroprotection, Cerebral ischemia–reperfusion, Oxidative stress, Rats

■ INTRODUCTION

Ischemic stroke is a major cause of death with high morbidity and mortality rates (19). Cerebral artery occlusion results in disturbed blood flow and neural death, which can be prevented by early reperfusion (8). However, reperfusion that occurs after ischemia leads to cerebral edema or hemorrhage, causing brain damage. This pathological disorder is known as cerebral ischemia/reperfusion (IR) damage (28). During an ischemic stroke attack, the stranglehold of glucose and oxygen provision causes an influx of calcium that activates mitochondrial passage and triggers cell death (31). Owing to the overproduction of reactive oxygen species (ROS) and cytochrome C released by mitochondria, caspases are

Mustafa Namik OZTANIR : 0000-0001-5709-1757
Muhammed Fatih DOGAN : 0000-0003-4628-2771
Nese BASAK TURKMEN : 0000-0001-5566-8321
Asli TASLIDERE : 0000-0003-3902-3210
Yasemin SAHIN : 0000-0003-4226-674X
Osman CIFTCI : 0000-0001-5755-3560

activated and cause apoptotic cell death (11). ROS play a role in the pathogenesis of neuronal cell death in chronic neurodegenerative diseases and acute central nervous system damage (24). The antioxidant capacity of tissues constitutes the primary endogenous defense mechanism against damage caused by ROS (13).

Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which act together with glutathione (GSH), protect against ROS production and lipid peroxidation in tissues (9). Li et al. suggested that oxidative stress plays an extremely pivotal role in the pathogenesis of cerebral IR injury and theaflavin attenuates neuronal injury in the rat model (16). A few studies have reported that rats with cerebral artery occlusion presented with increased ROS and malondialdehyde (MDA) levels and significantly reduced antioxidant activity (16,35). Wang et al. showed that impairment in the antioxidant defense system caused oxidative damage to brain lipids, proteins, and DNA, leading to neuronal cell death and brain dysfunction (34). Guo et al. demonstrated that protocatechualdehyde produced neuroprotective activity by protecting against cerebral IR-induced oxidative damage in rats (12). Therefore, it is evident that the protection of the cerebral antioxidant system is effective for alleviating cerebral IR damage caused by ROS.

Secukinumab is a high affinity human immunoglobulin G1 monoclonal antibody that selectively binds and neutralizes interleukin-17A and is effectively used in a number of immune-mediated inflammatory diseases, including psoriasis and ankylosing spondylitis (23). Interleukin-17A, an effective and potent proinflammatory cytokine, is considered to play a specific role in delayed phase of the inflammatory response and I/R injury (33). Camargo et al. suggested that anti-interleukin-17 can potentially attenuate and/or modulate inflammatory responses and oxidative stress pathways (7). Therefore, the present study aimed to investigate the neuroprotective effects of secukinumab on cerebral IR-induced oxidative damage in rats. For this purpose, we evaluated the ameliorating effects of secukinumab on oxidative stress both biochemically and histologically.

■ MATERIAL and METHODS

Animals and Experimental Protocol

This study was approved by the Institutional Animal Care and Use Ethics Committee on Animal Research of Inonu University (Protocol no. 2016/A-93) and was conducted according to National Institute of Health guidelines for the care and use of laboratory animals. Spraque-Dawley male 28 rats (8-10 weeks old, 230-250 g) supplied by İnönü University Laboratory Animal Research Center were housed in sterile polypropylene cages and fed an ad libitum diet consisting of standard commercial food pellets and water. All rats were housed on a 12:12 hour light: dark cycle at 55 ± 5% humidity and 22 ± 1°C ambient temperature. Rats were distributed into the following 4 groups (n=7 in each group). Groups in the study are as follow; 1) Sham group: Saline was administered intraperitoneally (i.p) to the rats once a week after the surgical procedure. 2) IR group:

The experimental IR model was created by surgical procedure in the rats and then i.p saline was administered once a week. 3) Secukinumab (SEC) group: Secukinumab (3 mg/kg, Verxant; Farmavona, Turkey) was administered i.p to rats once a week after the surgical procedure (6). 4) IR+SEC group: The experimental IR model was created by surgical procedure in the rats and then i.p SEC (3 mg/kg) was administered once a week.

On the 15th day of the study, the rats were euthanized under anesthesia (i.p, 10 mg/kg xylazine and 90 mg/kg ketamine) and the tissues of the rats were collected for biochemical analysis and histological examination.

Surgical Procedure

To create a universal cerebral ischemia, Yonekura et al.'s method was applied (37). The rats were anesthetized, and then cervical midlines of the rats were dissected. In the IR and IR+SEC groups of rats, bilateral common carotid arteries were simultaneously separated and blocked for 15 minutes using two vascular mini clips. The surgical procedure was similarly repeated for rats in the Sham and SEC groups, but their carotid arteries were not clipped. After the surgical procedure, all rats were kept in the operating room to awaken from anesthesia.

Biochemical Analyses

Oxidative parameters of brain tissues were determined by spectrophotometric methods. The reduced glutathione (GSH) level was analyzed by the method of Sedlak and Lindsay (30). It was measured at 412 nm and expressed as nanomoles per milliliter of tissue. The activity of superoxide dismutase (SOD) was analyzed by the method of Sun, Oberley, and Li (32). It was determined at 560 nm and expressed in units per milligram of protein. The activity of catalase (CAT) of the tissues was measured by Aebi's method (3). It was expressed as kU per milligram of protein. The activity of glutathione peroxidase (GPx) was measured in accordance with the procedure described by Paglia and Valentine (26). It was stated in units per milligram of protein. The level of MDA, an end product of the peroxidation of polyunsaturated fatty acids, was calculated in order to detect lipid peroxidation (10). It was estimated at 532 nm and expressed in nanomoles per milligram protein. The protein content of the brain tissue was determined in accordance with the Lowry method (22). Bovine serum albumin was employed as a standard ingredient.

Histopathological Examination

Brain tissues were fixed in 10% formalin, embedded in paraffin, and evaluated by light microscopy (Leica DFC280). Paraffin-embedded specimens were cut into 5 μm thick sections, mounted on slides, and stained with Hematoxylin-Eosin (H-E). Tissue sections examined by the microscope were evaluated with the Leica Q Win Image Analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

Thick sections were mounted on poly-l-ysine-coated slides for immunohistochemical analysis. Rehydrated sections were transferred to citrate buffer (pH 7.6) then heated in a microwave oven for 20 minutes. The sections were cooled at room temperature for 20 minutes and washed with

phosphate buffered saline (PBS). The sections kept in 0.3% H2O2 for 7 minutes were washed again with PBS and then incubated with primary rabbit-polyclonal Caspase-3 antibody (Abcam, Ab4051) for 2 hours. They were then rinsed in PBS and incubated for 10 minutes each at room temperature with biotinylated goat antipolyvalent and streptavidin peroxidase, respectively. Staining was completed with chromogen + substrate and slides were counterstained with Mayer's hematoxylin for 1 minute, respectively, rinsed in distilled water, and dehydrated.

For apoptotic cell determination, the Caspase-3 kit was employed in the examination based on the manufacturer's instructions.

Statistical Analyses

SPSS 18.0 (SPSS Inc., Chicago, IL) was used for statistical analysis of the means of the groups. One-way analysis of variance test and Tukey post hoc test were used for the comparison among multiple groups. When the p-value was <0.05, the result of statistical analysis was accepted as significant. All biochemical experiments were performed in triplicate to ensure the reliability of the results.

■ RESULTS

Biochemical Results

GSH, CAT, SOD, GPx, and MDA levels in the brain tissue are presented in Table I.

Global cerebral IR caused a significant increase in the MDA levels when comparing the Sham (p=0.01) and IR+SEC (p=0.01) groups. Secukinumab treatment remarkably reduced the MDA level in IR-induced brain injury.

CAT and SOD levels were significantly decreased in the IR group compared with both the Sham group (p<0.05). GSH and GPx levels decreased in the IR group but were not statistically significant as compared to the Sham group. Secukinumab treatment increased the GSH, GPx, CAT, and SOD levels in IR-induced brain injury. Secukinumab significantly increased the CAT and SOD levels (p=0.003, 0.001; respectively) in the IR+SEC group when compared with the IR group.

Histopathological Results

In Sham and SEC groups, the brain tissues showed normal histological appearance. Neurons of the cerebral cortex showed normal histological appearance in the Sham and SEC groups (Figure 1). In the IR group (Figure 2), some histological alterations in the brain tissue were observed. In this group, vascular congestion in the pia mater (thin black arrows) (Figure 2A, B, E), cytoplasm shrinkage and extensively dark pyknotic nuclei in the neurons of the cerebral cortex (Figure 2D), hemorrhage (Figure 2C), and cell infiltration (Figure 2A, B, E) were observed. In contrast, SEC treatment significantly attenuated the harmful effects. Histopathological damages were significantly decreased in the IR+SEC group (Figure 3). A small amount of cell infiltration (Figure 3B) and congestion (Figure 3A, B) in the pia mater was observed.

In the Sham (Figure 4A) and SEC (Figure 4B) groups, cerebellar cortex showed normal histological appearance. Purkinje cells showed no histological changes. In the IR group (Figure 4C), deeply stained, shrunken, and different-shaped Purkinje cells with pyknotic nuclei were observed. The number of these degenerative Purkinje cells were decreased in the IR+SEC group (Figure 4D).

Immunohistochemically, Caspase-3-stained cells were not observed in the cerebral cortex in the Sham (Figure 5A) and SEC (Figure 5B) groups. The percentage of Caspase-3-positive cells was high in the IR group (Figure 5C). The density of Caspase-3-stained cells immunohistochemically was minimal in the IR+SEC group (Figure 5D).

■ DISCUSSION

In this study, we evaluated the antioxidative effects of secukinumab, a recombinant monoclonal antibody, for the management of cerebral IR injury. Oxidative stress is a critically important pathological process in IR that contributes to neurocyte injury (15). Our findings show that secukinumab treatment markedly alleviates the brain damage of rats subjected to IR injury and has an ameliorative effect on both oxidative stress markers and histopathological alterations.

ROS products formed in tissues are effectively detoxified by antioxidant defense systems such as GSH, CAT, SOD, and GPx. Reactive free radicals interact with unsaturated fatty acids in the cell membrane and initiate lipid peroxidation, and lipid peroxides can break down and produce several secondary products, specifically MDA (25,27). A significant portion of cell death occurring after brain ischemia is caused directly or indirectly by oxidative damage to DNA and other

Table I: Antioxidant and Oxidant Parameters Values in Brain Tissues

	MDA (nmol/mg protein)	Reduced GSH (nmol/ml tissue)	CAT (kU/mg protein)	SOD (U/mg protein)	GPx (U/mg tissue)
Sham	8.73 ± 0.48[b,c]	103.04 ± 8.97[c]	0.831 ± 0.057[b]	10.79 ± 0.92[b,c]	46.39 ± 2.97[c]
IR	12.61 ± 1.65[a,d]	102.10 ± 14.52[c]	0.537 ± 0.067[a,c,d]	7.76 ± 0.60[a,c,d]	38.61 ± 10.49[c]
SEC	11.92 ± 0.58[a,d]	160.56 ± 7.57[a,b,d]	0.923 ± 0.096[b,d]	14.34 ± 1.12[a,b,d]	69.17 ± 5.38[a,b,d]
IR+SEC	8.68 ± 1.52[b,c]	103.32 ± 10.35[c]	0.727 ± 0.042[b,c]	11.21 ± 1.45[b,c]	46.99 ± 5.11[c]

*a: compared with Sham group, b: compared with IR group, c: compared with SEC group, d: c: compared with IR+SEC group. **MDA:** Malonaldehyde, **GSH:** Glutathione, **CAT:** Catalase, **SOD:** Superoxide dismutase, **GPx:** Glutathione peroxidase.*
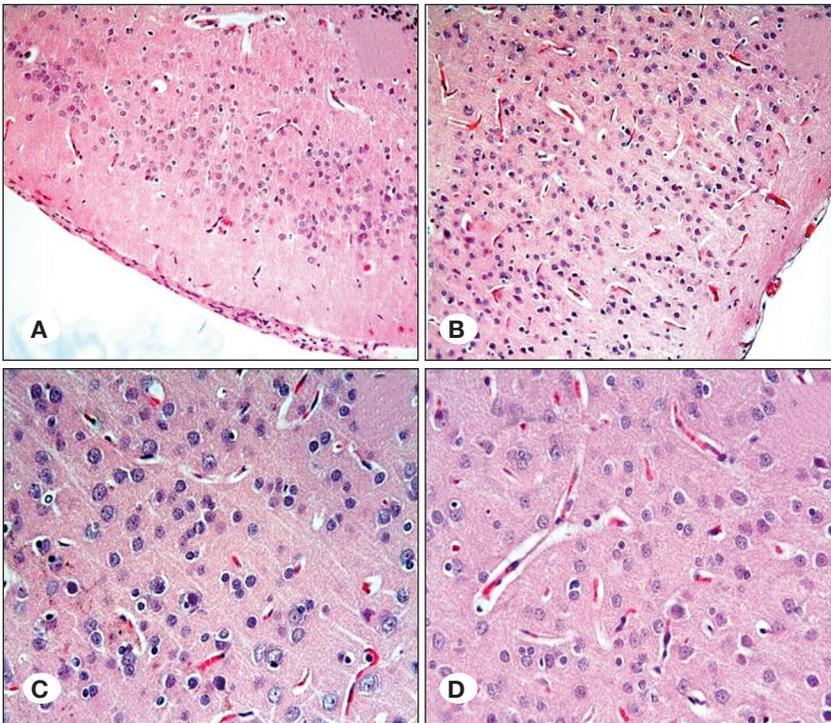
Figure 1: In Sham **(A, C)** and SEC **(B, D)** groups, brain tissue and neurons showed normal histological appearance (A, B: H-E; X20, C, D: H-E; X40).
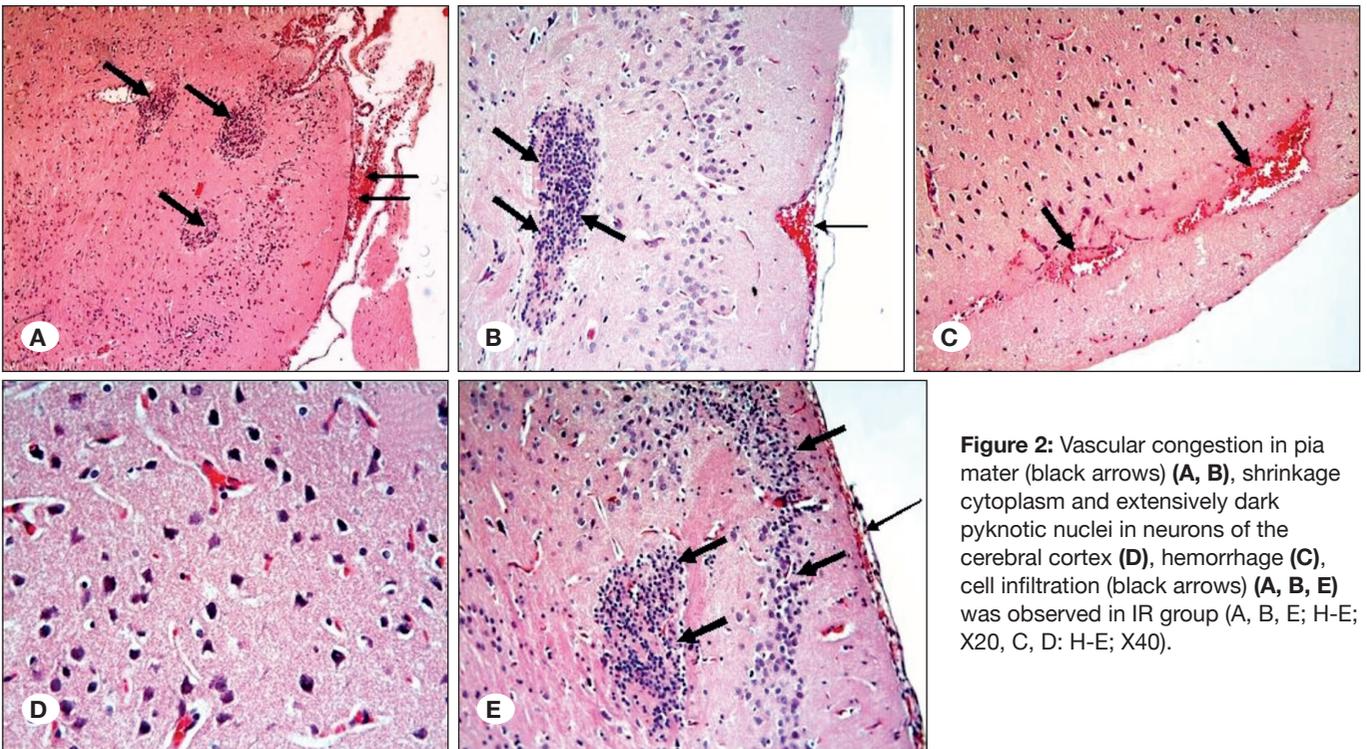

Figure 2: Vascular congestion in pia mater (black arrows) **(A, B)**, shrinkage cytoplasm and extensively dark pyknotic nuclei in neurons of the cerebral cortex **(D)**, hemorrhage **(C)**, cell infiltration (black arrows) **(A, B, E)** was observed in IR group (A, B, E; H-E; X20, C, D: H-E; X40).
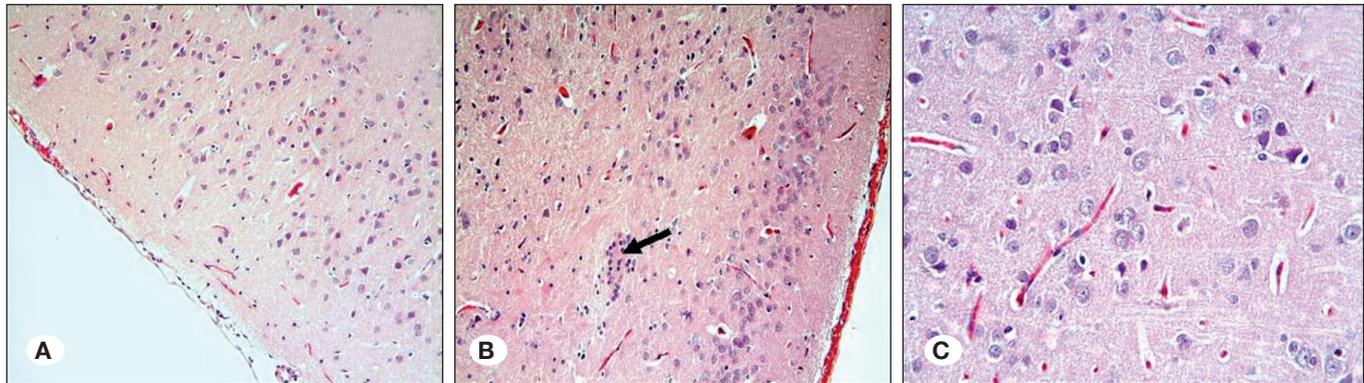
Figure 3: The histopathological damages were significantly decreased in IR+SEC group. A small amount of cell infiltration, **(B)** and congestion **(A, B)** in piamater was observed (A, B: H-E; X20, C:H-E; X40).
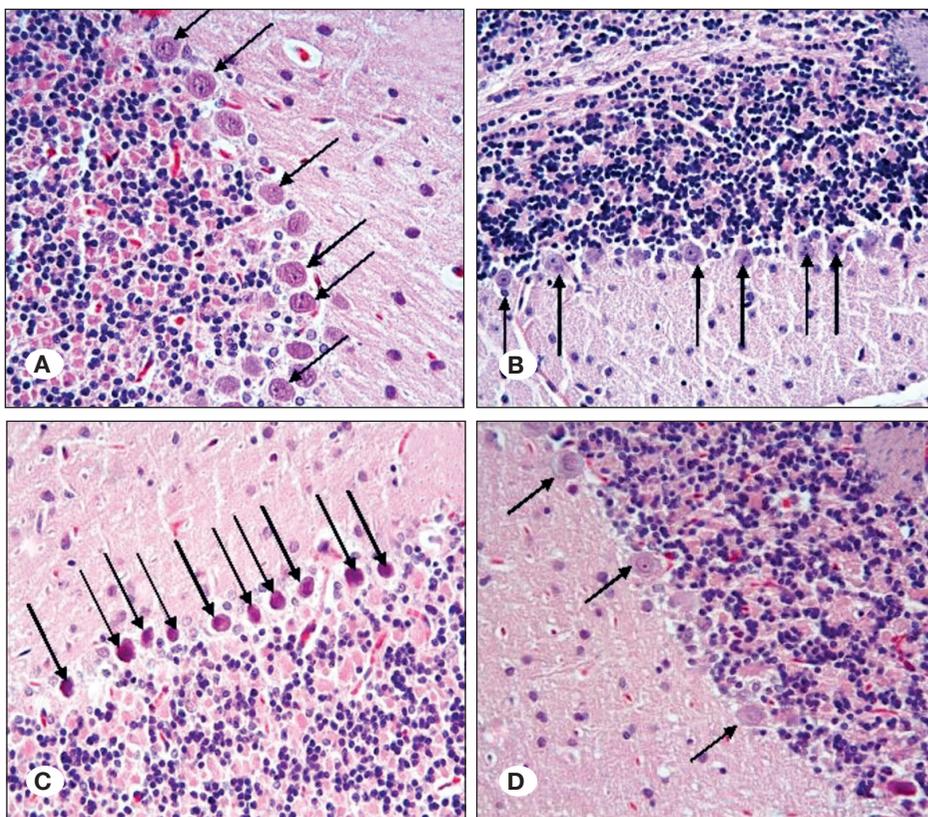

Figure 4: Purkinje cells in the cerebellum in all groups. Purkinje cells in normal histological appearance in Sham **(A)**, and SEC **(B)** groups (thin black arrows). Degenerated Purkinje cells in the IR group (thin black arrows) **(C)**. Reduction in the number of degenerate Purkinje cells in IR+SEC group compared with the IR group. Normal Purkinje cells were observed in the IR+SEC group (thin black arrows) (A, B, C, D: H-E; X40).

macromolecules (21) and not only caused by the temporary loss of oxygen and energy supply when the brain is deprived of blood flow but also from ROS produced through reactions with incoming oxygen during reperfusion (29). ROS, produced during cerebral ischemia, changes the expression and activity of various enzymes, such as SOD, GPx, and CAT, by excessively consuming endogenous antioxidant enzymes (2).

Our findings showed that IR significantly change the levels of oxidative stress markers, namely, GSH, CAT, SOD, GPx, and MDA, in the brain tissue. As shown in Table I, compared with the Sham group, MDA levels in the brain tissue in the IR group were significantly increased, and the SOD, GPx, CAT, and

GSH levels were significantly decreased. Conversely, when compared with IR group, the MDA level in the IR+SEC group was significantly decreased, whereas the GSH, SOD, GPx, and CAT levels were increased. Similarly, molecules that shift oxidant/antioxidant status in favor of oxidants during a cerebral damage were investigated in the cerebral IR injury model of rats. Li et al. demonstrated that icariside-II could be alleviated by reducing brain tissue oxidative stress (18). Bonfante et al. showed that stanniocalcin-1 reduced brain dysfunction associated with cerebral IR by decreasing oxidative stress parameters and brain barrier permeability (5). Yang et al. reported that mangiferin can play a certain protective role
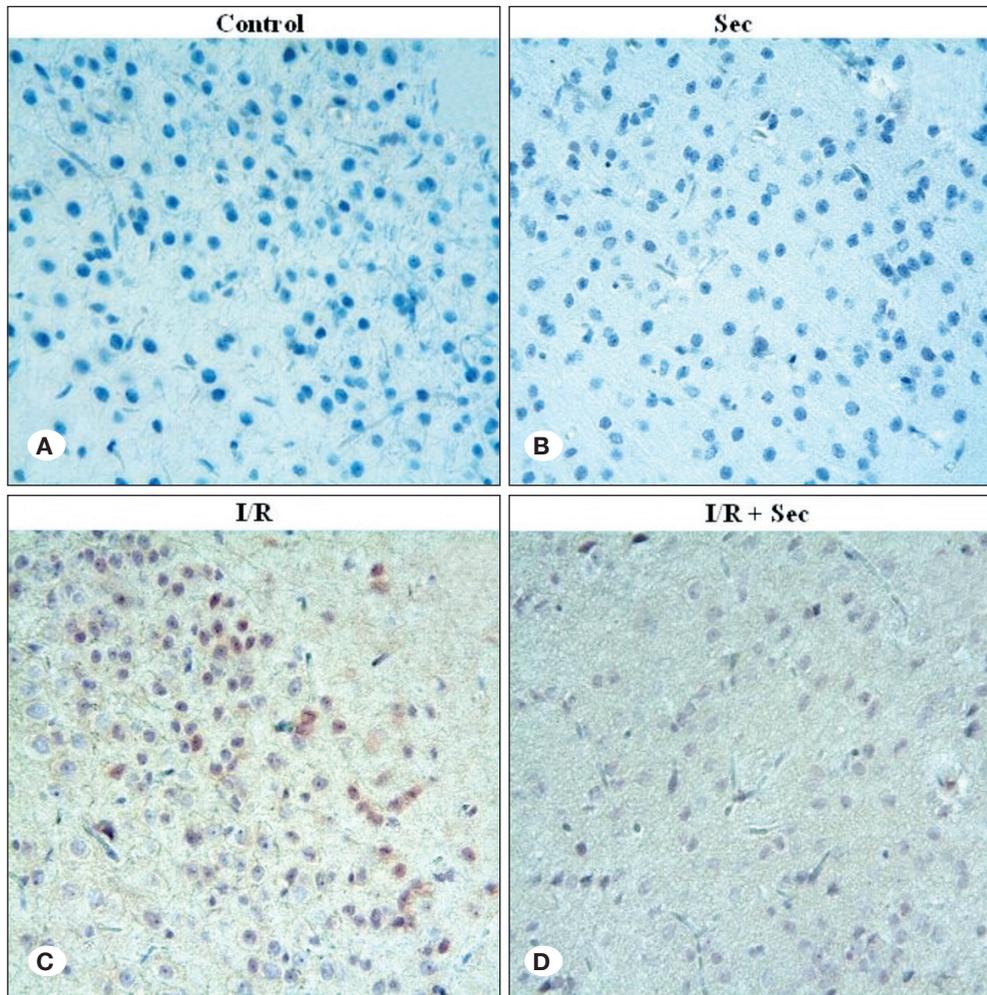
Figure 5: Caspase-3 immunostaining procedure in all groups. In the IR and IR+SEC groups, Caspase-3 positively stained cells were observed. In the IR+SEC group, positive stained cells were decreased compared with the IR group (Caspase-3; X40. A, B, C, D: Caspase 3; X 40).

in cerebral IR injury by improving the antioxidant capacity of the brain tissue (36). These results indicate that secukinumab helps alleviate cerebral IR damage by increasing antioxidant enzyme activities in the brain tissue, scavenging the ROS, and reducing lipid peroxidation.

Several experimental studies on IR reported that resveratrol (17) or ebselen (4) is protective against histopathological changes by ameliorating the oxidative stress induced by cerebral IR. As illustrated in Figure 2, we determined that IR injury caused histopathological changes in the brain tissue such as vascular congestion, cytoplasm shrinkage, extensively dark pyknotic nuclei, hemorrhage, and cell infiltration when comparing Sham with other groups in terms of the histological evaluation of brain tissues. As shown in Figure 3, these histopathological damages due to oxidative stress significantly decreased in the IR+SEC group. Hypoxia/reoxygenation induces extensive apoptosis of rat cerebral neurons in the IR model (14). In this study, apoptotic cells were determined using the Caspase-3 activity in the immunohistochemical method. We performed Caspase-3 immunoblotting and revealed that Caspase-3 was suppressed with the secukinumab treatment. The antioxidative activity of secukinumab is clearly noted in the brain tissue pathology. Secukinumab inhibits interleukin 17A, a member

of the cytokine family, and has an anti-inflammatory activity (20). Abdel-Maged et al. reported that secukinumab had neuroprotective and antioxidant effects besides its anti-inflammatory effects, and showed normal histological structure of the neurons in the cortex, hippocampus, and striatum in the mice experimental model of multiple sclerosis (1).

Therefore, the histopathological damage induced by IR in the brain tissue is considered to be caused by the imbalance between oxidant and antioxidant status. Our results showed that secukinumab treatment reversed the oxidative brain damage. Accordingly, the ameliorating effect of secukinumab treatment can be considered as an important result because it reduces oxidative stress and prevents histopathological damage in the brain tissue.

■ **CONCLUSION**

The present study demonstrates the therapeutic effect of secukinumab (3 mg/kg) in the rat experimental model of IR-induced brain injury that causes oxidative and histopathological damage. Secukinumab has antioxidative and neuroprotective effects; additionally, its protective effect can reduce the brain damage of oxidative stress in patients with ischemic stroke.

■ AUTHORSHIP CONTRIBUTION

Study conception and design: MNO, OC

Data collection: MFD, NBT, YS

Analysis and interpretation of results: MNO, AT, YS

Draft manuscript preparation: MFD, YS

Critical revision of the article: MFD, YS

Other (study supervision, fundings, materials, etc...): OC, AT, NBT

All authors (MNO, MFD, NBT, AT, YS, OC) reviewed the results and approved the final version of the manuscript.

■ REFERENCES

1. Abdel-Maged AES, Gad AM, Rashed LA, Azab SS, Mohamed EA, Awad AS: Repurposing of secukinumab as neuroprotective in cuprizone-induced multiple sclerosis experimental model via inhibition of oxidative, inflammatory, and neurodegenerative signaling. Mol Neurobiol 57(8):3291-3306, 2020

2. Adewole SO, Caxton-Martins EA, Ojewole JAO: Protective effect of quercetin on the morphology of pancreatic β-cells of streptozotocin-treated diabetic rats. African J Tradit Complement Altern Med 4(1):64-74, 2007

3. Aebi H: Catalase. In: Bergmeyer HU (ed), Methods of Enzymatic Analysis. New York: Academic Press, 1974:673-677

4. Aras M, Altas M, Meydan S, Nacar E, Karciołlu M, Ulutas KT, Serarslan Y: Effects of ebselen on ischemia/reperfusion injury in rat brain. Int J Neurosci 124(10): 771-776, 2014

5. Bonfante S, Della Giustina A, Danielski LG, Denicol T, Joaquim L, Biehl E, Scopel G, Carli RJ, Hubner M, Cardoso T, Tuon T, Generoso J, Barichello T, via Terra S, Petronilho F: Stanniocalcin-1 ameliorates cerebral ischemia by decrease oxidative stress and blood brain barrier permeability. Microvasc Res 128: 103956, 2020

6. Bruin G, Loesche C, Nyirady J, Sander O: Population pharmacokinetic modeling of secukinumab in patients with moderate to severe psoriasis. J Clin Pharmacol 57(7): 876-885, 2017

7. Camargo L do N, Righetti RF, Aristóteles LR de CRB, dos Santos TM, de Souza FCR, Fukuzaki S, Cruz MM, Alonso-Vale MIC, Saraiva-Romanholo BM, Prado CM, Martins M de A, Leick EA, Tibério I de FLC: Effects of anti-IL-17 on inflammation, remodeling, and oxidative stress in an experimental model of asthma exacerbated by LPS. Front Immunol 8:1835, 2018

8. Chen HM, Huang H Sen, Ruan L, He YB, Li XJ: Ulinastatin attenuates cerebral ischemia-reperfusion injury in rats. Int J Clin Exp Med 7(5):1483-1489, 2014

9. Ciftci O, Vardi N, Ozdemir I: Effects of quercetin and chrysin on 2,3,7,8-tetrachlorodibenzo-p-dioxin induced hepatotoxicity in rats. Environ Toxicol 28(3):146-154, 2011

10. Esterbauer H, Cheeseman KH: Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. Methods Enzymol 186:407-421, 1990

11. Gong L, Tang Y, An R, Lin M, Chen L, Du J: RTN1-C mediates cerebral ischemia/reperfusion injury via ER stress and mitochondria-associated apoptosis pathways. Cell Death Dis 8(10):3080, 2017

12. Guo C, Wang S, Duan J, Jia N, Zhu Y, Ding Y, Guan Y, Wei G, Yin Y, Xi M, Wen A: Protocatechualdehyde protects against cerebral ischemia-reperfusion-induced oxidative injury via protein kinase Cε/Nrf2/HO-1 Pathway. Mol Neurobiol 54(2): 833-845, 2017

13. Islekel S, Islekel H, Guner G, Ozdamar N: Alterations in superoxide dismutase, glutathione peroxidase and catalase activities in experimental cerebral ischemia-reperfusion. Res Exp Med 199(3):167-176, 1999

14. Kishimoto M, Suenaga J, Takase H, Araki K, Yao T, Fujimura T, Murayama K, Okumura K, Ueno R, Shimizu N, Kawahara N, Yamamoto T, Seko Y: Oxidative stress-responsive apoptosis inducing protein (ORAIP) plays a critical role in cerebral ischemia/reperfusion injury. Sci Rep 9(1): 13512, 2019

15. Li P, Shen M, Gao F, Wu J, Zhang J, Teng F, Zhang C: An antagomir to MicroRNA-106b-5p ameliorates cerebral ischemia and reperfusion injury in rats via inhibiting apoptosis and oxidative stress. Mol Neurobiol 54(4):2901-2921, 2017

16. Li R, Li X, Wu H, Yang Z, Li F, Jianhong Z: Theaflavin attenuates cerebral ischemia/reperfusion injury by abolishing miRNA-128-3p-mediated Nrf2 inhibition and reducing oxidative stress. Mol Med Rep 20(6):4893–4904, 2019

17. Li W, Tan C, Liu Y, Liu X, Wang X, Gui Y, Qin L, Deng F, Yu Z, Hu C, Chen L: Resveratrol ameliorates oxidative stress and inhibits aquaporin 4 expression following rat cerebral ischemia-reperfusion injury. Mol Med Rep 12(5):7756-7762, 2015

18. Li Y, Meng F: Effects of icariside II on brain tissue oxidative stress and nrf2/ho-1 expression in rats with cerebral ischemiareperfusion injury. Acta Cir Bras 34(2): e201900208, 2019

19. Liu T, Han S, Dai Q, Zheng J, Liu C, Li S, Li J: IL-17A-mediated excessive autophagy aggravated neuronal ischemic injuries via Src-PP2B-mTOR pathway. Front Immunol 10:1-14, 2019

20. Lockshin B, Balagula Y, Merola JF: Interleukin 17, inflammation, and cardiovascular risk in patients with psoriasis. Journal of the American Academy of Dermatology 79:345-352, 2018

21. Love S: Oxidative stress in brain ischemia. Brain Pathol 9(1):119-131, 1999

22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265-275, 1951

23. Mease PJ, McInnes IB, Kirkham B, Kavanaugh A, Rahman P, van der Heijde D, Robert Landewé R, Nash P, Pricop L, Yuan J, Richards HB, Mpofu S: Secukinumab inhibition of interleukin-17A in patients with psoriatic arthritis. N Engl J Med 373(14): 1329-1339, 2015

24. Nita DAI, Nita V, Spulber S, Moldovan M, Popa DP, Zagrean AM, Zagrean L: Oxidative damage following cerebral ischemia depends on reperfusion- A biochemical study in rat. J Cell Mol Med 5(2):163-170, 2001

25. Oguz F, Ciftci O, Aydın M, Timurkaan N, Beytur A, Altıntas R, Parlakpinar H: Aminoguanidine prevents testicular damage–induced- 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in male rats. Andrologia 45(4):225-231, 2012

26. Paglia DE, Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70(1):158-169, 1967

27. Palaniswamy KS, Vishwanadha VP, Ramalingam Singaravelu S: Fish oil rich in eicosapentaenoic acid protects against oxidative stress-related renal dysfunction induced by TCDD in Wistar rats. Cell Stress Chaperones 19(3):409-419, 2014

28. Radak D, Resanovic I, Isenovic ER: Link between oxidative stress and acute brain ischemia. Angiology 65:667-676, 2014

29. Rodrigo R, Fernandez-Gajardo R, Gutierrez R, Matamala J, Carrasco R, Miranda-Merchak A, Feuerhake W: Oxidative stress and pathophysiology of ischemic stroke: Novel therapeutic opportunities. CNS Neurol Disord-Drug Targets 12(5):698-714, 2013

30. Sedlak J, Lindsay RH: Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25:192–205, 1968

31. Shan HM, Zang M, Zhang Q, Shi RB, Shi XJ, Mamtilahun M, Liu C, Luo L, Tian X, Zhang Z, Yang GY, Tang Y, Pu J, Wang Y: Farnesoid X receptor knockout protects brain against ischemic injury through reducing neuronal apoptosis in mice. J Neuroinflammation 17(1):1-12, 2020

32. Sun Y, Oberley LW, Li Y: A simple method for clinical assay of superoxide dismutase. Clin Chem 34(3):497-500, 1988

33. Waisman A, Hauptmann J, Regen T: The role of IL-17 in CNS diseases. Acta Neuropathol 129(5):625-637, 2015

34. Wang M, Li YJ, Ding Y, Zhang HN, Sun T, Zhang K, Le Yang L, Guo YY, Liu SB, Zhao MG, W YM: Silibinin prevents autophagic cell death upon oxidative stress in cortical neurons and cerebral ischemia-reperfusion injury. Mol Neurobiol 53(2): 932-943, 2016

35. Wu J, Chen Y, Yu S, Li L, Zhao X, Li Q, Zhao J, Yong Z: Neuroprotective effects of sulfiredoxin-1 during cerebral ischemia/reperfusion oxidative stress injury in rats. Brain Res Bull 132:99-108, 2017

36. Yang Z, Weian C, Susu H, Hanmin W: Protective effects of mangiferin on cerebral ischemia-reperfusion injury and its mechanisms. Eur J Pharmacol 771:145-151, 2016

37. Yonekura I, Kawahara N, Nakatomi H, Furuya K, Kirino T: A model of global cerebral ischemia in C57 BL/6 Mice. J Cereb Blood Flow Metab 24(2):151-158, 2004