Vitamin D ameliorates memory function in association with reducing senescence and upregulating neurotrophin mRNA expression in transient global cerebral ischaemic injury model in rats

Tamina Melindah, M.Biomed[1,2], Dwi Cahyani Ratna Sari, PhD[3], Jajar Setiawan, PhD[1], Alex, M.Biomed[3,4], Micko Martha Thamrin, M.Biomed[3,5], Fathimiyah Zahra, M.Biomed[3], Nur Arfian, PhD[2,3]

[1]Department of Physiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, [2]Master program in Biomedical science, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, [3]Department of Anatomy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, [4]Department of Anatomy, Faculty of Medicine, Tanjungpura University, Pontianak, West Borneo, Indonesia, [5]Department of Anatomy, Faculty of Medicine, University Muhammadiyah Metro, Lampung, Indonesia

ABSTRACT

Introduction: Ischaemic stroke induces oxidative stress, mitochondrial damage, inflammation and senescence and the decrease of cognitive function. Vitamin D is a fat-soluble vitamin that has a neuroprotective effect to repair the function of the nervous system. The aim of this study is to investigate the effect of vitamin D on memory function, p16, p21 (senescence), and nerve growth factor (NGF) mRNA expression on the hippocampus after transient global cerebral ischemic.

Materials and Methods: The study was designed as quasi-experimental with a control group that only received post-tests. We performed in vivo study with an induction bilateral common carotid artery occlusion (BCCAO) model and vitamin D injection for 10 days. A total of 24 rats were divided into four groups (n = 6): Sham operation (SO [control]), BCCAO (transient global cerebral ischemic model not given vitamin D), VD1 (BCCAO + vitamin D 0.125 µg/kgBW), and VD2 (BCCAO + vitamin D 0.5 µg/kgBW). The spatial memory function was tested with the Morris water maze. We performed immunohistochemistry to localise p16 expression. p16, p21 and NGF mRNA expression were assessed by reverse transcriptase (RT-PCR) method.

Results: The vitamin D treatment group required shorter mileage to find the platform and probe test. The total time spent was longer in the target quadrant than in non-target. The Vitamin D-treated group had lower p16 and p21 mRNA expression and higher NGF mRNA expression than the BCCAO group. Immunostaining showed p16 signal in the pyramidal cell of CA1 area in the BCCAO group.

Conclusion: Vitamin D repairs memory function, senescence expression was lower and NGF was higher in the BCCAO model.

KEYWORDS:
Vitamin D, senescence, neurotrophin, memory, global cerebral ischaemic

INTRODUCTION

Stroke is a functional disorder of the brain caused by obstruction of blood flow to the brain due to bleeding (haemorrhagic stroke) or blockage (ischemic stroke).[1] The occlusion of the supplying artery blood to the brain affects a lack of adequate oxygen supply that disrupts cellular homeostasis which leads to death of nerve cells and impaired tissue function to a decrease in neurological function.[2] Cessation of brain tissue blood flow decreases the oxygen and glucose that are needed for ATP formation, resulting in a decrease Na+ K+ ATPase, which causes the membrane potential to decrease. K+ moves into space extracellular, while Na and Ca ions collect in the cell causing the cell surface to become more negative and triggering depolarisation of the membrane, resulting in structural changes in space and leading to tissue death in the brain.[2]

Ischemic injury-reperfusion results in disruption signalling of the oxidative stress response, which leads to mitochondrial damage, dysregulation of metabolic neuronal ca2+ homeostasis, dysfunction autophagy lysosome and proteasomes.[3] It also triggers activation of cellular senescence signalling that increases damage to mitochondrial structure and function, decreasing ATP production and disrupting normal cell metabolism.[4] Senescence incident will induce cessation of growth of cells competent in cell proliferation and differentiation. Senescence also triggers inflammation in neurons, loss of neuronal synapses and demyelination, resulting in cognitive impairment.[5]

Expression of cellular senescence can be characterised by the presence of changes in two major signalling pathways, namely cellular senescence markers p16 and p21.[6] Ischemic stroke research studies will accelerate the senescence-

This article was accepted: 19 July 2024
Corresponding Author: Dwi Cahyani Ratna Sari
Email: dwi.cahyani@ugm.ac.id

associated secretory phenotype (SASP). The transient middle cerebral artery occlusion (TMCAO) stroke model showed an increase in the expression of cellular senescence.[7] However, in the bilateral common carotid arteries occlusion (BCCAO) stroke model there are no research studies on increased expression of cellular senescence. Senescence in areas of the brain will impact on damage to the hippocampus, thereby disrupt factor signalling neurotrophins that play a role in cognitive function.[8,9]

Neurotrophin nerve growth factor (NGF) protein plays a role in synaptic and neuronal growth, myelination, differentiation and development neuronal. NGF is produced in hippocampus dentate gyrus, and pyramidal cells. NGF expression in the hippocampus is regulated by neural activity.[10,11] The hippocampus is a complex structure found in the temporal lobe which has important role in learning and memory. Previous research stated that an imbalance between reactive oxygen species, reactive nitrogen species and antioxidants triggers senescence cellular and decreased expression of NGF receptor in Alzheimer's animal model.[12,13] Furthermore, senescence triggers a decrease in nerve growth factors, resulting in a decline in cognitive function.[13]

Vitamin D is a fat-soluble vitamin that has the neuroprotective effect to repair the function of nervous system. Vitamin D has antioxidant properties that regulate cells differentiation, cells proliferation, peroxidation, neuroplasticity and axonal growth[14] moreover it plays a role in oxidative stress control. This study aims to elucidate vitamin D on spatial memory function, mRNA p16, mRNA p21 and mRNA NGF expression in a transient global cerebral ischemia model BCCAO.

MATERIALS AND METHODS

Study, Design, Location and Time

The study was designed as quasi-experimental with a control group that only received post-tests. The research was approved by Ethical Committee of Medical Research and Health of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada with number KE/FK/1486/EC/2022. A total of 24 rats (Rattus norvegicus), 150 to 300 gm body weight (BW), were used. The rats were adapted to the cage conditions for 7 days with access to food and water ad libitium before the rats divided into four groups (n = 6): SO (sham operation), BCCAO (transient global cerebral ischaemic model not given vitamin D), VD1 (BCCAO + vitamin D 0.125 µg/kgBW) and VD2 (BCCAO + vitamin D 0.5 µg/kgBW).

Transient Global Cerebral Ischaemic Injury Model Bilateral Common Carotid Arteries Occlusion

A transient global cerebral ischemic model was created using BCCAO.[15] The rats were anesthetised using ketamine 100 mg/kg intramuscular. After that, the rats were positioned supine on the operating table, then incised at the anterior median line of the neck for exploration and visualisation of the common carotid artery. The right and left common carotid arteries were clamped with non-traumatic vascular clamp for 20 minutes. Next, the neck wall was sutured, and the rats were returned to the cages for recovery. In the SO group, the same surgical procedure was performed to visualise the bilateral common carotid artery without clamping the arteries.

Vitamin D Treatment

This study used calcitriol (1a, 25-dihydroxy vitamin D) (Cayman®) as the active vitamin D that was diluted in ethanol 0.2% until a concentration of 1 mg/ml is obtained. Vitamin D was given at different doses, namely 0.125 µg/kgBW in the VD1 group and 0.5 µg/kgBW in the VD2 group. Vitamin D treatment was done by intraperitoneal injection once per day for 10 days, with a volume 0.1 ml/100 grams BW. The SO and BCCAO groups were intraperitoneal injected with ethanol 0.2% in equal volume.

Assessment of spatial memory function using Morris water maze

The spatial memory function assessment instrument in this study used the Morris water maze (MWM). The device for the test consisted of a white-painted rounded pool (1.5 m in diameter and 0.4 m in height). The pool was divided into four virtual quadrants titled A, B, C and D. There was a rounded, white-painted platform (13 cm in diameter and 16.5 cm in height) located in the middle of a randomly chosen quadrant and kept in the same location throughout the experiment for each rat. Eight starting points were marked on the outside of the pool wall. Several coloured pictures were placed around the pool. These pictures served as distal clues for the rats to find the platform. The pool was filled with water and added with milk (1.5 to 2.5 cm above the platform) until the platform was barely visible. The assessment in the learning phase was to judge the total mileage to find platforms and test probes as the total time spent in the target and non-target quadrants in each treatment group. The first assessment was the learning phase which was done using a pointer location platform around the pool in the form of a picture on the wall of the pool called a distal cue. Platforms are first hidden by adding a mixture of water and milk. The pool was divided into eight quadrants and platforms are placed in one quadrant permanently. On evaluation learning phase, the experimental animal was placed at the starting point, swim towards the quadrant target and rise to the platform. Applications MWM would record time (sec) and total distance (mm) to find platforms at quadrant target. The learning phase was performed four times for four consecutive days. The probe test was performed 24 hours after the trial's final learning phase. The test was carried out once for 120 seconds without the use of a platform. The result obtained is the total time measurement spent on the target and non-target quadrants of each treatment group. Every test performed would record using a video camera with applications MWM.

Termination

The rats were terminated on the 10 days. Rats were anesthetised using 80 to 100 doses of ketamine mg/kg intramuscular then the abdominal wall is incised to open the abdominal and thoracic cavities. 0.9% NaCl solution into the ventricles cynically are used for organ perfusion. Decapitation was carried out to retrieve the brain tissue of the rats with the right cerebral hemisphere on a normal buffer formalin for paraffin embedded tissue process, while the hippocampus separated from the left hemisphere in RNA preservation solution, stored at 20oC for mRNA extraction.

Immunohistochemical Staining

3-µm thick paraffin sections were deparaffinised, next the antigen was retrieved using heat-induced antigen retrieval methods, followed by blocking peroxidase with 3% H2O2 in the PBS solution. Then, the slides were incubated with blocking serum and incubated with mouse 1st polyclonal antibody anti-p16 (Invitrogen MA5-17142; 1:100) overnight. On the following days, the slides were incubated with antibodies and diaminobenzidine (DAB). The results were captured under a light microscope (Olympus CX22®) through the OptiLab software with 400x magnification.

RNA Extraction and cDNA Synthesis

The hippocampus of rats was extracted according to the procedural technique described by the manufacturer of the Genezol RNA Solution (GENEzol™, Cat. No. GZR100). Then, 3000 ng of total RNA was used to synthesis the cDNA. The synthesis of cDNA was performed using the cDNA Synthesis Kit (SMOBio, RP1400) with PCR condition of 25°C for 10 minutes, 42°C for 50 minutes, and 85°C for 5 minutes.

Reverse Transcriptase Polymerase Chain Reaction and Electrophoresis

Reverse transcriptase-PCR (RT-PCR) was performed to examine the expression of following genes: p16 (forward: 5′-CGTACCCCGATACAGGTGATG-3′, reverse: 5′-ATACCGCCAAATACCGCACGA -3′), p21 (forward: 5′-GTGATATGTACCAGCCACAGG -3′, reverse: 5′-CAGACGTAGTTGCCCTCCAG -3′), NGF (forward: 5′-CGAAGGGGAGCGCATCG-3′, reverse: 5′ GACATTACGCTATGCACCTCAGA -3′), and GAPDH (forward: 5′-GTTACCAGGGCTGCCTTCTC-3′, reverse: 5′-TCCCGTTGATGACCAGCTTC-3′) was used as housekeeping gene. NFW and Taq Master Mix (GoTaq® Green Master Mix, Cat No. M7122) was used. The PCR was performed using the following condition: initial denaturation at 94oC for 2 min, the following steps mere repeated for 35 cycles (denaturation at 94oC for 10 s, annealing 51oC for 1 min to expression p16, 61.3oC for 1 min to expression p21 and 56oC for 1 min to expression NGF, continued extension 72°C for 1 min, and ended with cycles elongation 72°C for 10 min). RT-PCR products were analysed on 2% Agarose-gel with DNA ladder (Bioron, Germany, Cat No. 306009). Gene expression was quantified then with densitometry analysis with ImageJ® software and GAPDH was used to normalise expression. Results of electrophoresis were performed by ultraviolet light transillumination using Geldoc Syngene Gbox Chemi XRQ series.

Analysis of Statistics

Data were analysed using the SPSS 24 software, and the normality test was performed by using Shapiro–Wilk. Normally distributed data were analysed using One-way ANOVA. The significant $p < 0.05$, then proceeds with the analysis post-hoc multiple comparisons.

RESULTS

Vitamin D Ameliorated Spatial Memory Function Based on Learning Phase and Probe Test of MWM

The results showed that the BCCAO group distance effect on reached platforms was longer than the other groups (Figure 1A).[16] First day assessment showed all groups required a long total distance to reach platforms. The BCCAO group (1440.93 ± 315.73 mm; p = 0.004) required significantly longer distance to achieve platforms than the SO group (970.20 ± 134.83 mm). The VD1 group (1159.61 ± 206.86 mm) and VD2 group (1165.33 ± 286.61 mm) have total distance longer than the BCCAO group, but not significantly different. Assessment on the second day showed the total distance to reached platforms of the BCCAO group (1472.91 ± 372.89 mm) significantly longer compared to the SO group (1017.92 ± 289.35 mm; p = 0.036) and VD2 (718.20 ± 319.95 mm; p = 0.001). BCCAO group tend to be longer than the VD1 group (1140.91 ± 407.08 mm), but not statically different. The third day showed that the BCCAO group (1439.36 ± 431.20 mm) required significantly longer compared to the SO group (746.75 ± 324.27 mm; p= 0.001); VD1 (959.46 ± 277.59 mm; p = 0.015) and VD2 (730.67 ± 156.08 mm; p = 0.001). The fourth day of evaluation demonstrated that the BCCAO group (1341.61 ± 335.79 mm) required the total distance reach platforms to be significantly longer than the SO group (714.86 ± 300.27 mm; p = 0.001); VD1 (821.69 ± 336.09 mm; p = 0.006) and VD2 (688.17 ± 160.09 mm; p = 0.001). There was no difference between the VD1 and VD2 group on the third and fourth days.

The SO, VD1 and VD2 groups have hippocampus dependent-allocentric swimming strategy that showed swimming direct path the location platforms. In contrast, the BCCAO group has a hippocampus-independent egocentric strategy swimming that showed with thigmotaxis swim, which affected most of the time was spent swimming towards the pool wall and difficulty finding the platforms. The finding is correlated with the swimming track pattern learning phase in BCCAO groups compared to the SO, VD1 and VD2 groups (1B).[16] Next, Probe test were performed for assessment of the total time spent in the target quadrant (Qtarget) compared to the quadrant was not the location platform (Figure 1C).[16] Probe test based showed that the total time spent in the quadrants target group SO; Qtarget was significantly longer than the non-target quadrant (Q1,Q2 and Q3; p = 0.000). Total time spent in the target group quadrant BCCAO; Qtarget significantly shorter than the quadrant non target Q1 but the total time spent in quadrant the target group of BCCAO is significantly longer than the non-target quadrant (Q2 and Q3; p = 0.000). Moreover, the assessment of total time spent in the target group VD1 and VD2 quadrant target was significantly longer than in the quadrant non-target (Q1, Q2 and Q3 p = 0.000) (Figure 1C).

Vitamin D Downregulated p16 mRNA and p21 mRNA Expression as Cellular Senescence Markers

The results of p16 mRNA and p21 mRNA expression in all groups were normally distributed according to the Shapiro-Wilk test (p > 0.05). RT-PCR revealed significantly higher of mRNA expression of p16 and p21 in BCCAO groups compare to the SO group. This finding demonstrates that the BCCAO group upregulated cellular senescence marker of p16 and p21 mRNA expression. On the other hand, vitamin D treatment affected downregulation of p16 mRNA expression which was demonstrated by significantly lower mRNA expression p16 in VD1 group (0.20 ± 0.01; p = 0.006) and VD2 (0.16 ± 0.03; p = 0.000) than the BCCAO group as well as p21. Vitamin D

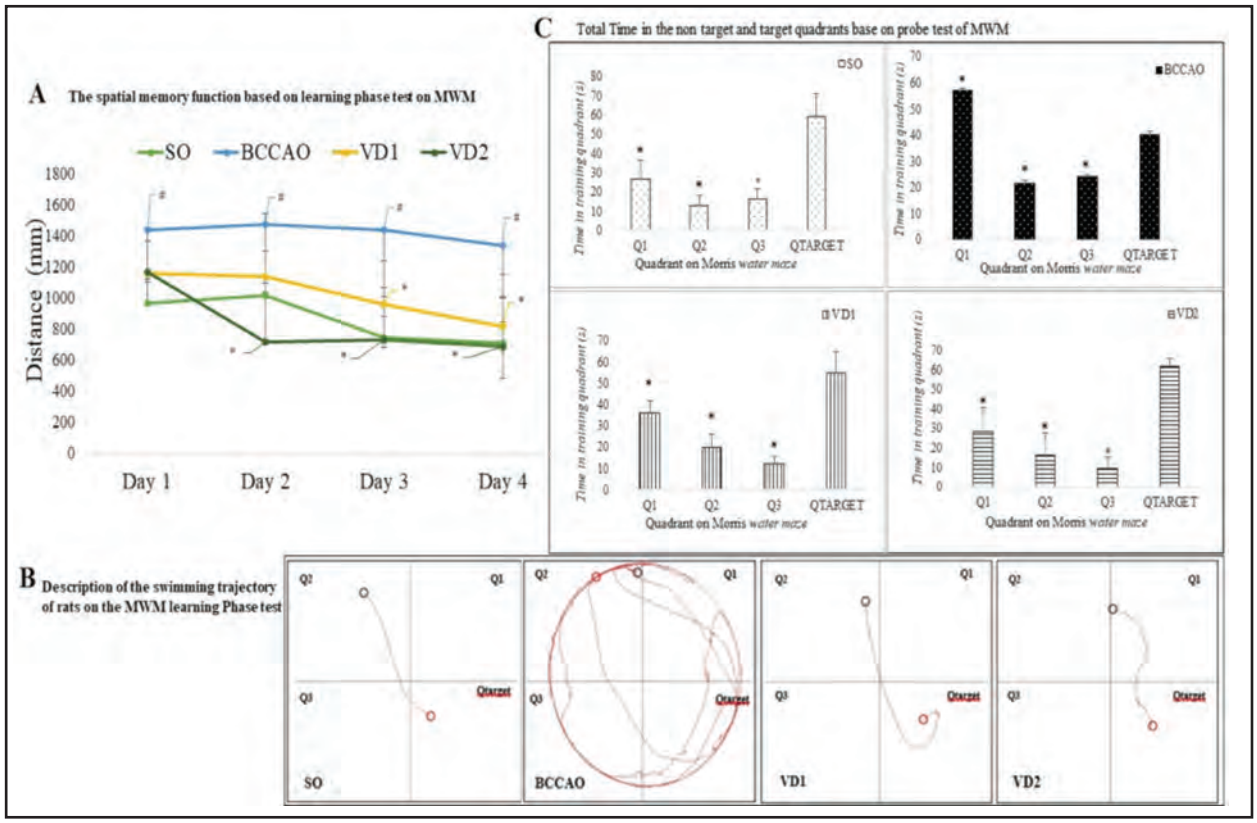

Fig. 1: Spatial memory function based on learning phase and probe test of MWM. (A) Mean ± SEM distance (mm) on learning phase of MWM by the group for 4 days. # $p < 0.05$ vs SO, * $p < 0.05$ vs BCCAO. (B) Swimming track pattern learning phase (total distance reached platforms on day 4). The black circle indicates the starting point, and the red circle indicates the endpoint swimming trajectory pattern based on the assessment learning phase of the SO, BCCAO, VD1 and VD2 rat groups. (C) The spatial memory probe test was assessed based on time spent in the target quadrant compared to the non-target quadrant. The data are presented in mean ± SEM. Probe test based on each group SO, BCCAO, VD1, VD2 quadrant target compared to quadrant non target (*$p < 0.05$).

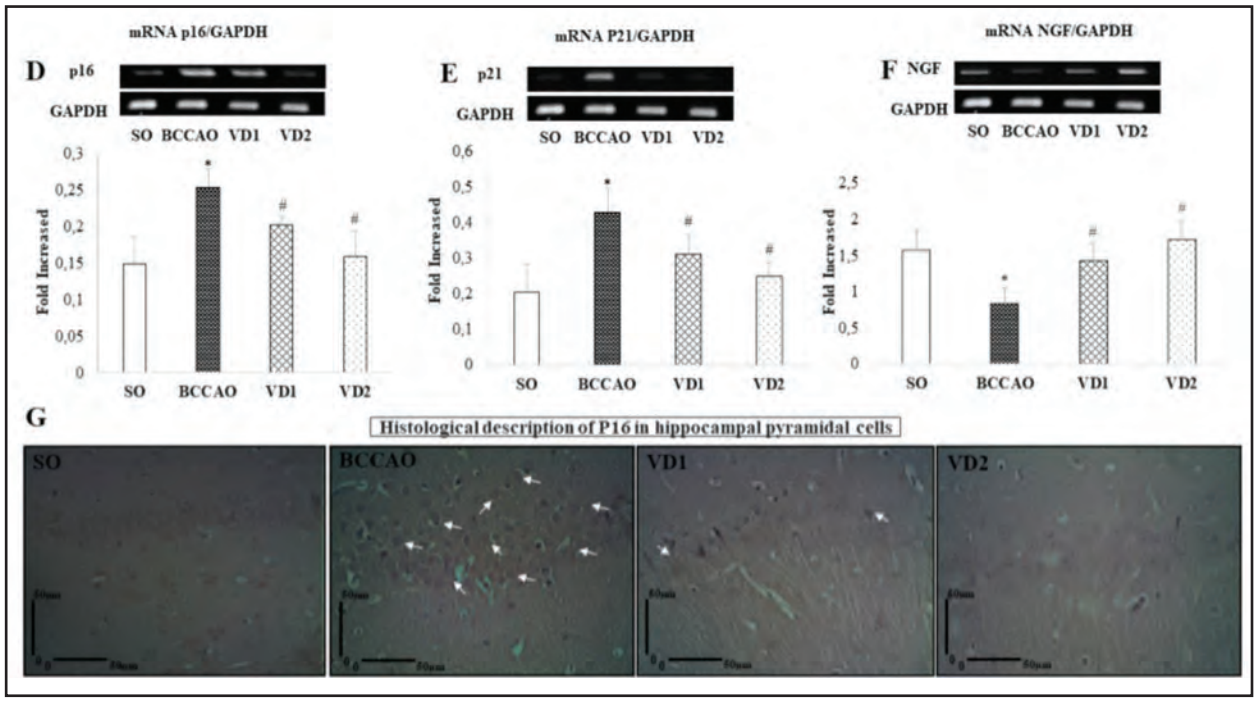

Fig. 2: (D) Representative image of electrophoresis results in RT-PCR products and mRNA expression of p16/GAPDH. (E) Representative image of electrophoresis results in RT-PCR products and mRNA expression of p21/GAPDH. (F) Representative image of electrophoresis results in RT-PCR products and mRNA expression of NGF/GAPDH. The data are presented in mean ± SEM. *$p < 0.05$ vs SO, # $p < 0.05$ vs BCCAO; (Analysed by one-way ANOVA, post-hoc LSD test). (G) Histological picture of the hippocampus. 400x magnification. (SO) Sham operation group; (BCCAO) BCCAO group; (VD1) BCCAO + Vitamin D 0.125µg group; (VD2) BCCAO + Vitamin D 0.5µg group. (White arrows: cellular senescence expression of p16).

administration showed p21 mRNA expression in VD1 group (0.33 ± 0.07; p = 0.024) and VD2 (0.25 ± 0.03; p = 0.000) significantly lower than the BCCAO group. Furthermore, p16 mRNA expression The VD1 group (0.20 ± 0.01; p = 0.006) was higher than the SO group. P21 mRNA expression the VD1 group (0.33 ± 0.01; p = 0.005) was higher than the SO group. In contrast, there are not significant difference between VD1 and VD2 groups (Figure 2D)[16] both in p16 and p21 mRNA expression (Figure 2E).[16]

Vitamin D Treatment Upregulated NGF mRNA Expression

The results of RT-PCR showed that NGF mRNA expression in the SO, BCCAO, VD1 and VD2 groups obtained data normally distributed based on the Shapiro-Wilk test (p > 0.05). NGF mRNA expression in the BCCAO group was significantly lower than the SO group (p = 0.000). mRNA expression in NGF group VD1 and VD2 were significantly higher than the BCCAO group (p = 0.000). The results of the treatment group VD1 with VD2 then VD1 and VD2 were compared with SO, but there was no significant difference (Figure 2F).[16]

Immunohistochemical Staining of p16 Protein Expression in Pyramidal cells of CA1 Area Hippocampus

p16 expression in the CA1 area of hippocampal pyramidal cells was observed by immunohistochemical staining. Immunopositive cells was indicated by colour browning of the pyramidal cells. In the BCCAO group, VD1, and VD2 have seen a brown colour is in the pyramidal cell CA1 area, showing that cellular senescence expression occurred in the hippocampus CA1 area. In contrast, in the SO group no brownish colour was seen in the CA1 area of hippocampal pyramidal cells (Figure 2G).[16] The cell membrane and cytoplasm are stained brown (severe sign white) is p16 expression, a marker of cellular senescence expression. The results of this observation indicated that global cerebral ischemic transient BCCAO model triggers the expression of cellular senescence at CA1 sites of hippocampal pyramidal cells.

DISCUSSION

This study revealed improvement in memory function in the Vitamin D treated group (Figure 1). MWM test results showed that memory function disruption in the BBCAO group occurred due to the induction of global cerebral ischaemic that led to damage the hippocampus CA1 area as an important region in processing learning and memory.[16] Transient global cerebral ischaemic BCCAO model affects damage to the hippocampus, resulting in cognitive dysfunction and significantly decreased function spatial memory.[15,16]

The BCCAO model caused a reduction of blood flow to most or all of the brain resulting in global ischaemic.[15] When blood flow is disrupted, oxidative stress (ROS) increases, affecting the expression of antioxidant enzymes[17] as the primary mechanism of nerve damage due to the transient global cerebral ischaemic.[18,19] Not only through the oxidative stress pathway but also inflammation response after ischemia, known as ischemic reperfusion injury (I/R injury), leads to neuronal damage effecting increase the expression toll-like receptors (TLR).[20] Increase in TLR results in an increase in factor activity transcription of NF-kB which is an inflammatory cytokine that is secreted when DNA damage occurs. NF-kB can directly activate p21 expression in response to DNA damage. The increased expression of p21 will trigger a senescence-associated SASP that will exacerbate the condition after ischemic stroke.[6,20,21]

The hippocampus is an important brain area for the formation, organization and storage of memories.[22] It is composed of pyramidal cells for projection of neurons spatial, contextual and emotional information.[22] Post-ischemia conditions lead to the direct changes in the synapse and vesicles of postsynaptic density that would affect the passage of impulse in the information delivery.[23,24] The increased of oxidative stress is a pathological disorder that triggers damage to the hippocampus. CA1 pyramidal cells in the hippocampus are one of the most vulnerable areas of damage due to transient global cerebral ischemic injury model, which decreases spatial memory function.[25,26]

The evaluation of the learning phase on the first day showed that all groups had trouble to finding the location platform. On the second day, BCCAO groups had difficulties continuing to find the platforms with the rats behaving in looking for wall contact on the edge of the pool and swimming in a manner thigmotaxis with its swimming strategy is the hippocampus-independent egocentric. In contrast, the vitamin D treatment groups showed the rat activity, which involved active swimming in locate behaviour platforms and swim direct-path reach soon platform with a swimming strategy in the form of hippocampus-dependent allocentric.[16,27,28] The evaluation of probe test in this study is based on the time spent in the target quadrant compared to the non-target quadrant. The analyses of probe test obtained that the vitamin D treatment group focused on the target quadrant, so the time spent in the target quadrant was longer than in a group of rats without vitamin D treatment (Figure 1C). Previous research by Moghaddasi's et al. using a cognitive function assessment (MWM test) showed that the cerebral hypoperfusion model damages the hippocampus of rat required a total mileage reach platform that is longer and time spent on fewer target quadrants than the control group.[29] Furthermore Curdt et al. research stated that a group of rats with damage to the hippocampus may experience memory loss and fail to form a strategy allocentric and finally depends on the strategy egocentric.[30]

An ischemic-reperfusion injury causes a degenerative state of cerebrum blood flow, followed by restoration of blood flow and oxygenation. Initial conditions of cerebral ischemia result in failure of pump Na+-K+ATPase, decreased tissue pH, depolarisation membrane, activation of Ca2+ channels causing an influx Ca2+ and releasing excitatory amino acids, especially glutamate, resulting in overactivation of glutamate receptors and increased intracellular Ca levels.[31] Reperfusion also results in tissue damage. Reperfusion will trigger increased oxidative stress and the production of excess reactive oxygen species (ROS) in the cerebral vessels. ROS leads to cell damage through DNA oxidation, peroxidation of cell membranes, and mitochondrial permeability transition pores (mPTPs) eventually triggering senescence activation cellular.[31,32] Continuous exposure to an injury can cause increasingly widespread cell senescence through activation of

the p16 and p21 pathways.[5] Cell senescence leads to progressive anatomical changes and atrophy also caused reduction of sensory perception in the central nervous system, then triggers further cell neurodegeneration and eventually results in decreased function cognitive.[5,6] Our study revealed that mRNA p16 and p21 expression in the BCCAO group was significantly higher than the SO group (Figure 2D and 2E). Similar result was also shown by Cheng et al. study that an increase in oxidative stress with manifestations of ROS production would trigger an increase in the expression of mRNA p16, mRNA p21 and mRNA p53 which was significantly higher than the control group.[32]

Neurotrophin factors are needed by neutrons for cellular processes, such as growth, neuronal differentiation, and cellular homeostasis in brain areas. Oxidative stress is one of the factors that decrease the production of neurotrophin.[33] In our study, the expression of NGF was lower in BCCAO group compared to the SO and VD groups. The BCCAO group experiencing stress or depression which significantly decrease the neurotrophin levels. Due to ischemic stroke, NGF is involved in development of brain structures, especially in the hippocampus and protected neurons. NGF can attenuate DNA damage, reduce ROS production and LDH levels and prevent apoptosis and neuronal senescence. Neurotrophin levels in older rats are significantly decreased compared to young rats.[16,33] Previous study examined the effect of stress on neurotrophin expression showed different results from this study that stress can increase neurotrophin expression (BDNF), possibly due to differences in stress-induced levels performed in experimental animal models.[34]

Vitamin D belongs to the group of fat-soluble vitamins metabolised by the body into an active form with various biological effects. The effectiveness of vitamin D has been widely published, including vitamin D as the regulator of calcium metabolism, hormone secretion, immune function, cell proliferation and differentiation processes, and as a membrane antioxidant that protects neurons against damage due to increased oxidative stress.[35,36] Effect of vitamin D administration on BDNF levels in young rats, middle age group and old age shows that vitamin D could increase BDNF levels by inhibiting oxidative stress signals.[36] Long-term therapy with calcitriol (1,25-dihydroxy-vitamin D) possibly inhibited the decreased density of hippocampal CA1 neurons due to aging cells by modulating the reduction of oxidative stress and inflammation. Vitamin D will induce protein synthesis which provides a neuroprotective effect against ROS-induced cytotoxicity.[37] Vitamin D induces SIRT protein, which plays a role in modulating the aging process, Klotho protein, which plays a role in anti-aging and Nrf-2 plays a role in factor inhibition NF-kB transcription resulting in decreased inflammation.[36,37,38]

The current research examines the effect of vitamin D on NGF in an experimental animal model of transient global cerebral ischemia, based on the results that NGF expression in the vitamin D treatment groups was higher than in the vitamin D untreated group. The results of this study are in accordance with the research Eriksdotter et al. reviewed the effect of vitamin D administration on NGF levels in rat hippocampus experimental animal models of Alzheimer's disease, NGF levels of the vitamin D given rat group were higher than those of the experimental animal model group not given vitamin D, high NGF levels will increase protection of neurons in the hippocampus.[12] The NGF levels increased in the hippocampus area will increase the growth of neurons, neurogenesis and functional recovery in a mouse model of stroke.[33,39] Spatial memory function test assessment in this study, the transient global ischemia BCCAO model given vitamin D 0.125 µg/kgBW (VD1) and 0.5 µg/kgBW (VD2), that the spatial memory function assessment was based on the learning phase and probe test, rats given vitamin D did better compared to the ischemic cerebral global transient model BCCAO group who didn't receive vitamin D. In this study, it was found that both doses of vitamin D had the same effect in attenuating senescence, increasing neurotrophins and were able to improve spatial memory function after transient global cerebral ischemia. This is aligned with the previous study which proved that both doses (0.125 µg/kgBW) also improve inflammation, and reduced epithelial cell apoptosis and fibroblast expansion in renal fibrosis.[40]

CONCLUSION

Vitamin D has neuroprotective effects on the hippocampus. Vitamin D improved memory function in a rat model of global cerebral ischaemic by attenuating cellular senescence and NGF, a cellular signal transduction that plays a role in the process of maintaining neuronal survival in the hippocampus.

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