Araştırma Makalesi (Research Article)

Hindistan Cevizi Yağının Travmatik Beyin Hasarı Olan Farelerde Nöroprotektif Etkisi

The neuroprotective effect of Coconut Oil in mice with Traumatic Brain Injury

Seyda CANKAYA[1], Mehmet Yalcin GUNAL[2], Mustafa Caglar BEKER[3,4], Ahmet Burak CAGLAYAN[3,4], Ekrem Musa OZDEMİR[4], Ozlem SAATCİ[5], Baris CANKAYA[6], Burak YULUG[1,4]

[1]Alaaddin Keykubat University Medical School, Department of Neurology, Alanya, Turkey

[2]Alaaddin Keykubat University Medical School, Department of Physiology, Alanya, Turkey

[3]Medipol University Medical School, Department of Physiology, Istanbul, Turkey

[4]Medipol University, Regenerative and Restorative Medicine Research Center, Istanbul, Turkey

[5]Istanbul Sancaktepe Teaching Hospital, Department of Otolaryngology, Istanbul, Turkey

[6]Marmara University, Pendik Education and Research Hospital, Department of Anaesthesiology, Istanbul, Turkey

Amaç: Bu çalışmada hindistan cevizi yağının travmatik beyin hasarı olan farelerde olası nöroprotektif etkisini değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Çalışma grubunda yer alan 10 adet erkek fare (C57BL/6), kontrol (n=5) ve hindistan cevizi yağı uygulanan gruba (n=5) rastgele ayrıldı. Bir hafta boyunca kontrol grubuna 250 ul salin, diğer gruba gruba 10 mg / kg/oral hindistan cevizi yağı verildi. Farelere 7. gün cold brain injury modeli uygulandı. Beyindeki enfarktüs volümü ve ödem, travmadan 24 saat sonra değerlendirildi.

Bulgular: Hindistancevizi yağı uygulanan grupta, enfarktüs volümü ve beyin ödeminin, kontrol grubuna göre anlamlı derecede daha düşük olduğu saptandı.

Sonuç: Bulgularımız, hindistancevizi yağının farelerde travmatik beyin hasarı sonrası hasarlı nöronlar üzerinde potansiyel bir nöroprotektif etkiye sahip olduğunu göstermiştir.

Anahtar kelimeler: Beyin hasarı, Hindistan cevizi yağı, Nöroproteksiyon

Objective: This study aimed to evaluate the possible neuroprotective effect of coconut oil in mice with traumatic brain injury.

Material and Methods: Ten adult male mice (C57BL/6) were randomly assigned into the control (n=5) and coconut oil administered group (n=5). The control group was received 250 µl saline/day and coconut oil was administered 10 mg/kg/day per os to the coconut oil group for a week. Brain coold injury model was produced on seventh day. 24 hours after the injury, infarction and oedema volume in the brain was measured in two groups.

Results: The coconut oil group mice were found to exhibit lower brain infarct volume and edema than the control group mice.

Conclusion: Our findings indicated that coconut oil has a potential neuroprotective effect on damaged neurons after traumatic brain injury in mice.

Key Words: Brain injury, Coconut oil, Neuroprotection

Yazışma Adresi: Şeyda ÇANKAYA, Alaaddin Keykubat Üniversitesi Tıp Fakültesi Nöroloji Ana Bilim Dalı, Alanya, Türkiye

Telefon: 05374777407, Mail:cankayaseyda@hotmail.com

ORCID No (Sırasıyla): 0000-0001-5309-0351, 0000-0001-7702-2441, 0000-0002-9476-8488, 0000-0002-6242-3709, 0000-0001-9416-7757, 0000-0002-3372-9514, 0000-0003-1635-413X, 0000-0002-9704-6173

Geliş Tarihi: 04.01.2021

Kabul Tarihi: 09.02.2021

DOI: 10.17517/ksutfd.853830

INTRODUCTION

Traumatic brain injury (TBI) is the common cause of mortality and morbidity leading to high financial costs worldwide. Unfortunately, there is no actual proven treatment for TBI. The main reason for this limitation is that central nervous system (CNS) has a limited capacity to regenerate. This is partly due that neurons have limited primary energy sources, such as glucose to overcome the injury state and initiate the neuro-regenerative response.

Coconut Oil (CoOil) is a well-known agent to increase the production of ketone bodies which are alternative energy sources for the brain(1, 2). Unlike many other dietary fats that contain high level long-chain fatty acids, CoOil includes medium-chain fatty acids (MCFA) which are well absorbed and easily converted by the liver to energy-providing ketone bodies(1, 3, 4). The main advantage of ketone bodies in this context is their availability as an internal energy source for mitochondria(5)which does not require to be absorbed from the circulation, and thus not blocked by the selectively permeable structure of brain blood barrier (BBB). TBI has a heterogeneous process and contains many critical steps which are overlapping with those of many other traumatic and neurodegenerative diseases (i.e. stroke, Alzheimer's disease) including direct tissue damage (i.e., vasogenic oedema) and indirect series of events (i.e., inflammation, anoxic neuronal cell depolarization, excitotoxic neuronal cell death and cytotoxic oedema formation)(6). Additionally, the anaerobic phase of TBI is associated with lactic acid accumulation due to impaired cellular energy states which is a challenge for effective neuroprotective treatment in humans with TBI (7). To overcome this hurdle, considerable research has focused on secondary injury mechanisms to develop neuroprotective treatment strategies. However, despite many promising preclinical studies, more than 30 phase III prospective clinical trials have failed to show any significance in their primary end point (8-10). However, in fact, there have been no clinical study that focused on the bioenergetic mechanisms in TBI making it logical to assume that treatment options that do not target bioenergetic mechanisms might be responsible for failed clinical study results in brain trauma patients. Consistently, several studies have suggested that TBI and other traumatic neurodegenerative diseases might include some major bioenergetic deficiencies (11). Additionally, there are pieces of evidence suggesting the role of repetitive brain trauma as a significant risk factor for the development of Alzheimer Disease (AD)(12). In this context, several preclinical and clinical studies have demonstrated that cortical glucose metabolism has been severely disturbed after brain trauma (13, 14). However, the bioenergetic role of ketone bodies has been recently confirmed by cultures co-treated with CoOil and amyloid-β (Aβ) peptide (15, 16). Overall, these studies have indicated that CoOil can have a great impact on neuronal survival by decreasing the Aβ secretion via modulating various secondary intracellular messengers, such as ADP-Ribosylation Factor 1 (ARF1) (17). Because CoOil has been found neuroprotective in various in-vivo and in-vitro models of AD(1, 16, 18), we aimed to assess the brain protective effect of CoOil in a brain trauma model. Based on the anti-inflammatory, pro-cognitive and bioenergetic role of CoOil, we further hypothesized that CoOil could exert a multifaceted brain protective effect in the TBI pathophysiology. From the clinical point of view, it can be assumed that ketone bodies might be a novel treatment option for people who are exposed to TBI with their potency to provide alternative energy sources for the injured brain. Following studies have also confirmed that CoOil exerted significant inhibitory effects on inflammationwhich is an essential step in TBI pathophsyiology.

MATERIAL and METHODS

Ethical approval

All applicable international, national and/ or institutional guidelines for the care and useof animals were followed. The study was approved by the Istanbul Medipol University Medical School Ethical Committee (approval number: 38828770-604.01.01-E.58829).

Animals

The study was performed at Meditam Research Laboratories of Istanbul Medipol University.

Ten male mice (C57BL/ 6 mice, 8–10 weeks old, weighing 25-30 g) were used for the study. The animals were maintained under a constant 12:12-h light-darkness regimen (with the lights on daily at 7.00 a. m.) and with ad libitum access to food and water. The mice were housed separately in cages after the operation.

Coconut oil treatment and experimental groups

A total of ten mice were randomly divided into two groups (n=5 per group), namely the control and CoOil treatment groups which were treated with 250 µl saline and 10 ml kg BW CoOil per os (p.o), respectively along a week of 7 days. As by previous literature,we have chosen the neuroprotective dose of coconut oil as 10 mg/kg which is equal to human doses (19, 20).

Cold injury

The brain injury was performed as previously described for a cryogenic trauma model(21, 22).All the mice were anaesthetized with intraperitoneally (i.p.) ketamine (60 mg/ kg) and xylazine (6 mg/ kg) and fixed in the stereotaxic device. A parietal craniotomy (3 mm diameter, 2.5 mm lateral, 2.5 mm posterior to the bregma) was done using a dental drill. The cold injury was performed using a liquid nitrogen-cooled copper probe (tip diameter 2.5 mm), which was placed on the dura for 60 s and then removed. After that, the scalp was sutured. The rectal temperature was continuously monitored and kept between 36.5 and 37°C with a homeothermic blanket during the procedure. The animals were then taken to the feeding room and the experimenters waited for the animals

to recover during the following 24 h post-trauma. At the end of this 24 h, the animals were anaesthetized again with high doses of i.p. xylazine (20 mg/ kg) and ketamine (100 mg/ kg). The mice were sacrificed, and their brains were dissected and put on dry ice. Coronal 18 µm-thick brain sections were taken from the frozen brain tissue for histopathological and protein analyses.

Cresyl violet staining

The sections were dried at room temperature for 30 min to remove the moisture, followed by fixation in a 4% paraformaldehyde solution for 7 min. After washing with distilled water, the sections were placed in a glass chamber containing phosphate-buffered saline (PBS) with subsequent shaking of the samples for 5 min at 140 rpm. Then, cresyl violet dye was applied to the sections for 15 min on a shaker with 80 rpm. After staining, the sections were rinsed three times with distilled water and they were dipped into four chambers containing sequentially increasing concentrations of ethanol (70%, 90%, 95% and 100%) for 20 s in each chamber. Finally, xylene was applied to the sections for 3 min at room temperature and the mounting medium was placed onto each slide (23).

Analysis of brain injury

Coronal brain sections from equidistant brain levels, 0.5 mm apart, were stained with cresyl violet staining according to a standard protocol(24).On the sections, the border between the injured and non-injured areas was outlined using an image analysis system (Image J; NIH, Bethesta, MD, USA) and the area of the injury was assessed by subtracting the area of the non-injured ipsilateral hemisphere from that on the contralateral side. The volume of the injury was calculated by integration of these lesion areas. Odema was calculated as the volume difference between the ischaemic and the non-ischaemic hemisphere and expressed as a percentage of the intact hemisphere(25).

Statistical analysis

For statistical data comparisons, a standard software package SPSS 18 for Windows (SPSS Inc., Chicago, IL, USA) was used. The differences between groups were analysed with independent t-test. All the values are given as mean ± SD with N values indicating the number of animals analysed. $p<0.05$ is considered significant.

RESULTS

Infarct Volume and Brain Swelling

To analyse the effects of CoOil on TBI, the damaged volume was measured. In vehicle-treated animals, reproducible brain infarcts were observed after 24 h as described by our previous literature (12). In the control-group (Group 1), the infarct volume **(Figure 1)** was measured as 33±8.5 mm^3 versus23±9.1mm^3 (mean ± SD; $p<0.05$) in coconut oil applied group (Group 2). When the alterations of brain swelling were analysed, a reduction was found in Group II (**Figure 2,** 87%; $p<0.05$).Shortly, these alterations of brain swelling and infarct were statistically significantly different between the groups.

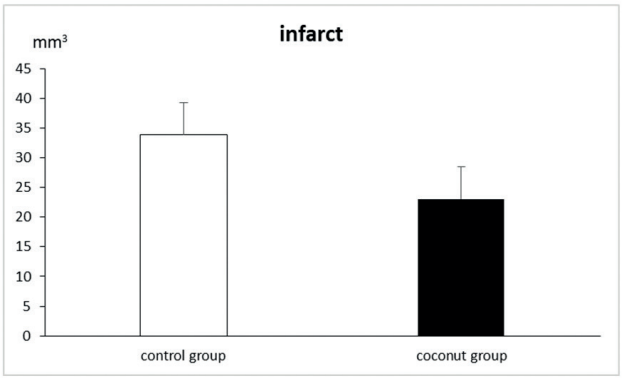

Figure 1. Infarct volume is lower in CoOil administered mice than in the control group 24 h after brain injury ($p<0.05$).

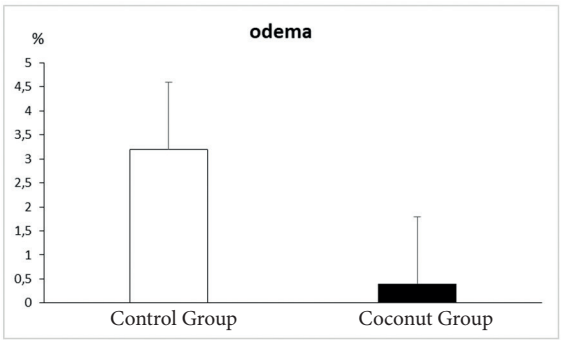

Figure 2. CoOil administered mice showing decreased brain swelling compared to the control group, 24 h after brain injury ($p< 0.05$). Distance bar = 2 mm, Group I: Control group; Group II: CoOil administered group

DISCUSSION

Despite the warnings of American Heart Association (AHA) (26) and the United States Department of Agriculture (USDA) (27) to consume CoOil due to increased cardiovascular system (CVS) side effects, there are rapidly replicating evidence showing that CoOil does not increase CVS side effects, suggesting further that it can be safely used in patients with neurodegenerative diseases (28, 29). Several studies further supported this recommendation by showing that CoOil might even slow the progression rate of PD and AD (30, 31).In investigating the neuroprotective effect of CoOil on the injured brain in mice, we have found that CoOi lreduced significantly the infarct and oedema volume. Despite many complex and interactive pathwaysinvolved in TBI, we have observed that 10 mg/kg CoOil sufficiently reduced infarct and oedema volumes, suggesting that in addition to major cascades (i.e., neuroinflammatory and oxidative), bioenergetic deficiencies,might play also an essential role in the pathophysiology of TBI. These results along with our current findings might strongly suggest that CoOil could play an important role in providing bioenergetic support by enhancing the utility of ketone bodies in TBI. Further, it is not

unreasonable to assume that the dosage applied in our study was sufficient to providesustainable energy for inducingthe repairing process after TBI. Our present results might also be relevant as they provide further evidence for the therapeutic role of CoOil in TBI-related dementia which is characterized with neuroinflammation, amyloid deposition and bioenergetic failures (32). In this regard, many studies have found that TBI might initiate multiple degenerative mechanisms, such as increased amyloid beta aggregation, leading to diffuse axonal injury (DAI) which is associated with increased risk of AD (33-36). Although our findings contribute to the literature in several ways, this study has some limitations that should be addressed. First, a long-term evaluation instead of a ‘‘snap-shot” histological analysis at post-injury 24 hours, could increase the power of the present study. Second, our sample size was small, and further investigation using more animals are necessary.

It should be noted, despite the small sample size we were able to show statistically significant differences between the groups regarding infarct and oedema volumes that support the potential neuroprotective role of CoOil in TBI-related dementia. Taken together, although some limitations, our study is the first study that tested the neuroprotective role of CoOil in TBI,and opens a new translational window for a new understanding the underlying bioenergetic and inflammatory mechanisms of the neuroprotective effect of CoOil in TBI.

Conflict of Interest and Financial Status

The authors declare that there is no conflict of interest to declare. The author(s) received no specific funding for this work

Research Contribution Rate Statement Summary

The authors declare that, they have contributed equally to themanuscript.

REFERENCES

1. Fernando WMADB, Martins IJ, Goozee K, Brennan CS, Jayasena V, Martins RN. The role of dietary coconut for the prevention and treatment of Alzheimer's disease: potential mechanisms of action. Br J Nutr. 2015;114(1):1-14.
2. Richer AC. Functional medicine approach to traumatic brain injury. Medical acupuncture. 2017;29(4):206-14.
3. Ghani NAA, Channip AA, Chok Hwee Hwa P, Ja'afar F, Yasin HM, Usman A. Physicochemical properties, antioxidant capacities, and metal contents of virgin coconut oil produced by wet and dry processes. Food Sci Nutr. 2018;6(5):1298-306. (doi: 10.1002/fsn3.671).
4. Ockner RK, Manning JA, Poppenhausen RB, Ho WK. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues. Science. 1972;177(4043):56-8.
5. Sato K, Kashiwaya Y, Keon C, Tsuchiya N, King MT, Radda GK et al. Insulin, ketone bodies, and mitochondrial energy transduction. The FASEB Journal. 1995;9(8):651-8.
6. Werner C, Engelhard K. Pathophysiology of traumatic brain injury. Br J Anaesth. 2007;99(1):4-9. (doi: 10.1093/bja/aem131).
7. Jha MK, Morrison BM. Glia-neuron energy metabolism in health and diseases: new insights into the role of nervous system metabolic transporters. Experimental neurology. 2018;309:23-31.
8. Narayan RK, Michel ME, Ansell B, Baethmann A, Biegon A, Bracken MB et al. Clinical trials in head injury. J Neurotrauma. 2002;19(5):503-57. (doi: 10.1089/089771502753754037).
9. Schouten JW. Neuroprotection in traumatic brain injury: a complex struggle against the biology of nature. Curr Opin Crit Care. 2007;13(2):134-42. (doi: 10.1097/MCC.0b013e3280895d5c).
10. Maas AI, Roozenbeek B, Manley GT. Clinical trials in traumatic brain injury: past experience and current developments. Neurotherapeutics. 2010;7(1):115-26. (doi: 10.1016/j.nurt.2009.10.022).
11. McKee AC, Daneshvar DH. The neuropathology of traumatic brain injury. Handb Clin Neurol. 127: Elsevier; 2015. p. 45-66.
12. Ramos-Cejudo J, Wisniewski T, Marmar C, Zetterberg H, Blennow K, de Leon MJ et al. Traumatic brain injury and Alzheimer's disease: the cerebrovascular link. EBioMedicine. 2018;28:21-30.
13. Mahar M, Cavalli V. Intrinsic mechanisms of neuronal axon regeneration. Nature Reviews Neuroscience. 2018;19(6):323-37.
14. Berry M, Ahmed Z, Morgan-Warren P, Fulton D, Logan A. Prospects for mTOR-mediated functional repair after central nervous system trauma. Neurobiology of disease. 2016;85:99-110.
15. Nafar F, Clarke J, Mearow K. Coconut oil protects cortical neurons from amyloid beta toxicity by enhancing signaling of cell survival pathways. Neurochemistry international. 2017;105:64-79.
16. Nafar F, Mearow KM. Coconut oil attenuates the effects of amyloid-β on cortical neurons in vitro. Journal of Alzheimer's Disease. 2014;39(2):233-7.
17. Bansal A, Kirschner M, Zu L, Cai D, Zhang L. Coconut oil decreases expression of amyloid precursor protein (APP) and secretion of amyloid peptides through inhibition of ADP-ribosylation factor 1 (ARF1). Brain research. 2019;1704:78-84.
18. Nafar F, Clarke JP, Mearow KM. Coconut oil protects cortical neurons from amyloid beta toxicity by enhancing signaling of cell survival pathways. Neurochem Int. 2017;105:64-79. (doi: 10.1016/j.neuint.2017.01.008).
19. Rahim NS, Lim SM, Mani V, Abdul Majeed AB, Ramasamy K. Enhanced memory in Wistar rats by virgin coconut oil is associated with increased antioxidative, cholinergic activities and reduced oxidative stress. Pharm Biol. 2017;55(1):825-32.
20. Yeap SK, Beh BK, Ali NM, Yusof HM, Ho WY, Koh SP et al. Antistress and antioxidant effects of virgin coconut oil in vivo. Exp Ther Med. 2015;9(1):39-42. (doi: 10.3892/etm.2014.2045).
21. Hermann DM, Kilic E, Kugler S, Isenmann S, Bahr M. Adenovirus-mediated glial cell line-derived neurotrophic factor (GDNF) expression protects against subsequent cortical cold injury in rats. Neurobiol Dis. 2001;8(6):964-73. (doi: 10.1006/nbdi.2001.0448).
22. Keskin I, Gunal MY, Ayturk N, Kilic U, Ozansoy M, Kilic E. Dose-dependent neuroprotective effect of enoxaparin on cold-induced traumatic brain injury. Neural Regen Res. 2017;12(5):761-4. (doi: 10.4103/1673-5374.206646).
23. Qu Y, Van der Gucht E, Massie A, Vandenbussche E, Vandesande F, Arckens L. In vivo microdialysis in the visual cortex of awake cat. III: histological verification. Brain Res Brain Res Protoc. 2001;7(1):52-60. (doi: 10.1016/s1385-299x(00)00062-3).
24. Alvarez-Buylla A, Vicario DS. Simple microcomputer system for mapping tissue sections with the light microscope. J

Neurosci Methods. 1988;25(2):165-73. (doi: 10.1016/0165-0270(88)90155-0).

25. Kilic U, Caglayan AB, Beker MC, et al. Particular phosphorylation of PI3K/Akt on Thr308 via PDK-1 and PTEN mediates melatonin's neuroprotective activity after focal cerebral ischemia in mice. Redox Biol. 2017;12:657-65. (doi: 10.1016/j.redox.2017.04.006).
26. Sacks FM, Lichtenstein AH, Wu JHY, et al. Dietary Fats and Cardiovascular Disease: A Presidential Advisory From the American Heart Association. Circulation. 2017;136(3):e1-e23. (doi: 10.1161/CIR.0000000000000510).
27. Siguel EN, Lerman RH. Role of essential fatty acids: dangers in the US Department of Agriculture dietary recommendations ("pyramid") and in low-fat diets. The American journal of clinical nutrition. 1994;60(6):973-4.
28. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am J Clin Nutr. 2010;91(3):535-46. (doi: 10.3945/ajcn.2009.27725).
29. Chowdhury R, Warnakula S, Kunutsor S, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. Ann Intern Med. 2014;160(6):398-406. (doi: 10.7326/M13-1788).
30. Mischley LK, Lau RC, Bennett RD. Role of diet and nutritional supplements in Parkinson's disease progression. Oxid Med Cell Longev. 2017;2017.
31. de la Rubia Ortí JE, García-Pardo MıP, Drehmer E, et al. Improvement of Main Cognitive Functions in Patients with Alzheimer's Disease after Treatment with Coconut Oil Enriched Mediterranean Diet: A Pilot Study. Journal of Alzheimer's Disease. 2018(Preprint):1-11.
32. Edwards III G, Moreno-Gonzalez I, Soto C. Amyloid-beta and tau pathology following repetitive mild traumatic brain injury. Biochemical and biophysical research communications. 2017;483(4):1137-42.
33. Yulug B, Cankaya S. Translational perspective: is cinnamon a suitable agent for cognitive impairment and Alzheimer's disease associated with brain trauma? Neural regeneration research. 2019;14(8):1372.
34. Bramlett HM, Dietrich WD. Quantitative structural changes in white and gray matter 1 year following traumatic brain injury in rats. Acta Neuropathol. 2002;103(6):607-14. (doi: 10.1007/s00401-001-0510-8).
35. Chen XH, Siman R, Iwata A, Meaney DF, Trojanowski JQ, Smith DH. Long-term accumulation of amyloid-beta, beta-secretase, presenilin-1, and caspase-3 in damaged axons following brain trauma. Am J Pathol. 2004;165(2):357-71. (doi: 10.1016/s0002-9440(10)63303-2).
36. Shively S, Scher AI, Perl DP, Diaz-Arrastia R. Dementia resulting from traumatic brain injury: what is the pathology? Archives of neurology. 2012;69(10):1245-51.