Traumatic_Brain_Injury-_An_Experimental_Study_in_Wistar_Rats.pdf

by

Submission date: 31-Jan-2023 12:05PM (UTC+0700)
Submission ID: 2003117301
File name: Traumatic_Brain_Injury-_An_Experimental_Study_in_Wistar_Rats.pdf (301.03K)
Word count: 3481
Character count: 18971

Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Aug 08; 10(A):1630-1633.
https://doi.org/10.3889/oamjms.2022.10469
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pharmacology*

Since 2002

OPEN ACCESS

The Effect of Minocycline on MMP-9 Levels in Traumatic Brain Injury: An Experimental Study in Wistar Rats

Darren Perdana[1], Andi Ihwan[2*], Andi Alfian Zainuddin[3], Andi Asadul Islam[2], Djoko Widodo[2], Nasrullah Nasrullah[2], Willy Adhimarta[2], Wahyudi Wahyudi[2], Robert Christeven[1], Muhammad Faruk[1]

[1]Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia; [2]Department of Neurosurgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia; [3]Department of Public Health and Community Medicine Science, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

Edited by: Ksenija Bogoeva-Kostovska
Citation: Perdana D, Ihwan A, Zainuddin AA, Islam AA, Widodo D, Nasrullah N, Adhimarta W, Wahyudi W, Christeven R, Faruk M. The Effect of Minocycline on MMP-9 Levels in Traumatic Brain Injury: An Experimental Study in Wistar Rats. Open Access Maced J Med Sci. 2022 Aug 08; 10(A):1630-1633. https://doi.org/10.3889/oamjms.2022.10469
Keywords: Traumatic brain injury; Minocycline; MMP-9; Experimental study
***Correspondence:** Andi Ihwan, Department of Neurosurgery, Faculty of Medicine, Hasanuddin University, Makassar, 90245, Indonesia.
E-mail: aihwan77@yahoo.com
Received: 19-Jun-2022
Revised: 05-Jul-2022
Accepted: 28-Jul-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Cerebral edema in traumatic brain injury (TBI) is caused by hyperpermeability of the bloodbrain barrier (BBB). One of the causes of impaired BBB permeability is the activation of matrix metalloproteinase 9 (MMP-9), which can be inhibited by the antibiotic minocycline.

AIM: This study aimed to investigate MMP-9 levels and the effect of minocycline administration in a Wistar rat model of traumatic brain injury.

METHODS: A modified Marmarou model of brain injury in rats was used in this study. The post-test control group design consisted of an untreated control group (Group 1), a group with traumatic brain injury (Group 2), and an injury group that received minocycline (Group 3). Sandwich ELISA was used to evaluate MMP-9 levels.

RESULTS: The mean level of MMP-9 in Group 2 was 0.610 ng/ml, compared with 0.519 ng/ml in the controls, and 0.552 ng/ml in Group 3. Statistical analysis showed a statistically significant relationship between the administration of minocycline and the decrease in MMP-9 levels in Wistar rats with traumatic brain injury ($p = 0.001$).

CONCLUSION: Administration of minocycline to Wistar rats with traumatic brain injury resulted in significantly lower levels of MMP-9. This result showed that minocycline has the potential to inhibit increased MMP-9 levels in traumatic brain injury.

Introduction

Traumatic brain injury is a complex disorder caused by external forces that cause changes in brain function or brain pathology [1]. As many as, 1.7 million people annually experience traumatic brain injury in the United States [2], [3]. Meanwhile in Indonesia, the prevalence of traumatic brain injury increased from 14.5% in 2007 to 14.9% in 2013 [4].

Recent studies have shown that overexpression of molecular matrix metalloproteinase-9 (MMP-9) occurs as a result of cerebral edema and damage to the blood-brain barrier (BBB) in traumatic brain injury [5], [6]. Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteolytic enzymes that play a role in the degradation and remodeling of the extracellular matrix in both physiological and pathological processes. A major function of MMPs is degradation of the extracellular matrix to facilitate cell migration and invasion. However, uncontrolled expression of MMPs can result in tissue injury and destruction. As MMP regulation is complex and tightly regulated, loss of this regulatory control is likely to play a role in the pathophysiology of BBB damage in cerebral edema. Therefore, MMP-9 inhibition is an important target in the treatment of traumatic brain injury [5].

Of the tetracycline derivatives capable of inhibiting MMP-2 and MMP-9, minocycline is the strongest non-specific MMP inhibitor, reducing invasion and migration in cancer cells and exerting anti-inflammatory effects in normal tissue [7], [8]. Therefore, researchers are interested in understanding the effects of minocycline administration on MMPs in the context of cerebral edema following traumatic brain injury. Until recently, there have been few studies on MMP-9 inhibitors. In addition, well-known MMP-9 inhibitors are not widely available and are unaffordable in developing countries. This study aimed to determine the impact of traumatic brain injury on MMP-9 levels as well as the effects of minocycline administration on MMP-9 in a Wistar rat model of traumatic brain injury.

Materials and Methods

This research was conducted on rats using an experimental laboratory method with a post-test control group design. The research was conducted at the Animal Laboratory, Faculty of Medicine, Hasanuddin University over a period of 2 weeks in September 2020. The research population consisted of rats with traumatic brain injury and a control group of uninjured rats. The sample size for each group was determined using Federer's formula [9], [10], [11], which identified the minimum number of experimental animals per group as nine (a total of 27 rats for this study). Inclusion criteria in this study were male Wistar rats (Rattus norvegicus) aged approximately 3–4 months, body weight approximately 300–400 g, and good health status. Rats that looked sick during the adaptation period (inactive) and showed weight loss > 10% during adaptation were excluded from the study. Experimental animals fulfilled the study dropout criteria if they died before the study was completed or the blood sample was damaged. Animals that met the inclusion criteria were included in the traumatic head injury model (craniectomy and traumatic brain injury). The rats were randomly assigned to three groups: Group 1 (control) = no head trauma applied with blood samples taken at the same time as Groups 2 and 3; Group 2 (TBI) = rats undergoing craniectomy and direct trauma to the brain with blood samples taken 24 h after treatment; and Group 3 (TBI+M) = craniectomy and direct trauma to the brain followed by administration of minocycline (10 mg/kg) and collection of blood samples 24 h after treatment. The blood samples were collected from the tail vein.

Minocycline was purchased from Dexa-Medica Pte.ltd. Levels of MMP-9 were determined using an ELISA kit (E0321Ra) from Bioassay Technology Laboratory (Shanghai Korain Biotech Co., Ltd, Shanghai, China).

Surgical procedure

The rats were anesthetized with 3% of sodium pentobarbital (50 mg/kg) and placed in a stereotaxic frame. A midline incision was made and the skin and temporal muscles were reflected before opening the skull. Craniectomy was performed with a diameter of 4.0 mm, using a dental bur on the left side. Briefly, a modified Marmarou weight drop model [12], [13] was used to apply load to the brain (tip diameter, 4.0 mm; cortical contusion depth, 5.0 mm; impact velocity, 20 m/s; residence time, 50.0 ms). After the injury, the skin incision was closed with nylon sutures 5/0. Minocycline [13], [14], [15] was administered as a single oral dose (10 mg/kg) to Group 3 (TBI+M) animals 12 h after the procedure.

Sample processing

The blood was centrifuged and the resulting supernatant (designated plasma) was immediately transferred into a clean polypropylene tube using a Pasteur pipette. The samples were then maintained at 2–8°C. Levels of MMP-9 were measured using the sandwich ELISA method. The levels of MMP-9 from each sample were compared between groups.

Statistical analysis

All data were processed and analyzed using SPSS version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Initially, data distribution was analyzed using the normality test (Shapiro–Wilk) for parametric data and the homogeneity test (Levene) for non-parametric data with $p > 0.05$. As the data were normally distributed, statistical analysis was continued to compare the mean differences between variables. This analysis used one-way analysis of variance (ANOVA) and the Fisher's least significant difference (LSD) *post hoc* test for parametric data.

The research was carried out after ethical clearance from the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University Makassar, under number 786/UN4.6.4.5.31/PP36/2020.

Results

Characteristics of the animal models used, such as body weight, are presented in Table 1. The Lavene's homogeneity test was used to determine the homogeneity of Wistar rats used as brain injury models, and $p > 0.05$ was found, indicating that there was no significant variation between each body weight.

Table 1: Wistar rats body weight

Body weight (g), mean ± SD	p
390.08 ± 10.58	0.155

SD: Standard deviation.

The experimental animals were divided into three groups, with nine rats per group. As the data were normally distributed, ANOVA was used for statistical analysis. The mean MMP-9 level in the injury group (TBI) was 0.610 ng/ml, while the mean value in the uninjured controls was 0.519 ng/ml (Table 2). In Group 3 (TBI+M), the MMP-9 level was 0.552 ng/ml. The differences were

Table 2: Comparison of matrix metalloproteinase 9 level between groups

Group	MMP-9 levels (ng/ml)				
	n	Mean ± SD	Minimum	Maximum	p
1 (control)	9	0.519 ± 0.024	0.483	0.562	0.001
2 (TBI)	9	0.610 ± 0.068	0.492	0.671	
3 (TBI+M)	9	0.552 ± 0.039	0.468	0.605	

TBI: Traumatic brain injury, MMP-9: Matrix metalloproteinase, 9SD: Standard deviation.

nificant, as shown by ANOVA (p = 0.001). Therefore, there was a statistically significant relationship between the administration of minocycline and the observed decrease in MMP-9 levels in cerebral edema in Wistar rats.

Table 3 shows the results of *post hoc* analysis using the LSD test. Analysis of MMP-9 lev in Group 2 (TBI) and the control group showed that the difference was statistically significant (p < 0.001). Meanwhile, the levels of MMP-9 in Group 2 (TBI) and Group 3 (TBI+M) were also significantly different (p = 0.033).

Table 3: *Post hoc* comparison using least significant difference

Groups	p < 0.05
Group 1 (control)	
p 3 (TBI+M)	0.029
Group 2 (TBI)	< 0.001
Group 3 (TBI+M)	
Group 2 (TBI)	0.033

TBI: Traumatic brain injury.

Discussion

In this study, we show that traumatic brain injury in Wistar rats resulted in increased serum levels of MMP-9. dministration of minocycline following injury led to a significant reduction in MMP-9. The injury was applied directly to the brain rather than the head to enable observation of the real impact of direct brain trauma on MMP-9 levels. Trauma given to the head can result in more diverse manifestations such as epidural hemorrhage, subdural hemorrhage, subgaleal hemorrhage, and skull fracture.

Marmarou's drop model (weight drop traumatic brain injury model) was sed to induce the effects of BBB damage caused by trau atic brain injury in Wistar rats [11], [16]. Levels of M P-9 were significantly increased in Group 2 rats 24 h after traumatic brain injury (impacted with a force of 571.17 Newton/mm^2 in the cranie y area), compared with levels in the control group. This is in line with the study of Hayashi *et al*. in 2009, which owed that MMP-9 levels were significantly upregulated 24 and 48 h after traumatic brain injury [17].

Overexpression of MMP-9 is induced by excitotoxicity, neuroinflammation, oxidative stress, and apoptosis as a result of ischemic reperfusion in secondar injury. Secondary injury occurs within minutes of traumatic brain injury and can ast for months to years, playing a major role in the morbidity and mortality resulting from traumatic brain injury [18]. It is important to recognize the point at which serum levels of MMP start to become unstable after traumatic brain injury, as this is the optimal time to administer MMP inhibitors [17].

Increased levels of MMP cause hyperpermeability of the BBB, leading to cerebral edema[19]. Therefore, suppression of MMP expression is the key to endogenous neuroprotection. MMP regulation can occur at multiple levels, including gene expression, post-translational processing, compartmentalization, and regulation by endogenous tissue inhibitors of matrix metalloproteinases [20]. Longstanding treatment techniques for traumatic brain injury associated with MMP inhibition include hyperbaric oxygen therapy and hypothermia [17]. In addition, traditional medicines such as rhubarb (dried roots and rhizomes) can reduce levels of MMP-9 [21]. Administration of tetracycline antibiotics such as minocycline and doxycycline can also reduce MMP-9 levels in traumatic brain injury [22].

In this study, minocycline was administered orally at 10 mg/kilogram body weight following brain injury to determine its eff on MMP-9 levels. *Post hoc* analysis revealed that the effect of minocycline on MMP-9 levels in the injured rats was significant. Thus, oral administration of minocycline reduced MMP-9 levels in this model of traumatic brain injury.

Minocycline is a member of the tetracycline group of antibiotics, which inhibit MMPs by affecting transcription and activation [23]. This is likely due to their ability to bind to zinc, which is required for the activation of MMPs [22]. Minocycline is the strongest non-specific tetracycline MMP inhibitor capable of inhibiting MMP-9 [7], [8]. Minocycline is highly lipophilic, meaning it can easily cross the BBB. Maximum concentration is observed within 2–3 h of ingestion, while its half-life (12–18 h) is longer than that of first-generation tetracyclines [24], [25], [26].

The limitation of our study was that it did not compare the effect of minocycline with standard MMP-9 inhibitors due to the lack of availability of such inhibitors in our institution. Therefore, further studies have been planned.

Conclusion

The levels of MMP-9 in an animal model of brain injury were significantly reduced by minocycline treatment. This result showed that minocycline has the potential to inhibit the MMP-9 inc se that occurs in traumatic brain injury. We suggest that further research is needed to identify other factors that may influence MMP-9 levels in this context. In ad n, research into the effects of minocycline on MMPs in human traumatic brain injury is required.

References

1. Winkler EA, Minter D, Yue JK, Manley GT. Cerebral edema in traumatic brain injury, neurosurg: Pathophysiology and

Prospective Therapeutic Targets. Neurosurg Clin N Am. 2016;27(4):473-88. https://doi.org/10.1016/j.nec.2016.05.008
PMid:27637397

2. Georges A, Das JM. Traumatic brain injury. In: StatPearls Publishing, Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459300/?report=classic [Last accessed on 2022 Mar 05].
3. Sabet N, Soltani Z, Khaksari M. Multipotential and systemic effects of traumatic brain injury. J Neuroimmunol. 2017;357:577619. https://doi.org/10.1016/j.jneuroim.2021.577619
PMid:34058510
4. Khan A, Prince M, Brayne C, Prina AM. Lifetime prevalence and factors associated with head injury among older people in low and middle income countries: A 10/66 Study. PLoS One. 2015;10(7):e0132229. https://doi.org/10.1371/journal.pone.0132229
PMid:26146992
5. Zeng JP, Jia LT, Jin X, Zheng JP, ZhangXW, Zhan RY. Emodin attenuates brain edema after traumatic brain injury in rats. Int J Clin Exp Med. 2017;10:15213-20.
6. Alluri H, Wilson RL, Shaji CA, Wiggins-Dohlvik K, Patel S, Liu Y, *et al*. Melatonin preserves blood-brain barrier integrity and permeability via matrix metalloproteinase-9 inhibition. PLoS One. 2016;11:e0154427. https://doi.org/10.1371/journal.pone.0154427
7. Nascimento GC, Rizzi E, Gerlach RF, Leite-Panissi CR. Expression of MMP-2 and MMP-9 in the rat trigeminal ganglion during the development of temporomandibular joint inflammation. Braz J Med Biol Res. 2013;46:956-67. https://doi.org/10.1590/1414-431X20133138
PMid:24270905
8. Shen LC, Chen YK, Lin LM, Shaw SY. Anti-invasion and anti-tumor growth effect of doxycycline treatment for human oral squamous-cell carcinoma--*in vitro* and *in vivo* studies. Oral Oncol. 2010;46(3):178-84. https://doi.org/10.1016/j.oraloncology.2009.11.013
PMid:20036604
9. Daud KR, Islam AA, Ihwan A, Zainuddin AA, Faruk M. Oral doxycycline on the level of matrix metalloproteinase 9 in rat models experiencing traumatic brain injury. IIUM Med J Malaysia. 2021;20:11-6. https://doi.org/10.31436/imjm.v20i1.1773
10. Prihantono P, Syamsu SA, Smaradhania N, Ahmad M, Siagian NA, Lubis K, *et al*. Application of *Scaevola taccada* (Gaertn.) Roxb. Reduce pro-inflammatory cytokines interleukin-1β in sprague dawley mice suffering from mastitis. Open Access Maced J Med Sci. 2020;8:423-7. https://doi.org/10.3889/oamjms.2020.4363
11. Nasution RA, Islam AA, Hatta M, Prihantono, Massi MN, Warsinggih, *et al*. Effectiveness of CAPE in reducing vascular permeability after brain injury. Med Clín Práct. 2021;4:100229. https://doi.org/10.1016/j.mcpsp.2021.100229
12. Nasution RA, Islam AA, Hatta M, Prihantono, Turchan A, Nasrullah, *et al*. Role of CAPE in Reducing Oxidative Stress in Animal Models with Traumatic Brain Injury. Ann Med Surg. 2020;57:118-22. https://doi.org/10.1016/j.amsu.2020.07.036
13. Higashida T, Kreipke CW, Rafols JA, Peng C, Schafer S, Schafer P, *et al*. The role of hypoxia-inducible factor-1α, aquaporin-4, and matrix metalloproteinase-9 in blood-brain barrier disruption and brain edema after traumatic brain injury. J Neurosurg. 2011;114:92-101. https://doi.org/10.3171/2010.6.JNS10207
PMid:20617879
14. Fan X, Lo EH, Wang X. Effects of minocycline plus tissue plasminogen activator combination therapy after focal embolic stroke in Type 1 diabetic rats. Stroke. 2013;44(3):745-52. https://doi.org/10.1161/STROKEAHA.111.000309
PMid:23422086
15. Yan P, Zhu A, Liao F, Xiao Q, Kraft AW, Gonzales E, *et al*. Minocycline reduces spontaneous hemorrhage in mouse models of cerebral amyloid angiopathy. Stroke. 2015;46(6):1633-40. https://doi.org/10.1161/STROKEAHA.115.008582
PMid:25944329
16. Dixon CE, Clifton GL, Lighthall JW, Yaghmai AA, Hayes RL. A controlled cortical impact model of traumatic brain injury in the rat. J Neurosci Methods. 1991;39(3):253-62. https://doi.org/10.1016/0165-0270(91)90104-8
17. Hayashi T, Kaneko Y, Yu S, Bae E, Stahl CE, Kawase T, *et al*. Quantitative analyses of matrix metalloproteinase activity after traumatic brain injury in adult rats. Brain Res. 2009;1280:172-7. https://doi.org/10.1016/j.brainres.2009.05.040
PMid:19464272
18. Webster KM, Sun M, Crack P, O'Brien TJ, Shultz AJ, Semple BD. Inflammation in epileptogenesis after traumatic brain injury. J Neuroinflammation. 2017;14(1):10. https://doi.org/10.1186/s12974-016-0786-1
PMid:28086980
19. Vajtr D, Benada O, Kukacka J, Průša R, Houstava L, Ťoupalík P, *et al*. Correlation of ultrastructural changes of endothelial cells and astrocytes occurring during blood brain barrier damage after traumatic brain injury with biochemical markers of BBB leakage and inflammatory response. Physiol Res. 2009;58(2):263-8. https://doi.org/10.33549/physiolres.931253
PMid:18380546
20. Grossetete M, Phelps J, Arko L, Yonas H, Rosenberg GA. Elevation of matrix metalloproteinases 3 and 9 in cerebrospinal fluid and blood in patients with severe traumatic brain injury. Neurosurgery. 2009;65(4):702-8. https://doi.org/10.1227/01.NEU.0000351768.11363.48
PMid:19834375
21. Yang Z, Fan R, Sun P, Cui H, Peng W, Luo J, *et al*. Rhubarb attenuates cerebral edema via inhibition of the extracellular signal-regulated kinase pathway following traumatic brain injury in rats. Pharmacogn Mag. 2018;14(53):134-9. https://doi.org/10.4103/pm.pm_218_17
PMid:29576714
22. Chang JJ, Kim-Tenser M, Emanuel BA, Jones GM, Chapple K, Alikhani A, *et al*. Pourmotabbed, Minocycline and matrix metalloproteinase inhibition in acute intracerebral hemorrhage: A pilot study. Eur J Neurol. 2017;24(1):1384-91. https://doi.org/10.1111/ene.13403
PMid:28929560
23. Hanemaaijer R, van Lent N, Sorsa T, Salo T, YrjŐ, Konttinen T, *et al*. Inhibition of matrix metalloproteinases (MMPs) by tetracyclines. In: Nelson M, Hillen W, Greenwald RA, editors. BT-tetracyclines in Biology, Chemistry and Medicine. Switzerland: Birkhäuser Basel; 2001. p. 267-81. https://doi.org/10.1007/978-3-0348-8306-1_11
24. Agwuh KN, MacGowan A. Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. J Antimicrob Chemother. 2006;58:256-65. https://doi.org/10.1093/jac/dkl224
PMid:16816396
25. Fouda AY, Newsome AS, Spellicy S, Waller JL, Zhi W, Hess DC, *et al*. Minocycline in acute cerebral hemorrhage: An early phase randomized trial. Stroke. 2017;48(10):2885-7. https://doi.org/10.1161/STROKEAHA.117.018658
PMid:28887388
26. Ding JY, Kreipke CW, Schafer P, Schafer S, Speirs SL, Rafols JA. Synapse loss regulated by matrix metalloproteinases in traumatic brain injury is associated with hypoxia inducible factor-1alpha expression. Brain Res. 2009;1268:125-34. https://doi.org/10.1016/j.brainres.2009.02.060
PMid:19285046

Traumatic_Brain_Injury-_An_Experimental_Study_in_Wistar_Rats.pdf

ORIGINALITY REPORT

25%	17%	19%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Vascular Mechanisms in CNS Trauma, 2014. Publication	2%
2	Chi Zhang, Jie Zhu, Jing Zhang, Haoyu Li, Zijin Zhao, Yiwei Liao, Xiangyu Wang, Jun Su, Shushan Sang, Xianrui Yuan, Qing Liu. "Neuroprotective and anti-apoptotic effects of valproic acid on adult rat cerebral cortex through ERK and Akt signaling pathway at acute phase of traumatic brain injury", Brain Research, 2014 Publication	2%
3	Pranay Saha, Parikshit Moitra, Urmimala Bhattacharjee, Santanu Bhattacharya. "Bimodal Turn‐On Fluorescent Probe for Photophysical and Electrochemical Detection of Human Serum Albumin in Clinical Samples", Advanced Materials Interfaces, 2022 Publication	1%
4	theses.gla.ac.uk Internet Source	

		1 %
5	uwe-repository.worktribe.com Internet Source	1 %
6	www.balkanmedicaljournal.org Internet Source	1 %
7	academic.oup.com Internet Source	1 %
8	www.mdpi.com Internet Source	1 %
9	Submitted to State Islamic University of Alauddin Makassar Student Paper	1 %
10	qspace.library.queensu.ca Internet Source	1 %
11	www.hindawi.com Internet Source	1 %
12	www.researchgate.net Internet Source	1 %
13	lirias.kuleuven.be Internet Source	1 %
14	scholar.unand.ac.id Internet Source	1 %
15	www.scielo.br Internet Source	

		1 %
16	Ethan A. Winkler, Daniel Minter, John K. Yue, Geoffrey T. Manley. "Cerebral Edema in Traumatic Brain Injury", Neurosurgery Clinics of North America, 2016 Publication	1 %
17	www.injury-lawyer-tn.com Internet Source	1 %
18	A. Kalela, V. Limnell, J. Aittoniemi, V. Virtanen, O. Jaakkola, T. Lehtimäki, S. T. Nikkari. "Serum matrix metalloproteinase - 9 and venous bypass graft occlusion", Scandinavian Journal of Clinical and Laboratory Investigation, 2009 Publication	<1 %
19	repository.umj.ac.id Internet Source	<1 %
20	Mahnaz Yadollahi, Mehrdad Karajizadeh, Najmeh Bordbar, Zahra Ghahramani. "Incidence and pattern of traumatic spinal cord injury in a single level I trauma center of southern Iran", Chinese Journal of Traumatology, 2023 Publication	<1 %
21	Ting Liu, Xing-Zhi Liao, Mai-Tao Zhou. "Ulinastatin alleviates traumatic brain injury	<1 %

by reducing endothelin-1", Translational Neuroscience, 2020
Publication

22	garuda.ristekbrin.go.id Internet Source	<1 %
23	jasbsci.biomedcentral.com Internet Source	<1 %
24	Marc Maegele, Ute Schaefer. "Stem cell - based cellular replacement strategies following traumatic brain injury (TBI)", Minimally Invasive Therapy & Allied Technologies, 2009 Publication	<1 %
25	journals.viamedica.pl Internet Source	<1 %
26	www.id-press.eu Internet Source	<1 %
27	www.iiste.org Internet Source	<1 %
28	www.spandidos-publications.com Internet Source	<1 %
29	"Abstracts", Differentiation, 10/2006 Publication	<1 %
30	Alexander Zlotnik. "Effect of Glutamate and Blood Glutamate Scavengers Oxaloacetate and Pyruvate on Neurological Outcome and	<1 %

Pathohistology of the Hippocampus after Traumatic Brain Injury in Rats :", Anesthesiology, 01/2012
Publication

31	Gary E. Stein. "New drugs to treat skin and soft tissue infections", Current Infectious Disease Reports, 09/2007 Publication	<1 %
32	Homsi, S.. "Minocycline effects on cerebral edema: Relations with inflammatory and oxidative stress markers following traumatic brain injury in mice", Brain Research, 20090921 Publication	<1 %
33	Szu-Fu Chen, Wei-Shen Su, Chun-Hu Wu, Tsuo-Hung Lan, Feng-Yi Yang. "Transcranial Ultrasound Stimulation Improves Long-Term Functional Outcomes and Protects Against Brain Damage in Traumatic Brain Injury", Molecular Neurobiology, 2018 Publication	<1 %
34	coek.info Internet Source	<1 %
35	e-journal.unair.ac.id Internet Source	<1 %
36	journal.frontiersin.org Internet Source	<1 %

37	link.springer.com Internet Source	<1 %
38	www.biorxiv.org Internet Source	<1 %
39	www.ncbi.nlm.nih.gov Internet Source	<1 %
40	www.societasim.it Internet Source	<1 %
41	Alice Giotta Lucifero, Nunzio Bruno, Sabino Luzzi. "Surgical management of thoracolumbar junction fractures: An evidence-based surgical algorithm", World Neurosurgery: X, 2023 Publication	<1 %
42	HOSGORLER, Ferda, KELEŞ, Didem, TANRIVERDI AKHISAROGLU, Serpil, İNANC, Seniz, AKHISAROGLU, Mustafa, CANKURT, Ülker, AYDOĞDU, Zekiye, UÇAR, Ahmet Deniz, ÇETİNAYAK, Oğuz, OKTAY, Gülgün and GÖNENÇ ARDA, Sevil. "Anti-inflammatory and Anti-apoptotic Effect of Valproic Acid and Doxycycline Independent from MMP Inhibition in Early Radiation Damage", Trakya Üniversitesi Tıp Fakültesi, 2016. Publication	<1 %

43	Jun-Yu Chen, Ming-Zhen Zhu, Tao Xiong, Zi-Yao Wang, Qing Chang. "Development and validation of a new prediction model for calcific aortic valve stenosis", Journal of Thoracic Disease, 2022 Publication	<1 %
44	Boxiao Yang, Rui Zhang, Qire Sa, Yanli Du. "Rhamnazin Ameliorates Traumatic Brain Injury in Mice via Reduction in Apoptosis, Oxidative Stress, and Inflammation", Neuroimmunomodulation, 2021 Publication	<1 %
45	James J Donkin, Robert Vink. "Mechanisms of cerebral edema in traumatic brain injury: therapeutic developments", Current Opinion in Neurology, 2010 Publication	<1 %
46	Xingzhi Jiang, Zhongxue Ye, Wen Yu, Qian Fang, Yafen Jiang. "Chemotherapy for ovarian cancer during pregnancy: A systematic review and meta‐analysis of case reports and series", Journal of Obstetrics and Gynaecology Research, 2021 Publication	<1 %
47	Yao Chen, Shu-ying Fang. "Potential genetic polymorphisms predicting polycystic ovary syndrome", Endocrine Connections, 2018 Publication	<1 %

48	Bornali Kundu, Al-Wala Awad, Min S. Park, Ramesh Grandhi, Toby Enniss, Gregory W.J. Hawryluk. "Characterization of Focal Brain Tissue Water Measurements in Human Traumatic Brain Injury", World Neurosurgery, 2020 Publication	<1%

Exclude quotes	On	Exclude matches	< 5 words
Exclude bibliography	On		