Effect Of Amlodipine In Amelioration Of Global Cerebral I/R Injury In Rat

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الخلاصة

ان الإصابة بنقص التروية الدماغية وإعادة الارواء (IRI) هي من العمليات المعقدة التي تؤدي إلى تلف الخلايا ثم موتها.أجريت هذه الدراسة لغرض التحقق من امكانية الأملوديبين , في حماية الأعصاب و تحسين حال الإصابة الدماغية الشاملة (IRI) في نموذج الجرذان.أربعةوعشرون جرذاً بالغاً استخدمت في هذه الدراسة, وقد تم توزيعهم بشكل عشوائي الى أربعة مجاميع. مجموعة التظاهر,مجموعة السيطرة,مجموعة المذيب والمجموعة المعالجة بالأملوديبين. أظهرت المستويات الدماغية ل (ICAM-1) زيادة معنوية ($p<0.05$) في مجموعة السيطرة عند المقارنة بمجموعة التظاهر. المجموعة المعالجة بالأملوديبين اظهرت انخفاضا معنويا($p<0.05$) في المستوى الدماغي ل (ICAM-1), اما بالنسبة للنتائج النسيجية فأن الأملوديبين تسبب في انخفاض معنوي($p<0.05$) بحدة الضررالدماغي الناتج بالجرذان نتيجة الأنسداد الثنائي المشترك للشريان السباتي. نتائج دراستنا اظهرت ان الأملوديبين قد خفض الضرر الناتج من الأصابة بنقص التروية الدماغية الشاملة / إعادة الإرواء.

Abstract

Background: Cerebral ischemia–reperfusion injury (IRI) is a complex process resulting in cellular damage and death. Ischemia and reperfusion in the brain, as in other organs, induces an inflammatory response which may exacerbate initial levels of tissue injury.

Objectives: This study was undertaken to investigate the possible neuroprotective activity of amlodipine in amelioration of global cerebral I/R injury in rat model.

Materials and Methods: Adult sprague-dawley rats were randomized in to 4 groups as follow: group I, sham group, rats underwent the same anesthesia and surgical procedure as the control group with out bilateral common carotid artery occlusion (BCCAO) ; group 2 control group (induced-untreated), rats underwent 30 min of global cerebral ischemia via (bilateral common carotid artery occlusion (BCCAO) followed by 1 hour of reperfusion ; group 3, Control – Vehicle, as control group but rats received daily for 10 days before the surgery the vehicle of amlodipine drug normal saline intraperitoneally (IP),the dose of vehicle was (0.9% Nacl) ,(1 ml/kg/day) ; group 4, amlodipine treated group, as control goup ,but rats received daily amlodipine intraperitoneally (IP), the dose of amlodipine was (10 mg/kg /day) for 10 days before the surgery .

Results: Compared with the sham group, levels of cerebral ICAM-1, increased significantly ($p<0.05$), amlodipine, significantly oppose the increase in cerebral level of ICAM-1 ($P < 0.05$). Histological analysis revealed that amlodipine markedly reduced ($P < 0.05$) the severity of brain injury in the rats underwent bilateral common carotid artery occlusion (BCCAO) .

Conclusions: The results of the present study revealed that pretreatment with amlodipine may ameliorate the global cerebral ischemia-reperfusion injury by anti-inflammatory effect.

Keywords: Global cerebral ischemia, cerebral reperfusion injury inflammation, amlodipine,ICAM-1.

Introduction

Acute ischemic stroke is the second leading cause of death in the world.In the Middle East and North Africa stroke is increasingly becoming a major health problem, with projections that deaths from it will nearly double by 2030 [1] . Three months following a stroke, 15-30% of stroke survivors are permanently disabled and 20% require institutional care [2].Deficits can include partial paralysis, difficulties with memory,thinking, language, and movements. Brain ischemia occurs when cerebral blood flow is reduced to a low level by certain pathological conditions, such as stroke or cardiac arrest [3,4,5].Global cerebral ischemia occurs commonly in patients who have a variety of clinical conditions including cardiac arrest (CA), shock, and asphyxia and in patients undergoing complex cardiac surgery [6,7,8].Cerebral ischemia and reperfusion initiates a complex cascade of pathological events, include excitotoxicity, peri-infarct depolarizations, inflammation and programmed cell death [9]**.** The cellular changes caused by a reduction in blood flow (i.e., the primary injury) include a reduction in oxygen delivery, a switch to anaerobic glycolysis, a progressive fall in high-energy phosphate compound i.e., adenosine triphosphate (ATP) , intracellular acidosis, and intracellular accumulation of sodium and calcium ,and loss of cell ion homeostasis , generation of arachidonic acid products, cytokine mediated cytotoxicity, activation of glial cells [10].Rapid reperfusion, although intended, contributes to secondary injury by a cascade of pathological processes including leukocyte infiltration, platelet and complement activation, postischemic hyperperfusion , hemodynamic disturbances, inflammatory processes, free radical formation and breakdown of the blood-brain barrier (BBB) [11,12] . When severely impaired, there is an increased risk of deleterious vasogenic edema, brain herniation and death [12] . Ischemia and reperfusion in the brain, as in other organs, induces an inflammatory response which may exacerbate initial levels of tissue injury [13] . Inflammation is characterized by the accumulation of inflammatory cells and mediators in the ischemic brain. In the acute phase (minutes to hours) of ischemic stroke, ROS and proinflammatory mediators (cytokines and chemokines) are released rapidly from injured tissue [14,15]

There is increasing evidence that cellular adhesion molecules (CAMs) play an important role in the pathophysiology of acute ischemic stroke [16].At the clinical level, increased sICAM-1 and sVCAM-1 have been documented in the plasma and cerebral spinal fluid of subjects with recent cerebral ischemic patients, and correlated to stroke severity [17,18,19]

Amlodipine, a third generation dihydropyridine calcium antagonist, is characterized by a higher vascular selectivity and a smaller negative inotropic effect compared to nifedipine [20].Dihydropyridine calcium-channel blockers are now being used to treat several disorders, such as hypertension, arrhythmia, angina pectoris, left ventricular diastolic dysfunction, myocardial infarction, Raynaud's phenomenon, and progressive systemic sclerosis [21,22].Amlodipine, besides being a Ca2+ channel blocker, has also antiinflammatory-antioxidant and antiapoptotic activity [23,24]**.** Umemoto et al. (2004)[25] showed in a stroke model of

hypertensive rats treated with amlodipine, brain tissue damage was low,and this effect was suggested to be associated with the increasing effect of amlodipine on superoxide dismutase (SOD) activity.That confirmed by Mogi et al. (2006)[26] who suggested that amlodipine treatment was reduces stroke size and neurologic deficit after focal brain ischemia, possibly through an increase in cerebral blood flow and inhibition of superoxide production .

Materials and Methods

Animals

A total of 24 Adult Sprague-Dawley rats weighing (150-220 g) were obtained from Animal Resource Center, the National Center for Drug Control and Researches. The animals were apparently healthy and they were housed in the animal house of College of Medicine/ University of Kufa, at temperature controlled environment (25±2°C) with ambient humidity. Lights were maintained on a 12 h light/dark cycle. The rats received standard chow diet with water. Rats in the study were maintained in accordance with the guidelines established by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Preparation of Amlodipine

Amlodipine was provided from (Pioneer Co. Sulaymanieyah/Kurdistan Iraq), Amlodipine prepared immediately before use by dissolving it in normal saline.

Experimental Groups

After one week of acclimatization, the rat were randomized into four groups (6 rats in each group) sham group rats underwent the same anesthetic and surgical procedures for an identical period of time ,but with out bilateral common carotid artery occlusion (BCCAO). Control group (induced untreated) rats underwent anesthesia and surgery with bilateral common carotid artery occlusion (BCCAO) for 30 min. and then reperfusion for 1 hour But without drugs.Control - Vehicle group for 10 days before surgery rats received daily intraperitoneally (IP) the vehicle of amlodipine, normal saline (0.9% Nacl) (1 ml/kg/day)[27].Then, anesthesia and surgery with bilateral common carotid artery occlusion (BCCAO) for 30 min. and later reperfusion for 1 hour.Amlodipine treated group rats received daily amlodipine intraperitoneally (IP) . The dose of amlodipine was (10 mg/kg /day) [28] for 10 days before the surgery ,then anesthesia and surgery with bilateral common carotid artery occlusion (BCCAO) for 30 min. and later reperfusion for 1 hour.

Induction of global brain ischemia

Induction of global ischemia by bilateral common carotid artery occlusion (BCCO) [29,30] rats were maintened at approx 37°C under a light bulb and under general anesthesia ketamine & xylazine (80mg/kg&5mg/kg intraperitoneally) [31].Animals were placed on the back in the supine position .A small median incision was made in the neck and both carotid arteries were separated from vagal nerves, then exposed bilaterally and occluded by using vascular clamps and clamped for 30 min. In the reperfusion, the clamp were removed after ischemia and reperfusion was allowed to take place for 1 hour.

Preparation of Samples

Tissue Preparation for ICAM-1 Measurement

Following decapitation, the brain was removed and washed in cold 0.9% saline, kept on ice and subsequently blotted on filter paper, then weighed and homogenised using a high intensity ultrasonic liquid processor and brain tissues were homogenized in ice-cold 1:10 (w/v) 0.1 M phosphate-buffered saline (PBS) (pH 7.4), containing protease

inhibitor cocktail and 0.2% Triton X-100 for 30 seconds [32] . The homogenates were centrifuged at 14,000×g for 20 min at 4◦C and the supernatant was collected for determination of ICAM-1 according to the manufacturer's instructions and guidelines using enzyme-linked immunosorbent assay (ELISA) kits (Quantikine®/R&D Systems.USA).

Tissue Sampling for Histopathology

Coronal brain sections from control and experimental groups of global ischemia were fixed with 10% formalin and embedded in paraffin wax the sections were stained with haemotoxylin and eosin dye (H&E) for histopathological observation[33].The histological observations (evaluated by a pathologist using a double-blind method) were scored using a pathological scoring scale as follows: 0,(normal) = no morphological signs of damage; 1,(slight) = edema or eosinophilic or dark neurons(pyknotic) or dark/shrunk cerebellar Purkinje cells; 2,(moderate)= at least two small hemorrhages and 3,(severe) = clearly infarctive foci (local necrosis).

Statistical Analysis

Statistical analyses were performed by using SPSS 17.0 for windows lnc. An expert advice was consulted for tests used. Data were expressed as mean ± SEM. Analysis of Variance (ANOVA) was used for the multiple comparisons among all groups followed by post-hoc tests using LSD method.Figures of error bar chart use logarithmic scale for further explanation the data.Pearson correlation coefficient was used to assess the associations between two variables of study parameters. Spearman correlation coefficient was used for non-parametric correlations.The histopathological brain changes are a non-normally distributed variable measured on an ordinal level of measurement; therefore non-parametric tests were used to assess the statistical significance involving this variable. The statistical significance of difference in total score between more than 2 groups was assessed by Kruskal-Wallis test, while Mann-Whitney U test was used for the difference between 2 groups. In all tests, $P < 0.05$ was considered to be statistically significant.

Results

Effect on Proinflammatory marker (ICAM-1)

At the end of the experiment, the levels of cerebral ICAM-1 were significantly ($P<0.05$) increased in control group as compared with sham group. The levels of cerebral ICAM-1 of amlodipine treated group were significantly ($p<0.05$) lower than that of control-vehicle group. The values of cerebral ICAM-1 are showed in table(1) and figures (1).

Table(1): Cerebral ICAM-1 levels (pg/ml) of the four experimental groups at the end of the experiment.

Group	ICAM-1
Sham	34.38± 4.99
Control	362.8 ± 26.8*
Control-Vehicle	377.4 ± 33.5
Amlodipine treated	64.98± 4.94**

The data expressed as mean ± SEM (N = 6 in each group). * P < 0.05 vs. sham group, ** P < 0.05 vs. control-Vehicle group.

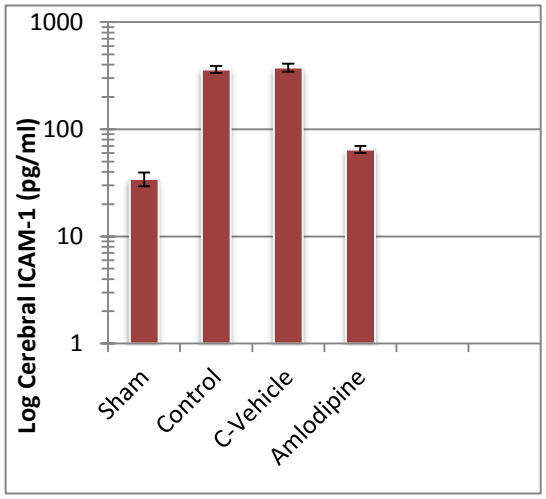

Figure (1): Error bar chart shows difference in mean ± SEM values of cerebral ICAM-1 level (pg/ml) in the four experimental groups.

Histopathological Findings

A cross section of sham rat's brain showed a normal appearance (100%) of rats in this group and also showed normal brain appearance as shown in table(2)and figures(2,3)**.**There was statistically significant difference between control group (II) and sham group (I) (P < 0.05) and the total severity scores of the control group showed severe cerebral injury (66.6%)**.** And it (33%) showed moderate injury as shown in table(2) and figures (2,3)**.**Treatment of rats with amlodipine improved cerebral injury score significantly (P < 0.05) as compared with control – vehicle group and the total severity scores mean of this group showed (16.7%) had normal histopathological appearance,and (66.6%) had slight cerebral injury ,and (16.6%) had moderate injury as shown in table (2) figure (2&3).

Table (2): The differences in histopathological scoring of abnormal brain changes among the four experimental groups.

Histopathological score	Study groups							
	Sham		Control		Control-vehicle		Amlodipine treated	
	N	%	N	%	N	%	N	%
Normal (0)	6	100	0	0	0	0	1	16.7
Slight (1)	0	0	0	0	0	0	4	66.6
Moderate (2)	0	0	2	33.3	3	50	1	16.7
Severe (3)	0	0	4	66.6	3	50	0	0
Total	6	100	6	100	6	100	6	100

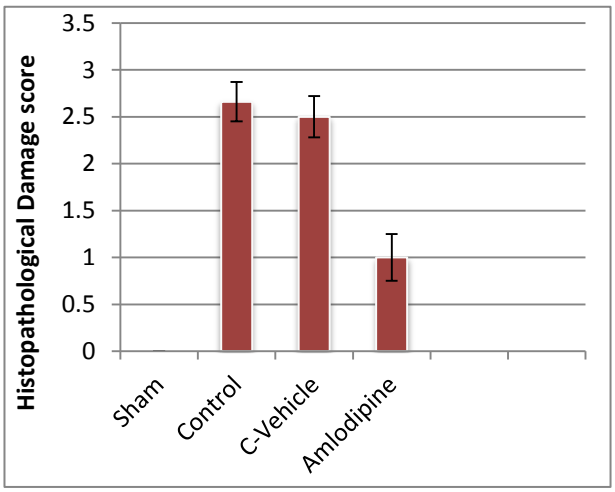

Figure (2): Error bar chart shows difference in mean ± SEM values of total severity scores in the four experimental groups.

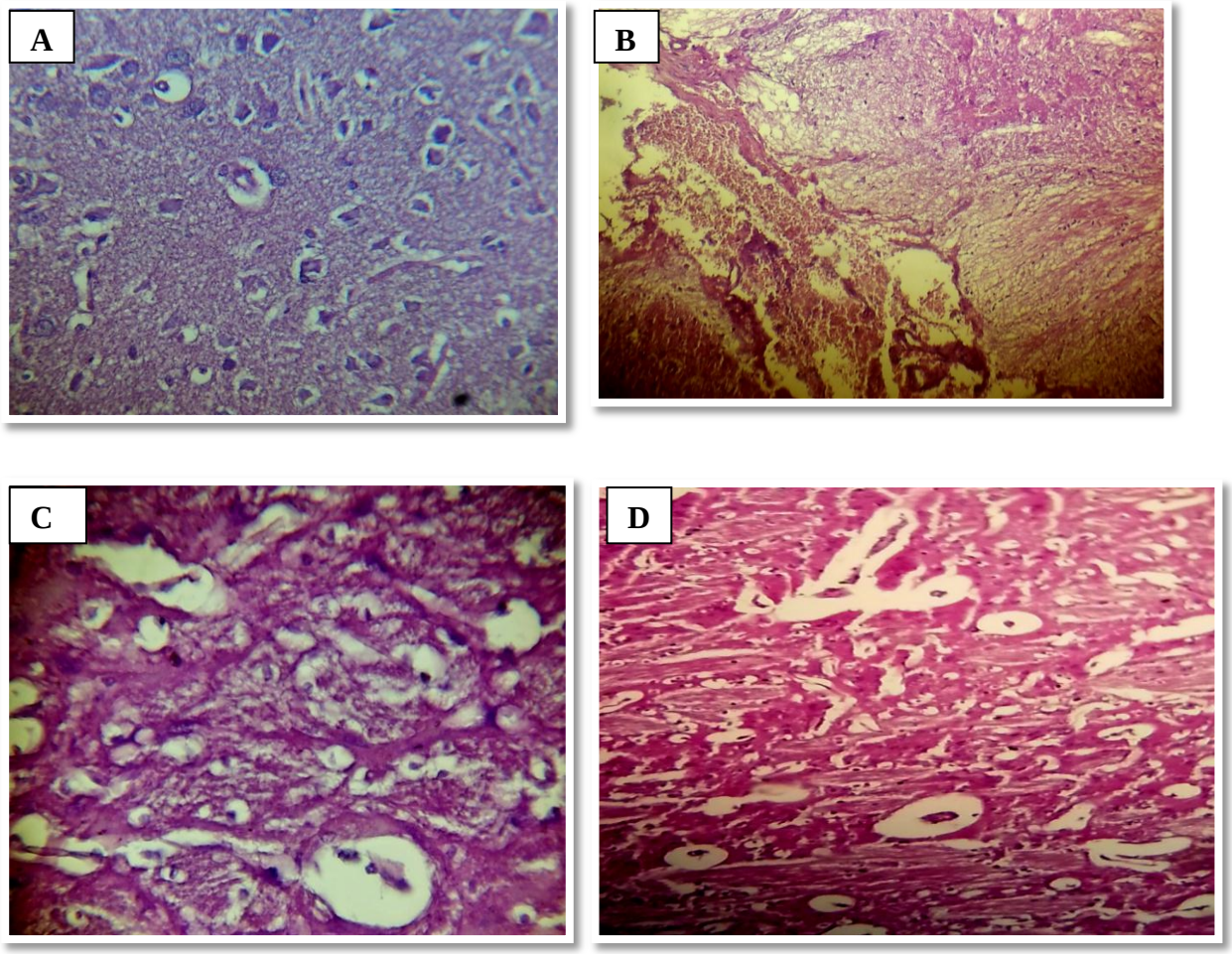

Figure (3): Photomicrograph represent the histopathological changes in rats. A: section of rat brain shows the normal architecture; B: cerebral section with moderate injury showed hemorrhage; C: cerebral section with severe injury showed necrosis (infarct foci)& hemorrhage; D: cerebral section in amlodipine treated group showed slight injury (eosinophilic neurons).Sections stained with H&E (X40).

Discussion:

Transient cerebral ischemia-reperfusion (IR) injury is a major complication in stroke, recovery, and perioperative period, in which 50–70% of survivors suffer from severe disabilities[34].Ischemia and reperfusion in the brain, as in other organs, induces an inflammatory response which may exacerbate initial levels of tissue injury [13].This inflammatory up-regulation occurs, anywhere from minutes to days after the ischemic event [35]

Effect of Global Cerebral I/R on Intercellular adhesion molecule-1 (ICAM-1)

In the present study a significant increase in Intercellular adhesion molecule-1 (ICAM-1) level ($P < 0.05$) was found in the I/R rats as compared with sham group. The results in the present study are in agreement with those reported by Staunton et al. (1988)[36] who found that transient cerebral ischemia induces expression of ICAM-1 as a homodimer on the membrane of inflamed cerebral endothelial cells. And Soriano et al. (1996)[37] showed that neutrophil adhesion in ischemic areas may be deleterious and that ICAM-1 deficiency reduces neurological damage after transient focal cerebral ischemia,also Connolly et al. (1996) & Kitagawa et al. (1998)[38,39] showed that ICAM-1-deficient mice have

smaller infarcts compared to wild-type mice following focal cerebral ischemia .

Effect of Global Cerebral Ischemia Reperfusion Injury on Brain Histopathology

There was statistically significant difference between control group and normal sham group ($P < 0.05$) . The score of the control group shows sever cerebral injury and moderate injury. Shah et al. (2005)[30] showed that in MCA/BCA occlusion for (30 min.) and then following reperfusion for (1 hour.), caused marked congestion of blood vessels**.**These effects were further augmented following reperfusion i.e.lymphocytic proliferation and neuronal necrosis.Chandrashekhar et al. (2010)[33] confirmed that the global cerebral ischemia on Sprague–Dawley rats by bilateral carotid artery (BCA) occlusion for 30 min followed by 1 hour reperfusion caused marked congestion of blood vessels and neutrophil infiltration and neuronal necrosis**.**

The Effect of treatment on Study Parameter

Effect of Amlodipine on Inflammatory Marker (ICAM-1)

We found that pretreatment with amlodipine for (10) days before cerebral ischemia result in significant ($p<0.05$) decrease in inflammatory mediator,such as intercellular adhesion molecule-1,ICAM-1.Yoshii et al. (2006)[23] showed regression of atherosclerosis, in apolipoprotein E–deficient(ApoEKO) mice by amlodipine through inhibitory actions on oxidative stress, inflammation and the production of adhesive molecules ,and found mice that were treated with amlodipine suppressed in ICAM-1**.**

Effect of Amlodipine on Brain Histopathology

In the present study the pretreatment with amlodipine for (10) days before cerebral ischemia caused improves the brain injury significantly ($P < 0.05$) as compared with control (induced untreated) group. The score of the control group shows sever cerebral injury while the score of amlodipine treated group shows normal and mild injury **.** That confirmed results reached by Halici et al. (2008)[40] who evaluated the effects of amlodipine as an antioxidant and analyze the histopathologic changes in experimental ischemic and ischemic-reperfusion (I/R) injury in rat ovaries,and their results showed that conservative treatment with amlodipine is effective in reducing tissue damage induced by ischemia, ischemic-reperfusion (I/R), or both in ovaries. Mogi et al. (2006)[26] found that amlodipine treatment reduces stroke size and neurologic deficit after focal brain ischemia, possibly through an increase in cerebral blood flow and inhibition of superoxide production.

References

1. Tran J, Mirzaei M, Anderson L, Leeder SR. *The epidemiology of stroke in the Middle East and North Africa.* J Neurol Sci. 2010 Aug 15;295(1-2):38-40.

2. American Heart Association: *Heart Disease and Stroke Statistics – 2005 Update* Dallas, Texas: American Heart Association; 2005.

3. Nedergaard, M., Diemer, N.H., 1988. *Experimental cerebral ischemia: barbiturate resistant increase in regional glucose utilization.* Journal of Cerebral Blood Flow& Metabolism 8, 763–766.

4. Neigh, G.N., Glasper, E.R., Kofler, J., Traystman, R.J., Mervis, R.F., Bachstetter, A.,DeVries, A.C., 2004.*Cardiac arrest with cardiopulmonary resuscitation reduces dendritic spine density in CA1 pyramidal cells and selectively alters acquisition of spatial memory*. Eur. J. Neurosci. 20, 1865–1872.

5. White, B.C., Grossman, L.I., Krause, G.S., 1993. *Brain injury by global ischemia and reperfusion: a theoretical perspective on membrane damage and repair.* Neurology 43, 1656–1665

6. Bernard SA, Gray TW, Buist MD, et al. *Treatment of comatose survivors of out-ofhospital cardiac arrest with induced hypothermia*. N Engl J Med 2002;346:557–63.

7. Salazar JD, Wityk RJ, Grega MA, et al. *Stroke after cardiac surgery: short- and long-term outcomes*. Ann Thorac Surg 2001;72:1195–201.
8. Nussmeier NA. *A review of risk factors for adverse neurologic outcome after cardiac surgery*. J Extra Corpor Technol 2002;34:4–10.
9. Dirnagl U, Iadecola C, Moskowitz MA. *Pathobiology of ischaemic stroke: an integrated view*. Trends Neurosci. (1999) 22, 391–397.
10. Woodruff TM, Thundyil J, Tang SC, Sobey CG, Taylor SM, Arumugam TV. *Pathophysiology, treatment, and animal and cellular models of human ischemic stroke.* Mol Neurodegener. 2011 Jan 25;6(1):11.
11. del Zoppo GJ. *Microvascular changes during cerebral ischemia and reperfusion.* Cerebrovasc Brain Metab Rev 1994;6:47–96.
12. Hacke W, Schwab S, Horn M, Spranger M, De Georgia M, von Kummer R. '*Malignant' middle cerebral artery territory infarction: clinical course and prognostic signs*. Arch Neurol 1996;53:309–315.
13. Lee JM, Grabb MC, Zipfel GJ, Choi DW. *Brain tissue responses to ischemia.* J Clin Invest. 2000 Sep;106(6):723-31.
14. Amantea D, Nappi G, Bernardi G, Bagetta G, Corasaniti MT. *Post ischemic brain damage: pathophysiology and role of inflammatory mediators.* FEBS J. 2009 Jan;276(1):13-26.
15. Kriz J (2006) *Inflammation in ischemic brain injury: timing is important.* Crit Rev Neurobiol 18, 145–157.
16. Yilmaz G, Granger DN: *Cell adhesion molecules and ischemic stroke. Neurol Res* 2008, 30:783-93.
17. Ehrensperger E, Minuk J, Durcan L, Mackey A, Wolfson C, Fontaine AM, Cote R (2005*) Predictive value of soluble intercellular adhesion molecule-1 for risk of ischemic events in individuals with cerebrovascular disease*. Cerebrovasc Dis 20:456-462.
18. Selakovic V, Colic M, Jovanovic M, Raicevic R, Jovicic A. *Cerebrospinal fluid and plasma concentration of soluble intercellular adhesion molecule 1, vascular cell adhesion molecule 1 and endothelial leukocyte adhesion molecule in patients with acute ischemic brain disease.* Vojnosanit Pregl2003;60:139–146.
19. Simundic AM, Basic V, Topic E, Demarin V, Vrkic N, Kunovic B, Stefanovic M, Begonja A (2004) *Soluble adhesion molecules in acute ischemic stroke*. Clin Invest Med 27:86-92.
20. Steffen H.-M.. *Amlodipine – a third generation dihydropyridine calcium antagonist. J Clin Basic Cardiol 1999; 2: 45.*
21. Abernethy DR, Schwartz JB. *Calcium-antagonist drugs.* N Engl J Medicine 1999; 341: 1447-57.
22. Michel T. *in Godman and Gilman's the pharmacological basis of therapeutics*. (ed. Brunton, L.) Mc Graw-Hill, New-York, 2006. pp.832-8.
23. Yoshii T, Iwai M, Li Z, Chen R, Ide A, Fukunaga S, Oshita A, Mogi M, Higaki J, Horiuchi M. *Regression of atherosclerosis by amlodipine via anti-inflammatory and anti-oxidative stress actions.* Hypertens Res. 2006 Jun;29(6):457-66.
24. Liu LL, Li QX, Xia L, Li J, Shao L. *Differential effects of dihydropyridine calcium antagonists on doxorubicininduced nephrotoxicity in rats*. Toxicology 2007;231:81-90.
25. Umemoto S, Tanaka M, Kawahara S, Kubo M, Umeji K, Hashimoto R et al. *Calcium antagonist reduces oxidative stress by upregulating Cu/Zn superoxide dismutase in stroke-prone spontaneously hypertensive rats*. Hypertens Res 2004; 27: 877-85.
26. Mogi M, Iwai M, Chen R, Iwanami J, Ide A, Tsukuda K, Yoshii T, Horiuchi M. *Amlodipine treatment reduces stroke size in apolipoprotein E-deficient mice.* Am J Hypertens. 2006 Nov;19(11):1144-9.
27. Gross GJ, Farber NE, Pieper GM. *Effects of amlodipine on myocardial ischemia-reperfusion injury in dogs.* Am J Cardiol. 1989 Nov 7;64(17):94I-100I.
28. Toklu .H, Deniz . M, Yüksel .M, Keyer-Uysal.M, Şener.G .*The protective effect of melatonin and amlodipine against cerebral ischemia/ reperfusion –induced oxidative brain injury in rats.*Marmara Medical Journal 2009;22(1);034-044
29. Farbiszewski R, Bielawski K, Bielawska A, Sobaniec W. *Spermine protects in vivo the antioxidant enzymes in transiently hypoperfused rat brain*. Acta Neurobiol Exp (Wars). 1995;55(4):253-8.
30. Shah ZA, Gilani RA, Sharma P, Vohora SB. *Cerebroprotective effect of Korean ginseng tea against global and focal models of ischemia in rats.* J Ethnopharmacol. 2005 Oct 3;101(1-3):299-307.
31. Lin TN, He YY, Wu G, Khan M, Hsu CY. *Effect of brain edema on infarct volume in a focal*

cerebral ischemia model in rats. Stroke. 1993 Jan;24(1):117-21.

32. Famakin B, Mou Y, Spatz M, Lawal M, Hallenbeck J. *Downstream Toll-like receptor signaling mediates adaptor-specific cytokine expression following focal cerebral ischemia.* J Neuroinflammation. 2012 Jul 16;9:174.

33. Chandrashekhar VM, Ranpariya VL, Ganapaty S, Parashar A, Muchandi AA. *Neuroprotective activity of Matricaria recutita Linn against global model of ischemia in rats.* J Ethnopharmacol. 2010 Feb 17;127(3):645-51.

34. Konig M., M. Klotz, L. Heuser et al., "*Perfusion CT in acute stroke: characterization of cerebral ischemia using parameter images of cerebral blood flow and their therapeutic relevance clinical experience,"* Electromedia, vol. 66, pp. 61–67, 1998.

35. Anthony L. D'Ambrosio, David J. Pinsky, and E. Sander Connolly. The *Role of the Complement Cascade in Ischemia/Reperfusion Injury:Implications for Neuroprotection* . Molecular Medicine 7(6): 367–382, 2001.

36. Staunton DE, Marlin SD, Stratowa C, Dustin ML, Springer TA (1988) *Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families*. Cell 52:925-933.

37. Soriano SG, Lipton SA, Wang YF, Xiao M, Springer TA, Gutierrez-Ramos JC, Hickey PR. *Intercellular adhesion molecule-1-deficient mice are less susceptible to cerebral ischemia-reperfusion injury.* Ann Neurol. 1996 May;39(5):618-24.

38. Connolly ES Jr, Winfree CJ, Springer TA, Naka Y, Liao H, Yan SD,Stern DM, Solomon RA, Gutierrez-Ramos J-C, Pinsky DJ. *Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion. Role of neutrophil adhesion in the pathogenesis of stroke. J Clin Invest*. 1996;97:209 –216.

39. Kitagawa K, Matsumoto M, Mabuchi T, Yagita Y, Ohtsuki T, Hori M, Yanagihara T(1998) *Deficiency of intercellular adhesion molecule 1 attenuates microcirculatory disturbance and infarction size in focal cerebral ischemia.* J Cereb Blood Flow Metab 18:1336-1345.

40. Halici Z, Karaca M, Keles ON, Borekci B, Odabasoglu F, Suleyman H, Cadirci E, Bayir Y, Unal B. *Protective effects of amlodipine on ischemia-reperfusion injury of rat ovary: biochemical and histopathologic evaluation.* Fertil Steril. 2008 Dec;90(6):2408-15.