Cerebral Ischemia-Reperfusion Injuries in Vanadyl-Treated Diabetic Rats

Hossein Ahmadi-Eslamloo, PhD; Seyed Mostafa Shid Moosavi, PhD; Gholam Abbas Dehghani, PhD

Department of Physiology, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence: Gholam Abbas Dehghani, PhD; Department of Physiology, School of Medicine, Zand Blvd., Shiraz, Iran
Tel: +98 917717966
Fax: +98 71 32302026
Email: dehghang@sums.ac.ir
Received: 09 January 2017
Revised: 03 April 2017
Accepted: 09 April 2017

Abstract

Background: Ischemic stroke recovery is poor in diabetic mellitus (DM). Vanadium compounds (vanadium) relieve DM signs, but their influences on cerebral ischemia/reperfusion injury (I/RI) are inconclusive. Herein, the intensity of I/RI was inspected in vanadium-treated DM rats.

Methods: Rats made diabetic with a single intravenous dose of streptozocin (39 mg/kg). Normal and DM rats used water or vanadyl solution for 45 days. Under isoflurane anesthesia, right middle cerebral artery occlusion was performed for 60 minutes and 12 hours reperfusion. Ischemic rats were divided into untreated-control normal (ICN) and diabetic (ICD), vanadium-treated normal (IVTN) and diabetic (IVTD) groups (n=14 each). After neurological deficit score (NDS) test, the rats were sacrificed and their brain removed and stained with triphenyltetrazolium chloride (TTC) to measure cerebral infarct volume (CIV, mm$^3$) or Evans blue extravasation (EBE, μg/g wet-tissue). Data analysis was performed using one-way ANOVA and Tukey’s test (SPSS software, version 21.0) and P values <0.05 were considered statistically significant.

Results: Blood glucose (BG, mg/dL) was similar in ICN and IVTN, elevated in IVTD and ICD (245±6 vs. 344±2, P<0.001). The increased CIV in ICN and IVTN was similar (48±2 and 34±5), very high in ICD but lower in IVTD (249±37 vs. 110±16, P<0.001). EBE was absent in non-lesioned hemispheres, similarly increased in lesioned hemispheres of ICN and IVTN (14±1 and 13±1). EBE in IVTD was significantly lower than ICD (21±2 vs. 33±5, P=0.01).

Conclusion: I/RI was moderate in normoglycemia and did not change with vanadium. Hyperglycemia robustly intensified I/RI. Vanadium ameliorated hyperglycemia and reduced I/RI. Nonetheless, more investigations are required to link the mechanisms of vanadium on DM and stroke injuries.

Keywords ● Diabetes mellitus ● Cerebrum ● Ischemia/Reperfusion ● Injury ● Vanadium ● Rat

Introduction

Diabetic mellitus (DM) is a metabolic disorder with long-term complications.1 Hyperglycemia, the outcome of DM, is a well-known risk factor and may reduce the chance of recovery if present during ischemic stroke.2,3 The deleterious influence of hyperglycemia on the ischemic stroke injury usually comes with
higher mortality and slower recovery.\textsuperscript{4,5} Animal studies have reiterated that the alleviation of hyperglycemia with insulin therapy would reduce the potency of stroke injury in DM.\textsuperscript{6,7} Vanadium compounds (vanadium) are heavily investigated for their insulin-like activities in diabetic patients and diabetic rats.\textsuperscript{8,9} Vanadium has been found to improve the abnormalities of carbohydrate and lipid metabolism in isolated tissues taken from DM patients or animals.\textsuperscript{10,11} Although the anti-diabetic effects of vanadium compounds are well documented,\textsuperscript{11} data about its beneficial influences in reducing stroke injury in diabetes conditions are insufficient or almost missing.

Literature indicates that the control of glycemic status in diabetic animals has prevented further damages that are usually initiated in ischemic stroke.\textsuperscript{12} In this condition, one may hypothesize that vanadium would indirectly alleviate hyperglycemia and the outcome would be a reduction of acute ischemic brain injury in DM rats. Therefore, in the present study, we attempted to elucidate the intervention of hyperglycemia with brain injuries initiated with 60 minutes MCA occlusion followed by 12 hours reperfusion in chronic DM rats. Again, the same procedure was used in 45-day vanadyl treated normal and diabetic rats and the outcome of I/RI that occur during an acute stroke was investigated. The results would shed some light on the contribution of vanadium due to its insulin-like activity in reviving the injuries that occur during acute focal cerebral ischemia in acute or chronic diabetic rats.

Materials and Methods

Animals

Male adult Sprague-Dawley rats (180-210 g) were obtained from the animal house of Shiraz University of Medical Sciences, Shiraz, Iran. All procedures were followed according to the guidelines of the Institutional Animal Ethics Committee of Shiraz University of Medical Sciences (Shiraz, Iran). The animals were housed in separate cages under controlled room temperature (22-24 °C), humidity (40-60%), 12-h light/dark cycle, and had access to rat chow (Pars Dam, Iran) and water.

Fluid Solutions

The drinking water (base solution) contained 3 g/L NaCl in distilled water to overcome natruresis in diabetic rats.\textsuperscript{9} Vanadyl solution contained 0.1 to 0.5 mg/mL vanadyl sulfate (VOSO$_4$+5H$_2$O, Merck, Germany) dissolved in the base solution. The concentration of vanadyl solution, depending on the daily fluid consumption, was chosen such that each animal receives the same amount of vanadium (mgkg$^{-1}$day$^{-1}$). All solutions were freshly prepared every 3-5 days and stored in a dark, cold room (4 °C) until use.

Routine Measurements

Daily Fluid Consumption: The type of bottle used for drinking solutions was virtually dripless. Thus, the measured bottle volume specified the amount of fluid used by each animal.

Blood Samples: The animals were lightly anesthetized with anesthetic ether (Merck, Germany). One drop of blood was collected from the tip of cut tail and the blood glucose (BG) was measured with glucose monitoring system (Glucocard 01-Mini, Japan).

Body Weight: The body weight measurements were done daily after each maneuver and then in 10 days interval until the completion of treatments.

Arterial Systolic Blood Pressure: The tail cuff holder and Power Lab system (AD Instrument, Australia) were used to measure arterial systolic pressure (ASBP) noninvasively.

Induction of Diabetes and Animal Care

Diabetes mellitus was induced by a single intravenous injection of 39 mg/kg freshly prepared streptozotocin solution (STZ, Sigma) in normal saline through the lateral tail vein. Diabetes was confirmed 48-72 hours after STZ injection by observing hyperglycemia (BG 300-350 mg/dL), polydipsia (fluid intake ≥100 mL/day), and polyuria (wet-cage). The normal animals (control) received the same volume of normal saline. The rats were kept in separate cages and treated accordingly.

Surgical Procedures

The animals fasted overnight. Anesthesia was conducted through a face mask with isoflurane (15-30% in 30% oxygen and 70% nitrous oxide). Core temperature was recorded during surgery, ischemia, and the first 15 minutes of reperfusion period. The temperature was kept at 37±1 °C with a heating lamp or an ice bag.

Regional Cerebral Blood Flow (rCBF) Measurements

A laser Doppler electrode was placed on the surface of the right side of the skull and connected to a flow meter (model ML191, AD Instrument, Australia) and tested as described previously.\textsuperscript{13} The rCBF trace was continuously recorded during each maneuver.\textsuperscript{13}
**Induction of Focal Cerebral Ischemia**

Transient focal cerebral ischemia was induced according to the method of Longa; as modified by Vakili et al. The occlusion of MCA was confirmed by observing a sharp decline in the rCBF trace (75-80%) of the right cranium. Reperfusion started 60 minutes after MCA occlusion by gently pulling out the thread. Then, all instruments were removed, incisions sutured, and the animal was returned to a warm cage for 12 hours recuperation.

**Evaluation of Neurological Deficit Score (NDS) Test**

Rats that survived the anesthesia, neck surgery, 60 minutes cerebral ischemia, and 12 hours reperfusion period were prepared for NDS test and blindly evaluated 12 hours after neck surgery in the sham group or 12 hours after MCA reopening in the ischemic group by a five-point NDS test as described previously.

**Measurements of Cerebral Infarct Volume (CIV)**

CIV was measured according to the method of Swanson et al. After the NDS test, rats were deeply anesthetized with intraperitoneal injection of chloral hydrate and sacrificed by guillotine. The brain was removed, cleaned, washed, and solidified in cold saline solution (4 °C). The prepared coronal sectioned slices were stained with 2% TTC (2,3,5-triphenyltetrazolium chloride, Sigma) fixed in 10% buffer formalin solution. The brain was removed, cleaned, washed, and solidified in cold saline solution (4 °C). The prepared coronal sectioned slices were stained with 2% TTC (2,3,5-triphenyltetrazolium chloride, Sigma) fixed in 10% buffer formalin solution. The optical density values of the solutions were measured with a Gen5 microplate reader (BioTek, Germany) at 620 nm wavelength and the results were calculated and reported accordingly.

**Preparation of Animals for Blood-Brain Barrier (BBB) Integrity Evaluation**

Evans blue solution (4 ml/kg, from freshly prepared EB solution contained 2% EB in normal saline, Sigma) was injected through the femoral venous cannula in the sham and ischemic groups. After the evaluation of NDS test, animals were sacrificed under deep chloral hydrate anesthesia. The chest was opened with 2%TTC (2,3,5-triphenyltetrazolium chloride, Sigma) fixed in 10% buffer formalin solution. The brain was removed, cleaned, washed, and solidified in cold saline solution (4 °C). The prepared coronal sectioned slices were stained with 2% TTC (2,3,5-triphenyltetrazolium chloride, Sigma) fixed in 10% buffer formalin solution. The optical density values of the solutions were measured with a Gen5 microplate reader (BioTek, Germany) at 620 nm wavelength and the results were calculated and reported accordingly.

**Evaluation of Brain Edema**

The tissue swelling of the ipsilateral lesioned hemispheres was analyzed accordingly. In this context, as presumed systematically by many investigators, we also assumed that the contralateral (left) hemispheres were not affected by the right MCA occlusion. Subsequently, these calculated values were considered as edema formation that usually occurs in the ischemic hemispheres.

**Experimental Design**

Initially, 4 groups (n=21 each) of normal and DM rats were prepared and treated for 45 days, namely:
- Control normal (CN): Rats used base solution for drinking.
- Vanadyl-treated normal (VTN): Rats used vanadyl sulfate solution orally.
- Control diabetic (CD): Rats used base solution similar to the CN group.
- Vanadium-treated diabetic (VTD): Rats used vanadyl solution similar to the VTN group.

In the VTN and VTD groups, depending on the daily-consumed fluid and rats' body weight, the concentration of vanadyl was adjusted so that each rat received a similar amount of vanadyl sulfate (table 1). After 45 days of treatment, the rats in each group were further subdivided into 5 subgroups and the experiment followed accordingly.

1. Sham groups: Seven rats from each group, as mentioned above, were randomly selected for neck surgery. The right common carotid artery without MCA occlusion was exposed to perform TTC staining or EB extravasation technique as described previously.
   - Vanadium-treated diabetic (VTD): Rats used vanadyl solution similar to the VTN group.

2. Ischemic control normal group (ICN, n=14): The first part of the surgical procedure was similar to the sham group. Then, acute cerebral ischemia was induced by 60 minutes right MCA occlusion followed by 12 hours reperfusion.
3. Ischemic vanadyl-treated normal group (IVTN, n=14): The surgical procedures for the induction of acute cerebral ischemia were exactly similar to the ICN group.
4. Ischemic control diabetic group (ICD, n=14): The procedure for the induction of acute cerebral ischemia was exactly similar to the ICN group.
5. Ischemic vanadyl-treated diabetic group (IVTD, n=14): The neck surgery and induction of acute cerebral ischemia were exactly similar to the ICD groups.

**Statistical Analysis**

All values are presented as means±SEM. The analysis was performed using the SPSS software (version 21.0). One way ANOVA with Turkey’s post hoc test was used to evaluate changes that occurred during the 45-day of the
Stroke injuries in vanadium-treated diabetic rats

Experiment in BG, daily drinking water, daily vanadyl consumption, body weight, ASBP, rCBF, CIV, and BBB permeability in the sham and ischemic groups. The Kruskal-Wallis H test was also used to assess variations observed in the NDS test. Overall, P<0.05 was considered statistically significant.

Results

The overall survival rates of the sham groups were 100%. The survival rate in the ICN, IVTN, and IVTD groups were 90%, but it was 60% in the ICD group. The data presented in table 1 are confirmed by earlier reports.21,22 VTN and VTD rats statistically used the same amount of vanadyl (25.8±2.1 and 27.4±2.8 mg kg⁻¹ day⁻¹, respectively). Oral vanadyl did not alter BG in VTN rats, but significantly reduced the elevated BG of the VTD groups (P<0.001). While the induction of diabetes alone significantly elevated ASBP (table 1), but vanadyl did not affect ASBP in the VTN or VTD groups. The overall variations of body weight growth, as presented in table 1, indicated that STZ-diabetes significantly reduced the body weight, but vanadyl VTD rats significantly elevated the body weight (P<0.001).

Regional Cerebral Blood Flow

The alterations of rCBF (percentage from baseline) during surgery in the sham and ischemic rat groups are presented in figure 1. Sixty minutes MCA occlusion similarly reduced the rCBF of lesioned side (75-85%) of the 4 ischemic groups. MCA reopening swiftly returned rCBF back to its pre-occluded levels.

Neurological Disability Test

Rats that survived anesthesia, neck surgery (sham), 60 minutes cerebral ischemia, and 12 hours reperfusion (ischemic groups) were prepared for the NDS test. The NDS results of the sham and ischemic groups are presented in figure 2. It shows that NDS in the ICN and IVTN groups were significantly lower than the ICN group (P<0.001) and vanadium therapy did not affect the NDS of diabetic rats.

Cerebral Infarction Volume

A sample photograph of coronal sections of the rat brain stained with TTC is shown in figure 3. The evenly dark red color of the slices

Table 1: Blood glucose, body weight, daily water consumption (water), daily vanadyl consumption (vanadyl), arterial systolic blood pressure (ASBP) of rats treated for 45 days (n=19)

| Group   | Blood glucose (mg/dL) | Body weight (g) | Water (mL/day) | Vanadyl (mg/kg) | ASBP (mmHg) |
|---------|-----------------------|-----------------|----------------|-----------------|-------------|
| Day 10  | 85±4                  | 84±3            | 83±2           | 80±2            | 185±4       |
| Day 20  | 84±3                  | 82±8            | 81±3           | 81±2            | 189±4       |
| Day 30  | 315±12***     | 321±11***   | 332±9***       | 344±2***       | 189±3       |
| Day 45  | 317±4***     | 311±3***   | 269±13±***     | 245±6±***      | 192±6       |
| Data are presented as mean±SEM. CN: Control normal; VTN: Vanadyl-treated normal; CD: Control diabetic; VTD: Vanadyl-treated diabetic; ***P<0.001 versus CN; *P<0.05 versus CN; †P<0.001 VTD versus VTN; ‡P<0.001 VTD versus CD
from both hemispheres of sham rats reinstated that anesthesia and neck surgery did not induce cerebral injury. The appearance of white color together with dark reddish color in the right hemispheres of ischemic rats indicates that 60 minutes MCA occlusion had induced various degrees of cerebral infarctions, while this maneuver did not affect the left hemispheres.

The quantitative values of infarct size of the lesioned hemispheres of the ischemic groups are presented in figure 4. Their comparisons indicated that the infarct size in the ICN and IVTN groups were low and not significantly different from each other (P=0.98). However, the significant increases observed in the infarct size of ICD rats were substantially lower in IVTD (P<0.001).

**Blood Brain Barrier (BBB) Permeability**

A photograph of the rats’ brain that received an intravenous infusion of EB is shown in figure 5. The absence of blue color on the surface of both hemispheres of the sham group and the left (non-lesioned) hemispheres of the ischemic group indicated that anesthesia, neck surgery, or right MCA occlusion did not affect the integrity of cerebral vasculature of the left sides of the ischemic groups. Similarly, the quantitative EB concentrations of these hemispheres were close to zero (figure 6). EB concentrations of the lesioned hemispheres of both groups of ICD and IVTD were significantly higher than the non-diabetic groups (figure 6). Figure 6 shows that
 Stroke injuries in vanadium-treated diabetic rats

Long-term vanadium therapy has helped type 1 diabetic patients to have a better control over their blood glucose and daily insulin injection. Oral vanadium in chronic DM rats has reduced the damages arising in the myocardium and kidney induced by hyperglycemia. Extensive investigations performed in recent years have ratified that soon vanadium would be used as a new antidiabetic drug for relieving hyperglycemia and its complicated signs in chronic DM patients. Although our information about the functional activity of vanadium in protecting peripheral organs obtained from chronic diabetic patients and DM animals is adequate, we have little knowledge about the insulin-like actions of vanadium in the recovery of brain injury after acute ischemic stroke. Therefore, prior to the use of vanadium as a conventional antidiabetic drug, learning its benefits and harms in diabetic patients with high risk of stroke deserves extensive investigations.

In rats, the right middle cerebral artery regularly nourishes the lateral surface of the cortical and subcortical areas of the right hemisphere. This area mainly participates in the process of sensorimotor information that helps the balancing abilities of the animal during standing or moving. Severe reduction of blood flow to this region could seriously damage neurons and their synaptic electrophysiological information originated from or proceeding through this region and consequently would impair the balancing capability of the animal. For this reason, investigators traditionally use the method of right MCA occlusion/reopening to evaluate post-ischemic recovery scores, similar to what happens in human, to find out the symptoms of ischemic stroke after recovery at various conditions. Hence, evaluation of NDS test would help to judge the degree of stroke injury and its post-ischemic recovery in living conditions.

In the present study, evaluation of NDS test was done blindly by a person who was not aware of rats’ conditions. NDS in the sham groups indicated that the injection of STZ, oral vanadyl therapy, anesthesia, and neck surgery did not restrict the balancing ability and free movements of the rats. Whereas, comparison of NDS in the ICN and ICD groups indicated that hyperglycemia profoundly impaired motoneuron functions and post-ischemic recovery in the ICD group (figure 3). This notion is confirmed by some clinical and experimental reports retreating that hyperglycemia highly sensitizes the brain to stroke injury. It is worth noticing that while vanadium therapy improved

even though EB concentration in the IVTD group is significantly lower than the ICD group (P=0.01), but this reduction is still significantly higher than either of the ICN or IVTN groups (P<0.001).

Brain Edema

The calculated tissue swelling (edema) of the lesioned hemispheres of ischemic rats is presented in figure 7. Although the variations of brain edema in the ICN and IVTN groups were not statistically significantly different from each other, it was extensively elevated in diabetes groups (figure 7). Brain edema of the IVTD group was significantly lowered compared to the ICD group (figure 7, P=0.01), but still was significantly higher than the obtained edema in the ICN or IVTN group (figure 7, P<0.001).

Discussion

The results show that 60 minutes occlusion of the MCA in the presence of hyperglycemia intensified cerebral infarction, BBB disruption, and edema in the ICD group (Figures 4, 6, and 7). In contrast, vanadium ameliorated hyperglycemia significantly lowered the cerebral infarct size, BBB disruption, and edema in the IVTD group (Figures 4, 6, and 7). These results are endorsed by previous reports stating that improved hyperglycemia, due to insulin-like actions of vanadium, helped the recovery of the damaged brain from ischemia. Nonetheless, our data could not specify if the improved signs of acute ischemic brain injury and better survival rates observed in IVTD rats were due to direct insulin-like activity of vanadium, amelioration of hyperglycemia, or both. Based on these results, we are unable to explain how vanadium revived DM rats from stroke injury.

Figure 7: Different levels of edema are seen in the right (lesioned) hemisphere of the 4 ischemic groups, being highest in ICD rats. The calculated brain edema (%) are presented as Mean±SEM. ICN: Ischemic control normal; IVTN: Ischemic vanadyl-treated normal; ICD: Ischemic control diabetic; IVTD: Ischemic vanadyl-treated diabetic. ***P<0.001 versus ICN; *P<0.05 IVTD versus IVTN.
hyperglycemia, reduced cerebral infarct size, partially diminished BBB disruption and edema, it is not clear why post-ischemic recovery scores were not improved in the IVTD group.

The blood-brain barrier is a diffusion barrier segregating the central nervous system from the systemic circulation. The foremost function of BBB is to limit paracellular movement of solutes, ions, and water to modulate the intracellular and extracellular signaling pathways. The occurrence of BBB disruption during ischemic stroke and subsequent reperfusion increases vascular-derived substances into the brain. The destruction and/or dysfunction of brain cells are the ultimate outcome of ischemic stroke leading to neurological deficits.

The EB extravasation technique is used to precisely evaluate the intensity of BBB disruption after cerebral ischemia. Regional measurement of EB extravasation would precisely evaluate the intensity of BBB disruption in focal cerebral ischemia. The results of the present study indicated that hyperglycemia highly sensitized the brain of chronic DM rats to acute ischemia-induced with 60 minutes of MCA occlusion. EBE was greatest in the presence of hyperglycemia in ICD compared to normoglycemic rats of the ICN group (Figure 5). At the same time, amelioration of hyperglycemia during vanadium therapy extensively reduced EBE in the IVTD rats compared to ICD group (Figures 5 and 6).

Cerebrovascular endothelial cells are normally involved in the regulation of transcapillary fluid movement. The inability of endothelial microvascular beds, known as BBB disruption, develops brain edema during acute ischemic stroke. The results of the present study indicated that hyperglycemia exceedingly intensified ischemic BBB breakdown (figure 6) and augmented brain edema (figure 7) in the ICD group. This has the support of other investigators who illustrated that hyperglycemia directly modifies the sensitivity of endothelial cells to stroke. Conversely, the improved hyperglycemia in response to the insulin-like activity of vanadium in diabetic rats of the IVTD group diminished BBB disruption (figure 6) and brain edema (figure 7). At present, due to the lack of literature, we can only conclude that diminished cerebral infarction (figure 4), less BBB breakdown (figure 6), and reduced amounts of brain edema (figure 7) observed in vanadyl-treated diabetic rats were directly due to diminished hyperglycemia. Nevertheless, further investigation is required to differentiate the exact mechanisms of vanadium therapy in the prevention of ischemic stroke injury.

Conclusion

These results demonstrated that oral vanadyl pretreatment of normoglycemic rats per se did not alter the extent of acute stroke injury. The concurrence of diabetes with ischemic stroke, occurring during IR injury, degraded blood brain integrity, induced edema, intensified stroke symptoms, and mortality rate. Nonetheless, stroke rehabilitation requires extensive investigations to identify the medications involved in reviving hyperglycemia. The outcome would lessen post-ischemic stroke symptoms in DM patients.

Acknowledgment

The authors cordially appreciate the financial support from the Vice Chancellor for Research of Shiraz University of Medical Sciences (grant number: 926730). This article was part of a thesis written by Hossein Ahmadi-Eslamloo, a Ph.D. student in physiology.

Conflict of Interest: None declared.

References

1. Yanardag R, Tunali S. Vanadyl sulfate administration protects the streptozotocin-induced oxidative damage to brain tissue in rats. Mol Cell Biochem. 2006;286:153-9. doi: 10.1007/s11010-005-9107-1. PubMed PMID: 16532257.

2. Lipska K, Sylaja PN, Sarma PS, Thankappan KR, Kutty VR, Vasan RS, et al. Risk factors for acute ischaemic stroke in young adults in South India. J Neurol Neurosurg Psychiatry. 2007;78:959-63. doi: 10.1136/jnnp.2006.106831. PubMed PMID: 17220290; PubMed Central PMCID: PMC2117871.

3. Bruno A, Liebeskind D, Hao Q, Raychev R, Investigators US. Diabetes mellitus, acute hyperglycemia, and ischemic stroke. Curr Treat Options Neurol. 2010;12:492-503. doi: 10.1007/s11940-010-0093-6. PubMed PMID: 20848328; PubMed Central PMCID: PMC2943579.

4. Li PA, Siesjo BK. Role of hyperglycaemia-related acidosis in ischaemic brain damage. Acta Physiol Scand. 1997;161:567-80. doi: 10.1046/j.1365-201X.1997.00264.x. PubMed PMID: 9429666.

5. Gisselsson L, Smith ML, Siesjo BK. Hyperglycemia and focal brain ischemia. J Cereb Blood Flow Metab. 1999;19:288-97. doi: 10.1097/00004647-199903000-00007.
Stroke injuries in vanadium-treated diabetic rats

6. Lukovits TG, Mazzone TM, Gorelick TM. Diabetes mellitus and cerebrovascular disease. Neuroepidemiology. 1999;18:1-14. PubMed PMID: 9831810.

7. Hung LM, Huang JP, Liao JM, Yang MH, Li DE, Day YJ, et al. Insulin renders diabetic rats resistant to acute ischemic stroke by arresting nitric oxide reaction with superoxide to form peroxynitrite. J Biomed Sci. 2014;21:92. doi: 10.1186/s12929-014-0092-0. PubMed PMID: 25223305; PubMed Central PMCID: PMCPMC4266964.

8. Soveid M, Dehghani GA, Omrani GR. Long-term efficacy and safety of vanadium in the treatment of type 1 diabetes. Arch Iran Med. 2013;16:408-11. doi: 013167/AIM.009. PubMed PMID: 23808778.

9. Dehghani GA, Sotoodeh M, Omrani GR. Trophic effects of vanadium on beta-cells of STZ-induced insulin dependent diabetic rats & evidence for long-term relief of diabetes mellitus. Indian J Med Res. 1999;110:70-5. PubMed PMID: 10573657.

10. Mehdi MZ, Pandey SK, Theberge JF, Srivastava AK. Insulin signal mimicry as a mechanism for the insulin-like effects of vanadium. Cell Biochem Biophys. 2006;44:73-81. doi: 10.1385/CBB:44:1:073. PubMed PMID: 16456236.

11. Srivastava AK, Mehdi MZ. Insulino-mimetic and anti-diabetic effects of vanadium compounds. Diabet Med. 2005;22:2-13. doi: 10.1111/j.1464-5491.2004.01381.x. PubMed PMID: 15606684.

12. Li PA, Gisselsson L, Keuker J, Vogel J, Smith ML, Kuschinsky W, et al. Hyperglycemia-exaggerated ischemic brain damage following 30 min of middle cerebral arterial occlusion is not due to capillary obstruction. Brain Res. 1998;804:36-44. PubMed PMID: 9729262.

13. Mohammadi MT, Shid-Moosavi SM, Dehghani GA. Contribution of nitric oxide synthase (NOS) in blood-brain barrier disruption during acute focal cerebral ischemia in normal rat. Pathophysiology. 2012;19:13-20. doi: 10.1016/j.pathophys.2011.07.003. PubMed PMID: 21852076.

14. Vakili A, Nekoeoeian A, Dehghani G. L-NNAME and 7-Nitroindazole reduces brain injuries in transient focal cerebral ischemia in rat. Iranian Journal of Medical Sciences. 2015;29:109-15.

15. Hoda MN, Li W, Ahmad A, Ogbi S, Zemskova MA, Johnson MH, et al. Sex-independent neuroprotection with minocycline after experimental thromboembolic stroke. Exp Transl Stroke Med. 2011;3:16. doi: 10.1186/2040-7378-3-16. PubMed PMID: 22177314; PubMed Central PMCID: PMCPMC3289711.

16. Swanson RA, Morton MT, Tao-Wu G, Savalos RA, Davidson C, Sharp FR. A semiautomated method for measuring brain infarct volume. J Cereb Blood Flow Metab. 1990;10:290-3. doi: 10.1038/jcbfm.1990.47. PubMed PMID: 1689322.

17. Qian Y, Tang X, Guan T, Li Y, Sun H. Neuroprotection by Combined Administration with Maslinic Acid, a Natural Product from Olea europaea, and MK-801 in the Cerebral Ischemia Model. Molecules. 2016;21. doi: 10.3390/molecules21081093. PubMed PMID: 27548129.

18. Mohammadi MT, Shid Moosavi SM, Dehghani GA. Contribution of nitric oxide synthase (NOS) activity in blood-brain barrier disruption and edema after acute ischemia/reperfusion in aortic coarctation-induced hypertensive rats. Iran Biomed J. 2011;15:22-30. PubMed PMID: 21725496; PubMed Central PMCID: PMCPMC3639734.

19. McBride DW, Klebe D, Tang J, Zhang JH. Correcting for Brain Swelling’s Effects on Infarct Volume Calculation After Middle Cerebral Artery Occlusion in Rats. Transl Stroke Res. 2015;6:323-38. doi: 10.1007/s12975-015-0400-3. PubMed PMID: 25933988; PubMed Central PMCID: PMCPMC4765329.

20. Mohammadi MT, Dehghani GA. Acute hypertension induces brain injury and blood-brain barrier disruption through reduction of claudins mRNA expression in rat. Pathol Res Pract. 2014;210:985-90. doi: 10.1016/j.prp.2014.05.007. PubMed PMID: 24996562.

21. Dehghani GA, Atapour N, Sotoodeh M, Omrani GR. Document The influence of vanadyl sulphate on islet cells, blood glucose and insulin levels of normal and STZ-induced diabetic rats. Iranian Journal of Medical Sciences. 1992;17:167-72.

22. Pirmoradi L, Mohammadi MT, Safaei A, Mesbah F, Dehghani GA. Does the relief of glucose toxicity act as a mediator in proliferative actions of vanadium on pancreatic islet beta cells in streptozocin diabetic rats? Iran Biomed J. 2014;18:173-80. PubMed PMID: 24842144; PubMed Central PMCID: PMCPMC4048482.

23. Yanardag R, Bolkent S, Karabulut-Bulan O, Tunali S. Effects of vanadyl sulfate on kidney in experimental diabetes. Biol Trace Elem Res. 2003;95:73-85. doi: 10.1385/BTER:95:1:73. PubMed PMID: 14555801.
24. Clark TA, Deniset JF, Heyliger CE, Pierce GN. Alternative therapies for diabetes and its cardiac complications: role of vanadium. Heart Fail Rev. 2014;19:123-32. doi: 10.1007/s10741-013-9380-0. PubMed PMID: 23430125.

25. Bhuiyan MS, Fukunaga K. Cardioprotection by vanadium compounds targeting Akt-mediated signaling. J Pharmacol Sci. 2009;110:1-13. PubMed PMID: 19423951.

26. Trevino S, Velazquez-Vazquez D, Sanchez-Lara E, Diaz-Fonseca A, Flores-Hernandez JA, Perez-Benitez A, et al. Metforminum Decavanadate as a Potential Metallopharmaceutical Drug for the Treatment of Diabetes Mellitus. Oxid Med Cell Longev. 2016;2016:6058705. doi: 10.1155/2016/6058705. PubMed PMID: 27119007; PubMed Central PMCID: PMCPMC4826921.

27. Boden G, Chen X, Ruiz J, van Rossum GD, Turco S. Effects of vanadyl sulfate on carbohydrate and lipid metabolism in patients with non-insulin-dependent diabetes mellitus. Metabolism. 1996;45:1130-5. PubMed PMID: 8781301.

28. Li SH, McNeill JH. In vivo effects of vanadium on GLUT4 translocation in cardiac tissue of STZ-diabetic rats. Mol Cell Biochem. 2001;217:121-9. PubMed PMID: 11269655.

29. Andersen CS, Andersen AB, Finger S. Neurological correlates of unilateral and bilateral “strokes” of the middle cerebral artery in the rat. Physiol Behav. 1991;50:263-9. PubMed PMID: 1745668.

30. Corbett D, Nurse S. The problem of assessing effective neuroprotection in experimental cerebral ischemia. Prog Neurobiol. 1998;54:531-48. PubMed PMID: 9550190.

31. Zausinger S, Hungerhuber E, Baethmann A, Reulen H, Schmid-Elsaesser R. Neurological impairment in rats after transient middle cerebral artery occlusion: a comparative study under various treatment paradigms. Brain Res. 2000;863:94-105. PubMed PMID: 10773197.

32. Panahpour H, Dehghani GA. Attenuation of focal cerebral ischemic injury following post-ischemic inhibition of angiotensin converting enzyme (ACE) activity in normotensive rat. Iran Biomed J. 2012;16:202-8. PubMed PMID: 23183619; PubMed Central PMCID: PMCPMC3600966.

33. Martin A, Rojas S, Chamorro A, Falcon C, Bargallo N, Planas AM. Why does acute hyperglycemia worsen the outcome of transient focal cerebral ischemia? Role of corticosteroids, inflammation, and protein O-glycosylation. Stroke. 2006;37:1288-95. doi: 10.1161/01.STR.0000217389.55009.f8. PubMed PMID: 16601221.

34. Dietrich WD, Alonso O, Busto R. Moderate hyperglycemia worsens acute blood-brain barrier injury after forebrain ischemia in rats. Stroke. 1993;24:111-6. PubMed PMID: 8418533.

35. Behrends M, Martinez-Palli G, Niemann CU, Cohen S, Ramachandran R, Hirose R. Acute hyperglycemia worsens hepatic ischemia/reperfusion injury in rats. J Gastrointest Surg. 2010;14:528-35. doi: 10.1007/s11605-009-1112-3. PubMed PMID: 19997981; PubMed Central PMCID: PMCPMC2820661.

36. Belayev L, Busto R, Zhao W, Ginsberg MD. Quantitative evaluation of blood-brain barrier permeability following middle cerebral artery occlusion in rats. Brain Res. 1996;739:88-96. PubMed PMID: 8955928.

37. Schalkwijk CG, Stehouwer CD. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci (Lond). 2005;109:143-59. doi: 10.1042/CS20050025. PubMed PMID: 16033329.