Pakistan J. Zool., pp 1-7, 2021. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20201110091145

Effect of Extended Window Thrombectomy on Acute Cerebral Infarction and Granulocyte Colony-Stimulating Factor Expression and Postoperative Brain Function

Xiuli Wang[1], Cuilan Hu[2] and Sen Ye[3]*

[1]*Internal Medicine, Jinan Second People's Hospital, Jinan, 250022, China*
[2]*Pharmacy Department, Jiyang People's Hospital of Jinan, Jinan, 251400, China*
[3]*Medical Records Department, Jinan Central Hospital, Jinan, 250013, China*

ABSTRACT

The aim of this study was to investigate the relationship between extended time window thrombectomy on acute cerebral infarction and granulocyte colony-stimulating factor (GCSF) expression, postoperative brain function. Eighty SD rats were randomly divided into 4 groups: control group, infarction group, 4.5h thrombolysis group and 6h thrombolysis group. The postoperative body weight loss rate and neurological function change of each group were observed by body weight test and neurological function scores. The infarct volume and microvessel density of each group were detected by TTC staining and immunohistochemistry. The effects of thrombectomy on brain wave fluctuation and GCSF in rats at different time points were measured according to electroencephalogram and RT-PCR. We found that the neurological function scores of rats decreased with the prolongation of thrombectomy time, and the scores of neurological function were lower in the 4.5h thrombolysis group than in the 6h thrombolysis group at the same time point. At the same time, the infarct volume of rats decreased in both 4.5h thrombolysis group and 6h thrombolysis group. On the 3rd and 7th postoperatively, compared with the acute infarction group, 4.5h thrombolysis group and 6h thrombolysis group showed increased microvessel density which grew gradually over time, while brain waves tended to flatten. RT-PCR results showed that the infarction group, 4.5h thrombolysis group and 6h thrombolysis group had higher GCSF in brain tissue than the normal group, and both reached the lowest peak on 3d and increased on 7d to a level higher than 1d. At the same time, 6h thrombolysis group had lower mRNA expression of GCSF in the brain tissue than 4.5h thrombolysis group on 1d, 3d and 7d. Extended time window thrombectomy on acute cerebral infarction can promote brain function repair and increase the expression of GCSF in brain tissue.

Article Information
Received 10 November 2020
Revised 29 November 2020
Accepted 04 December 2020
Available online 24 August 2021

Authors' Contribution
XW collected the samples. CH analysed the data. SY conducted the experiments and analysed the results. All authors discussed the results and wrote the manuscript.

Key words
Acute cerebral infarction, Extended time window thrombectomy, Granulocyte colony-stimulating factor, Microvessel density

INTRODUCTION

Acute cerebral infarction, a type of disease with high morbidity and mortality, poses a serious threat to the safety of human life. The main reason for its occurrence is the local blood circulation disorder in the brain tissue, causing tissue ischemia and hypoxia, and thereby ceased cell activity in the ischemic area, changes in tissue structure, cell necrosis, etc. which then induces the formation of neurological damage. In severe cases, it can lead to softening lesion and ischemic necrosis of brain tissue (Wu *et al*., 2016). In 1981, Astru first discovered that there is a reversible ischemic penumbra in the limited infarct area of the patient's brain, believing that the surrounding tissues of the patient's occluded artery infarct area do not die immediately, but requires a process to transit from reversible ischemia to irreversible infarction (Kwon *et al*., 2007). The main treatment goal of acute cerebral infarction is to rescue the reversible ischemic zone in the patient brain area (Aizawa *et al*., 2018). The key factor in treatment is the start time, as beyond a certain time range, the patient's ischemic damage area becomes irreversible, and then no measure will produce any therapeutic effect (Duan *et al*., 2018). However, if appropriate measures can be taken within the effective time range, the tissue that is about to become infarct area can be saved, thus reducing the infarct area. This effective time range is known as treatment time window. Studies believe that whether there is an ischemic penumbra in the brain of thrombolytic patients is the key

* Corresponding author: yesen770138@163.com
0030-9923/2021/0001-0001 $ 9.00/0

to a good prognosis. According to research, the key to the treatment of acute cerebral infarction is ultra-early thrombolysis. The best treatment time window is within 6 h. However, in real life, there are few opportunities for thrombolysis within the effective time window, so how to appropriately extend the time window in active rescue of most patients' ischemic penumbra under certain conditions has become an urgent problem at present (Ibaraki *et al*., 2005). At the same time, studies have reported that not all patients have improved neurological function after treatment within a time window of 6-8 h. When patients are treated beyond the time window, it is found that some patients have recovered their functions. Hence, treatment in extended time window is not ineffective (Moore and Ling, 1995). Studies believe that arterial thrombectomy is currently an effective treatment approach for patients with acute cerebral infarction, and the time period required to initiate thrombolysis may have relation to the incidence of complications of ischemia reperfusion injury (Möhlenbruch *et al*., 2012). Granulocyte colony stimulating factor (GCSF) is one commonly used mobilization agent for bone marrow stem cells in clinical practice (Naumenko *et al*., 2011). Bone marrow stem cells have characteristic of homing to the ischemic tissues of the body. When the body is ischemic, the body mobilizes homing of its own bone marrow stem cells. This phenomenon is a self-repair response of the body to the ischemic injury site (Velickovic *et al*., 2018). Studies believe that GCSF can be used to down-regulate the expression of adhesion factors secreted by stromal cells in the bone marrow microenvironment and those produced on the surface of hematopoietic stem cells, thereby improving the interaction between bone marrow stromal and bone marrow stem cells in the body, so that stem cells enter the circulating blood stream through the body's stromal membrane and vascular endothelial tissue, which eventually leads to an increased number of rod cells in the body's circulating pool (Mishra *et al*., 2016). This experiment investigated the effects of extended time thrombectomy in rats with acute cerebral infarction on GCSF expression in the body and postoperative brain function.

MATERIALS AND METHODS

Experimental animals

The eighty SD rats with a weight of 200 g were purchased from Animal Experimental Center of Dalian Medical University. During the experiment, the rats were given adequate food and water, 12 h alternate light and dark every day.

Experimental grouping

The eighty SD rats were randomly divided into 4 groups, namely the control group, the infarction group, the 4.5h thrombolysis group, and the 6h thrombolysis group, with 20 rats in each group. In the 4.5h and 6h thrombolysis groups, 10 mg/kg of r-tPA was injected via femoral vein after the infarction model was established. Each group was sacrificed at 1d, 3d, and 7d after cerebral infarction to extract the brains.

Establishment of acute cerebral infarction model in rats

Thrombin and venous blood were well mixed, then injected into a PE tube, let it stand at room temperature for 4h, and then cut into 1 mm fragments of thrombus under the microscope. After the rats were anesthetized, the external carotid artery, the right internal carotid artery, the common carotid artery, the superior thyroid artery, and the occipital artery were found from the neck opening. The distal end of the superior thyroid artery, occipital artery, ECA, and PPA, as well as the ICA and CCA were clamped and then the proximal end of the ECA was placed in the PE tube. Then, the ICA venule clamp was loosened, the thrombus was inserted, the ECA was quickly ligated and the CCA venule clamp was loosened.

The symptoms of the rat cerebral infarction model were scored with reference to the Longa 5-point method. The scoring criteria are shown in Table I.

Postoperative neurological function and body weight detection of brain tissue in each group

Before thrombolysis and at 1, 3, and 7d after thrombolysis, the weight of rats was measured to calculate the weight loss rate and the neurological function was scored.

Calculation of infarct volume in rats

Brain tissue was taken out at specified time point and frozen at -20°C for 30 min. The brain tissue was then cut into 2mm segments and stained by TTC staining. Afterwards, Image J software was used to analyze and calculate the infarct volume of the brain slice.

Postoperative EEG detection in rats

After the rat was anesthetized, a hole was drilled in the middle of the rat's skull, and an insulated electrode was placed except the tip to the dura. In addition, a similar electrode was embedded in the middle of the forehead front as a ground electrode to be fixed with coagulant and recorded as a single two-lead EEG.

Dynamic changes of rat microvessel

The brain tissue was embedded, sectioned, deparaffinized, hydrated and repaired by

immunohistochemistry, the microvessel density in ischemic penumbra of cerebral infarction in rats was observed under a microscope, and the microvessel count was calculated at the same time. When counting, three areas were randomly selected for manual counting, with the average value as the microvessel density, and microvessel density of each group of slices was counted at each time point.

Expression of GCFS in rat brain tissue

On 1, 3, and 7 d of cerebral infarction, the brain tissues of each group of rats were quickly frozen with liquid nitrogen. After tissue RNA extraction using RNA extraction kit, reverse transcription was quickly performed. The reverse transcription product was subjected to RT-PCR reaction according to the preset RT-PCR reaction system and reaction program. GCSF expression was examined with β-actin as an internal reference in the experiment.

Statistical analysis

The data in this study were all processed by SPSS20.0 statistical analysis software (IBM, USA); the measurement data were expressed as "mean ± standard deviation" ($\bar{x}$±s), and comparison between groups was performed by one-way analysis of variance or repeated measures analysis of variance. LSD-t test was used for pairwise comparison between groups; the count data was expressed by percentage (%), and the comparison between groups was analyzed by χ^2; $P<0.05$ indicates statistically significant difference.

RESULTS

Neurological function score results after extended time window thrombectomy on acute cerebral infarction

Thrombus extraction on d 1, 3 and 7 after operation affected the neurological function scores of rats in each group. It was found that the neurological function scores of rats continuously decreased with the extension of thrombus extraction time, and 4.5h thrombolysis group had lower neurological function score than 6h thrombolysis group at the same time point (Table II).

Body weight measurement after extended time window thrombectomy on acute cerebral infarction

Table III shows the body weights of rats in each group after extended time window thrombectomy on acute cerebral infarction. It can be seen that before the operation, there is no significant difference in body weight of the rats in each group, and compared with the control group, infarction group and thrombolysis group have increased body weight loss rate, and as time prolongs, body weight of rats in the thrombolysis group gradually recovers (Table III).

Table I. Scoring criteria for acute cerebral infarction in rats.

Score	Behavioral Standards
0	No obvious neurological deficit symptoms
1	There is a slight defect in nerve function, and the right forepaw cannot fully extend
2	There is a moderate defect in focal neurological function, the rat rotates to the right when walking
3	There is severe defect in focal neurological function, and the rat falls to the right
4	The rat is unconscious or unable to walk

Table II. Neurological function scores after extended time window thrombectomy on acute cerebral infarction.

Group	1d after operation	3d after operation	7d after operation
4.5h thrombolysis group (n=20)	3.64±0.51	1.86±0.24	0.60±0.01
6h thrombolysis group (n=20)	4.25±0.36	3.17±0.34	1.00±0.06
t value	6.985	7.025	6.127
P value	0.005	0.002	0.009

Table III. Postoperative body weight measurement results of rats in each group.

Group	Preoperative body weight	Loss rate % at 3d after operation	Loss rate % at 7d after operation
Control group (n=20)	284.13±24.34	0.98±0.06	2.14±0.11
Infarction group (n=20)	282.34±12.37	-10.34±1.67	-16.73±2.36
4.5h thrombolysis group (n=20)	286.31±26.44	-1.27.±0.13	2.28±0.74
6h thrombolysis group (n=20)	284.34±22.19	-5.31±0.09	-3.97±0.16
F value	1.253	36.257	21.520
P value	0.521	0.001	0.001

Changes in infarct volume of rats in each group

The infarct volume changes of rats in each experimental group are shown in Table IV. The right brain infarct volume of rats in the infarction group exceeds 50%, the infarct volume decreases in both 4.5h thrombolysis group and 6h thrombolysis group, and 4.5h thrombolysis group has the smallest infarct volume (Table IV).

EEG test results of rats in each group

The EEG results of rats in each group at different time points fluctuated greatly in 6h thrombolysis group, showing greater fluctuation than 4.5h thrombolysis group, and the waveform gradually flattened with time.

Dynamic changes of rat microvessel density

Microvessel density changes of rats in each group are shown in Table IV. There is no difference in microvessel density between the acute infarction group, 4.5h thrombolysis group and 6h thrombolysis group on 1d after the operation. On 3d and 7d after the operation, compared with the acute infarction group, 4.5h thrombolysis group and 6h thrombolysis group have increased microvessel density in rats, and the microvessel density gradually increases with time.

Effect of extended time window thrombectomy on GCSF expression in brain tissue

Gel electrophoresis shows that each lane is single, indicating that the product has no specificity and primer dimers. Table IV shows that infarction group, 4.5h thrombolysis group and 6h thrombolysis group have lower GCSF expression level in the brain tissue than the control group. Meanwhile, compared with the control group, both 4.5h thrombolysis group and 6h thrombolysis group show increased expression of GCSF in the rat brain tissue, where, 4.5h thrombolysis group has higher increase in expression of GCSF than the 6h thrombolysis group.

The 6h thrombolysis group has lower mRNA expression of GCSF in the rat brain tissue than 4.5h thrombolysis group on d1, d3 and d7.

Table IV. Effect of time window thrombectomy on right brain infarct volume, microvessel density and GCSF expression in brain of rats of each experimental group.

Group	1d after operation (%)	3d after operation (%)	7d after operation (%)
Infarct volume			
Infarction group (n=20)	82.16±1.21	85.36.±2.11	86.33±3.14
4.5h thrombolysis group (n=20)	40.23±0.09[a]	50.09±2.14	56.35±1.96
6h thrombolysis group (n=20)	51.28±1.76	62.34±2.71	70.13±3.16
F value	52.207	35.725	38.358
P value	0.001	0.001	0.001
Microvessel density			
Infarction group (n=20)	12.06±1.31	12.46.±1.93	15.62±2.96
4.5h thrombolysis group (n=20)	13.28±1.64	19.24±3.15	28.34±3.19
6h thrombolysis group (n=20)	13.98±2.11	22.13±2.32	29.39±4.13
F value	26.957	18.623	19.305
P value	0.003	0.005	0.009
GCSF expression			
Control group (n=20)	1.36±0.02	1.43±0.12	1.39±0.09
Infarction group (n=20)	0.36±0.01	0.23±0.01	0.43±0.03
4.5h thrombolysis group (n=20)	0.69±0.01	0.53±0.13	0.93±0.16
6h thrombolysis group (n=20)	0.46±0.03	0.39±0.06	0.76±0.07
F value	22.326	25.309	15.573
P value	0.002	0.001	0.007

DISCUSSION

Acute cerebral infarction is a disease in which cerebral ischemia causes cell necrosis, ceased cell activity, or loss of nerve function in some regions or tissues of the brain. In severe cases, it can lead to brain tissue necrosis or softening lesion. Studies have showed that in the natural course of disease, cerebral infarction area gradually spreads from the infarction center to the surrounding area. Therefore, if the blood perfusion in the ischemic area can be controlled in time, the necrosis of nerve cells in this area can be avoided to save the patient's incompletely necrotic tissue, reduce the area of brain tissue infarction, and then alleviate the neurological damage, which means great significance for clinical treatment of acute stroke.

We have found that with the extension of thrombus extraction time, the nerve function scores of 4.5 h thrombolysis group and 6h thrombolysis group continually decreased, with microvessel density continually increased. At the same time, both 4.5h thrombolysis group and 6h thrombolysis group had reduced infarct volume and higher expression of GCSF in brain tissue than the control group. The expression reached the lowest peak on 3d, and increased on 7d to a level higher than 1d.

Arterial thrombolysis has a significant clinical effect in the treatment of acute cerebral infarction, which is ideal for the treatment of acute cerebral infarction (Page *et al*., 2018). Jin *et al*. (2004) reported that the transition of reversible ischemia to irreversible ischemia in brain cells is a dynamic development process, which is subjected to influence of the circulatory system, occlusion location, intracerebral blood flow rate, and local cerebral blood flow. Infarct usually occurs 24 h after the vascular occlusion, but the infarction rate will not reach its peak even after a longer period of time. In the treatment of patients with acute cerebral infarction, it was found that after arterial thrombolytic therapy, the patient's neurological function was significantly improved, with 6h effective rate of the thrombolysis group at 88.1%, marked effective rate at 71.4% and vessel repass rate at 69%; the 6-24h effective rate of thrombolysis group was 75%, with marked effective rate at 50%, and vessel repass rate at 69% (Kim *et al*., 2012). According to the results, it can be seen that extended time window arterial thrombolysis still has fine therapeutic effect and high vessel repass rate (Wang *et al*., 2020). In a study on the treatment of patients with aortic occlusion-induced acute cerebral infarction in extended time window, it was found that 15 out of 18 patients with acute cerebral infarction showed vessel repass, 1 had perioperative cerebral hemorrhage, and 4 had reocclusion of blood vessels. In follow-up observation after 3 months, 9 patients had MRS scores below 3, 5 cases died, 4 cases had MRS score higher than 3, which proved that extended time window treatment can increase the patient's vessel repass rate and reduce bleeding complications as an effective treatment choice for aortic occlusion with good prognosis effect (Rahman *et al*., 2018).

GCSF as a mobilizing agent of bone marrow stem cells can significantly increase the number of stem cells in the peripheral blood, migrate to the cerebral ischemic area, and differentiate into nerve cells to repair the damaged tissues in the patient's brain, which thus has certain neuroprotective effect (Kateja *et al*., 2017). Peng *et al*. (2010) confirmed that in the rat model with transient or permanent cerebral artery occlusion, compared with the control group, GCSF treatment group had reduced IL-1β level, significantly reduced infarct volume and edema in the brain tissue, and significantly improved neurological function in the rats. In a study of acute ischemia-reperfusion rats. Chen *et al*. (2019) found that at 30 min after ischemia in rats, intravenous injection of GCSF could significantly reduce infarct volume of the body after 24 h. The mechanism of action is mainly to up-regulate STAT-3 signaling pathway in the ischemic penumbra of brain tissues. Chang *et al*. (2018) found that GCSF can mobilize MSC homing to the brain ischemic injury area of the body in a chemically activated manner to promote the repair of brain function and nerve function. At the same time, MRI results showed that the body's lesions were significantly reduced and double indirect immunohistochemistry results showed that Brdu-positive cells in the peripheral area of the ischemic tissue had the expression of GFAP, MAP-2, and Willebrand factors. Dong *et al*. (2018) found that MSC accumulation was higher in cerebral ischemic areas than in non-ischemic areas, indicating that damaged brain tissue can specifically absorb bone marrow-derived cells.

In summary, extended time window thrombectomy on acute cerebral infarction can promote brain function restoration and increase the expression of GCSFs in brain tissue.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Aizawa, Y., Nakai, T., Saito, Y., Monno, K., Morikawa, T., Kogawa, R., Hatta, T., Tamaki, T., Kato, M. and Arimoto, M., 2018. Calcified amorphous tumor-induced acute cerebral infarction. *Int. Heart J.,* **59**: 240-242. https://doi.org/10.1536/ihj.17-020

Albers, G.W., Amarenco, P., Easton, J.D., Sacco, R.L. and Teal, P., 2004. Antithrombotic and thrombolytic therapy for ischemic stroke. The Seventh ACCP

Conference on Antithrombotic and Thrombolytic Therapy. *Chest,* **126**: 483S-512S. https://doi.org/10.1378/chest.126.3_suppl.483S

Cagnazzo, F., Derraz, I., Dargazanli, C., Lefevre, P.-H., Gascou, G., Riquelme, C., Bonafe, A. and Costalat, V., 2020. Mechanical thrombectomy in patients with acute ischemic stroke and ASPECTS≤ 6: A meta-analysis. *J. NeuroInterv. Surg.,* **12:**350-355. https://doi.org/10.1136/neurintsurg-2019-015237

Chang, Y.-C., Soriano, M., Hahn, R.A., Casillas, R.P., Gordon, M.K., Laskin, J.D. and Gerecke, D.R., 2018. Expression of cytokines and chemokines in mouse skin treated with sulfur mustard. *Toxicol. appl. Pharmacol.,* **355:** 52-59. https://doi.org/10.1016/j.taap.2018.06.008

Chen, Y., Lin, L., Tao, X., Song, Y., Cui, J. and Wan, J., 2019. The role of podocyte damage in the etiology of ischemia-reperfusion acute kidney injury and post-injury fibrosis. *BMC Nephrol.,* **20:** 106. https://doi.org/10.1186/s12882-019-1298-x

Dong, H., Li, G., Shang, C., Yin, H., Luo, Y., Meng, H., Li, X., Wang, Y., Lin, L. and Zhao, M., 2018. Umbilical cord mesenchymal stem cell (UC-MSC) transplantations for cerebral palsy. *Am. J. Transl. Res.,* **10:** 901.

Duan, M., Gu, J., Wang, X., Li, Y., Zhang, S., Yin, Y. and Zhang, R., 2018. Effects of genetically modified cotton stalks on antibiotic resistance genes, intI1, and intI2 during pig manure composting. *Ecotoxicol. Environ. Saf.,* **147:** 637-642. https://doi.org/10.1016/j.ecoenv.2017.09.023

Ibaraki, T., Imahori, S., Kubo, M., Masuda, T., Uno, T. and Yagiura, M., 2005. Effective local search algorithms for routing and scheduling problems with general time-window constraints. *Transp. Sci.,* **39:** 206-232. https://doi.org/10.1287/trsc.1030.0085

Jin, X.M., Li, L., Peng, M.Y. Yin, X.D. and Ma, Y.H., 2020. To explore the risk factors of outcome after mechanical thrombectomy in acute large area infection. *Chinese J. Magn. Resonan. Imag.,* **11**: 730-734.

Kateja, N., Agarwal, H., Hebbi, V. and Rathore, A.S., 2017. Integrated continuous processing of proteins expressed as inclusion bodies: GCSF as a case study. *Biotechnol. Prog.,* **33:** 998-1009. https://doi.org/10.1002/btpr.2413

Kim, J., Song, T.-J., Park, J.H., Lee, H.S., Nam, C.M., Nam, H.S., Kim, Y.D. and Heo, J.H., 2012. Different prognostic value of white blood cell subtypes in patients with acute cerebral infarction. *Atherosclerosis,* **222:** 464-467. https://doi.org/10.1016/j.atherosclerosis.2012.02.042

Kwon, Y.-H., Lee, C.-H., Ahn, S.H., Lee, M.Y., Yang, D.S., Byun, W.M., Park, J.-W. and Jang, S.H., 2007. Motor recovery via the peri-infarct area in patients with corona radiata infarct. *NeuroRehabilitation,* **22:** 105-108. https://doi.org/10.3233/NRE-2007-22205

Mishra, V.V., Choudhary, S., Sharma, U., Aggarwal, R., Agarwal, R., Gandhi, K. and Goraniya, N., 2016. Effects of granulocyte colony-stimulating factor (GCSF) on persistent thin endometrium in frozen embryo transfer (FET) cycles. *J. Obstet. Gynecol. India,* **66**(Suppl 1): 407-411. https://doi.org/10.1007/s13224-015-0775-9

Möhlenbruch, M., Seifert, M., Okulla, T., Wüllner, U., Hadizadeh, D., Nelles, M., Greschus, S., Wilhelm, K., Schild, H. and Klockgether, T., 2012. Mechanical thrombectomy compared to local-intraarterial thrombolysis in carotid T and middle cerebral artery occlusions. *Clin. Neuroradiol.,* **22:** 141-147. https://doi.org/10.1007/s00062-011-0099-9

Moore, J. and Ling, H., 1995. Super resolved time frequency processing of surface wave mechanisms contained in wideband radar ECHO. *Microw. Opt. Technol. Lett.,* **9:** 237-240. https://doi.org/10.1002/mop.4650090504

Naumenko, N., Pollari, E., Kurronen, A., Giniatullina, R., Shakirzyanova, A., Magga, J., Koistinaho, J. and Giniatullin, R., 2011. Gender-specific mechanism of synaptic impairment and its prevention by GCSF in a mouse model of ALS. *Front. cell. Neurosci.,* **5:** 26. https://doi.org/10.3389/fncel.2011.00026

Page, P.S., Khattar, N.K., White, A.C., Cambon, A.C., Brock, G.N., Rai, S.N. and James, R.F., 2018. Intra-arterial thrombolysis for acute central retinal artery occlusion: A systematic review and meta-analysis. *Front. Neurol.,* 9**:** 76. https://doi.org/10.3389/fneur.2018.00076

Peng, K., Wu, M., Deng, F., Song, J., Dong, C., Wang, H. and HU, Z., 2010. Identification of protein–protein interactions of the occlusion-derived virus-associated proteins of Helicoverpa armigera nucleopolyhedrovirus. *J. Gen. Virol.,* **91:** 659-670. https://doi.org/10.1099/vir.0.017103-0

Rahman, I.A., Zahrani, M., Chow, C.-M., Abramson, B. and Yanagawa, B., 2018. Abdominal aortic occlusion following large left atrial mass embolization. *CASE: Cardiovas. Imag. Case Rep.,* **2:** 273. https://doi.org/10.1016/j.case.2018.07.006

Velickovic, K., Leija, H.A.L., Bloor, I., Law, J., Sacks, H., Symonds, M. and Sottile, V., 2018. Low temperature exposure induces browning of bone

marrow stem cell derived adipocytes *in vitro*. *Scient. Rep.,* **8:**1-12. https://doi.org/10.1038/s41598-018-23267-9

Wang, H., Shu, H.S. and Yang, G., 2020. A comparative study of influence of age on prognosis of patients with cerebral infection undergoing mechnical thrombectomy. *J. clin. Neurosurg.,* **17:** 212-215.

Wu, W., Guan, Y., Xu, K., Fu, X.-J., Lei, X.-F., Lei, L.-J., Zhang, Z.-Q., Cheng, Y. and Li, Y.-Q. 2016. Plasma homocysteine levels predict the risk of acute cerebral infarction in patients with carotid artery lesions. *Mol. Neurobiol.,* **53:** 2510-2517. https://doi.org/10.1007/s12035-015-9226-y