Int J Clin Exp Med 2019;12(1):251-261
www.ijcem.com /ISSN:1940-5901/IJCEM0073765

Original Article

Hyperhomocysteinaemia in conjunction with hypertension, hemodynamic variations and estrogen deficiency: a novel rat model of intracranial aneurysm

Hao Fu[1*], Zhengchao Li[1*], Yang Liu[2*], Lingyan Jiang[2*], Xiaochu Chen[1], Haiyang Yan[1], Jing Gui[1], Chao Ye[3], Shengkai Sun[1], Zhihong Wang[1]

[1]Tianjin Key Laboratory of Neurotrauma Repair, Affiliated Hospital of Logistics University of Chinese People's Armed Police Force, Tianjin, P. R. China; [2]Department of Neurology, Shanghai Fourth People's Hospital, Shanghai, P. R. China; [3]Department of Outpatient, Headquarters of PAP, Beijing, P. R. China. [*]Equal contributors.

Received January 31, 2018; Accepted October 11, 2018; Epub January 15, 2019; Published January 30, 2019

Abstract: Establishing a sensitive and comprehensive experimental model is one of the most important issues for the study of intracranial aneurysm (IA). Hyperhomocysteinaemia (HHcy) is involved in multiple cerebrovascular diseases. Interestingly, the vascular damage induced by HHcy coincides highly with the pathologic changes of IA. The purpose of this study was to explore whether HHcy facilitated the IA formation in rats. Female SD rats (8-12 weeks) underwent left common carotid artery and posterior branches of bilateral renal arteries ligation and bilateral ovariectomies traditionally. 3% methionine was added in the diet after surgery to establish a new IA model. Rats were sacrificed and conducted vascular proplasm at 6 months post modeling. The IA formation was viewed under electron microscope. The addition of 3% methionine in the diet dramatically increased the rate of IA formation, especially secondary and tertiary IA with 50% rise. Methionine treatment also significantly elevated MMP-9, iNOs and SOD gene expression in peripheral blood which were measured by RT-PCR. In summary, the addition of methionine in the diet obviously contribute to the IA formation, probably owing that hyperhomocysteine increase the systemic inflammatory level and act synergistically with hypertension and hemodynamic compromise.

Keywords: Intracranial aneurysm, hyperhomocysteinaemia, hypertension, hemodynamics, estrogen, animal model

Introduction

An intracranial aneurysm (IA) is a tumour-like protrusion because of the pathological localized dilation or ballooning of intracranial vascular walls. The morbidity of IA is about 1%-5% [1]. The biggest threat which IA patients face is the subarachnoid hemorrhage (SAH) result from the rupture of an IA. Once SAH happens, it will seriously endanger the patients' life, and the mortality can reach up to 30%-45%, meanwhile, half of survivors will remain permanent neurological dysfunction [2].

Scholars have tried hard to reveal the pathogenesis of IA over the years through a large number of clinical trials and epidemiological research, however, there is a major bottleneck in the clinical study of this disease, the pathogenesis of IA has not yet been elucidated so far for reasons as follow. Firstly, the IA tissue is difficult to obtain. Current treatments do not require Most of the patient do not want to get involved in the clinic trials because both of the two IA treatment prescriptions don't need to excise the tissue, which make sources the tissue even more fewer. Second of all, there is too many confounding factors. The formation of an IA is easily affected by so many aspects and factors, which make great disturbance while analyzing the cause of formation of IA. Thirdly, researchers can't analyze the whole process of the IA pathogenesis continuously, but only can evaluate the IA sample in a certain time due to the limitation of human experiment, which brings a major inconvenience in conducting the experiment. At last, human experiment requires a complete set of standardized and complex procedures for review which will extend the experiment remarkably. Therefore, the con-

struction of animal models has become an important tool for the study of the pathogenesis of IA.

Nagata et al [3] succeeded inducing the IA in rats for the first time by making hypertension and hemodynamic variations, each of which respectively result from the ligation of the posterior branches of bilateral renal arteries and the unilateral ligation of carotid arteries. This model also verifies that hypertension and localized variations of hemodynamics are indispensable factors in the establishment of a model in rats to study the IA.

Since then, this model is considered to be the classic model of IA rat model and it has also become the basic model of each improved model. However, the IA formation rate of this model is rather low at only about 30% [4]. In 2009, Eldawoody et al [5] successfully constructed an IA rat model based on the classic model by introduced the bilateral oophorectomy to prevent oestrogen from maintaining vessels. The improved model did increase the formation rate of IA in model rats, but still less than 50%. Xu et al [6] simplified Nagata team's model in which rats were treated with the unilateral ligation of carotid arteries and a methionine diet so as to increase the level of Hcy in the blood. However, not enough attention has been paid to this model nor further popularized because of that the incidence of IAs is quite low in stage III. In addition, some researchers attempted to replace rats with other animals. It proves that mice and rabbits are far less susceptible to the IA than rats. Although monkeys are much more susceptible to the IA, they are expensive and significantly prolong the cycle of studies. Since dramatic physical differences between dogs and humans have developed diverse natural causes of the IA for these two species respectively, researchers merely study on dogs in terms of hemodynamics and intravascular therapies [7-9].

On account of the above research, we considered to construct a new IA model based on the classic rat model. This model should have a rather high morbidity of IA and more suitable on IA's pathogenesis for human being. Recently, a clinical research with large sample came into our view again which is about the dependency of Hcy and apoplexy. The research revealed that patients with hyperhomocystinemia (HHcy) are tend to be more easily attacked by aneurysmal subarachnoid hemorrhage (SAH) [10]. Another research showed that HHcy combined with the change of hemodynamics can promote the formation of IA. With the synergistic effect of the above factors, our research will firstly make IA model according to Eldawoody's method [5], and then the rats are fed diet with extra methionine, which can transfer to Hcy by demethylation, and an overdose of methionine will elevate the plasma level of Hcy and cause HHcy. We hope that the overdose of Hcy thereby can act as a mediator to produce a synergistic mechanism of HHcy, hypertension, hemodynamic variations and estrogen deficiency, and eventually trigger the IA in rats. Meanwhile the model conducted with multiple factors can be more similar to IA's pathogenesis for human being which can provide strong and firm support for our research of lucubrating in IA's pathogenesis.

Materials and methods

All surgical and experimental procedures were carried out in accordance with the guide from the National Institutes of Health for the Care and Use of Laboratory Animals, and were approved by the Ethics Committee of the Affiliated Hospital of the Logistics University of PAP.

IA model and treatment

36 SPF female Sprague-Dawley rats, 8-10 weeks, weight 250-300 g which were bought from the Laboratory Animal Center of Academy of Military Medical Sciences, Beijing were took as experimental animal. The License number is SCXK-(Jun) 2012-0004. The rats were fed under 12 hour light condition every day, 4 rats per cage with standard diet. All of the rats were acclimatized for 1 week. 36 rats were randomly divided into 3 groups (n=12): Control group, Traditional Model group and Novel Model group. The rats were anesthetized by intraperitoneal injection of 1% pentobarbital sodium 40 mg/kg and the dose was increased if necessary. The construction of rat model was begun right after the anesthesia.

The Traditional Model group and Novel Model group rats were given the following operations (**Figure 1**). ① Ligation of the right CCA. Make an incision of neck and separate the subcuta-

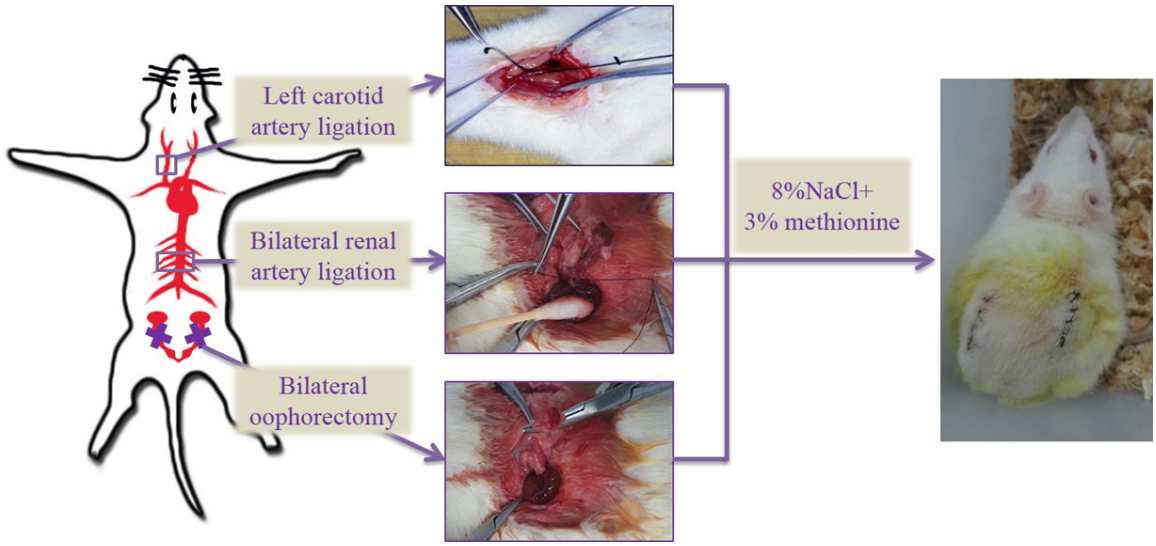

Figure 1. Model construction diagram.

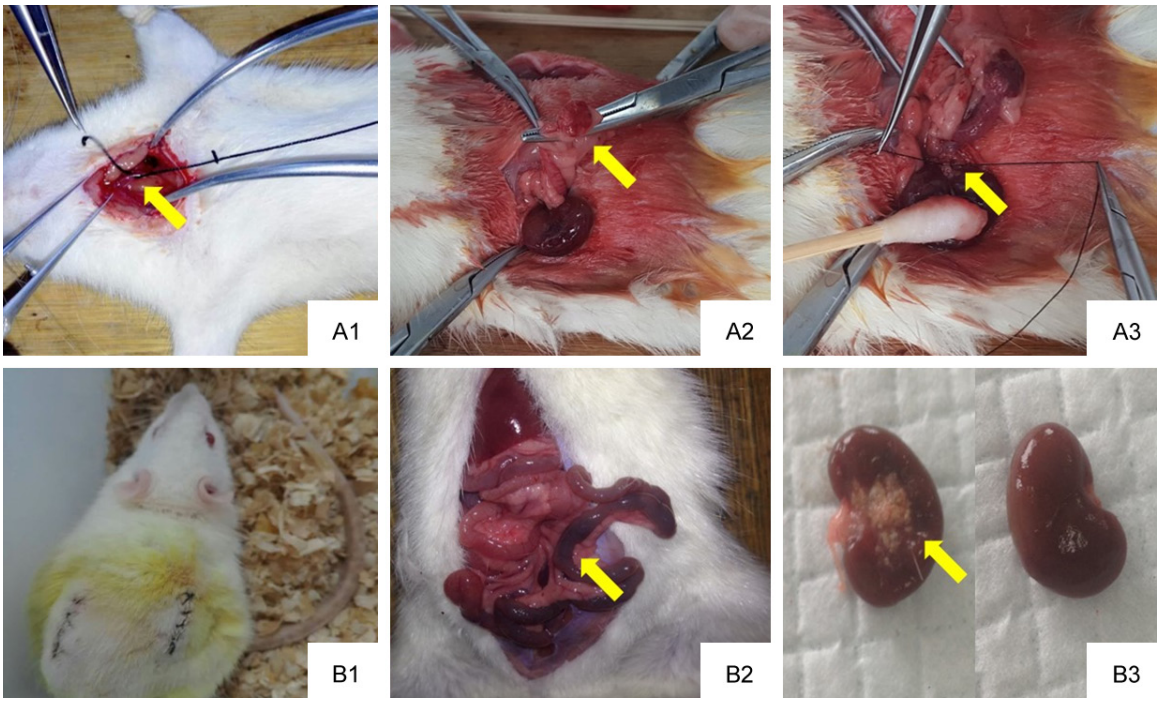

Figure 2. Operations during the IA model construction. A1. Unilateral common carotid artery. A2. Bilateral oophorectomy. A3. Ligations of the posterior branches of both renal arteries. B1. Postoperative recovery was well. B2. Death due to intestinal obstruction. B3. Ischemic necrosis of nephridial tissue due to ligations of the posterior branches of both renal arteries.

neous connective tissue. On the right side of trachea, the connective tissue was downward isolated beside the lateral border midpoint of sternohyoid muscle, then the nervi vagus and pulsatile CCA can be seen. Ligate the right CCA with 5-0 surgical suture in order to change the intracranial hemodynamic (**Figure 2A1**). ② Bilateral oophorectomy. The thumb was placed at the lower edge of the back rib of the rat. The index finger and middle finger were placed in the middle of the abdomen, and slid the three fingers together to one side to find the kidney. Then make a longitudinal incision for about 1.5 cm long according to the position of kidney, open the abdominal cavity via the back approach. Pull out the adipose tissue carefully

Table 1. Primers used in this study

Primer Name	Primer sequence (5'-3')		Final concentration
MMP-9	Forward	ACAACGTCTTTCACTACCAA	400 nmol/L
	Reverse	CAAAAGAGGAGCCTTAGTTC	400 nmol/L
iNOS	Forward	TTCAGGTATGCGGTATTTGG	400 nmol/L
	Reverse	GTTGGAAGTGTAGCGTTTCG	400 nmol/L
MCP-1	Forward	CACTCACCTGCTGCTACTCAT	400 nmol/L
	Reverse	CTACAGCTTCTTTGGGACACCT	400 nmol/L
SOD	Forward	GGCCAAGGGAGATGTTACAA	400 nmol/L
	Reverse	GCTTGATAGCCTCCAGCAAC	400 nmol/L
GAPDH	Forward	TACACTGAGGACCAGGTTG	400 nmol/L
	Reverse	CCCTGTTGCTGTAGCCATA	400 nmol/L

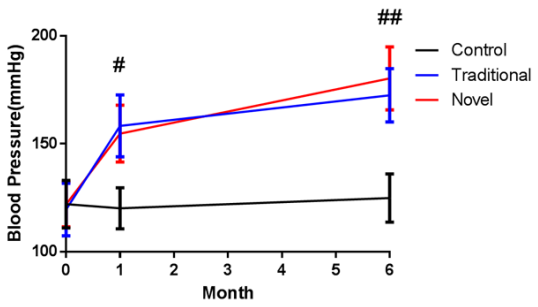

Figure 3. Variation of blood pressure. #*P*<0.05 when Traditional Model group or Novel Model group compared with the Control group. ##*P*<0.05 when Novel Model group compared with Traditional Model group or Control group.

and find the ovary, ligate the ovary from the root and cut it down. The opposite side was under the same operation (**Figure 2A2**). ③ Ligations of the posterior branches of both renal arteries. After the bilateral oophorectomy, the surgeon uses the index finger and middle finger to push the kidneys out of the back incision, and separate the renal capsule. The assistant uses two swabs to clamp the kidneys and pull them out to expose the renal vein. Ligate the posterior branches of both renal arteries which lie posterior and superior to the renal artery by 7-0 surgical suture under the 10 times operative microscopy. Flush the bilateral abdominal cavity with enough normal saline to prevent ankylenteron, and then suture the muscularis and the skin in turn (**Figure 2A3**). 12 rats that expose the right CCA, bilateral ovary and renal arterie for 20 min were served as Control group.

After the operation, each groups conduct rehabilitative feeding (**Figure 2B1**). If the rats don't eat or died in 3 days, a supplement was added. If the rats don't eat during this stage, they may get adhesive ileus and finally die. Therefore, intraperitoneal injection were conducted with excessive anesthetic to execute the rats in order to decrease their pain (**Figure 2B2**). After 1 week of regular food and rehabilitative feeding, the Traditional Model group were fed the diet with 8% sodium chloride. The Novel Model group were fed by the diet with 8% sodium chloride and 3% methionine. And the Control group were fed by the normal diet.

Blood pressure determination

Using the tail-cuff auto-pickup method for determining systolic arterial pressure in unanesthetized rats with a continuous measurement of 3 days per time point, measured 1 times a day at three time points which were before operation, 1 month and 6 month after operation. The data of the third measurements were used as the systolic arterial pressure in order to let the rats to adapt the blood pressure measurement environment and avoid the fluctuation of blood pressure caused by the struggle. In addition, renal hypertension can be verified again by observing the morphology of the kidney after the final execution (**Figure 2B3**).

Blood indicator determination

6 months after operation, the rats were anesthetized by ether gas, then cut off one side of the rat's whiskers. The capillary glass tube which the diameter is 0.9 mm were cut into the length of 1.2 cm and Infiltrated by 1% heparin sodium. Then the glass tube was piercing rotary through the outer canthus into the posterior orbital of the rats. The operator had better to put on PE gloves in order to make the rotary movement easier to conduct. 1 mL blood was taken by procoagulant tube and EDTA anticoagulant tube separately. The blood samples in procoagulant tubes were delivered to our clinical laboratory department (Mai Rui automatic biochemical test system (BS-480)) for the determination of estradiol and Hcy concentrations. The Total RNA extraction from blood, cDNA synthesized and real-time fluorescent quantitative PCR reaction was carried out

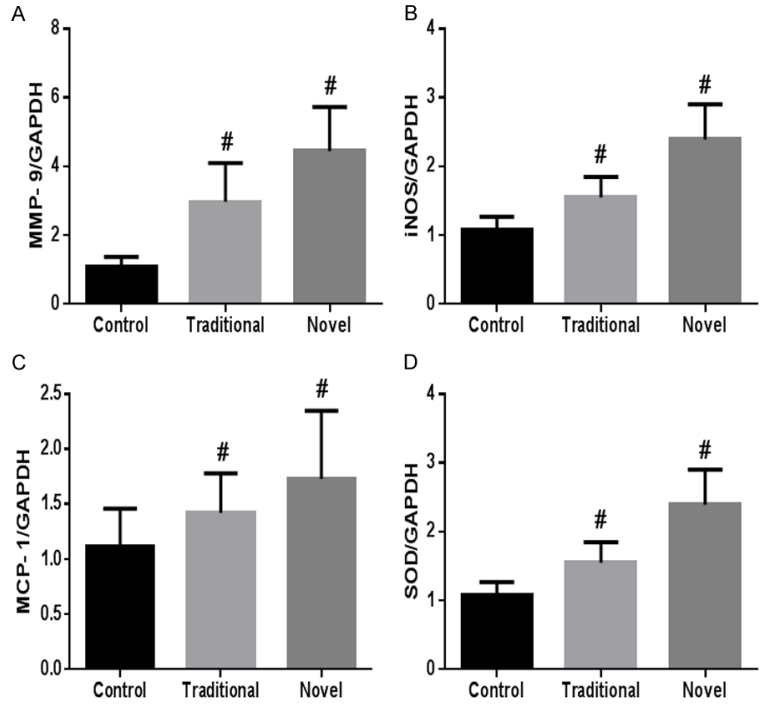

Figure 4. The relative gene expression in each group. RT-PCR analysis for MMP-9 (A), iNOS (B), MCP-1 (C), and SOD (D) expression in peripheral blood 6 months after surgery. #$P<0.05$ versus the Control group.

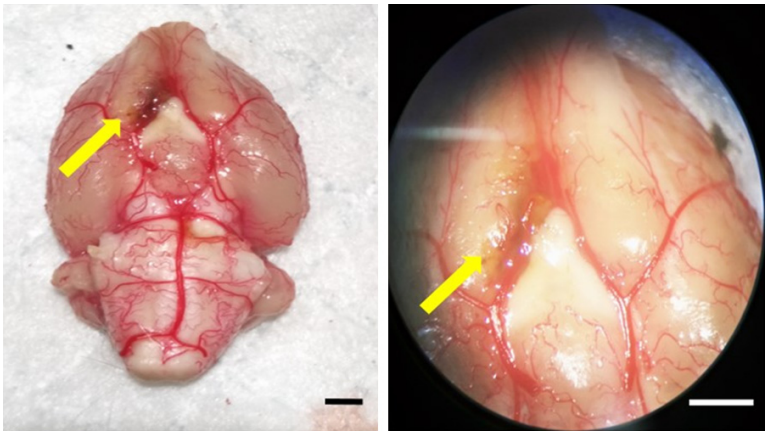

Figure 5. Right ACA-OA site exist oboslete hemorrhage. Bar=50 mm.

according to the method and steps marked in the kit instructions of the blood samples in anticoagulant tubes. Relative expression of matrix metalloproteinase-9 (MMP-9), inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein-1 (MCP-1) and superoxide dismutase (SOD) were calculated by 2-ΔΔCt method (**Table 1**).

Vascular cast

Vascular corrosion casts were produced as reported previously [11]. After induction of anesthesia, the rats underwent laparotomy and thoracotomy. The gastric lavage needle (16-gauge caliber with a length of 6 cm) was inserted into the left ventricle and secured in the ascending aorta with a forceps. After the right atrium was cut for drainage, the rats were perfused with 50 mL normal saline. This procedure was followed by manual injection of 10 ml Batson No. 17 plastic which compounded according to the instructions before perfusing normal saline. After 2 h for polymerization at room temperature, the entire brain was removed and digested in saturated KOH with intermittent shaking. Shake one times per 2 hour during the day and change the solution once every 24 hours. A complete gel casted cerebral artery circle (Willis cycle) can be obtain after about 3 days. Finally, scanning electron microscopy was used to observe. The IA classification criteria are as follows. Roughened apical intimal pad with irregularly shaped imprints (stage I). Shallow fusiform elevation at the apical intimal pad covered with abnormal imprints (stage II). Saccular aneurysm covered with abnormal imprints (stage III).

Statistical analysis

Two independent groups were compared by the unpaired t-test. Three or more independent groups were compared by one-way ANOVA and LSD test. Categorical data for groups were tested using the Chi-square test. Statistical analyses were performed on a computer running

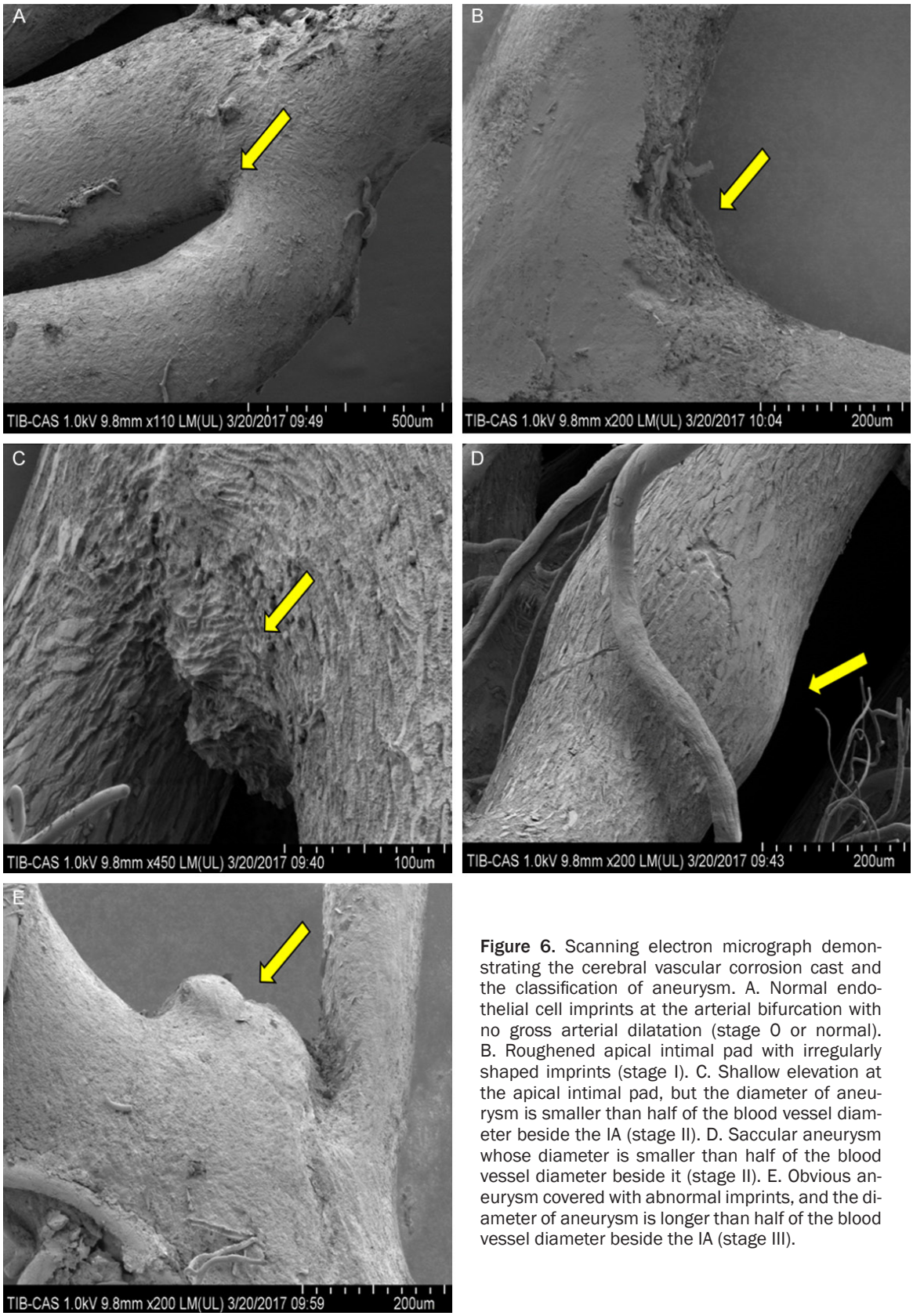

Figure 6. Scanning electron micrograph demonstrating the cerebral vascular corrosion cast and the classification of aneurysm. A. Normal endothelial cell imprints at the arterial bifurcation with no gross arterial dilatation (stage 0 or normal). B. Roughened apical intimal pad with irregularly shaped imprints (stage I). C. Shallow elevation at the apical intimal pad, but the diameter of aneurysm is smaller than half of the blood vessel diameter beside the IA (stage II). D. Saccular aneurysm whose diameter is smaller than half of the blood vessel diameter beside it (stage II). E. Obvious aneurysm covered with abnormal imprints, and the diameter of aneurysm is longer than half of the blood vessel diameter beside the IA (stage III).

Table 2. The number, staging and location of IA

Factors	Groups		
	Traditional Model group	Novel Model group	Control group
Number of rats (%)			
Total number of rats	12 (100%)	12 (100%)	12 (100%)
Rats with lesions	8 (67%)	12 (100%)	0 (0%)
Rats with multiple lesions	2 (17%)	5 (42%)	0 (0%)
Number of IA (%)			
Total number of lesions	10 (100%)	17 (100%)	-
Stage I	5 (50%)	6 (35%)	-
Stage II	3 (30%)	4 (24%)	-
Stage III	2 (20%)	7 (41%)	-
Number of lesions per rat (mean ± SEM)#	0.83±0.71	1.42±0.51	0
Number of Stage II/III lesions per rat (mean ± SEM)##	0.42±0.51	0.92±0.67	0
Site			
ACA-OA bifurcation	7 (70%)	14 (82%)	-
ACA	1 (10%)	2 (12%)	-
ACOMA	2 (20%)	1 (6%)	-

ACA: anterior cerebral artery, OA: olfactory artery, ACOMA: anterior communicating artery. #: There were significant differences in the IA formation rate between Traditional Model group and Novel Model group (P=0.032). ##: There was an increased trend of stage II/III IA in Novel Model group when compared with Traditional Model group and (P=0.052).

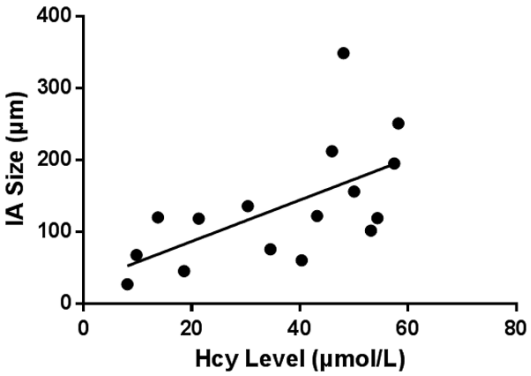

Figure 7. Correlation between Hcy level and IA size. r=0.603, P=0.013.

statistical software SPSS17.0. Differences were considered statistically significant with a probability value of less than 0.05.

Results

Variation of blood pressure

The systolic pressure has no statistical significance among the three groups (P>0.05). 1 month after molding, the pressure of rat's in Traditional Model group and Novel Model group were both higher than the Control group (P<0.05). 6 months after molding, the pressure of rats in Novel Model group became much higher than the rats in Traditional Model group, and the difference was statistically significant (**Figure 3**).

Variation of Hcy

6 months after the molding, the Hcy of Novel Model group (46.87±9.09 μmol/L) is significantly greater than the Traditional group (14.32±5.61 μmol/L) and the Control group (15.29±4.90 μmol/L), and the difference was statistically significant (P<0.05).

Variation of serum estrogen

6 months after the molding, the serum estrogen of the Novel Model group (103.16±7.20 pmol/L) and the Traditional Model group (95.43±8.28 pmol/L) has no obvious difference (P>0.05). But the Hcy of them was lower than the Control group (176.72±11.41 pmol/L), and the difference was statistically significant (P<0.05).

Variation of gene expression

The expression of MMP-9, iNOS, MCP-1 and SOD was up-regulated in the Traditional Model

group rats when compared with the Control group. And the expression in Novel Model group was much higher than the Traditional group (P<0.05) (**Figure 4**).

Formation of intracranial aneurysm

1 case of SAH was found in the Novel Model group (**Figure 5**), but this rat didn't appear any abnormal behavior nor any clinical manifestation just like human being. Vessel casting was observed through microscope, 10 IAs in 12 rats was found in Traditional group, 5 of them were in stage II or stage III, while 17 IAs in Novel Model group was found, 11 of them were in stage II or stage III (**Figure 6** and **Table 2**).

Sizes of intracranial aneurysm

The sizes of IAs in stage II or stage III in the Novel Model group were significantly larger than those in the Traditional group (164.39± 81.52 μm vs. 75.84±45.37 μm, P<0.05).

Correlation between Hcy level and IA size

There is a positive correlation between the Hcy level and the size of IA, the rats with higher level of Hcy tend to have a larger IA in stage II or stage III (r=0.603, P=0.013) (**Figure 7**).

Discussion

On the basis of traditional IA model methods, we used the diet containing 3% methionine to cause HHcy. The result indicated that Hcy can act as a mediator to produce a synergistic effect of HHcy, hypertension, hemodynamic variations and estrogen deficiency, and eventually promote the formation of IA in stage II or stage III as well as increase the size of IA. This novel model makes up the low formation rate of IA in traditional modeling methods, and provides a more reliable basic model for the mechanism research of IA.

The result shows that the systolic blood pressure (SBP) of Traditional and Novel Model group were over average 150 mmHg after ligating the posterior branches of rat's renal arteries combining with high salt diet in 1 month after molding, at 6 months after modeling, the SBP of the two model groups have increased further with an average value of over 170 mmHg. Hence, we can prove the operation above can establish a stable renal hypertension model. This result is in agreement with the experiment results of Nagata and Eldawoody et al [3, 5].

Meanwhile the unilateral carotid artery was ligated in this experiment, which can alter the hemodynamics of intracranial vessels and change the wall shear stress (WSS). Studies prove that the value of WSS to maintain normal cellular forms is around 2Pa. An abnormally high value of WSS may degrade, dilate, or even expand local vascular walls, and eventually accelerate the formation and the growth of arterial aneurysms. In contrast, by damaging endothelial intercellular spaces and paralysing regulatory functions of antioxidants and anti-inflammatory mediators, an extremely low value of WSS may promote endothelial remodelling, degeneration, and eventually apoptosis [12]. In this experiment, iNOS and MMP-9 were both rose remarkably in two model groups, which indicates that the variation of hemodynamics has occurred and it caused vascular endothelial damage and degradation of extrscellular matrix. We consider that the two factors above can promote the physical mechanics change in the blood vessel of brain. which can damage the cerebrovascular endothelial cell through a series of chemical changes. They can damage media vascular to make the chemical change on arterial wall [13]. Hence, changes of blood pressure and hemodynamics are the basic conditions of intracranial aneurysm model.

The result also reveals that serum estrogen in the Traditional and the Novel Model group was dramatically declined compared with the Control group, which is similar to Eldawoody's research. The decline of serum estrogen can effectively reduce the protection on vessel and increase the incidence of IA [5]. At the same time, many studies have shown that the occurrence of IA is obviously promoted in menopausal women due to the significantly drop of their Estrogen levels [14]. Therefore, we believe that this intervention of remove the protection of estrogen conforms to the etiological mechanism of IA in human beings, which can also improve the success rate of IA rat model. Therefore, it should be one of the necessary conditions of building the IA rat model.

In this experiment we used the diet containing 3% methionine to feed the rats in the Novel Model group. After 6 months, their Hcy

increased to three times as much as the other two groups. The consequent symptoms are similar to those accompanying hereditary HHcy, which is attributable to the lack of cystathionine β-synthase [15]. This approach is with the same principle as Xu's research of feeding rats water with 1 g/kg/day methionine, but adding methionine to drinking water may produce an unpleasant, pungent odor which make the some rats circumvented by minimizing the intake of water, and eventually cause the serum level of Hcy to fluctuates greatly [6].

According to the result, the success rate of molding was markedly improved after rat taking extra methionine. Moreover, the formation rate of IA in stage III was significantly increased, which shows that HHcy can promote the formation of IA in rats. This result is correspond with a cohort study. Semmler et al [16] found that the polymorphism of Hcy metabolism related genes were relevant to Caucasian getting IA. Huo Y et al [10] studied on randomized clinical trials from the China Stroke Primary Prevention Trial (CSPPT) and found out a close bond between the increase in the plasma Hcy and the IA.

The promoting effect of HHcy on inducing IA in rats may be related to the following reasons. ① The pathophysiological changes caused by HHcy are closely related to the histological features of IA formation and rupture. One of the main histological features coupled with the formation of the IA is the attenuation of the tunica media and vascular remodelling. This feature is induced by the synergistic mechanism of vascular endothelial injuries, extracellular matrix deterioration and vascular smooth muscle apoptosis [15]. As found by Eberhardt RT et al [17], moderate amplification of the Hcy level can deactivate nitric oxide by generating reactive oxygen species, and hence trigger endothelial dysfunction. A higher level of Hcy can also contribute to the increased expression of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) which stimulates extracellular matrix degradation and vascular smooth muscle cell apoptosis [6, 18]. It hereby can be concluded that these pathophysiological alterations due to an increased level of Hcy are strongly connected with that main histological feature. ② Hcy can deterioate the inflammation in the vessel. In this experiment, the expression of MCP-1 and SOD in Novel Model group were significantly increased. This indicates that the level of inflammation was obviously increased, and the oxidative stress was aggravated In the HHcy rats. Evidences also suggest that Hcy is linked with various kinds of inflammatory mediators. Vilas-Boas et al [19] identified that mice with an elevated level of Hcy are plagued with a chronic inflammation, and the levels of tumour necrosis factor α (TNF-α) and the vascular cell adhesion molecule 1 (VCAM-1) escalate significantly. Likewise, Akalin et al [20] validate that the oxidative stress induced by HHcy can trigger a cascade reaction of inflammation. These mediators including MMP, the tumour necrosis factor, and oxygen radicals generate endothelial cell injuries or dysfunctions by oxidative stresses and inflammatory cell infiltration. Injured adhesion molecules of the endothelial expression and multiple chemokines further hasten the apoptosis of endothelial cells and the remodelling of vessels via mononuclear cell infiltration [20]. The final result was IA take shape. In addition, research indicates that the apoptosis of vascular smooth muscle cells (VSMC) in IA tissues can be clearly observed. The oxidative stresses of inflammatory mediators play a prominent role in inducing this symptom [21]. Which means HHcy may cause damage to cerebrovascular through inflammatory medium-oxidative stress mechanisms and promote IA come into being. ③ HHcy has synergistic injury effect with hypertension and hemodynamic changes. In Xu's experiment, IA can be promoted by the coexistence of HHcy and intracranial hemodynamic changes [6]. Another result reveals that this coexistence of hypertension and HHcy can promote each other and increasing the damage to vessel which finally promoting IA [17]. We think there are two reasons, for one thing, end-stage renal disease can be caused by prolonged high blood pressure, making glomerular filtration rate (GFR) reduced [22]. When the GFR is reduced, the excretion of Hcy will subsequently be lowered. Correspondingly, the level of Hcy will rise, so the vascular damage led by Hcy will deteriorate. For another, HHcy can paralyse the glomerulus and decrease the GFR by the activation of redox signalling pathways and DNA methylation, and further escalate blood pressure so as to make intracranial hemodynamic variations. The thorough mechanism forms a vicious circle to worsen vessels [23, 24]. These results add credence to the synergistic effect.

After 6 months molding, systolic blood pressure (SBP) in Novel Model group using methionine were higher than the Traditional Model group, which shows that HHcy can promote the blood pressure getting higher when HHcy and hypertension both exist.

Summary and prospect

In summary, a novel IA rat model was successfully constructed on the basis of Eldawoody's method by inducing HHcy, which enhanced the cooperative interaction between each hazard.

On the point of model preparation, this method accomplished ovary and renal arteries ligation through bilateral back incision instead of median abdominal incision, which can avoid the contact between incision and padding, and reduce the risk of infection. Meanwhile all the operation was completed in one surgery, which can avoid the risk of repeated surgical operation. Besides, the new way of feeding could control the methionine and serum Hcy intake of rat, which can reduce the workload, decreases the technical difficulties and expedite the recovery of the rats. Judging from the results, this novel method can improve the formation rate of induced IA as well as the incident of IA in stage III through the interaction of HHcy with hypertension, hemodynamic changes and estrogen deficiency.

Overall, this novel model is stable, reliable and with rather better reproducibility. Moreover, this modeling method is much closer to IA's forming process in human body which can help us conduct in-depth study. However the formation and rupture of IA are extremely complex and are closely related to age, smoke, obesity and atherosclerosis and so on [25-27]. The limitations of the article is that we didn't get involved in the above aspects in this experiment. Meanwhile, all the building factors considered in our research were all related to IA and had a interaction effect, but the theory was not distinct and the impact was not explicit, which need to be discussed and lucubrate further.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (1167-2332); the Scientific Research Project of Tianjin Health and Family Planning Commission (2015155); Tianjin Science and Technology Planning Project (15ZXLCSY00040, 16ZXHLSY00120); the Basic Research Project of Logistics University of PAP (WHJ201721, WHJ201728, WHJ201729); Tongji University "Chinese Universities Scientific Fund" (NO. 2212-0180282) and Medical Research Project of Hongkou District Health and Family Planning Commission (NO. HongWei 1802-06).

Disclosure of conflict of interest

None.

Address correspondence to: Shengkai Sun and Zhihong Wang, Tianjin Key Laboratory of Neurotrauma Repair, Affiliated Hospital of Logistics University of Chinese People's Armed Police Force, 220 Chenglin Road, Hedong, Tianjin 300162, P. R. China. Tel: +86-22-60578186; Fax: +86-22-60578186; E-mail: sskrui@163.com (SKS); wangzhihongyjb@163.com (ZHW)

References

[1] Vlak MH, Algra A, Brandenburg R and Rinkel GJ. Prevalence of unruptured intracranial aneurysms, with emphasis on sex, age, comorbidity, country, and time period: a systematic review and meta-analysis. Lancet Neurol 2011; 10: 626-636.

[2] Connolly ES Jr, Rabinstein AA, Carhuapoma JR, Derdeyn CP, Dion J, Higashida RT, Hoh BL, Kirkness CJ, Naidech AM, Ogilvy CS, Patel AB, Thompson BG and Vespa P. Guidelines for the management of aneurysmal subarachnoid hemorrhage: a guideline for healthcare professionals from the American heart association/American stroke association. Stroke 2012; 43: 1711-1737.

[3] Nagata I, Handa H, Hashimoto N and Hazama F. Experimentally induced cerebral aneurysms in rats: part VI. Hypertension. Surg Neurol 1980; 14: 477-479.

[4] Jamous MA, Nagahiro S, Kitazato KT, Satomi J and Satoh K. Role of estrogen deficiency in the formation and progression of cerebral aneurysms. Part I: experimental study of the effect of oophorectomy in rats. J Neurosurg 2005; 103: 1046-1051.

[5] Eldawoody H, Shimizu H, Kimura N, Saito A, Nakayama T, Takahashi A and Tominaga T. Simplified experimental cerebral aneurysm model in rats: comprehensive evaluation of induced aneurysms and arterial changes in the circle of Willis. Brain Res 2009; 1300: 159-168.

[6] Xu Y, Tian Y, Wei HJ, Dong JF and Zhang JN. Methionine diet-induced hyperhomocysteinemia accelerates cerebral aneurysm formation in rats. Neurosci Lett 2011; 494: 139-144.
[7] Hashimoto N, Kim C, Kikuchi H, Kojima M, Kang Y and Hazama F. Experimental induction of cerebral aneurysms in monkeys. J Neurosurg 1987; 67: 903-905.
[8] Morimoto M, Miyamoto S, Mizoguchi A, Kume N, Kita T and Hashimoto N. Mouse model of cerebral aneurysm: experimental induction by renal hypertension and local hemodynamic changes. Stroke 2002; 33: 1911-1915.
[9] Gao L, Hoi Y, Swartz DD, Kolega J, Siddiqui A and Meng H. Nascent aneurysm formation at the basilar terminus induced by hemodynamics. Stroke 2008; 39: 2085-2090.
[10] Huo Y, Li J, Qin X, Huang Y, Wang X, Gottesman RF, Tang G, Wang B, Chen D, He M, Fu J, Cai Y, Shi X, Zhang Y, Cui Y, Sun N, Li X, Cheng X, Wang J, Yang X, Yang T, Xiao C, Zhao G, Dong Q, Zhu D, Wang X, Ge J, Zhao L, Hu D, Liu L and Hou FF. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: the CSPPT randomized clinical trial. JAMA 2015; 313: 1325-1335.
[11] Jamous MA, Nagahiro S, Kitazato KT, Tamura T, Kuwayama K and Satoh K. Role of estrogen deficiency in the formation and progression of cerebral aneurysms. Part II: experimental study of the effects of hormone replacement therapy in rats. J Neurosurg 2005; 103: 1052-1057.
[12] Shimada K, Furukawa H, Wada K, Wei Y, Tada Y, Kuwabara A, Shikata F, Kanematsu Y, Lawton MT, Kitazato KT, Nagahiro S and Hashimoto T. Angiotensin-(1-7) protects against the development of aneurysmal subarachnoid hemorrhage in mice. J Cereb Blood Flow Metab 2015; 35: 1163-1168.
[13] Penn DL, Witte SR, Komotar RJ and Sander CE Jr. The role of vascular remodeling and inflammation in the pathogenesis of intracranial aneurysms. J Clin Neurosci 2014; 21: 28-32.
[14] Vlak MH, Rinkel GJ, Greebe P, Greving JP and Algra A. Lifetime risks for aneurysmal subarachnoid haemorrhage: multivariable risk stratification. J Neurol Neurosurg Psychiatry 2013; 84: 619-623.
[15] Rasmussen K and Moller J. Total homocysteine measurement in clinical practice. Ann Clin Biochem 2000; 37: 627-648.
[16] Semmler A, Linnebank M, Krex D, Gotz A, Moskau S, Ziegler A and Simon M. Polymorphisms of homocysteine metabolism are associated with intracranial aneurysms. Cerebrovasc Dis 2008; 26: 425-429.
[17] Eberhardt RT, Forgione MA, Cap A, Leopold JA, Rudd MA, Trolliet M, Heydrick S, Stark R, Klings ES, Moldovan NI, Yaghoubi M, Goldschmidt-Clermont PJ, Farber HW, Cohen R and Loscalzo J. Endothelial dysfunction in a murine model of mild hyperhomocyst(e)inemia. J Clin Invest 2000; 106: 483-491.
[18] Aoki T, Kataoka H, Morimoto M, Nozaki K and Hashimoto N. Macrophage-derived matrix metalloproteinase-2 and -9 promote the progression of cerebral aneurysms in rats. Stroke 2007; 38: 162-169.
[19] Vilas-Boas W, Veloso Cerqueira BA, Figueiredo CV, Santiago RP, da Guarda CC, Pitanga TN, Santana SS, Dias Zanette AM, Goncalves Mde S. Association of homocysteine and inflammatory-related molecules in sickle cell anemia. Hematology 2016; 21: 126-31.
[20] Akalin A, Alatas O and Colak O. Relation of plasma homocysteine levels to atherosclerotic vascular disease and inflammation markers in type 2 diabetic patients. Eur J Endocrinol 2008; 158: 47-52.
[21] Zhang HF, Zhao MG, Liang GB, Song ZQ and Li ZQ. Expression of pro-inflammatory cytokines and the risk of intracranial aneurysm. Inflammation 2013; 36: 1195-1200.
[22] Tozawa M, Iseki K, Iseki C, Kinjo K, Ikemiya Y and Takishita S. Blood pressure predicts risk of developing end-stage renal disease in men and women. Hypertension 2003; 41: 1341-1345.
[23] Lee ME and Wang H. Homocysteine and hypomethylation. A novel link to vascular disease. Trends Cardiovasc Med 1999; 9: 49-54.
[24] Yi F, Zhang AY, Li N, Muh RW, Fillet M, Renert AF and Li PL. Inhibition of ceramide-redox signaling pathway blocks glomerular injury in hyperhomocysteinemic rats. Kidney Int 2006; 70: 88-96.
[25] Rinkel GJ, Djibuti M, Algra A and van Gijn J. Prevalence and risk of rupture of intracranial aneurysms: a systematic review. Stroke 1998; 29: 251-256.
[26] Gu YX, Chen XC, Song DL, Leng B and Zhao F. Risk factors for intracranial aneurysm in a Chinese ethnic population. Chin Med J (Engl) 2006; 119: 1359-1364.
[27] Tromp G, Weinsheimer S, Ronkainen A and Kuivaniemi H. Molecular basis and genetic predisposition to intracranial aneurysm. Ann Med 2014; 46: 597-606.