ARTICULO ORIGINAL

Comparison of hemorheology and plasma contents of TXB2, 6-Keto-PGF1 alpha in model rats with three kinds of cerebral ischemia

Li Le-Jun,1,3 Tian Jin-Zhou,1,2 Yin Jun-Xiang,3 Shi Jing,1 Zhang Liu-Tong,2 Yang Chen-Zhi,1 Chen Yu-Jing,3 Zhang Lei-Ming,3

1 BUCM Neurology Centre, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China.
2 Department of Preclinical Medicine, Hubei College of Traditional Chinese Medicine, Wuhan, China
3 Laboratory of Pharmacology, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China.

Resumen

Objetivo: Para comparar características cambiantes hemorreológicas de la alfa TXB2 y 6 keto-PGF1 plasmáticas en modelo de ratas, de tres clases, con isquemia cerebral. **Materiales y métodos:** 128 ratones adultos del tipo Sprague-Dawley fueron divididos aleatoriamente en 4 grupos: uno con oclusión de la arteria cerebral media con hilo de rosca intraluminal (grupo MCAO); un grupo con ligadura bilateral de la arteria carótida común (grupo BCCA); un grupo con ligadura unilateral de la arteria carótida común (grupo UCCA) y un grupo normal de control (grupo NC). La sangre para la prueba hemorreológica de todas las ratas fue tomada de la aorta abdominal 24 hs posterior a isquemia cerebral y el índice hemorreológico fue calculado. El contenido plasmático de la alfa TXB2 y 6 keto-PGF1 fue detectado por radio-inmunidad. **Resultados:** El valor de la viscosidad de la sangre completa, el valor de la viscosidad del plasma, y el hematocrito fueron más altos en el grupo MCAO, seguido por los grupos BCCA y UCCA. El índice de deformidad del eritrocito en el grupo MCAO fue significativamente más bajo que el del grupo normal. Hubo diferencias significativas de los niveles plasmáticos de 6-keto-PGF1a, TXB2 entre el grupo MCAO y el control normal, pero no hubo diferencia entre los tres grupos modelos ($p>0.05$). **Conclusión:** El grupo MCAO fue el que mayormente contribuyó en los cambios hemorreológicos y en el contenido plasmático de TXB2, 6-keto-PGF1 alfa entre los tres modelos de rata con isquemia cerebral.

Palabras clave: Obstrucción de la arteria cerebral media, Ligadura bilateral de la arteria carótida común, Hemorreología, Tromboxano B2, 6 –keto-PGF1 alfa, Isquemia Cerebral.

Abstract

Objective: To compare changing features of hemorheological, TXB2 and 6-keto-PGF1 alpha in plasma in three kinds of model rats with cerebral ischemia. **Materials and Methods:** 128 adult male Sprague-Dawley rats were randomly divided into four groups: a middle cerebral artery occlusion with intraluminal thread group (MCAO-group), a bilateral common carotid artery ligation group (BCCA-group), a unilateral common carotid artery ligation group (UCCA-group) and a normal control group (NC-group). Blood for hemorheological testing of all rats was taken from abdominal aorta 24h following cerebral ischemia and hemorheological index was determined. Plasma contents of TXB2 and 6-keto-PGF1 alpha were detected by radio-immunity. **Results:** The whole blood viscosity value, plasma viscosity value, and hematocrit were higher in MCAO-group among three model groups, followed by BCCA-group and UCCA-group. The deformity index of RBC in MCAO-group was significantly lower than that in normal-group. There were significant differences for plasma contents of TXB2 and 6-keto-PGF1 alpha among the three model groups and the normal group. There were significant differences for plasma level of 6-Keto-PGF1a, TXB2 between MCAO-group and the normal group, but no difference among the three model groups($p>0.05$).**Conclusion:** MCAO was the greatest in contribute to changes of hemorheology and plasma contents of TXB2, 6-keto-PGF1 alpha among three rat models with cerebral ischemia.

Keywords: Middle cerebral artery occlusion (MCAO), Bilateral common carotid artery ligation (BCCA),Hemorheology, Thromboxane B2, 6-keto-PGF1 alpha, Cerebral ischemia.

Introducción

In previous studies, we have investigated enhanced thromboxane (TX) biosynthesis, as reflected by the urinary excretion of a major TXA2 metabolite, 11-dehydro-TXB2, in patients with acute ischemic stroke. Increased TX production was found to occur episodically during the first 2 to 3 days after the onset of ischemic stroke,1,3 a noninvasive index of platelet activation, was present in the chronic phase after a transient ischemic attack (TIA) or stroke.1,3.

It has been demonstrated that plasma indexes of 6-Keto-PGF1a/TXB2 disequilibrium or hemorrheological

abnormalities possibly participate in nosogenic patho-physiological mechanisms of cerebrovascular disease.4,8

For the restriction of clinical research, rat models with cerebral ischemia has become an indispensable tool in the study of mechanism and prevention of cerebral disease. It is important for hemorrheological detection in the onset, prognosis and prevention of cerebrovascular disease (CVD).9,10 As is known to now, TXB2 is one Vol. 18, No1-2, 2009 / Revista Ecuatoriana de Neurología 33 of the strongest vaso-excitor material(VEM) and platelet aggregation agent that can promote thrombosis. As a platelet functional inhibitor, 6-Keto-PGF1 alpha has protective effect for vasospasm caused by platelet aggregation. They keep balance under physiological status.4,5

The purpose of the study is to monitor hemorrheology and plasma contents of TXB2 and 6-Keto-PGF1 alpha 24 hours after cerebral ischemia using middle cerebral artery occlusion (MCAO) model, bilateral common carotid artery ligation (BCCA) model, and unilateral common carotid artery ligation (UCCA) model, to find difference between them and to screen the most similar clinical feature model.

Materials and methods

Experimental Procedures.

Animal protocols were approved by the Stanford University Administrative Panel on Laboratory Animal Care. Institutional guidelines were followed in all protocols. All animal experiments were conducted in accordance with the NIH guide for the care and use of laboratory animals (NIH publication 80-23). All efforts were made to minimize animal suffering, and only the smallest numbers of animals were used to generate reliable scientific data.

Animals and Experimental groups. Adult male Sprague-Dawley rats, weighing 200-250 g, were obtained from the Experimental Animal Center of Beijing University, China. They were maintained under controlled lighting (lights on 07:00–19:00 h) and temperature (22º C) and given free access to water and the commercial laboratory rodent diet. Twelve hours prior to experiment, the rats were fasted, but allowed free access to water. They were randomly divided into four groups (32 rats per group): MCAO group, BCCA group, UCCA-group and normal group.

Middle cerebral artery occlusion model. Male Sprague-Dawley rats weighing between 290 and 320 g (Charles River, Wilmington, Del) were anesthetized with 3% halothane by facemask and were subsequently maintained with 1% halothane in 200 ml/min oxygen and 800 ml/min air. Depth of anesthesia was assessed every 15 min by hind-limb pinch. A thermistor probe was inserted 50 mm into the rectum and rectal temperature was maintained between 36.5ºC and 37.5ºC during ischemia. ECG leads were placed to monitor heart rate and respirations. Physiological parameters were monitored every 15 min and maintained in the normal range throughout surgery. The MCA was occluded using an intraluminal suture pre viously used by our lab.11,12 In brief, a midline incision was made in the neck to expose the common carotid (CCA), external carotid (ECA), internal carotid (ICA), and pterygopalatine (PPA) arteries. The CCA, ECA, and PPA were ligated with a 6-0 silk suture. Ischemia was induced by inserting an uncoated, 30-mm long segment of 3-0 nylon monofilament suture (tip rounded by flame) 19-20 mm from the bifurcation of the CCA to induce ischemia in the arterial territory supplied by the MCA. After 2 h of ischemia, the suture was removed and the animal was allowed to recover. After recovering from anesthesia, the rats were allowed free access to food and water. Behavioral tests were performed in rats by Zea longa 5 cent method of preparation, the method for assessing praxiology of rat, animals with 1-3 cents will be selected.13

Bilateral common carotid artery ligation model. All procedures were performed under anesthesia with the intraperitoneal injection of pentobarbital (50mg/kg). For chronic bilateral occlusion, five days prior to angiography, the left CCA was exposed through a midline cervical incision
under anesthesia and then it was ligated by 6-0 nylon suture, and cut by microscissors. The wound was thereafter
closed with a suture.14

After recovering from anesthesia, the rats were allowed free access to food and water. All rats were observed at room temperature for 24h.

Unilateral common carotid artery ligation model. All procedures were performed under anesthesia with the intraperitoneal injection of pentobarbital (50mg/kg). For acute unilateral occlusion, the right ICA was carefully exposed through a midline cervical incision, and a microsurgical clip (Zen temporary clip, Oowa-Tsusho, Tokyo, Japan) was applied to the proximal part of the ICA to acquire the images of immediately after occlusion. After recovering from anesthesia, the rats were allowed free access to food and water.14

Statistical analysis. Statistical analyses for continuous data were performed using a one-way analysis of variance followed by a multiple comparison procedure (Bonferroni post-hoc test). All data are expressed as mean ±S.E.M. $P < <0.05$ was considered significant.

Main instruments and reagent. Heparin (Jiangsu wanbang biochemistry medicine limited company, Batch No: 0507110,12500u),red cell deformity reagent (Peking sidi scientific instrument company , Batch No: 060628, 100ml/bot). The LG- R-80 type blood viscosity tester (Peking sidi scientific instrument company), the LG- B-190 type cytomorphosis/ aggregation tester (Peking sidi scientific instrument company), GL-20 type completely automated high speed refrigerated centrifuge (xiangxi instrument gauge total factory), thromboxanB2 (TXB2) and 6-keto-prostacyclinF1a radio-immunity kit (Peking kemeidongya biotechnology limited company).

Hemorheological detection. Blood samples were collected from abdominal aorta 24h after cerebral ischemia. The index of hemorheolgy included the whole blood viscosity, reduced viscosity, plasma viscosity, hematocrit, the index of red cell deformity.

item / group	whole blood viscosity ($mPa\cdot s/150s^{-1}$)	whole blood viscosity ($mPa\cdot s/5s^{-1}$)	plasma viscosity ($mPa\cdot s$)	HCT (%)	Index of RBC deformation	Index of RBC aggregation
Normal	4.14±0.341	16.82± 1.091	1.56± 0.165	41.0± 3.51	0.51± 0.018	1.21±0.161
UCCA	5.22±0.435*	17.88± 1.489	1.79± 0.168*	47.1± 5.18*	0.48± 0.015*	1.36±0.197
BCCA	4.56±0.392*	19.76± 1.251*	1.80± 0.184*	47.4± 3.44*	0.48± 0.037*	1.35±0.195
MCAO	6.06±0.578*	27.12± 2.342**	1.81± 0.195*	47.5± 5.28*	0.47± 0.072*	1.38±0.199

Table 1: Comparison of hemorheological index after cerebral ischemia 24h between 3 model groups with normal group. Mean±SEM. n=8. *P<0.05, **P<0.01 vs normal group

All rats abstained from food but could not refrain from water 24h before taking blood sample. They were anesthetized with 10% Chloral Hydrate intraperitoneal injection the next day, taking blood sample from abdominal aorta. 6ml blood sample was kept in an anticoagulation cuvette with heparin 100μl inside, with LG- R-80 type blood viscosity tester and LG- B-190 type cytomorphosis/aggregation tester , at 37 ºC ± 0.1. Whole blood hypsi-tomy coefficient of viscosity (shear rate 150s-1),hypo-tomy coefficient of viscosity (shear rate 5s-1), plasma viscosity, index of red blood cell deformation and aggregation were detected. The HCT determination used decigram method centrifugation in common temperature with rotary speed 3000 r/min for 30 min.

Determination plasma contents of TXB2, 6-Keto-PGF1a. 24h after cerebral ischemia, blood samples from abdominal aorta were taken from all rats. Each group of rats abstained from food but could not refrain from water 24h before taking blood sample. Next day, 10%Chloral Hydrate was used to anesthetize. Through abdominal cavity,
abdominal aorta blood sample was taken: 3ml in anticoagulation
cuvette with 0.2 ml 10%EDTA. After misce bene, GL-20 type completely automated high speed refrigerated centrifuge was used , 4°C, 3500 r/min, for 15 min, and conserved at -20°C for use. Determination for plasma contents of TXB2 and 6-Keto-PGF1a with radio-immunity proceeded according to description of kit.

Results

1.Comparison of hemorheological index after 24 hours of cerebral ischemia between 3 model groups and normal group.

There were significant differences for indexes of rats between the three cerebral ischemia groups and the normal group($P<0.05$). The whole blood viscosity value, plasma viscosity value and hematocrit of rats in MCAO- roup were the highest among the three model groups, their values were (19.29±2.996,mPa•s/5s-1, (1.812±0.348) mPa.s and (47.5±5.28)%. Meanwhile the deformity index of RBC 0.47±0.017, of rats in MCAO-group was significant lower than that of rats in normal-group, ($P<0.05$).

The results showed that the expression of Erythrocyte aggregation in 3 model groups was enhanced but there was no difference between 3 model groups. ($P>0.05$). There were no significant differences for other index of hemorheological change in rats among the three cerebral ischemia groups ($P>0.05$). Results are listed in Table1.

2. Comparison of plasma contents of TXB 2, 6-Keto-PGF1a after 24 hours of cerebral ischemia, between 3 model groups and normal group.

The results showed that the expression of TXB2 in 3 model groups was enhanced, but plasma contents of 6-Keto-PGF1 was lower. There was significant differece between 3 model groups and normal group (P<0.05). The content of TXB2 of MCAO-group was the highest in the three cerebral ischemia groups. There was significant difference for plasma contents of TXB2 between MCAO-group and UCCA group (P<0.05), but no significant difference for plasma contents of TXB2 between MCAO-group and BCCA group (P>0.05).

There was no significant difference for plasma contents of 6-Keto-PGF1a between three cerebral ischemia groups (P>0.05). Results are listed in Figure 1.

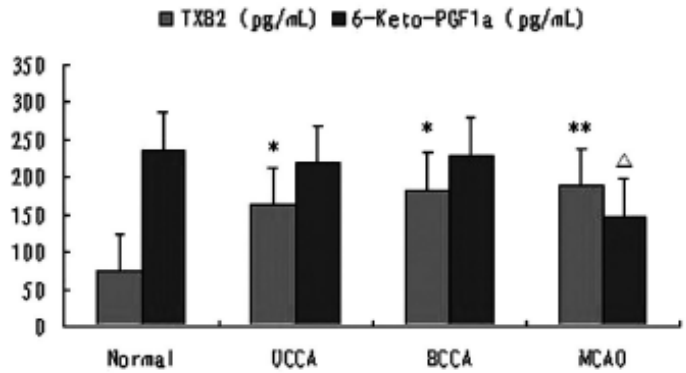

Figure1: Comparison of plasma contents of TXB2, 6-Keto-PGF1a after cerebral ischemia 24h between 3 model groups with normal group. Mean ± SEM. n=8. *P<0.05, **P<0.01 vs normal group.

MCAO-group vs UCCA-group, ˆP<0.05.

3. Comparison of plasma indexes of 6-Keto-PGF1a/TXB2 after 24 hs of cerebral ischemia between 3 model groups with normal group.

Based on results of plasma contents of TXB2,6-Keto-PGF1a 24hs after cerebral ischemia, ratio of 6-Keto-PGF1a and TXB2 was figured out. Results showed that plasma indexes of 6-Keto-PGF1a/TXB2 of three cerebral ischemia groups were lower than that of normal group, but there was significant difference of plasma indexes of 6-Keto-PGF1a/TXB2 between MCAO-group and normal group (P<0.05). There was no significant difference among the three cerebral ischemia groups (P>0.05). Result are shown in Figure 2.

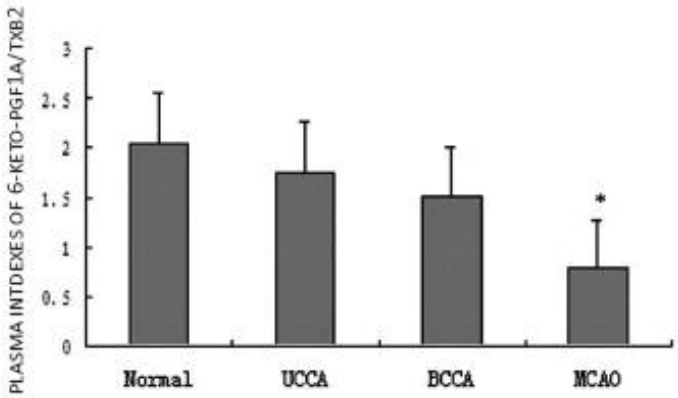

Figure 2: Comparison of plasma indexes of 6-Keto-PGF1a/TXB2 24h after cerebral ischemia between 3 model groups with normal group.

Mean±SEM. n=8. *P<0.05, **P<0.01 vs normal group.

Discussion

Hemorheological detection is important in the course of disease, including nosogenesis, development, prognosis, turnover, as well as curative effect assessment, especially for prevention of cardiocerebrovascular disease. Brain microcirculation slow down or stagnation is the main pathological change at the beginning of cerebral ischemia4 TXB2 is one of the strongest vaso-excitor material (VEM) and platelet aggregation agent at present. 6-Keto-PGF1a is the platelet function inhibitor, have protective effect for vasospasm caused by platelet aggregation, they keep balance under physiological status.4,5,15 Disequilibrium of TXA2 and PGI2 caused by cerebral ischemia is one of the reasons of platelet aggregation, vasospasm and thrombogenesis.

There were significant differences between the three cerebral ischemia groups and normal group for indexes of whole blood viscosity value, plasma viscosity value, hematocrit and plasma contents of TXB2, 6-Keto-PGF 1a, plasma indexes of 6-Keto-PGF1a/TXB2($P<0.05$). Results showed that disequilibrium of TXA2 and PGI2 is included in the of cerebrovascular disease, which is similar with that of abroad6,8 The whole blood viscosity value, plasma viscosity value, hematocrit and plasma contents of TXB2 of rats in MCAO-group were the highest among the three model groups. Index of red blood cell deformation is the lowest in MCAO group. There is significant difference of rats between MCAO-group and normal group with plasma indexes of 6-Keto-PGF1a/TXB2 ($P<0.05$). This may happen because model of MCAO bring damage to the brain most, but there were no significant difference for indexes of hemorrheology and plasma contents of TXB2,6-Keto-PGF1a between the three cerebral ischemia groups ($p>0.05$). Although different models bring out different degree of injury to the brain, all 3 models have effect on hemorrheology, plasma of TXB2 and 6-the Keto-PGF1a. It has been demonstrated that hemorrheology abnormality or plasma indexes of 6-Keto-PGF1a/TXB2 disequilibrium are important in cerebrovascular disease,4,5,15 to display the abnormal hemorrheological changes: TXB2 obviously to heighten, but 6-Keto-PGF1a to reduce or change slightly.

Results showed that abnormal change of hemorrheology – increased TXB2 and reduced plasma indexes of 6-Keto-PGF1a/TXB2- after cerebral ischemia, is similar to domestic and abroad result,6,7,16,17 Cerebrovascular diseases often affect latero-vascular territory. MCAO-model (middle cerebral artery occlusion model) has been accepted by international clinics, therefore being the most suitable cerebral ischemia model, which shows obvious changes in hemorheology and plasma contents of TXB2, 6-Keto-PGF1a and has characteristic features of cerebrovascular disease.

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