Original Article
The serum levels of MMP-9, MMP-2 and vWF in patients with low doses of urokinase peritoneal dialysis decreased uremia complicated with cerebral infarction

Shu-Jin Wang[1*], Zhong-Sen Qu[2*], Qing-De Zhang[3*], Liang Li[4], Feng Wang[2], Bin Zhang[2], Bang-Li Wu[1], Yu-Wu Zhao[2]

[1]Department of Neurology, The First Hospital of Zibo Affiliated Weifang Medical University, Zibo 25520, China; [2]Department of Neurology, Shanghai Jiao Tong University Affiliated The Sixth People's Hospital, Yi Shan Road 600, Shanghai 200233, China; [3]Department of Internal Medicine, Heze High Medical School, Heze 274030, China; [4]Department of Neurology, Qingdao University Affiliated The Central Hospital, Qingdao 266042, China. *Equal contributors.

Abstract: To investigate the effect of MMP-9, MMP-2 and vWF in patients with low doses of urokinase peritoneal dialysis decreased uremia complicated with cerebral infarction. 112 cases of uremia complicated with cerebral infarction were randomly divided into the peritoneal dialysate with urokinase treatment group (66 cases) and the conventional treatment group (46 cases). At the same time, 50 cases of healthy people who were more than 45 years old were enrolled in the control group. The basic treatment in both treatment groups was the same. In urokinase therapy group based on the conventional treatment, urokinase was added into peritoneal dialysis fluid, and changes of serum MMP-9, MMP-2 and vWF were observed by drawing blood at different time points within 8 weeks. The changes of serum MMP-2, MMP-9 and vWF were detected by enzyme-linked immunosorbent assay. At the time of the onset of uremia complicated with cerebral infarction patients the serum MMP-9, MMP-2, vWF were significantly higher ($P<0.05$, $P<0.05$, $P<0.01$). Conventional antiplatelet therapy in brain protection only reduce MMP-9 to the normal range ($P>0.05$) within 8 weeks. But the MMP-2 and vWF cannot be reduced to the normal range ($P<0.01$, $P<0.01$). Low doses of urokinase can reduce MMP-9 (7 d) and MMP-2 (14 d) to the normal range ($P>0.05$, $P>0.05$) at the early stage and decrease the vWF to a normal range within 8 weeks ($P>0.05$). At the time of the onset of uremia complicated with cerebral infarction patients the serum MMP-9, MMP-2 and vWF increased significantly. Low doses of urokinase dialysis can reduce serum MMP-9, MMP-2, and vWF in acute uremia complicated with cerebral infarction without recurrence of cerebral infarction and cerebral hemorrhagic transformation, indicating that low dose of urokinase peritoneal dialysis may have a certain effect on the early treatment of this disease.

Keywords: Uremia, infarction, urokinase, matrix metalloproteinases, von Willebrand factor

Introduction

Endothelial dysfunction can promote the occurrence of cerebral infarction. Matrix metallo proteinases (MMPs) plays an important role in maintaining endothelial stability, which is the important medium for the degradation and remodeling of extracellular matrix (ECM). Especially MMP-2 and MMP-9, can dissolve collagen ingredients, increase plaque instability, degrade ECM, destroy the integrity of cerebrovascular and cause serious damage to the blood brain barrier, playing an important role in the pathogenesis of cerebral vascular disease

[1]. Research confirmed that: in patients with acute cerebral infarction in cerebral artery area, plasma MMP-9 levels were significantly increased within 24 h after onset [2]. And MMP-9 levels were related with disease severity and infarct size [3]. In addition, Reynolds et al suggested that: S-100 protein, B-nerve growth factor, vWF, MMP-9 and monocyte chemoattractant factor-1 had the closest correlation with acute cerebral infarction diagnosis. MMP-9 and vWF combined with CT examination can be used as sensitive index for early diagnosis of ischemic stroke [4]. As for the MMP-9 in Human Kidney aspects: Ebihara et al demonstrated

that: MMP-9 mRNA expression in peripheral blood mononuclear cells increased in patients with HD and CAPD and increased more obvious in HD patients, which may be associated with atherosclerosis [5]. In CKD patients (stage 3 and 4), serum MMP-9 level was significantly elevated, while it was reduced in patients with CKD of stage 5 [6]. The changes of MMP-9 in patients uremia combined with cerebral infarction and in CAPD process are unclear.

Data have confirmed that MMP-2 plays an important role in the BBB opening at early stage of cerebral ischemia [7]. In cultured human umbilical vein endothelial cells, in four hours of hypoxia, followed by 4 h or 24 h reoxygenation process, secretion of MMP-2 and its inhibitor TIMP-2 was significantly increased, suggesting that they played an important role in ischemia- reperfusion injury [8]. Another study confirmed that in MMP-2 knockout, or MMP-2/MMP-9 knockout mice with artery occlusion, cerebral infracted cortex and striatum area decreased, and the cerebral hemorrhage and bleeding volume were reduced in ischemia-reperfusion, indicating that: at early stage of cerebral ischemia and/or reperfusion, MMP-2 or MMP-2/MMP-9 inhibition could reduce hemorrhagic transformation more effectively than MMP-9 inhibition [9]. In terms of kidney disease, MMP-2 activity and expression in CKD patients were higher than that in the control group [10]. Moreover, microvascular MMP-2 levels in dialysis patients were higher than those in CKD patients and healthy donors [11]. In addition, in non-dialysis CKD patients, serum MMP-2 was significantly increased in patients with carotid artery plaque compared with those without plaque, and systolic blood pressure and serum levels of MMP-2 were independently related with carotid intima-media thickness (IMT) [12]. The changes of serum MMP-2 in patients with uremia cerebral infarction and CAPD patients are also unclear, which are also concerned in this study.

In addition, in the acute phase of ischemic stroke, due to the endothelial dysfunction and thrombosis, vWF increased [13]. In patients with carotid and aortic arch calcified TIA and ischemic stroke, serum levels of VWF significantly increased. In ischemic stroke patients with artery atherosclerosis, vWF was significantly higher than that in patients with other TOAST subtypes [14]. In type 2 diabetes pati-

ents with CAPD, carotid intima-media thickness was increased compared with patients with non-type 2 diabetes, and vWF levels were aso increased [15]. Moreover, in patients with kidney disease needing dialysis, vWF, intercellular adhesion molecule 1 (ICAM 1) and thrombomodulin increased [16]. Therefore, we speculated that vWF would play an important role in the pathogenesis of uremia combined with cerebral infarction.

In summary, we speculate that MMP-9, MMP-2 and vWF might play an important role in uremia combined with cerebral infarction. In the treatment of cerebral infarction, t-PA is the only drug for acute treatment approved by FDA, but the time window is only 3-4 hours; after t-PA thrombolysis, recanalization will further activate MMP-9, leading to the conversion of ischemia stoke to hemorrhagic stroke. The application of t-PA after infarction enlarges the overall levels of MMP-9 [17]. Thus, MMP-9 inhibitor will play an important role in the treatment of acute ischemic stroke. In rat models with embolic cerebral artery occlusion, Lipitor combined with t-PA will extend the therapeutic time window to six hours, without increase in hemorrhagic transformation. And this will reduce the t-PA therapy-induced MMP-9 upregulation [18]. Urokinase has long been reported in the treatment of acute cerebral infarction [19]. And intravenous urokinase combined with low-dose tPA is effective in cerebral infarction [20]. The mechanism is not clear how. Whether there is a role of inhibition of MMPs is our interest. Recent studies have confirmed that: In the progressed plaque cells, serum uPA and uPAR were highly expressed. On the cell surface, uPA binds to urokinase receptor with high affinity, and provides accurate positioning for ECM proteolysis, indicating that urokinase and its receptor may play a role in atherosclerotic disease [21]. In addition, in a successful venous thrombolysis-reperfusion period, VWF antagonists in combination with tPA can prevent microvascular thrombosis and reduce thrombosis recurrence or a second stroke [22].

Above studies suggest that after tPA thrombolysis, the increased MMPs and VWF will increase the risk of brain hemorrhage; application of MMPs and VWF inhibitors could prolong therapy time window, reduce thrombosis, and release cerebral edema. However, uremia is a kind of thrombolysis contraindications in China;

is there other alternative therapeutic approach? Therefore, this study examined the the dynamic changes of serum MMP-2, MMP-9 and vWF before and after low dose of urokinase PD in patients with uremic cerebral infarction, to explore the effect of urokinase on MMP-2, MMP-9 and vWF and to explore its possible role in the treatment of uremic cerebral infarction.

Materials and methods

Clinical data

In this study 112 cases of uremia complicated with cerebral infarction patients from January 2013 to December 2013 were enrolled according to the clinical diagnostic criteria. Which were approved by both hospital ethics committee and patients or their families. According to the symptoms, signs and cranial CT and MRI diagnosed brain infarction, cerebral infarction was confirmed onset for the first time [23], and they were randomly divided into conventional treatment group and urokinase treatment group. Balanced inspections were carried on sex, age, clinical manifestations, renal function and NIHSS score and there was no statistical significance (P>0.05).

Exclusion criteria

Intracranial infection, cerebral hemorrhage and subarachnoid hemorrhage, without cancer, without blood diseases and bleeding tendency, no history of rheumatic heart disease and atrial fibrillation, no history of liver disease and respiratory system, one month before the experiment no surgery and history of trauma, no history of without autoimmune disease but taking immunization inhibitor drugs, and no history of taking anti-free radicals drugs were excluded.

We chose at the age of 45-year-old normal 50 cases of healthy people at same stage for control group. Clotting 4 items, blood pressure, blood lipids, blood glucose, liver and kidney function and heart and lung function were normal. We only detect the corresponding value as a control without making any deal with the healthy control group.

Methods

Treatment: Tenchoff tubes were used in all patients with peritoneal dialysis. Catheter incision were carried out conventionally. American

Baxter's O-pipes and peritoneal fluid were used for dialysis. Intermittent peritoneal dialysis (IPD) treatment were carried out for the first 3~5 d at the beginning. Continuous ambulatory peritoneal dialysis (CAPD) was then carried out. Each group was 2 000 ml and 4 times a day. In the conventional treatment group peritoneal dialysis was carried out and did antiplatelet therapy for cerebral protection (Aspirin 100 mg/qn/d, Nimotop 30 mg/tid/d) in order to control blood pressure, diuretic swelling, maintain water, electrolyte, acid-base balance; Based on conventional therapy, peritoneal dialysate with 100,000 IU urokinase was added to urokinase treatment group. Twice in daily treatment and eight weeks for a cycle treatment in both groups.

Sample collection: In the healthy control group, fasting 12 h before blood sampling and take 2 ml blood in the morning. Take fasting blood 2 ml 24 h, 3 d, 7 d, 14 d, 8 w later in the morning. In the experimental group onset we took 4 ml fasting venous blood onset within 4.5 h and 6 h, 12 h, 24 h, 3 d, 7 d, 14 d, 8 w after onset. 2 ml was taken into the coagulant tubes (BD Company) with 1500 g centrifugation 10 min at 4°C. They were frozen at -20°C refrigerator and then reserved at -80°C refrigerator.

Serum MMP-2, MMP-9, vWF detection: Take serum samples of patients which were stored in -80°C refrigerator freezer and detected MMP-2, MMP-9, vWF by enzyme-linked immunosorbent assay (The kit belongs to R & D System, USA). Serum MMP-2, MMP-9, vWF were measured onset within 4.5 h and 6 h, 12 h, 24 h, 3 d, 7 d, 14 d, 8 w after onset.

Statistical analysis

All data were expressed by mean ± standard deviation and analyzed using SPSS 13.0 software package. The average number was compared using ANOVA. Paired t-test was used for comparison on different time points within groups. The level of significance was set at P<0.05.

Results

Patients' basic clinical condition in the three groups

In conventional treatment and urokinase group, carotid plaques, fasting glucose (P<0.05), Total

Table 1. Patients' basic clinical condition in three groups

Group	Control group (A)	Conventional treatment group (B)	Urokinase therapy group (C)
Male/female	28/22	29/17	41/25
Age	52.4 ± 5.2	56.7 ± 6.6	58.1 ± 7.8
Glucose (mmol/L)	5.28 ± 0.88	8.67 ± 1.82*	7.84 ± 1.67*
SBP (mmHg)	128.6 ± 12.1	168.9 ± 21.4**	176.7 ± 19.6**
DBP (mmHg)	73.4 ± 9. 2	96.3 ± 11. 8*	94.2 ± 10.9*
Total cholesterol (mmol/L)	5.13 ± 0.82	7. 24 ± 1.46*	6. 94 ± 1.27*
Triglycerides (mmol/L)	1. 58 ± 0.32	2. 43 ± 0.49*	2.89 ± 0.57*
WBC (×10^9/L)	6. 12 ± 1.35	9.96 ± 2.48*	11.01 ± 2.24*
The number of carotid artery plaque	2	26	34
NIHSS score	0	7.4 ± 2.2	8.7 ± 2.6

*$P<0.05$, **$P<0.01$ compared with the control group.

Table 2. Dynamic changes of serum MMP-9 levels in patients with cerebral infarction ($\bar{x} \pm s$) (µg/L)

Group	Control group	Conventional treatment group	Urokinase therapy group
	134.76 ± 47.34	-	-
<4.5 h	-	164.41 ± 54.62*	159.35 ± 49.14#
6 h	-	208.48 ± 86.34**,★★	189.64 ± 78.53##,▲
12 h	-	275.65 ± 116.79**,★★	211.44 ± 91.68##,▲▲,△△,◆◆
24 h	136.24 ± 43.42	298.54 ± 123.17**,★★	246.85 ± 101.45##,▲▲,△△,◆◆
3 d	134.37 ± 42.39	323.69 ± 138.76**,★★	229.38 ± 98.96##,▲▲,△△,◆◆
7 d	141.38 ± 39.46	284.32 ± 121.24**,★★	160.35 ± 70.14△△
14 d	131.97 ± 36.38	217.43 ± 91.51**,★★	152.35 ± 52.45△△
8 w	137.19 ± 41.32	151.46 ± 49.18	146.43 ± 39.52

*$P<0.05$, #$P<0.05$ compared with the health control group, ▲$P<0.05$ compared with 4.5 h and 8 w groups, **$P<0.01$, ##$P<0.01$ compared with the health control group, ★★$P<0.01$ compared with 4.5 h group, △△$P<0.01$ compared with the conventional control group, ▲▲$P<0.01$ compared with 4.5 h group, ◆◆$P<0.01$ compared with 4.5 h, 7 d, 14 d, 8 w groups.
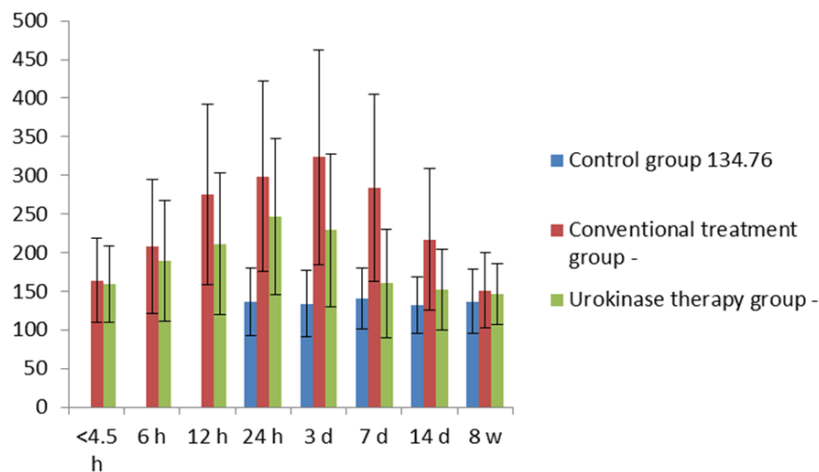

Figure 1. Dynamic changes of serum MMP-9 levels in patients with cerebral infarction. ($\bar{x} \pm s$) (µg/L).

cholesterol ($P<0.05$), triglycerides ($P<0.05$) levels and WBC counts were higher than the control group. Both systolic and diastolic blood pressure were significantly higher than the control group ($P<0.05$, $P<0.01$, **Table 1**).

Dynamic changes of serum MMP-9 level in patients with cerebral infarction

This study showed that at the time of the onset of uremia complicated with cerebral infarction patients (<4.5 h) the serum MMP-9 level increased significantly ($P<0.05$). The serum MMP-9 level in the conventional group continuously increased from 4.5 h onset to 14 d ($P<0.05$, $P<0.01$), and it decreased to normal range at 8 w onset ($P>0.05$). Compared with the healthy group, in urokinase treatment group the serum MMP-9 level increased significantly at the onset from 4.5 h to 3 d ($P<0.05$, $P<0.01$), and it

Table 3. Dynamic changes of serum MMP-2 level in patients with cerebral infarction ($\overline{x} \pm s$) (μg/L)

Group	Control group	Conventional treatment group	Urokinase therapy group
	128.34 ± 34.35	-	-
<4.5 h	-	163.94 ± 56.12*	158.42 ± 48.35#
6 h	-	170.79 ± 74.84*	154.53 ± 51.36#
12 h	-	182.49 ± 86.56**	163.48 ± 77.52#
24 h	120.47 ± 36.49	243.19 ± 101.23**,★★	186.21 ± 84.47##,△△,▲,◆◆
3 d	132.73 ± 33.32	346.53 ± 128.68**,★★	232.78 ± 97.69##,△△,▲▲
7 d	122.85 ± 29.43	376.92 ± 131.29**,★★	254.96 ± 112.84##,△△,▲▲
14 d	131.64 ± 41.38	268.36 ± 107.89**,★★	148.66 ± 46.32△△
8 w	128.19 ± 38.32	186.46 ± 89.64**	138.84 ± 39.86△△

*P<0.05, #P<0.05 compared with the health control group. ▲P<0.05 compared with 4.5 h, 6 h and 14 d group. **P<0.01, ##P<0.01 compared with the health control group, ★★P<0.01 compared with 4.5 h, 6 h, 12 h and 8 w groups, △△P<0.01 compared with the conventional control group, ◆◆P<0.01 compared with 8 w group, ▲▲P<0.01 compared with 4.5 h, 6 h, 12 h, 14 d, 24 h and 8 w groups.
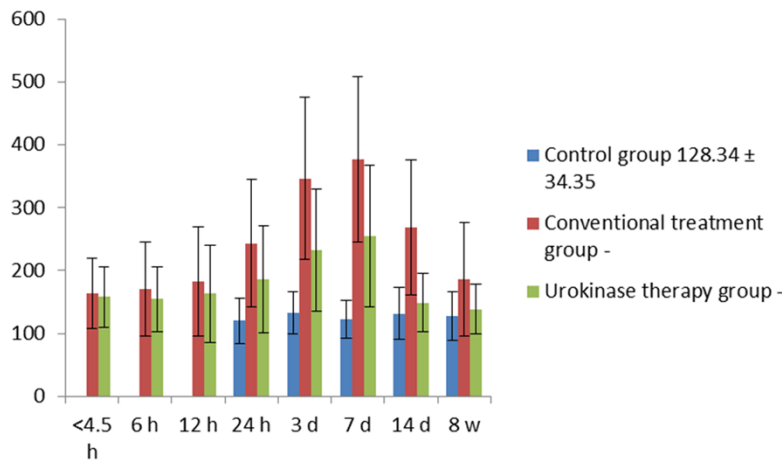

Figure 2. Dynamic changes of serum MMP-2 level in patients with cerebral infarction ($\overline{x} \pm s$) (μg/L).

was significant lower than the conventional group between 12 h and 7 d (P<0.01). At the onset 6 h-3 d it was significant increased compared with that at 4.5 h (P<0.05, P<0.01). It decreased to the same as the control group level at 7 d (P>0.05). This results showed that compared with the conventional group, peritoneal dialysate with urokinase can decrease the serum MMP-9 level at an early stage (**Table 2**; **Figure 1**).

Dynamic changes of serum MMP-2 level in patients with cerebral infarction

This study showed that at the time of the onset of uremia complicated with cerebral infarction

patients (<4.5 h) the serum MMP-2 level increased significantly (P<0.05). The serum MMP-2 level in the conventional group continuously increased from 4.5 h onset to 8 w (P<0.05, P<0.01). Compared with the healthy group, in urokinase treatment group the serum MMP-2 level increased significantly at the onset from 4.5 h to 7 d (P<0.05, P<0.01), and it was significant lower than the conventional group between 12 h and 8 w (P<0.01). At the onset 6 h-3 d it was significant increased compared with that at 4.5 h (P<0.05, P<0.01). It reached its peak at 3 d-7 d, which was its highest point (P<0.05, P<0.01) and it decreased to normal range at 14 d (P>0.05). This results showed that compared with the conventional group, peritoneal dialysate with urokinase can decrease the serum MMP-2 level at an early stage (**Table 3**; **Figure 2**).

Dynamic changes of serum vWF level in patients

This study showed that at the time of the onset of uremia complicated with cerebral infarction patients (<4.5 h) the serum vWF level increased significantly (P<0.05). The serum vWF level in the conventional group continuously increased from 4.5 h onset to 8 w (P<0.05, P<0.01) and it increased to its peak at 12 h-3 d. Compared with the healthy group, in urokinase treatment group the serum vWF level increased significantly at the onset from 4.5 h to 14 d (P<0.05, P<0.01), and it was significant lower than the conventional group between 6 h and 8 w (P<0.01). It reached its peak at 12-24 and decreased to normal range at 8 w (P>0.05). This results showed that compared with the conventional group, peritoneal dialysate with urokinase can decrease the serum vWF level at an early stage (**Table 4**; **Figure 3**).

Table 4. Dynamic changes of serum vWF level in patients ($\overline{x} \pm s$) (μg/L)

Group	Control group	Conventional treatment group	Urokinase therapy group
	91.67 ± 28.36	-	-
<4.5 h	-	154.37 ± 64.53**	151.52 ± 53.23##
6 h	-	203.14 ± 79.72**,★★,♦	160.44 ± 61.47##,△
12 h	-	242.32 ± 91.84**,★★,♦♦	184.56 ± 67.52##,▲,△△
24 h	102.42 ± 35.68	248.48 ± 99.36**,★★,♦♦	191.83 ± 74.91##,▲,△△
3 d	97.34 ± 32.53	212.24 ± 85.57**,★★,♦	169.78 ± 65.16##,△△
7 d	94.82 ± 33.28	202.47 ± 82.74**,★★,♦	157.48 ± 57.94##,△△
14 d	103.14 ± 35.21	187.68 ± 71.39**,★	132.94 ± 46.46#,△△,▲▲
8 w	96.35 ± 29.84	162.54 ± 58.48**	109.76 ± 36.69△△,▲▲

#P<0.05 compared with the control group, △P<0.05 compared with the conventional control group, ▲P<0.05 compared with 4.5 h, 6 h and 7 d groups, ♦P<0.05 compared with 12 h and 24 h groups. **P<0.01, ##P<0.01 compared with the healthy control groups, ★★P<0.01 compared with 4.5 h and 8 w groups, △△P<0.01 compared with the conventional control groups, ♦♦P<0.01 compared with 4.5 h, 14 d and 8 w groups. ▲▲P<0.01 compared with 12 h and 24 h groups, ♦P<0.05 compared with 4.5 h, and 8 w groups, ★P<0.05 compared with 4.5 h groups.
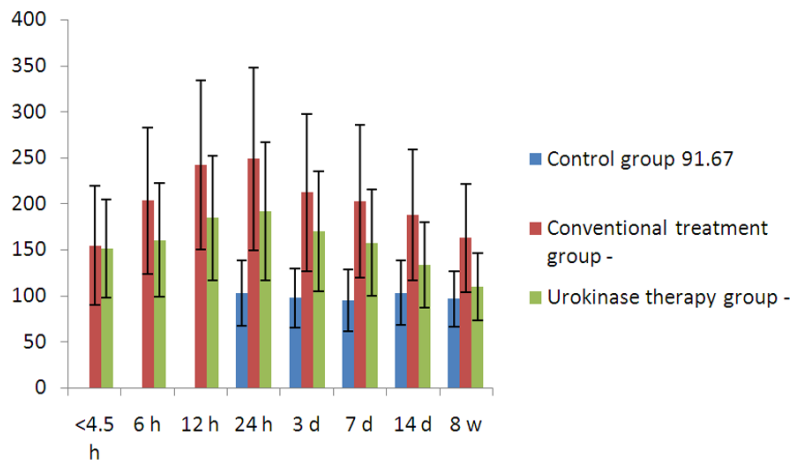

Figure 3. Dynamic changes of serum vWF level in patients ($\overline{x} \pm s$) (μg/L).

Adverse reactions

In the urokinase drug treatment group, four cases of dizziness with mild symptoms, tolerance without affecting medication were found and gradually adopt the medication process. One case of bleeding gums was found and DIC and platelet test showed no abnormalities with complete treatment.

Discussion

Uremic cerebral infarction cases increase significantly. But CAPD patients are more prone to stroke than HD patients [24]. No matter for PD or HD patients, risk of cerebrovascular disease has increased [25]. Diabetes and hypertension are risk factors for uremia [26]. This study also confirmed that: glucose and blood pressure in uremic patients with cerebral infarction were significantly higher than those in the control group (P<0.05, P<0.01), and blood lipid levels were also elevated (P<0.05); the number of patients with carotid artery plaque was also significantly increased, indicating that risk factors for infarction were still risk factors for uremic cerebral infarction.

Atherosclerosis and endothelial dysfunction play important roles in the pathogenesis of cerebral infarction. Matrix metalloproteinases (MMPs) plays an important role in maintaining endothelial stability, which are important mediators for extracellular matrix (ECM) degradation and remodeling. Cerebral infarction activates MMPs system, leading to upregulation of MMPs and degradation of extracellular matrix, further leading to secondary brain edema and brain injury; in patients with acute cerebral artery territory infarction, plasma MMP-9 levels were significantly increased in 24 h after onset [2]. Rosenberg et al. [7] demonstrated that after 4 h of middle cerebral artery occlusion, MMP-9 levels increased [27]. In this study, the serum level of MMP-9 in patients with uremic cerebral infarction was higher than that in the control group in 4.5 h after pathogenesis (P<0.05); MMP-9 level in the first 24 h of pathogenesis was progressively increased, and it was consistent with BBB injury after infarction, suggesting that serum MMP-9 levels in uremic patients with cerebral infarction elevated in acute phase.

In conventional treatment group, serum MMP-9 level continued to rise from the onset of 4.5 h to 14 d ($P<0.05$, $P<0.01$), and reduced to normal range in the pathogenesis of 8 w. It remained high in 14 d in conventional treatment group, suggesting that clopidogrel and Nimotopine cannot reduce serum MMP-9 levels in acute phase; their inhibitory effect was small. It may be related with the increased oxidative stress stimulated by peritoneal dialysis, the release of MMP-9 in acute phase induced by leukocytosis, and the complicated carotid plaques in such patients. In addition, studies have confirmed that plasma MMP-9 remained to be elevated in 7 d after cerebral infarction, which was also related to the severity of the disease [28]. Plasma MMP-9 in the acute phase of stroke increased, which was related with disease severity and infarct area [29]. In this study, the subjects were patients with uremic cerebral infarction, so the elevated serum MMP-9 was not only related with cerebral infarction, but also probably related with uremia.

T-PA is the only drug for the treatment of acute ischemia stroke approved by FDA; after t-PA thrombolysis, recanalization will further activate MMP-9, leading to the conversion of ischemia stroke to hemorrhagic stroke [17]. In the cerebral ischemia rat model with focal or cerebral middle artery occlusion, minocycline or Lipitor in combination with t-PA can reduce plasma MMP-9 levels, infarct size and cerebral hemorrhagic transformation, and extend the therapeutic window to 6 hours [30, 31]. Thus, MMP-9 inhibitors may play an important role in the treatment of acute ischemic stroke. This study confirmed that: in urokinase treatment group, serum MMP-9 level between 12 h and 7 d was significantly lower than that in the conventional treatment group ($P<0.01$), prompting that a small dose of urokinase dialysis can reduce serum levels of MMP-9 in the acute phase, and the MMP-9 level was reduced to normal range in 7 d, which had no significant difference with control group ($P>0.05$). It indicated that small dose of urokinase can inhibit the serum MMP-9 levels in acute phase, suggesting that small dose of urokinase dialysis can inhibit the activity of MMP-9 in the acute phase and has a therapeutic effect on uremic cerebral infarction, without significant hemorrhagic transformation.

Zlokovic confirmed that: in the transient MCAO model, MMP-9 activity and microvascular endothelial cells were co-located in 24 h, but 7-14 days later, MMP-9 signal was transferred to the surroundings of cortical infarction, and located on neurons and glial cells [32]. And Zhao et al considered that in 7 d after ischemic stroke, extensive MMPs inhibitor could cause damage to mouse brain repair, and reduce neurovascular regeneration in ischemic penumbra [33]. Therefore, the effect of small doses of urokinase dialysis on MMP-9, brain neurons and glial cells and blood vessels in uremic patients with cerebral infarction still require to be confirmed by additional experiments.

Pasterkamp et al found that in 36 cases of coronary arteries with atherosclerosis, immunization coloration of MMP-2 and MMP-9 were increased in plaques with expanded lumen, suggesting that matrix metalloproteinases not only play an important role in plaque fragility, but also participate in the reconstruction of atherosclerosis [34]. And some studies confirmed that: in MMP-2 knockout, or MMP-2/MMP-9 knockout mice with arterial occlusion, cortex and striatum cerebral infarction area was reduced, and cerebral hemorrhage and bleeding volume both reduced in reperfusion [9].

There are also studies confirmed that: olmesartan can reduce angiotensin 2 in cerebral ischemic area to reduce MMP-2, MMP-9 and membrane type 1-MMP, and to improve ischemic score, infarct size and cerebral edema [35].

These studies suggest that MMP-2 will also play an important role in the pathogenesis of cerebral infarction. We use a small dose of urokinase peritoneal dialysis to observe changes in serum MMP-2 level in patients. The results showed that in uremic patients with acute cerebral infarction, in acute onset (<4.5 h) phase, serum MMP-2 levels were significantly increased ($P<0.05$), which was consistent with the above findings. And the blood pressure and carotid artery plaque were significantly higher than those in control group; it was consistent with the finding of Nagano M, who confirmed that in non-hemodialysis patients with CKD, serum MMP-2 in patients with carotid artery plaque was significantly increased compared with those without plaque [36].

In conventional treatment group of our study, serum MMP-2 level continued to rise from the onset of 4.5 h to 8 w (P<0.05, P<0.01), prompting that conventional treatment was not able to reduce serum levels of MMP-2 within 8 w. In urokinase treatment group, serum levels of MMP-2 peaked in the pathogenesis of 3 d-7 d (P<0.05, P<0.01), and reduced to normal range on the 14th d (P>0.05). And there was no significant bleeding, suggesting that a small dose of urokinase dialysis can reduce serum levels of MMP-2 at early stage, with less bleeding tendency. Combined with the effect of a small dose of urokinase dialysis on serum MMP-9, this study confirmed that it can reduce early MMP-9 and MMP-2 levels in uremic patients with acute cerebral infarction, and has a certain therapeutic value for such disease.

In addition, the study of MMPs and their inhibitors in hemodialysis patients confirmed that MMP-2/TIMPs levels were increased, and Cu/Zn SOD levels were also increased; both increase were related with carotid intima-media thickness [37]. In CAPD patients with coronary heart disease, concentrations of MMP-2 and its inhibitor TIMP-2, oxidative stress product Cu/Zn SOD, kynurenic acid and its metabolite quinolinic acid were significantly increased compared with CAPD patients [38]. In hemodialysis patients with carotid atherosclerosis, serum levels of MMP-2, Cu/Zn SOD and OxLDL increased, which can independently predict intima thickness [39]. These studies suggest that oxidative stress may contribute to serum levels of MMP-2 in patients with uremia and cerebral vascular disease or arteriosclerosis; the impact of oxidative stress on MMP-2 in the present study still requires to be confirmed by additional experiments.

The conventional therapy cannot completely reduce serum levels of MMP-2 in 8 w; it was considered to be associated with oxidative stress and kidney disease itself, because MMPs play an important role in the pathogenesis of glomerulosclerosis and glomerular nephritis [40].

vWF is mainly present in endothelial cells, megakaryocytes and platelets; adhesion, activation and aggregation of platelets on the exposed subendothelial ECM will cause clots. Glycoprotein GPIb-V-IX bound to vWF to initiate the adhesion between platelet and ECM. In artery and moderate narrow blood vessels, platelets in circulating blood bound to ECM via collagen connected-vWF and GPIbα [41]. Plasma high-level VWF predicts the increased risk of stroke [42]. In the acute phase of ischemic stroke, due to endothelial cell dysfunction and thrombosis, VWF increased [43]. And in patients with carotid and aortic arch calcified TIA and ischemic stroke, serum levels of VWF significantly increased. The VWF in ischemic stroke patients with artery atherosclerosis was significantly higher than that in patients with other TOAST subtypes [14]. This study showed that at the time of the onset of uremia complicated with cerebral infarction patients (<4.5 h) the serum vWF level increased significantly (P<0.05), which was consistent with the above result. The serum vWF level in the conventional group continuously increased from 4.5 h onset to 8 w (P<0.05, P<0.01), indicating that conventional anti-platelet and neuroprotective treatment cannot put serum down to the normal range within 8 w, indicating that conventional anti-platelet and neuro protective treatment cannot put the level of serum vWF level down to the normal range within 8 w. The study showed that the serum vWF level in urokinase therapy group was significant lower than the conventional group between 6 h and 8 w (P<0.01) and decreased to normal level at 8 w (P>0.05). This results indicated that compared with the conventional group, low doses of urokinase dialysis can reduce serum vWF level. Study confirmed that in successful thrombolytic reperfusion period, vWF antagonists in combination with tPA can prevent microvascular thrombosis, reduce thrombosis recurrence or a second stroke [22].

This study demonstrated that in 8 w low dose of urokinase peritoneal dialysis therapy, in addition to inhibit serum vWF, there was no recurrence of cerebral infarction and significant cerebral hemorrhage transformation, indicating that the therapy may be safe and effective for treatment of cerebral infarction complicated with uremia.

There were also studies confirmed that in patients with acute cerebral infarction and 3-month follow-up, plasma VWF was increased. Compared with the acute time, although it decreased, it was much higher than that in the control group. Whether conventional therapy can reduce serum vWF levels in these patients

in a long time still need to extend the duration of treatment for further observation [44].

Conclusion

This study confirmed that at the time of the onset of uremia complicated with cerebral infarction patients the serum MMP-9, MMP-2 and vWF increased significantly and conventional anti-platelet, neuroprotective treatment cannot put them down to the normal range within 8 w. Low doses of urokinase dialysis can reduce serum MMP-9, MMP-2, and vWF in acute uremia complicated with cerebral infarction without recurrence of cerebral infarction and cerebral hemorrhagic transformation, indicating that low dose of urokinase peritoneal dialysis may have a certain effect on the early treatment of this disease.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Shu-Jin Wang, Department of Neurology, The First Hospital of Zibo Affiliated Weifang Medical University, Zibo 25520, China. E-mail: shujinw@126.com; Zhong-Sen Qu and Yu-Wu Zhao, Department of Neurology, Shanghai Jiao Tong University Affiliated The Sixth People's Hospital, Yi Shan Road 600, Shanghai 200233, China. E-mail: quzsen@163.com (ZSQ); zywuls@163.com (YWZ)

References

[1] Rosell A, Ortega-Aznar A, Alvarez-Sabín J, Fernández-Cadenas I, Ribó M, Molina CA, Lo EH and Montaner J. Increased brain expression of matrix metalloproteinase-9 after ischemic and hemorrhagic human stroke. Stroke 2006; 37: 1399-1406.

[2] Cojocarui IM, Cojocaru M, Sapira V, Socoliuc G, Hertea C and Paveliu S. Changes in plasma matrix metalloproteinase-9 levels in patients with acute ischemic stroke. Rom J Intern Med 2012; 50: 155-158.

[3] Demir R, Ulvi H, Özel L, Özdemir G, Güzelcik M and Aygül R. Relationship between plasma metalloproteinase-9 levels and volume and sever-ity of infarct in patients with acute ischemic stroke. Acta Neurol Belg 2012; 112: 351-356.

[4] Reynolds MA, Kirchick HJ, Dahlen JR, Anderberg JM, McPherson PH, Nakamura KK, Laskowitz DT, Valkirs GE and Buechler KF. Early biomarkers of stroke. Clin Chem 2003; 49: 1733-1739.

[5] Ebihara I, Nakamura T, Tomino Y, Shimada N and Koide H. Metalloproteinase-9 mRNA expression in monocytes from patients with chronic renal failure. Am J Nephrol 1998; 18: 305-310.

[6] Pawlak K, Mysliwiec M and Pawlak D. Peripheral blood level alterations of MMP-2 and MMP-9 in patients with chronic kidney disease on conservative treatment and on hemodialysis. Clin Biochem 2011; 44: 838-843.

[7] Rosenberg GA, Estrada EY and Dencoff JE. Matrix metalloproteinases and TIMPs are associated with blood-brain barrier opening after reperfusion in rat brain. Stroke 1998; 29: 2189-2195.

[8] Cavdar Z, Oktay G, Egrilmez MY, Genc S, Genc K, Altun Z, Islekel H and Guner G. In vitro reoxygenation following hypoxia increases MMP-2 and TIMP-2 secretion by human umbilical vein endothelial cells. Acta Biochim Pol 2010; 57: 69-73.

[9] Suofu Y, Clark JF, Broderick JP, Kurosawa Y, Wagner KR and Lu A. Matrix metalloproteinase-2 or -9 deletions protect against hemorrhagic transformation during early stage of cerebral ischemia and reperfusion. Neuroscience 2012; 212: 180-189.

[10] Chang HR, Yang SF, Li ML, Lin CC, Hsieh YS and Lian JD. Relationships between circulating matrix metalloproteinase-2 and -9 and renal function in patients with chronic kidney disease. Clinica Chimica Acta 2006; 3669: 243-248.

[11] Chung AW, Yang HH, Kim JM, Sigrist MK, Chum E, Gourlay WA and Levin A. Upregulation of matrix metalloproteinase-2 in the arterial vasculature contributes to stiffening and vasomotor dysfunction in patients with chronic kidney disease. Circulation 2009; 120: 792-801.

[12] Nagano M, Fukami K, Yamagishi S, Ueda S and Kaida Y. Circulating matrix metalloproteinase-2 is an independent correlate of proteinuria in patients with chronic kidney disease. Am J Nephrol 2009; 29: 109-115.

[13] Arslan Y, Yoldaş TK and Zorlu Y. Interaction between vWF levels and aspirin resistance in ischemic stroke patients. Transl Stroke Res 2013; 4: 484-487.

[14] Sonneveld MA, van Dijk AC, van den Herik EG, van Loon JE, de Lau LM, van der Lugt A, Koudstaal PJ, de Maat MP and Leebeek FW. Relationship of Von Willebrand Factor with ca-

rotid artery and aortic arch calcification in ischemic stroke patients. Atherosclerosis 2013; 230: 210-215.

[15] Rašić S, Hadžović-Džuvo A, Rebić D, Valjevac A and Unčanin S. Bosn J Endothelial dysfunction in uremic patients on continuous ambulatory peritoneal dialysis (CAPD). Basic Med Sci 2011; 11: 153-157.

[16] Malyszko J, Malyszko JS, Pawlak K and Mysliwiec M. Thyroid function, endothelium, and inflammation in hemodialyzed patients: possible relations? J Ren Nutr 2007; 17: 30-37.

[17] Wang X, Lee SR, Arai K, Lee SR, Tsuji K, Rebeck GW and Lo EH. Lipoprotein receptor-mediated induction of matrix metalloproteinase by tissue plasminogen activator. Nat Med 2003; 9: 1313-1317.

[18] Zhang L, Chopp M, Jia L, Cui Y, Lu M and Zhang ZG. Atorvastatin extends the therapeutic window for tPA to 6 h after the onset of embolic stroke in rats. J Cereb Blood F Met 2009; 29: 1816-1824.

[19] Fletcher AP, Alkjaersig N, Lewis M, Tulevski V, Davies A, Brooks JE, Hardin WB, Landau WM and Raichle ME. A pilot study of urokinase therapy in cerebral infarction. Stroke 1976; 7: 135-142.

[20] Chen H, Zhu G, Liu N and Zhang W. Low-dose tissue plasminogen activator is as effective as standard tissue plasminogen activator administration for the treatment of acute ischemic stroke. Curr Neurovasc Res 2014; 11: 62-67.

[21] Fuhrman B. The urokinase system in the pathogenesis of atherosclerosis. Atherosclerosis 2012; 222: 8-14.

[22] del Zoppo GJ and Koziol JA. Recanalization and stroke outcome. Circulation 2007; 115: 2602-2605.

[23] Diagnosis of stroke was based on the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM, (codes 433.X1, 434.X1, or 436).

[24] Toyoda K, Fujii K, Ando T, Kumai Y, Ibayashi S and Iida M. Incidence, etiology, and outcome of stroke in patients on continuous ambulatory peritoneal dialysis. Cerebrovasc Dis 2004; 17: 98-105.

[25] Kes P, Basić-Kes V, Basić-Jukić N and Jurić I. A risk factors for stroke in the patients with chronic kidney disease. Acta Med Croatica 2011; 65: 67-77.

[26] Wang HH, Hung SY, Sung JM, Hung KY and Wang JD. Risk of Stroke in Long-term Dialysis Patients Compared With the General Population. Am J Kidney Dis 2014; 63: 604-611.

[27] Rosenberg GA, Navratil M, Barone F and Feuerstein G. Proteolytic cascade enzymes increase in focal cerebral ischemia in rat. Cereb Blood Flow Metab 1996; 16: 360-366.

[28] Lucivero V, Prontera M, Mezzapesa DM, Petruzzellis M, Sancilio M, Tinelli A, Di Noia D, Ruggieri M and Federico F. Different roles of matrix metalloproteinases-2 and -9 after human ischaemic stroke. Neurol Sci 2007; 28: 165-170.

[29] Demir R, Ulvi H, Özel L, Özdemir G, Güzelcik M and Aygül R. Relationship between plasma metalloproteinase-9 levels and volume and severity of infarct in patients with acute ischemic stroke. Acta Neurol Belg 2012; 112: 351-356.

[30] Machado LS, Sazonova IY, Kozak A, Wiley DC, El-Remessy AB, Ergul A, Hess DC, Waller JL and Fagan SC. Minocycline and tissue-type plasminogen activator for stroke assessment of interaction potential. Stroke 2009; 40: 3028-3033.

[31] Zhang L, Chopp M, Jia L, Cui Y, Lu M and Zhang ZG. Atorvastatin extends the therapeutic window for tPA to 6 h after the onset of embolic stroke in rats. J Cereb Blood F Met 2009; 29: 1816-1824.

[32] Zlokovic BV. Remodeling after stroke. Nat Med 2006; 12: 390-391.

[33] Zhao BQ, Wang S, Kim HY, Storrie H, Rosen BR, Mooney DJ, Wang X and Lo EH. Role of matrix metalloproteinases in delayed cortical responses after stroke. Nat Med 2006; 12: 441-445.

[34] Pasterkamp G, Schoneveld AH, Hijnen DJ, de Kleijn DP, Teepen H, van der Wal AC and Borst C. Atherosclerotic arterial remodeling and the localization of macrophages and matrix metalloproteases 1, 2 and 9 in the human coronary artery. Atherosclerosis 2000; 150: 245-53.

[35] Hosomi N, Nishiyama A, Ban C, Naya T, Takahashi T, Kohno M and Koziol J. Angiotensin type 1 receptor blockage improves ischemic injury following transient focal cerebral ischemia. Neuroscience 2005; 134: 225-231.

[36] Nagano M, Fukami K, Yamagishi S, Ueda S, Kaida Y, Matsumoto T, Yoshimura J, Hazama T, Takamiya Y, Kusumoto T, Gohara S, Tanaka H, Adachi H and Okuda S. Circulating matrix metalloproteinase-2 is an independent correlate of proteinuria in patients with chronic kidney disease. Am J Nephrol 2009; 29: 109-115.

[37] Pawlak K, Pawlak D and Mysliwiec M. Extrinsic coagulation pathway activation and metalloproteinase-2/TIMPs system are related to oxidative stress and atherosclerosis in hemodialysis patients. Thromb Haemost 2004; 92: 646-653.

[38] Pawlak K, Tankiewicz J, Mysliwiec M and Pawlak D. Systemic levels of MMP2/TIMP2 and cardiovascular risk in CAPD patients. Nephron Clin Pract 2010; 115: c251-258.

[39] Pawlak K, Pawlak D and Mysliwiec M. Serum matrix metalloproteinase-2 and increased oxidative stress are associated with carotid ath-

erosclerosis in hemodialyzed patients. Athero-sclerosis 2007; 190: 199-204.

[40] Lods N, Ferrari P, Frey FJ, Kappeler A, Berthier C, Vogt B and Marti HP. Angiotensin-converting enzyme inhibition but not angiotensin II receptor blockade regulates matrix metalloproteinase activity in patients with glomerulonephritis. J Am Soc Nephrol 2003; 14: 2861-2872.

[41] Savage B, Almus-Jacobs F and Ruggeri ZM. Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow. Cell 1998; 94: 657-666.

[42] Roldán V, Marín F, García-Herola A, Lip GY. Correlation of plasma von Willebrand factor levels, an index of endothelial damage/dysfunction, with two point-based stroke risk stratification scores in atrial fibrillation. Thromb Res 2005; 116: 321-325.

[43] Arslan Y, Yoldaş TK and Zorlu Y. Interaction between vWF levels and aspirin resistance in ischemic stroke patients. Transl Stroke Res 2013; 4: 484-487.

[44] Hanson E, Jood K, Karlsson S, Nilsson S, Blomstrand C and Jern C. Plasma levels of von Willebrand factor in the etiologic subtypes of ischemic stroke. J Thromb Haemost 2011; 9: 275-281.