Int J Clin Exp Med 2015;8(1):1333-1341
www.ijcem.com /ISSN:1940-5901/IJCEM0003968

Original Article

Impact of low-dose urokinase in peritoneal dialysis on serum oxidative stress, nitric oxide and endothelin in cerebral infarction complicated with uremia

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Received November 19, 2014; Accepted January 9, 2015; Epub January 15, 2015; Published January 30, 2015

Abstract: Background: Cerebrovascular accident is an important cause of death in patients with chronic renal failure. Methods: This study evaluated the interference of low-dose urokinase in peritoneal dialysis solution on uremic serum superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO) and endothelin (ET) dynamics in patients with a cerebral infarction complicated by uremia. Results: Both the urokinase and conventional treatment groups showed decreased SOD activities, increased MDA content, and elevated serum NO and ET levels at the initiation stage of treatment. Antiplatelet and cerebral protection therapy slightly reduced body MDA content and increased SOD activity at the early stage of treatment, and its effects on reducing serum NO and ET-1 are also limited. Conclusion: Our results revealed that a small amount of urokinase in peritoneal dialysis can reduce body MDA content, increase SOD activity and decrease serum levels of NO and ET-1 at the early stage of cerebral infarction complicated by uremia. We also found that continuous treatment for 8 weeks may provide a potential treatment of cerebral infarction complicated with uremia.

Keywords: Low-dose urokinase, peritoneal dialysis, oxidative stress, nitric oxide, cerebral infarction

Introduction

The prevalence of chronic kidney diseases is increasing. Chronic kidney diseases may be complicated by nervous system diseases such as epilepsy, stroke, peripheral neuropathy, and uremic encephalopathy [1]. The risk of cerebrovascular disease is significantly higher in peritoneal dialysis (PD) and hemodialysis (HD) patients [2]. Continuous ambulatory peritoneal dialysis (CAPD) patients are more likely to have a stroke than HD patients [3]. PD patients are prone to atherosclerosis, which is attributed to their common risk factors, and also to anemia, hypoalbuminemia, oxidative stress, and inflammatory response, which are implicated in vascular remodeling [4]. Patients with chronic renal failure generally show increased oxidative stress [5, 6], and dialysis can further increase the oxidative stress, the response to oxidative stress [7], and coagulation abnormalities involved in endothelial dysfunction and thrombosis. ET and NO play an important role in the regulation of endothelial function. In chronic renal failure patients, whether they are undergoing HD or CAPD, plasma ET is significantly higher than that in healthy controls [10]. Also, excessive release of NO is associated with uremia in both animal and human patients [11]. Recent reports have revealed that serum NO levels are significantly elevated in CAPD patients compared with the control group, while endothelin levels showed no significant difference between these two groups [12]. NO bioactivity abnormalities, as well as malnutrition and infection, can increase arterial thrombosis in patients with uremia [13].

Alterations in oxidative stress and endothelial function in patients with cerebral infarction complicated with uremia are not fully understood. To date, there is no particular treatment for this disease in China. PD, a renal replacement therapy, is an ideal treatment for chronic

renal failure. It is effective in preventing bacterial peritonitis in patients with chronic renal failure [14]. Urokinase used to treat acute cerebral infarction, however, was reported decades ago [15]. Recent studies have shown that a small dose of tissue-type plasminogen activator (tPA) combined with urokinase is effective for treatment of cerebral infarction [16]. Analysis of 229 cases of acute cerebral infarction complicated with uremia onset within 4.5 h has indicated that the effect of thrombolytic therapy with recombinant tPA is more significant in older patients with higher National Institutes of Health Stroke Scale (NIHSS) score and is negatively affected by renal dysfunction [17]. In the permanent middle cerebral artery occlusion rat model of cerebral ischemia, dialysis at 2.5 h after cerebral ischemia onset can reduce the plasma level of glutamic acid and the cerebral infarction size. Application of blood oxygenation level dependent (BOLD) fMRI has found that brain functions can be partially restored in these rats after dialysis [18].

Since uremia is contraindicated with intravenous thrombolysis in China, is there any alternative treatment for such patients for whom intravenous thrombolysis is not suitable? We speculated that low-dose urokinase may have a therapeutic effect in cerebral infarction complicated by uremia. Therefore, this study examined the serum SOD, MDA, ET, and NO dynamic changes in patients with cerebral infarction complicated with uremia and explored the role of low-dose urokinase in the treatment of the disease.

Materials and methods

Ethics

The present study has been performed with the approval of the ethics committee of Shanghai Jiao Tong University and was in compliance with the Helsinki Declaration. The informed consents of the study were collected from all the candidate subjects.

Clinical data

This study comprised 130 patients diagnosed with cerebral infarction complicated with uremia who were admitted to hospital from June 2012 to October 2013. This study was approved by the hospital ethics committee and the patients or their guardians provided informed consent. The patients were randomly divided into a conventional treatment group and a urokinase treatment group, based on symptoms, signs, cranial CT, MRI diagnosis infarction, and first onset of cerebral infarction [19]. The selection criteria were: patients had no cerebral hemorrhage and subarachnoid hemorrhage, no serious systemic complications, no bleeding disorders or bleeding tendency, no history of rheumatic heart disease or atrial fibrillation, no history of liver disease or respiratory system disease, no obvious infection, no surgery or trauma one month before the experiment, no autoimmune disease.

Blood clotting (prothrombin time, activated partial thromboplastin time, thrombin time and fibrinogen) were determined before treatment. There were no differences in the gender, age, clinical presentation, renal function, and NIHSS score between patients in the urokinase treatment group and those in the conventional treatment group ($P > 0.05$).

Sixty volunteers over the age of 40, which was the same age range as the experimental group, were selected as the healthy control group. Blood clotting tetrachoric levels, blood pressure, blood lipids, blood glucose, liver and kidney function and heart and lung function were all normal in the control group. The healthy control group without any treatment provided a corresponding reference value for each test.

Methods

Treatment: Tenchoff tubes were inserted after conventional catheter incision for all patients with peritoneal dialysis. O-pipes and peritoneal dialysis fluid (Baxter, USA) were used for dialysis. All patients were treated using intermittent peritoneal dialysis (IPD) for the first 3-5 days, followed by continuous ambulatory peritoneal dialysis (CAPD), using 2000 ml each, 4 times per day. The conventional treatment group received conventional treatment with peritoneal dialysis (clopidogrel 50 mg/qn/d, Nimotop 30 mg/tid/d), for control of blood pressure, diuretic swelling, kidney protection, water maintenance, and electrolyte and acid-base balance. Based on the conventional treatment, the urokinase treatment group was given peritoneal dialysis fluid that also contained urokinase (100,000 IU) twice daily. Each course of treatment lasted eight weeks for both groups.

Table 1. Clinical features of patients in the three groups

Group	Healthy control (A)	Conventional treatment (B)	Urokinase treatment (C)
Male/Female	37/23	37/20	45/28
Age (yr)	50.6±4.6	50.9±4.7	51.3±4.9
Blood sugar (mmol/L)	4.66±0.38	5.43±0.54	5.82±0.46
SBP (mmHg)	123.7±13.8	172.1±20.2**	164.6±18.8**
DBP (mmHg)	76.3±8.5	93.4±10.7*	96.8±10.4*
Total cholesterol (mmol/L)	4.84±0.68	6.12±1.15*	6.58±1.26*
Triglyceride (mmol/L)	1.63±0.27	2. 86±0.48*	2.54±0.52*
NIHSS score	0	8.5±2.1	7.4±2.6

*$P < 0.05$, **$P < 0.01$ compared with healthy control.

Table 2. Change in serum MDA content in the three groups

Group	Healthy control	Conventional treatment	Urokinase treatment
Pre-treatment	4.86±0.65	8.14±1.32**	8.26±1.18##
< 4.5 h	-	8.31±1.46**	8.42±1.23##
6 h	-	8.37±1.24**	8.28±1.26##
12 h	-	7.96±1.21**	7.74±1.14##
24 h	4.92±0.78	7.92±1.22**	7.46±1.22##,▲
3 d	4.96±0.82	7.85±1.12**	5.27±0.91ΔΔ,▲▲
7 d	4.82±0.74	7.72±1.28**	4.93±0.75ΔΔ,▲▲
14 d	4.74±0.77	6.24±0.94*,★	4.58±0.79Δ,▲▲
8 w	4.88±0.78	6.27±0.96*,★	4.22±0.68Δ,▲▲

Note: units are nmol/ml and results are presented as $\bar{x}$ ±S. *$P < 0.05$ compared with healthy control, Δ$P < 0.05$ compared with conventional treatment group, **$P < 0.01$, ##$P < 0.01$ compared with healthy control, ★$P < 0.05$ compared with time points before 7 d, ▲▲$P < 0.01$ compared with time points after 24 h, ▲$P < 0.05$ compared with time points after 12 h, ΔΔ$P < 0.01$ compared with conventional treatment group.

Sample collection: Alcohol and high-fat diet were forbidden for the healthy control group 24 h before blood sampling. Venous blood (2 ml) was collected after fasting 12 h at 8 AM, and blood was also collected at the same time in 24 h, 3 d, 7 d, 14 d, and 8 w after the first sampling. The experimental groups (conventional and urokinase treatment groups) fasting venous blood samples (2 ml) were taken at arrived hospital, and at 4.5 h, 6 h, 12 h, 24 h, 3 d, 7 d, 14 d, 8 w after the onset of the disease. The blood samples were collected in a procoagulant tube (BD company, Franklin Lakes, New Jersey, USA) and centrifuged at 4°C and 1500 *g* for 10 min. The serum was then collected, frozen at -20°C and stored at -80°C for future use.

Serum SOD, MDA determination: The serum MDA content and SOD activity levels were determined using UV spectrophotometric colorimetry. SOD activities were examined using the xanthine oxidase method, and MDA content was determined using the thiobarbituric acid method (kits provided by Nanjing Institute of Biological Engineering, Zhonghua road, Nanjing, China).

Endothelin and nitric oxide detection: Endothelin-1 (ET-1) levels were determined using ET radioimmunoassay kits (provided by the People's Liberation Army General Hospital, Technology Development Center, Fuxing road, Beijing, China). NO levels were detected using nitrate reductase assay kits (provided by Nanjing Institute of Biological Engineering, Zhonghua road, Nanjing, China).

Statistical analysis

All data were presented as the mean ± standard deviation ($\bar{x}$ ±S) and were analyzed using the SPSS 13.0 software package. The average values were compared using a one-way ANOVA and different time points were compared using paired t-tests within groups. A *t*-test or a one-way ANOVA procedure followed by Student-Newman-Keuls test was used to determine differences between the means among the groups. Correlations were determined using Pearson's linear regression analysis. The level of significance was set at $P < 0.05$.

Results

Clinical features of patients

The conventional treatment and the urokinase treatment groups showed significantly higher levels of systolic ($P < 0.01$) and diastolic blood pressure ($P < 0.05$), total cholesterol ($P < 0.05$), and triglyceride levels ($P < 0.05$) compared with the healthy control group. Fasting blood glucose showed no significant difference among the three groups ($P > 0.05$; **Table 1**).

Table 3. Change in SOD activity in the three groups

Group	Healthy control	Conventional treatment	Urokinase treatment
Pre-treatment	99.34±10.12	72.86±7.78$^{**,\bigstar}$	70.34±7.82$^{\#\#}$
< 4.5 h	-	78.48±8.16$^{**,\bigstar}$	76.46±8.46$^{\#\#}$
6 h	-	77.37±8.79$^{**,\bigstar}$	80.38±9.06$^{\#\#}$
12 h	-	80.56±8.31$^{**,\bigstar}$	85.34±8.94$^{\#\#}$
24 h	103.25±11.46	79.84±8.42$^{**,\bigstar}$	91.76±10.22$^{\#,\Delta,\blacktriangle}$
3 d	101.22±9.69	83.58±10.14**	96.27±11.36$^{\Delta,\blacktriangle\blacktriangle}$
7 d	101.28±9.92	83.76±10.59**	106.63±10.75$^{\Delta\Delta,\blacktriangle\blacktriangle}$
14 d	102.36±10.24	86.34±9.82**	112.96±13.44$^{\#,\Delta\Delta,\blacktriangle\blacktriangle}$
8 w	101.78±9.44	91.79±9.86*	115.32±12.59$^{\#,\Delta,\blacktriangle\blacktriangle}$

Note: units are ng/ml. $^{*}P < 0.05$, $^{\#}P < 0.05$ compared with healthy control, $^{\Delta}P < 0.05$ compared with conventional treatment group, $^{**}P < 0.01$, $^{\#\#}P < 0.01$ compared with healthy control, $^{\bigstar}P < 0.05$ compared with 8 w, $^{\blacktriangle\blacktriangle}P < 0.01$ compared with 4.5 h and 6 h, $^{\blacktriangle}P < 0.05$ compared with 4.5 h and 6 h, $^{\Delta\Delta}P < 0.01$ compared with conventional treatment group.

Table 4. Change in serum ET-1 levels in the three groups

Group	Healthy control	Conventional treatment	Urokinase treatment
Pre-treatment	85.47±23.59	158.24±41.95**	154.63±44.76$^{\#\#}$
< 4.5 h	-	156.48±42.58**	160.32±48.27$^{\#\#}$
6 h	-	152.58±35.72**	158.59±39.56$^{\#\#}$
12 h	-	154.49±41.86**	149.81±37.43$^{\#\#}$
24 h	86.78±28.54	146.26±40.39**	132.47±35.12$^{\#\#,\blacktriangle\blacktriangle,\Delta}$
3 d	85.36±24.42	141.52±28.89**	126.98±33.32$^{\#\#,\blacktriangle\blacktriangle,\Delta}$
7 d	87.73±25.12	134.89±26.53$^{**,\bigstar\bigstar}$	120.78±29.54$^{\#\#,\blacktriangle\blacktriangle,\Delta}$
14 d	89.64±27.65	136.48±29.36$^{**,\bigstar\bigstar}$	118.58±26.34$^{\#,\blacktriangle\blacktriangle,\Delta}$
8 w	87.18±25.38	127.79±27.58$^{**,\bigstar\bigstar}$	109.46±24.64$^{\#,\blacktriangle\blacktriangle,\Delta}$

Note: units are pg/ml and results are presented as $\bar{x}$±S. $^{\#}P < 0.05$ compared with healthy controls, $^{\Delta}P < 0.05$ compared with conventional treatment group, $^{**}P < 0.01$, $^{\#\#}P < 0.01$ compared with healthy control, $^{\bigstar\bigstar}P < 0.01$ compared with time points before 7 d, $^{\blacktriangle\blacktriangle}P < 0.01$ compared with healthy controls before 24 h.

Changes in serum MDA content and SOD activity

At the initiation stage of treatment, MDA contents in the conventional treatment group and the urokinase treatment group were significantly higher than the healthy control group ($P < 0.01$), whereas there was no significant difference between the urokinase treatment group and the conventional treatment group ($P > 0.05$). MDA content in the conventional therapy group started to decrease at 14 d ($P < 0.05$), and the content was lower than 7 d or the time points before 7 d to the end of the 8-week treatment ($P < 0.05$); however, the MDA content was consistently higher than the healthy control group ($P < 0.05$). The MDA content in urokinase treatment group patients showed no significant decrease for the first 2 days ($P > 0.05$), but was significantly decreased at 3 d compared with the conventional treatment group ($P < 0.01$). The MDA content in urokinase treatment group at 14 d and 8 w was significantly lower than itself after the first 24 hours ($P < 0.01$), whereas after 24 hours, the differences were no longer statistically significant ($P > 0.05$) compared with the healthy control group. These results suggested that addition of urokinase into peritoneal dialysis fluid can significantly reduce serum MDA content compared to conventional anti-platelet and cerebral protection therapy. The results are summarized in **Table 2**.

At the initial stage of treatment, SOD activities were significantly reduced in both the urokinase treatment group and the conventional treatment group compared with the healthy control group ($P < 0.01$), but there was no difference between the urokinase and the conventional treatment groups ($P > 0.05$). At 8 w, SOD activity had been restored in conventional treatment group, but remained significantly lower than pre-treatment levels ($P < 0.05$), and levels in the urokinase treatment or healthy control groups ($P < 0.05$). SOD activity in the urokinase treatment group began to be restored at 24 h, but the value was significantly lower than pre-treatment levels ($P < 0.05$). SOD activities at any time point after 3 d were significantly higher than at 4.5 h and 6 h ($P < 0.01$). Although it was still lower than the healthy control group, SOD activity in the urokinase treatment group at 3 d showed a much better restoration of activity than did the conventional treatment group ($P < 0.05$). In addition, after 14

Table 5. Change in serum NO levels in the three groups

Group	Healthy control	Conventional treatment	Urokinase treatment
Pre-treatment	52.67±8.42	65.38±8.56*	62.24±8.63$^{\#}$
< 4.5 h	-	64.85±8.49*	66.60±9.51$^{\#}$
6 h	-	68.56±9.67*	72.58±10.15$^{\#}$
12 h	-	90.32±15.28$^{**,\bigstar\bigstar}$	82.47±13.85$^{\#\#,\blacktriangle}$
24 h	50.45±7.98	96.48±14.54$^{**,\bigstar\bigstar}$	78.31±12.22$^{\#\#,\blacktriangle,\Delta}$
3 d	53.78±7.56	85.26±13.53$^{**,\bigstar\bigstar}$	71.98±9.74$^{\#,\Delta}$
7 d	54.36±7.29	78.89±10.26$^{**,\bigstar}$	65.39±8.85$^{\#,\Delta}$
14 d	52.16±8.14	67.18±8.39*	52.67±6.81$^{\Delta}$
8 w	51.94±7.74	63.17±7.84*	46.16±6.18$^{\Delta}$

Note: units are μmol/L and results are presented as $\bar{\chi}$±s. $^{*}P < 0.05$, $^{\#}P < 0.05$ compared with healthy control, $^{\Delta}P < 0.05$ compared with conventional treatment group, $^{\blacktriangle}P < 0.05$ compared with time points before 6 h, $^{\bigstar}P < 0.05$ compared with < 4.5 h, 14 d and 8 w, $^{**}P < 0.01$, $^{\#\#}P < 0.01$ compared with healthy control, $^{\bigstar\bigstar}P < 0.01$ compared with time points before 6 h.

days of urokinase treatment, the SOD activity was significantly increased compared with the healthy control group ($P < 0.05$). These results suggested that while the currently-used conventional treatment somewhat improve SOD activity in patients with cerebral infarction complicated with uremia, urokinase treatment increased SOD activity in the acute phase, and continually maintains SOD bioactivity. The results are summarized in **Table 3**.

Changes in serum ET-1 level

Elevated ET-1 levels were observed before treatment in the urokinase and conventional treatment groups compared with the healthy control group ($P < 0.01$), while there was no significant difference between the urokinase and conventional treatment groups ($P > 0.05$). In the conventional treatment group, serum ET-1 levels were significantly reduced after 7d, compared to the treatment group at any time point before 7 d ($P < 0.01$). The increase in ET-1 levels during conventional treatment was greater than in the healthy control group ($P < 0.01$). ET-1 levels started to decrease at 24 h after disease onset in the urokinase treatment group ($P < 0.01$), which was significantly lower than in the conventional treatment group ($P < 0.05$). The reduction in ET-1 in the urokinase treatment group was more significant than in the conventional treatment group after 24 h ($P < 0.05$). Although there were differences in ET-1 values compared with the healthy control group ($P < 0.05$) at 14 d and 8 w, the values tended to decrease. These results indicate that compared with conventional treatment, low-dose urokinase in peritoneal dialysis fluid can significantly reduce serum ET-1, which may improve endothelial functions in cerebral infarction complicated by uremia. The results are summarized in **Table 4**.

Correlation analysis of the initial serum ET-1, SOD and MDA levels were performed. In patients with chronic kidney disease, MDA content was positively correlated with ET-1 ($r = 0.672$, $P < 0.01$), while SOD activity was negatively correlated with ET-1 ($r = -0.598$, $P < 0.05$).

Changes in the serum NO level

The serum NO level increased significantly at the onset of acute cerebral infarction complicated with uremia (< 4.5 h, 6 h; $P < 0.05$), and this increase remained in the conventional treatment group 12 hours after the onset of the disease ($P < 0.01$) and until 7 d ($P < 0.01$), when it subsequently decreased. NO levels at and after 14 d were no longer higher than pretreatment levels ($P > 0.05$), but the differences were still significant compared to healthy controls ($P < 0.05$), suggesting that conventional anti-platelet and cerebral protection therapy do not return the high serum NO levels at the onset of cerebral infarction back to normal levels. Although the NO level in the urokinase treatment group was significantly higher than in the healthy control group at 12 h after disease onset ($P < 0.01$), there was a downward trend compared to the conventional therapy group. As shown in **Table 5**, the NO level started to decrease at 24 h. Although there were significant differences compared to the healthy control group ($P < 0.01$), NO levels in the urokinase treatment group were significantly decreased compared with the conventional treatment group ($P < 0.05$), and thereafter were significantly lower than the conventional therapy group ($P < 0.05$). At 14 d and thereafter, comparison with the healthy control group showed

no significant difference ($P > 0.05$). The results suggest that addition of urokinase in peritoneal dialysis solution contributes to early reduction of serum NO and provides significantly better treatment effect than conventional therapy. The results are summarized in **Table 5**.

Correlation analysis of the initial serum NO, SOD and MDA levels were performed. In patients with chronic kidney disease, MDA content was positively correlated with NO ($r = 0.726$, $P < 0.01$), while SOD activity was negatively correlated with NO ($r = -0.654$, $P < 0.01$).

Adverse reactions

Three patients in the urokinase treatment group had abdominal discomfort, which was mild and did not affect the drug treatment; this adverse reaction gradually resolved during the treatment process. There were two reports of bleeding gums, but no disseminated intravascular coagulation (DIC) or platelet abnormalities and the patients successfully completed the treatment course.

Discussion

Cerebrovascular accident is an important cause of death in patients with chronic renal failure. Oxidative stress plays an important role in cerebral ischemia-reperfusion injury. In patients with cerebral infarction, the level of oxidative stress is increased [20]. In uremia and hemodialysis patients, polymorphonuclear leukocytes and monocytes are increased, thereby inducing oxidative stress [7, 21]. Using macrophage-derived foam cells from end stage renal disease (ESRD) patients, Gonçalves *et al.* discovered that the MDA content was increased in the supernatant of the medium, which confirmed the onset of inflammation and oxidative stress, and the formation of atherosclerotic plaques was promoted [22]. This study also demonstrated that serum MDA was increased at the initial treatment stage of cerebral infarction complicated with uremia ($P < 0.01$), which is consistent with the results of *in vitro* studies by Gonçalves *et al*. This suggests that elevated levels of serum MDA are associated with uremia itself, as well as cerebral infarction. Fourteen days of conventional treatment can reduce serum MDA levels, but within the 8-week treatment duration, the serum oxidative stress products cannot be removed completely. MDA content at 3 d in the urokinase treatment group was significantly lower ($P < 0.01$), and at 14 d there was no significant difference compared with the healthy control group ($P > 0.05$). This suggests that, compared with conventional treatment, a small amount of urokinase in peritoneal dialysis fluid can significantly reduce the serum MDA content, eliminating the effect of oxidative stress products.

An increase in the plasma MDA levels has been reported to be related to superoxide anion produced by neutrophils, whereas erythrocyte SOD activities are not significantly changed in HD patients [23]. Other studies report that vitamin E can reduce the plasma MDA content in HD and CAPD patients, but not erythrocyte SOD activity [24]. The results of this study demonstrate the presence of serum SOD activity reduction at the onset of cerebral infarction complicated by uremia ($P < 0.01$), which is in contrast to the previous finding that erythrocyte SOD activity is not changed, and suggests that the ability to scavenge *in vivo* oxygen free radicals is repressed when cerebral infarction is complicated by uremia. After 8 w of the conventional treatment for uremia complicated by cerebral infarction, there was a recovery of SOD activity compared with its level at the initial treatment ($P < 0.05$), but this SOD level was still significantly lower than in the urokinase treatment group or the healthy control group ($P < 0.05$). These results indicate (indicated?) that conventional treatment partially restores SOD activity within 8 weeks. The SOD activity in the urokinase treatment group started to be restored at 24 h ($P < 0.05$). After 14 days of treatment, SOD activity was higher than the control group ($P < 0.05$). These results suggest that low-dose urokinase in the peritoneal dialysis solution improves SOD activity and continually enhances the effect of SOD activity, which reduces the body's oxidative stress response in cerebral infarction complicated by uremia.

Increased oxidative stress and coagulation abnormalities are involved in endothelial dysfunction and thrombosis [5-9]. ET plays an important role in the regulation of endothelial function. When cerebral infarction occurs, damage to local cerebral vessels, tissue ischemia and the body's hypoxic stress response cause elevated ET levels. In chronic renal failure patients, regardless of HD or CAPD, plasma ET levels are higher than healthy controls [10]. The results of this study show that the initial serum

ET-1 levels in patients with cerebral infarction complicated by uremia were higher than in the healthy control group ($P < 0.01$). Our results are consistent with previous *in vivo* studies, but different from the finding by Kovačević *et al*., that serum ET levels are not changed in CAPD patients [12]. This might be attributed to both uremia and cerebral infarction. Moreover, correlation analysis at the onset of the disease found that ET-1 is positively correlated with MDA content ($P < 0.05$), but negatively correlated with SOD activity ($P < 0.05$). These results suggest that the *in vivo* oxidative stress response increases in patients with cerebral infarction complicated with uremia, and cause elevated ET-1 levels, which thereby cause toxic effects inside the body.

In addition, another study suggests that in patients with scleroderma renal failure, ET-1 expression is increased in glomerulus and arterioles, suggesting that ET-1 may be a therapeutic target for these diseases [25]. Our results showed that serum ET-1 levels in the conventional treatment group decreased after 7d, but that levels were still higher than those in the healthy control group ($P < 0.01$). At various time points after 7 d, ET-1 levels were still higher than the healthy control group ($P < 0.01$). This suggests that conventional treatment can reduce serum ET-1 levels in patients with cerebral infarction complicated with uremia within a certain time window, but the effect is rather limited. Urokinase serum ET-1 levels began to decrease at 24 h after the onset ($P < 0.01$), which was significantly lower than the conventional therapy group until 8 w ($P < 0.05$). Although differences remain between the healthy control group at 14 d and 8 w and the urokinase treatment group ($P < 0.05$), the levels tended to decrease. Based on these results, low-dose urokinase can significantly reduce serum ET-1 compared with conventional treatment and thereby improve vascular endothelial function in cerebral infarction complicated by uremia.

The results of this study demonstrate that a small amount of urokinase could not reduce serum ET-1 to a normal level within 8 weeks in patients with cerebral infarction complicated by uremia. This might be attributed to a lower ET (B) receptor expression in the kidney itself and in systemic arteries [26], increased glycation end products (AGEs) [27], coagulation abnormalities and fibrinolytic system dysfunction [8, 9]. However, further research is needed to extend treatment duration and examine more indicators.

NO is synthesized in kidney endothelial cells by L-arginine and oxygen molecules effected by NOS. NOS isoforms include the endothelial (eNOS), inducible (iNOS) and neuronal (nNOS) types. NO regulates kidney function by regulating vascular tension and renal sodium excretion, and also maintains cerebral vascular tension and regulating cerebral blood flow. Abundant iNOS expression in inflammatory cells produces excessive amounts of NO, triggering neurotoxic effects [28]. Elevated iNOS and eNOS expression promotes excessive NO release from systemic vascular lumen in uremic patients [11]. *In vivo* NO levels are increased in HD patients [29]. Long-term PD patients also show an elevated serum NO level [30]. Our results found that serum NO levels are significantly increased when acute cerebral infarction is complicated by uremia (< 4.5 h, 6 h; $P < 0.05$), which is consistent with previous findings.

Studies have confirmed that CAPD patients are more likely to have a hypercoagulable state than HD patients [31]. PD patients with atherosclerosis have higher levels of tPA and PAI-1 than patients who do not have atherosclerosis [32]. Coagulation also increases in the dialysate of PD patients [33]. The roles of coagulation and fibrinolysis warrant further experimental confirmation. Given that cerebral infarction patients with atrial fibrillation were excluded and patients with moderate NIHSS score were selected in this study, future studies should expand the research scope on cerebral infarction.

In conclusion, this study has demonstrated that *in vivo* oxidative stress levels are significantly increased in patients with cerebral infarction complicated by uremia. This increase is accompanied by elevated levels of ET-1 and NO, suggesting that endothelial dysfunctions can be triggered by an elevated oxidative stress response when cerebral infarction is complicated by uremia. Conventional antiplatelet and cerebral protection therapy can partially reduce *in vivo* oxidative stress levels and improve endothelial function; low-dose urokinase in uremic dialysis fluid can significantly reduce *in vivo* lev-

els of oxidative stress at an early stage of treatment, greatly improve endothelial function, and have a role in treatment of cerebral infarction complicated by uremia.

Acknowledgements

This work was supported by Grants from Project of Shandong Province Higher Educational Science and Technology Program (J11LF78), and by Grants from China Postdoctoral Science Foundation (20060390635).

Disclosure of conflict of interest

None.

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