

Study on the clinical efficacy of Human Urinary Kallikrein in the treatment of acute cerebral infarction according to TOAST classification

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Abstract: To observe and evaluate the clinical efficacy of Human Urinary Kallikrein in the treatment of acute cerebral infarction (ACT) according to TOAST (The Trial of Org 10172 in Acute Stroke Treatment) classification. In accordance with randomized controlled trial, 110 patients with acute cerebral infarction were randomly assigned to kallikrein treatment group (55 cases) and control group (55 cases). TOAST classification and basic treatment were administrated on patients between two groups respectively. 0.15 PNA unit of Human Urinary Kallikrein injection plus 100 mL saline in intravenous infusion was performed in the kallikrein group, with once a day for 14 consecutive days. The National Institutes of Health Stroke Scale (NIHSS) scores in two groups were analyzed before and after the treatment. No difference was shown in the NIHSS scores before treatment among patients between two groups ($P>0.05$). While after the treatment, the NIHSS scores in both groups were reduced ($P<0.05$) and the NIHSS scores in the kallikrein treatment group were less than those in control group ($P<0.05$). Moreover, after the treatment, the NIHSS scores for large-artery atherosclerosis subtype (L) and small-artery occlusion lacunar subtype (S) as two subtypes of TOAST classification in the two groups were both reduced ($P<0.05$). After the treatment, NIHSS scores for L subtype in the kallikrein treatment were less than those in the control group ($P<0.05$). After the treatment, NIHSS scores for S subtype in the kallikrein treatment were less than those in the control group, without statistically significant difference. Comparisons on clinical efficacy indicated differences on the S subtype between two groups ($P<0.05$). The standardization effective rate was calculated, indicating 81.82% in the kallikrein treatment group and 54.55% in the control group, respectively. In TOAST classification, Human Urinary Kallikrein is able to remarkably improve the NIHSS scores for L subtype and S subtype patients with acute cerebral infarction and help to enhance the clinical efficacy.

Keywords: Cerebral infarction; Drug therapy; Human Urinary Kallikrein; Efficacy; TOAST classification.

INTRODUCTION

At present, the major approach to treat cerebral infarction is thrombolytic therapy, which has a strict time window limitation and relatively numerous contraindications, with many bleeding complications after treatment (Cohen and Leker, 2011). Searching for safe and effective therapeutic methods on cerebral infarction is still the current research and development trend. Human Urinary Kallikrein, as a glycoprotein extracted from urine, is a sort of the first class national new drugs (Tang *et al.*, 2012). Clinical studies have showed that it is able to activate its kininogenase kallikrein-kinin system (Campbell, 2011), expand cerebral small artery, improve the blood supply and oxygen supply in the ischemia cerebral tissues, as well as promote the blood vessel and nerve regeneration in ischemic regions (Xia *et al.*, 2006; Chao and Chao, 2006; Li *et al.*, 2005). According to etiological factors, pathogenesis, clinical types, onset time and other aspects, the therapeutic regimens on cerebral infarction, with

strong pointedness, are ascertained and the individualized treatments, at the core of classification and stage, are implemented (Rao, 2007). TOAST classification is an etiological classification, widely accepted in international (Chen, 2011). This study, through the observation on the clinical efficacy of Human Urinary Kallikrein in the treatment of acute cerebral infarction according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification, was to analyze the mechanism of Human Urinary Kallikrein in the treatment of acute cerebral infarction.

MATERIAL AND METHODS

Clinical information

Study subjects

One hundred and ten patients with acute cerebral infarction who were admitted in the neurology department of the First Affiliated Hospital of Zhengzhou University, from January 2012 to December 2014 were taken as study subjects. They were in accordance with the diagnostic criteria of cerebral infarction approved by the fourth

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national cerebrovascular academic conference (1995) (Chinese Neuroscience Society, Chinese Neurosurgical Society, 1996), and confirmed by head CT or MRI. Inclusion criteria for cases: (1) Ages ranging from 18 to 75 years. (2) Patients with the first onset or with a history of cerebral infarction but no sequelae left; (3) Onset time less than 48 h; (4) Patients without bleeding disorder or bleeding trends in latest one month; (5) Patients without incomplete hepatic and renal function; (6) Patients without the medical history of peptic ulcer, hemorrhagic stroke, brain tumor and brain trauma; (7) Patients or their relatives had signed the informed consent.

Grouping

According to randomized controlled trial, 110 patients were assigned into two groups: 55 cases in the kallikrein treatment group (38 male and 17 females; an average age at (63.23 ± 9.09) years old; 36 cases together with hypertension, 9 cases with diabetes and 10 cases with coronary heart disease; (6.54 ± 6.53) value for the NIHSS scores on hospitalization). 55 cases in the control group (36 male and 19 females; an average age at (63.13 ± 8.43) years old; 37 cases together with hypertension, 10 cases with diabetes and 8 cases with coronary heart disease; (7.56 ± 6.31) value for the NIHSS scores on hospitalization). Baseline comparisons on ages, gender compositions, past medical history, NIHSS scores on hospitalization indicated that there was no statistically significant difference ($P > 0.05$).

According to TOAST classification, the cerebral infarction patients between two groups were divided into large-artery atherosclerosis (L), small-artery atherosclerosis (S), cardiogenic cerebral embolism (C), other determined etiology (O) and undetermined causes (U) [10]. In the kallikrein treatment group, there were 19 cases (34.5%) in L type, 21 cases (38.2%) in S type, 6 cases (10.9%) in C type, 2 cases (3.6%) in O cases and 7 cases (12.7%) in U cases; While in the control group, there were 21 cases (38.2%) in L type, 20 cases (36.4%) in S type, 6 cases (10.9%) in C type, 2 cases (3.6%) in O cases and 6 cases (10.9%) in U cases. Comparisons on the TOAST classification between two groups showed no statistical significance in difference ($P > 0.05$) and were comparable.

Therapeutic methods

Basic treatment was performed among patients in both two groups according to disease condition, with antiplatelet therapy, statins preparation and citicoline neuroprotective agents. The dehydrating agent to alleviate the cerebral edema was selected appropriately according to the infarction areas. During combining the basic diseases, corresponding expectant treatment should be conducted, such as controlling the blood pressure and blood glucose. The above mentioned basic treatment was performed on the control group. On that basis, 0.15 PNA

unit of Human Urinary Kallikrein injection (Trade name: Kailikang, Guangdong Techpool Bio-Pharma Co., Ltd. With approved medicine of H20052065) plus 100mL saline in intravenous infusion was taken in the kallikrein group, with once a day for 14 consecutive days. In the kallikrein group, during 24 h before medication and in the treatment period, angiotensin converting enzyme inhibitor, steroid drugs and other therapeutic drugs on cerebral infarction were forbidden.

Monitoring indexes

NIHSS scores was performed before treatment and on the 14 day after treatment among patients in the two groups respectively. The blood pressure was monitored during the treatment process. Moreover, before treatment and on the 14 day after treatment, blood urine routine, blood biochemistry, blood coagulation, electrocardiogram and others were examined. Head CT and MRI were reexamined in necessity. Drug-related bleeding events and adverse effect were observed.

Efficacy evaluation and safety evaluation

Criteria in efficacy evaluation

According to the improvement degree of clinical NIHSS scores, it was divided as: basic cure (NIHSS score was decreased more than 90%); significant improvement (NIHSS score was decreased by 46%-89%); improvement (NIHSS score was decreased by 18%-45%); no-change (NIHSS score was decreased or increased no more than 18%); deterioration (NIHSS score was increased more than 18%); death. The basic cure, significant improvement and improvement were regarded effectivity, while no-change, deterioration and death were regarded inefficiency (Chinese Neuroscience Society, Chinese Neurosurgical Society, 1996).

Safety evaluation

Patients' blood pressure was closely observed during the treatment. Experimental indicators before and after treatment as well as various adverse effect events were recorded in details. Those who quitted midway due to adverse effect should also participate the safety evaluation (Tan *et al.*, 2011).

STATISTICAL ANALYSIS

Statistical software SPSS 16.0 was adopted for statistical analyses. Mean \pm standard deviation ($\bar{x} \pm s$) was shown for the results. Independent sample *t* test was used for mean comparison in the two samples in measurement data. Meanwhile, paring *t* test was used for paired data and χ^2 test was adopted for enumeration data comparison. The direct method was applied to the standard and effective calculation. Two-sided test was taken for all statistics. $P < 0.05$ was considered statistical significance.

RESULTS

NIHSS scores for patients between two groups before and after treatment

No statistically significant difference was shown in the comparisons of NIHSS scores before treatment among patients between two groups ($P > 0.05$). While in the 14 day after the treatment, the NIHSS scores in both group were reduced ($P < 0.05$). The NIHSS scores after treatment in the kallikrein group was less than those in control group, showing statistically significant difference. Those suggested that the effect of Human Urinary Kallikrein in improving patients' neurological impairment in the kallikrein treatment group was superior to that in the control group (Table 1).

In the 5 subtypes of TOAST classification, NIHSS scores comparison before treatment for patients in treatment group and control group showed no statistically significant difference ($P > 0.05$). Moreover, after the treatment, the NIHSS scores for both large-artery atherosclerosis subtype (L) and small-artery occlusion lacunar subtype (S) in two groups were reduced ($P < 0.05$). After the treatment, NIHSS scores for L subtype in the kallikrein treatment were less than those in the control group, indicating statistically significant difference ($P < 0.05$). After the treatment, NIHSS scores for S subtype in the kallikrein treatment were less than those in the control group, without statistically significant difference. There was no distinct change in NIHSS scores of C, O and U types before and after treatment (table 2).

Efficacy evaluation for patients between two groups

In the 14 day after treatment, there were 7 cases in basic cure, 13 cases in significant improvement, 21 cases in improvement, 9 cases in no-change, 15 cases in deterioration and 0 case in death in the kallikrein treatment, while there were 2 case in basic cure, 12 cases in significant improvement, 12 cases in improvement, 23 cases in no-change, 3 cases in deterioration and 3 cases in death in the control group. χ^2 test was applied to compare the effectivity of two samples and the efficacies between two groups were compared, showing a statistically significant difference ($\chi^2 = 8.59$, $P = 0.01$).

In the 5 subtypes of TOAST classification, the clinical efficacy for patients in treatment group and control group was compared, respectively. That of S subtype showed a statistically significant difference; while that of other subtypes indicated no statistically significant difference ($P > 0.05$) (table 3).

The 5 subtypes of TOAST classification presented a different distribution proportion in the kallikrein treatment group and control group. The standard effective rates for the two groups were calculated respectively as 81.82% in the kallikrein treatment group and 54.55% in the control group, suggesting that the total effective rate in the kallikrein treatment group was larger than that in the control group.

Safety analyses

Human Urinary Kallikrein in intravenous infusion was

Table 1: Comparisons of National Institutes of Health Stroke Scale (NIHSS) before and after treatment

Grouping	n	Before treatment	After treatment	Pairing <i>t</i> test	<i>P</i> value
Kallikrein treatment group	55	6.54±6.53	3.93±4.26	4.36	0.01
Control group	55	7.56±6.31	6.47±7.51	2.21	0.02
Independent sample <i>t</i> test		-0.51	-2.21		
<i>P</i> value		0.54	0.04		

Table 2: NIHSS comparisons of different TOAST subtypes between two groups before and after treatment

Grouping	Subtype	NIHSS scores	
		Before treatment	After treatment
Kallikrein treatment group (n = 55)	L(n=19)	7.01±8.12	2.49±3.36*#
	S(n=21)	5.23±2.87	2.75±2.29*
	C(n=6)	14.21±9.36	9.98±8.97
	O(n=2)	4.48±0.69	3.01±1.39
	U(n=7)	5.64±3.21	3.42±2.23
Control group (n = 55)	L(n=21)	8.14±5.01	6.48±4.64*
	S(n=20)	5.49±3.61	4.31±3.74*
	C(n=6)	14.35±10.54	16.21±15.13
	O(n=2)	3.46±0.68	2.48±0.69
	U(n=6)	6.13±7.21	4.51±7.21

Note: *Compared that before treatment, $P < 0.05$; # Compared that in control group, $P < 0.05$

implemented on patients in the kallikrein group. At the same time, during 24 h before medication and in the medication process, angiotensin converting enzyme inhibitor (ACEI) antihypertensive drug was forbidden to patients in the kallikrein group. There was no hypotension in this study. On the 12 day with the application of Human Urinary Kallikrein treatment, bleeding cerebral infarction occurred on the 1 cerebral infarction patient of C subtype, on whom drug discontinuance in advance was administrated, finally the disease condition was still in progress after drug discontinuance. Furthermore, there was no remarkable adverse reaction occurring on other patients. The examination of blood urine routine, blood chemistry and electrocardiogram for the rest patients in the kallikrein treatment group before and after the medication indicated on abnormal change.

DISCUSSION

Human Urinary Kallikein, as a glycoprotein preparation extracted from human urine, is a sort of the first class national new drugs (Ding, 2005). Through kallidin generated by activated kininogen and a nine-peptide regulator generated by the action of kininase 1, thus acting targetedly on the β_1 receptor induced specifically by ischemia/injure, it can cause the effect of the relaxation of vascular smooth muscles and others. Pharmacodynamics study has showed that Human Urinary Kallikein is able to selectively expand the cerebral small artery in the ischemia regions, enhance the blood supply and oxygen supply in the ischemia cerebral tissues, suppress platelet aggregation, reduce the apoptosis of nerve cells and neuroglial cells and the infiltration of inflammatory cells, as well as promote the generation of new blood vessel and the regeneration of nerve cells (Li *et al.*, 2005). Clinical experiments results at II and III stages indicated that it is safe and effective for Human Urinary Kallikein used in the treatment of acute cerebral infarction.

This study found that NIHSS scores for patients could be significantly improved at the 14 day after Human Urinary Kallikein in the treatment of acute cerebral infarction. Meanwhile, the effect was superior to that in the control

group and the total effective rate was higher than that in the control group. On this basis, considering that different etiological factors in acute cerebral may affect its efficacy and prognosis (Chang, *et al.*, 2009; Zhen *et al.*, 2011), this study was to further analyze the impact of different TOAST classification on the efficacy of Human Urinary Kallikrein. The results indicated that those with effective treatment and significant improvement in NIHSS scores in the kallikrein treatment group were most L and S subtype; No significant improvement in NIHSS scores and uncertain clinical efficacy were shown in C, O and U subtype before and after the treatment. Through the injection of glass beads in carotid artery to induce the cerebral embolism of rabbits and the study in the impacts of Urinary Kallikrein on microcirculation. Nagano H *et al* (Nagano and Hayashim, 1992) indicated that Urinary Kallikrein had a selectivity on the dilation of blood vessel. The sequences from strong to weak were smaller thin arterioles, larger thin arterioles and arterioles. The majority of S subtype was small vessel lesions, with relatively small infarct lessons. Moreover, in the acute stage of cerebral infarction, Urinary Kallikrein had the ability to expand thin arterioles effectively, help the recanalization of pathological vessels or promot the formation of the collateral circulation in ischemic regions, improve the blood supply and oxygen supply of ischemic cerebral tissues as well as improve the NIHSS scores of S subtype patients of acute cerebral infarction; L subtype was occupying more than 50% in the luminal stenosis of large artery caused by atherothrombosis. It was always clonic onset, accompanied with collateral circulation openness. Besides, using Urinary Kallikrein at the acute stage of cerebral infarction was able to promote the neovascularization. In animal experients, it had been confirmed that Urinary Kallikrein was able to promote the formation of new vessels in ischemic regions, reduce nerve cell apoptosis and play the neuroprotection role. Through transgenic mehtod in the cerebral ischemia/reperfusion mice model, Liu *et al* (2009) studied the neuroprotection mechanism of human tissue-type kininogenase and found that human tissue-type kininogenase, through activating ERK1/2 signal pathway rather than JNK and P38 signal pathway, reduced ROS generation, inhibited caspase-3 activity and played the

Table 3: Comparisons of clinical efficacy after the treatment of different TOAST subtypes between two groups

Grouping	L		S		C		O		U	
	Effecti- vity	Ineffec- tiveness	Effecti- vity	Ineffec- tiveness	Effecti- vity	Ineffec- tiveness	Effecti- vity	Ineffec- tiveness	Effecti- vity	Ineffectiv- eness
Kallikrein treatment group	16	3	19	2	3	3	1	1	6	1
Control group	13	8	11	7	2	5	1	1	3	4
χ^2 value	1.48		4.57		0.00		0.00		2.87	
P value	0.11		0.04		1.00		1.00		0.07	

neuroprotection role. Su et al (Su, *et al.*, 2011), on the reearch basis of Liu, had a further find that human tissue-type kininogenase, through the activation of Homer 1b/c signal pathway, enabled the phosphorylation of ERK 1/2 and Akt-GSK 3 β , thus activating ERK 1/2 signal pathway. C subtype was always the relatively large artery in cardiogenic embolus embolism cranium, referring a complicated mechanism (Montaner *et al.*, 2008; Licata *et al.*, 2009; Tuttolomondo *et al.*, 2012). Furthermore, the function and mechanism of Human Urinary Kallikrein in different TOAST classification needed the further studies.

Through dependent sample t test, it was obtained in this study that the effect of L subtype in the improvement of NIHSS scores in kallikrein treatment group was superior to that in the control group. With the utilization of tests, it was gained that the clinical efficacy of S subtype in the kallikrein group was superior to that in the control group. Those suggested that the etiological factor was not the only cause impacting the clinical efficacy of Human Urinary Kallikrein in the treatment of acute cerebral infarction and the efficacy may be impacted by multi-factor jointly. TOAST classification was merely referred to etiological diagnosis, with limitations in itself. Moreover, due to few samples, short-time research and other reasons, the results can't be regarded as the only basis to guide the medicine selection of Human Urinary Kallikrein and the mechanism of Human Urinary Kallikrein still can't be confirmed. Specific to the scarcity of TOAST classification, Gao et al (Gao *et al.*, 2011; Amarenco *et al.*, 2009) put forward the CISS (Chinese ischemic stroke subclassification) classification, a new typing method combined with pathogenesis.

It is concluded from this study that the treatment of Human Urinary Kallikrein in acute cerebral infarction is effective and safe. Furthermore, the therapeutic time window not only can be prolonged, but also enables more acute cerebral infarction patients without thrombolytic therapy to receive a favorable treatment effect and to improve the prognosis.

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Study on the clinical efficacy of human urinary kallikrein

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