Human Urinary Kallidinogenase Improves Outcome of Stroke Patients by Shortening Mean Transit Time of Perfusion Magnetic Resonance Imaging

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Background: Improving cerebral perfusion remains a good option for ischemic stroke for restoring cerebral blood flow. Human urinary kallidinogenase has been shown promising in treating stroke patients. To investigate whether human urinary kallidinogenase's efficacy in treating stroke patients has relationship with improving cerebral perfusion and possible mechanism. *Methods:* Fifty-eight stroke patients in Nanjing Drum Tower Hospital were enrolled in this prospective study. Of them, 29 received human urinary kallidinogenase, while the other 29 were selected as control. National institute health stroke scale, modified Rankin Scale and activities of daily living score were used to determine patient outcome. Cerebral perfusion in patients was determined by perfusion magnetic resonance imaging. Serum apelin and vascular endothelial growth factor were measured by enzyme-linked immunosorbent assay. *Results:* We confirmed that human urinary kallidinogenase improved stroke outcome in patients. Cerebral perfusion was elevated by human urinary kallidinogenase 12 days after therapy. Human urinary kallidinogenase enhanced vascular endothelial growth factor and APJ expression in stroke patients. The reduced mean transit time was related with favorable outcome analyzed by univariate logistic regression. *Conclusions:* Human urinary kallidinogenase facilitated stroke recovery and enhanced cerebral reperfusion through up-regulating vascular endothelial growth factor, apelin/APJ pathway. **Key Words:** Human urinary kallidinogenase—ischemic stroke—cerebral perfusion—angiogenesis—VEGF—apelin/APJ.

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Stroke brought great burden to the world with dissatisfactory conventional treatment available to date.[1] For stroke patients, improving cerebrovascular perfusion, such as thrombolysis, dilation of the arteriole, and formation of new vessels in the penumbra region, remains a good option to rescue injured tissue and alleviate neurologic deficits.[2]

The KKS system consisting of kinins, kallikreins, and kininogens have been shown to protect against ischemic stroke in patients[3] and animal studies.[4] A case–control study on 1268 stroke patients and 1210 controls confirmed that plasma tissue kallikrein level is negatively related to first-ever stroke and stroke recurrence.[5] Human urinary kallidinogenase (HUK) is a positive regulatory substance in KKS by producing kallikrein. It has been approved by Chinese State Food and Drug Administration and used clinically in China to treat stroke patients for more than 4 years.[3] However, the efficacy and relative mechanisms are still not well understood.

Kallikrein has been identified to protect against ischemic brain injury through multiple signaling pathways including anti-inflammation, antiapoptosis, promoting angiogenesis and neurogenesis.[4,6] In addition to ischemic brain injury, research on hind limb ischemia, cardiac infarction, and renal ischemia further confirmed kallikrein was a novel angiogenic factor currently.[7] However, whether HUK, a commercially available KKS-regulating medicine, is capable to promote cerebral perfusion has not been reported yet.

Recently, growing interest is concern on the signaling mechanisms through which tissue kallikrein promotes angiogenesis after ischemic injury. Apelin is a novel angiogenic factor identified as the endogenous ligand of its receptor APJ. Apelin and APJ are expressed widely on heart, brain, kidneys, and lungs.[8] Kallikrein has been shown to promote neovascularization through kinin-mediated activation of the Akt–endothelial nitric oxide synthase pathway and display vascular endothelial growth factor (VEGF) dependency.[6] However, no data are available to show that kallikrein could promote other proangiogenic factors like apelin.

In this study, we aim at providing additional evidence on the efficacy and safety of HUK in the application of stroke patients. We will further evaluate the angiogenic roles of HUK and confirm whether HUK could enhance cerebral perfusion in the penumbra region. Finally, we intend to find out the underlying targets through which HUK exerts its angiogenic function.

Methods

Patient Selection

An open-label, control, prospective study was performed. This study was approved by the ethics committee of the Affiliated Drum Tower Hospital, Nanjing University Medical School, and carried out in 58 stroke patients at the Department of Neurology, Drum Tower Hospital, between May 2012 and May 2013, which were divided into 2 groups randomly with (HUK group) or without (control group) HUK using a computer-generated random sequence. Eligible patients were men and women aged 18-80 years who had a clinical diagnosis of ischemic stroke and at 6 to 72 hours after stroke onset. All the enrolled patients signed the informed consent and belonged to anterior circulation infarction according to magnetic resonance imaging (MRI) scanning. Patients were excluded if they had the following situations: (1) signs of cerebral hemorrhage on computed tomography scan; (2) National Institutes of Health Stroke Scale (NIHSS) score of less than or equal to 3 or greater than or equal to 21; (3) hypertension at the time of admission: blood pressure 200/110 mm Hg or more or 90/60 mm Hg or less; (4) pregnancy, lactation, or parturition within the previous 30 days; (5) presence of platelet abnormalities (PLT $<100 \times 10^9$/L or PLT $>300 \times 10^9$/L) and anemia (Hemoglobin < 90 g/L); (6) presumed cardiogenic embolism or mental disorders; (7) previous known pneumonia or multiorgan dysfunction; (8) recent (within 2 weeks) thrombolysis and anticoagulation treatment; (9) receiving angiotensin converting enzyme inhibitors (ACEI) drugs within 24 hours; and (10) with MRI contraindication including a history of heart valve replacements, pacemakers, or cranial artery clipping.

Procedures

Antiplatelet, antihypertension, and hypoglycemic drugs were regularly given to HUK and control groups. All patients were given daily ozagrelum 80 mg/day and 75 mg/day clopidogrel for 10 days, then aspirin 100 mg/day for long term. MRI was performed to evaluate the perfusion, and NIHSS and activities of daily living (ADL) score were used to calculate the efficacy of HUK on the first day and 12th day after HUK treatment. Moreover, VEGF and apelin were also tested at first and 12th day after HUK treatment. Blood pressure was all recorded to evaluate the safety of HUK before and after treatment every day. At 3-month follow-up, ADL and modified Rankin Scale (mRS) scores were assessed by telephone.

Magnetic Resonance Imaging

MRI data were collected at 3 T using an 8-channel phased array head coil (Achieva 3.0 T TX dual-source parallel RF excitation and transmission technology; Philips Medical Systems, Best, The Netherlands). The MRI protocol included axial diffusion-weighted imaging (DWI), fluid-attenuated inversion recovery (FLAIR) imaging, MR angiography (MRA), and the dynamic susceptibility contrast-enhanced perfusion-weighted imaging (DSC-PWI).

DSC-PWI scans were acquired using a fast-field echo combined with echo-planar imaging with an intravenous