ABSTRACT

Purpose: To investigate the effect of human urinary kallidinogenase (HUK) on neurological deficit, plasma fibrinogen and apoptosis-related factors in patients with acute cerebral infarction (ACI).

Methods: Patients with ACI (96 cases) were recruited over a 1-year period for this study, and were randomly assigned to two groups of 48 patients each: the control group and the observation group. They consisted of 53 males and 43 females aged 55 to 76 years (mean age = 64.9±7.8 years). The course of disease ranged from 5 to 19 h (mean duration = 11.9±3.0 h). The control group received aspirin (100 mg/kg body weight, bwt/day), atorvastatin (20 mg/kg bwt/day), and edaravone injections (30 mg/kg bwt, twice a day). In addition to treatment in the control group, the observation group received HUK injection (0.15 PNA HUK dissolved in 100 ml of physiological saline)/day for 2 weeks. Clinical effectiveness and neurological deficits were assessed based on the National Institutes of Health Stroke Scale (NIHSS). The level of plasma fibrinogen was measured using the Biuret method, while serum levels of B-lymphocyte tumor-2 protein (Bcl-2) and cytochrome C (Cyt C) were measured using enzyme-linked immunosorbent assay (ELISA) kits.

Results: After treatment, total effectiveness was significantly higher in the observation group (91.67%) than in control group (77.08%), while the neurological deficit score and infarct size were significantly reduced (p<0.05). The levels of plasma fibrinogen were significantly reduced in the observation group relative to the control group at weeks 1 and 2, and 1 month after treatment (p<0.05). The level of plasma fibrinogen were significantly reduced in the observation group relative to the control group at weeks 1 and 2, and 1 month after treatment (p<0.05). The level of Bcl-2 at day 14 after treatment was significantly higher in the observation group than in the control group (p<0.05). In the observation group, serum levels of Cyt C were significantly reduced at day 14 and 3 months after treatment, when compared to the levels at day 1 after treatment. At day 1 after treatment, the levels were significantly higher than those in the control group. However, at day 14 and 3 months after treatment, Cyt C was significantly lower than the corresponding control level (p<0.05). There was only 1 case of hypotension in the observation group which was not related to HUK. There was no significant difference in the total incidence of adverse reactions between the two groups (p>0.05).

Conclusion: The results obtained in this study have shown that HUK effectively ameliorates neurological deficits, reduces plasma fibrinogen levels and inhibits neuronal apoptosis in ACI patients.

Keywords: Acute cerebral infarction, human urinary kallidinogenase, neurological deficit, plasma fibrinogen, Apoptosis-related factors.

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Introduction

Acute cerebral infarction (ACI) is an acute cerebrovascular disease common in the elderly, and characterised by rapid progression, high incidence, high disability and high mortality. At present, its clinical treatment focuses mainly on saving dying brain cells and reducing the infarct area. Anticoagulation, fluid supplementation, thrombolysis and improvement in cerebral circulation are the conventional methods for treating ACI. However, the clinical effectiveness is limited, and treatments are accompanied by a high incidence of adverse reactions. Human urinary kallidinogenase (HUK), a drug often used for treating ACI, dilates blood vessels, improves perfusion, effectively protects brain neurons and reduces infarct size. Previous studies have shown that the combination of conventional treatment with the use of neuro-protective drugs can significantly ameliorate the clinical symptoms.
of ACI\(^3\). The aim of this study was to investigate
the effects of HUK on neurological deficit, and lev-
eels of plasma fibrinogen and apoptosis-related fac-
tors in patients with ACI.

Methods

Patients and general information
Patients with ACI (96 cases) were recruited
over a 1-year period for this study and randomly
assigned to two groups of 48 patients each:
Control group and observation group. They
consisted of 53 males and 43 females aged 55 to
76 years (mean age = 64.9±7.8 years). The course
of disease ranged from 5 to 19 h (mean duration =
11.9±3.0 h).

Their baseline medical history was:
Diabetes (27 cases), coronary heart disease
(18 cases), hypertension (36 cases) and hyperlipi-
daemia (15 cases).

According to the location of lesion, the pa-
tients were classified thus:
Basal ganglia lesion (53 cases), cerebral lobe
lesion (27 cases), cerebellum lesion (11 cases) and
pons lesion (5 cases).

The inclusion criteria were:
• Patients who met the diagnosis criteria for
ACI based on the Guidelines for Preventing Cere-
brovascular Diseases\(^4\), and confirmed by MRI and
CT scan;
• Patients who saw the doctor less than 72 h
from time of onset;
• Patients aged 40 to 76 years;
• Patients with different degrees of neurolog-
ical deficits;
• Patients who did not receive anticoagulant
thrombolyis before enrolment;
• Patients who signed written informed con-
sent with their family members.

The exclusion criteria were:
• Patients with haemorrhagic cerebral infarction,
brain tumours and other cerebral organic diseases;
• Patients who had infectious or autoimmune
diseases;
• Patients who had severe heart, liver and kid-
ney dysfunctions;
• Patients who had allergies, especially those
who were allergic to HUK;
• Pregnant and lactating women. There were
no significant differences in age, sex and lesion lo-
cation between the two groups (Table 1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male/female</td>
</tr>
<tr>
<td></td>
<td>Observation</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Age (year)</td>
<td>Mean age</td>
</tr>
<tr>
<td></td>
<td>Observation</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Course of disease</td>
<td>11.2±2.1</td>
</tr>
<tr>
<td>Baseline medical history</td>
<td>64.6±7.3</td>
</tr>
<tr>
<td></td>
<td>65.1±7.2</td>
</tr>
<tr>
<td>Baseline medical history</td>
<td>Diabetes</td>
</tr>
<tr>
<td></td>
<td>Observation</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Baseline medical history</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td></td>
<td>Observation</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Baseline medical history</td>
<td>Hypertension</td>
</tr>
<tr>
<td></td>
<td>Observation</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Baseline medical history</td>
<td>Hyperlipidaemia</td>
</tr>
<tr>
<td></td>
<td>Observation</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Lesion location</td>
<td>Basal ganglia</td>
</tr>
<tr>
<td></td>
<td>Observation</td>
</tr>
<tr>
<td>Lesion location</td>
<td>Cerebral lobe</td>
</tr>
<tr>
<td></td>
<td>Observation</td>
</tr>
<tr>
<td>Lesion location</td>
<td>Cerebellum</td>
</tr>
<tr>
<td></td>
<td>Observation</td>
</tr>
<tr>
<td>Lesion location</td>
<td>Pons</td>
</tr>
<tr>
<td></td>
<td>Observation</td>
</tr>
</tbody>
</table>

Table 1: General clinical data of patients.

Treatment regimen
Control group:
Aspirin (100 mg/kg bwt/day), atorvastatin (20
mg/kg bwt/day), and edaravone injection (30 mg/
kg bwt, twice a day) were administered.

Observation group:
In addition to treatment in the control group,
the observation group received HUK injection
(0.15 PNA HUK dissolved in 100 ml of physio-
logical saline)/day for 2 weeks. The dripping speed
was controlled at 30 - 40 drops/min.

Observation indices

Assessment of clinical effectiveness
Clinical effectiveness was assessed based on
the National Institutes of Health Stroke Scale (NI-
HSS)\(^5\) before and after treatment, and classified
into five: Recovery, remarkably effective, effec-
tive, ineffective and deterioration.

Recovery:
NIHSS score reduced by 90-100 %, with
grade 0 degree of disability;
Remarkably effective:
NIHSS score decreased by 45-89 %, with de-
gree of disability between grades 1 and 3;
Effective:
NIHSS score decreased by 18-45 %;
Ineffective:
NIHSS score reduced by about 18 %;
Deterioration:
NIHSS score increased by about 18 %.
The total effectiveness was calculated thus:

\[
\text{Total effectiveness (\%)} = \left( \frac{\text{recovery} + \text{remarkably effective} + \text{effective}}{\text{Total number of cases}} \right) \times 100
\]

Assessment of neurological deficits
This was performed using the NIHSS and the
patients were scored. The lower the score was, the
better the neurological status of the patient.
Determination of levels of plasma fibrinogen
Plasma fibrinogen was measured using Biuret method.

Determination of levels of apoptosis-related factors
The serum levels of Bcl-2 and Cyt C were measured using ELISA kits.

Statistical analysis
Data are expressed as mean ± SEM, and the statistical analysis was performed using SPSS (19.0). Groups were compared using Student t-test, and values of p<0.05 were considered statistically significant.

Results

Clinical effectiveness between the two groups
After treatment, total effectiveness was significantly higher in the observation group (91.67%) than in control group (77.08%) (p<0.05). The results are shown in Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Recovery</th>
<th>Remarkably effective</th>
<th>Effective</th>
<th>Ineffective</th>
<th>Deterioration</th>
<th>Total effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>48</td>
<td>13 (27.08%)</td>
<td>24 (50.00%)</td>
<td>7 (14.58%)</td>
<td>4 (8.33%)</td>
<td>0 (0.00%)</td>
<td>44 (91.67%)</td>
</tr>
<tr>
<td>Control</td>
<td>48</td>
<td>10 (20.83%)</td>
<td>19 (39.58%)</td>
<td>8 (16.67%)</td>
<td>9 (18.75%)</td>
<td>2 (4.16%)</td>
<td>37 (77.08%)</td>
</tr>
</tbody>
</table>

*p<0.05, when compared to control group.

Degree of neurological deficit and infarction size
Before treatment, there was no significant difference in neurological deficit score and infarct size between two groups (p>0.05). However, after treatment, neurological deficit score and infarct volume were significantly lower in the treatment group than in the control group (p<0.05). These results are shown in Table 3.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Neurological deficit score</th>
<th>Infarction size (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Observation</td>
<td>48</td>
<td>23.1±3.5</td>
<td>15.2±3.1</td>
</tr>
<tr>
<td>Control</td>
<td>48</td>
<td>23.1±3.0</td>
<td>10.2±2.6</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>0.076</td>
<td>6.942</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*p<0.05, when compared to the value before treatment.

Levels of plasma fibrinogen
There was no significant difference in the level of plasma fibrinogen between the two groups before treatment (p>0.05). However, its levels were significantly lower in the observation group than in control group at weeks 1 and 2, and at 1 month after treatment (p<0.05: Table 4).

Table 4: Comparison of the levels of plasma fibrinogen between the two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before treatment</th>
<th>1 week after treatment</th>
<th>2 weeks after treatment</th>
<th>1 month after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>3.80±0.25</td>
<td>1.21±0.96*</td>
<td>1.69±0.71*</td>
<td>1.90±0.81*</td>
</tr>
<tr>
<td>Control</td>
<td>3.80±0.91</td>
<td>3.56±1.23*</td>
<td>3.49±0.81*</td>
<td>3.46±0.88*</td>
</tr>
<tr>
<td>r</td>
<td>0.038</td>
<td>8.772</td>
<td>9.412</td>
<td>7.424</td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*p<0.05, when compared to the value before treatment.

Levels of apoptosis-related factors
In the observation group, serum Bcl-2 was significantly higher at day 14 after treatment than at day 1 and 3 months after treatment (p<0.05). At day 14 of treatment, it was significantly higher in the observation group than in the control group (p<0.05). However, in the control group, there were no significant differences in the levels of Bcl-2 at the different time points (p>0.05: Table 5).

Table 5: Comparison of serum levels of Bcl-2 between the two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>1 day after treatment</th>
<th>14 days after treatment</th>
<th>3 months after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>48</td>
<td>4.83±0.26</td>
<td>5.48±0.25*</td>
<td>4.99±0.25*</td>
</tr>
<tr>
<td>Control</td>
<td>48</td>
<td>4.58±0.25</td>
<td>4.35±0.26</td>
<td>4.15±0.29</td>
</tr>
</tbody>
</table>
| *p<0.05, when compared to control group; #p<0.05, when compared to day 1 and 3 months after treatment.

In the observation group, the serum levels of Cyt C were significantly reduced at day 14 and 3 months after treatment when compared to levels on day 1 after treatment (p<0.05). At day 1 after treatment, it was significantly higher than the corresponding level in the control group (p>0.05). However, at day 14 and 3 months after treatment, the levels were significantly lower than the corresponding levels in the control group (p<0.05). In the control group, the level of Cyt C was significantly higher at day 14 after treatment than at day 1 and 3 months after treatment (p<0.05: Table 6).

Table 6: Comparison of serum levels of Cyt C between the two groups.
During the treatment period, one patient in the observation group suffered a reduction in blood pressure, which was relieved after adjustment. There were 3 cases of liver function damage in the control group, which were significantly improved after discontinuing the use of statins. There was no significant difference in the total incidence of adverse reactions between the two groups (p>0.05), and the adverse reactions were not related to HUK.

**Discussion**

A sudden decrease or seizure of local blood supply to the brain tissue results in brain ischaemia and hypoxia, and ultimately leads to irreversible brain damage due to brain tissue necrosis and softening. This impairs, to varying degrees, the regulatory function of vascular nerves and hypothalamus, leading to neurological dysfunction and ACI(6-7). In practice, thrombolysis and neuro-protective therapy are the two main strategies for the treatment of ACI. However, thrombolysis produces adverse reactions which have negative impacts on prognosis. The commonly used vasodilators dilate blood vessels so that blood flow in the lesion area is diverted to normal vessels, a phenomenon described as “vascular access steal syndrome”(8).

Human urinary kallidinogenase (HUK), also known as human uro-kininogenase, is a glycoprotein isolated from the urine of healthy humans. Immediately following cerebral infarction, this enzyme reverses the sharp drop in the level of glucose in the brain, thereby preventing the development of cerebral ischaemic dysfunction and protecting brain function. Studies have shown that treatment of cerebral infarction using HUK can significantly improve vasoconstriction, tissue blood supply, oxygen supply and haemodynamics(9).

In the present study, total effectiveness was significantly higher in the observation group than in control group, and there was only 1 case of hypotension which was not related to HUK. There was no significant difference in the total incidence of adverse reactions between the two groups. These results suggest that HUK may effectively improve clinical symptoms of ACI, and are in agreement with those previously reported(10).

In this study, neurological deficit score and infarct size after treatment were significantly reduced relative to their values before treatment, an indication that HUK may significantly improve cerebral blood circulation and promote neurological recovery. This may not be unconnected with the fact that HUK inhibits the generation of superoxide anion, inflammation, and activity of reductase-type coenzyme II; prevents the occurrence of ischaemia-reperfusion injury, protects brain neuron, and alleviates neurological deficits(11). As an acute phase reaction protein, fibrinogen participates in platelet aggregation and blood coagulation. It makes the visible components in blood adhere to the walls of damaged blood vessels by increasing blood viscosity and slowing down blood flow, thereby aggravating the damaged state and promoting cerebral infarction(12). In this study, after treatment, the levels of plasma fibrinogen were significantly reduced in observation group when compared to the control group. The possible explanation for this is that HUK may block red blood cell (RBC) and platelet aggregation, and reduce peripheral vascular resistance, thereby reducing blood viscosity and improving vascular microcirculation.

Studies have shown that the development of cerebral infarction is closely associated with neuronal apoptosis. In a previous study involving rats with cerebral infarction, it was shown that necrotic cells exist mainly in the central area of infarction, while neuronal apoptosis occur mainly in the ischaemic penumbra, and that para-infarction lesion neurons are mainly apoptotic in the early stage of cerebral infarction(13). Apoptosis has been shown to be involved in the pathogenesis of diseases, and mitochondria-mediated apoptotic pathway plays an important role in the development of cerebral infarction. Upon the stimulation of cells, the mitochondrion releases a huge amount of apoptosis-related factors such as Cyt C and apoptosis inducing factor (AIF), which activate downstream effector caspase-3 and initiate the caspase cascade reaction, ultimately leading to cell apoptosis(14-15).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>1 day after treatment</th>
<th>14 days after treatment</th>
<th>3 months after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>48</td>
<td>275.74±12.56*</td>
<td>218.45±12.95*</td>
<td>234.15±13.25*</td>
</tr>
<tr>
<td>Control</td>
<td>48</td>
<td>256.42±13.71</td>
<td>268.52±12.37</td>
<td>258.28±13.51</td>
</tr>
</tbody>
</table>

Table 6: Comparison of serum levels of Cyt C between the two groups.

*p<0.05, when compared to control group; *p<0.05, when compared to day 1 after treatment.
B-Lymphocyte tumor-2 protein (Bcl-2), a membrane integrin, stabilises the levels of pro-apoptotic proteins in cells, prevents the release of Cyt C, and inhibits apoptosis(16).

In this study, the level of Bcl-2 at day 14 of treatment was significantly higher in the observation group than in control group. In the observation group, the serum levels of Cyt C were significantly reduced at day 14 and 3 months after treatment when compared to its level at day 1 after treatment. At day 1 after treatment, it was significantly higher than the corresponding level in control group. However, at day 14 and 3 months after treatment, Cyt C levels were significantly lower than the corresponding levels in the control group. These results appear to suggest that HUK may effectively regulate the levels of apoptotic factors and exert anti-apoptotic effects.

Conclusion

The results obtained in this study have shown that HUK effectively ameliorates neurological deficits, reduces the level of plasma fibrinogen and inhibits neuronal apoptosis in ACI patients.

References


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