Value of contrast-enhanced ultrasonography for evaluating the angiogenesis of carotid plaque in patients with cerebral infarction and its correlation with the changes of plaque properties

Juan Lyu, Jing Wan

Department of Ultrasound, Xinjiang Uygur Autonomous Region People’s Hospital, Urumchi, Xinjiang, 830000

1. Introduction

Cerebral infarction is one of the most common cerebrovascular diseases in China. The formation, rupture and shedding of carotid atherosclerotic plaques are the main mechanisms leading to intracranial arterial embolism. Early detection of carotid plaques and judgment of the plaque properties helps to early conduct secondary prevention and treatment and reduce the risk of cerebral infarction[1,2]. Carotid artery ultrasound is a common auxiliary examination method to detect carotid plaques and determine the plaque properties in clinical practice. Although routine ultrasound examination can detect carotid plaques in a timely manner, the judgment of plaque properties is quite limited, and the echo of plaques alone cannot accurately determine the plaque properties. Recent studies on the pathological characteristics of carotid plaques have shown that angiogenesis within plaques is an important link that leads to the changes in the plaque properties and the decrease in its stability, and the assessment of angiogenesis within plaques can provide a basis for the judgment of plaque properties[3]. Contrast-enhanced ultrasound (CEUS) is a method to evaluate angiogenesis.

In the following studies, we specifically analyzed the value of CEUS in evaluating the angiogenesis in carotid plaques in patients with cerebral infarction and its correlation with the changes in the plaque properties.
2. Case information and research methods

2.1 General case information

The patients with carotid low-echo plaque detected by carotid artery ultrasound in the Ultrasound Department of Xinjiang Uygur Autonomous Region People’s Hospital between January 2014 and January 2016 were chosen as the research subjects, and the patients with previous history of myocardial infarction or cerebral infarction as well as those combined with autoimmune diseases or malignant tumors were excluded. A total of 142 patients were included, all followed up for at least 2 years and divided into two groups according to the occurrence of cerebral infarction or not during the follow-up. Cerebral infarction group developed cerebral infarction during the follow-up period, there were a total of 58 cases that were 43-67 years old and included 34 males and 24 females, and they were followed up for 24-41 months; control group did not develop cerebral infarction during the follow-up period, there were a total of 84 cases that were 42-66 years old and included 46 males and 38 females, and they were followed up for 25-43 months. There was no significant difference in the general data between the two groups (P>0.05).

2.2 CEUS methods

GE LOGIQ E9 color Doppler diasonograph was used for CEUS, regular ultrasound scanning was conducted at first to determine the position of the carotid plaques, then ultrasound contrast agents were injected through the vein, the contrast enhancement in carotid plaques was continuously recorded, and the dynamic images were input in software to calculate peak intensity (Peak) and time to peak (TP).

2.3 Serum index detection methods

After they were enrolled, fasting venous blood was collected from the two groups of patients and centrifuged to isolate serum, and the instructions of Elisa kit were followed to detect the contents of VEGF, HGF, Ang-1, PDGF-BB, VE-cadherin, FKN, IL-4, IL-10, IL-17, ICTP, MMP10, TIMP1, Vaspin and Caspase-3.

2.4 Statistical methods

SPSS 19.0 software was used to input data, t test was performed for the analysis of measurement data between the two groups, and Pearson test was performed for the analysis of correlation. P<0.05 showed statistical significance in the differences.

3. Results

3.1 CEUS parameters of the two groups of patients

The CEUS parameters Peak and TP levels of cerebral infarction group were (35.21±5.23) dB and (53.49±6.62) s respectively; the CEUS parameters Peak and TP levels of control group were (20.36±3.73) dB and (86.12±10.25) s respectively. The t test analysis of the differences in CEUS parameters between the two groups of patients showed that the Peak level of cerebral infarction group was higher than that of control group whereas TP level was lower than that of control group (P<0.05).

3.2 Serum angiogenesis index contents of the two groups of patients

The t test analysis of the differences in serum angiogenesis indexes VEGF (ng/mL), HGF (ng/mL), Ang-1 (pg/mL) and PDGF-BB (ng/mL) contents between the two groups of patients showed that serum VEGF, HGF, Ang-1 and PDGF-BB contents of cerebral infarction group were significantly higher than those of control group (P<0.05). Pearson test analysis of the correlation between CEUS parameters and serum angiogenesis indexes in cerebral infarction group showed that serum VEGF, HGF, Ang-1 and PDGF-BB contents were positively correlated with Peak level and negatively correlated with TP level.

Table 1.

Comparison of serum angiogenesis indexes between the two groups of patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>VEGF (ng/mL)</th>
<th>HGF (ng/mL)</th>
<th>Ang-1 (pg/mL)</th>
<th>PDGF-BB (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral infarction</td>
<td>58</td>
<td>178.3±20.3</td>
<td>103.3±13.6</td>
<td>22.52±2.94</td>
<td>28.61±3.35</td>
</tr>
<tr>
<td>Control group</td>
<td>84</td>
<td>73.3±9.3</td>
<td>40.2±6.2</td>
<td>10.33±1.52</td>
<td>13.26±1.85</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 2.

Comparison of serum inflammatory cytokines between the two groups of patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>VE-cadherin (ng/mL)</th>
<th>FKN (ng/mL)</th>
<th>IL-4 (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
<th>IL-17 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral infarction</td>
<td>58</td>
<td>7.85±0.93</td>
<td>89.3±11.3</td>
<td>82.3±10.3</td>
<td>37.4±5.2</td>
<td>25.5±3.4</td>
</tr>
<tr>
<td>Control group</td>
<td>84</td>
<td>3.02±0.45</td>
<td>37.7±5.2</td>
<td>162.1±20.3</td>
<td>75.1±8.9</td>
<td>11.3±1.5</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
properties, the arterial endothelial and subcutaneous components are separated from lumen, blood supply is reduced and relative hypoxia occurs; hypoxia stimulation will increase the number of new blood vessels in the plaque and provide more changes for blood components, especially the lipids and inflammatory mediators to contact with the subcutaneous matrix, thus promoting the increase of lipid core volume in plaque and the decrease of plaque stability[4]. Contrast-enhanced ultrasonography is a method to evaluate angiogenesis. The number of new blood vessels can be determined by using contrast agent to determine the parameters Peak and TP[5]. In the above study, we analyzed the relationship between the changes of CEUS parameters of carotid plaques and the occurrence of cerebral infarction, and it was found that the Peak level of cerebral infarction group was higher than that of control group whereas TP level was lower than that of control group. Changes in the CEUS parameters Peak and TP reveal increased angiogenesis in carotid plaques in patients with cerebral infarction, and massive angiogenesis in plaques will increase the risk of cerebral infarction.

The formation of new blood vessels in carotid atherosclerotic plaques is mediated by a variety of cytokines, and the hypoxia condition in plaques promotes the formation of angiogenesis cytokines. VEGF is a cytokine specifically acting on endothelial cells, which can bind with VEGFR on the cell membrane to stimulate the growth of endothelial cells and form new vascular structures[6]; HGF is a cytokine with extensive growth-promoting effect, which promotes the proliferation of endothelial cells and smooth muscle cells, and can not only increase the number of new blood vessels in plaques, but also increase the stenosis of arterial lumen[7]; Ang-1 is the Ang family member involved in the maintenance of new vascular structures, which can reduce vascular permeability and mature the new vascular structures[8]; PDGF-BB is composed of two B chains of PDGF and has strong pro-cell division activity, which can enable endothelial cells to infiltrate the site of angiogenesis and promote the process of angiogenesis[9]. In the study, the changes of the above-mentioned angiogenesis molecules in the serum of patients with carotid atherosclerosis showed that serum VEGF, HGF, Ang-1 and PDGF-BB contents of cerebral infarction group were obviously higher than those of control group. This suggests that increased generation of angiogenesis molecules may increase the risk of cerebral infarction in patients with carotid atherosclerosis. Further analysis of the relationship between the changes of CEUS parameters and angiogenesis molecules showed that serum VEGF, HGF, Ang-1 and PDGF-BB contents of cerebral infarction group were positively correlated with Peak level and negatively correlated with TP level. This suggests that changes in the CEUS parameters Peak and TP can directly reflect the vitality of angiogenesis in the body. The increased formation of angiogenesis molecules will further affect the stability of plaques, and increase the risk of plaque rupture, detachment and

### Table 3.
Comparison of serum collagen metabolism indexes between the two groups of patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>ICTP (ng/mL)</th>
<th>MMP10 (ng/mL)</th>
<th>Caspase-3 (pg/mL)</th>
<th>TIMP1 (pg/mL)</th>
<th>Vaspin (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral infarction group</td>
<td>58</td>
<td>38.9±5.2</td>
<td>8.71±0.95</td>
<td>32.3±4.2</td>
<td>213.5±22.9</td>
<td>0.41±0.07</td>
</tr>
<tr>
<td>Control group</td>
<td>84</td>
<td>20.3±3.5</td>
<td>3.84±0.55</td>
<td>14.1±1.8</td>
<td>367.2±42.9</td>
<td>0.79±0.09</td>
</tr>
<tr>
<td>t</td>
<td>12.383</td>
<td>18.492</td>
<td>15.686</td>
<td>10.383</td>
<td>11.496</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
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<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

### 4. Discussion

Carotid atherosclerosis is the main cause of transient cerebral ischemic attack and cerebral infarction. After rupture and detachment, the atheromatous plaque will enter the intracranial artery with blood circulation, resulting in interruption of intracranial artery blood flow and cerebral infarction. Color Doppler ultrasonography is the preferred auxiliary examination method for clinical diagnosis of carotid atherosclerosis and evaluation of the plaque stability. It can more accurately detect carotid plaques, but the accuracy of judging the plaque properties by the echo characteristics within the plaques is not ideal. During the change of the carotid atherosclerotic plaque...
formation of thromboembolism.

The formation of new blood vessels in carotid plaques can increase the infiltration of inflammatory cells in the plaques and then damage the stability of the plaques by inflammatory reactions[10,11]. After a large number of inflammatory cell infiltrate in plaques, the secretion of VE-cadherin, FKN, IL-17 and other pro-inflammatory factors increases while the secretion of IL-4, IL-10 and other anti-inflammatory factors decreases, which leads to the excessive activation of inflammatory response, and then can on the one hand, directly damage the plaque integrity and increase the risk of plaque rupture, and on the other hand, affect the collagen metabolism, promote collagen hydrolysis within plaque fibrous cap and cause plaque ruptures[12-15]. Type I collagen is the main collagen component in the plaque fibrous cap. Its hydrolysis under the action of protease MMP10 and Caspase-3 leads to increased generation of hydrolysis product ICTP, and also destroys the integrity of the fibrous cap[16,17]; TIMP1 and Vaspin are molecules with protease-inhibitory activity, which can inhibit the hydrolysis of plaque fibrous cap by protease and enhance plaque stability[18]. In the study, analysis of the changes of the above inflammatory cytokines and collagen hydrolysis indexes in the serum of patients with carotid atherosclerosis revealed that serum VE-cadherin, FKN, IL-17, ICTP, MMP10 and Caspase-3 contents of cerebral infarction group were obviously higher than those of control group whereas IL-4, IL-10, TIMP1 and Vaspin contents were obviously lower than those of control group. Further analysis of the relationship of the changes of CEUS parameters with inflammatory cytokines and collagen hydrolysis indexes showed that serum VE-cadherin, FKN, IL-17, ICTP, MMP10 and Caspase-3 contents of cerebral infarction group were positively correlated with Peak level and negatively correlated with TP level; serum IL-4, IL-10, TIMP1 and Vaspin contents were negatively correlated with Peak level and positively correlated with TP level. It means that the changes of CEUS parameters Peak and TP are related to the inflammation activation and collagen metabolism during the change of plaque properties, and further indicates that increased angiogenesis can cause the activation of inflammation and the enhancement of collagen hydrolysis, reduce plaque stability and raise the risk of plaque rupture, detachment and formation of thromboembolism.

The above results show that the changes of CEUS parameters Peak and TP have predictive value for cerebral infarction in patients with carotid atherosclerosis; the changes of Peak and TP are correlated with the changes of angiogenesis molecules, inflammatory cytokines and collagen metabolism indexes. CEUS can evaluate the angiogenesis and plaque property change within the plaques.

References


