Research of expression quantity of serum miR-146a and miR-146b in patients with acute cerebral infarction before and after the intervention of rosuvastatin

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Objective: To study the expression quantity of serum miR-146a and miR-146b in patients with acute cerebral infarction before and after the intervention of rosuvastatin and its correlation with toll-like receptor 2 (TLR2) and TLR4 signaling pathways.

Methods: A total of 65 patients with acute cerebral infarction treated in our hospital from December 2015 to August 2016 were selected for prospective study. They were treated with lipid-lowering rosuvastatin, and peripheral blood samples were collected at 8th week before and after treatment, respectively. Serum was separated and expression quantity of miR-146a and miR-146b and contents of TNF-α, interleukin (IL)-1β, IL-6 and IL-17 were determined. Peripheral blood mononuclear cells were isolated and fluorescence intensities of TLR2, TLR4, myeloid differentiation primary response gene 88 (MyD88), interleukin-1 receptor-associated kinase 1 (IRAK-1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) were measured.

Results: At 8th week of intervention of rosuvastatin, expression quantity of serum miR-146a [(0.762 ± 0.092) vs. (0.346 ± 0.053)] and miR-146b [(0.714 ± 0.088) vs. (0.317 ± 0.047)] in patients with acute cerebral infarction was significantly higher than those before the intervention. Fluorescence intensities of peripheral blood mononuclear cells such as TLR2 [(10.34 ± 1.27) vs. (16.94 ± 1.94)], TLR4 [(11.37 ± 1.54) vs. (24.35 ± 3.26)], IRAK [(9.34 ± 0.92) vs. (15.32 ± 1.82)], MyD88 [(4.42 ± 0.56) vs. (9.41 ± 1.03)] and NF-kB [(6.65 ± 0.78) vs. (13.49 ± 1.76)] and contents of inflammatory factors such as TNF-α [(64.26 ± 8.29) μg/L vs. (106.39 ± 13.84) μg/L], IL-1β [(37.91 ± 5.24) μg/L vs. (64.23 ± 8.33) μg/L], IL-6 [(34.28 ± 4.85) ng/L vs. (82.46 ± 1.97) ng/L] and IL-17 [(56.75 ± 7.49) ng/L vs. (98.31 ± 11.36) ng/L] of serum were all significantly lower than those before the intervention. Expression quantity of serum miR-146a and miR-146b had a negative correlation with fluorescence intensities of TLR2, TLR4, IRAK, MyD88 and NF-kB. Fluorescence intensities of TLR2 and TLR4 in peripheral blood mononuclear cells had a positive correlation with contents of TNF-α, IL-1β, IL-6 and IL-17 in serum.

Conclusions: Treatment with rosuvastatin can up-regulate the expression quantity of serum miR-146a and miR-146b in patients with acute cerebral infarction and further inhibit the secretion of IRAK, MyD88, NF-kB, TNF-α, IL-1β, IL-6 and IL-17 mediated by TLR2 and TLR4.

1. Introduction

Acute cerebral infarction is a common cardiovascular and cerebrovascular disease in China, which has high fatality rate, disability rate and recurrence rate[1-3]. Anti-platelet, anticoagulant, and lipid-lowering therapies are the conventional treatments of acute cerebral infarction, which have the preventive effect on both the occurrence and recurrence of cerebral infarction. Statins are the conventional lipid-lowering drugs for patients with acute cerebrovascularinfarction. Rosuvastatin is one of the statins commonly used in clinic, which has double lipid-lowering and anti-inflammatory effects and inhibiting effect on both the pathological process of atherosclerosis and activation, aggregation of blood platelet[4,5]. The mechanism of rosuvastatin exerting the effect of lowering lipid is selectively inhibiting the hydroxymethyl glutaric acid independently. A reductase and the endogeneous synthesis of cholesterol, which can also increase the number of low-density lipoprotein cholesterol receptor on the surface of hepatocyte and improve the liver intake...
of low-density lipoprotein cholesterol and catabolism. However, the specific mechanism of anti-inflammatory effect of rosuvastatin is not completely clear at present. The secretions of various inflammatory factors in body are all regulated and controlled by rosuvastatin, but the specific methods of regulation and control have not yet been illuminated.

MicroRNA (miRNA) is a newfound non-coding small molecule RNA in recent years, with the length of 17–25 bp. It is affected by various upstream factors and can targeted regulate the expression of various genes, which has wide biological effects[6,7]. miR-146a and miR-146b are two kinds of miRNA closely correlated with cardiovascular and cerebrovascular diseases. There was a research that has proved that miR-146a and miR-146b tend to show abnormal low expression in the body of patients with various cardiovascular and cerebrovascular diseases and can affect the process of inflammatory response[8,9]. The abnormal low expression of miR-146a and miR-146b in the body of patients with acute cerebral infarction will lead to a decrease of inhibition of TLR2 and TLR4 by miRNAs and further up-regulate the expression of TLR2 and TLR4 and increase the secretion of downstream inflammatory factors, by which we can conclude that rosuvastatin may affect the secretion of inflammatory factors mediated by TLR2 and TLR4 through regulating the expression of miR-146a and miR-146b, which further plays an anti-inflammatory role in the treatment process of acute cerebral infarction. To verify the above conclusions, we analyzed the expression quantity of serum miR-146a and miR-146b and downstream TLR2 and TLR4 in patients with acute cerebral infarction before and after the intervention of rosuvastatin in the following study.

2. Materials and methods

2.1. Subjects

Patients with acute cerebral infarction treated in our hospital from December 2015 to August 2016 were selected as study subjects for prospective study. The inclusive criteria were as follow: (1) diagnostic criteria of acute cerebral infarction were accordant with the Fourth National Cerebrovascular Disease Seminars set; (2) patients were admitted to hospital within 48 h after onset; (3) cerebral infarction lesions were confirmed, magnetic resonance imaging after admitting to hospital; (4) treatments with statins were not taken for patients before 4 weeks recruited; (5) lipid-lowering rosuvastatin therapy was taken after admitting to hospital. Patients suffering from malignant tumor combined with serious infection and autoimmune disease were excluded. The present study was approved by Hospital Ethics Committees and the informed consents were obtained from patients tested. There were 65 cases recruited including 42 cases of male and 23 cases of female, with age of (57.31 ± 7.48) years.

2.2. Study methods

A total of 10 mL peripheral blood sample was collected after admitting to hospital and after receiving rosuvastatin treatment for 8 weeks, respectively, which was divided into three samples and detected by the following methods: (1) miRNA from one of the blood samples was extracted and inverse transcribed into cDNA by using miRcute miRNA extraction and separation kit and miRcute miRNA cDNA the first strand synthesis kit (Tiangen Company, Beijing), and then the expression quantity of miR-146a and miR-146b was determined by using miRcute miRNA fluorescence quantitative detection kit FP401; (2) lymphocyte separation medium was added into another blood sample and then density gradient was centrifuged. After peripheral blood mononuclear cells were isolated, fluorescent antibodies of toll-like receptor 2 (TLR2), TLR4, myeloid differentiation primary response gene 88 (MyD88), interleukin-1 receptor-associated kinase (IRAK) and nuclear factor kappa-lightchain-enhancer of activated B cells (NF-kB) were incubated, and the fluorescence intensities of TLR2, TLR4, MyD88, IRAK and NFkB were measured using flow cytomtery; (3) the other blood sample was kept static and then centrifuged. Serum was separated and the contents of TNF-α, IL-1β, IL-6 and IL-17 were detected using ELISA.

2.3. Statistical methods

Software SPSS version 19.0 was used to input and analyze data. Measurement data were expressed using mean ± SD. Student’s t-test was used to analyze measurement data before and after treatment. The correlation between the two variables was tested using Pearson’s correlation analysis. Difference had statistically significance at P < 0.05.

3. Results

3.1. Expression quantity of serum miR-146a and miR-146b

At 8th week of intervention of rosuvastatin, expression quantity of serum miR-146a (0.762 ± 0.092 vs. 0.346 ± 0.053) and miR-146b (0.714 ± 0.088 vs. 0.317 ± 0.047) in patients with acute cerebral infarction was significantly higher than those before the intervention. Difference of expression quantity of serum miR-146a and miR-146b in patients with acute cerebral infarction before and after the treatment had statistically significance (P < 0.05).

3.2. Expression quantity of TLRs in peripheral blood mononuclear cells

At 8th week of intervention of rosuvastatin, fluorescence intensities of TLR2 (10.34 ± 1.27 vs. 16.94 ± 1.94), TLR4 (11.37 ± 1.54 vs. 24.35 ± 3.26), IRAK (9.34 ± 0.92 vs. 15.32 ± 1.82), MyD88 (4.42 ± 0.56 vs. 9.41 ± 1.03) and NF-kB (6.65 ± 0.78 vs. 13.49 ± 1.76) in peripheral blood mononuclear cells of patients with acute cerebral infarction were all significantly lower than those before the intervention. Difference of fluorescence intensities of TLR2, TLR4, MyD88, IRAK and NF-kB in peripheral blood mononuclear cells of patients with acute cerebral infarction before and after the treatment had statistically significance (P < 0.05).

3.3. Contents of inflammatory factors of serum

At 8th week of intervention of rosuvastatin, contents of inflammatory factors TNF-α [(64.26 ± 8.29) μg/L vs. (106.39 ± 13.84) μg/L], IL-1β [(37.91 ± 5.24) μg/L vs. (64.23 ± 8.33) μg/L], IL-6 [(34.28 ± 4.85) ng/L vs. (82.46 ± 11.97) ng/L] and IL-17 [(56.75 ± 7.49) ng/L vs. (98.31 ± 11.36) ng/L] of serum were all significantly lower than those before the intervention. Difference of
contents of inflammatory factors such as TNF-\(\alpha\), IL-1\(\beta\), IL-6 and IL-17 of serum in patients with acute cerebral infarction before and after the treatment had statistically significance \((P < 0.05)\).

3.4. Correlation analysis

Correlation analysis of expression quantity of miR-146a, miR-146b and fluorescence intensities of TLR2, TLR4, MyD88, IRAK and NF-kB was presented in Table 1. Expression quantity of serum miR-146a and miR-146b had a negative correlation with fluorescence intensities of TLR2, TLR4, IRAK, MyD88 and NF-kB. Correlation analysis of fluorescence intensities of TLR2, TLR4 and inflammatory factors such as TNF-\(\alpha\), IL-1\(\beta\), IL-6 and IL-17 were presented in Table 2. Fluorescence intensities of TLR2 and TLR4 in peripheral blood mononuclear cells had a positive correlation with contents of TNF-\(\alpha\), IL-1\(\beta\), IL-6 and IL-17 of serum.

### Table 1

<table>
<thead>
<tr>
<th>Fluorescence intensities</th>
<th>miR-146a</th>
<th>(r)</th>
<th>(P)</th>
<th>miR-146b</th>
<th>(r)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2</td>
<td>-0.671</td>
<td>&lt; 0.05</td>
<td></td>
<td>-0.739</td>
<td>&lt; 0.05</td>
<td></td>
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<tr>
<td>TLR4</td>
<td>-0.596</td>
<td>&lt; 0.05</td>
<td></td>
<td>-0.665</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>MyD88</td>
<td>-0.703</td>
<td>&lt; 0.05</td>
<td></td>
<td>-0.715</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>IRAK</td>
<td>-0.761</td>
<td>&lt; 0.05</td>
<td></td>
<td>-0.604</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>NF-kB</td>
<td>-0.642</td>
<td>&lt; 0.05</td>
<td></td>
<td>-0.676</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Inflammatory factors</th>
<th>TLR2</th>
<th>(r)</th>
<th>(P)</th>
<th>TLR4</th>
<th>(r)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-(\alpha)</td>
<td>0.714</td>
<td>&lt; 0.05</td>
<td></td>
<td>0.625</td>
<td>&lt; 0.05</td>
<td></td>
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<tr>
<td>IL-1(\beta)</td>
<td>0.586</td>
<td>&lt; 0.05</td>
<td></td>
<td>0.708</td>
<td>&lt; 0.05</td>
<td></td>
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<tr>
<td>IL-6</td>
<td>0.642</td>
<td>&lt; 0.05</td>
<td></td>
<td>0.651</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>IL-17</td>
<td>0.661</td>
<td>&lt; 0.05</td>
<td></td>
<td>0.593</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

4. Discussion

Rosuvastatin is the secondary prevention drug commonly used on patients with acute cerebral infarction which has lipid-lowering and anti-inflammatory pharmacology effect. The mechanism of rosuvastatin for lowering lipid has already been confirmed, yet the mechanism of anti-inflammatory effect has not been illuminated. miRNA is a kind of newfound non-coding RNA with the length of 17–25 bp in recent years which can target towards 3’UTR region of various genes mRNA, induce the degradation of mRNA or inhibit the mRNA translation, and further produce a relevant biological effect by changing the expression of genes\([10]\). There is a research which has proved that the change of contents of various miRNAs was correlative with inflammatory response in patients with cardiovascular and cerebrovascular diseases, and miR-146a and miR-146b tend to show a low expression in the body of patients with various cardiovascular and cerebrovascular diseases\([11-14]\). To confirm whether rosuvastatin produces anti-inflammatory effect by regulating the expression of miR-146a and miR-146b, we firstly compare the expression quantity of serum miR-146a and miR-146b before and after the rosuvastatin treatment, and the results showed that the expression quantity of serum miR-146a and miR-146b in patients with acute cerebral infarction after the treatment was significantly higher than those before the treatment, which indicated that rosuvastatin has regulating effect on expression quantity of serum miR-146a and miR-146b in patients with acute cerebral infarction and it can produce relevant biological effect by regulating the expression of miR-146a and miR-146b.

TLRs is a kind of pattern recognition receptor expressed by immune cells and inflammatory cells in body. There are totally 13 classes of TLRs in the body of mammal that have been found, which are TLR1-TLR13. In the known TLRs molecules, the expression of TLR2 and TLR4 is regulated and controlled by miRNAs, while the change of TLR2 and TLR4 express can regulate the process of inflammatory response. MyD88 is mutual adapter protein in the downstream channel of TLR2 and TLR4. When TLR2 and TLR4 are not activated, MyD88, IL-IR relative kinase (IRAK) combined with MyD88 regulating protein-toll relevant protein. When TLR2 and TLR4 are activated, toll relevant protein separated with MyD88 and IRAK, and then the phosphorylation of IRAK will result in the transfer of NFkB into the nuclear, which further activate the expression of various inflammatory factors\([15-18]\). There was research which reported that miR-146a and miR-146b can targeted regulate the expression of various inflammatory factors\([25,26]\). We can sum up by analyzing the correlation of fluorescence intensities of TLR2 and TLR4 by miR-146a and miR-146b, we analyzed the expression quantity of TLRs in peripheral blood before and after the rosuvastatin treatment and its correlation with miR-146a and miR-146b. The results showed that fluorescence intensities of TLR2, TLR4, IRAK, MyD88 and NFkB in peripheral blood mononuclear cells of patients with acute cerebral infarction after the treatment were all significantly lower than those before the treatment and also had a negative correlation with expression quantity of miR-146a and miR-146b, which indicated that rosuvastatin treatment can increase the expression of miR-146a and miR-146b in the body of patients with acute cerebral infarction and further inhibit the inflammatory response by targeted inhibiting the expression of TLR2 and TLR4.

Inflammatory factors regulated and controlled by TLR2 and TLR4 through MyD88/NF-kB include TNF-\(\alpha\), IL-1\(\beta\), IL-6 and IL-17, etc. And the inflammatory response mediated by them plays an important role on the damage process of neurological function after cerebral infarction\([21]\). TNF-\(\alpha\) can directly cause the inflammatory injury on neuron and gliocyte, which has also the chemotaxis of inflammatory cell\([22]\). IL-1\(\beta\) and IL-17 have toxic effect on neuron and gliocyte around the infarction focus, and they can lead to the cellular damage and necrocytosis\([23,24]\). While IL-6 has the effect of endogenous chemotactic factor, which can recruit the inflammatory cells at local focus and mediate the cascade amplification of inflammatory response\([25,26]\). We can sum up by analyzing the contents of the above inflammatory factors of serum before and after the rosuvastatin treatment that the contents of TNF-\(\alpha\), IL-1\(\beta\), IL-6 and IL-17 of serum in patients with acute cerebral infarction after treatment were significantly lower than those before the treatment. To further confirm the regulation and control effect produced by TLR2 and TLR4 on inflammatory factors in the body of patients with acute cerebral infarction, we analyzed the correlation between expression quantity of TLR2 and TLR4 in peripheral blood mononuclear cells and the contents of TNF-\(\alpha\), IL-1\(\beta\), IL-6 and IL-17 of serum, and the results showed that fluorescence intensities of TLR2 and TLR4 in peripheral blood mononuclear cells had a positive correlation with contents of TNF-\(\alpha\), IL-1\(\beta\), IL-6 and IL-17 of serum, which...
indicated that the up-regulation effect of rosuvastatin treatment on the expression of miR-146a and miR-146b in the body of patients with acute cerebral infarction can reduce the synthesis and secretion of inflammatory factors including TNF-α, IL-1β, IL-6 and IL-17 by inhibiting the expression of TLR2 and TLR4, which further reduces the degree of inflammatory response in body.

In conclusion, rosuvastatin treatment can up-regulate the expression quantity of serum miR-146a and miR146b in patients with acute cerebral infarction and further inhibit the secretion of IRAK, MyD88, NF-Kb, TNF-α, IL-1β, IL-6 and IL-17 mediated by TLR2 and TLR4.

Conflict of interest statement

The authors report no conflict of interest.

References


