行政院國家科學委員會專題研究計畫  期中進度報告

與肺炎披衣菌感染的相關研究

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Abstract

There is increasing evidence that infective pathogens such as *Chlamydia pneumoniae* linked to atherosclerosis of cerebral vessels. As an independent contributing factor, the CD14 receptor-lipopolysaccharide complex plays an important role in activating inflammatory reactions. In particular, the C(-260)→T polymorphism in the promoter of CD14 gene have been reported to regulate the density of CD14 expression on monocytes which is an important mediator for the activation of monocytes to secret inflammatory cytokines and to be implicated in atherosclerotic diseases. In this study, we investigated a possible association between *C pneumoniae* infection and the polymorphism of CD14, and ischemic stroke. A total of 450 patients with ischemic stroke and 450 age- and sex-matched controls were included in the study. *C pneumoniae* serologic status and the CD14 genotype were determined in both patients and controls. *C pneumoniae* seropositivity was more common in the stroke patients than in the controls (74.2% vs. 57.1%, *P* < 0.001). Persistent infection (*C pneumoniae* IgG titers ≥32) was demonstrated to be higher in stroke patients (53.3% vs 43.3%, *P* = 0.014). The genotype distributions of C(-260)→T polymorphism in the promoter of CD14 gene were similar in the controls (TT, 32.3%; CT, 54.2%; CC, 13.5%) and stroke patients (TT, 36.1%; CT, 47.2%; and CC, 16.7%). There was no significant difference between these CD14 genotype distributions. However, the frequency of persistent *C pneumoniae* infection was significantly different in CC genotype between stroke patients and controls (61.3% vs 44.3%, *P* = 0.05). The odds ratio of persistent *C pneumoniae* infection was 2.43 (95% CI=1.34-4.43; *P*<0.05) in stroke patients.
Atherosclerosis is a multifactorial disease. The pathogenesis of atherosclerosis is diverse and involves many factors. Recently, there is mounting evidence indicating a strong association of chronic infection with an ubiquitous respiratory pathogen, Chlamydia pneumoniae (C pneumoniae), a Gram-negative obligate intracellular bacterium with atherosclerosis. Most persons have their first C pneumoniae infection before age 20 and reinfection is common. C pneumoniae first establishes persistent infection in the lungs. The persistent infected lung macrophages then disseminate infection to normal arteries and arteries with preexisting atheromatous lesions. The association has been demonstrated by seroepidemiological studies, examination of atheromatous plaque specimens by molecular and isolation method, in-vitro experiments and animal models and recently preliminary antichlamydial antibiotic intervention studies.

The CD14, pattern recognition receptor, is a membrane glycoprotein expressed specifically on monocytes and macrophages. It has a unique ability to discriminate nonself lipoglycans of infectious pathogen. At the molecular level, CD14 acts by transferring lipopolysaccharide (LPS) and other bacterial ligands from circulating LPS-binding protein to Toll-like receptor 4/MD-2 signaling complex. Engagement of this complex resulted in the activation of innate host defense mechanisms such as release of inflammatory cytokines, and in up-regulation of co-stimulatory molecules, thus providing cues that are essential to directing adaptive immune response. Recently, a polymorphism in the promoter region of the CD14 gene has been studied. This polymorphism is within the Sp1 transcription factor binding site and consists of a single base exchange (C→T) at position –260 with C introducing a HaeIII restriction site. The C (-260)→T polymorphism in the promoter of CD14 gene have been reported to regulate the density of CD14 expression on monocytes. The T variants of the –260 polymorphism can promote CD14 gene transcription and cause higher expression of CD14 on monocytes, which leads to an enhanced inflammatory response. Therefore, CD14 polymorphism could be a genetic factor responsible for interindividual differences in the susceptibility to C pneumoniae infection, and subsequently for the incidence of its associated diseases.

Age, gender, race, ethnicity, and vascular factors have been identified as risk factors for stroke. Chronic infectious diseases and genetic factors may also play an important role in causing stroke and atherosclerotic diseases. Specifically, there is increasing evidence of associations between stroke and various persistent bacterial or viral agents, such as Helicobacter pylori, Chlamydia pneumoniae, or cytomegalovirus, and clinical conditions, such as dental disease, which may be associated with persistent infection. Several putative mechanisms may play at least a partial role in the development of atherosclerotic disease and stroke. One
of the mechanisms underlying atherosclerotic disease and stroke starts with an inflammatory cell reaction. Endotoxin-activated monocytes produce proinflammatory cytokines, promote procoagulant activity, and may have a marked impact on the development of atherosclerosis. Because genetic background is important in determining whether certain bacteria infection for an individual, we hypothesized that the polymorphism of the promoter of CD14 gene may influence the susceptibility to *C pneumoniae* infection. In this study, we investigated the association of (1) *C pneumoniae* seropositivity and ischemic stroke and (2) *C pneumoniae* infection and C(-260)→T polymorphism in the promoter of the CD14 gene.

**Results**

Table 1 summarizes the baseline characteristics in patients and controls. The age and sex distributions did not differ significantly between the two groups. The risk factors including hypertension, diabetes mellitus, and smoking were significantly more common in the stroke patients than control subjects. *C pneumoniae* serologic status and the distributions of genotypes of the CD14 polymorphism in patients and controls are given in Table 2. *C pneumoniae* seropositivity was more common among the stroke patients than the controls (IgG ≥ 16; 74.2% vs. 56.9%, respectively, *P* <0.001; IgG ≥ 32; 53.2% vs.45.1%, respectively, *P*=0.05). The distributions of genotypes and the allelic frequencies of the polymorphism in the CD14 promoter in the stroke and control groups are shown in Table 2. There was no significant difference between the CD14 genotypes of the stroke (TT, 36.1%; CT, 47.2%; CC, 16.7%) and the controls (TT, 32.3%; CT, 54.2%; and CC, 13.5%; *P*=0.068). The T allele frequency was similar among controls and cases (59.3% vs 59.6%; *P*=0.81).

The interaction between the CD14 polymorphism and *C pneumoniae* serologic status was examined by assessing the distribution of CD14 polymorphism after stratification by *C pneumoniae* serologic status. We found that the distribution of CD14 polymorphism was significantly different between the two subtypes, irrespective of *C pneumoniae* persistent infection. *C pneumoniae* IgG titers ≥32 were measured in 43.3% of control subjects and 53.3% of the patients (*P*=0.05). Of the 450 control subjects, 195 had *C pneumoniae* persistent infection, and TT homozygotes were higher than CC genotypes (51.0% vs 44.3%, *P*=0.135). In stroke patients, the prevalence of persistent *C pneumoniae* infection (IgG ≥32) in CC homozygotes was higher than TT genotypes (61.3% vs 54.3%, *p*=0.084) (Table 3). We further analyzed the CD14 polymorphism and status of *C pneumoniae* persistant
infection. The frequency of persistent *C pneumoniae* infection was significantly different in CC genotype between stroke patients and controls (61.3% vs 44.3%, \( P=0.05 \)) (Table 3).

The magnitude of the association between *C pneumoniae* serologic status and CD14 polymorphism and stroke was assessed by estimating the odd ratios (OR) of *C pneumoniae* serologic status and CD14 polymorphism for stroke patients (Table 4). In logistic regression analysis, age was significantly associated with persistent *C pneumoniae* infection in stroke patients (OR, 2.43; 95% confidence interval (CI), 1.34 to 4.43; \( P<0.05 \); Table 4). CC genotype was significantly associated with persistent *C pneumoniae* infection in stroke patients (OR, 3.246; 95% CI, 1.228 to 8.585; \( P=0.018 \); Table 4).

**Discussion**

Accumulating evidence has indicated that in addition to the well-established risk factors, infection, particularly *C pneumoniae* infection, is strongly associated with the initiation/progression of atherosclerosis and subsequently with myocardial infarction and cerebrovascular disease.\(^1\)\(^2\)\(^5\)\(^13\) The present study is the first to examine the relationship between CD14 promoter gene polymorphisms and *C pneumoniae* infection, and their association with stroke. CD14 is a glycosyl-phosphadidylinositol anchored cell surface molecule that has been identified as a receptor for LPS, the cell wall component of gram-negative bacteria.\(^16\) Upon LPS binding to CD14 the production and release of proinflammatory cytokines including tumor necrosis factor (TNF), interleukin (IL) 1, IL-6 and IL-8 as well as the anti-inflammatory mediators IL-10 and transforming growth factor-\(\beta\), oxygen radicals, and nitric oxide are triggered.\(^17\) With the infection of *C pneumoniae* and with the increased production of their endotoxins (LPSs), which stimulate the synthesis of interleukins and other growth factors in monocytes\(^28\)\(^30\) and endothelial cells\(^3\) that may lead to chronic inflammatory reaction with increased adhesion of monocytes, leukocytes and platelets to the vessel wall and ultimately to atherosclerosis and thrombosis.

Although CD14 promoter polymorphism has been associated with myocardial infarction,\(^18\)\(^19\) two recent studies failed to find the association with stroke.\(^31\)\(^32\) In this study, the allele and genotype distribution in the promoter of CD14 gene polymorphism tested were similar among the controls and the stroke patients. Our results found that the C(-260)→T polymorphism in the CD14 promoter gene per se is not associated with risk of ischemic stroke. Several studies have found an association between *C pneumoniae* and stroke,\(^2\)\(^35\)\(^37\) but the results were
In this study, there was significant difference in *C pneumoniae* infection (IgG $\geq 16$ and IgG $\geq 32$) in control and stroke groups. These results suggest that CD14 promoter polymorphisms appear to influence the susceptibility to *C pneumoniae* infection, and the stroke patient with CC genotype of CD14 promoter gene are more likely to have persistent *C pneumoniae* infection. This study indicates that this polymorphism may be directly linked to progression of atherosclerotic lesions and plaque vulnerability and rather than initiation of atherosclerosis. It may be one of the genetic risk factors associated with stroke.

In conclusions, this study provides evidence of an association between C (-260)$\rightarrow$T polymorphism in the promoter of CD14 gene and persistent *C pneumoniae* infection in patients with ischemic stroke. The mechanism underlying an association between *C pneumoniae* infection and stroke remains uncertain. It is possible that *C pneumoniae* uses monocytes as a transport system for systemic dissemination and enters a persistent state. The circulating monocytes carrying a pathogen with reduced antimicrobial susceptibility might initiate re-infection or promote atherosclerosis by the release of proinflammatory mediators. There may be an association between *C pneumoniae* infection and serum cholesterol levels or the presence of antibodies to heat-shock protein, which induces an immune reaction and might play a role in the pathogenesis of atherosclerosis.

Therefore, it is thought that there is an underlying mechanism linking chronic *C pneumoniae* infection with atherosclerosis, and this mechanism may involve a low-grade inflammatory response or the induction of a prothrombotic state. Previous studies have yielded varying results with regard to the influence of stroke etiology. In studies were not consistent and some are even controversial. Therefore, the genetic risk factors could not always be identified with certainty. Our findings provide a potential explanation for conflicting evidence regarding the role of *C pneumoniae* infection in stroke patients. Genetic polymorphisms for the CD14 genotypes may modulate the risk of stroke by influencing individual susceptibility to *C pneumoniae* infection.
References

Table 1. Clinical characteristics of stroke patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 450)</th>
<th>Stroke patients (n = 450)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, %</td>
<td>56.0</td>
<td>55.1</td>
<td>NS</td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>63.2±12.3</td>
<td>64.2±12.7</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>16.9</td>
<td>41.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>27.7</td>
<td>74.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>16.3</td>
<td>33.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>13.9</td>
<td>17.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

χ² tests were used to compare values of stroke patients and controls for all parameters except for age, which was compared by Student’s t test; NS, not significant.

Table 2. *C. pneumoniae* serologic status and CD14 [C(-260)→T] polymorphism genotype frequencies in control subjects and stroke patients.

<table>
<thead>
<tr>
<th></th>
<th>Controls, n (%)</th>
<th>Stroke patients, n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. pneumoniae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG ≧ 16</td>
<td>257 (57.1%)</td>
<td>334 (74.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgG ≧ 32</td>
<td>195 (43.3%)</td>
<td>240 (53.3%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>145 (32.2%)</td>
<td>162 (36%)</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>244 (54.2%)</td>
<td>213 (47.3%)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>61 (13.6%)</td>
<td>75 (16.7%)</td>
<td>0.068</td>
</tr>
<tr>
<td>Allele frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T allele</td>
<td>59.3%</td>
<td>59.6%</td>
<td></td>
</tr>
<tr>
<td>C allele</td>
<td>40.7%</td>
<td>40.4%</td>
<td>0.81</td>
</tr>
</tbody>
</table>
Table 3. Frequency of Chlamydial persistent infection in stroke patients and controls of different CD14 promoter polymorphisms

<table>
<thead>
<tr>
<th>CD14 genotype</th>
<th>Controls</th>
<th>Stroke</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>27/61 (44.3)*</td>
<td>46/75 (61.3)</td>
<td>0.050</td>
</tr>
<tr>
<td>CT</td>
<td>115/244 (47.1)</td>
<td>118/213 (55.4)</td>
<td>0.078</td>
</tr>
<tr>
<td>TT</td>
<td>74/145 (51.0)</td>
<td>88/162 (54.3)</td>
<td>0.617</td>
</tr>
<tr>
<td>CC+CT</td>
<td>142/305 (46.6)</td>
<td>164/288 (56.9)</td>
<td>0.010</td>
</tr>
<tr>
<td>TT+CT</td>
<td>189/389 (48.5)</td>
<td>206/375 (54.9)</td>
<td>0.076</td>
</tr>
</tbody>
</table>

*No (%) positive for C pneumoniae IgG ≥32

Table 4. Odds ratio for age, TT genotype and C pneumoniae persistent infection in stroke patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke (n=450)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.011</td>
<td>0.983-1.040</td>
<td>.452</td>
</tr>
<tr>
<td>C. pneumoniae IgG ≥32</td>
<td>2.43</td>
<td>1.34-4.43</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CD14 CC genotype</td>
<td>3.246</td>
<td>1.228-8.585-3.153</td>
<td>.018</td>
</tr>
</tbody>
</table>

Age, sex, hypertension, diabetes, hypercholesterolemia, and smoking were adjusted.