Relationship between Smoking and Responsiveness to Clopidogrel in Non-cardiogenic Ischemic Stroke Patients

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Abstract

Objective Clopidogrel is used to prevent the recurrence of non-cardiogenic ischemic stroke, but individual responsiveness to the drug varies. Moreover, it is known that smoking, which is a risk factor for ischemic stroke, affects the drug’s pharmacokinetics. The objective of the present study was to investigate a possible relationship between smoking and responsiveness to clopidogrel in non-cardiogenic ischemic stroke patients.

Methods The study involved 209 non-cardiogenic ischemic stroke patients who were administered oral clopidogrel at a dosage of 75 mg/day for at least 1 week. Platelet aggregation in response to adenosine diphosphate (20 μM) was measured in each patient using the VerifyNow P2Y12 Assay. Platelet aggregation and the incidence of resistance to clopidogrel were compared between a smokers group (70 patients) and a non-smokers group (139 patients). Clopidogrel resistance was defined as a P2Y12 Reaction Units (PRU) value >230 and/or % inhibition <20%.

Results The mean PRU was 128.3±85.5 in the smokers group and 167.7±86.6 in the non-smokers group (p=0.002). The incidence of PRU >230 was 12.9% (9 patients) in the smokers group and 25.9% (36 patients) in the non-smokers group (p=0.033). The mean % inhibition was 48.6±30.7% in the smokers group and 36.9±27.6% in the non-smokers group (p=0.009). The incidence of patients with % inhibition <20% was 24.3% (17 patients) in the smokers group and 34.5% (48 patients) in the non-smokers group (p=0.155).

Conclusion The incidence of clopidogrel resistance was lower in the non-cardiogenic ischemic stroke patients who were smokers, thus indicating that these patients’ responsiveness to this drug may be enhanced.

Key words: clopidogrel resistance, ischemic stroke, VerifyNow P2Y12 Assay, smoking

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Introduction

Smoking is a known risk factor for stroke, especially for cerebral infarction and subarachnoid hemorrhage (1-3). In Japan, smoking has been reported as a risk factor for lacunar and atherothrombotic infarction (4, 5). Smoking is also known to affect drug pharmacokinetics (6). Clopidogrel is a prodrug of the thienopyridine class, and it is recommended for the prevention of recurrence of non-cardiogenic ischemic stroke in Japan. In earlier studies, we measured platelet aggregation in non-cardiogenic ischemic stroke patients and found that 8-29% showed clopidogrel resistance (7, 8). Various factors have been identified as causing individual variability in the antiplatelet effect of clopidogrel, including genetic polymorphism of cytochrome P450 (CYP) enzymes (9, 10), and our group reported the involvement of chronic kidney disease (11). However, little is known regarding whether a relationship exists between smoking and the antiplatelet effect of clopidogrel in non-cardiogenic ischemic stroke patients. Accordingly, the VerifyNow P2Y12 Assay (Accumetrics Inc., San Diego, USA) was used to measure platelet aggregation in non-cardiogenic ischemic stroke patients, and the possible relationship between smok-
Materials and Methods

Subjects

The study included 209 non-cardiogenic ischemic stroke patients (168 men, 41 women; mean age 65.6±9.6 years) who were referred to the Stroke Center of our hospital between October 2009 and October 2013 and administered oral clopidogrel (75 mg/day for at least 1 week). The clinical type of stroke was atherothrombotic infarction in 140 patients, lacunar infarction in 46 patients, and transient ischemic attack in 23 patients. None of the patients was co-administered any other antiplatelet drug.

Seventy of the patients who were smokers at the time they came to the hospital were included in the smokers group. The number of cigarettes smoked was 10-60/day (mean 19.8/day), and the duration of smoking was 14-63 years (mean 43.2 years). The data for the baseline clinical and laboratory characteristics of the smokers group (70 patients) and the non-smokers group (139 patients) are indicated in the Table. No significant differences were found between the two groups in regard to any of the following parameters: age, sex, body mass index (BMI), underlying diseases (diabetes mellitus, hypertension, dyslipidemia), HbA1c, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, estimated glomerular filtration rate, concomitant medications (angiotensin II receptor blockers, proton pump inhibitors, statins, insulin, sulfonylureas, dipeptidyl peptidase-4 inhibitors, glinides, biguanides, α-glucosidase inhibitors, thiazolidinediones), or clinical stroke type.

Blood sample preparation

A 21-G or larger blood collection needle was used to draw 1.8 mL of blood from each subject into a vacuum blood collection tube containing 0.2 mL of 3.2% sodium citrate. Within 10 minutes to 4 hours after collection of each blood sample, platelet aggregation in response to adenosine diphosphate (ADP, 20 μM) was measured using the VerifyNow P2Y12 Assay. Platelet aggregation and the incidence of resistance to clopidogrel were compared between the smokers group and the non-smokers group.

Definition of clopidogrel resistance

Since publication of the Gauging Responsiveness With a VerifyNow P2Y12 Assay: Impact on Thrombosis and Safety (GRAVITAS) trial (12) in 2011, there has been an increasing trend to use the P2Y12 Reaction Units (PRU) value as an index of clopidogrel resistance. There are also multiple reports of the use of % inhibition. For this study, clopidogrel resistance was defined as a PRU value >230 (12-14) and/or % inhibition <20% (15-17).

Table. Baseline Clinical and Laboratory Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Smokers group (n=70)</th>
<th>Non-smokers group (n=139)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.9±8.6</td>
<td>66.1±10.2</td>
<td>0.110</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>60 (85.7)</td>
<td>108 (77.7)</td>
<td>0.199</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1±3.3</td>
<td>24.0±3.2</td>
<td>0.663</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>29 (41.2)</td>
<td>45 (32.4)</td>
<td>0.222</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>46 (65.7)</td>
<td>100 (71.9)</td>
<td>0.425</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>50 (71.4)</td>
<td>92 (66.2)</td>
<td>0.530</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.1±1.1</td>
<td>6.1±1.6</td>
<td>0.149</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>194.8±42.7</td>
<td>187.7±32.9</td>
<td>0.479</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>49.9±14.8</td>
<td>47.9±15.9</td>
<td>0.329</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>119.7±42.2</td>
<td>111.3±30.4</td>
<td>0.361</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>162.4±78.4</td>
<td>152.4±76.6</td>
<td>0.347</td>
</tr>
<tr>
<td>eGFR (mL/min/m²)</td>
<td>73.5±15.7</td>
<td>69.8±22.8</td>
<td>0.157</td>
</tr>
</tbody>
</table>

The PRU and % inhibition data were compared using the Mann-Whitney U test or Fisher’s exact test. Statistical analysis

In this study, smoking was associated with the efficacy of clopidogrel. That is, non-cardiogenic ischemic stroke patients who smoked showed strong inhibition of platelet aggregation by clopidogrel, and the incidence of such patients who could be thought to show clopidogrel resistance was reduced.

Few studies looking at the relationship between smoking and the platelet aggregation inhibitory effect of clopidogrel in patients with ischemic stroke have so far been conducted,
but a number of studies have been conducted in patients with coronary artery disease and peripheral arterial disease (18-25). However, no such studies have been conducted in Japan. Price et al. (18) used the VerifyNow P2Y12 Assay to measure platelet aggregation in 377 patients with coronary artery disease who had undergone percutaneous coronary intervention with a sirolimus-eluting stent followed by oral clopidogrel (75 mg/day) and then investigated for factors that affected the drug’s antiplatelet activity. They found that PRU was significantly lower in smokers than in non-smokers, while the incidence of clopidogrel resistance was also lower, and a multivariate analysis also showed an association between smoking and antiplatelet activity. Moreover, the clinical benefit of clopidogrel in preventing cardiovascular events was greater in smokers than in non-smokers, thus leading to the concept of “smokers’ paradox” (26-28).

It is thought that CYP enzymes are involved in the enhanced antiplatelet activity of clopidogrel in smokers. Clopidogrel is a prodrug that is converted to its active form in the liver via two metabolic steps involving CYP 1A2, 2B6, 2C9, 2C19, 3A4, and 3A5 (29, 30). Among these CYP enzymes, it is known that smoking induces a higher activity of CYP 1A2 and 2B6 (31, 32). Accordingly, it is thought that the active form of clopidogrel is increased in smokers resulting in more potent clopidogrel activity.

The mechanism of induction of CYP1A2 by smoking is thought to be as follows (33). The smoke generated by smoking includes polycyclic aromatic hydrocarbons, which are carcinogenic substances. Binding of these substances to aryl hydrocarbon receptor (AhR), which is present in the cell cytoplasm and is a ligand-dependent transcription regulatory factor, leads to activation of the AhR, which then translocates into the nucleus. The translocated AhR then forms heterodimers with AhR nuclear translocator (ARNT). These heterodimers then bind to an enhancer sequence, called the xenobiotic responsive element (XRE), on DNA and induce transcription of the CYP1A2 gene.

The induction of CYP2B6 by smoking involves the constitutive androstane receptor (CAR) (32, 34). CAR, located in the cell cytoplasm, translocates into the nucleus when it is activated and there it forms heterodimers by binding to retinoid X receptor (RXR). These heterodimers then bind to the phenobarbital-responsive enhancer module (PBREM) and/or xenobiotic-responsive enhancer module (XREM) on DNA and induce transcription of the CYP2B6 gene.

This study is associated with a number of limitations. First, the study size was small, thus limiting our ability to make comparisons based on the number of cigarettes smoked per day. The activity of CYP1A2 was previously reported to correlate with the number of cigarettes smoked per day (35). Future studies investigating the relationship between the number of cigarettes smoked per day and the responsiveness to clopidogrel are warranted. Second, this study compared only the presence/absence of smoking and platelet aggregation, however, the activities of CYP enzymes must be evaluated. Third, the precise definition of clopidogrel resistance when using the VerifyNow P2Y12 Assay has not yet been established in Japanese patients. The incidence of clopidogrel resistance varies greatly depending on the testing method and the cut-off value used. Such variation is the reason that the definition of clopidogrel resistance has not been established. Lastly, the genetic polymorphisms of CYP enzymes relating to metabolism of clopidogrel were not confirmed. It is known that CYP2C19 genetic polymorphisms are involved in the efficacy of clopidogrel (10), and specific polymorphisms are more prominent in certain geographical regions. Asians have higher incidences of CYP2C19*2 and CYP2C19*3 polymorphisms, which are reduced-function mutations and include more poor metabolizers (‘2/2’, ‘2/3’, ‘3/3’) (36). In addition, CYP1A2 genetic polymorphisms are related to induction of CYP1A2 activity by smoking, and patients carrying CYP1A2*1F (163C>A mutation) have elevated CYP1A2 activity (22, 33, 37). Therefore, future studies are also needed to study the effects of genetic polymorphisms of CYP enzymes.

### Conclusion

The incidence of clopidogrel resistance was lower in the non-cardiogenic ischemic stroke patients who were smokers, thus indicating that these patients’ responsiveness to this drug may be enhanced.

The authors state that they have no Conflict of Interest (COI).

### References