Prevalence and Risk Factors for Aspirin Resistance in Elderly Patients with Type 2 Diabetes

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Original Article

1. Introduction

Elderly people with diabetes have twice the prevalence of mortality of age-matched controls without diabetes. The principal killer is macrovascular diseases. Aspirin is currently recommended by the American Diabetes Association for diabetic patients aged older than 40 years without cardiovascular disease.

However, some studies have suggested a lower effect of primary prevention of cardiovascular disease with low-dose aspirin in diabetic patients compared with other risk factors for cardiovascular disease. Lack of adequate inhibition of platelets from aspirin therapy is known as “aspirin resistance.” The prevalence of aspirin resistance is 10%–40% for patients with diabetes.

Results of studies investigating whether aspirin resistance is increased in diabetic patients compared with nondiabetic patients are inconsistent, but the consequences of insufficient inhibition of platelets during aspirin treatment could be substantial. Some studies suggested an association of aspirin resistance with smoking, hypertension, hypercholesterolemia, HbA1c levels, body mass index, levels of C-reactive protein, age, and being female. Interestingly, scholars revealed that hyperhomocysteinemia is an independent risk factor for macroangiopathy and mortality in diabetic patients, and that homocysteine (Hcy) is also associated with aspirin resistance.

According to the above hypothesis, we undertook this study to determine the prevalence and related risk factors for aspirin resistance in elderly patients with Type 2 diabetes.
2. Patients and methods

2.1. Ethical approval of the study protocol

This study complied with the Declaration of Helsinki. It was approved by the Scientific and Ethics Review Board of the First Geriatric Cardiology Division, Chinese PLA General Hospital, Beijing. All participants provided written informed consent to be included in the study.

2.2. Participants

We enrolled 140 patients from April 2008 to June 2010. Patients were recruited from the Wangshoulu area of Beijing. Patients were aged 60 years or older and were being treated for Type 2 diabetes; all patients were on regular treatment with aspirin (75–100 mg daily over 1 month). The exclusion criteria were hypersensitivity to aspirin; the use of clopidogrel, ticlopidine, dipyridamole, or other nonsteroidal anti-inflammatory drugs, heparin, or low–molecular weight heparin; a major surgical procedure within 1 week before study enrollment; family or personal history of bleeding disorders; platelet count lower than 150,000/μL or higher than 450,000/μL; hemoglobin level lower than 8 g/dL; history of myeloproliferative disorders; or a history of drug-induced thrombocytopenia.

2.3. Blood sampling

Blood samples were obtained from patients between 7 AM and 9 AM, 2–12 hours after ingestion of the last dose of aspirin to eliminate the effects of circadian platelet function. The first 2 mL of blood drawn by venipuncture through a 21-gauge needle were discarded. Tubes containing 3.2% sodium citrate were used for light transmission aggregometry (LTA) and thrombelastography (TEG). One tube that contained lithium heparin was also used for TEG. In addition, one tube that contained a clavicular tilt angle difference (a mixture of citrate, theophylline, adenosine, and dipyridamole) was collected for measurement of CD62P (P-selectin) and PAC-1 (activated GPIIb/IIIa receptors). Moreover, four conventional tubes were used for the percentage activity of HbA1c, high-sensitivity C-reactive protein, Type B natriuretic peptide, Hcy, and protein C; the percentage activity of antithrombin III; routine measurement of blood components and blood lipids; and other biochemical measurements. All assays were processed within 2 hours of blood sampling.

2.4. Light transmission aggregometry

Platelet aggregation was assessed in platelet-rich plasma at 37°C by LTA. Samples were centrifuged at 800 revolutions per minute for 5 minutes to obtain native platelet-rich plasma. The platelet count was assessed using a standard cell counter. Platelet-poor plasma was obtained by centrifugation of the remaining blood at 4,000 revolutions per minute at room temperature for 8 minutes. Aggregation was measured with a ChronoLog Aggregometer (Chronolog, Havertown, PA, USA). Aggregation was expressed as the maximal percentage change in light transmittance from baseline after the addition of arachidonic acid (AA; 0.5 mM) and adenosine diphosphate (ADP; 10 μM) using platelet-poor plasma as the reference.

2.5. TEG platelet-mapping assay

The TEG platelet-mapping assay from Haemoscope Corporation (Niles, IL, USA) relies on the measurement of clot strength to enable quantitative analyses of platelet function. AA (1mM/L) was added to activator F to measure the degree of thromboxane A2-induced platelet aggregation. This methodology is described elsewhere.

2.6. Markers of platelet reactivity

Platelet activation was determined by assessing platelet surface expression of PAC-1 and CD62P after natural activation using flow cytometry (BD Biosciences, San Jose, CA, USA) as previously described. The antibodies of R-phycoerythrin-conjugated anti-CD62P (recognizes P-selectin), fluorescein isothiocyanate-conjugated PAC-1 (recognizes the active GPIIb/IIIa receptor), and R-phycoerythrin-conjugated CD61 (recognizes the total population of GPIIb/IIIa receptors) were obtained from BD Biosciences. Results of CD62P and PAC-1 were expressed as percentages.

2.7. Definition of aspirin resistance

The definitions of aspirin resistance were 20% or higher AA-induced and 70% or higher ADP-induced aggregation according to LTA. Aspirin semiresponders were defined as meeting one (but not both) of the criteria described above. By TEG, aspirin resistance was defined as 50% or higher aggregation induced by AA.

2.8. Statistical analyses

Continuous variables are mean ± standard deviation. For continuous variables, analyses of univariate tests or Kruskal-Wallis tests (if the distribution was not normal) were used to compare the three groups defined by aggregation. The Student t test or Mann-Whitney U two-sample tests (if the distribution was not normal) were used to compare the continuous variables between the two groups. Categorical data and proportions were analyzed using the χ2 test. A p value less than 0.05 was considered significant. Parameters significantly related with the presence of aspirin resistance were determined using binary logistic regression analyses (SPSS Windows, version 14.0; SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Patient characteristics

Demographics, as well as comparison of aspirin-resistant patients, aspirin semiresponders, and aspirin-sensitive patients by LTA and TEG are provided in Tables 1 and 2. Aspirin resistance, aspirin semiresponders, and a combined group of aspirin resistance plus aspirin semiresponders as measured by LTA were not associated with significant differences with regard to age; being female; being a current smoker; having hypertension, coronary artery disease, cerebrovascular disease, or peripheral arterial occlusive disease; and baseline platelet count. Serum Hcy levels were high in patients with aspirin resistance. Aspirin semiresponders were less likely to take angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) than aspirin-sensitive patients (p = 0.020). Moreover, there were fewer patients in the combined group of aspirin-resistant patients and aspirin semiresponders receiving ACEIs or ARBs than aspirin-sensitive patients (p = 0.011).

By TEG, there were no significant differences between the aspirin-resistant and aspirin-sensitive group when comparing age; being a current smoker; having hypertension, coronary artery disease, cerebrovascular disease, or peripheral arterial occlusive disease; and baseline platelet count. There were significantly more women in the aspirin-resistant group (p = 0.004). Aspirin-resistant patients had higher levels of Hcy in serum, triglyceride levels, and
Aspirin-resistant patients were more likely to take statins than aspirin-resistant patients (p = 0.012). PAC-1 levels were high in the aspirin-resistant group, but this difference did not reach statistical significance (p = 0.224).

3.2. Testing of platelet aggregation

By LTA, six patients (4.3%) were aspirin resistant. An additional 44 patients (31.4%) were aspirin semiresponders. By TEG, 31 patients (22.1%) were aspirin resistant. Of the 31 patients who were aspirin resistant by TEG, 3 were aspirin resistant by LTA. In addition, eight of 44 semiresponders by LTA were aspirin resistant by TEG.

4. Discussion

In the present study, we demonstrated, for the first time, that the prevalence of aspirin resistance by LTA was 4.3% in elderly people with diabetes, and that the proportion of semiresponders to aspirin treatment was 31.4%. These findings implied that only 64.3% of patients with diabetes may benefit from aspirin therapy. Dichiara et al suggested that a low prevalence (0–2%) of aspirin resistance was observed with all aspirin doses (81 mg/d, 162 mg/d, and 325 mg/d for 4 weeks) in 120 patients with stable coronary artery disease as determined by AA-induced LTA. Lordkipanidze et al demonstrated that the prevalence of aspirin resistance in 201 patients with stable coronary artery disease varied according to the assay used: 2.8% for LTA AA and LTA ADP. Despite these differences, in a relatively large study by Gum et al, 5.5% of patients were aspirin resistant by LTA AA and LTA ADP in 325 patients with stable coronary artery disease. This figure is similar to that in the present study.

We also demonstrated that TEG might be a more sensitive test for aspirin resistance than LTA. In a study of 120 patients with stable coronary artery disease taking aspirin, 9% were aspirin-resistant by TEG. In the present study, 31 patients (22.1%) were aspirin resistant according to TEG. Similar results with respect to the prevalence of aspirin resistance in diabetic patients using the platelet function analyzer (PFA-100) were reported by Cohen et al (23%) and Fateh-Moghadam et al (21.5%). A particular advantage of the TEG Platelet
Mapping system is that, in addition to measuring platelet function, it specifically measures the platelet contribution to clot strength. Hence, the TEG Platelet Mapping system has been extensively used in clinical practice not only for evaluation of platelet function but also in the management of hemostasis.

Using a multivariable logistic regression analysis, we demonstrated that female sex and Hcy levels were independent risk factors for aspirin resistance in diabetic patients. One study revealed that, in patients with Type 2 diabetes, Hcy levels were significantly increased compared with healthy individuals. Hyperhomocysteinemia is an independent risk factor for macroangiopathy, microangiopathy, and mortality. Gonzalez et al. subsequently revealed that Hcy levels higher than 16.7 μmol/L were associated with an increased risk of mortality in blood samples (relative risk 2.30 [95% CI: 1.02–5.17]) from elderly patients (>60 years). The present study demonstrated that Hcy levels were independent risk factors for aspirin resistance in diabetic patients. This may be because of the association with endothelial damage, proliferation of smooth muscle cells, and enhancement of coagulation. Furthermore, Zanin et al. suggested that Hcy decreased extracellular nucleotide hydrolysis in rat platelets and Hcy treatment increased platelet aggregation induced by ADP. These findings suggest that we should pay more attention to serum Hcy levels and aspirin resistance, and whether lowering serum Hcy levels may improve the antplatelet effects of aspirin.

Several studies reported a relatively higher prevalence of aspirin resistance in women compared with men. The present study was in accordance with those studies. Nevertheless, in a large study by Becker et al., women demonstrated the same (or greater) decreases in platelet reactivity after aspirin therapy, retaining slightly more platelet reactivity compared with men. There could be two reasons for these inconsistent results. First, a lack of association of aspirin resistance with clinical events in these studies (including the present study) has yielded different results regarding sex in platelet aggregation-related phenotypes. Second, different demographics among studied populations may play a part in these differing results. For example, the study by Becker et al. comprised patients who were younger, with a higher body mass index, and in which there were more colored patients than those in the present population.
Study. Further study should focus on the events of aspirin resistance with evaluation of platelet function in the female population.

Recently, some studies implied that certain agents (e.g., ACEIs, ARBs, statins, and calcium-channel blockers) may decrease platelet aggregation. In the present study, the number of patients receiving ACEIs or ARBs therapy with aspirin sensitivity was more than that of patients who were aspirin semiresponders and aspirin-resistant patients. These findings suggest us that ACEI or ARB therapy may improve the antiplatelet effects of aspirin. An interesting finding of the present study was that more aspirin-sensitive patients took statins than aspirin-resistant patients. A study by Santos et al. revealed that atorvastatin combined with aspirin early in the onset of the acute event significantly reduced persistent thromboxane A2-dependent aspirin resistance in aspirin-free patients with hypercholesterolemia. These results indicate that statin therapy in patients with dyslipidemia may ameliorate the antiplatelet effects of aspirin.

CD62P and PAC-1 are used as markers of platelet activation. Investigations have suggested that diabetic patients have increased platelet reactivity (P-selectin expression and PAC-1 binding) compared with nondiabetic individuals on combined aspirin and clopidogrel treatment. Lev et al. revealed that comparison of baseline platelet reactivity showed a trend toward higher baseline P-selectin levels in aspirin-resistant patients than in aspirin-sensitive patients. In the present study, PAC-1 levels were high in the aspirin-resistant group by TEG. These results indicate that increasing platelet reactivity in diabetic patients may be associated with aspirin resistance. This may be the result of reduction in the level of endothelial nitric oxide, dyslipidemia, and low-grade inflammation in diabetic patients.

The present study had one important limitation. The prevalence of aspirin resistance in elderly patients with diabetes was valid for the dose of 75–100 mg/d of aspirin, but we did not investigate the other suggested doses of 162 mg/d and 325 mg/d.

In conclusion, these findings suggest that a significant number of elderly patients with Type 2 diabetes who have been prescribed aspirin therapy may not obtain the intended platelet inhibition. We found that the prevalence of aspirin resistance was considerably higher in elderly female patients and in elderly patients with higher serum levels of Hcy. These findings suggest that the antiplatelet effect of low-dose aspirin may be decreased or lost in these patients. We should pay more attention to antiplatelet therapy in these patients. Further studies are needed to ascertain the mechanism of aspirin resistance and strategies for antiplatelet therapy in elderly diabetic patients with aspirin resistance.

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