CLINICAL RESEARCH

A randomized comparison of platelet reactivity in patients after treatment with various commercial clopidogrel preparations: The CLO-CLO trial

Étude randomisée comparant la réactivité plaquettaire des patients traités par diverses préparations commerciales de clopidogrel. Étude CLO-CLO

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Summary
Background. — The salt linked to the clopidogrel molecule in generic preparations is suspected to affect its clinical efficacy. There is a lack of information about inhibition of platelet reactivity by generic preparations.
Aims. — To compare the effect of original clopidogrel (clopidogrel bisulphate [Plavix®]), generic clopidogrel preparations (clopidogrel hydrochloride [Clopidogrel-Mepha®]; clopidogrel besylate [Clopidogrel Sandoz®]) and prasugrel (Efient®) on platelet reactivity in patients with coronary artery disease.
Methods. — Patients with coronary artery disease treated with stents received, in a random sequence, original clopidogrel bisulphate, clopidogrel hydrochloride and clopidogrel besylate. Platelet function was assessed with the Multiplate analyser after an initial loading dose (600 mg) and at day 10 after each treatment period. Prasugrel was given for another 10 days. An adenosine diphosphate (ADP) test value < 46 antiaggregation units (U) was defined as therapeutic platelet inhibition.
Results. — Sixty patients (mean age 69 ± 10 years; 50 men) were randomized. Original clopidogrel bisulphate, clopidogrel hydrochloride and clopidogrel besylate provided similar inhibition.

Abbreviations: ADP, adenosine diphosphate; PCI, percutaneous coronary intervention; U, antiaggregation units.
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of platelet reactivity with values of 31±25, 33±28 and 28±23 U, respectively (P not significant). Prasugrel provided better inhibition of platelet function (10±11 vs. 31±25 U for clopidogrel bisulphate; P<0.001). An ADP test value > 46 U was measured in 11 patients (18%) with clopidogrel bisulphate, 13 (22%) with clopidogrel besylate and 13 (22%) with clopidogrel hydrochloride compared with only one (2%) with prasugrel. Conclusion Generic clopidogrel preparations provided similar inhibition of platelet reactivity to original clopidogrel bisulphate, although prasugrel was more efficient.

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Background

Dual antiplatelet therapy with aspirin and thienopyridine is essential after coronary intervention and stent placement [1–3]. The level of on-treatment platelet reactivity is associated with long-term adverse cardiovascular events after percutaneous coronary intervention (PCI) [4]. The efficacy of such treatment is largely influenced by interindividual variability in the pharmacodynamic response to clopidogrel [5,6]. In addition, clopidogrel recently became generic; several commercial preparations are now available in which clopidogrel is linked to a salt that might change its clinical efficacy. None of these generic preparations has been validated other than by pharmacodynamic tests. In this prospective trial, we use a platelet function test [7] to compare, in routine clinical practice, platelet reactivity after randomized administration of two new generic clopidogrel preparations (clopidogrel besylate [Clopidogrel Sandoz®] and clopidogrel hydrochloride [Clopidogrel-Mepha®]) and the original preparation (clopidogrel bisulphate [Plavix®]).

Methods

Patient selection and study design

Consecutive patients with ischaemic heart disease undergoing PCI with stent implantation were considered for enrolment in this trial. PCI was performed using standard techniques by the femoral or radial route. Exclusion criteria were: cardiogenic shock; pregnancy; intolerance to aspirin, thienopyridine or contrast media; poor compliance; active bleeding; inability to give informed consent; anaemia; thrombocytopenia; moderate-to-severe renal failure (defined as creatinine clearance of 30 to 60 mL/min and < 30 mL/min, respectively); planned surgery; or inability to have follow-up information. Patients already treated

Résumé

Objectifs. — Le but de cette étude est de comparer l’effet sur l’agrégation plaquettaire de la molécule originale de clopidogrel (clopidogrel bisulfate [Plavix®]) aux molécules génériques (clopidogrel besylate [clopidogrel Sandoz®] et clopidogrel hydrochloride [clopidogrel-Mepha®]) et au prasugrel (Efient®).

Historique. — On suspecte que le sel utilisé pour lier la molécule de clopidogrel pourrait changer l’efficacité du médicament et par là même avoir un impact sur son activité d’antiagrégant plaquettaires. Aucune information n’est actuellement disponible.

Méthode. — Des patients avec maladie coronaire traités par angioplastie et mise en place de stent ont été inclus dans cette étude. Ils ont reçu de manière randomisée les trois molécules de clopidogrel, à savoir le clopidogrel bisulfate (Plavix®), le clopidogrel hydrochloride (Clopidogrel-Mepha®) et le clopidogrel besylate (Clopidogrel Sandoz®) pour une durée de dix jours chacune. Après cette période de 30 jours les malades étaient placés sous prasugrel pour une nouvelle période de dix jours. L’agrégation plaquettaire a été mesurée après la dose de charge (600 mg) et après chaque intervalle de traitement de dix jours. Un appareil de type Multiplate Analyzer a été utilisé pour évaluer la fonction plaquettaire. Une valeur d’ADP inférieure à 46 U représentait une antiagrégation plaquettaire efficace.

Résultats. — Soixante patients (50 hommes) avec un âge moyen de 69±10 ans ont été inclus. Il n’y avait pas de différence significative de l’activité antiagrégante entre les trois préparations de clopidogrel, le clopidogrel bisulfate, le clopidogrel hydrochloride et le clopidogrel besylate avec des valeurs d’ADP de, respectivement, 31±25 U, 33±28 et 28±23 U (P = N5). Le prasugrel est significativement plus efficace que l’ensemble des clopidogrel ; 10±11 U vs. 31±25 U. Une valeur d’ADP supérieure à 46 U a été observée chez 11 malades avec le clopidogrel bisulfate (19.6%), 12 malades (21.1%) avec le clopidogrel besylate et 13 malades (23.2%) avec le clopidogrel hydrochloride. Seul un patient (2,3%) avec le prasugrel avait une valeur d’ADP supérieure à 46 U.

Conclusion. — Le niveau d’antiagrégation plaquettaire obtenu avec les préparations génériques de clopidogrel est comparable à celui obtenu avec la forme originale alors que le prasugrel est plus efficace.

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with thienopyridines were equally excluded. All patients gave their written informed consent and the protocol was approved by the hospital’s ethical committee. The control group was composed of 50 healthy medication-free adults. The subjects in the control group did not receive any antiplatelet therapy. The rationale behind having a control group was to confirm the validity of the platelet aggregation test.

This was a single-blind randomized trial with complete crossover (Fig. 1). The primary endpoint was therapeutic antiaggregation defined as a value of < 46 antiaggregation units (U) on whole blood platelet function testing. Randomization was achieved with envelopes, the patients being assigned to one of the three clopidogrel groups for 10 days, then crossed over to another clopidogrel molecule, as shown in Fig. 1.

**Percutaneous coronary intervention and antiplatelet therapy management**

At the time of PCI, all patients received 500 mg of aspirin in addition to either unfractionated or low-molecular-weight heparin. A loading dose (600 mg) of clopidogrel was given at the end of the procedure. The clopidogrel molecule was chosen at random between original clopidogrel bisulphate, clopidogrel hydrochloride or clopidogrel besylate. After treatment initiation, the clopidogrel preparation was given for 10 days at a dose of 75 mg/day. At days 10 and 20, the clopidogrel molecule was changed to a new molecule, so that after 30 days all patients had received all clopidogrel preparations for 10 days. After this initial 30-day period, a loading dose (60 mg) of prasugrel was given, followed by a maintenance dose of 10 mg/day for 10 days. After these 40 days, the patient was left for 1 year on the drug that provided the lowest platelet reactivity.

**Platelet reactivity assessment**

Platelet reactivity testing of clopidogrel and prasugrel was performed using the Multiplate analysyer (Dynabyte, Munich, Germany). This method has been approved for human use by the United States Food and Drug Administration. The assay is made up of two distinct silver electrode sensors. The changes in the electrodes’ impedance after platelet adhesion is detected by the sensor and allows aggregation units to be calculated. The variables evaluated are: maximal aggregation, velocity (steepness of the curve) and area under the curve (AUC, AU min), calculated from the mean values of the two curves. The final result is expressed in units (U), with 1 U defined as 10 AU min. Platelet reactivity was assessed 6 hours after the loading dose of clopidogrel and every 10 days at the time of the switch of the clopidogrel preparation. Efficient platelet inhibition was defined as an adenosine diphosphate (ADP) test value < 46 U. Patients with values > 46 U received a loading dose of another clopidogrel preparation on the same day, with repeated platelet function measurement after 6 hours.

For the trial, the operator determining platelet reactivity was blinded to the treatment arm, patient characteristics and biomarkers.

**Statistical analyses**

We performed a power analysis that concluded the inclusion of 60 patients at resistance rates of 15% for original clopidogrel and 40% for the generic clopidogrel preparations would yield a statistical power of 88% at a significance level of alpha equal to 0.05 for a two-tailed analysis. The power analysis was performed for proportions of paired samples.

Continuous variables are presented as mean ± standard deviation and after confirmation of a normal distribution (QQ plot). To compare antiaggregation units achieved with
the different clopidogrel preparations, the paired t test was employed. Categorical variables are presented as numbers and percentages. We considered a P value < 0.05 as significant. SPSS software, version 18 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Results

Sixty patients (50 men and 10 women) with a mean age of 69 ± 10 years were included. The control group was composed of 50 healthy adults (22% men) who were not taking any medication and had a mean age of 39 ± 10 years.

Patient characteristics are shown in Table 1. Briefly, the patients had the usual risk factors expected in patients with ischaemic heart disease. Baseline coagulation variables and platelet reactivity were normal for all patients (platelet count 225 ± 57 g/L, prothrombin time 92 ± 13%, activated partial thromboplastin time 33 ± 4 seconds).

After the loading dose, platelet reactivity was the same for all clopidogrel preparations, with a mean value of 17 ± 15 U for clopidogrel bisulphate, 23 ± 16 U for clopidogrel hydrochloride and 21 ± 16 U for clopidogrel besylate (P = 0.43; Fig. 2). After 10 days of treatment, there were no significant differences between the clopidogrel preparations, with a mean value of 31 ± 25 U for clopidogrel bisulphate, 33 ± 28 U for clopidogrel hydrochloride and 28 ± 23 U for clopidogrel besylate (P = 0.69; Table 2; Fig. 3). There were no statistical differences between the groups: clopidogrel bisulphate vs. clopidogrel besylate (P = 0.58); clopidogrel bisulphate vs. clopidogrel hydrochloride (P = 0.34); and clopidogrel hydrochloride vs. clopidogrel besylate (P = 0.14) (Fig. 3). For all clopidogrel preparations, the higher platelet reactivity inhibition was obtained after the loading dose, as shown in Table 2. Eleven (18%) patients showed values > 46 U for clopidogrel bisulphate, 13 patients

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<th>Table 1 Patient characteristics.</th>
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</tr>
<tr>
<td>Men</td>
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<td>Age (years)</td>
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<td>Body mass index (kg/m²)</td>
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<td>Current smoker</td>
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<td>Renal failure</td>
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<td>Stable angina</td>
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<td>Silent ischaemia</td>
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Data are mean ± standard deviation or number (%). STEMI: ST-segment elevation myocardial infarction.

Discussion

Stent thrombosis remains a partially unsolved problem, associated with a high rate of morbidity and mortality after PCI. A significantly higher rate of stent thrombosis in patients with higher on-treatment platelet reactivity has been reported, with an incidence of up to 3% [8]. Also, using the P2Y12 point-of-care assay, high platelet reactivity (P2Y12 reactivity units > 230) was associated with higher rates of death, myocardial infarction or stent thrombosis. Thus, as clopidogrel has become generic, with several commercial preparations now available, it is of critical importance to demonstrate that similar inhibition of platelet reactivity can be achieved with these preparations.
Table 2  Mean antiaggregation values for the treated population and controls (adenosine diphosphate test).

<table>
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<th>Mean U ± standard deviation</th>
<th>% of patients with ADP test value &lt; 46 U</th>
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<tr>
<td></td>
<td></td>
<td>No treatment</td>
<td>Loading dose</td>
</tr>
<tr>
<td>Clopidogrel bisulphate (Plavix®)</td>
<td>60</td>
<td>17 ± 15</td>
<td>31 ± 25</td>
</tr>
<tr>
<td>Clopidogrel besylate (Clopidogrel Sandoz®)</td>
<td>60</td>
<td>21 ± 16</td>
<td>28 ± 23</td>
</tr>
<tr>
<td>Clopidogrel hydrochloride (Clopidogrel-Mepha®)</td>
<td>60</td>
<td>23 ± 16</td>
<td>33 ± 28</td>
</tr>
<tr>
<td>Prasugrel (Efient®)</td>
<td>60</td>
<td>6 ± 16</td>
<td>10 ± 11</td>
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<tr>
<td>Control group (no treatment)</td>
<td>50</td>
<td>61 ± 17</td>
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</tbody>
</table>

ADP: adenosine diphosphate; U: antiaggregation units.

In this prospective trial, we showed that all preparations of clopidogrel tested provide similar inhibition of platelet reactivity and that generic preparations can be used safely after PCI. We found 36 patients (21.9%) taking any clopidogrel who had an ADP test value > 46 U; they have to be considered as clopidogrel resistant. This is in line with previous reports showing clopidogrel resistance in 23 to 40% of patients [9]. Our results show a wide response variability; thus a clear cut-off value to define true clopidogrel resistance has still to be determined, as in other reported trials [10]. Furthermore, platelet reactivity is not the only variable involved in stent thrombosis-implantation technique, angiographic result and clinical status (such as acute coronary syndrome or comorbidities such as diabetes) also affect the rate of stent thrombosis and morbidity [11].

It has also been recently shown that the incidence of major adverse clinical events, including stent thrombosis, is higher when platelet reactivity is still present at a high value (> 46 U) when the Multiplate analyser is used [4,8].

Our data confirm the results of two previous trials comparing original clopidogrel with generic clopidogrel preparations in healthy subjects [12,13]. These trials showed similar safety profiles and met the criteria for pharmacokinetic bioequivalence. Only healthy volunteers were included in these studies, which is a limitation because patients with atherosclerotic disease have a higher degree of variability in their response to clopidogrel due to comorbidities and multiple medications. Our trial, by comparison, was conducted in a real-life setting, with patients who had a clinical indication for antiplatelet therapy.

In addition, our data confirm the value of prasugrel as a potent antiplatelet agent. Prasugrel, like clopidogrel, requires conversion to an active metabolite before binding to the platelet P2Y12 receptor to confer antiplatelet activity. Prasugrel inhibits ADP-induced platelet aggregation more rapidly, more consistently and to a greater extent than standard and higher doses of clopidogrel in healthy volunteers and in patients with coronary artery disease undergoing PCI [14–16]. When antiaggregation is of critical importance (patients with multiple stenting, multivessel disease or left main PCI, diabetic patients, etc.), clopidogrel resistance can be avoided with prescription of prasugrel, along with very efficient inhibition of platelet reactivity.

**Study limitations**

The main limitation of this trial was the small number of patients. However, it seems obvious that a significant difference between the original and generic clopidogrel preparations would require a considerable number of patients to show very little difference. Moreover, any benefit favouring one of the preparations would not necessarily be correlated with better clinical outcome. As the platelet reactivity test has limitations, its routine use in clinics also has limitations and results should be interpreted in the setting of the clinical situation. Finally, there are no large-scale trials that show clinical outcome improvement after adaptation of antiplatelet therapy using the platelet reactivity test [8].

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.

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**References**


