Thrombosis Research 129 (2012) 700–703

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

The effect of clopidogrel besylate and clopidogrel hydrogensulfate on platelet aggregation in patients with coronary artery disease: A retrospective study

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ARTICLE INFO

Article history:
Received 9 June 2011
Received in revised form 2 August 2011
Accepted 15 August 2011
Available online 15 September 2011

Keywords:
Clopidogrel besylate
Cardiovascular disease
Aggregometry
Antiplatelet therapy

ABSTRACT

Background: Recently several alternative forms of the original clopidogrel hydrogensulfate (CHS) were spread worldwide. A large amount of such drugs turned out to be clopidogrel besylate (CB). Only three studies, involving healthy volunteers, investigated the antiplatelet effect of CB, whereas its attribute remained unexplored in the case of patients with cardiovascular diseases. This retrospective study aimed to evaluate the difference between the antiplatelet effects of two clopidogrel formulas, CHS and CB, on patients with coronary artery diseases.

Methods: Data of 150 patients with previous CHS treatment were investigated. According to the documentations, the CHS therapy was shifted to CB. 94 patients of the selected population received dual antiplatelet therapy, clopidogrel and aspirin. The antiplatelet effects of CHS and CB were compared by ADP induced platelet aggregation measurements using light transmission aggregometry.

Results: Irrespective of the therapeutic combinations the performed statistical investigations failed to show significant difference ($p = 0.30$) between the effect of CB ($AGGmax_{CB}$: $27.6 \pm 13.7\%$) or CHS ($AGGmax_{CHS}$: $29.0 \pm 15.3\%$) on the ADP induced platelet aggregation. Insignificant deviations were found in both forms of clopidogrel salts, either in the lack ($AGGmax_{CB}$: $32.5 \pm 14{,}2\%$; $AGGmax_{CHS}$: $34{,}0 \pm 16{,}1\%$; $p = 0{,}29$) or in the presence of aspirin ($AGGmax_{CB}$: $24.7 \pm 12{,}5\%$; $AGGmax_{CHS}$: $26{,}0 \pm 14{,}1\%$; $p = 0{,}31$).

Conclusion: Our results indicated that both CB and CHS had an identical inhibitory effect on ADP induced platelet aggregation in patients with cardiovascular diseases. Moreover their efficiency showed no overall significant difference in the case of dual antiplatelet therapy with aspirin as well. However there might be an inter- and intraindividual variability between the two clopidogrel formulas.

Introduction

The World Health Organization (WHO) predicts that in the next decade cardiovascular diseases will remain the leading causes of mortality worldwide. One of the major pillars of the prevention is the antithrombotic drug therapy. Alone or in combination with aspirin, the efficacy and security of clopidogrel in the secondary prevention of cardiovascular and cerebrovascular diseases have been demonstrated by several large scale studies (CAPRIE, CURE, CLARITY and COMMIT) [1–7].

Clopidogrel is a prodrug modified through oxidation in the liver in two steps and its developing active metabolites are responsible for inhibiting platelet aggregation. The active metabolites are selectively and irreversibly connected to the platelet adenosine diphosphate (ADP) receptor (P2Y12). Consequently, the platelet aggregation inhibition is maintained during the whole life-span of platelets (cc. 7–10 days) and the normal operation is only restored following the formation of new platelets. The clopidogrel molecule is instabile as a base so it is released in salt forms as a peroral drug: clopidogrel hydrogensulfate (CHS) and clopidogrel besylate (CB).

The present clinical recommendations are based on the results of those studies, which were carried out with the original CHS [1–9]. The cost of clopidogrel has been known as a factor in the premature discontinuation of therapy resulting in high risk for adverse events. Several lower-cost alternative forms of the original CHS containing antiplatelet drug were released worldwide. They were released decisively in two kinds of salt forms: CHS and CB. Theoretically, the two different salt forms may have an influence on the pharmacokinetic

Abbreviations: ACE-I, Angiotensin-converting enzyme inhibitors; ADP, Adenosine diphosphate; ARB, Angiotensin receptor blocking agents; BB, Beta adrenergic receptor blocking agents; CB, clopidogrel besylate; CCB, calcium channel blocker; CHS, clopidogrel hydrogensulfate; DBP, diastolic blood pressure; HPR, highy platelet reactivity; HR, heart rate; LTA, Light transmission aggregometry; NSTEMI, Non-ST elevation myocardial infarction; PCI, Percutaneous coronary intervention; PPI, proton pump inhibitor; PPP, platelet-poor plasma; PRP, platelet-rich plasma; SBP, systolic blood pressure; STEMI, ST Elevation myocardial infarction; VASP, vasodilator stimulated phosphoprotein.

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doi:10.1016/j.thromres.2011.08.013

and pharmacodinamic phenomena as well as on the tolerability of the drug. In spite of the fact that CB has captured a large market share in many European countries, only three studies involving healthy, medication free volunteers investigated the above mentioned hypothesis. Although these trials failed to demonstrate significant differences either in the measure or the tolerability of platelet aggregation [10–12]. In the case of patients with known cardiovascular diseases and receiving adequate cardioprotective drug therapy the question remained unanswered. Therefore this retrospective study aimed to perform a statistical evaluation of the difference between the antiplatelet effects of two clopidogrel salt formulas, such as CB and CHS, on patients with coronary heart diseases.

Methods

Patients and study design

Due to the clopidogrel price differences both the doctors and the patients were encouraged to switch to the low cost alternative clopidogrel products such as e.g. CB from the formerly received innovator CHS. Thus, in our retrospective study those patients' data were studied whose antiplatelet therapies were switched from the original CHS (Sanofi Pharma Bristol-Myers Squibb SNC, Paris, France) to the alternative CB (Pharmaten S.A. Attiki, Greece). Altogether 150 patients (79 male and 71 female, mean age 61.2 ± 9.9 years) with previous cardiovascular events (unstable angina, STEMI, NSTEMI, with or without PCI and stent implantation) were analyzed (Table 1). The patients had normal liver and kidney functions and a normal platelet count. According to the patients' documentations all subjects received the original CHS after the cardiovascular event. 94 patients received a dual platelet aggregation inhibition therapy (clopidogrel and aspirin) and 56 patients were on clopidogrel monotherapy (without aspirin). The indication of aspirin as part of dual therapy was the history of PCI in less than 12 months. Patients receiving clopidogrel monotherapy had a previous history of aspirin intolerance. The conversion from CHS to CB occurred a minimum of 30 days following the cardiovascular event. All patients received 75 mg of clopidogrel (CHS or CB) and in case of dual therapy, 100 mg of aspirin on a daily basis. Blood samples were drawn, as part of our clinical routine care, just before (under CHS effect) and 21–40 days after switching to CB. All the patients received cardioprotective drug therapy (ACE-I or ARB, statin, BB) according to the recent cardiovascular disease prevention guidelines [13]. In the examined period there were no other changes in the concomitant therapy or it represented an exclusion criterion for the study. The study was approved by the local Ethics Committee at the University of Pecs (3727.316-3255/2010).

Table 1
Baseline characteristics of the study population.

Number of patients	150
Age (years)	61.2 ± 9.9
Male, n (%)	79 (53)
Body mass index (kg/m^2)	28.2 ± 5.2
SBP (mmHg)	137 ± 16
DBP (mmHg)	82 ± 12
HR (beat/min)	75 ± 10
Arterial hypertension, n (%)	135 (90)
Diabetes mellitus, n (%)	48 (32)
Active smokers, n (%)	27 (18)
Hypercholesterolaemia, n (%)	111 (74)
Platelet count, x10^3/μL	221.4 ± 70
Dual therapy with aspirin, n (%)	94 (63)
PPI, n (%)	34 (23)
Statin, n (%)	123 (82)
BB, n (%)	114 (76)
ACEI, n (%)	109 (73)
CCB, n (%)	49 (33)

Data are presented as mean ± SD.
SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; PPI: proton pump inhibitor; BB: beta blocker; ACEI: angiotensin converting enzyme inhibitor; CCB: calcium channel blocker.

Aggregometry

For research purposes ADP induced light transmission aggregometry (LTA) or measurement of vasodilator stimulated phosphoprotein (VASP) phosphorylation are recommended tests in evaluating the clopidogrel antiplatelet effect [14]. Since a retrospective study could only investigate the available clinical documentations, the daily routine data of CHS and CB on ADP induced platelet aggregation were examined by light transmission aggregometer (Carat TX4, Carat Diagnostics, Budapest, Hungary). Besides its cost-effectiveness, the advantage of using this method lies in its relatively widespread application. Platelet aggregation was calculated from the optical density ratio of platelet-rich (PRP) and platelet-poor plasma (PPP) as previously described [15–17]. PRP was obtained from the supernatant of plasma that had been centrifuged at 900 g for 4 minutes at room temperature. Further spinning at 2300 g for 10 minutes resulted in PPP in the supernatant. Platelet count in the PRP varied from 250 to 350 x 10^9/L. All samples were processed within one hour. The efficacy of clopidogrel therapy was expressed using ADP 5 μM, while epinephrine 10 μM was used to refer to the efficacy of aspirin therapy. Maximal aggregation was calculated in every measurement and compared to PPP as the reference (AGGmax, %) at 37 °C. Based on the previous studies using light transmission aggregometry an aggregation value of more than 40% was defined as a low response to clopidogrel [15,18,19].

Statistics

Inhibition of ADP-induced platelet aggregation was expressed as the % difference of optical density ratio of platelet-rich and platelet-poor plasma. The comparison between the altered platelet function of the efficiency of CB and CHS administration was analyzed by paired Student *t*-test and Spearmann correlation (r). A P value <0.05 was considered statistically significant. N represents the number of patients. All values are reported as mean ± SD. Statistical analysis was carried out with SPSS 16.0 software (SPSS, Chicago, IL, USA).

Results

The mean values of ADP induced platelet aggregation did not show a significant alteration (p = 0.30, r = 0.68) following the conversion of CHS ($AGGmax_{CHS}$: 29.0 ± 15.3%) to CB ($AGGmax_{CB}$: 27.6 ± 13.7%) (Figs. 1 and 2/a). On the other hand markedly high interindividual as well as intraindividual variability was found in the case of both clopidogrel salts. In the presence of CHS, out of 150 patients 30 had

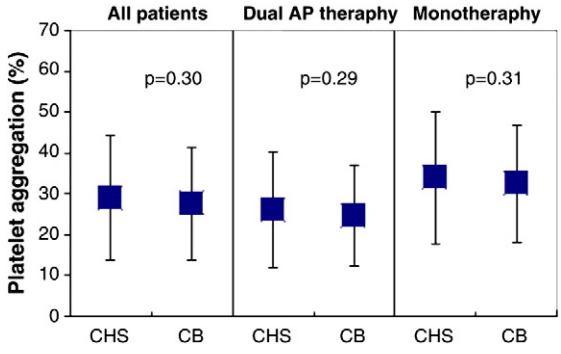

Fig. 1. Mean and ± SD values of ADP induced platelet aggregation in patients under CHS and the following CB therapy, with a history of a cardiovascular event: irrespective of the presence of aspirin (p = 0.30; n = 150); under dual (aspirin + clopidogrel) antiplatelet therapy (p = 0.29; n = 94) and in the lack of aspirin - monotherapy (p = 0.31; n = 56).