REVIEWS

Adv Clin Exp Med 2013, **22**, 6, 875–885
ISSN 1899–5276

Marcin Protasiewicz[1, 3, A, D, F], Paweł Szymkiewicz[1, 3, A, B, D, G],
Wiktor Kuliczkowski[2, D, E], Andrzej Mysiak[1, 3, D–F], Wojciech Witkiewicz[1, 4, 5, E]

Modern Antiplatelet Therapy – Opportunities and Risks

Nowoczesne leczenie przeciwpłytkowe – szanse i zagrożenia

[1] Wrovasc – Integrated Cardiovascular Centre, Regional Specialist Hospital in Wrocław, Research and Development Centre, Poland
[2] 3rd Department of Cardiology with Clinical Unit, Silesian Centre for Heart Diseases, Zabrze, Poland
[3] Department of Cardiology, Wroclaw University Hospital, Poland
[4] General and Vascular Surgery Unit, Regional Specialist Hospital, Research and Development Centre in Wrocław, Poland
[5] Department of Animal Physiology and Biostructure, Wrocław University of Environmental and Life Sciences, Poland

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Resistance to clopidogrel has been extensively investigated and described by researchers from many countries. Subsequent studies have contributed to a better understanding of the complex mechanisms in which clopidogrel is metabolised and have confirmed the negative effects on prognosis, both in a group of patients with low and excessive response to the drug. To prevent the dramatic consequences of an abnormal response to clopidogrel, 2 different strategies were adopted. The 1st employs the identification of patients based on platelet function tests, and genotyping and increasing the drug dose until the required aggregation parameters are achieved. The 2nd strategy is focused on developing new generation drugs whose efficacy will show fewer individual variations. So far diagnostic methods and tailored therapy have not been conclusively accepted for the treatment of patients, because novel pharmaceuticals have emerged providing more rapid action and beneficial effects, but without the disadvantages of clopidogrel. Still, there is a group of patients for whom clopidogrel remains the preferred choice, and considering the economic aspect, this drug can play a significant role in the treatment of many patients **(Adv Clin Exp Med 2013, 22, 6, 875–885).**

Key words: clopidogrel resistance, platelet aggregation, antiplatelet therapy.

Streszczenie

Oporność na klopidogrel to zjawisko szeroko badane i opisywane na całym świecie. Kolejne prace przyniosły lepsze zrozumienie złożonych mechanizmów metabolicznych przemian klopidogrelu i potwierdziły niekorzystny wpływ na rokowanie zarówno w grupie chorych prezentujących słabszą, jak i nadmierną odpowiedź na leczenie tym lekiem. W celu zapobiegnięcia dramatycznym następstwom nieprawidłowej reakcji na klopidogrel przyjęto 2 odmienne strategie postępowania: opartą na identyfikacji chorych na podstawie testów płytkowych oraz genetycznych i intensyfikacji dawkowania leku aż do uzyskania oczekiwanych parametrów agregacji lub stworzenie nowej generacji leków, których skuteczność działania nie będzie tak silnie osobnicza. Obecnie diagnostyka i terapia indywidualna nie zostały jednoznacznie zaakceptowane w leczeniu chorych z uwagi na pojawienie się nowych farmaceutyków, które oferują szybsze i skuteczniejsze działania oraz nie mają wad klopidogrelu. Pozostaje jednak grupa chorych, w której klopidogrel, wydaje się, jest wciąż preferowany w leczeniu, a uwzględniając kontekst ekonomiczny, może odgrywać znaczącą rolę w terapii wielu chorych **(Adv Clin Exp Med 2013, 22, 6, 875–885).**

Słowa kluczowe: odporność na klopidogrel, agregacja płytek krwi, leczenie przeciwpłytkowe.

Antiplatelet agents have been the major type of pharmacological treatment in atherosclerotic cardiovascular diseases for many years. After decades of aspirin (ASA) being used alone, it was adjoined with thienopyridine derivatives, particularly clopidogrel. The high efficacy of clopidogrel was confirmed in a number of randomized clinical trials. In combination with aspirin this drug became a part of routine pharmacological regimes in the treatment of patients with acute coronary syndrome (ACS) and those who had percutaneous coronary interventions (PCI). Over time, reports were published on patients who had suboptimal responses to treatment with clopidogrel. Initially, this problem was named as resistance to clopidogrel and later a more precise definition was adopted: high on-treatment platelet reactivity. According to different authors this reaction is found in 5–44% of patients [1]. There is substantial evidence that low platelet inhibition demonstrated by laboratory tests in patients during treatment with clopidogrel is associated with a higher risk of adverse cardiovascular events compared with patients who adequately respond to clopidogrel [2]. Assuming that globally over 40 m people are taking clopidogrel, the problem of low response to this drug has become particularly important. Potential tools for the identification of low responders to clopidogrel include genetic tests and *in vitro* platelet function tests. These tests, however, have not been conclusively standardized and accepted. According to current opinion, even a partial evaluation of responsiveness to treatment with clopidogrel requires the use of a relevant *in vitro* test and patient genotyping. According to the current guidelines of the European Society of Cardiology (ESC, 2011) on the diagnosis and treatment of patients with acute coronary syndrome without persistent ST-segment elevation, such a procedure is recommended as a diagnostic element only in selected cases (class of recommendation IIb, level of evidence *C*). While trying to classify patients with low response to antiplatelet treatment scientists sought alternative methods to treatment with clopidogrel. After investigations and clinical trials two new antiplatelet agents were introduced in treatment – prasugrel and ticagrelor, and in 2011 they were included in the ESC guidelines, referred to above. The question of whether the popularity of clopidogrel is coming to an end can be raised. Will clopidogrel, after a period of matchless success, end like ticlopidine, which has recently been relegated to a minor position?

The common use of thienopyridine is linked with the dynamic development in the percutaneous coronary intervention (PCI) procedure, which, after introduction, has become the most popular method of coronary revascularization [3]. This invasive method was proven to be useful in the treatment of various atherosclerotic lesions, depending on the type of expanded vessel (native vessel or bypass) and clinical status of a patient [4]. Thrombosis was found as a negative consequence of damage to vessel walls and potential stent placement associated with coronary angioplasty. The 1st studies evaluating pharmacological prevention of thrombosis demonstrated the best outcome in patients who had dual treatment with ASA plus ticlopidine. A regimen combining these two drugs, defined as a dual antithrombotic treatment, was demonstrated to be superior to ASA alone and ASA plus warfarin in a group of patients who had undergone PCI with stent placement. However, the same study demonstrated that ticlopidine is associated with side effects, i.e. a 2.4% rate of neutropenia (WBC < 1.200/mm3). In addition, thrombotic thrombocytopoenic purpura (TTP) is estimated to occur in about 1 in 4.800 treated patients [5]. Also, the pharmacological profile of ticlopidine showed a significant delay in the onset of the drug's action, and significant differences in individual response to treatment. These limitations were considerably reduced by the use of clopidogrel, a 2nd generation thienopyridine. The CAPRIE trial (Clopidogrel vs. Aspirin in Patients at Risk of Ischemic Events) confirmed the clinical usefulness of clopidogrel in preventing ischaemic events in patients with diagnosed atherosclerosis [6]. Clopidogrel was also found to be much safer than ticlopidine. According to the CAPRIE trial, the rate of neutropenia is reduced to 0.1% in patients taking clopidogrel, the onset of action is more rapid, and the once-a--day dosing regimen is more convenient [6]. Therefore, the role of ticlopidine in clinical practice was marginalized.

Clopidogrel

Clopidogrel is a thienopyridine class drug inhibiting the P2Y12 receptor. The receptor plays a key role in the activation and aggregation of thrombocytes induced by adenosine diphosphate (ADP) [7]. The inhibition of P2Y12 has a pleiotropic effect; it suppresses aggregation, affects pro-coagulant platelet properties and thrombin synthesis, modulates the release of many pro-inflammatory markers, e.g. CRP, TNF-α, and reduces the plasma level of CD40L and p-selectin level [8, 9]. Clopidogrel is an oral prodrug with a complicated metabolic pathway. In the first stage it requires intestinal absorption determined by p-glycoprotein (p-gp), a membrane protein encoded by the ABCB1 gene from the class of multidrug resistance genes

(MDR-1). Almost 85% of clopidogrel absorbed from the intestines to the bloodstream is rapidly hydrolyzed to a non-active form by plasma esterases. Only about 10–15% of the absorbed drug is metabolised to 2-oxyclopidogrel by liver enzymes of the P450 cytochrome and the glycoprotein Paraoxonase-1 (POX-1). CYP2C19, CYP3A4 and CYP3A5 are the major and best studied enzymes of metabolic pathway for clopidogrel. At the 2nd stage of oxidation a new thiol group is formed which finally binds with cystine and selectively blocks the P2Y12 receptor. Clopidogrel has a persistent antiplatelet effect which requires only a single daily dose with no need for continuous monitoring.

Use of Clopidogrel

Today clopidogrel is the most popular P2Y12 inhibitor. The short-term and long-term efficacy of treatment with this drug was demonstrated in a group of patients who had PCI, due both to ACS and as an elective procedure. The CURE trial (Clopidogrel in Unstable Angina to Prevent Recurrent Ischemic Events) was carried out on patients with ACS without ST-segment elevation. This study, carried out on a group of over 2.000 patients, demonstrated that clopidogrel, compared to ASA alone, introduced before the PCI procedure, reduced in a 30-day follow-up the relative risk (RRR) of cardiac death, myocardial infarction or repeat revascularization by 30% (6.4% vs. 4.5%, HR 0.70; 95% CI 0.50–0.97; p = 0.03) and RRR by 31% (12.6% vs. 8.8%, HR 0.69; 95% CI 0.54–0.87; p < 0.002) in overall follow-up [10]. Patients with ACS and ST-segment elevation were the subjects of a few randomized prospective clinical trials, e.g. CLARITY–TIMI (Clopidogrel as Adjunctive Reperfusion Therapy–Thrombolysis In Myocardial Infarction), for which patients undergoing fibrinolytic treatment were recruited. One group was given clopidogrel (including a 300 mg loading dose), and the 2 was given a placebo. In a 30-day follow-up RRR for recurrent myocardial infarction was about 30% (5.9% vs. 4.1%; HR 0.69; p = 0.02) and RRR for fatal cardiovascular event, recurrent myocardial infarction or ischaemia with a need for urgent revascularization was 20% (11.6% vs. 14.1%; HR 0.80; p = 0.03). Importantly, this treatment did not increase the rates of major bleeding (2.0% vs. 1.9%; p = 0.99) [11]. Similar conclusions were made in the COMMIT – CCS trial (Clopidogrel and Metoprolol in Myocardial Infarction Trial/Second Chinese Cardiac Study), which confirmed the efficacy of treatment with clopidogrel without increase in the rate of major bleeding [12]. In the CREDO trail (Clopidogrel for the Reduction of Events During Observation) analysis of results suggested the high efficacy of clopidogrel, both in pre-treatment and in combination with aspirin as a long-term treatment of patients with stable coronary disease undergoing elective PCI. According to the study protocol, patients were randomized to a group treated with ASA and clopidogrel for 12 months, or to a placebo group, where after 28 days of dual antiplatelet treatment patients continued taking ASA alone. The CREDO trial, carried out on 2.116 patients demonstrated that dual antiplatelet treatment was associated with 27% RRR (11.5% vs. 8.5%; HR 0.73; 95% CI 3.9–44.4; p = 0.02) with reference to the combined endpoint – death, myocardial infarction or stroke in a 12-month follow-up period. Similarly, the effect on the risk of bleeding was found to be statistically insignificant (6.7% vs. 8.8%; p = 0.07) [13]. Helton et al. published an interesting paper in which they presented results of meta-analysis covering about 80.000 patients recruited for CURE, CREDO, CLARITY, CHARISMA and COMMIT trials. It was found that clopidogrel combined with ASA reduced all-cause mortality when compared to treatment with ASA alone (6.3% vs. 6.7%; p = 0.026). The incidence of recurrent myocardial infarction was significantly higher in the group of patients treated with ASA alone than in the group treated with two antiplatelet drugs (3.3% vs. 2.7%; p = 0.026) in contrast to the rate of major bleeding (1.3% vs. 1.6%; p = 0.0001). Importantly, no statistically significant difference was found for the rates of fatal bleeding events (0.27% vs. 0.28%; p = 0.79 respectively) [14]. Results of the CAPRIE trial suggest that the use of clopidogrel should be extended in selected cases and introduced in patients who do not qualify for treatment with antithrombotic drugs as well as in secondary stroke prevention, peripheral artery disease, and after myocardial infarction [15].

Treatment and Dosing Regimens of Clopidogrel

About 5 days are required for a single 75 mg dose of clopidogrel to produce an inhibitory effect on the P2Y12 receptor. This long period, greatly inconvenient in the treatment of ACS, was reduced through the use of loading doses, which produced a more rapid and potent antiplatelet effect. Initially, a 300 mg loading dose of clopidogrel was considered to be conventional, but later it was used only before elective PCIs. Then a double 600 mg dose was introduced to the standard treatment of ACS, as it demonstrated more rapid onset of action (3–5 h) and more efficient platelet inhibition with more stable effects. This contributed to reduced rates of

periprocedural mycoardial damage and improved short-term prognosis for patients without significant increase in the risk of bleeding (ARMYDA-2-Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty) [16]. Similar observations were made in the CURRENT-OASIS 7 trial (Clopidogrel and Aspirin Optimal Dose Usage to Reduce Recurrent Events – Seventh Organization to Assess Strategies in Ischemic Symptoms). According to the study protocol, patients were given a 600 mg loading dose followed by a 150 mg dose of clopidogrel a day for 7 days. Statistical analysis was carried out for data obtained from over 17.000 patients who underwent PCI. Results were in favour of the group treated with higher doses of clopidogrel with respect to the combined end-point, which included fatal cardiac event, myocardial infarction and stroke 3.9% vs. 4.5% (HR 0.86; 95% CI 0.74–0.99; p = 0.039). This result was mainly affected by reduced incidence of myocardial infarction (2.0% vs. 2.6%; HR 0.69; 95% CI 0.56–0.87; P = 0.001) because the incidence of stroke and fatal cardiac events was comparable in both groups. The risk of stent thrombosis was reduced by 50% in the group of patients treated with the higher dose of clopidogrel. The risk of stent thrombosis was reduced both on day 2 of observation (0.2% vs. 0.4%; HR 0.49, 95% CI 0.27– 0.89, p = 0.018) and on days 3 to 10 (0.4% vs. 0.6%; 0.58, CI 0.37– 0.90, p = 0.016) [17]. This clearly suggests that both the higher loading dose and higher dose given in the first 7 days after randomization contributed to the reduced risk of stent thrombosis. A significantly higher rate of major bleeding was observed in the group of patients taking a double dose of clopidogrel (2.5% vs. 2.0%; HR 1.24; 95% CI 1.05–1.46; p = 0.01). However, there was no difference in fatal bleeds, intercranial haemorrhage (ICH) or CABG-related bleeds. Interestingly, the risk of stent thrombosis is higher in non-compliant patients who discontinue the drug. Such effects of interrupted treatment were more frequently found in a group of patients who had drug-eluting stents (DES) [18]. The REGINA survey was designed to evaluate how the interruption of dual antiplatelet treatment is managed in the 'real world'. Over 2.000 specialist physicians and dentists completed the interview. Answers obtained from 93% of respondents demonstrated that an unjustified total interruption of dual antiplatelet treatment was much more frequent than expected (22%). Low-molecular-weight heparin was the substitution therapy in over 2/3 of scenarios and was associated with longer periods of antiplatelet therapy interruption [19].

Despite the very good clinical outcome after using clopidogrel along aspirin, many clinicians still registered a certain number of patients who developed on therapy stent thrombosis after PCI. Based on this observation a definition of incomplete platelet inhibition and later clopidogrel resistance was formulated. The first studies focused on this problem pointed out individual variations of platelet inhibition [20]. It was suggested, in one of the first clinical trials carried out on 105 patients that non-responders to platelet inhibitors identified based on platelet aggregometry during PCI are at risk of developing early stent thrombosis [21]. Further studies provided similar findings, also concerning complications that develop in long-term follow-up, regardless of the primary clinical status of the patient and type of laboratory tests used. Based on these findings a hypothesis of clopidogrel resistance was formulated with subsequent attempts to explain this problem [22]. However, this proved to be a challenging task, and the definition of clopidogrel resistance remains unclear and is debated by researchers worldwide. The di-fferences and inaccuracies result from the complicated nature of thrombotic processes, lack of reliable, simple and inexpensive laboratory diagnostic methods, lack of conclusive cut-off values for measurements obtained in platelet tests, complicated genetic background responsible for the absorption and activation of clopidogrel, and a number of factors affecting its metabolism. These problems explain significant differences in the reported incidence of clopidogrel resistance (5–44%). However, the core of the problem is still the reactivity of platelets after determining the final dose of clopidogrel and during its use. Defining clopidogrel resistance in this way, we consider patients both directly after coronary interventions and patients on long-term treatment. This assumption corresponds with the final and intended effect of platelet inhibition, regardless of the limiting factors. Importantly, an increasing number of scientific reports support the fact that unsatisfactory platelet inhibition can influence long-term prognosis for the patient [23].

Factors Affecting Clopidogrel Responsiveness

Clinical implications concerning ineffective clopidogrel treatment were proven by demonstrating that low responsiveness to clopidogrel is associated with an increased risk of adverse cardiovascular events [23]. A conclusive explanation of reasons for low responsiveness to clopidogrel and identification of all factors is very complicated. Factors responsible for low antiplatelet effect include: poor compliance, variable hepatic metabolism of the drug, variable intestinal absorption, drug-to-

drug interactions, increased baseline platelet activity [24] and many other factors, e.g. cox-1 conformational change, cox-a polymorphism, receptor gf2b3a mutations, increased platelet consumption in sepsis, advanced atherosclerosis, diabetes and type of hypoglycaemic treatment, chronic renal disease, or tobacco smoking.

Platelet Function Tests

The most popular method for measuring the antiplatelet effect of clopidogrel is ADP-induced thrombocyte aggregation level in platelet-rich plasma. This method evaluates the inhibition of P2Y12 and P2Y1 receptors induced by ADP based on platelet aggregation measured with light transmission aggregometry (LTA). Most tests record the peak value for platelet aggregation after the use of 5 μmol/L, 10μmol/L and 20μmol/L ADP concentrations. Currently there is no established consensus among researchers working on the pathophysiology of platelets with respect to the use of any ADP concentration in particular. In addition, no precise cut-off values were defined for platelet aggregation which would indicate nonresponsiveness to clopidogrel. In the most important study on the clinical usefulness of LTA, Breet et al. proposed limit values for 5 μmol/L ADP > 42.9% and > 64.5% for 20 μmol/L ADP. AUC (area under curve) defining test accuracy was 63% and 62% respectively [25]. Gurbel et al. also suggested > 46% for 5 μmol/L ADP and 59% for 20 μmol/L ADP with AUC 77% and 78% [26]. The presented data clearly indicates groups of patients not presenting a complete response to clopidogrel, but also in this case it is extremely difficult to propose a precise cut-off value. In addition, results from LTA have low repeatability, and the test itself is time-consuming, requiring qualified laboratory personnel and is dependent on the availability of a laboratory. Nevertheless, LTA remains a very popular test and so far has been considered the gold standard in the evaluation of platelet function. Low suitability for practical use and, most of all, the complicated process of acquiring results from LTA forced researchers to find novel platelet activity tests which would be easy to carry out and offer bedside monitoring. The VerifyNow system is a solution responding to these needs. This test is a modified rapid and easy version of optical aggregometry known for its simplicity and convenience of use. Platelet aggregation is evaluated *in vitro* and is based on degree of light transmittance through an agonist stimulated blood sample. The VerifyNow system is a point-of-care solution for bedside use. All the blood is analysed with no need for using fixing agents potentially interfering with the test result, which is an unquestionable advantage of this system. VerifyNow was found to be an efficient tool in the study by Lev et al. and the low response to clopidogrel detected by this assay correlated with the higher number of periprocedural complications (myocardial infarction) in patients who had PCI [27]. There are also other laboratory diagnostic methods with a significant value for clinical practice. The VASP assay (VAsodilator-Stimulated Phosphoprotein) is based on flow cytofluorometry. VASP is an intraplatelet protein phosphorylated in response to the activation of the P2Y12 receptor. The level of VASP is measured after incubation with ADP and prostoglandin-1 (PGE-1), antibodies specific for the phosphorylated intraplatelet form of VASP. The following step is cytofluorometric analysis that correlates with the level of inhibition of the P2Y12 receptor. Interestingly, unlike previously described methods, the VASP assay does not consider the contribution of the P2Y1 receptor. The suitability of the VASP assay in clinical practice as a method for the monitoring and modification of treatment with clopidogrel was demonstrated by Bonello et al. [28]. The Multiplate Analyzer is an aggregometer that employs modified impedance aggregometry. Activated platelets adhering to sensor wires inside the device cause an increase in electrical resistance between the wires. The Multiplate apparatus continuously registers platelet aggregation in comparison to increased electrical resistance and quantifies it in aggregation units (AU) plotted against time. The most important evaluated parameter is the area under the curve (AUC), which depends on the slope and height of the aggregation curve. These calculations are correlated with platelet activity and demonstrate good repeatability. Thromboelastography mapping (mTEG) is a somewhat forgotten testing method but it is useful in clinical practice. This test employs thromboelastography to evaluate the effect of antiplatelet drugs on blood clotting. Tests carried out with TEG apparatus involve quantitative analysis of platelet function based on the strength/resistance of the formed thrombin-induced platelet-fibrin clot. Advantages of this test include rapid performance and general evaluation of clopidogrel activity reflected in coagulation parameters, general tendency of clot formation and evaluation of response to ASA. Each of these laboratory tests proves to be valuable in platelet function testing, and the obtained results enable the modification of therapeutic decisions. However, so far none of them is an ideal test, i.e. inexpensive, easy, rapid, specific in identifying a group of patients with low response to clopidogrel in correlation with long-term prognosis,

and offering easy monitoring and optimised treatment modification.

Genetic Testing

As already mentioned, clopidogrel is taken as a prodrug, and its further effect is determined by transformation into an active form. Two key stages of this process are intestinal ABCB1 gene-dependent absorption, and hepatic metabolism determined by liver enzymes encoded by genes of the P450 cytochrome. CYP2C19, CYP3A4 and CYP3A5 are, so far, the best-studied enzymes. One of the most crucial studies, carried out by Mega et al., on the effect of genes on the hepatic metabolism of clopidogrel demonstrated that in a group of healthy volunteers subjected to testing, the carriers of a mutant allele of the CYP2C19 gene had a 32.4% ($p < 0.001$) lower plasma level of clopidogrel and a relative average platelet aggregation reduced by 25% [29]. In the same paper the authors reported results obtained from patients recruited for the TRITON-TIMI trial. According to the data presented patients-carriers of a mutation in allele CYP2C19 had lower levels of active clopidogrel metabolite, reduced platelet inhibition, and increased risk of myocardial infarction, stroke or death. Data obtained in the study (12.1% vs. 8.0%; HR 1.53; 95% CI, 1.07 to 2.19; $p = 0.01$) indicate a more than doubled increase of risk for the established end-points. Additionally, the risk of stent thrombosis in examined patients was more than 3 times higher (2.7% vs. 0.8%; HR, 3.33; 95% CI, 1.28 to 8.62; $p = 0.004$). Genetic variability concerns a much wider spectrum of CYP class genes. Variability of CYP3A4 and CYP3A5 also has an influence on reduced response to clopidogrel treatment [30, 31]. The effect of these genes on long-term prognosis has not, however, been confirmed conclusively.

The effective plasma level of clopidogrel is also determined by *P* glycoprotein (P-gp) – a membrane protein found, for example, in gastrointestinal cells responsible for the excretion of excessive amounts of absorbed drugs, thus regulating the level of xenobiotics, including clopidogrel, in the human body. Point mutations within this protein create an obstacle for achieving therapeutic concentration of many drugs. P-gp is also called ABCB1 (ATP-binding cassette sub-family *B* member 1), MDR1 or PGY1. For clopidogrel the level of active metabolite is thus determined by a mutation in C3435T. Polymorphism within a single gene considerably reduces intestinal absorption, both in heterozygotes and homozygotes. Such findings were made in a study carried out by Simon et al. This study demonstrated that adverse cardiovascular events (death, non-fatal myocardial infarction or stroke within one year following myocardial infarction) were more frequent by about 50% in a group of patients with identified homozygous mutation (TT in position 3435) when compared to wild-type homozygotes (CC in position 3435) (15.5% vs. 10.7%; HR 1.72; 95% CI 1.20–2.47) [32]. It was also found that the coexistence of 2 mutations (2 alleles in CYP2C19 or at least 1 in ABCB1) is associated with a 5 times higher risk of adverse cardiovascular events compared to wild-type homozygotes. The analysis of genetic polymorphism on the efficiency of clopidogrel treatment is difficult because it is difficult to conclusively and fully define the effect of described phenotypes as well as potential simple and complex combinations of genetic variants. In addition, the effect of other genes, including those not associated with liver cytochromes, cannot be ruled out. Moreover, genetic testing is expensive, and so far there is no common and rapid bedside test available. However, it has unquestionable value for the understanding of many processes and requires further studies.

Methods for Overriding Clopidogrel Resistance

Resistance to antiplatelet drugs led to 2 strategies in science for solving this problem. One of them is focused on the analysis of factors reducing the level of the active clopidogrel metabolite and choosing tailored therapy *h* based on current laboratory tests.

This method of treatment would help to achieve a long-term satisfactory loading level of clopidogrel through the modification of a daily dose of the drug based on the level of thrombocyte inhibition. However, this method was found to be expensive, very time-consuming, and required much involvement from the patient and physician. Today, there is no standardized platelet function test which would identify low-responders to clopidogrel in an easy and conclusive way, enabling further monitoring of the effects of the chosen therapy. Numerous scientific reports concerning tests for the analysis of platelet aggregation are inconclusive about the identification of patients presenting low response to clopidogrel. Additionally, the evaluation of response to clopidogrel greatly depends on the type of chosen test. In an interesting study carried out in a group of patients with ACS and presenting unsatisfactory platelet inhibition index identified by VASP, despite a 1st loading dose of clopidogrel, patients received an additional bolus of the drug (600 mg). Then patients from

the 1st group were assigned to have tailored therapy (VASP-guided serial clopidogrel doses), and patients from the 2nd (control) group were given a loading dose only twice. The study provided surprising results. Patients who were on a tailored treatment with an adjusted dose had lower rates of adverse cariovascular events and stent thrombosis compared to the control group. However, in 10% of patients the expected treatment outcome was not achieved despite serial loading with 2.4 g of clopidogrel a day [28]. This study shows that "tailored treatment" is an alternative solution for clopidogrel resistance and enables the achievement of the expected outcome, but is expensive, inconvenient, time-consuming and, most importantly, not fully effective. In addition, it has not been explained how to maintain an effective clopidogrel loading in long-term treatment. Moreover, Pena et al. found in their study that some patients demonstrated an unsatisfactory response to treatment with clopidogrel despite receiving a 300 mg daily dose and presented with side effects like gastric disorders and arthralgia [33]. Novel antiplatelet drugs were found to be an alternative to overriding clopidogrel resistance. The most popular among them are prasugrel and ticagrelor, which should be the preferred choice according to ESC recommendations released in 2011.

New Antiplatelet Drugs

Prasugrel is, like clopidogrel, a thienopyridine class drug. It gained its superiority due to its better pharmacodynamic profile, reflected by more rapid onset of action (30 min for a loading dose), and, most importantly, a lack of evident individual variations in the efficacy of treatment. In the TRITON TIMI-38 trial (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis in Myocardial Infarction) 13.608 patients with ACS treated with PCI were randomized to receive clopidogrel or prasugrel. The novel drug was found to be more effective than clopidogrel. A 15-month follow-up demonstrated over 20% RRR for the number of myocardial infarction events (9.7% vs. 7.4% HR 0.76; 95% CI, 0.67 to 0.85; P < 0.001) and a 52% RRR (2.4% vs. 1.1% HR 0.48; 95% CI, 0.36 to 0.64; P < 0.001) for stent thrombosis. In the group on prasugrel 2.4% of patients were found to have at least 1 major bleeding non-CABG-related compared to 1.8% in the group on clopidogrel (HR 1.32; p = 0.03). Based on this study prasugrel was accepted by global societies of cardiology in the treatment of patients with ACS. According to TRITON-TIMI subanalyses, prasugrel presented particular therapeutical value in patients with ACS with ST-segment elevation treated with PCI, patients with diabetes, and in episodes of stent thrombosis. However, less beneficial results were found in elderly patients (> 75 y.o.), patients with body weight lower than 60 kg, and patients with a history of stroke [34]. The TRITON TIMI 38 trial suggests that benefits from using prasugrel are particularly clear in patients receiving invasive treatment who have a low risk of bleeding. Patients who have a higher risk of bleeding may not benefit from the treatment, and in some cases, e.g. history of stroke, treatment may be harmful. Ticagrelor is the 1st reversibly binding antagonist of the P2Y12 receptor. It is an oral drug and requires no metabolic activation. One of its strong advantages is the rapid onset of action and reversibility of effects, enabling complete (and very important) recovery of platelet function with no need for their regeneration in bone marrow. Pharmacodynamic analyses demonstrated that ticagrelor achieved greater, more rapid and more stable inhibition than clopidogrel. Studies also showed that after 30 min ticagrelor produced the same effect in platelet inhibition as a 600 mg dose of clopidogrel. One hour after administration, the inhibition of platelet receptors was significantly greater in patients with coronary disease taking ticagrelor than in those treated with clopidogrel [35]. In the PLATO trial, 18.624 patients were randomized to receive ticagrelor or clopidogrel within 24 h following the onset of ACS symptoms. The study demonstrated a 16% reduction in the combined end-point, i.e. cardiovascular death, myocardial infarction or stroke in a 1-year follow-up (9.8% vs. 11.7%; HR 0.84; 95% CI 0.77– 0.92; p < 0.001), 23% (1.3% vs. 1.9%, p = 0.009), reduced stent thrombosis and, most importantly, a 17% reduction in total mortality (10.2% vs. 12.3%, p < 0.001). The PLATO trial provided the very important conclusion that reduction in mortality was statistically significant in patients treated with ticagrelor within the overall follow-up period. Interestingly, ticagrelor was the 1st drug found to be superior to clopidogrel. In addition, the subanalysis of patients subjected to CABG within 7 days following the withdrawal of ticagrelor demonstrated a statistically significant reduction in total mortality (9.7% vs. 4.7%; HR 0.49; 95% CI 0.32–0.77; p < 0.01) and mortality due to CV event (7.9% vs. 4.1%; HR 0.52; 95% CI 0.32–0.85; p < 0.01) in comparison to clopidogrel. Ticagrelor was not found to be associated with increased total rate of bleeding, but long-term treatment was associated with an increased number of episodes not related to intervention procedures [36]. Because of excellent results ticagrelor was introduced in the treatment of patients with

acute coronary syndrome and found a strong position in the current guidelines of the European Society of Cardiology for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation.

Bleeding

Bleeding is one of the major complications in treatment with antiplatelet drugs. Regardless of the adopted score describing this adverse event, there is a direct correlation between the used drug dose and the risk of death and/or major bleeding [37]. A number of clinical trials, such as ACUITY or OASIS-5, refer to this problem and associate it with a 4 times increased risk of death, 5 times increased risk of recurrent myocardial infarction, and 3 times increased risk of stroke in a 30-day follow-up [38]. The REPLACE-2 trial demonstrated that both in patients with ACS and elective PCI bleeding was an independent factor responsible for mortality in a 12-month follow-up. These trials also showed that even minor bleeding episodes have a negative effect on prognosis [39]. According to the bleeding criteria adopted in the CURE trial the risk of major bleeding in patients on clopidogrel was 2.01%, while in the placebo group the risk was estimated at 1.54% in a 30-day follow-up. At the end of follow-up (mean 8 months) the scores were 1.75% and 1.18% for clopidogrel and placebo, respectively [10]. This data suggests that clopidogrel, both in 30-day and long-term follow-up, was associated with an increased rate of major bleeding. Another important fact is that no statistically significant differences in mortality were found (0.83% vs. 0.91%). In addition, the estimated bleeding rate based on TIMI and GUSTO trials was about 2 times lower than that estimated according to the CURE criteria. In the TRITON-TIMI trial prasugrel was associated with a 32% increase in major bleeding according to the TIMI score (2.4% vs. 1.8%; HR 1.32; 95% CI 1.03–1.68; p = 0.03). This increase concerned life-threatening bleeding events, and also a significant increase in the rate of fatal bleeding. As mentioned before, treatment with prasugrel is associated with an increased rate of major bleeding in elderly patients (> 75 y.o.), patients with body weight lower than 60 kg and in those with a history of stroke. Bleeding rate was also significantly higher in the group of patients qualified for CABG (13.4% vs. 3.2%; HR 4.73; 95% CI 1.9–11.82; p < 0.001) [34]. In the PLATO trial clopidogrel was also compared with ticagrelor. There was no statistically significant difference in the rate of major bleeding in the main patient groups, both with respect to methodology adopted in the trial (11.6% vs.11.2%; p = 0.43) and the TIMI score (7.9% for ticagrelor and 7.7% for clopidogrel, P = 0.57). In addition, no differences were found in life-threatening bleeding events and fatal bleeding events (5.8% in both groups; p = 0.70). However, the incidence of major non-GABG-related bleeding was similar to that for patients on prasugrel found in the TRITON-TIMI 38 trial (4.5% vs. 3.8%, P = 0.03) and according to the TIMI score (2.8% vs. 2.2%, p = 0.03). The use of ticagrelor was associated with a higher rate of intracranial haemorrhages (0.3% vs. 0.2% P = 0.06), including fatal events (0.1% vs. 0.01%, p = 0.02) [36].

Despite differences in objectives and study protocols for the above-mentioned trials, it is clear that risk rates for the TIMI classification system with respect to standard doses of clopidogrel were similar for high doses of clopidogrel, prasugrel and ticagrelor.

When compared to clopidogrel, novel antiplatelet drugs such as prasugrel and ticagrelor elicit increased platelet inhibition and, at the same time, can contribute to an increased risk of bleeding in certain patient groups. Therefore, it would be reasonable to consider the individual risk of bleeding in patients before introducing treatment with specific antiplatelet drugs. We should also remember that the increased risk of major bleeding is associated with independent factors identified based on studies and registry data, such as older age, female sex, history of bleeding, declining baseline haemoglobin level, diabetes and renal diseases [37]. The risk of bleeding also depends on concomitant medical treatment with such drugs like oral anticoagulants, P2Y12 receptor inhibitors and GP Iib/IIIa, as well as the type of access site during angioplasty (femoral instead of transradial) [40].

Conclusions

Tests aimed at the identification of patients resistant to clopidogrel and methods of managing "tailored treatment" based on genetic and platelet function tests have not been conclusively accepted. Today, novel antiplatelet drugs are the major solution in overriding clopidogrel resistance. Considering the lack of conclusive methods for the evaluation of clopidogrel resistance and the demonstrated greater efficacy of novel antiplatelet drugs, the latest ECS guidelines reasonably recommend ticagrelor and prasugrel as the first-line drugs in ACS. Thus, is the popularity of clopidogrel coming to an end? With time this drug will probably be marginalized. A hope for clopidogrel might come from rivaroxaban – a direct factor Xa inhibitor. As shown in a revolting trial ATLAS ACS TIMI 51,

the addition of a small dose of this anticoagulant to a standard, double antiplatelet therapy was associated with the reduction of all-cause mortality and cardiovascular mortality. Head-to-head comparison with the new antiplatelet drugs needs further investigation. Today it cannot be said for sure if experience gained in using platelet tests will have a significant clinical value or just an added value as a part of objective monitoring of a patient's compliance. Currently in Poland clopidogrel remains the major drug used for this treatment because of regulations on drug refunds and cost of therapy with novel antiplatelet agents. Because of that, the identification of patients resistant to clopidogrel in order to choose adequate modified treatment for them is becoming particularly important. However, this is not an optimal solution. Recent studies demonstrated that there are more efficient and safer drugs, which are even able to reduce mortality in patients with acute coronary syndrome (e.g. ticagrelor). Treatment with such drugs should be made available to a wide group of patients in the shortest possible time.

This publication is part of the project "Wrovasc – Integrated Cardiovascular Centre", co-financed by the European Regional Development Fund, within Innovative Economy. Operational Program, 2007–2013 implemented at the Regional Specialist Hospital, Research and Development Centre in Wroclaw (Grants for Innovation).

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Address for correspondence:

Paweł Szymkiewicz
Department of Cardiology
Borowska 213
50-556 Wrocław
Poland
Tel.: 784 028 356
E-mail: pawel.szymkiewicz@gmail.com

Conflict of interest: None declared

Received: 16.08.2012
Revised: 9.04.2013
Accepted: 26.11.2013