Aspirin and clopidogrel are well established as antiplatelet medication in the treatment of atherothrombotic vascular disease. However, despite treatment, a substantial number of patients experience recurrent ischemic episodes, referred to as aspirin or clopidogrel treatment failure. Various laboratory techniques are available with which to evaluate the effectiveness of antiplatelet drugs. Interestingly, the agreement between the results of the different tests may be poor. The term aspirin or clopidogrel resistance denotes those conditions in which an inadequate inhibitory efficacy of the given antiplatelet agent is detected by an in vitro assay of platelet function. It has been estimated that on average some 30% of patients treated with aspirin, and 20% on clopidogrel, do not achieve an appropriate level of efficacy as concerns platelet activity.

**Keywords:** aspirin, clopidogrel, vascular diseases

Correspondent (levelező szerző): László VÉCSEI, MD, PhD, DSc, University of Szeged, Albert Szent-Györgyi Clinical Center, Faculty of Medicine, Neurology Department; H-6725 Szeged, Semmelweis u. 6. E-mail: vecsei@nepsy.szote.u-szeged.hu, phone: (06-62) 545-351, fax: (06-62) 545-597


www.elitmed.hu

Cardiovascular and cerebrovascular diseases rank among the leading causes of morbidity and mortality in the developed countries. Although some of the traditional risk factors can be influenced effectively, either medically (in hypertension, diabetes or hyperlipidemia) or nonmedically (smoking, obesity or lack of exercise), and besides the primary prevention, several products are available for the secondary prevention, these diseases still cause major problems worldwide. The pathophysiological basis of the complications of atherosclerotic vascular diseases involves the development of critical stenosis limiting blood flow in the affected vessel, and atherothrombotic and thromboembolic ischemic complications. The platelets play a key role in these processes. In this paper we wish to discuss at
the two most widely-used drugs that inhibit the platelet function, such as aspirin (acetylsalicylic acid) and clopidogrel, as regards their antiplatelet effect and the phenomenon of resistance to these drugs. We review current knowledge on aspirin and clopidogrel resistance and the clinical relevance of such resistance1-4.

Pathophysiology of thrombotic events

Thrombus formation is influenced by three elementary factors: the state of the vascular endothelium, rheological disturbances and hypercoagulability. Thrombus formation may have both hereditary and acquired causes. A thrombus may be occlusive or nonocclusive, and the clots dislodging from it may cause embolism. Thrombi can evolve in the arterial system, in the venous system, or in the heart, as mural thrombi. The basis of arterial thrombosis is atherosclerotic plaque or turbulent blood flow. The platelet reaction and coagulation activation play important roles in the development of arterial thrombosis. Venous thrombosis occurs mainly at stasis, and the coagulation cascade is of greater importance than the platelet reaction in this process. Pulmonary embolism and cardio- or cerebrovascular diseases are the most severe complications of atherothrombotic events5.

Platelet reaction

In primary hemostasis, a hemostatic plug is normally formed as a physiologic product subsequent to the platelet-blood vessel interaction. If this reaction is triggered by a pathological event (e.g. damaged atherosclerotic plaque), the platelet activation could lead to atherothrombotic complications. The platelet reaction involves the adhesion, secretion and aggregation of platelets. Platelet activation is regulated by various positive and negative signals and feedback mechanisms. A number of extracellular molecules, membrane receptors, membrane-associated enzymes and G-proteins, second messengers and intracellular enzymes are involved in these processes6.

As the first step of the platelet reaction, platelets bind to the subendothelial collagen via their surface glycoproteins (GPs). This collagen-induced platelet adhesion is facilitated by membrane proteins. Glycoprotein Ia-IIa (GPla-IIa) is one of the most important binding site for collagen. The polypeptides of the GP Ib-IX-V complex are members of the leucine-rich GP family. This receptor serve as an adhesion receptor by binding the platelet to the subendothelial matrix through the von Willebrand factor (vWF) (Figure 1A, B).

Inositol triphosphate (IP3) and diacylglycerol (DAG) are two of the main second messengers in platelet activation. These messengers are cleaved from the membrane proteins by phospholipase C (PLC). The formation of IP3 leads to intracellular Ca²⁺ release and hence to intracellular Ca²⁺ level regulation, while the formation of DAG, by activating protein kinase C (PKC), leads to secretion, membrane rearrangement and aggregation (Figure 2A, B). An increased intracellular Ca²⁺ level is an important factor in platelet activation. Intracellular Ca²⁺ can originate either from the intracellular tubular system (e.g. by IP3), or through the function of Ca²⁺ channels in the plasma membrane (by agonist stimulation, e.g. by thrombin, serotonin (5-HT), adenosine diphosphate (ADP) or thromboxane A₂ (TXA₂)) (Figure 3). In the activation of PKC, besides DAG, an elevated Ca²⁺ level is important. Activated PKC plays an important role in cytoskeletal reorganization. The cytoskeletal rearrangement is accompanied by rearrangement of the membrane, and domains that were previously concealed,
Simultaneously with the activation of the platelets, mechanisms inhibiting activation and aggregation are also initiated. The two main inhibitory elements in platelet activation are adenosine 3’5’-cyclic monophosphate (cAMP) and the guanosine 3’5’-cyclic monophosphate (cGMP). These elements are produced from adenosine or guanosine triphosphate (ATP or GTP) by adenylyl or guanylyl cyclase. cAMP and cGMP inhibit adhesion, secretion and aggregation too. One of the most important effects of cAMP is the antagonism of Ca2+-dependent platelet-activating reactions. Probably the most effective activator of adenylyl cyclase is prostacyclin (PGI2), which is an endothelial cell-derived eicosanoid. The nitrogen monoxide (NO) released by the endothelial cells increases the intracellular cGMP level, which also acts against activation.

One of the key steps in activation is that phospholipase A2 (PLA2) (located in the platelet membrane) becomes activated, and cleaves arachidonic acid (AA) from the membrane lipids. The AA is transformed into endoperoxides (e.g., PGH2) by COX. From these, thromboxane synthase produces TXA2.

Figure 1. Adhesion (A), TXA2 formation (B)
A: The GPIa-IIa complex on the platelet surface binds directly to the surface of the collagen fibers. The GPIb-IX-V complex binds to the collagen fibers through the vWF.
B: PLA2 becomes activated after adhesion and through the elevated intracellular Ca2+ level. It cleaves AA from the membrane lipids. The AA is transformed into endoperoxides (e.g., PGH2) by COX. From these, thromboxane synthase produces TXA2.

Figure 2. IP3 production (A), membrane rearrangement (B)
A: PLC is activated in the signal transduction system and as a result DAG and IP3 are produced. IP3 leads to Ca2+ release form the tubular system.
B: PKC activated by DAG and by the increased Ca2+ level results in cytoskeletal reorganization. Following the membrane rearrangement, hidden domains emerge to the surface, with secretion processes and exocytosis (e.g., ADP, 5-HAT and TXA2). and thus the activation is amplified.

Simultaneously with the activation of the platelets, mechanisms inhibiting activation and aggregation are also initiated. The two main inhibitory elements in platelet activation are adenosine 3’5’-cyclic monophosphate (cAMP) and the guanosine 3’5’-cyclic monophosphate (cGMP). These elements are produced from adenosine or guanosine triphosphate (ATP or GTP) by adenylyl or guanylyl cyclase. cAMP and cGMP inhibit adhesion, secretion and aggregation too. One of the most important effects of cAMP is the antagonism of Ca2+-dependent platelet-activating reactions. Probably the most effective activator of adenylyl cyclase is prostacyclin (PGI2), which is an endothelial cell-derived eicosanoid. The nitrogen monoxide (NO) released by the endothelial cells increases the intracellular cGMP level, which also acts against activation.

One of the key steps in activation is that phospholipase A2 (PLA2) (located in the platelet membrane) becomes activated, and cleaves arachidonic acid (AA) from the membrane lipids. The AA is transformed into eicosanoids through the cyclooxygenase (COX) and the lipoxygenase pathway. This results in prostaglandin H2 (PGH2) and prostaglandin G2 (PGG2); and from these elements, through thromboxane synthase, the TXA2 formed activates additional platelets (Figure 1B). TXA2 activates PLC, thereby initiating the IP3 and DAG pathway. ADP binds to the purinergic receptors of the platelets (P2X1, P2Y1 and P2Y12). The receptor P2X1 has a role in the shape change of the platelets, whereas P2Y1 is involved in early activation, and P2Y12 in long-term activation and aggregation.
The final step of the platelet reaction is platelet aggregation. As a result of the membrane rearrangement, the GPIIb-IIIa complex reaches the surface of the platelets, and takes part in a specific protein-protein interaction with fibrinogen, which makes possible the aggregation between platelets.

**Aspirin**

Aspirin and clopidogrel are currently among the most widely used antiplatelet agents. Aspirin irreversibly inhibits the COX activity of PGH synthase 1 and PGH synthase 2 (COX-1 and COX-2). It exerts a 50 to 100 times stronger effect on COX-1 than on COX-2. These enzymes produce PGH2, from the AA in the membrane of the platelet. PGH2 is the precursor of other PGs and TXA2. Aspirin blocks the AA pathway (thromboxane synthesis). Since there is no protein synthesis in platelets, the inhibition lasts for their entire life (7-10 days). It follows that almost complete inhibition can be achieved with daily regular administration, with 10% of the platelets normally functioning because of the continuous platelet exchange. Aspirin also inhibits COX-2 in nucleated cells, but these cells are capable of protein synthesis (and thus the effect is not irreversible), and the sensitivity of this to aspirin is decreased. This is the explanation for the much higher aspirin doses and the shorter dosing interval for the anti-inflammatory effect, as opposed to the platelet inhibitory effect.

The production of PGI2, which is a vasodilator and a platelet aggregation inhibitor, can originate from vascular endothelial cells by the action of COX-1 and COX-2. The COX-2 pathway is mainly aspirin-insensitive at regular doses of the drug, which may result in residual PGI2 synthesis at the daily antiplatelet doses of aspirin. The usual dose, if antiplatelet therapy is indicated, is 75 to 150 mg per day. In cases of acute indication, a loading dose can be used (150-300 mg). Aspirin is absorbed in the stomach and in the upper intestinal tract, the peak plasma concentration is reached about 30 to 40 min after administration, and the platelet inhibitory effect occurs after 1 h. The oral bioavailability of aspirin is 40 to 50%. Its circulatory half-life is 15 to 20 min. Aspirin is broken down in the plasma by hydrolysis, and yields salicylic acid. The biotransformation of salicylic acid takes place mainly in the liver. The serum half-life of salicylic acid is dose-dependent; thus, the larger the dose employed, the longer it will take to reach the steady-state. The most common adverse effects of aspirin are bleeding complications, intolerance, asthma, hepatotoxicity, rashes and renal toxicity.

**Clopidogrel**

Clopidogrel (clopidogrel hydrogensulfate) is a thienopyridine derivative, which irreversibly inhibits the ADP receptor and ADP-induced platelet aggregation. Its current oral dose as an antiplatelet agent is 75 mg per day. In accordance with the information given above about aspirin, a single daily dose results in almost complete inhibition because of the irreversible effect. Steinhubl reported that a wide range of the cytochrome P450 isoenzymes (CYPs) are responsible for the metabolism of the drug. Only a fraction of clopidogrel is metabolized by CYPs, the rest is hydrolyzed by esterases to an inactive carboxylic acid derivative and thus clopidogrel itself is not detectable in the plasma. The inhibition of the ADP-P2Y12 receptor interaction results in an increase in the level of cAMP. The inhibition of the GPIIb-IIIa receptor activation, thereby leading to the inhibition of platelet aggregation. Its possible adverse effects...
are gastrointestinal problems, bleeding complications, rash and neutropenia. Aspirin and clopidogrel are nowadays the most commonly used anti-platelet drugs.

**Clinical benefits of aspirin and clopidogrel**

Aspirin and clopidogrel are the most widely used and most extensively studied antithrombotic drugs today. Their efficacy has been proven in several large, multicentric trials:

A meta-analysis of 287 randomized antiplatelet trials by the Antithrombotic Trialists’ Collaboration documented that aspirin reduced the overall risk of serious vascular events (acute myocardial infarction, ischemic stroke, unstable or stable angina and peripheral arterial disease) in high-risk patients by 22% (odds reduction) as compared with the controls. In another respect, according to the same meta-analysis, 40 out of 1000 patients with myocardial infarction avoid a serious vascular event in the first month of their antiplatelet therapy, and another 40 patients avoid such an event during the subsequent years. The study by Johnson et al. (a meta-regression analysis of 11 randomized, placebo-controlled trials) indicated that aspirin reduced the risk of stroke by 15% (relative risk reduction) in patients with previous stroke or transient ischemic attack (TIA).

A meta-analysis of 51,342 subjects demonstrated that aspirin use in primary prevention reduced the occurrence of myocardial infarction in males and of ischemic stroke in females, thereby decreasing the overall rate of cardiovascular events. The ASA and Carotid Endarterectomy trial found that the rates of stroke, myocardial infarction and fatal vascular complications in the first 3 months after carotid endarterectomy were significantly lower if patients were taking lower doses of aspirin (81 mg or 325 mg) instead of higher doses (650 mg or 1300 mg). The side-effects of aspirin are dose-dependent, and gastrointestinal bleedings occur less frequently at a lower dose.

The CAPRIE study lead to the result that the long-term administration of clopidogrel to patients with atherosclerotic vascular disease gives rise to a significant, 8.7% relative reduction in the risk of vascular events as compared with aspirin treatment. This study showed that the long-term administration of clopidogrel was more effective than aspirin in reducing the combined vascular complications in patients with atherosclerotic vascular disease. In 2009, Berger et al. performed a meta-analysis of blinded randomized clinical trials (CURE, CREDO, CLARITY-TIMI 28, COMMIT and CHARISMA) comparing clopidogrel in combination with aspirin to aspirin alone in a total of 79,613 patients at high risk for cardiovascular events, and concluded that clopidogrel significantly reduced (a 14% proportional reduction) the risk of cardiovascular events, similarly in both sexes.

Various studies have indicated that the administration of clopidogrel in combination with aspirin reduces the mortality and morbidity rates in patients following an acute myocardial infarction. Bhattacharya et al. believes that 9-12 months of dual antiplatelet therapy without bleeding complications makes further major bleeding episodes unlikely, even if dual antiplatelet therapy is continued. Doubt has been cast in this dual therapy e.g. by the CHARISMA study, which suggested that the combination of clopidogrel and aspirin was not significantly more effective than aspirin alone in reducing the rates of myocardial infarction, stroke or death from cardiovascular causes in patients with stable cardiovascular disease or multiple cardiovascular risk factors, and that dual antiplatelet therapy should not be administered to patients without a history of established vascular disease. Reaume et al. also came to the conclusion that there is no evidence to support this combination (aspirin and clopidogrel) for the primary prevention of coronary artery disease or atherothrombotic ischemic events, the secondary prevention of stable coronary artery disease, or the prevention of cardioembolic stroke in patients with atrial fibrillation. The MATCH study concluded that dual antiplatelet therapy was accompanied by an increased risk of major bleeding, whereas there was no evidence of a significant reduction of major vascular events in high-risk patients with recent ischemic stroke or TIA. In summary, combined antiplatelet therapy has strict indications in myocardial infarction and post-stenting conditions and, since it may be associated with a higher risk of bleeding complications, a thorough consideration should always precede the administration of this combination.

**Aspirin resistance**

Aspirin is unable to prevent 81% of recurrent serious vascular events in high-risk patients, and 12.9% of high-risk patients experience a recurrent vascular event in the following two years despite taking aspirin. Vascular events that occur during aspirin treatment have various causes. Inadequate compliance (not taking the medication), an incorrect diagnosis
(not atherothrombotic vascular disease), a low dosage (therapeutic error), drug interactions (other medications that interfere with the antithrombosis effect of aspirin), different pathways (aspirin-insensitive TX biosynthesis; alternative pathways of platelet activation), increased platelet turnover, and genetic factors (platelet GP polymorphisms) may all be responsible for recurrent vascular events during aspirin therapy. Aspirin ‘treatment failure’, and aspirin ‘nonresponsiveness’ are widely-used terms. ‘Aspirin resistance’, maybe the most popular term, alludes to the occurrence of vascular events during regular and proper aspirin intake. After laboratory tests, Weber et al. classified aspirin resistance into three subtypes (pharmacokinetic, pharmacodynamic and pseudo-resistance types). The semantic complexity of this phenomenon has not yet been exactly clarified. Aspirin resistance is one of the most extensively studied problems today. It has many definitions, depending on whether it is approached from laboratory or clinical perspectives, and it describes different phenomena. Among the most commonly used definitions of aspirin resistance: Aspirin resistance means the inability of aspirin (a) to protect individuals from thrombotic complications, i.e. classical clinical aspirin resistance; (b) to reduce TXA₃ production; or (c) to produce an antiplatelet effect in one or more in vitro tests. These definitions cover almost every approach to aspirin resistance. Let us consider these definitions point by point.

(a) Among others, Eikelboom and Hankey described that several large trials had confirmed that vascular events recur in numerous vascular patients taking aspirin, and that the prevalence of aspirin resistance varies in a wide range. The pilot study by Grotemeyer et al. on 180 post-stroke patients indicated that the incidence of vascular events in patients with laboratory aspirin resistance was 40%, as compared with 4.4% in patients who responded to the drug (classical and laboratory aspirin resistance).

(b) The definition that aspirin resistance is the inability of aspirin to decrease TXA₂ production in platelets is a biochemical definition, and approaches the problem more accurately. Numerous laboratory tests can detect TX production of platelets, and its degree in vivo. Eikelboom et al. measured the level of urinary 11-dehydro-TXB₂ (a marker of in vivo TX generation) in patients treated with aspirin who had vascular events during a 5-year follow-up period, and in controls. They found that the urinary concentration of 11-dehydro-TXB₂ (its elevated level) was a possible marker of aspirin resistance.

(c) The failure of aspirin to produce an expected response in one or more laboratory measurements of platelet activation and aggregation is a laboratory diagnosis, which approaches aspirin resistance via several in vitro reactions. Gum et al. demonstrated the correlation between aspirin resistance diagnosed through optical platelet aggregometry and clinical unresponsiveness. Numerous methods are available for the measurement of aspirin resistance, and laboratories have used and still use different methods.

Despite this large amount of information, or for this very reason, a uniform definition of, and thus a clear clinical consensus on, aspirin resistance has still not been achieved because of the lack of a standardized laboratory definition and the contradictory data on prevalence.

Aspirin resistance does not work in an all-or-nothing manner, and it could change over time, both intra- and interindividually, so aspirin resistance is rather a ‘continuum’. The intraindividual changes in aspirin resistance are a result of various common factors, e.g. cigarette smoking stimulates platelet aggregation and blunts the effect of aspirin in men with coronary artery disease, or commonly used medications, such as nonsteroidal anti-inflammatory drugs (NSAIDs), e.g. ibuprofen, reduce the efficacy of aspirin.

The dose may also have an effect on the therapeutic efficacy of aspirin. A study of 468 patients with stable coronary disease found that low-dose aspirin therapy (<100 mg/day) was an independent risk factor for aspirin resistance. However, a dose of about 1000 mg/day may result in an inverse reaction.

Helgason et al. demonstrated that the antiplatelet effect of a fixed dose of aspirin is not constant over time. In their study, the extent of platelet aggregation inhibition was examined (after two weeks and approximately six months) in 306 patients with previous ischemic stroke, while increasing doses of aspirin were administered (from 325 to 1300 mg/day). 32.7% of the patients with complete inhibition at initial testing converted to partial inhibition without change in dosage on repeated testing. 67.3% of the patients with partial inhibition had a complete response at the same dosage. 22.8% of these later reverted to partial inhibition. Overall, aspirin resistance was found in 8.2% of the patients at the end of the study with 1300 mg/day aspirin administration. A study by Bozic-Mijovski et al. on 20 healthy subjects indicated that the variation in optical platelet aggregometry and closure time is significant, and has a great influence on the determination of aspirin resistance. They found that the change from aspirin-resistant to aspirin-responder or vice versa appeared.
in 25 and 30% (according to the laboratory test), and it could happen from one day to another. Harrison et al.\textsuperscript{42} studied the platelet function analyser 100 (PFA-100), the VerifyNow-ASA, and the light transmission aggregation (LTA) methods for the determination of aspirin resistance during constant aspirin therapy one year after the first testing. They found poor agreement between the three tests in identifying aspirin nonresponsiveness. Reproducibility over time was poor. Harrison et al.\textsuperscript{42} considered that these deficiencies make these tests unreliable for prediction the risk of recurrent vascular events. The latter three studies call attention to the inconsistencies of the methods used for the determination, and thus to the difficulty in assessing clinical relevance of aspirin resistance.

Several studies have been conducted on the relationship between clinical outcomes and aspirin resistance: Mueller et al.\textsuperscript{43} demonstrated a relationship between failed platelet inhibition with aspirin and the risk of vascular reocclusion in 100 patients with claudication, who had been subjected to percutaneous peripheral angioplasty. Gum et al. concluded that aspirin resistance increases the risk of major vascular events regardless of age, gender or conventional vascular risk factors\textsuperscript{34}. Eikelboom et al. also reported that aspirin resistance is an independent predictor of cardiovascular complications\textsuperscript{28}.

However, we have found that the results of optical platelet aggregometry are not good indicators of the risk of recurrent vascular events in patients taking aspirin, and conventional risk factors are more important predictors\textsuperscript{2}. In their meta-analysis of 20 studies involving a total of 2,930 patients with cardiovascular disease, Krasopoulos et al.\textsuperscript{44} reported a positive correlation between in vitro aspirin resistance and the risk of cardiovascular morbidity. We may conclude, however, that aspirin resistance is associated with an increased occurrence of vascular events.

**Clopidogrel resistance**

Clopidogrel reduces the risk of cardiovascular complications, but in some cases cardiovascular events still occur. These events may be explained by clopidogrel resistance, which predisposes patients to recurrent cardiovascular events\textsuperscript{45}. Gurbel et al.\textsuperscript{46} demonstrated that patients after percutaneous coronary intervention with subacute stent thrombosis have a higher platelet reactivity than patients without stent thrombosis, and high post-treatment platelet reactivity and incomplete P2Y\textsubscript{12} receptor inhibition are risk factors for this event.

The inhibition of platelet aggregation with clopido-
REFERENCES

37. Davis JW, Hartman CR, Lewis HD Jr, Shelton L, Eigen-


