Ph.D. THESIS

ANALYSING OF DIFFERENT SCHEMES OF CLOPIDIGREL THERAPY AFTER INTRACORONARY STENT IMPLANTATION

Tibor Szűk MD

Tutor:

Prof. István Édes MD, PhD

University of Debrecen
Institute of Cardiology

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Introduction

1. Epidemiology and importance of ischaemic heart disease

The most frequent diseases responsible for early mortality in the well-developed countries and Hungary are the cardiovascular diseases (CVD), including ischaemic heart disease. CVD is the leading cause of death worldwide, which requires more than 17,5 millon lives and some 7,6 million people die of heart attack. The late complications of acute myocardial injuries have similarly high importance as the primary events, because they basically influence the late prognosis and quality of life of patients, and mortality of the secondary diseases (chronic heart failure, left ventricular aneurysm etc.). These late complications can be prevented, as the revascularisation of the viable myocardium has been performed. In 2002, the cardiovascular diseases caused 51% of total mortality in Hungary, in 2005, the cardiovascular mortality did not change significantly, it was 52,3%.

According to WHO facts regarding Hungary in 2002, cardiovascular mortality was 616,9/100000, mortality of ischaemic heart disease was 296,5/100000, which is extremely high compared to those of the well-developed countries.

In 2005, the incidence of ischaemic heart disease among the population being older than 19 years was 8354/100000 in men and 9369/100000 in women. 8337 patients were hospitalized with a main diagnosis of acute myocardial infarction, 10,2% of which was treated with thrombolysis. The number of aortocoronary bypass grafting in ischaemic heart disease was 2230. 40092 coronarography, 14957 PCI and 13710 stent implantation were performed, these numbers could approach only the means of EU and US.

Based on these facts, improvement of the very poor mortality rates of our country is a primary question and the cardiovascular mortality has an outstanding importance. Among the most frequent and effective treatments of the already developed disease causing symptoms and potentially resulting in death are the revascularisation of the stenotic or occluded coronary artery by coronary artery bypass grafting or the ever increasing and widespread use of catheter technics.

2. Function of the platelets and the activation markers

The thrombocyte as one of the smallest particule of the blood and having no nucleus, has an important role in the development of stent thrombosis, an early and life-threatening

complication of coronary stent implantation,. The outer plasma membrane contains several transmembrane protein complexes, they mainly play a part as receptors and adhesion molecules.

The injury of the endothelium (e.g. due to rupture or erosion of the surface of the vulnerable and atheromatous plaque) and thus the contact of platelets with subendothelial structures triggers the complex adhesion and aggregation process of the thrombocytes.

The glycoprotein (GP) Ib-IX-V (CD 42) complex plays the primary role in the adhesion and aggregation of platelets, approximately 25000 complexes are situated on the surface of a single platelet. This receptor complex is connected to subendothelial structures by binding of the von Willebrand factor and also contains a binding epitope for thrombin. The platelets are activated after the adhesion, which triggers the release-reaction.

The connection of platelets with each other, and the platelet aggregation process is achieved by the GP IIb/IIIa (CD41) complex. This complex is the primary fibrinogen-binding receptor. A single platelet contains 40000-80000 complexes on its surface, the level of expression depends on the intensity of the inductor provoking aggregation.

The GP IV (CD 36 or GP IIIb) plays a role in the initial steps of collagen binding to platelets and it serves as a receptor of thrombospondin.

The role of the CD9 membrane protein is not fully elucidated, probably it has a facilitating role in adhesion and aggregation and it serves as a receptor of fibronectin.

The GP VI and the GP Ia/IIa is the receptor of collagen, the GP Ic/IIa is the receptor of fibronectin and the GP IX (CD 42a) is the receptor for vWF and thrombin, all of them are cell surface proteins. The platelets can be detected by the labelling of the GP IX during flow cytometry.

The activation of platelets can be induced by several molecules through specific membrane receptors e.g. thrombin, thromboxan A2, ADP, collagen, epinephrin and serotonin. These receptors are activating the GP IIb/IIIa complex usually through the classic G-protein mediated intracellular messenger system, so the GP IIb/IIIa receptor can bind the fibrinogen by the induced comformational change. The storing granules empty their content into the extracellular space due to Ca^{2+} released by the secunder messenger system mentioned above in response to platelet inductors and the adhesion process. This leads to significant increase of activation and aggregation. The alpha-granules contain procoagulant and adhesive proteins, like vWF, fibrinogen, fibronectin, vitronectin, V-factor etc. P-selectin (CD62) is an adhesive-protein being situated in the membrane of the alpha-granules, it plays a role in the platelet-white blood cell and other cell-cell interactions and it appears on the surface membrane

during the activation process. Therefore the appearance of P-selectin on the outer membrane of the platelets is an important marker of platelet activation. In resting state a receptor count of 500/cell, and approximately that of 25000/cell can be detected on the cell surfaceafter activation

The lisosomal proteins have also great importance, especially LAMP-3 (Lysosome Associated Membrane Protein-3). It is part of the lisosomal membrane and gets to the outer plasma membrane during the activation process similarly to P-selectin. Its appearance on the plasma membrane is probably simply the consequence of the fusion of the lisosomal and plasma membranes.

3. History of platelet aggregation inhibition and stent thrombosis

A few months after the enthusiasm caused by the first stent implantations followed the period of disillusion. The main reason of which was the high rate of acute and subacute stent occlusions and the occurance of frequent and severe bleeding complications. Acute stent thrombosis is defined by occlusion of the implanted stent within 24 hours, usually caused by deposition of the stent, while subacute stent thrombosis is characterized by thrombotic occlusion between 24 hours and 1 month after stent deployment. The reason in that latter case is usually the suboptimal level of the platelet aggregation inhibition. Among the first generation self-expandible stents early stent thrombosis rate was 24-25%, high dose aspirin and unfractionated heparin was applied to treat the complication. In the early 90s, aggressive antithrombotic therapy was introduced, which contained aspirin, unfractionated heparin, dextrane and dipyridamol and, later warfarine could be added. At those times, the stent thrombosis rate decreased to 3,4-5,4%, but the frequency of bleeding complications increased to an unacceptably high degree, 5-15%. Based on the activity of Antonio Colombo, the concept of optimal stent deployment was introduced, according to which symmetrical complete expansion of the stent can be acheived by application of high deployment pressures up to 14-22 bar. This concept significantly decreased the rate of acute stent thrombosis, principally. In 1995-96, TASTE, French Registry, ISAR, FANTASTIC and STARS trials proved that combination of aspirin and ticlopidine dramatically decreased the rate of subacute stent thrombosis, thus the prevalence decreased to 0,4-2,8%, nevertheless, bleeding complications were also minimalized to 0,5%. The CLASSICS trial published in 2001 showed that combination of aspirin and another ADP-receptor antagonist, clopidogrel is as effective as the earlier aspirin+ticlopidine combination in lowering of stent thrombosis rates (clopidogrel: 3,9%, ticlopidine:4,6%, P: not significant) However, the side effects were less

frequent and the onset of effect was quicker. The CREDO study published in 2003 indicated, that clopidogrel loading (300 mg) is more effective when applied 3-6 hours before intervention because it decreases the occurance of subacute stent thrombosis at a degree being close to significant when compared to clopidogrel administration without applying a loading dose. The frequency of stent thrombosis varies between 1,3-6,47%, as several trials showed.

4. The mode of action of ASA and clopidogrel

ASA:, acetylsalicic acid, the active compound of aspirin, irreversibly inhibits the 1st subtype (COX-1) of the cyclooxigenase enzyme in megakaryocytes and platelets by the acetylation of serin in the active centre of the enzyme. COX-1 is responsible for the oxidation of arachidonic acid, then unstable prostaglandin endoperoxids (PGG_2, PGH_2) arise from arachidonic acid. PGH_2 transforms into the strong aggregation inductor and vasoconstrictive thromboxane A_2 (TX A_2). by the thromboxane synthase. ASA is absorbed quickly, it reaches its maximum plasma level in 15-20 minutes, but the inhibitory effect on platelet aggregation prevails during the whole lifespan of the platelet, that means approximately 10 days.

Clopidogrel: the thienopyridine-derivate clopidogrel is absorbed quickly from the gastrointestinal tract, than transforms into an active metabolite by the hepatic cytochrome P450 (CYP) 3A4 enzyme. The onset of inhibitory effect on aggregation depends on the starting dose: in case of 75 mg it is 4-7 days, in case of 300-600 mg loading doses, it is 3-24 hours. The drug irreversibly inhibits the $P2Y_{12}$ receptor, a subtype of the ADP-receptors of the platelet membrane. The platelet surface contains approximately 800-1000 ADP-receptors. The agonist of the purinergic P1-receptors is the adenosisn, that of the P2-receptors are the ATP and ADP. The P2Y receptor family exerts its intracellular effect through the G_i-protein, the $P2Y_{12}$ subtype inhibits the adenyl-cyclase enzyme, which transforms ATP into cAMP and this intracellular signalling leads to Ca^{++}-liberation, cytoskeletal changes, GP IIb/IIIa expression and release reaction. Besides, G_i-protein activates the PI3K (phosphatidilinositol 3-kinase) enzyme and the potassium chanels and also activates the fibrinogen receptor through unknown signalisation pathways. The PI3K phosphorilates the 3-hydroxyl group of the inositol ring of phosphatidilinositol and activates the GPIIb/IIIa receptor through the RAP1B GTPase and AKT/PKC (proteinkinase C) enzymes.

5. Measuring of ASA and clopidogrel nonresponsiveness, current guidelines

Drug resistance, which plays an unequivocal role in stent thrombosis, is a target of widespread scientific research. The rate of ASA resistance is 24%, the frequency of clopidogrel resistance is about 21%. The definition of nonresponsivesess is not clear either, since it can mean clinical ineffectiveness, sudden thrombotic event under the effect of the normal dose of drug or it can even mean the result of one particular laboratory test. Several methods are used for detecting resistant patients by objective laboratory tests, eg. the most reliable aggregometry, PFA-100 equipment, measurement of degradation products of ASA metabolism such as measuring TXAB2 levels from urine or the VASP (vasodilator stimulated phosphoprotein) phosphorilation of platelets, which correlates well with the rate of bindings of the GPIIb/IIIa receptor to fibrinogen. 25-54% of patients show laboratory nonresponsiveness. 8% of these patients have complete nonresponsiveness, which can not be improved by applying even higher doses of drugs.

Only fewer facts are available about clopidogrel nonresponsiveness. The most reliable laboratory test is again aggregometry or measurement of urine cAMP-level and VASP phophorilation, the role of PFA-100 is contradictory. According to metaanalysis of several studies, nonresponsiveness after a 300 mg loading dose is about 20%, but as individual trials show, it can vary between 25 and 6%.

Scientific literatural facts show that clopidogrel nonresponsiveness is more frequent among patients with ASA resistance, than among those in the normal population. 47,4% of ASA resistant patients has poor reactivity to clopidogrel, either. This patient population is in extreme danger of thrombotic complications, as none of the members of the generally used antiplatelet combinations have satisfactory effect.

The recent international guidelines suggest to give 300-500 mg ASA orally 2-3 hours before an elective coronary stent implantation. 300 mg of clopidogrel has to be administered at least 6 hours before the procedure, if it is not possible, a 600 mg loading dose has to be given. The clinical benefit to administer doses higher than 300 mg is not clearly established, the ongoing CURRENT-OASIS-7 study is expected to clear this question unequivocally. According to the current practice, the patients are frequently not pretreated simultaneously with both antipaletelet drugs, especially clopidogrel, because: 1. the urgent procedures performed in unstable conditions are frequent, 2. after clopidogrel loading the possibility of a severe bleeding complication is high during a potentially urgent heart surgical treatment, 3. the cost of the drug is also high. The risk of heart surgery under clopidogrel effect is extremely high, especially when the patient was administered the drug within 48 hours of

surgery. The risk of inhospital mortality, severe arrhytmias and reintubation, and the need of postoperative balloon counterpulsation is markedly higher in patients with clopidogrel therapy before the surgery. The real danger of this risk is established in case of the coronary interventions which always carry the possibility of an urgent heart surgery.

So, in recent clinical practice, clopidogrel loading having been performed at the time of stent implantation is more frequent than it should be reasonable according to current guidelines. A question arises from these facts, namely to what extent this practice threatens our patients' lives, increases the rate of complications, especially that of stent thrombosis compared to pretreatment?

Aims

1. To evaluate the effectivity of different clopidogrel loading strategies (pretreatment vs. „ad hoc" loading) by prospective clinical data collection.
2. To analyse the safety of the different loading schemes.
3. To compare our results with the international guidelines.
4. To assess the influence of different clopidogrel loading strategies on activation of different platelet markers after stent implantation.
5. To detect the most sensitive activation markers of clopidogrel.
6. To evaluate, if in vitro marker tests can detect the clinical effectivity of clopidogrel.
7. To assess the influence of ASA nonresponsiveness on activation markers.

Patients and methods

Clinical study design

In the prespecified time interval, between March 2002 and February 2004, all consecutive patients with bare metal (any of the commercially available noncoated stainless steel) stent implantations were prospectively entered in the Clopidogrel Registry at the Institute of Cardiology, University of Debrecen, Hungary (study initiating and coordinating center). All patient data were stored and analysized in an institution being independent on the centers performing the enrollment, and where the applied therapeutic protocol of the participating individuals was unknown. The participating centers were the following: the

Departments of Cardiology and Emergency Medicine, University of Vienna, Austria, and the 3rd Department of Medicine (Cardiology and Emergency Medicine), Wilhelminenhospital, Vienna, Austria.

Complete medical history was recorded for all patients. The patients eligible for entry into the registry were clinically controlled at 1 month after stent implantation.

Stent implantation was performed via 6F sheath and guiding catheter from femoral approach in accordance with the recent clinical protocols.

Venous blood samples for analyses of serum creatine kinase (CK) and its myocardial fraction (CK-MB) were obtained immediately before diagnostic angiography and 8 to 12 hours after cessation of the coronary procedure. Serum levels of CK (normal, ≤80 U/L) and CK-MB (normal, ≤10.0 U/L) were determined by use of standard routine laboratory tests.

Inclusion criteria

1. presence of stable angina (SA), unstable angina or non–ST-segment elevation acute myocardial infarction (UA/NSTEMI, defined in accordance with the current guidelines)
2. coronary intervention with bare metal stent implantation
3. treatment with clopidogrel either 6 to 24 hours before coronary intervention or immediately after stent implantation

In accordance with the nature of the registry, patients with chronic total coronary occlusion or a partially decreased coronary blood flow (TIMI grade <3) and patients with multivessel stenting or stenting of left main or major bifurcations, vein or mammary grafts were also included.

Exclusion criteria

1. permanent treatment with a clopidogrel maintenance dose of 75 mg due to high risk of acute or subacute stent thrombosis or previously documented stent thrombosis
2. pretreatment with clopidogrel loading dose more than 24 hours or less than 6 hours (n = 253),
3. clinical or laboratory signs of an ST-segment elevation acute myocardial infarction (AMI)
4. contraindication of antithrombotic or antiplatelet therapy

5. high risk of bleeding

6. use of glycoprotein (GP) IIb/IIIa inhibitors within 48 hours before PCI (n = 187) in patients with UA/ NSTEMI

7. implantation of drug-eluting stents (DES)

8. no coronary stenting with or without clopidogrel pretreatment for any reason (no significant coronary stenosis, no possibility for PCI, and decision to perform urgent coronary bypass surgery).

Patients

Data on a total of 4160 patients were analyzed. The patients were divided into 2 groups: group 1 consisted of patients (n = 2679) who received a loading dose of clopidogrel (300 mg) immediately after stenting in the catheterization laboratory, and group 2 consisted of patients (n = 1481) who received clopidogrel (loading dose, 300 mg) 6 to 24 hours

before the intervention.

Intravenous unfractionated heparin was administered to all patients during the invasive procedure to achieve an activated clotting time between 250 and 300 seconds. Heparin was usually discontinued at the end of the procedure. Stent implantations were carried out by experienced invasive cardiologists who perform more than 300 procedures per year. Provisional GPIIb-IIIa inhibition (with an agent selected by the operator) could be provided during PCI at any time at the discretion of the operator in case of abrupt closure, flow limiting dissection, side-branch closure, distal embolization, slow flow or any other clinical or angiographic instability and bailout conditions. After stent implantation, all patients received a daily maintenance dose of 75 mg clopidogrel for at least 1 month. All patients received aspirin (100 mg) daily at least one day before and continuously after the intervention.

The study was approved by the institutional ethical committee on human research in each institution, and written informed consent was obtained from all patients.

End points

The primary 30-day triple composite end point of the prospective multicenter Clopidogrel Registry was the composite of AMI, all-cause death, and urgent repeated TVR.

The secondary end points were broken down into primary outcome events (all-cause death, AMI, and TVR), angiographically proven stent thrombosis, major bleeding (safety end point), and need for procedural GPIIb-IIIIa inhibition.

The prespecified secondary analysis included the assessment of the primary end points in patients with SA and UA/NSTEMI separately.

Definitions

- Death was defined as all-cause death
- The AMI end point was determined as evidence of typical chest pain with new significant Q waves in ≥2 contiguous electrocardiographic leads, or CK/CKMB elevation ≥3 times above the upper limit of normal
- TVR was regarded as any repeated PCI of the previously stented artery after entry into the registry
- Major bleeding was defined as clinically significant hemorrhage (intracranial, intraocular, retroperitoneal, or with hemoglobin level decrease of >4 g/dL), or overt bleeding with haemoglobin level decrease of >3 g/dL, or necessity of transfusion of ≥2 U of blood
- Stent thrombosis was diagnosed if the stent was thrombotically occluded within 30 days after stenting (either acute or subacute stent thrombosis, within the first 24 hours or between 24 hours and 30 days after stenting, respectively)

Platelet aggregometry

In 28 randomly chosen pateients („ad hoc" loaded patients n=19, pretreated patients n=9) optical aggregometry was performed. We used Carat TX (Entec Gmbh, Ilmenau, Germany) 4 channel, computer controlled aggregometer. 10 ml (9/10 part blood, 1/10 part 3,3% sodium-citrate) mix was used. We performed the measurement within 2 hours after the blood sampling. We got platelet rich plasma (PPR) with centrifugation on 150 g for 10 minutes and the platelet poor plasma (PPP) with centrifugation on 2000g for 10 minutes. The platelet number of PRP was adjusted to 250-350 G/L. The platelet number of PPP had to be

below 10 G/L. We used the inductor kit of Theracont TE-3 (Carat Diagnostic Gmbh, Budapest) for reagent, which contains 5 mM ADP as inductor solution. The compound of the system was: 0,45 ml PRP + 0,05 ml aggregator solution. The PPP was used for the calibration before the examination. The principal of the measurement was the method described by Born, optical aggregometry with infrared detection. Hundred percent was the optical density of PPP, zero percent was the optical density of PRP. The aggregation curve showes the change in light transparency of the PRP in point of the aggregation inhibition after applying the inductor. The greater the rate of aggregation, the greater the amount of transmitted light. The measurement is followed for 8 minutes. The results were expressed in relative percent.

Investigation of platelet markers

Forty patients undergoing elective coronary stent implantation were studied to evaluate the efficacy of different clopidogrel treatment regimens. Exclusion criteria were the followings: acute coronary syndrome within 48 hours, presence of coagulopathies, drug or alcohol abuse, serious liver or kidney disorders, platelet count <100 G/l, stroke within 3 months, previous surgical treatment within 1 month, treatment with GPIIb/IIIa antagonist or acenocoumarol, participation in other investigational drug trials.Two similarly designed stent types were used to get homogenous patient populations in this respect: Medtronic S660 and S670. Optiray 350 non-ionic contrast media was used in all patients. Patients were split into two groups: group 1. included 20 subjects, who were not pretreated with clopidogrel, but were given a 300 mg loading-dose at the time of implantation (loading group), while 20 patients in group 2. were pretreated with 300 mg clopidogrel at least 6 hours prior to the intervention (pretreated group). Heparin and clopidogrel therapy was continued in accordance with the clinical study, ASA administration was started minimum 7 days before the intervention. Blood samples from 61 cardiac symptom free, age and sex-matched control subjects with normal ECG and echocardiography were used in the study.

Venous blood was drawn from patients in the following time points: immediately at the time of stent implantation and at 1, 2, 4, and 24 hours. All blood samples were obtained from the antecubital vein via a 21-gauge needle directly into vacutainer tubes containing 0.105M sodium citrate (Becton Dickinson, San Jose, CA) with minimal venous stasis. Blood sampling conditions were designed to avoid artefactual activation of platelets during phlebotomy. Within 2 hours of collection, 40 µl of all samples were fixed in 1 ml 1 % paraformaldehyde and kept at room temperature (RT) for minimum 1 hour. Platelet count was

determined in each case by Advia 120 Hematology System (Bayer Diagnostics, Tarrytown, NJ, USA). Fixed whole blood samples were centrifuged at 1300 g for 15 minutes at RT. The pellet was washed in 1 ml of phosphate-buffered saline (PBS), then centrifuged as above and finally resuspended in PBS.

Platelets were identified by a FITC-conjugated monoclonal antibody to GPIX (CD42a). Platelet activation was detected by phycoerythrin (PE)-labeled anti-P-selectin (CD62-PE, Becton Dickinson, San Jose, CA), by Lysosome Associated Membrane Protein (CD63-PE, Immonotech, France) and by anti-GPIIb/IIIa (CD41-PE, Dako, Glostrup, Denmark). Fixed platelets (40 μl) were incubated with saturating concentrations of FITC- and PE-labeled antibodies for 20 minutes in the dark at RT. 10 000 dual-color labeled platelet events were acquired on a FACSCalibur flow cytometer by using the CELLQuest 3.1 software (Becton Dickinson, San Jose, CA). Results were expressed as percentage of double positive platelets as well as by providing the fluorescence intensity values. Estimation of the number of GPIIb/IIIa receptors was based upon the mean fluorescence intensity (MFI) of anti-CD41 (PE) labelling of platelets.

Statistics

In our clinical study the sample size calculation was based on the previous meta-analysis of Bhatt et al, including 10 studies with different timings of the loading dose of clopidogrel. The sample size calculation was based on the following assumptions: 1. presence of an absolute incidence of the composite end point in group 1 or 2 of at least 2% (based on a previous meta-analysis of Bhatt et al) and 2. probability of 0,80 of detecting a decrease of 30-day primary end point events from 4,04% to 2,1%.

The sample size calculation using the formula for comparing 2 proportions with a 1:2 allocation ratio (the size of group 1 was expected to be larger than that of group 2, according to the local institutional PCI

standard therapy in the inclusion period), with 5% of the participants lost to follow-up, resulted in a sample size of 2030 in group 1 and 1015 in group 2.

Primary and secondary end points

The assessment of primary and secondary end points was based on a comparison

between the 2 treatment groups by means of chi^2 tests. The absolute risk, the differences between the absolute risks, and the 95% CIs of the differences were calculated. Continuous variables are presented as means ± SD, and an intergroup comparison was performed with the unpaired t tests.

In the prespecified subgroup analysis of the separate patient collectives of SA and UA/NSTEMI, the absolute risk reduction was calculated only for primary end point, as the number of patients in the subgroups did not fully reach the sample size required for subgroup analyses to be reliable.

A Cox proportional hazard model was used to examine the association between the 2 therapeutic regimens and the 30-day triple primary end point. The assumption of proportional hazards was assessed graphically by comparing the hazard ratios by log-rank test. Logistic regression analysis was carried out to identify independent correlates for the 30-day primary end point. Statistical analysis was carried out with the SAS program (version 8.1, SAS Institute, Cary, NC).

Platelet marker studies

In the case of investigation of platelet markers, continuous variables were presented as mean ± standard deviation. Categorical variables were presented as percentages and compared using chi-square test. Kolmogorov-Smirnov test was used for the evaluation of normality of the data. Student′s unpaired t-test by two-tailed analysis was used for comparison of independent variables between groups and paired t-test was used to compare differences in a given parameter before and after the intervention. Analysis was performed by using SPSS 13. software program. A P value of <0,05 was considered to be significant.

Results

Baseline clinical and angiographic data, aggregometry

Except for the higher proportion of the patients with SA in group 1 and UA/NSTEMI in group 2, no significant difference was found between the 2 groups as regards the baseline clinical and lesion characteristics and the coronary intervention data. The mean time of clopidogrel pretreatment was 18 ± 4 hours.

The ADP-induced aggregation in the „ad hoc" loaded group did not differ significantly 4 hours after the administration of the loading dose from the pretreated patients, investigated in 28, randomly choosed patients for aggregometry. After 6 hours, the two aggregation curve were completely similar.

Outcomes (end points)

Pretreatment with clopidogrel 6 to 24 hours before coronary stenting was significantly more effective in reducing the primary triple composite end points; the composite of all-cause death, AMI, and urgent TVR occurred in 127 (4,74%) of the 2679 patients in group 1 and in 41 (2,77%) of the 1481 patients in group 2 (difference in absolute risk 1,97, 95% CI 0,81-3.13, P = 0,002). The primary end point events occurred mostly in the first few days in both groups.

Among the secondary end point events, the incidence of AMI (2,99% vs 1,76%, P =0,018) was lower in group 2, whereas the all-cause death and urgent TVR were only slightly less frequent in group 2. Although group 2 consisted of more patients with UA/NSTEMI, stent thrombosis was significantly less common after pretreatment with 300 mg clopidogrel 6 to 24 hours before stenting (2,09% vs. 1,08%, P = 0,018). A trend toward a reduced need for procedural GPIIb-IIIa inhibition was also observed in group 2. In contrast, the safety end point, major bleeding, was recorded significantly more frequently in group 2 (0,41% vs 1,35%, P = 0,001, in group 1 vs 2). Logistic regression analysis indicated treatment with clopidogrel loading dose at the time of coronary stenting (P < 0,001), UA/NSTEMI (P = 0,001), age (P = 0,017), and higher number of implanted stents (P = 0,004) as significant independent predictors for the occurrence of the primary end point.

Primary end points and cardiac events in patients with SA or UA/NSTEMI

The indication of coronary angiography was SA in 2598 (62,5%) patients and UA/NSTEMI in 1562 (37,5%) patients. No differences in baseline variables were found between the patients with SA or UA/NSTEMI in groups 1 and 2. The mean time of clopidogrel pretreatment was 21 ± 3 hours in group 2 patients with SA, and 16 ± 5 hours in group 2 patients with UA/NSTEMI. Clopidogrel pretreatment 6 to 24 hours before coronary stenting resulted in a significant decrease of the combined primary end point events both in patients with SA (4,04% vs 2,38%, difference in absolute risk 1,66, 95% CI 0,28-3,04,

P =0,.031) and in those with UA/NSTEMI (6,08% vs 3,28%, difference in absolute risk 2,80, 95% CI 0,74-4,87, P = 0,012) in groups 1 and 2, respectively. In patients with UA/NSTEMI, the outcome events were considerably more frequent than in the
patients with SA.

In patients with SA, a trend toward higher frequency of AMI (2,5% vs 1,55%, P = 0,152), all-cause death (0,57% vs 0,24%, P = 0,358), urgent TVR (0,97% vs 0,6%, P = 0,492), stent thrombosis (1,54% vs 0,71%, P = 0,092), and need of procedural GPIIb-IIIa inhibition (8,7% vs 6,55%, P = 0,064) was observed in group 1 compared with group 2. In contrast, pretreatment was associated with significantly more major bleeding events (0,46% vs 1,19%, P = 0,043, in groups 1 vs 2).

In patients with UA/NSTEMI, the pretreatment with clopidogrel 6 to 24 hours before coronary stenting decreased the occurrence of AMI within the first 30 days significantly (3,91% vs 2,03%, P = 0,039, in groups 1 and 2). A trend toward a clinical benefit of the pretreatment mode was documented in the incidences of stent thrombosis (3,04% vs 1,56%, P = 0,067), all-cause death (0,87% vs 0,62%, P = 0,771), urgent TVR (1,3% vs 0,62%, P = 0,213), and need for procedural GPIIb-IIIa inhibition (13,25% vs 11,86%, P = 0,44) in groups 1 vs 2, respectively. In contrast, pretreatment was associated with significantly more frequent major bleeding (0,34% vs 1,56%, P = 0,01, in groups 1 and 2).

Platelet marker studies

Twenty patients were studied in both groups. Demographic, interventional and medication characteristics were comparable. Hypertension, diabetes mellitus, previous MI and tobacco use were common in both groups. A single stent was usually used in all study patients and the features of stents were comparable. There was a slightly higher rate of three vessel disease in group 2. The most frequent target vessel was the left anterior descending coronary artery (LAD) in group 1 and the right coronary artery (RCA) in group 2. Haematological parameters, creatine kinase values, liver and renal function tests and serum lipid characteristics did not differ significantly in the study groups. The distribution of medications was similar with slight differences. No patient received GPIIb/IIIa receptor inhibitors. One patient suffered subacute stent thrombosis with non-Q wave myocardial infarction in group 2, and none of them in group 1. Q wave myocardial infarction and death did not occur in either group within 30 days after stent implantation. The two groups were highly homogenous and

comparable, since no significant difference could be observed in any of the investigated baseline characteristics.

Receptor expression

The expression of platelet granule proteins were significantly elevated immediately after stent implantation compared to 24 hour values in group 1. This emphasizes that stent implantation is a remarkable platelet activating procedure despite aspirin treatment.

CD62. P-selectin expression was inhibited by clopidogrel pretreatment at the time of stent implantation as shown in group 2. In the loading group, the expression of P-selectin was significantly higher at this time point. After 1 hour, the expression of CD62 was markedly inhibited by clopidogrel loading, the inhibition was similar and did not differ from that of group 2. At subsequent time points, the expression levels were similar in both groups and in the loading group were significantly decreased at all time points compared with the baseline. In contrast, no significant change was noted in the pretreatment group during time course.

CD63. The lysosome associated antigen expression was similar at baseline in the two groups. In the pretreated group the surface exposure of CD63 remained basically unchanged. In contrast, in the loading group the expression significantly decreased after 1 hour and remained diminished compared with the baseline value. However, the difference between the patient groups were not significant at any sampling time.

CD41. The number of GPIIb/IIIa receptors on the surface of platelets at baseline was higher in group B, but the difference between groups was not significant. Moreover, at 1 hour, the MFI value was also reduced significantly in the pretreated group. At subsequent sampling times the receptor expressions were slightly, but significantly decreased in both groups. There was no difference in the values between the groups of patients at any investigated time point.

CD42a. The expression of GPIX was not affected by clopidogrel in either group and there was no difference in the MFI values between the study groups at any sampling times.

Platelet count

The number of circulating platelets was similar in the two groups and was in the reference range at all sampling times. At 4 and 24 hours the platelet counts were slightly, but significantly increased compared to the baseline in the loading group. No change was noted in the clopidogrel pretreated group over time.

Aspirin non-responder patients

Baseline data from both groups were pooled together and platelet activation markers were compared in aspirin responder and non-responder patient groups. The cut-off point for aspirin resistance was defined at below 250 seconds closure time for the collagen-epinephrine cartridge as measured by PFA-100. There was no significant difference in any of the studied receptor expressions at baseline, nor in platelet count between the aspirin resistant and responsive patients in both study groups.

Discussion

Intracoronary stenting has a widespread application in the management of ischaemic heart disease, which plays a significant role in the early mortality of the active population, thereby ischaemic heart disease is regarded as a population disease. What concerns the supplementary drug treatment of intracoronary stenting, both the European and American cardiological associations have their own therapeutic guidelines. Regarding clopidogrel treatment, data of the CREDO study having been published in 2003 represent the standard. Based on these data, clopidogrel, which is applied in order to effectively reduce the risk of premature thrombotic occlusion of stents, is necessary to be administered possibly at least 6 hours before the planned procedure at a loading dose of 300 mg. If clopidogrel loading takes place at an earlier time than previously mentioned related to the time of the procedure, the recommendations of the two guidelines slightly diverge from each other. The European guideline suggests administration of double dose clopidogrel, 600 mg, while the American guideline regards administration of the 600 mg dose 2 hours prior to stent implantation as clinically well-founded. However, it does not consider the advantages of the applied higher doses as unambiguous and it recommends administration of glycoprotein IIb/IIIa receptor blockers until build-up of clopidogrel effect. Nevertheless, based on the experience of cardiological centers performing these ordinary procedures in great numbers, the application of clopidogrel loading dose very often takes place at the same time of the procedure. The primary cause of this observation is the ever increasing prevalence of stent implantations performed as an emergency. In this case, the necessary 6 hours before the procedure to administer the loading dose is not available. Moreover, the possibility of a potential urgent-

immediate heart surgery always may emerge, mainly in the patient population of the old with plenty of comorbidities. In that latter case, clopidogrel pretreatment involves the significant risk of bleeding, thus increasing perioperative mortality. According to these facts, the real everyday practice and current therapeutic recommendations do not often overlap with each other, and the suggested recommendations may not be entirely followed due to actual problems and clinical practice.

Other working groups generally tested patients whose clopidogrel loading dose had been administered at least 3 hours prior to stent implantation, or clopidogrel maintenance doses of 75 mg were applied at the same time of the procedure. The multicentric Clopidogrel Registry having been created by us was to compare the efficacy and safety of the two clopidogrel loading treatment schemes (pretreatment with a loading dose of 300 mg 6-24 hours prior to stenting, or loading with a dose of 300 mg at the time of stenting), those having been applied parallerly depending on the clinical circumstances and necessities on a varied patient population with a large case number and being consistent with the everyday clinical practice. Results of the Clopidogrel Registry confirmed the clinical advantages of 300 mg clopidogrel pretreatment in respect to adverse events presenting within the first 30 days over patients being in the non-pretreated group. The primary 30-day clinical end point of the trial consisted of a composite endpoint of myocardial infarction, death and urgent revascularisation, respectively. Reviewing data of subgroup of patients with stable or unstable angina or non ST-segment elevation myocardial infarction separately, the same results could be gained, too. What regards secondary endpoints, the prevalence of myocardial infarction and subacute stent thrombosis was significantly higher among patients non-pretreated with clopidogrel. However, the difference in absolute value between end point events of the two patient groups is minimal, it ranged between 1.97 (combined primary endpoint) and 1.01 (stent thrombosis) %. The price of this minimal advantage was the increased prevalence of major bleeding events (safety end point) in the pretreated patient group, the difference of absolute risks is 0.94 %. Largely similar results were observed in the TRITON-TIMI-38 trial having been published recently. Based on the results of the trial, the new generation ADP-receptor blocker prasugrel decreased the number of ischaemic events (9,9 vs. 12,1 %) during the 6-15 month follow-up period following stent implantation in patients with acute coronary syndrome compared to clopidogrel, also including stent thrombosis (1,1 vs. 2,4%). However, the frequency of significant, occasionally fatal bleeding events parallerly increased (1,4 vs. 0,9%, illetve 0,4 vs. 0,1%).

A possible advantage of clopidogrel loading at least 6 hours prior to the planned coronary intervention can be the prevention of early thrombotic occlusion of the stented coronary artery within 2-6 hours after the intervention. Within this timeframe full-dose unfractionated heparin being applied during the intervention has antiocoagulant effect primarily. Nevertheless, it has only moderate effect on platelet aggregation by the inhibition of thrombin. Moreover, enhanced platelet activity can be observed among patients having had stent implantation. Similarly to other authors, we made the observation that prevalence of acute and subacute stent thrombosis is highest during the first few days following coronary intervention and the majority of adverse events took place also during the first few days.

Unequivocally preferential trend could be observed for the favour of pretreated patients in comparison with patients with „ad hoc" loading on analyzing secondary endpoint events. This observation was made in spite of the fact that the number of patients having unstable angina or non-ST-segment elevation myocardial infarction was significantly higher in the previous patient group. Difference of significant degree was only reached in subgroup of patients with acute myocardial infarction and stent thrombosis. The non-significant advantage of clopidogrel pretreatment in comparison with „ad hoc" loading in terms of death and urgent revascularisation may correlate with the time of clopidogrel loading or mode of its administration.

Based on the results of pharmacokinetic studies and CREDO trial, we excluded patients from our trial to whom clopidogrel loading dose was administered within less than 6 hours related to the time of coronary intervention on account of the indeterminate effect of clopidogrel. The average application time of loading doses during clopidogrel pretreatment proved to be 18±4 hours pre-PCI in our trial, which is identical with the result of the subgroup analysis in the CREDO trial.

Loading treatment administered at least 6 hours prior to PCI guarantees to reach the best and fastest therapeutic effect, especially in patients with unstable angina or non-ST-segment elevation myocardial infarction. Thus, administration of 600 mg loading dose is recommended as a solution to this problem. In vitro platelet function tests indicated nearly complete inhibition of platelet aggregation 2 hours after administration of the 600 mg doses. The 600 mg loading dose proved to be more effective than that of 300 mg in terms of inhibition of aggregation in patient groups of unstable angina/non-ST-segment elevation myocardial infarction. It also increased the proportion of patients responding adequately to clopidogrel and reduced the rate of non-responders in patients having high platelet reactivity post-PCI. The ARMYDA-2 trial verified the clinical advantage of applying a loading dose of

600 mg 6 hours prior to PCI compared to a dose of 300 mg. No further clinical advantages were observed on analysis of 30-day events occuring at loading doses having been administered more than 2-3 hours pre-PCI. These data were obtained in the ISAR REACT trial in patients having low or moderate cardiovascular risk (the patients were randomized into subgroups based on time of administration of 600 mg loading dose prior to PCI: 2-3, 3-6, 6-12 and more than 12 hours pre-PCI). These data confirmed the complete inhibitory effect of the 600 mg dose observed 2 hours after its administration.

Based on latest recommendations of ESC and ACC/AHA, and following update of the latter recommendation, administration of a clopidogrel loading dose of 300 mg is suggested at least 6 hours prior to stent implantation in patients with stable angina (class I. indication, „B" level of evidence). In fact, immediate clopidogrel loading (ESC: 600 mg, ACC/AHA: 300 or 600 mg, the exact mode of administration is yet to be clarified) is proposed for patients having unstable angina or non-ST-segment elevation myocardial infarction (class I. indication, „C" level of evidence). Our registry demonstrated that clopidogrel loading treatment having been administered 6-24 hours prior to coronary stenting significantly decreased the occurance of the primary combined end point both in patients with stable angina and those suffering from unstable angina or non-ST-segment elevation myocardial infarction. The advantage of pretreatment was more pronounced among patients having unstable angina/non-ST-segment elevation myocardial infarction, since the loading dose of 300 mg having been administered 16±5 hours prior to PCI on average led to significant reduction of events including myocardial infarction and stent thrombosis during the 30-day follow-up.

However, the clinical advantages of clopidogrel pretreatment can be achieved in price of having higher rates of bleeding complications among patients receiving dual platelet antiaggregation treatment, especially when use of GP IIb/IIIa blockers is necessitated during stent implantation or when urgent heart surgery is indicated. One trial indicated a 5-fold increase in prevalence of bleeding complications among patients having had heart surgery who were administered clopidogrel treatment within 5-7 days prior to surgery. It was demonstrated in another trial that the risk of heart surgery having been performed in clopidogrel effect was outstandingly high, especially when the drug was administered for the patients within 48 hours before surgery. In-hospital mortality, need of postoperative ballon counterpulsation and possibility of severe arrhytmia, stroke and reintubation is markedly higher among patients receiving clopidogrel treatment preoperatively in comparison with heparin or aspirin administration. On the contrary, Cannon et al could observe only

moderately increased risk of bleeding during heart surgery in patients having received dual antiplatelet treatment. Nevertheless, they experienced a favourable effect on early graft occlusion and on incidence of perioperative stroke. Regarding our trial, the 300 mg loading dose having been administered 6-24 hour pre-PCI significantly increased the risk of bleeding complications among our patients and also in the individual patient subgroups. To evaluate the prognostic significance of these data requires further investigations.

The major limitation of the Clopidogrel Registry was its non-randomized modality, since the selection of proper clopidogrel loading time was an individual decision of the treating physician on each occasion. However, this limitation proves to be the merit of the registry at the same time, while, arising from the nature of the registry, patients had been included from a non-selected and non-reduced patient pool representing a wide spectrum of patient types (stable angina, unstable angina or non-ST-segment elevation myocardial infarction) and coronary lesions. Therefore, the registry well illustrates the real character of everyday interventions. There is another limiting factor, namely patients having had drug-eluting stent (DES) implantation had not been randomized into the trial, since this stent type had not had a well-defined place in the everyday clinical practice and their use had well been below 50 % in the majority of the interventional centers on onset of the registry.

In summary, based on the Clopidogrel Registry, pretreatment using a loading dose of 300 mg and having been administered 6-24 hours prior to the planned PCI has minimal effectivity over loading treatment having been applied at time of the procedure, this mild superiority is gained in price of higher prevalence of drug-induced bleeding events. Our results coincide with findings of the TRITON TIMI-38 trial having been published recently, which compared the efficacy and safety of prasugrel related to clopidogrel. Both in vitro data and marked inhibition of platelet aggregation experienced 4 hours after loading dose administration and observed during optical aggregometry support the relative efficacy of „ad hoc" treatment. This efficacy, however, on examination of combined endpoints lags slightly behind the efficacy of pretreatment. Based on all these data, clopidogrel pretreatment at least 6 hours pre-PCI is mainly suggested for patients having stable angina, while it is no sense postponing the PCI for a later date after termination of coronary angiography due to the satisfactory relative efficacy of „ad hoc" loading.

We previously described that patients with enhanced thrombotic tendency (e.g. stroke and diabetes) display significantly elevated levels of indirect platelet activation markers like monocyte tissue factor expression and monocyte-platelet heterotypic aggregate formation.

In our study we set out to investigate direct platelet activation markers in stented patients and to evaluate the effect of salycilate non-responsiveness. Other groups usually investigated patients with loading doses administered minimally 3 hours before the stent implantation or used a 75 mg maintenance dose from the time of implantation, while we compared the effects of a single 300 mg loading dose of clopidogrel administered just at the time of stenting compared to conventional pretreatment.

The benefit of pretreatment with clopidogrel loading before coronary intervention is the prevention of acute or subacute thrombotic occlusion of the stented artery, since the loading dose of 300 mg at the time of intervention reaches its maximal antiplatelet efficacy 4-6 hours after drug administration.

Data obtained from the CURE (Clopidogrel in Unstable Angina to Prevent Recurrent Event) and CREDO (Clopidogrel for the Reduction of Events During Observation) studies also supported these findings. Although, the increased risk of bleeding up to 1.6% may be a considerable complication, and limit the benefit of pre-treatment, especially in the patient subgroups with GPIIb/IIIa receptor blocker administration or urgent coronary bypass surgery. In some previous studies platelet aggregation was significantly inhibited 2 hours after administration of 300 mg clopidogrel, but after 4 hours there was no significant difference between the two regimens.

It is not straightforward that clopidogrel pretreatment is useful. Studies could not demonstrate efficacy of pre-treatment with clopidogrel at 1-month and 6-month follow-up in patients with stable angina in prevention of major cardiac events or found no correlation between post-PCI myonecrosis and the degree of platelet aggregation inhibition.

Under physiological conditions platelets circulate in a resting state, but after activation caused by vessel injury or implantation of endovascular prothesis, undergo several changes, such as biochemical, morphological and immunological alterations. Platelet activation by intracoronary stents is induced by mechanical trauma, flow disturbances, exposure to arteficial surface and shear forces. Flow cytometry is an advanced technique to investigate surface antigens by using monoclonal antibodies. P-selectin (CD62) and lysosome associated membrane protein-3 (CD63) are released from α-granules and lysosomes, respectively. Previous studies demonstrated, that surface expression of CD62 antigen was significantly increased after intracoronary stenting due to extended α-granule secretion and liberation of biologically active compounds, which trigger stent thrombosis. Two in vitro studies showed, that CD62 and CD63 surface expression are elevated within 2 minutes after exposure to the stent surface and thereafter the level of activation slowly increased in the next 10 minutes.

Our study suggests, that P-selectin and lysosome associated membrane protein-3 expressions are significantly inhibited for 24 hours by a 300 mg clopidogrel loading dose administered at the time of stent implantation. At 0 hour, the P-selectin expression of the loading group was significantly higher compared to the pretreated group, but after 1 hour the expression of this antigen was similar in the two groups and remained similar during the 24 hours of the study. The CD63 antigen expression did not show significant difference between the study groups at any sampling time, however in the loading group a significant decrease in surface CD63 expression has been observed already after 1 hour similarly to P-selectin. The initial decrease in the surface expression of P-selectin among patients with „ad hoc" loading has to be due to the lack of a next activation stimulus (stent implantation), while the decrease measured at the later timepoints has to be due to the clopidogrel loading. The stent implantation causes an immediate platelet activation within seconds, which expressed just at the 1st blood sampling.

The platelet surface antigens, such as GPIIb/IIIa receptor (CD41) and GPIX (CD42a) are higly expressed on resting platelet surface. In an in vitro study these conformational antigens did not show changes in the surface expression, probably due to high expression even on resting platelets. Some previous trials demonstrated similar findings while others, detected variable decrease in GPIIb/IIIa receptor expression after stenting due to combined therapy with aspirin and an ADP receptor blocker. In the majority of former trials - not utilizing ADP receptor blockers - an elevation or constantly high expression have been observed in various platelet activation parameters during stenting.

Our investigations noted, that the number of CD41 molecules significantly decreased in both loading and pretreated groups. The finding parallels with the decrease in P-selectin expression and suggests a deactivation of platelets as exemplified by other markers. In the loading group similarly to the previously described parameters the platelet count was significantly lower at time of stenting and was increased after 4 hours. Using the same regimen and analysis period Weltermann et al found that the pretreatment with clopidogrel did not result in a pronounced inhibition of the platelet and the coagulation system. The number of GPIX did not show any changes in our study groups, nor in the same group in the different sampling times, though a study of Gawaz et al. revealed increased binding of von Willebrand factor to platelets after stent implantation. Here, there is no disaggreement with our findings, since we studied the absolute number of GPIX after stent implantation, the binding to its substrate could be increased beside the normal surface receptor number.

Aspirin responders and non-responders did not show any difference at baseline in the studied platelet receptors, nor in platelet count. The mechanism of aspirin resistance is

complex, and involves possible variations in COX-2 expression, GPIIIa polymorphism, cell-cell interactions and von Willebrand factor levels. Thus, there is no connection between the pathophysiological mechanisms of aspirin non-responsiveness and the surface receptor expression levels of the examined proteins in our trial. The rate of ASA nonresponsive patients measured by PFA-100 equipment (27,5%) is similar to previous studies.

There are some limitations to our study, so the observations has to be interpreted with caution. The sample size in both study arms was relatively low, however the groups were quite homogenous in regard to the baseline characteristcs, the differences in target vessel and in the number of diseased vessel was not significant. The study was designed as a prospective trial, but no randomisation and blinding were used. Our baseline samples at 0 hour were drawn from patients just at the time of stent implantation, thus these data show the characteristics of platelets immediately activated by stents.

In conclusion, our data demonstrated that, in patients with stable angina or stabilization after ACS, who have undergone successful intracoronary stent implantation, the administration of a 300 mg clopidogrel loading dose at the time of intervention effectively and promptly inhibit platelet activation markers and as effective as the pretreatment. P-selectin and LAMP-3 expression were significantly inhibited by clopidogrel loading dose after 1 hour, the number of GPIIb/IIIa receptors decreased after 2 hours, and the initially depressed platelet count normalized after 4 hours. We also found that our results were not biased by aspirin non-responsiveness. These data point to the beneficial effect of clopidogrel treatment in reducing platelet reactivity during stenting and to the variable sensitivity of different platelet activation markers towards ADP receptor blockers.

When the results of our clinical and laboratory examinations are evaluated, the fact had to be noticed, that the first analyzes the reaction to different clopidogrel administrations of a complex biological system which can be measured by clinical endpoints, while the second detects the changes of certain in vitro parameters of that complex system, in our study the changes in the surface markers of platelets. The reaction of a complete biological system (the living human body) to a drug is influenced by uncountable factors, the platelet markers were the object of our study trom these, as one of the most important element, which can influence the final biological, clinical outcome, as we presumed. The decrease of platelet surface expression of p-selectin and LAMP-3 within 1 hour after the clopidogrel loading means the inhibition of fusion of the membrane of the α-granules and lisosomas, which indirectly sign the deactivation of platelets. The inhibition of emptying of the storage granules leads to decrease of the concentration of the stored, activation (further aggregation of

platelets) enhancing and inducing agents, so it decrease the possibility of intrastent and intracoronary thrombus formation. Besides these, some other mechanisms play role in that severe, life threatening complacation. The minimal advantage for the pretreatment in our clinical study, which is consistent with the actual guidelines, attracts attention to other facts playing important role in stent thrombosis. But the fact has to be emphasised again, that the little advantage for pretreatment in the clinical composite endpoint and stent thrombosis can be observable beside the higher frequency ot bleeding complications and in the case of a diagnostic catheterisation indicating coronary intervention, the loading at the time of sten implantation can be acceptable, as far as possible with 600 mg clopidogrel and the postponement of the intervention for hours after the diagnostic procedure is not rationale.

Summary

Based on data of the Clopidogrel Registry, pretreatment using a loading dose of 300 mg and having been administered 6-24 hours prior to the planned PCI has minimal effectivity over loading treatment having been applied at time of the procedure, this effectivity is reached at the expense of the increase of drug-induced bleeding events. Our results match with the current therapeutic recommendations. Both in vitro data and marked inhibition of platelet aggregation experienced 4 hours after loading dose administration and observed during optical aggregometry support the relative efficacy of „ad hoc" treatment. This efficacy, however, on examination of combined endpoints lags slightly behind the efficacy of pretreatment. Based on all these data, clopidogrel pretreatment at least 6 hours pre-PCI is mainly suggested for patients having stable angina, while it is no sense postponing the PCI for a later date after termination of diagnostic coronary angiography due to the satisfactory relative efficacy of „ad hoc" loading.

The results of platelet marker tests clearly proved that in patients with stable angina or after stabilization of those ACS patients, who have undergone successful intracoronary stent implantation, the administration of a 300 mg clopidogrel loading dose at the time of intervention effectively and promptly inhibits platelet activation markers and is as effective as the pretreatment. P-selectin and LAMP-3 expression were significantly inhibited by the clopidogrel loading dose of 300 mg after 1 hour, the number of GPIIb/IIIa receptors decreased

after 2 hours, and the initially depressed platelet count normalized after 4 hours. We also found that our results were not biased by aspirin non-responsiveness. Our results emphasize the variable sensitivity of different platelet activation markers toward ADP receptor blockers, primarily inhibition of expression of cell surface markers (p-selectin, LAMP-3) could be observed having been released from intracellular storing granules during platelet activation. Our data also point to the beneficial effect of clopidogrel treatment in reducing platelet reactivity during stenting.

The minimal advantage observed in our clinical trial for the favour of pretreatment and thus being consistent with the current therapeutic recommendations call attention to the emphatic role of other factors bearing a part in developing stent thrombosis.

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