Potent low dose platelet inhibitory effects of clopidogrel and aspirin on coronary thrombus formation in an animal model of acute unstable angina

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Summary

Application of clopidogrel before percutaneous coronary intervention in patients with acute coronary syndrome reduces the risk of cardiac events. Clopidogrel administration before surgery increases bleeding complications after CABG. Therefore, the antithrombotic effect of the low-dose combination of clopidogrel and aspirin was investigated in an *in vivo* pig model of coronary artery thrombus formation with cyclic flow reductions. The platelet inhibitory effect was determined by platelet aggregation and CFR, according to the methodology described by Folts. CFR were initiated by endothelial damage and placement of a constrictor around the LAD. 30 min after CFR were established, clopidogrel (0.1 mg/kg or 5 mg/kg), aspirin (1 mg/kg or 7 mg/kg) or LDC (0.1 mg/kg clopidogrel and 1 mg/kg aspirin) were administered orally. CFR-frequency was determined for further 240 min.

CFR-frequency (CFR/30 min) was significantly reduced at 60 min in response to aspirin (7 mg/kg, –48%, $p<0.05$), and at 120 min in response to clopidogrel (5 mg/kg, –65%, $p<0.05$) but not at low doses of either compound. In contrast, LDC of clopidogrel (0.1 mg/kg) plus aspirin (1 mg/kg) resulted in a complete and rapid abrogation of CFR at 90 min (-70%, $p<0.05$). Furthermore, LDC led to reduction of platelet aggregation when CFR-frequency was already significantly decreased. In contrast, high dose groups presented a significant reduction of platelet aggregation prior to CFR-frequency decrease. Low dose combination of clopidogrel plus aspirin demonstrates a potent over additive anti-thrombotic effect *in vivo* with a significant reduction in thrombus formation early after drug application. The effect occurs before inhibition of platelet aggregation is detectable.

Keywords

Aspirin, clopidogrel, platelets, acute coronary syndrome, coronary artery bypass grafting

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Introduction

According to the guidelines, percutanous coronary intervention (PCI) is the treatment of choice in acute coronary syndrome (ACS). It has been shown that patients benefit from an early invasive approach (1, 2). To prevent early stent thrombosis, clopidogrel (300mg) is frequently administered before PCI is performed (3).

Clopidogrel treatment is indicated in patients with unstable angina and non-ST-segment elevation myocardial infarction. It should not be used in patients who are potential candidates for urgent coronary artery bypass grafting (CABG) (4).

Several studies have clearly demonstrated the beneficial role of acetylsalicylic acid (ASA, Aspirin®) in preventing thrombotic complications in cardiovascular diseases (5–8). Previous studies have demonstrated a synergistic effect of the combination therapy with ASA and clopidogrel under experimental and clinical conditions (9, 10). Further trials confirmed the need for dual antiplatelet therapy after coronary artery stenting (3, 11, 12).

However, recent publications documented that preoperative clopidogrel therapy is associated with increased mediastinal bleeding, reoperation for bleeding and transfusion requirements as well as prolonged ICU and hospital length of stay (13–18).

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In regard to the documented complications after CABG under clopidogrel pretreatment, the issue of a low-dose combination therapy and its effect on *in vivo* platelet function is of utmost interest and suggests a dose reduction. Therefore, it was tested in an *in vivo* model of coronary artery thrombus formation i). whether a combination therapy with clopidogrel and ASA at dosages which are ineffective as single therapy can still prevent coronary thrombus formation *in vivo*, and ii). if *in vivo* observation can be confirmed by *ex vivo* platelet aggregation tests.

Material and methods

Experimental model

Thirty male Landrace pigs weighing 30 ± 4 kg were premedicated with the sedative azaperon intramuscularly (7.5 mg/kg). Anesthesia was initiated with an intravenous sodium thiopental bolus (5 mg/kg) and then maintained by intravenous infusion of sodium thiopental (10 mg/kg/h). After intubation, pigs were mechanically ventilated with a Dräger respirator Servo 900b (oxygen-air: FiO_2 0.35; Pco_2 controlled) and arterial and central venous lines were introduced via the femoral artery and vein (19). For 'peroral' drug administration a catheter was placed in the jejunum through a small paramedian laparotomy. Prior to femoral artery preparation, a 7.5 mg bolus of the analgetic piritramid was given intravenously and during left thoracotomy piritramid was infused continuously (0.25 mg/kg/h). After removal of the fourth rib, the heart was suspended in a pericardial cradle and a Doppler flow probe (ART, Active Redirection Transit Time Flow Probe, Triton Technology Inc., San Diego, USA) was placed around the pulmonary artery. A TIP-catheter was inserted into the left ventricular apex. Approximately 1.5 cm of the left anterior descending coronary artery (LAD), proximal to its first diagonal branch, were carefully dissected from surrounding tissue and a pulsed ultrasonic Doppler flow probe (Triton Technology Inc., San Diego, USA) was placed around the vessel for continuous flow measurement.

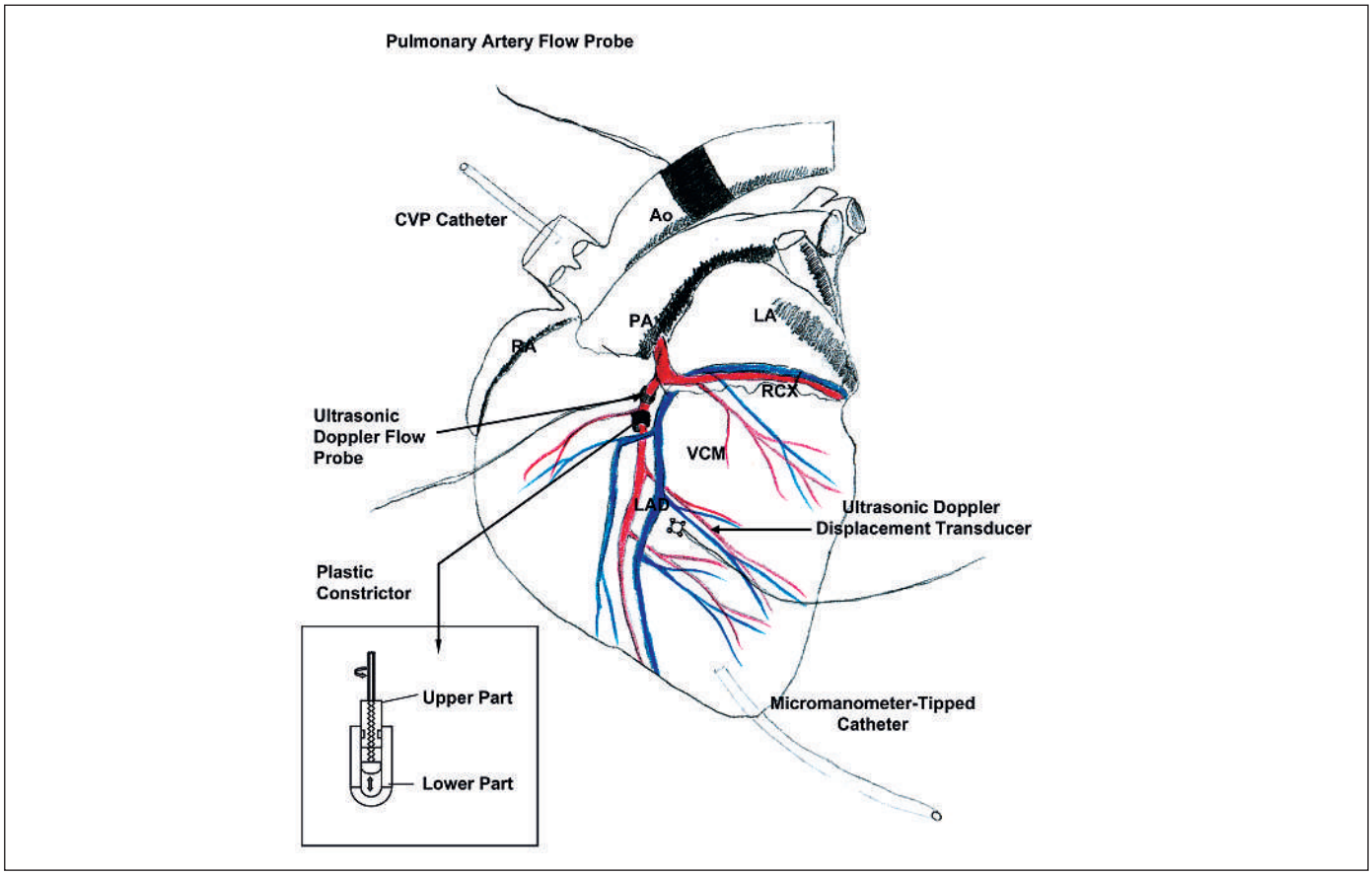

Figure 1: Scheme of the pig model showing a new method for producing critical coronary artery stenosis. To consider the fragility of pig coronary arteries, intimal damage in pigs must be produced very carefully. Also, shaking the constrictor to embolize the thrombus may increase the amount of intimal damage and may not return to the same point on the artery, which could reduce the degree of stenosis. To avoid these problems, Folts et al. used the combination of an oversized plastic occluder and a balloon of a percutaneous transluminal coronary angioplasty catheter, but over time the loss of pressure affects this system adversely. Therefore, we designed a new plastic constrictor which can be adjusted very easily and more exactly via a scale on the turning handle. The U-shaped lower part of the plastic constrictor was placed around the left anterior descending coronary artery and then connected with the upper part. The upper part was connected to a flexible shaft, which transmitted the movement to a thread to shift a plunger to narrow the coronary artery. Critical coronary artery stenosis was achieved by continuous reduction of the internal diameter of the constrictor. LAD, left anterior descending coronary artery; VCM, vena cordis magna, Ao, aorta; RCX, circumflex coronary artery; PA, pulmonary artery; LA, left atrium; CVP, central venous pressure.

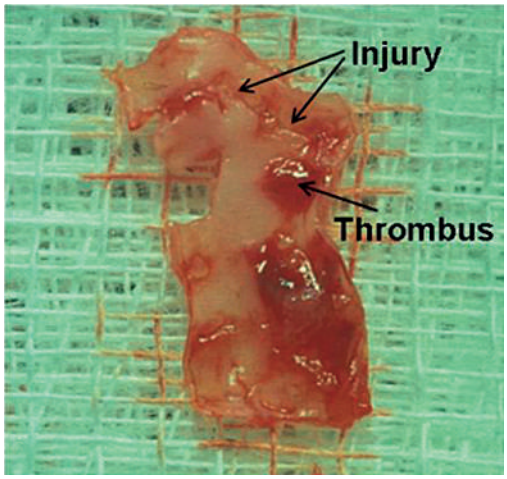

Figure 2: Explanted injured coronary artery with fresh thrombus distal to the site of injury.

Preparation and administration of drugs

Clopidogrel was dissolved in non-pyrogenic aqua injectabile (Sanofi Recherche, Toulouse, France) and Aspisol® (Bayer AG, Leverkusen, Germany) was dissolved as instructed by the manufacturer. All drugs were prepared just prior to use. Clopidogrel and ASA or a combination of these were administered via a jejunal catheter to mimic 'peroral' administration, which corresponds to the routine way of administration in humans.

Study protocol

Baseline hemodynamics, including heart rate, systolic, diastolic and mean arterial pressure, central venous pressure (CVP), left ventricular pressure (LVP), cardiac output and mean coronary blood flow velocities were continuously recorded with a System 6 unit (Triton Technology Inc., San Diego, USA). Cyclic flow reductions were induced as described by Folts et al. (20, 21) by carefully squeezing the LAD distal to the pulsed ultrasonic Doppler flow probe with a smoothed forceps to damage the endothelium. In pigs, however, intimal damage should be performed in a well-controlled manner, because of the fragility of porcine coronary arteries (22). Therefore, we designed a new plastic constrictor with a flexible shaft used to reproducibly narrow the coronary artery (Fig. 1). This special 4-mm plastic constrictor was placed around the injured segment of the artery. Critical coronary artery stenosis was achieved by continuous reduction of the internal diameter of the constrictor, until the reactive hyperemic response to a temporary 15 sec occlusion was nearly abolished. The combination of endothelial damage and critical stenosis resulted in spontaneous repetitive cyclic flow reductions which were monitored for a time interval of 30 min duration as baseline CFR. Thereafter, drug or vehicle were administered ('peroral') via a jejunal catheter. Coronary blood flow and hemodynamics were then measured for another period of 240 min. Wall thickening was measured before and after drug administration every 30 min until the end of the experiment. After CFR were abolished, epinephrine (0.6 µg/kg/min) was infused intravenously for 30 min at the end of the observation time. No systemic heparin was given at any time during the experiment.

According to previous examinations in pigs using clopidogrel, we started at a dose of 5mg/kg (11, 23). Pilot experiments with dose reduction of single administered ASA (7, 3 and 1 mg/kg) and clopidogrel (5, 3, 1, and 0.1 mg/kg) led to the ineffective *in vivo* dosage. Thereafter, the combined application of these ineffective low doses was investigated.

Subsequently, pigs were divided into six groups of 5 animals each: group 1 received saline 0.9%, group 2 and 3 received ASA (Group 2: 7 mg/kg, Group 3: 1 mg/kg), group 4 and 5 received clopidogrel (Group 4: 5 mg/kg, Group 5: 0.1 mg/kg) and group 6 received a combination of ASA 1mg/kg and clopidogrel 0.1 mg/kg.

For the analysis of the antithrombotic effects of ASA, clopidogrel or the drug combination, respectively, each animal was used as its own control by comparing CFR prior to and post drug treatment (Fig. 2).

All investigative procedures and animal facilities conformed with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee.

Methods

Evaluation of cyclic flow reduction (CFR)

Thrombus formation at the site of injury in the left anterior descending coronary artery and induction of the critical stenosis led to blood flow reduction and decrease of blood velocity, respectively. To assess the severity of CFR, the area under the blood flow velocity curve over time is determined by planimetry using a personal computer based software system (Photoshop 6.0, Adobe Systems Incorporated, San Jose, USA) and calculated as cm^2/ 30 min until cessation of CFR according to the publication of Just et al. (24). Therefore, severity is zero after CFR cessation. In regard to previous publications both "severity" of cyclic flow reduction and the number of cycles per 30 min were examined (20, 21, 24). Derived from the single chart of CFR in each animal, we counted the 50% reduction of CFR to baseline in each group. Time was measured when 50% reduction was reached and data were compared between groups. The existence of thrombus was documented by explantation of the injured artery segment at the nadir of CFR in pilot experiments (Fig. 2). Additionally, the complete thrombotic occluded coronary artery was rapidly explanted in animals with evolving myocardial infarction whereas a fresh thrombotic lesion was observed.

Platelet aggregation studies

Blood samples were collected in polypropylene tubes containing 3.8% trisodium citrate solution (9:1 v/v) before CFR and every 30 min until 240 min after 'peroral' drug administration. Platelet rich plasma (PRP) was obtained by centrifugation of the blood (300xg, 10 min, 20°C), whereas blood was centrifuged at 1800xg (10 min, 20°C) to obtain platelet poor plasma (PPP). Platelet aggregation was measured by the turbidometric method of Born and Cross (25) on a LABor APACT aggregometer (LABor, Ahrensburg, Germany), and PRP was equilibrated at 37°C for 1 min under constant stirring (1000 rpm). Aggregation was induced by ADP 3 or 10 µM (Adenosin 5' diphosphate sodium salt,

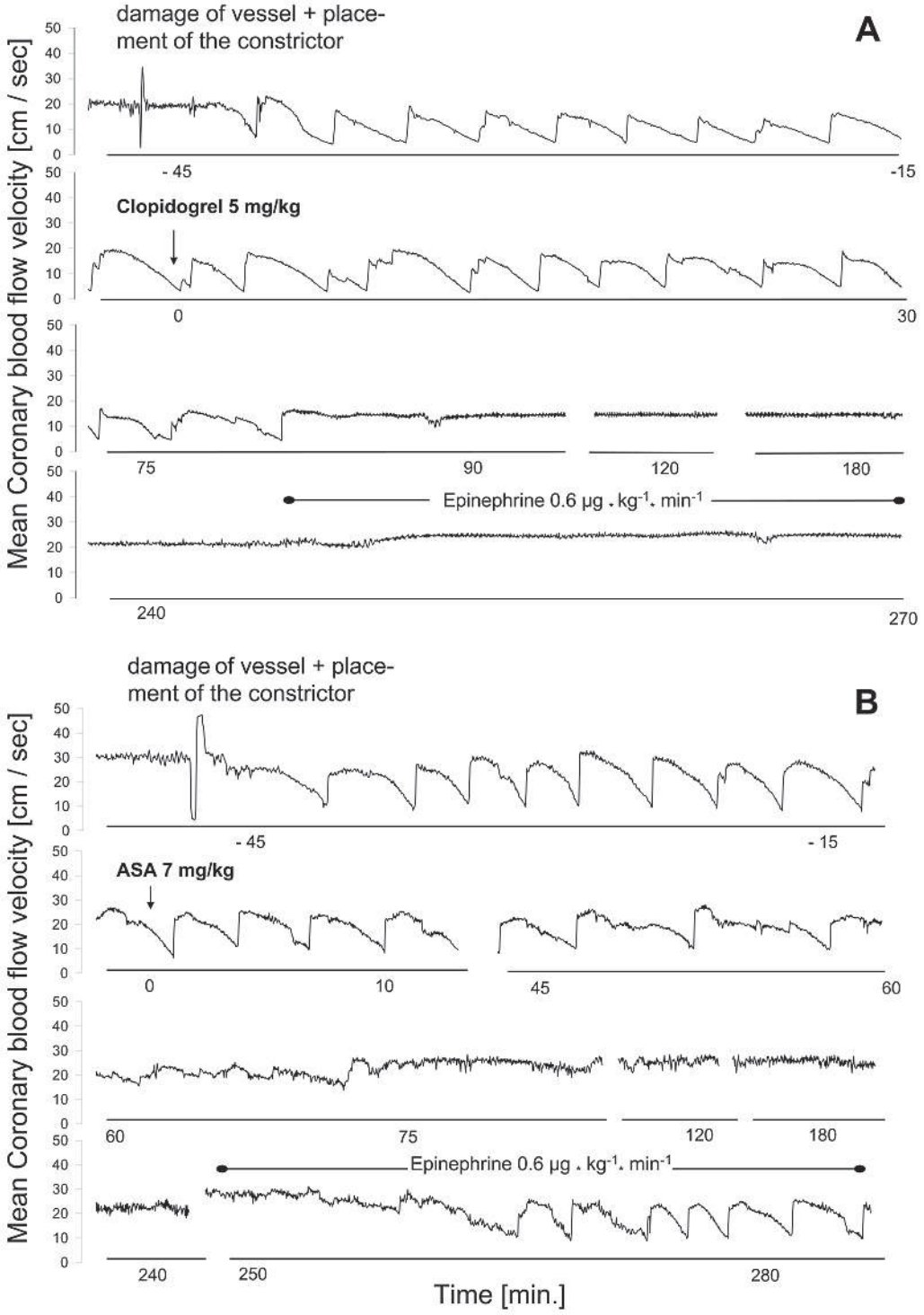

Figure 3: Representative recordings of cyclic flow reductions in mean blood flow velocity in the left descending pig coronary artery caused by a critical stenosis and vessel damage. A) Cyclic flow reductions disappeared 80 min after oral application of 5 mg/kg clopidogrel and were non-recurrent by epinephrine infusion. B) Cyclic flow reductions abolished 60 min after oral application of 7mg/kg ASA and renewed by epinephrine infusion.

Table 1: Effect of ASA, clopidogrel and their combination on the occurrence and reoccurrence of CFR.

Drug	Inhibition of CFR	Administration of epinephrine	Reoccurrence of CFR	Primary end-point (infarction/ death)
Saline 0.9%	0/5	0/5	-	2
ASA 7 mg/kg	5/5	5/5	3/5	0
ASA 1 mg/kg	0/5	0/5	-	2
Clopidogrel 5 mg/kg	5/5	5/5	0/5	0
Clopidogrel 0.1 mg/kg	0/5	0/5	-	1
ASA 1 mg/kg + Clopidogrel 0.1 mg/kg	4/5	4/5	2/4	0

Epinephrine infusion (0.6 µg/kg/min) was performed at the end of 4 hours observation time after drug administration to cause the reoccurrence of CFR. Death or infarction were observed during the experiment in three groups. Numbers present incidences to total number of animals studied in each group.

Sigma Chemie, Heidelberg) or 3 or 10 µg/mL collagen (Collagen Reagent, Chrono-Par, Nobis Labordiagnostik GmbH, Endingen, Germany). The extent of aggregation was estimated by quantitatively measuring the maximum height above baseline of the curve.

Platelet count

Before initiating CFR and at the end of the experiment, blood samples (2 mL) were collected in polypropylene tubes containing EDTA (EDTA-Monovette®, Braun AG, Melsungen, Germany). 20 µL of EDTA-blood were added into a Thrombo plus® tube (Sarstedt AG, Nürnbrecht, Germany), where hemolysis occurred immediately. The results were obtained by visual counting in a microscope using a Neubauer chamber and were calculated as platelets/µl.

Statistical analyses

All data are expressed as mean ± standard error of mean. The n values indicate the number of animals studied. In aggregation studies the responses are expressed as inhibition of control aggregation. Comparisons between variables were made using one way ANOVA. For repeated measurements, ANOVA with the multiple comparison method versus control group (Dunnett's test) was used. Probability values $p<0.05$ were considered to indicate statistical significance.

Results

Cyclic flow reductions

Endothelial damage of the left anterior descending coronary artery and placement of the coronary constrictor resulted in recurrent CFR, with mean frequencies ranging from 6 to 10 per 30 min observation interval in the various groups. An example of CFR and cessation after drug administration is given in Figures 3 A and B. Mean severity of CFR at baseline in all groups ranged from 48 to 64 cm² / 30 min observation interval. Baseline and follow-up data of severity are given in Table 2.

After saline administration (group 1), the CFR continued with a slightly but not significantly lower frequency and with unaltered severity compared to the baseline period. Two pigs of this group died due to infarction 90 and 120 min, respectively, after drug administration (Table 1 and Fig. 4A).

ASA (7 mg/kg, group 2) showed a tendency for CFR-frequency reduction at 30 min and significantly diminished CFR at 60 min (-48%, $p<0.05$ vs. pretreated CFR-frequency) and at 90 min (-94%, $p<0.01$). An example for CFR cessation 60 min after ASA 7mg/kg administration is given in Figure 3B. CFR were abolished after 90 min in all pigs (Fig. 4B). CFR reoccurred in 3 of 5 animals caused by epinephrine infusions at the end of the experiment (Table 1). The severity was significantly reduced at 90 min (-90%, $p<0.01$) after drug administration.

CFR-frequency of group 3 (ASA 1mg/kg) continued unabated ranging from 6 to 9 / 30 min during the 4 hours of observation (Fig. 4C). In this group, one of two pigs died due to infarction.

Following clopidogrel administration (5 mg/kg, group 4). CFR frequency showed a trend to decrease at 90 min. A significant reduction occurred at 120 min (-65%, $p<0.05$) and at 150 min (-94%, $p<0.01$). In this group, CFR were abolished after 180 min (Fig. 4D). An original recording with CFR termination 80 min after drug administration is shown in Figure 3A. Epinephrine was infused in five pigs without restoring CFR (Table 1). In this group, the severity was significantly reduced 120 min (-80%, $p<0.05$) after drug administration.

However, the CFR-frequency continued unabated following clopidogrel administration at 0.1 mg/kg (group 5), with its severity also not being significantly altered until the end of the experiment. One pig in group 5 developed infarction (120 min).

The combination therapy (group 6) of clopidogrel 0.1 mg/kg and ASA 1 mg/kg reduced CFR-frequency by 46% at 60 min. At

Table 2: Severity of cyclic flow reductions.

Drug	baseline	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Saline	48.2±8.6	49.5±8.0	43.2±5.5	43.2±7.2	48.6±7.8	43.8±9.6	42.4±7.6	41.5±7.1	42.6±5.6
ASA 7 mg/kg	53.0±5.6	54.2±6.3	40.7±11.7	5.5±2.9*	0±0*	0±0*	0±0*	0±0*	0±0*
Clopidogrel 5 mg/kg	52.9±4.2	44.6±2.1	37.2±10.1	30.3±10.6	19.1±12.0*	8.7±5.3*	0±0*	0±0*	0±0*
ASA 1 mg/kg	52.7±2.8	49.2±2.5	49.2±2.8	47.9±3.6	45.5±3.3	51.4±3.0	48.5±1.4	45.5±1.7	47.0±2.0
Clopidogrel 0.1 mg/kg	61.0±2.5	62.0±5.3	58.5±3.6	53.9±2.2	51.9±2.1	55.4±2.6	50.5±3.3	50.6±2.7	51.0±1.3
ASA 1 mg/kg + Clopidogrel 0.1 mg/kg	63.5±5.6	55.8±9.5	54.3±15.3	47.0±15.8	23.5±12.5*	17.4±11.0*	10.9±10.9*	16.5±11.2*	12.0±12.0*

Data present severity of CFR as means ± SEM (cm^2/30min) in each group measured prior and every 30 min after drug administration until cessation of CFR. (*$p < 0.05$ compared to baseline)

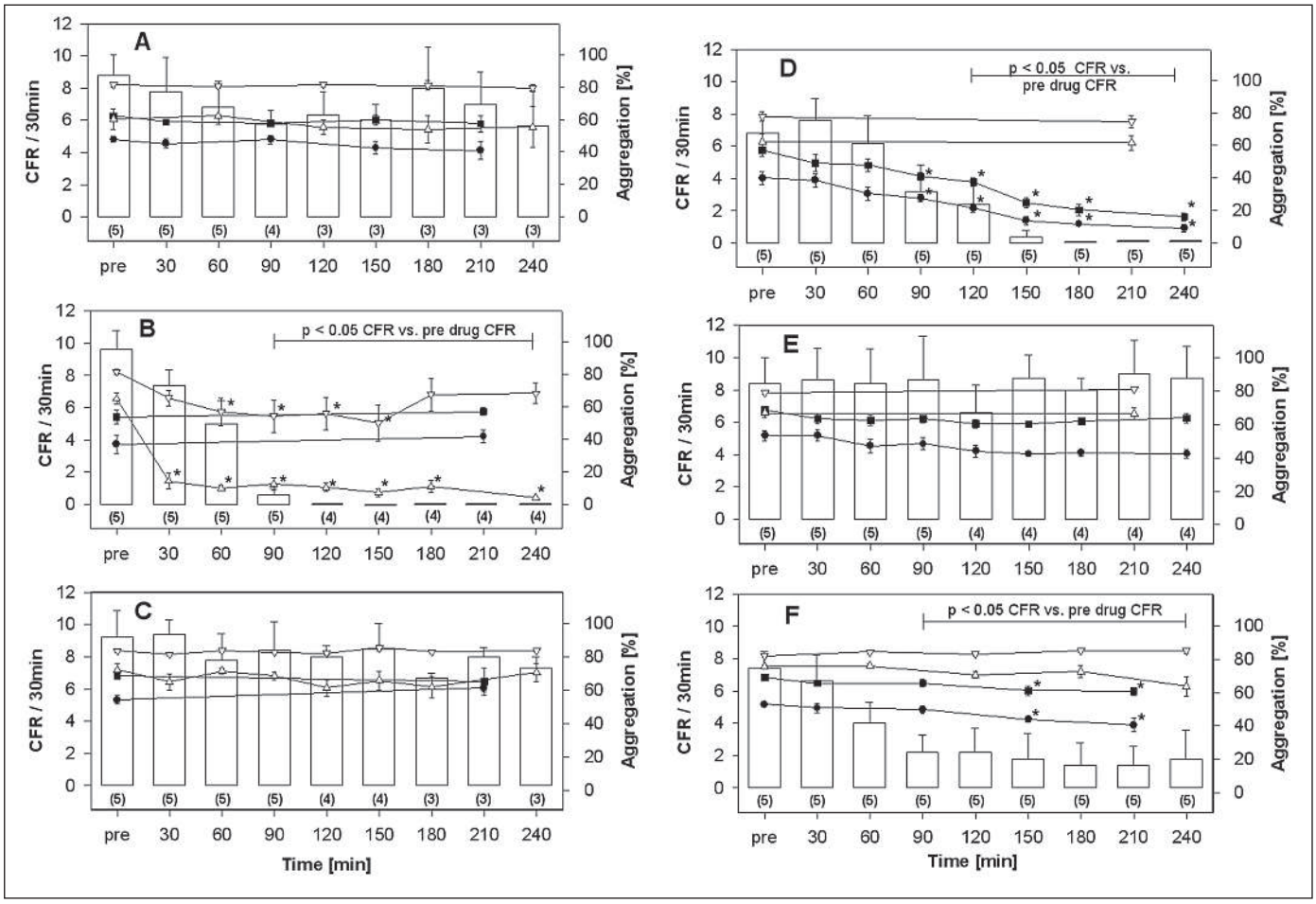

Figure 4: Relationships between frequency of cyclic flow reductions [CFR per 30 min, white bars] and *ex vivo* platelet aggregation with ADP [3 µmol (●), 10 µmol (■)] and collagen [3 µg/ml (Δ), 10 µg/ml (∇)], respectively, in pigs with critical stenosis of left descending coronary artery before and every 30 min until 4 hours after drug administration. A) Saline 0.9% (group 1), B) ASA 7 mg/kg (group 2), C) ASA 1mg/kg (group 3), D) clopidogrel 5 mg/kg (group 4), E) clopidogrel 0.1 mg/kg (group 5), F) clopidogrel 0.1 mg/kg + ASA 1 mg/kg (group 6, combination). Values are means ± SEM. Numbers in parentheses give numbers of animals per group at the timepoint. * Signifies p<0.05 vs. pre-drug aggregation.

90 min after drug administration, CFR reduction (-70%, p<0.05) was significant until the end of the experiment (-70 to –80%, p<0.05). The severity of the flow reductions was also significantly reduced after 120 min (-63%, p<0.05). However, in one pig the CFR continued with only slight reductions between 30 and 120 min following drug administration. Therefore, CFR were abolished in four pigs (Fig. 4F). CFR were reintroduced in two of these animals after epinephrine infusion (Table 1).

The reduction in the number of CFR by 50% compared to baseline was accomplished after 60 min in the ASA group (7 mg/kg) and after 65 min in the combination group (clopidogrel 0.1 mg/kg + ASA 1mg/kg). This is in contrast to clopidogrel alone, where a 50% reduction was observed 90 min after drug administration (5 mg/kg, p<0.05 vs. ASA 7mg/kg and combination). The other groups (clopidogrel 0.1mg/kg, ASA 1 mg/kg and control) did not show CFR reduction at all.

The stimulation of CFR under epinephrine presents the reintroduction of platelet activation by the adrenergic system. This was completely abolished under 5mg/kg clopidogrel alone.

Platelet aggregation

The antiaggregating effects of clopidogrel and ASA are shown in Figures 4A-F.

ADP-induced platelet aggregation was clearly and significantly inhibited in PRP by clopidogrel 5 mg/kg (Fig. 4D) and was slightly reduced by clopidogrel 0.1 mg/kg (Fig. 4E), but not by ASA 7 mg/kg and 1 mg/kg, respectively. On the other hand, collagen-induced aggregation was significantly inhibited by ASA 7 mg/kg (Fig. 4B), whereas ASA 1mg/kg (Fig. 4C) and clopidogrel had no effect. Normal saline treatment of the animals had no effect on ADP- and collagen-induced platelet aggregation (Fig. 4A). The combination of low dose clopidogrel (0.1 mg/kg) and ASA (1mg/kg) produced no effects initially and exerted a minor but statistically significant impact on ADP-induced aggregation after 150 min without any effect on collagen-induced aggregation (Fig. 4F).

Platelet count

The number of platelets as mean of all groups (n=30) was 357.000 ± 20.000/µl at baseline and 350.000 ± 26.000/µL at the

end of the experiment, with a range from 172.000/µl to 658.000/µl. No statistically significant difference between groups could be observed. The decrease in platelet count after 4 hours of cyclic flow reductions amounted to a maximum of 12% in individual animals.

Hemodynamic and laboratory parameters

Mean arterial pressure, heart rate, cardiac output, CVP and LVdp/dt displayed no statistically significant differences between the six groups at any time. All laboratory and respiratory parameters remained within physiological ranges. There were no statistically significant differences between groups at any time.

Discussion

The present study demonstrates the over additive antithrombotic effect of low-dose clopidogrel and ASA in an experimental animal model of coronary artery thrombus formation. Our results present the *in vivo* antithrombotic effect occuring at clopidogrel and ASA doses that do not inhibit or only slightly reduce *ex vivo* platelet aggregation induced by a single proaggregatory mediator. In addition, the onset of action in the low-dose combination group is remarkable, being already present 60–90 min following 'peroral' drug administration.

ASA is the most widely used antithrombotic agent in acute coronary syndromes and chronic coronary artery disease (26). It has been shown to be highly beneficial in the treatment of unstable angina (8, 27). However, ASA fails to be preventive in a certain number of patients, which may be partly due to the fact that ASA inhibits just one of the several existing platelet activation pathways (8). The different and complementary ADP-dependent platelet activation and amplifying pathways are irreversibly inhibited by clopidogrel which was previously published for CFR inhibition in femoral arteries (23). After oral administration, clopidogrel is absorbed from the gastrointestinal tract and rapidly metabolized in the liver. Therefore, the antiaggregatory effect of clopidogrel in humans is detectable *ex vivo* within 2 to 4 hours after administration of the loading dose (300 mg).

Under our experimental conditions, platelet aggregation data present a significant reduction at ASA 7 mg/kg and with clopidogrel 5mg/kg. Inhibition of platelet aggregation at 0.1 mg/kg was attenuated and none was found at ASA 1 mg/kg. However, in the low-dose combination group inhibition of platelet aggregation was significantly reduced but close to the group with administration of 0.1 mg/kg clopidogrel.

In contrast to the data of *ex vivo* platelet aggregation, inhibition of *in vivo* CFR was much more pronounced at low-dose combination of ASA and clopidogrel versus either compound alone. However, CFR could not be completely abolished in one animal and were reintroduced by epinephrine infusions in 2 of 4 animals but also in 3 of 5 animals at ASA 7 mg/kg. This observation may indicate that other stimuli, i.e. changes in hemodynamics, activation of the adrenergic system or an increase in stenosis severity causing an increase in shear stress, may overcome the anti-thrombotic action of ASA (28, 29). CFR were reintroduced in 3 of 5 pigs at ASA 7 mg/kg. The underlying mechanisms have been shown in previous studies (30–33).

These experimental findings are supported by previously published examinations in a stent model of *in vivo* arterial thrombosis in pigs. In these experiments clopidogrel plus ASA changed the thrombus structure and destabilized the thrombus without complete abolishment of platelet reactivity (34). Additionally, in regard to the *in vivo* process of arterial thrombosis caused by a vessel wall injury leading to subendothelial matrix exposure and endothelial mediator release, the *in vitro* initiation of platelet aggregation is achieved by supraphysiological concentration of a single inductor. Begent and Born demonstrated *in vivo* that 10 to 180 x 10^{-14} mol ADP are sufficient to induce a platelet thrombus (35). In comparison to these physiological concentrations, it seems obvious that an effective inhibition just sufficient to eliminate platelet thrombus formations *in vivo* is overcome by supraphysiological inductor concentrations *in vitro* (3 and 10 µM ADP). Thus, the use of a single mediator for platelet aggregation *in vitro* or *ex vivo* testing applied in higher concentrations seems to be of limited value to predict *in vivo* anti-thrombotic efficacy (inhibition of CFR) within coronary arteries.

The present findings go along with several previous studies demonstrating that the combination of ASA and clopidogrel *in vivo* and *ex vivo* is superior in preventing stent thrombosis as compared to therapy with ASA alone (12, 22). Furthermore, Herbert et al. showed that ASA potentiates the antithrombotic activity of clopidogrel in rabbits (9). In addition, a combination of high dose clopidogrel, ketanserin and thromboxane synthase inhibitor ridogrel reduced CFR and neointimal proliferation under coronary flow variations after angioplasty in dogs (36). Similar to our findings, clopidogrel treatment alone in dogs prevented CFR at high dosage (37).

In contrast to these high dose regimen studies, there are no published clinical or experimental data investigating the antithrombotic effect of a low-dose combination of ASA and clopidogrel as presented in this study.

Previous publications concerning coronary artery bypass grafting under combination of clopidogrel plus ASA reported increased postoperative bleeding, blood product transfusions, reoperations for bleeding and morbiditiy (13–18). These findings raise the concern regarding routine treatment of combined high dosage ASA and clopidogrel before definite coronary stent implantation, resulting in the recommendation not to administer these drugs in patients who are potential candidates for urgent CABG (4). On the other hand, the results of the Clopidogrel for the Reduction of Events during Observation (CREDO) trial pointed to a better outcome for clopidogrel plus ASA versus ASA and placebo when given six or more hours prior to percutaneous intervention (3). Therefore, further experimental and clinical trials for dose response relationship of pharmacological platelet inhibition with clopidogrel and ASA before CABG should be performed.

In conclusion, in this animal model of coronary thrombosis due to platelet-induced thrombus formation, ineffective dosages of clopidogrel and ASA alone turned out to be potent in combination. The *in vivo* effect was not mirrored by the *ex vivo* platelet aggregation.

Limitations

For measurement of the anti-aggregant effect of ASA and clopidogrel, collagen and ADP dependent platelet activation was used. According to our data, we observed the time dependent inhibition of *in vitro* platelet aggregation. Indeed, one study limitation could be the unknown metabolism of clopidogrel and its active metabolite in pigs. In regard to previous publications in pig models, none of the authors measured the pharmacokinetics of clopidogrel. Concerning clopidogrel plasma concentrations in pigs, neither Sanofi Research nor Bristol Meyers Squibb have performed any measurements (personal communications J.M. Herbert, Sanofi-Aventis, Toulouse).

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