Is cardiopulmonary bypass a reason for aspirin resistance after coronary artery bypass grafting?☆

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Objective: 'Off-pump' coronary artery bypass grafting (OPCAB) is an alternative to conventional coronary artery bypass grafting (CABG) using cardiopulmonary bypass (CPB). While midterm results after OPCAB have become available, systematic studies of changes in platelet function after OPCAB are still missing. Since we have previously shown that oral aspirin treatment (100 mg) does not achieve sufficient platelet inhibition in the majority of patients operated on with CPB, we hypothesized that bypass surgery without CPB (off-pump coronary artery bypass, OPCAB) causes less impairment of platelet inhibition by aspirin. The aim of this study was to investigate platelet function and the antiplatelet effect of aspirin after off-pump coronary artery bypass grafting in comparison with conventional on-pump surgery. Methods: We compared platelet function (in vitro aggregation and thromboxane formation) before and at days 1 and 5 after coronary artery bypass grafting, performed with (n = 15) or without (n = 14) CPB. Oral aspirin treatment (100 mg/day) was started at day 1 after surgery. Results: After a 5 day oral treatment with aspirin, platelet aggregation was inhibited significantly in OPCAB-patients to 55.7 ± 16.3% of control before surgery (P < 0.05), whereas aggregation remained unchanged after CPB (105.8 ± 26.9% of control before surgery; P > 0.05). Since aspirin primarily inhibits platelet thromboxane formation, thromboxane was determined after in vitro aggregation. According to platelet aggregation, thromboxane formation was only inhibited significantly after OPCAB (29.2 ± 13.0% of control before surgery, P < 0.05), but not after CPB (74.5 ± 21.4% of control before surgery, P > 0.05). This resistance to aspirin after CPB may be caused by an increased release of new platelets which are competent to form thromboxane, since the number of platelets decreased from 237 ± 11 × 10^3/μl before CPB to 174 ± 13 × 10^3/μl at day 1 after surgery and increased significantly the following days reaching 303 ± 17 × 10^3/μl at day 5. Platelet counts of patients operated on without CPB showed no significant changes (236 ± 16 × 10^3/μl before OPCAB, 220 ± 16 × 10^3/μl at day 1 and 266 ± 31 × 10^3/μl at day 5 after surgery). Conclusions: The antiplatelet effect of aspirin is largely impaired after CPB, but not after CABG without CPB. Hence, increased platelet turnover after CPB seems to contribute to aspirin resistance, since an increased number of platelets might be competent to form thromboxane within the dosing intervals.

Keywords: Off-pump coronary artery bypass grafting; Aspirin; Aspirin resistance; Platelet function

1. Introduction

'Off-pump' coronary artery bypass grafting (OPCAB) is an alternative to conventional coronary artery bypass grafting (CABG) using cardiopulmonary bypass (CPB) in the surgical treatment of coronary artery disease. OPCAB poses particular procedural and technical demands and sewing on a moving target may be a source of additional surgical tissue trauma. However, the differences between OPCAB and traditional CABG by far exceed the surgical techniques.

Unlike conventional CPB, OPCAB does not trigger a systemic inflammatory response [1,2]. It avoids hemodilution and blood components are not traumatized by the heart-lung machine. On the other hand, a better preserved hemostasis increases the risk of thrombosis, which is of particular importance for the patency of coronary anastomosis [3,4]. OPCAB even appears to induce a temporary state of hypercoagulation [5]. Accordingly, thromboembolic complications seem to be more frequent following OPCAB than after conventional CABG [6]. Hence, adequate antithrombotic strategies are of particular importance for patients who undergo off-pump cardiac surgery. Specifically, it is not known whether the conventional antiplatelet treatment, as established for on-pump procedures, is optimal for OPCAB-patients. Standards for the choice of antiplatelet drug,
dosage and proper timing of dosage following OPCAB have not been developed.

A large meta-analysis demonstrated that patients after CABG generally derive less benefit from antithrombotic treatment, mostly performed with aspirin (acetylsalicylic acid) than other patient subgroups with cardiovascular disease [7]. Moreover, we have previously shown that conventional aspirin treatment (100 mg) does not achieve sufficient platelet inhibition in the majority of patients within 10 days after CABG [8,9]. While mid-term results of (nonrandomized) clinical trials comparing OPCAB and conventional CABG have become available [10,11], systematic studies of changes in platelet function after OPCAB are still missing, in particular with respect to antplatelet therapy.

We hypothesized that the impaired aspirin-effect after conventional CABG is at least in part due to extracorporeal circulation. Hence, we investigated patients with coronary heart disease operated on with or without CPB and the aim of the present study was to compare platelet function and the antiplatelet effect of aspirin after off-pump coronary artery bypass grafting to conventional on-pump surgery.

2. Material and methods

2.1. Subjects and treatment

The study was conducted in agreement with the Declaration of Helsinki and was approved by the local ethical committee. Twenty-nine patients requiring elective CABG procedures were consecutively included in a prospective manner; the operation technique (OPCAB or conventional CABG with CPB) was chosen according to localisation and severity of coronary vessel disease.

Informed written consent was obtained from each patient. Fifteen of the patients underwent CABG using CPB; in 14 patients, the OPCAB technique was applied. Anaesthesia was performed using enflurane, fentanyl, pancuronium and thiopental. Both groups showed comparable demographic characteristics (CPB-group: age 62.7 ± 2.5 years, 12 male + 3 female; OPCAB-group: age 59.7 ± 2.5 years, 12 male + 2 female). Previous treatment with aspirin was routinely terminated at least 10 days before CABG. Aspirin was re-administered beginning at day 1 after surgery. With the exception of heparin (3 × 7500 IU/d, injected subcutaneously), no additional antithrombotic drugs were given after surgery.

Samples (20 ml) of venous blood for aggregation experiments were collected in the morning (before aspirin treatment) one day before and at days 1 and 5 after CABG in citrated (0.125 M sodium citrate) vacutainers. Platelet counts were determined daily by automated counting.

2.2. Platelet aggregation and thromboxane formation

Platelet rich plasma (PRP) was prepared immediately after blood collection by centrifugation at 250 × g for 10 min at room temperature. Platelet aggregation was measured in a two-channel aggregometer (L.ear, Hamburg, Germany). PRP (250 μl) was preincubated for 6 min at 37 °C. Thereafter, platelets were stimulated by addition of 1 mM arachidonic acid. The concentration of arachidonic acid was chosen sufficiently high to achieve substrate saturation of cyclooxygenase, the target of aspirin.

In order to assess the inhibitory effect of aspirin on aggregation of platelets, aspirin (at final concentrations of 30 or 100 μM) was added 5 min prior to stimulation. Aggregation was recorded by determination of change in light transmission over 5 min after stimulation at constant stirring (1200 rpm, 37 °C). Thereafter, the platelet aggregates were sedimented by centrifugation and the supernatants stored at −20 °C for determination of TXB2 by radioimmunoassay.

2.3. Substances and solutions

Reagents used for the experiments were as follows: aspirin lysine salt (Aspisol, Bayer, Leverkusen, Germany). Arachidonic acid was purchased from Oxford Biomedical Research (Oxford, MI, USA). All other reagents were from Merck (Darmstadt, Germany) or Sigma (Deisenhofen, Germany).

2.4. Statistical analysis

All data are mean ± standard errors of the mean (SEM). Comparisons between groups were performed by Student’s t-test and one-way analysis of variance, followed by post hoc analysis (Dunnett), as required. P-values < 0.05 were considered significant.

3. Results

Aspirin was well tolerated by all patients. In particular, thrombotic or bleeding complications did not occur.

3.1. Platelet counts (see Fig. 1)

The mean platelet counts before surgery (control) were comparable in both groups (CPB-group: 237 ± 11 × 10³/μl; OPCAB-group: 236 ± 16 × 10³/μl). The platelet counts...
showed no significant change after off-pump surgery (220 ± 16×10^3/μl at day 1 and 266 ± 31×10^3/μl at day 5 after surgery). In contrast, due to hemodilution during extracorporeal circulation, the platelet counts decreased in the CPB-group to 174 ± 13×10^3/μl at day 1 after surgery (P<0.05 vs. control) and recovered at day 5 (303 ± 17×10^3/μl), which exceeded significantly the control values obtained before surgery (P<0.05).

3.2. Platelet aggregation (see Fig. 2)

Arachidonic acid-stimulated platelet aggregation was comparable in both groups before surgery; platelets showed a normal sensitivity to aspirin in all patients, since the addition of aspirin (100 μM) in vitro inhibited platelet aggregation nearly completely.

One day after surgery, platelet aggregation was reduced significantly in both groups (CPB-group: 37.8 ± 10.8% and OPCAB-group: 42.6 ± 10.5%). At day 5 after surgery, aspirin inhibited significantly platelet aggregation in OPCAB-patients to 55.7 ± 16.3% of control value before surgery (P<0.05). By contrast, the 5 day aspirin treatment did not result in a significant inhibiton of platelet function (105.8 ± 26.9% of control value before surgery; P>0.05). Even the in vitro effect of aspirin was attenuated remarkably after CPB, reflecting platelet ‘resistance’ to aspirin. In the presence of 100 μM aspirin in vitro, a concentration probably exceeding the peak plasma level after oral aspirin treatment [12], platelet aggregation decreased only to 72.0 ± 17.2% of control value before surgery (P>0.05). Aspirin in vitro reduced aggregation of platelets from OPCAB-patients to 17.7 ± 6.3% (P<0.05). Hence, the sensitivity of platelets from OPCAB-patients to aspirin in vitro is significantly higher than after CPB.

3.3. Platelet thromboxane formation (see Fig. 3)

Since platelet thromboxane formation is the primary target of aspirin, thromboxane (TXB_2) biosynthesis was measured as marker for the activation of platelets. Similar to platelet aggregation, arachidonic acid-stimulated TXB_2 formation was comparable in both groups before surgery and the addition of aspirin (100 μM) in vitro inhibited the TXB_2 synthesis nearly completely, indicating a normal sensitivity to aspirin.

At day 1 after surgery, there was a significant decrease in TXB_2 formation in both groups to 42.8 ± 11.8% (OPCAB) and 39.4 ± 10.1% (CPB) of control value before surgery (P<0.05). After a 5 day treatment with aspirin, thromboxane formation was inhibited to 29.2 ± 13.0% of control before surgery (P<0.05) in patients without CPB, whereas thromboxane biosynthesis recovered substantially in the CPB-group and reached values no more significantly different from control (74.5 ± 21.4%; P>0.05). Hence, oral aspirin did not inhibit significantly platelet cyclooxygenase at day 5 in these patients. Only the addition of very high doses of aspirin in vitro (100 μM) achieved a significant inhibition to 25.2 ± 3.8% (P<0.05) after CPB. In contrast, thromboxane formation of platelets from OPCAB-patients was inhibited nearly completely in the presence of 100 μl/l aspirin in vitro (5.5 ± 2.5% of control before surgery, P<0.05), showing a high sensitivity to aspirin.

4. Discussion

The major finding of the present work is that platelets from patients after CABG with CPB exhibit a poor response to aspirin, as shown by the absence of the expected inhibition of arachidonic acid-induced aggregation and thromboxane formation after a 5 day oral aspirin treatment, whereas aspirin achieved a significantly better inhibition after OPCAB.

Off-pump coronary artery bypass offers many potential advantages along with the avoidance of CPB. For example, CPB induces coagulation disorders of multifactorial aetiology, but transient platelet dysfunction is considered to be of the most important reasons [13]. On the other hand, thrombembolic complications seem to appear more frequently following OPCAB than with conventional on-pump surgery [6]. Even though postoperative hypercoagulability is a recognized phenomenon that occurs often following major surgeries [14], the hypercoagulant activity associated with
OPCAB is discussed controversially, especially with respect to the role of platelets. An increased platelet function [15,16], as well as a procoagulant activity independent of platelet activation [3] are described after off-pump surgery.

However, since platelets might contribute at least in part to hypercoagulability after OPCAB, platelet function and sufficiency of antiplatelet therapy have to be examined in order to prevent graft thrombosis. Thus, we investigated the effect of aspirin after OPCAB in comparison to CABG using CPB.

Before surgery, platelets aggregated sufficiently (after stimulation with arachidonic acid) in all patients enrolled in the study. As expected, both groups showed a normal sensitivity to aspirin before surgery, since the platelet function was inhibited nearly completely in the presence of high concentrations of aspirin (100 μM) in vitro.

At day 1 after surgery, platelet aggregation and platelet thromboxane formation were significantly reduced in both groups, OPCAB- and CPB-patients. This inhibitory effect seems to be independent of the use of CPB and might be explained by interactions of drugs used in anaesthesia with platelets, since our patients received amongst other drugs thiopental. Although the effect of anaesthetics on platelet function is discussed controversially and results of many studies have been conflicting [17], it appears that halothane, sevoflurane and propofol inhibit platelet function in a reversible and dose-related manner at concentrations used clinically, whereas isoflurane, enflurane, desflurane, etomidate, opioids and muscle relaxants seem to have negligible effects on platelets at therapeutic concentrations [18]. Thiopental, a drug commonly used in cardiac anaesthesia, inhibits at therapeutic concentrations platelet activation in patients undergoing elective CABG; this effect begins just after the administration of the anaesthetic and persists for 48 h after surgery [19].

At day 5 after surgery, when anaesthetics are probably eliminated, platelet function was inhibited by oral aspirin treatment only in the OPCAB-group, without a sufficient antiplatelet effect after CPB. Even in the presence of aspirin at a concentration (100 μM), which exceeds those normally found in plasma after oral antiplatelet treatment [12], platelet function was inhibited nearly completely after OPCAB, but only in part after CPB-surgery (aggregation: 17% vs. 72%, thromboxane formation: 5% vs. 25%). It is important to recall, in this context, that platelet thromboxane synthesis needs to be blocked to less than 10% to achieve efficient platelet inhibition [20]. Moreover, as shown previously, a 5-day treatment with oral aspirin (100 mg/d) is sufficient to inhibit significantly platelet aggregation in healthy volunteers [8].

The lower sensitivity to aspirin after CPB might at least in part be explained by an increased platelet turnover due to platelet depletion after extracorporeal circulation. In the present study this resulted in a fall of the platelet count by about 25% at the first day after CPB. Thereafter, platelet counts rapidly recovered and at day 7 after CPB, platelets rose to more than 50% above the value before surgery, indicating an augmented platelet regeneration within the first days after CPB. Since the plasma half-life of aspirin is ~20 min [12], circadian inhibition of platelet function may be incomplete and a significant amount (>10%) of circulating platelets can be competent for thromboxane formation. Thus, the enhanced post-surgical regeneration of platelets with active cyclooxygenase, that could not be acetylated by circulating aspirin, may be one reason for aspirin resistance after CPB.

Although the present work gives new insight into the antiplatelet effect of aspirin after myocardial revascularisation the exact pathophysiological mechanism of aspirin resistance is still not known. According to Weber, aspirin resistance can be classified into three major categories [21], of which one (type 1) includes the inhibition of platelet thromboxane formation by aspirin in vitro but not in vivo (pharmacokinetic resistance), while type 2 resistance is characterized by the inability of aspirin to inhibit platelet thromboxane formation in vitro (pharmacodynamic type). Type 3 is characterized by thromboxane - independent platelet aggregation (pseudoresistance). According to this classification, the type of aspirin resistance described here in patients after CPB is a least in part consistent with type 1 due to the increased platelet turnover and the short plasma half-life of aspirin. Moreover, type 2-resistance may contribute to the attenuated antiplatelet effect of aspirin, since the in vitro effect of high dose aspirin is also attenuated after CPB, but not after OPCAB.

Our study has some limitations: The groups were small in size, but sufficient to indicate differences concerning platelet function, according to sample size calculations basing on the expected differences found in previous investigations [8,9]. Moreover, the patients were not randomized; this is reflected in the severity of coronary vessel disease. It is obvious that patients in the on-pump group were in a more advanced state with respect to number and localisation of diseased coronary vessels. This is no real limitation of our results, since we focused on platelet function but not on myocardial revascularisation itself.

In conclusion, our study indicates that platelets are inhibited more by aspirin in the postoperative period after OPCAB than after CABG with CPB, but further research has to be directed to the development of perioperative anticoagulation standards and the optimal use of antiplatelet agents after cardiac surgery without CPB.

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References


Appendix A. Conference discussion

Dr A. Hassan (Halifax, Canada): Did you look at these patients in terms of their platelet response to aspirin prior to the operation?

Dr Kurt: Of course. In our presentation we showed for the surgeon that every patient was not aspirin-resistant, and currently we investigated in our laboratory in cooperation with pharmacology the reason of the aspirin resistance.

One point to the question. A very interesting additional finding is that aspirin-resistant platelets are also resistant towards the different inhibition of cyclo-oxygenase. Our current hypothesis, this is only hypothesis, is that this type of aspirin resistance is located at the enzyme of the cyclo-oxygenase itself.

Dr R. Poston (Baltimore, MD, USA): I was interested in the fact that the platelet counts didn’t drop after off-pump CABG but the platelet function did. I wonder if you have an explanation for why that was the case?

Dr Kurt: Why the platelets get turned over you mean?

Dr Poston: On off-pump surgery your platelet counts didn’t drop, however, the platelet function, the aggregation and the thromboxane formation, even in the absence of aspirin, on the initial day after surgery, it dropped. What is the explanation for that?

Dr Kurt: The first point is that after cardiopulmonary bypass, the platelet counts get depleted, first, and the second one is before surgery we stop aspirin treatment and after surgery, on the first day after surgery, we give every patient aspirin, and aspirin makes acceleration of the platelets and this is irreversible, and the platelet recovery by regeneration after a few days after surgery. First they get depletion and go up and then they increased after a few days.