Role of Dipyrone in the High On-Treatment Platelet Reactivity amongst Acetylsalicylic Acid-Treated Patients Undergoing Peripheral Artery Revascularisation

Jan Hartinger^a Robert Novotny^b Jana Bilkova^d Tomas Kvasnicka^d
Petr Mitas^c Martin Sima^a Jaroslav Hlubocky^c Jan Kvasnicka^d Ondrej Slanar^a
Jaroslav Lindner^c

^aDepartment of Clinical Pharmacology and Pharmacy, Institute of Pharmacology, General University Hospital and 1st Faculty of Medicine, Charles University in Prague, Prague, Czech Republic; ^bTransplant Surgery Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; ^c2nd Department of Cardiovascular Surgery, General University Hospital and 1st Faculty of Medicine, Charles University in Prague, Prague, Czech Republic; ^dThrombotic Centre, General University Hospital, Prague, Czech Republic

Significance of the Study

- This study evaluated the effects of dipyrone (metamizole) on the effectiveness of acetylsalicylic acid in patients with peripheral arterial disease after surgical revascularisation on the lower extremities. It demonstrates that in patients with peripheral arterial disease, dipyrone can induce resistance to acetylsalicylic acid. This is important as many vascular centres use dipyrone as a postoperative analgesic.

Keywords

High on-treatment platelet reactivity · Vascular surgery · Peripheral arterial disease · Dipyrone

Abstract

Objective: To evaluate the effects of dipyrone on sensitivity to aspirin (acetylsalicylic acid [ASA]) in patients who underwent peripheral artery vascular reconstruction. **Subjects and Methods:** Impedance aggregometry and light transmission aggregometry were used to determine the effects of dipyrone on ASA treatment in 21 patients. Blood samples were drawn in a 7-day period after the surgery. The cut-off value for high on-treatment platelet reactivity (HTPR) was set at <

65% of aggregation inhibition for impedance aggregometry. For light transmission aggregometry the cut-off value for arachidonic acid-induced aggregation was set at >20% of aggregating platelets, and the cut-off value for epinephrine-induced aggregation was >44% of aggregating platelets. The cut-off value for each method was derived from a large number of patients treated with a daily dose of 100 mg of ASA. **Results:** We found HTPR in 14 (67%) of the 21 patients. None had primary resistance to ASA, i.e., after the addition of ASA in vitro all samples showed antiplatelet efficacy. Regression analysis showed a possible correlation between lower efficacy of ASA treatment and higher daily doses of dipyrone ($p = 0.005$ for impedance aggregometry, $p = 0.04$ for light transmission aggregometry), higher platelet count

($p = 0.005$ for impedance aggregometry), and shorter time from surgery ($p = 0.03$ for impedance aggregometry). **Conclusion:** HTPR occurs in 67% of ASA-treated patients after lower limb vascular surgery. The occurrence of HTPR correlates with the daily dose of dipyrrone. Therefore, dipyrrone should not be used as a postoperative analgesic in ASA-treated patients after peripheral artery revascularisation due to its influence on the effectiveness of ASA.

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Introduction

Low-dose aspirin (acetylsalicylic acid [ASA]) therapy reduces by 40% the incidence of coronary and peripheral artery closures after surgical revascularisation [1]. Despite the high efficacy of aspirin in the prevention of secondary cardiovascular events, up to 27–28% of patients do not respond to aspirin antiplatelet therapy. This high on-treatment platelet reactivity (HTPR) is associated with significantly higher risks of developing acute coronary syndrome, vascular graft failure, cerebrovascular events, and higher mortality [2, 3].

Even though HTPR is not strictly defined and its definition varies between different studies, high platelet reactivity despite ASA treatment has been repeatedly described as a negative prognostic factor for the further clinical course [3–7]. A meta-analysis by Krasopoulos et al. [2] showed an odds ratio of 4.06 for acute coronary syndrome, 3.78 for stroke, and 5.99 for overall mortality in HTPR patients; the odds ratio for any cardiovascular event is 3.8 [2, 7]. Patients diagnosed with HTPR early after coronary artery bypass grafting had the worst prognosis in the year following the surgery [8, 9], and the perioperative HTPR also correlated with venous coronary bypass obliteration [6].

Only one study [5] examined the incidence and clinical significance of aspirin resistance in patients with peripheral arterial disease (PAD). After balloon angioplasty of lower extremity arteries, a correlation between aspirin resistance and throughput-affected arteries was confirmed during annual monitoring. ASA resistance was defined as a lack of inhibition of platelet aggregation upon induction with collagen (COL) and adenosine diphosphate (ADP) measured by impedance aggregometry. In a group of 100 patients studied, there were 8 occlusions of arteries where laboratory findings confirmed ASA resistance. The patients were checked 4 times during a year. During each measurement performed on these patients, changes in the sensitivity to antiplatelet therapy were no-

ticed in terms of both increases and decreases in ASA effectiveness.

Dipyrrone (metamizole) and other non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to interfere with the efficacy of ASA by inducing HTPR [10–14]. The objective of this study was to evaluate the incidence of HTPR as well as the effect of dipyrrone on antiplatelet treatment in patients who underwent peripheral artery revascularisation.

Subjects and Methods

Patient Selection

A total of 21 patients (5 women, 16 men) with PAD who had undergone vascular procedures with no complications were selected for our study. The inclusion criterion was a daily dose of 100 mg of aspirin at least 6 days before testing for platelet reactivity. The exclusion criterion was a hospital stay <6 days. The average age of the patients was 68.9 ± 4.8 years. All patients were tested within the first 30 days after revascularisation of a lower extremity. Fifteen patients had undergone a bypass procedure (prosthetic, venous, or allograft bypass) and 6 patients had undergone other types of surgery/intervention (patch, thrombectomy, transmetatarsal amputation, percutaneous transluminal angioplasty, or angiography).

Investigation of Platelet Activity

Two different methods were used for the evaluation of platelet function: light transmission aggregometry (LTA) (PAP 8E Analyzer; Bio/Data Corporation, USA) and impedance aggregometry (Multiplate Analyzer; Roche, Munich, Germany) [15, 16]. For LTA, arachidonic acid (ARA), epinephrine (EPI), ADP, and COL were used as aggregation inducers.

COL was not used for HTPR determination because of its lower specificity for inhibition of aggregation induced by ASA when compared to ARA or EPI. ADP results were not used because the results of ADP-induced aggregation are influenced by concomitant clopidogrel use, which was common in our patients. Based on results obtained from a large group of ASA-treated patients, the cut-off value for HTPR was set at <65% platelet aggregation inhibition in the impedance aggregometry tests. For LTA, the cut-off value for ARA-induced aggregation was set at >20% of aggregating platelets, and the cut-off value for EPI-induced aggregation was set at >44% of aggregating platelets.

Impedance aggregometry was performed using whole blood. The TRAP assay with the inducer TRAP 6 was used for checking the total reactivity of platelets. TRAP is an activating peptide for thrombin receptors. This test is independent of antiplatelet therapy with ASA. Two tests were used to monitor the effectiveness of ASA: the ASPI test and the ASPI ASA test. The inducer in these tests is ARA (Roche; final concentration 0.5 mM). In this assay, the addition of 30 mg/mL ASA in vitro was used as a control test for the detection of primary resistance to ASA. The results are expressed as percent efficacy of ASA. For clinical use, efficiency is determined using a cut-off value of average efficiency at a daily dose of 100 mg of ASA. Patients identified as sensitive to aspirin therapy have a 65–100% effectiveness of therapy.

Table 1. Patient medical history and characteristics (*n* = 21)

Subgroup	ASA sensitive (<i>n</i> = 7, 33%)	HTPR (<i>n</i> = 14, 67%)	<i>p</i>
Female	1 (14)	5 (36)	0.608
Bypass surgery	3 (43)	12 (86)	0.124
Other revascularisation (PTA, thrombectomy, patch)	4 (57)	2 (14)	0.124
Active smoking	2 (29)	5 (36)	1.000
Diabetes mellitus	1 (14)	3 (21)	1.000
Arterial hypertension	4 (57)	11 (79)	0.608
Chronic kidney disease ¹	1 (14)	3 (21)	1.000
Coronary artery disease	2 (29)	6 (43)	0.874
Carotid artery disease ²	2 (29)	4 (29)	1.000

ASA, acetylsalicylic acid; HTPR, high on-treatment platelet reactivity; PTA, percutaneous transluminal angioplasty. ¹ Including 1 patient in the chronic haemodialysis programme. ² Including 1 patient after ischaemic stroke.

Table 2. Pharmacotherapeutic information (*n* = 21)

Subgroup	ASA sensitive (<i>n</i> = 7, 33%)	HTPR (<i>n</i> = 14, 67%)	<i>p</i>
Concomitant clopidogrel	5 (71)	7 (50)	0.640
Clopidogrel resistant ¹	3 (60)	2 (28)	0.621
Concomitant anticoagulation	5 (71)	12 (85)	0.844
Concomitant dipyrene	5 (71)	14 (100)	0.189
Acetaminophen	1 (14)	5 (36)	0.608
Opioids (dihydrocodeine, tramadol, oxycodone)	0 (0)	5 (36)	0.204
Proton pump inhibitors	3 (43)	5 (36)	1.000
HMG-CoA reductase inhibitors (statins)	1 (14)	2 (14)	1.000
Allopurinol	2 (29)	1 (7)	0.508
Calcium channel blockers	0 (0)	7 (50)	0.072
ACE inhibitors	2 (29)	5 (36)	1.000
ARBs (sartans)	0 (0)	3 (21)	0.508
Diuretics (e.g., indapamide, hydrochlorothiazide, furosemide, spironolactone, amiloride, and combinations)	2 (29)	6 (43)	0.874
β-Blockers	3 (43)	7 (50)	1.000

ASA, acetylsalicylic acid; HTPR, high on-treatment platelet reactivity. ¹ Clopidogrel users only.

If tested samples showed discrepant results, an additional platelet function test on a PFA-200 (Siemens, Germany) or VerifyNow (Accumetrics, San Diego, CA, USA) was performed in order to determine platelet function. Correlations between continuous variables and the percentage of platelet inhibition measured by LTA with ARA as an inducer, LTA with EPI as an inducer, and impedance aggregometry were examined. The impact of postoperative HTPR on individual clinical outcomes was not monitored.

Statistical Analysis

All variables (listed in Tables 1 and 2) were tested for normality via tests on skewness and kurtosis, as well as by Kolmogorov-Smirnov and Shapiro-Wilk tests. To verify statistically significant correlations between variables pairwise, the Pearson correlation coefficient was used; χ^2 analysis was used with Yates's correction for categorical variables. The Student *t* test was used for comparison of platelet counts between ASA non-responders and ASA responders. Linear regression analysis was used for continuous variables. For visualisation of the results, GraphPad Prism 3.02 (GraphPad Software, Inc., La Jolla, CA, USA) was used.

Fig. 1. Linear regression between the average daily dipyrene dose during the 6 days before blood sampling and the acetylsalicylic acid-induced antiaggregation effect measured by impedance aggregometry or light transmission aggregometry (LTA) ($n = 19$). **a** Impedance aggregometry. **b** LTA arachidonic acid. **c** LTA epinephrine.

Results

Detailed patient characteristics are summarised in Table 1. More patients had HTPR despite ASA treatment after vascular bypass grafting than after other procedures (80 vs. 33%, $p = 0.1$). Gender, smoking habits, and concomitant diseases (diabetes mellitus, arterial hypertension, chronic kidney disease, coronary artery disease, and carotid artery disease) were equally distributed among HTPR patients and patients with effective antiplatelet ASA treatment (Table 1). The use of clopidogrel, anticoagulants, proton pump inhibitors, statins, allopurinol, calcium channel blockers, ACE inhibitors, ARBs, diuretics, and β -blockers was not significantly different between HTPR patients and patients with effective antiplatelet treatment (Table 2).

Platelet counts were examined in 16 of the 21 patients. Only 6 patients, with known platelet counts at the time when blood samples were drawn, had an adequate response to ASA treatment. These patients had significantly lower platelet counts than the HTPR patients (274.8 ± 31.9 vs. 436.5 ± 40.7 , $p = 0.01$).

With the use of linear regression analysis, age, weight, and BMI did not significantly influence ASA sensitivity expressed as the percentage of aggregating platelets detected by impedance aggregometry and LTA. A significant correlation between platelet counts and the results of impedance aggregometry was found ($p = 0.005$), while LTA showed no such relationship. The longer the period after surgery, the higher was the efficacy of ASA treatment as measured by impedance aggregometry ($p = 0.03$). The higher the average dipyrene daily dose, the lower was the ASA effectiveness as measured by impedance aggregometry ($p = 0.005$) and LTA when EPI was used as an inducer ($p = 0.04$). The correlation between LTA results and an average daily dose of dipyrene was not significant when ARA was used as an inducer (Fig. 1). The results of impedance aggregometry correlated with the LTA results when ARA ($p = 0.001$) or EPI ($p = 0.04$) was used as an inducer. The pharmacotherapeutic details are summarised in Table 2.

No association was found between the tested variables and LTA results when COL was used as an inducer. In all, 57% of the patients were treated with dual antiplatelet therapy. ADP was also tested as an inducer in LTA tests. The results did not significantly correlate with daily dipyrene dose, time from surgery, and platelet counts in the whole patient group as well as in the patients treated with dual antiplatelet therapy [16] or aspirin alone [17]. The correlation missed statistical significance for platelet

count ($p = 0.070$) and time from surgery ($p = 0.079$) in the patients not treated with clopidogrel. Nevertheless, the number of patients tested was too small to detect a significant difference between clopidogrel users and non-users in LTA ADP tests ($p = 0.069$). The ASPI results in multiple tests did not differ significantly between the patients treated with dual antiplatelet therapy and those treated with aspirin alone ($p = 0.314$).

Discussion

Our study demonstrates that HTPR occurs despite treatment with ASA in two-thirds of patients with PAD. This high number of HTPR patients is probably caused by recent surgical intervention, which is known to be a significant contributor to the development of HTPR in surgical patients. A possible reason for transient post-surgical HTPR is a higher platelet turnover in this clinical setting or higher COX-2 expression in platelets after surgical interventions [18–21]. Although several reports have described resistance to antiplatelet therapy in patients undergoing a percutaneous intervention or coronary arterial bypass grafting or after stroke, limited data describing resistance to aspirin amongst patients with PAD are available [2, 5, 17–19, 22–24]. We have found only one study which examined the incidence and clinical significance of HTPR in patients with PAD [5].

Regression analysis showed a negative effect of a higher platelet count and a higher average daily dose of dipyrrone on platelet reactivity in ASA-treated patients detected by impedance aggregometry. The negative correlation of ASA efficacy with average daily dipyrrone dose was also significant in LTA testing if EPI was used as the inducer of platelet aggregation. No such correlation was observed when ARA was used as the inducer of platelet aggregation in LTA testing. This suggests that the difference between impedance aggregometry and LTA shows different predictive values for various platelet function tests [25, 26]. Several reports have shown that analgesic therapy with NSAIDs have a significant effect on the efficacy of aspirin [10–14]. The significance of this interaction was proven in healthy volunteers for ibuprofen and dipyrrone, which are widely used as perioperative analgesics in Central Europe [10]. Also, a few observational studies have confirmed the clinical importance of adverse interactions between ASA and NSAIDs in clinical settings [12, 13, 27]. In patients with coronary artery disease, dipyrrone was shown to nullify the antiplatelet efficacy of ASA soon af-

ter coronary artery bypass graft surgery [14]. This effect can be overcome by administering aspirin before dipyrrone [28]. We have shown that medication with dipyrrone should be weighed carefully against the risk of a lower effectiveness of ASA therapy in patients after lower extremity vascular surgery. We did not study the effect of other NSAIDs used in different clinical settings [29, 30], since only dipyrrone, acetaminophen, and opioids are used in our department.

All patients receiving angiotensin receptor blockers, calcium channel blockers, and opioids had HTPR, but this was most likely due to the small number of patients using these medications. Concomitant clopidogrel therapy did not significantly affect the results of the impedance aggregometry tests. The LTA test results with ADP as an aggregation inducer did not show any statistical significance when compared to clopidogrel users and non-users. The impedance aggregometry results of the ASPI test did not differ between patients treated with dual antiplatelet therapy and those treated with aspirin alone. Therefore, we can assume that clopidogrel did not affect the findings.

Our results did not show any correlation between the administration of proton pump inhibitors, higher BMI values, diabetes mellitus, and chronic kidney disease – which have been reported to be risk factors for the development of HTPR on ASA treatment [23, 31–33] – and LTA or impedance aggregometry results. This is probably caused by the small number of subjects in our study.

Conclusions

The present study shows an association between the use of dipyrrone and HTPR in patients with PAD. Our data suggest that a causal relationship exists between HTPR and dipyrrone administration. However, this study does not allow us to draw any firm conclusion due to the small number of patients tested. Thus, prospective randomised trials are needed to confirm our findings. The importance of this finding is stressed by the fact that treatment of postoperative pain with the use of dipyrrone is common in many countries.

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Statement of Ethics

This study was approved by the institutional ethics committee. Written consent was obtained from all patients.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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