Cardiovasc Drugs Ther (2014) 28:323–329
DOI 10.1007/s10557-014-6536-7

ORIGINAL ARTICLE

Laboratory Aspirin Resistance Reversibility in Diabetic Patients: a Pilot Study Using Different Pharmaceutical Formulations

Roberto Grimaldi · Marta Bisi · Enrica Lonni · Eloise Beggiato · Alessandra Valpreda · Marco Francesco Lococo · Enrico Dosio · Davide Giacomo Presutti · Milena Tagliabue · Fiorenzo Gaita

Published online: 2 July 2014

Abstract
Purpose Aspirin resistance occurs most frequently in diabetic patients and is associated with poor prognosis. The purpose of this study was to evaluate the prevalence of aspirin resistance in a cohort of diabetic patients and whether it can be reversed using more bioavailable aspirin formulations.
Methods Platelets function of 163 diabetic patients taking acetyl salicylic acid (ASA) 100 mg daily has been evaluated with PFA100 and VerifyNow. Patients found resistant by at least one test received an infusion of 288 mg of lysine acetylsalicylate (Flectadol®) corresponding to ASA 160 mg. Platelets function was measured again after 1 and 24 h. Patients whose the resistance was reversed received 288 mg of soluble salt of lysine acetylsalicylate (Cardirene 160®) corresponding to ASA160 mg instead of aspirin and their aggregation status was re-evaluated after 1 month of therapy.
Results Prevalence of aspirin resistance in our population was 18,4 % (30/163). In 27 out of 30 patients (90 %) aspirin resistance was reversed within 24 h from the infusion. 25 out of 27 patients (92 %) were found fully aspirin-sensitive after 1 month of oral therapy with soluble salt; two patients were found with borderline value. No adverse reactions were observed.
Conclusions A significant number of diabetic patients are resistant to aspirin therapy. A single intravenous dose of lysine acetylsalicylate can reverse the platelet hyper-aggregability and laboratory aspirin resistance in large majority of patients. The efficacy of antiaggregation can be maintained by chronic therapy with an oral drug with a more favourable pharmacokinetic profile.

Keywords Platelet responsiveness · Diabetes mellitus type 2 · Aspirin resistance · Point of care testing

Abbreviations

ACEi	angiotensin converting enzyme inhibitor
ACS	acute coronary syndrome
ARB	angiotensin receptor blockers
ASA	acetyl salicylic acid
BB	beta-blockers
CAD	coronary artery disease
COX 1	cyclooxygenase 1
CT	closure time
LAS	lysine acetylsalicylate
OH	oral hypoglycaemic
PFA	Platelet Function Analyzer
PPI	proton pump inhibitor
ROS	reactive oxygen species
SPECT	single-photon emission computed tomography
TIA	transient ischaemic attack

R. Grimaldi · M. Bisi (✉) · E. Lonni · M. F. Lococo · D. G. Presutti · F. Gaita
Division of Cardiology, Department of Internal Medicine, Città della Salute e della Scienza, Turin, Italy
e-mail: martadoro@hotmail.com

E. Beggiato · A. Valpreda · E. Dosio
Division of Haematology and Oncology, Department of Internal Medicine, Città della Salute e della Scienza, Turin, Italy

M. Tagliabue
Division of Endocrinology,Diabetology and Metabolism, Department of Internal Medicine, Città della Salute e della Scienza, Turin, Italy

Introduction

Cardiovascular disease is the leading cause of mortality in patients with type 2 diabetes mellitus, accounting for an estimated 65–80 % of deaths in these patients [1]. Although there have been substantial reductions in cardiovascular

related morbidity and mortality in the general population over the last 40 years attributed to improved treatment of cardiovascular risk factors and disease, the same benefit has not been observed in those with diabetes mellitus [2, 3].

A landmark observational study suggested that patients with diabetes without prior myocardial infarction had a similar risk of coronary heart disease to patients with prior myocardial infarction without diabetes [4].

Numerous pathophysiological mechanisms have been proposed to explain the exceptionally poor outcome of patients with diabetes and coronary artery disease including aggressive atherosclerosis [5], abnormal endothelial function [6, 7], impaired fibrinolysis, platelet hyperactivity [8]. Increased platelet activity plays a major role in the pathogenesis of thromboischaemic complications in diabetic patients.

Significant interest emerged for the widespread implementation of interventions for lowering cardiovascular risk in patients with diabetes, such as the use of aspirin therapy.

Several studies have demonstrated a beneficial role of antiplatelet therapy with aspirin in primary and secondary prevention of coronary heart disease in patients with diabetes [9].

Subgroup analysis of a diabetic cohort of the US Physicians Health Study demonstrated a reduction in the myocardial infarction rate ranging from 10.2 % in the placebo treated group to 4.0 % for the aspirin-treated group [10].

However, aspirin's antiplatelet effect is not uniform in all patients and reduced antiplatelet activity has been reported between 20 and 30 % of patients [11, 12]. This group seems to have a greater than threefold increase in the risk of major adverse events in stable coronary artery disease [13, 14].

The frequency of this phenomenon varies significantly, depending on the applied detection method and the investigated group of patients. Various study have investigated the effectiveness of antiplatelet therapy and, in their conclusion, several acquired conditions are supposed to play a role in the aspirin responsiveness [15], such as glycaemic control [16–18], hypertension, hypercholesterolemia, smoking or drugs [19–21], age [22] as well as genetic polymorphisms [23, 24].

Beside these factors, different modes of ASA administration might affect the rate of ASA absorption and, consequently, platelet response [25, 26] in fact a reduced absorption of acetyl salicylic acid could compromise the response to antiaggregation activity. Especially in diabetic patients, in which the peripheral circulation, even in the splanchnic area, is frequently affected, but also in patients with chronic gastroenteric disorders, on gastroprotective therapy or simply with diffuse vascular disease, an insufficient adsorption can be the main factor that makes antiaggregation inefficient.

The aim of our study was to investigate in a cohort of diabetic patients, with and without known cerebral or cardiovascular disease, the prevalence of aspirin resistance and whether this resistance can be reversed by using a more bioavailable formulation of salicylic acid such as the intravenous via and or soluble salt.

Methods

We prospectively enrolled 163 diabetic patients between November 2011 and December 2012. The patients were recruited from consecutive patients presenting to the outpatient clinic for follow-up treatment of type 2 diabetes who were chronically taking acetyl salicylic acid 100 mg daily. We enrolled both male and female over 18 years old with type 2 diabetes diagnosed at least 12 months earlier on pharmacological or insulin therapy and that were taking ASA 100 mg at least for 30 days before the enrolment. Compliance on aspirin was determined by patient interview both at study enrolment and follow-up. Patients taking oral anticoagulation therapy, with a personal history of haemorrhagic disorder, on chronic treatment with non-steroid anti-inflammatory drugs, under administration of heparin or low-molecular-weight heparin within 24 h before enrolment, acute coronary syndrome (ACS) or major cerebral vascular event or hospitalization for any cause within the 30 days before, excessive low or high platelet count (<130,000/μl or >450.000/μl) and history of haematological disorder have been excluded. The assumption of other anti aggregation therapy acting on P2Y12, such as clopidogrel, was not considered an exclusion criterion since the tests used to evaluate the aggregation status are specific for COX1 inhibition. Enrolled patients were taking ASA both in primary prevention, in order to reduce the risk of cardiovascular event in a high risk population, and in secondary prevention after ACS or transient ischaemic attack (TIA)/stroke. We also enrolled those patients with coronary artery disease (CAD) diagnosed by Treadmill test, myocardial single-photon emission computed tomography (SPECT) or coronary angiography.

This study complied with the Declaration of Helsinki and was approved by the Ethical Committee of the San Giovanni Battista Hospital (Turin, Italy); all patients signed an informed consent.

In all enrolled patients, platelets aggregation was assessed a preliminary test using PFA100 and VerifyNow between 8.00 and 10.00 am and the last assumption of enteric coated ASA was between 12.00 am and 2.00 pm of the previous day. Patients found resistant by at least one of the tests were called to receive and infusion of 288 mg of lysine acetylsalicylate (Flectadol®) corresponding to 160 mg of acetylsalicylic acid in 100 ml of saline solution between 10.00 and 12.00 am before the daily dose administration. All patients were instructed not to take aspirin for the following 24 h. Blood samples both for PFA100 and for VerifyNow were collected 1 and 24 h after the end of the infusion. Patients in whom the

resistance have been reversed received 288 mg of soluble salt of lysine acetylsalicylate (Cardirene 160®) corresponding to 160 mg of acetylsalicylic acid instead of aspirin as their chronic antiaggregation treatment. In our study the dosage was chosen based on the availability in Italy, at the time of enrolment, of the only dosages of 160 mg and 300 mg of oral lysine acetylsalicylate.

The platelet function of these patients was re-tested 1 month after the beginning of oral lysine acetylsalicylate (LAS).

Efficacy of anti-aggregation therapy was assessed using the platelet function analyser system (PFA 100) and the VerifyNow aspirin test.

In the PFA-100 device (Siemens, Marburg, Germany) the citrate whole blood is sucked at high shear rates in a capillary closed by a membrane with a micro opening. Some platelet agonists, collagen/epinephrine (first reaction cartridge) and collagen/ADP (second cartridge), trigger platelet activation and aggregation on the membrane's opening. This gradually slows down the blood's flow until the complete occlusion of the opening. PFA-100 measures the closure time (CT) from the start test [27, 28]. Aspirin usually prolongs only the collagen/epinephrine-induced CT [29]. Resistance to aspirin was defined as a normal collagen/epinephrine CT. In our laboratory, normal ranges for the first cartridge CT are 85–160 s. Patients with CTs >160 were defined as aspirin responders; patients with CTs <160 were defined as aspirin resistant.

Studies using PFA100 show that the resistance to the ASA stands in a range between 10 and 15 % [19, 30]. In the subgroup of aspirin resistant it has been detected a higher rate of adverse cardiovascular events [14].

In VerifyNow device (Accumetrics, San Diego, CA) the citrate whole blood is put in contact with fibrinogen-coated beads tied to the receptors on the platelet surface. Arachidonic acid used as agonist, induces platelet aggregation on the beads. The test measures the aggregation with a optical turbidimetric method [31]: the increase of the light transmittances is directly proportional to the degree of platelet aggregation. VerifyNow aspirin test measures platelet aggregation as aspirin reaction unit (ARU) with a cutoff for aspirin resistance at 550 ARU. Patients with ARU<550 were defined as aspirin responders; patients with ARU>550 were defined as aspirin resistant.

This test is highly specific for aspirin action on COX1, since there is a different cartridge for testing P2Y12 inhibitors, such as clopidogrel. Using the VerifyNow test the resistance rate to the aspirin range from 6 to 20 % [32, 33].

We considered to be resistant to ASA those patients who resulted resistant in at least one of the two tests used.

Statistics

Statistical significance was considered at the 5 % level (p= 0.05). Continuous variables are presented as mean±standard deviation and categorical variables are presented as frequencies and percentages. Differences between groups were evaluated by using independent samples 2-sided Student's t-test for continuous variables and Fisher's exact test for categorical variables. The agreement between the aspirin resistance status assessed by the various platelet function tests was evaluated with the use of the Cohen's k coefficient.

Results

One hundred sixty-three patients with diabetes mellitus were enrolled; 60 female (37 %) and 103 male (63 %) with a mean age of 68±7 years. All patients were taking aspirin at least for 30 days before the enrolment, 89 patients (55 %) in primary prevention while the remaining 74 patients (45 %) as secondary prevention (34 % for CAD and 11 % for stroke/TIA); the mean duration of diabetes mellitus was 18±9 years with a mediocre metabolic control (HbA1c 62±11,9 mmol/mol), the mean BMI was 29,01±6,01 which shows poor control of body weight.

Assessment of platelet function in basal sample showed a prevalence of aspirin resistance of 18,4 % (30 patients) in at least one of the two tests used; among these subjects 10 were resistant in both tests, whereas 12 only with PFA100 and 8 only with VerifyNow, showing a moderate concordance between assays (k=0,5).

There were no statistically significant differences in clinical characteristics between aspirin resistant and aspirin sensitive patients (Table 1), apart from BMI, higher in resistant population (in PFA100 and VerifyNow resistant group respectively 32,5±6,3 and 31,0±9,8, and 29,1±3,8 in the sensitive group; p=0,008) with no difference between those resistant to VerifyNow and PFA100, and a slightly higher prevalence of clopidogrel assumption between resistant patients.

No adverse reactions occurred during or following the infusion.

In 27 out of 30 patients (90 %) the aspirin resistance was reversed within 24 h from the infusion (in 22 at 1 h and in 5 at 24 h) using both platelets function tests. All the values of PFA100 and VerifyNow recorded at each point are listed in Tables 2–3; the trends of aggregation tests for each resistant patients are shown in Figs. 1 and 2.

Three patients (one resistant with PFA100 and two with VerifyNow) were found resistant with that assay after the infusion at 1 and 24 h. All patients whose aspirin resistance had been reversed were treated with LAS 288 mg (corresponding to ASA 160 mg) daily for 1 month.

All 27 patients were tested 1 month after the infusion on daily therapy with Cardirene and 25 patients have been found fully aspirin-sensitive, while two patients, both found resistant with PFA100, have been found with borderline value (150 and 153 s).

Table 1 Clinical characteristics compared between resistant and sensitive patients

Clinical characteristics compared between resistant and sensitive patients	PFA-100 resistant (22)	VerifyNow resistant (18)	Aspirin sensitive (133)
Age +/− SD (years)	67,4 +/− 9,5	68,9 +/− 9,0	68,5 +/− 9,0
Sex [F (%)/M(%)]	6 (27)/16 (73)	6 (35)/11 (65)	52 (39)/81 (61)
DM duration +/− SD (years)	19,4 +/− 9,8	20,9 +/− 9,0	17,6 +/− 9,1
HbA1c [mmol/mol +/− SD (%)]	65 +/− 11,1	57 +/− 7,7	62 +/− 11,9
	(8,1 +/− 1,4)	(7,4 +/− 1,0)	(7,8 +/− 1,5)
BMI +/− SD§	32,5 +/− 6,3	31,0 +/− 9,8	29,1 +/− 3,8
Smoker active n° (%), ex n° (%)	5 active (22)	3 active (17)	12 active (9)
	2 ex (9)	3 ex (17)	22 ex (16)
Hypertension n° (%)	19 (86)	12 (71)	115 (86)
Cholesterol:			
LDL +/− SD (mg/dl)	LDL 103,9 +/− 51,2	LDL 106,8 +/− 53,7	LDL 93,9 +/− 34,8
HDL +/− SD (mg/dl)	HDL 47,2 +/− 17,2	HDL 50,9 +/− 21,5	HDL 34,7 +/− 14,1
Creatinine (mg/dl)	1,07 +/− 0,4	1,06 +/− 0,5	1,01 +/− 0,3
ASA Indication:	13 (59)	11 (65)	75 (54)
Primary prevention (%)	9 (41)	6 (35)	58 (44)
Secondary prevention (%)	7 CAD 3 TIA/stroke	6 CAD 2 TIA/stroke	44 CAD 20 TIA/ stroke
Concomitant medications (%):			
- Thienopyridines	2 (9)	1 (6)	2 (1, 5)
- PPI	8 (36)	6 (35)	34 (26)
- ACEi	12 (54)	6 (35)	63 (47)
- ARB	9 (41)	6 (35)	47 (35)
- BB	9 (41)	6 (35)	57 (43)
- Diuretics:			
loop/thiazide/	8 (36)/4 (18)/	5 (29)/3 (18)/	24 (18)/29 (22)/
k spearing	3 (14)	1 (6)	16 (12)
- Statins	16 (72)	11 (65)	80 (60)
- Insulin	11 (50)	7 (41)	56 (42)
- OH	14 (64)	9 (53)	102 (77)

§ p=0,008

Discussion

In our study the prevalence of aspirin resistance among diabetic patients was 18 %, as reported in previous studies [12]. In our cohort it does not seem influenced by age, sex, duration of diabetes or metabolic control, smoking, hypertension, dyslipidaemia, creatinine level, or by the presence of a previous ischaemic event [30]; also the therapy associated with aspirin was found to be irrelevant. In previous studies some of these characteristics were reported to be related with a higher prevalence of aspirin resistance, particularly metabolic control [18, 19], but data are still controversial.

In our population we found the resistant group to have higher BMI than sensitive group. As described in previous papers (17, 34) a greater volume of distribution could influence the efficacy, and increasing the dose could have played a role in reversing ASA resistant in this group. Nevertheless also in the sensitive group there are obese patients and in the resistant group there are patients with BMI <25, showing as there is not a direct correlation between BMI and ASA responsiveness and increasing the dose can not be considered the only explanation to the reversibility obtained.

The relative higher prevalence of patients assuming clopidogrel in the resistant group is probably an incidental finding considering the very small overall amount of patient assuming thienopyridines in our population, since patients with very recent ACS were excluded. Alternatively the use of clopidogrel could define a group of patients with higher risk for cardiovascular events.

The high prevalence of aspirin resistance in a population already with higher risk than general population could have important clinical implication [13, 14, 35]. Abnormal platelet function may be related to several mechanisms, including metabolic alterations, oxidative stress and endothelial dysfunction. Platelets in diabetic subjects are characterized by enhanced thromboxane synthesis; furthermore hyperglycaemia induces

Table 2 VerifyNow values for resistant patients

VerifyNow R	basal	1 h	24 hs	30 days
#1	573	387	370	420
#2	563	399	444	421
#3	577	479	489	524
#4	638	405	428	385
#5	651	400	415	408
#6	641	553	407	528
#7	599	563	423	535
#8	564	427	415	534
#9	562	437	428	518
#10	583	571	512	417
#11	594	415	487	467
#12	586	432	410	452
#13	589	405	408	470
#14	570	562	576	565
#15	579	559	560	556
#16	581	587	384	539
#17	553	533	367	440

Table 3 PFA 100 values for resistant patients

PFA 100 R	basal	1 h	24 hs	30 days
#2	109	203	243	213
#3	101	>300	>300	>300
#4	112	>300	>300	>300
#5	123	261	289	>300
#6	139	>300	>300	>300
#7	127	235	>300	>300
#9	128	>300	218	>300
#13	111	>300	300	>300
#15	137	210	>300	173
#18	119	>300	>300	>300
#19	107	>300	>300	180
#20	117	>300	>300	>300
#21	140	>300	>300	>300
#22	99	81	>300	150
#23	141	>300	>300	274
#24	133	187	167	153
#25	143	>300	164	>300
#26	121	>300	>300	168
#27	128	294	291	>300
#28	119	151	95	120
#29	138	>300	300	>300
#30	129	>300	291	>300

The same number corresponds to the same patient in both Tables 2 and 3. The patients included in both tables are those resistant to both tests, those included in only one table are resistant only to the test of that table

Values over (for VerifyNow) or under for PFA 100) the cut off value are written in bold

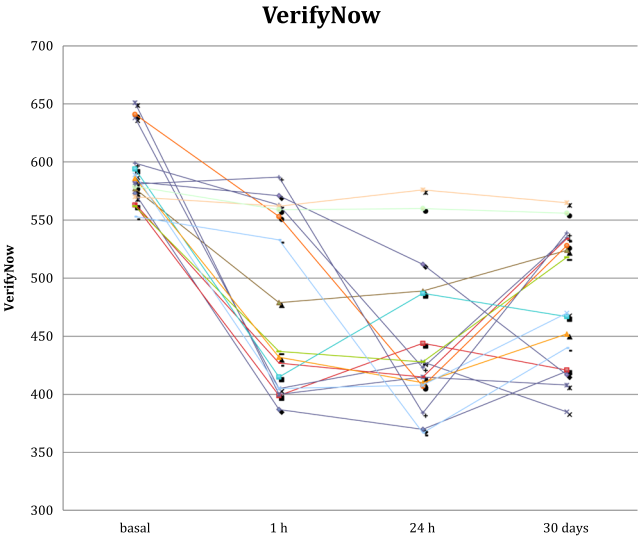

Fig. 1 VerifyNow values trends. Cut off value for resistance is over 550 ARU

reactive oxygen species (ROS) inducing oxidative stress. ROS lead to activation of transcription factors, such as NF-kB and activator protein-1, and subsequent production of pro-inflammatory cytokines and adhesion molecules and to reduction of synthesis of nitric oxide [36]. These mechanisms are implicated in a reduced response to aspirin activity, but also other mechanisms have been proposed for aspirin resistance [37, 38].

In our study we provide a support for the assumption that delayed or impaired intestinal absorption plays and important role in the absence of laboratory platelet response to aspirin in diabetic patients. In particular we focused on the pharmaceutical formulation since the absorption can be deeply altered by different formulations [26, 39]. This is also underlined by the persistence of the reversion of aspirin resistance not only after the infusion but also after 1 month of oral assumption of soluble salt at the same dose of the infusion instead of

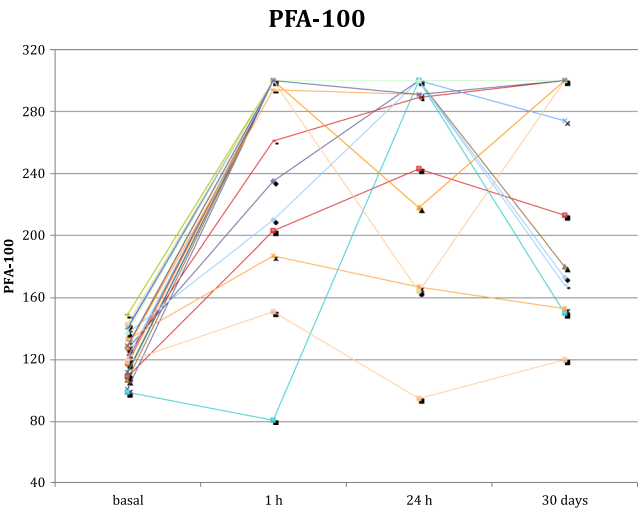

Fig. 2 PFA 100 values trends. Cut off value for resistance is under 160 s

enteric-coated pills, in most of the patients who had their resistance reversed. This formulation has shown not to increase the adverse events or gastric intolerance or compliance [40, 41]. This observation could provide a further option for improving the antiaggregation therapy in high-risk patients, as diabetic ones, tailoring the therapy on the basis of quick and easy test available as point of care in many centres.

Nevertheless there is still a small percentage (10 %) of patients that are non-responsive to antiaggregation therapy even after endovenous administration confirming the important role of the others mechanisms previously described.

One contributing cause for the efficiency of intravenous aspirin may be the, even slightly, higher dose, chosen based on the commercial availability. Several studies demonstrated that the inhibition of thromboxane formation was dose-dependent [42, 43], but other studies were unable to reveal any dose-effect relationship using aspirin doses between 30 and 325 mg per day [44]. Some authors have also hypothesized that an accelerated platelet turnover or the interindividual variability in the recovery of platelet cyclooxygenase activity during the dosing interval may limit the duration of the antiplatelet effect of low-dose aspirin in patients with diabetes mellitus and it might explain an incomplete laboratory response to aspirin therapy. Following these assumptions, they have demonstrated improved platelet antiaggregation with an administration twice daily at low dose (75 mg) compared to single daily administration at high dose (320 mg) [45, 46].

Our study, in agreement with previous findings, suggests that different platelet function assays that are suitable for detecting aspirin resistance cannot be used interchangeably since there is only a moderate concordance between the tests used (K=0,5) [47–49]. Furthermore the prevalence of aspirin resistance might have been overestimated by the use of tests on whole blood due to enhanced interaction, in diabetic patients, between platelets and red blood cells, which release large contents of adenosine diphosphate (ADP) y. The tests used have been chosen on the basis of accessibility, economic sustainability, ease and rapidity in their execution among tests validated.

This study has several important limitations; we did not measure the blood salicylate or serum/plasma thromboxane A2 metabolite levels, number of cases was small, the laboratory follow up was at short time and the assessment of compliance only by interview.

Despite such limitations our study highlight a new promising approach to reduce the prevalence of laboratory aspirin resistance.

In addition, our study is an observational study, for this reason no formal sample size determination was performed and all patients belong to a sequential single-arm cohort,-depending on their presentation at our outpatient clinic.

As this study was planned as proof of concept, a further prospective controlled clinical trial with two arms for comparison between the standard therapy and the "reversed resistance" with adequate sample size needs to be performed.

In conclusion, diabetic patients show markedly augmented platelet function despite regular oral intake of 100 mg of aspirin daily. A single intravenous dose of lysine acetylsalicylate, corresponding to 160 mg of acetyl salicylic acid, instead of the standard oral 100 mg of the enteric-coated formulation, can reverse the augmented platelet aggregability and laboratory aspirin resistance in the large majority of patients found resistant with standard therapy. The chronic therapy with an oral drug with a more favourable pharmacokinetic profile, such as the soluble salt, has shown to maintain the efficacy of antiaggregation in short follow-up in those who had their resistance reversed. Additional clinical studies are needed to find the optimal dosing and pharmaceutical formulation and to confirm the clinical significance of reversion of laboratory aspirin resistance in diabetic patients.

Acknowledgment We would like to thank Roberto Passera PhD, for his assistance during the study.

Declaration of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med. 1998;4:229–34.
2. Gu K, Cowie CC, Harris MI. Diabetes and decline in heart disease mortality in US adults. JAMA. 1999;14:1291–7.
3. Kahn R, Robertson RM, Smith R, Eddy D. The impact of prevention on reducing the burden of cardiovascular disease. Diabetes Care. 2008;8:1686–96.
4. Bulugahapitiya U, Siyambalapitiya S, Sithole J, Idris I. Is diabetes a coronary risk equivalent? systematic review and meta-analysis. Diabet Med. 2009;2:142–8.
5. Aronson D, Bloomgarden Z, Rayfield EJ. Potential mechanisms promoting restenosis in diabetic patients. J Am Coll Cardiol. 1996;27:528–35.
6. Donatelli M, Hoffmann E, Colletti I, et al. Circulating endothelin-1 levels in type 2 diabetic patients with ischaemic heart disease. Acta Diabetol. 1996;33:246–8.
7. Williams SB, Goldfine AB, Timimi FK, et al. Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. Circulation. 1998;97:1695–701.
8. Colwell JA, Nesto RW. The platelet in diabetes: focus on prevention of ischemic events. Diabetes Care. 2003;26:2181–8.
9. Antithrombotic Trialists Collaboration. Collaboration meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ. 2002;324:71–86.
10. The Physicians' Health Study. Aspirin for the primary prevention of myocardial infarction. N Engl J Med. 1988;318:924–6.
11. Patrono C. Aspirin resistance: definition, mechanisms and clinical read-outs. J Thromb Haemost. 2003;1:1710–3.

12. Fateh-Moghadam S, Plöckinger U, Cabeza N, et al. Prevalence of aspirin resistance in patients with type 2 diabetes. Acta Diabetol. 2005;43:99–103.
13. Krasopoulos G, Brister SJ. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. BMJ. 2008;336:195–8.
14. Snoep JD, Hovens MM, Eikenboom JC, van der Bom JG, Huisman MV. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events a systematic review and meta-analysis. Arch Intern Med. 2007;167:1593–9.
15. Cambria-Kiely JA, Gandhi PJ. Possible mechanisms of aspirin resistance. J Tromb Thrombolysis. 2002;13:49–56.
16. Watala C, Golanski J, Pluta J, et al. Reduced sensitivity of platelets from type 2 diabetic patients to acetylsalicylic acid (aspirin);its relation to metabolic control. Thromb Res. 2004;113:101–13.
17. Cohen HW, Crandall JP, Hailpern SM, Billett HH. Aspirin resistance associated with HbA1c and obesity in diabetic patients. J Diabetes Complicat. 2008;22:224–8.
18. Ertugrul DT, Tutal E, Yildiz M, et al. Aspirin resistance is associated with glycaemic control, the dose of aspirin, and obesity in type 2 diabetes mellitus. J Clin Endocrinol Metab. 2010;95:2897–901.
19. Kim H, Lee HK, Han K, Jeon HK. Prevalence and risk factors for aspirin and clopidogrel resistance in patients with coronary artery disease or ischemic cerebrovascular disease. Ann Clin Lab Sci. 2009;39:289–94.
20. Würtz M, Grove EL, Kristensen SD, Hvas AM. The antiplatelet effect of aspirin is reduced by proton pump inhibitors in patients with coronary artery disease. Heart. 2010;96:368–71.
21. Bauriedel G, Skowasch D, Schneider M, Andrié R, Jabs A, Lüderitz B. Antiplatelet effects of angiotensin converting enzyme inhibitors compared with aspirin and clopidogrel: a pilotstudy with whole-blood aggregometry. Am Heart J. 2003;145(2):343–8.
22. Maree AO, Curtin RJ, Dooley M, et al. Platelet response to low-dose enteric-coated aspirin in patients with stable cardiovascular disease. J Am Coll Cardiol. 2005;46:1258–63.
23. Xu ZH, Jiao JR, Yang R, Luo BY, Wang XF, Wu F. Aspirin resistence: clinical significance and genetic polymorphism. J Int Med Res. 2012;40:282–92.
24. Sharma V, Kaul S, Al-Hazzani A, Alshatwi AA, Jyothy A, Munshi A. Association of COX-2 rs20417 with aspirin resistance. J Thromb Thrombolysis. 2013;35:95–9.
25. Grosser T, Fries S, Lawson JA, Kapoor SC, Grant GR, FitzGerald GA. Drug resistance and pseudoresistance: an unintended consequence of enteric coating aspirin. Circulation. 2013;127:377–85.
26. Feldman M, Cryer B. Aspirin absorption rates and platelet inhibition time with 325-mg buffered aspirin tablets (chewed or swallowed intact) and buffered aspirin solution. Am J Cardiol. 1999;84:404–9.
27. Kratzer MA, Born GV. Simulation of primary haemostasis in vitro. Haemostasis. 1985;15:357–62.
28. Kundu SK, Heilmann EJ, Sio R, Garcia C, Davidson RM. Ostgaard RA description of on in vitro platelet function analyzer – PFA 100. Semin Thromb Hemost. 1995;21:106–12.
29. Jilma B. Platelet function analyzer (PFA-100): a tool to quantify congenital or acquired platelet dysfunction. J Lab Clin Med. 2001;138:152–63.
30. Van Werkum JW, Harmsze AM, Elsenberg EH, Buoman HJ, Ten Berg JM, Hackeng CM. The use of the verifyNow system to monitor antiplatelet therapy: a review of the current evidence. Platelets. 2008;19:479–88.
31. Gum PA, Kottke-Marchant K, Poggio ED, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. Am J Cardiol. 2001;88:230–5.
32. Lordkipanidzé M, Diodati JG, Schampaert E, Palisaitis DA, Pharand C. Prevalence of unresponsiveness to aspirin and/or clopidogrel in patients with stable coronary heart disease. Am J Cardiol. 2009;104:1189–93.
33. Karnabatidis D, Spiliopoulos S, Pastromas G et al. Prevalence of nonresponsiveness to aspirin in patients with symptomatic peripheral arterial disease using true point of care testing. Cardiovasc Intervent Radiol 2013
34. Peace A, McCall M, Tedesco T, et al. The role of weight and enteric coating on aspirin response in cardiovascular patients. J Thromb Haemost. 2010;8:2323–5.
35. Hobikoglu GF, Norgaz T, Aksu H, et al. The effect of acetylsalicylic acid resistance on prognosis of patients who have developed acute coronary syndrome during acetylsalicylic acid therapy. Can J Cardiol. 2007;23:201–6.
36. Angiolillo DJ. Antiplatelet therapy in type 2 diabetes mellitus. Curr Opini Endocrinol, Diabetes Obes. 2007;14:124–31.
37. Cipollone F, Rocca B, Patrono C. Cyclooxygenase-2 expression and inhibition in atherothrombosis. Arterioscler Thromb Vasc Biol. 2004;24:246–55.
38. Valles J, Santos MT, Aznar J, Velert M, Barberá G, Carmena R. Modulatory effect of erythrocytes on the platelet reactivity to collagen in IDDM patients. Diabetes. 1997;46:1047–53.
39. Cox D, Maree AO, Dooley M, Conroy R, Byrme MF, Fitzgerald DJ. Effect of enteric coating on antiplatelet activity of low-dose aspirin in healthy volunteers. Stroke. 2006;37:2153–8.
40. Majluf-Cruz A, Chavez-Ochoa AR, Majluf-Cruz K, et al. Effect of combined administration of clopidogrel and lysine acetylsalicylate versus clopidogrel and aspirin on platelet aggregation and activated GpIIb/IIIa expression in healthy volunteers. Platelet. 2006;17:105–7.
41. Gurfinkel EP, Altman R, Scazziota A, Heguilen R, Mautner B. Fast platelet suppression by lysine acetylsalicylate in chronic stable coronary patients. Potential clinical impact over regular aspirin for coronary syndromes. Clin Cardiol. 2000;23:697–700.
42. Fitzgerald GA, Oates JA, Hawiger J, et al. Endogeneous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. J Clin Invest. 1983;71:676–88.
43. Hart RG, Leonard AD, Talbert RL, et al. Aspirin dosage and thromboxane synthesis in patients with vascular disease. Pharmacotherapy. 2003;23:579–84.
44. Dippel DW, Van Kooten F, Leebeek FW, et al. What is the lowest dose of aspirin for maximum suppression of in vivo thromboxane production after a transient ischemic attack or ischemic stroke? Cerebrovasc Dis. 2004;17:296–302.
45. Rocca B, Santilli F, Pitocco D, et al. The recovery of platelet cyclooxygenase activity explains interindividual variability of responsiveness to low-dose aspirin in patients with and without diabetes. J Thromb Haemost. 2012;10:1220–30.
46. Spectre G, Arnetz L, Ostenson CG, Brismar K, Li N, Hjemdahl P. Twice daily dosing of aspirin improves platelet inhibition in whole blood in patients with type 2 diabetes mellitus and micro- or macrovascular complications. Thromb Haemost. 2011;106(3):491–9.
47. Harrison P, Segal H, Silver L, Syed A, Cuthbertson FC, Rothwell PM. Lack of reproducibility of assessment of aspirin responsiveness by optical aggregometry and two platelet function tests. Platelets. 2008;19:119–24.
48. Chakroun T, Addad F, Abderazek F, et al. Screening for aspirin resistance in stable coronary artery patients by three different tests. Thromb Res. 2007;112:413–8.
49. Karon BS, Wockenfus A, Scott R, et al. Aspirin responsiveness in healthy volunteers measured with multiple assay platforms. Clin Chem. 2008;54:1060–5.