Thrombosis Research 130 (2012) 350–354

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Aspirin insensitive thromboxane generation is associated with oxidative stress in type 2 diabetes mellitus

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ARTICLE INFO

Article history:
Received 8 November 2011
Received in revised form 6 March 2012
Accepted 27 March 2012
Available online 20 April 2012

Keywords:
Thromboxane
Platelet inhibition
Aspirin resistance
Oxidative stress
Diabetes

ABSTRACT

Introduction: Aspirin (ASA) irreversibly inhibits platelet cyclooxygenase-1 (COX-1) leading to decreased thromboxane-mediated platelet activation. The effect of ASA ingestion on platelet activation, thromboxane generation, oxidative stress and anti-oxidant biomarkers was studied in type 2 diabetes mellitus (DM).
Material and methods: Baseline and post-ASA samples (100/325 mg x 7 days) were obtained from 75 DM patients and 86 healthy controls for urinary 11-dehydro-thromboxane B2 (11dhTxB2), 8-iso-prostaglandin-F2α (8-isoPGF2α) and serum sP-Selectin, nitrite (NO_2^-), nitrate (NO_3^-) and paraoxonase 1 (PON1) activity.
Results: Compared to baseline controls, baseline DM had higher mean levels of 11dhTxB2 (3,665 ± 2,465 vs 2,450 ± 1,572 pg/mg creatinine, $p = 0.002$), 8-isoPGF2α (1,457 ± 543 vs 1,009 ± 412 pg/mg creatinine, $p < 0.0001$), NO_2^- (11.8 ± 7.3 vs 4.8 ± 5.3 μM, $p < 0.0001$), NO_3^- (50.4 ± 39.3 vs 20.9 ± 16.7 μM, $p < 0.0001$) and sP-Selectin (120.8 ± 56.7 vs 93.0 ± 26.1 ng/mL, $p = 0.02$), and the same held for post-ASA levels ($p < 0.0001$). ASA demonstrated no effect on 8-isoPGF2α, NO_2^-, NO_3^-, sP-Selectin or PON1 activity in either DM or controls. Post ASA inhibition of urinary 11dhTxB2 was 71.5% in DM and 75.1% in controls. There were twice as many ASA poor responders in DM than in controls (14.8% and 8.4%) based on systemic thromboxane reduction. Urinary 8-isoPGF2α excretion was greater in DM ASA poor responders than good responders ($p < 0.009$).
Conclusions: This suggests that oxidative stress may maintain platelet function irrespective of COX-1 pathway inhibition and/or increase systemic generation of thromboxane from non-platelet sources.
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Introduction

Acetylsalicylic acid (ASA) is widely prescribed as an aid in the prevention of cardiovascular and cerebrovascular disease (CVD) because it irreversibly acetylates platelet cyclooxygenase-1 (COX-1) for the entire life cycle of the platelet; this results in the inhibition of platelet thromboxane A2 generation (TxA2), a potent vasoconstrictor and promoter of platelet aggregation [1]. Despite its widespread use, not all individuals respond to ASA in the same way [2]. In various arterial thrombotic diseases low dose ASA blocks over 95% of platelet COX-1 activity thus reducing the risk of CVD [3]. Moreover, ASA effectiveness is limited because 10-20% of patients with arterial thrombosis may develop recurrent vascular events while on ASA treatment. Inadequate (poor) response to therapeutic doses based on laboratory tests has been referred to as aspirin resistance [4]. Poor response to aspirin treatment has been described in healthy populations as well as in patients with diabetes (DM) or CVD though the exact mechanisms responsible for this clinical state remain numerous and unclear.

Genetic variations, increased platelet turnover, platelet activation by alternative pathways, alternative/additional sources of TxA2 production such as COX-2 in macrophages/monocytes and issues with drug bioavailability have all been implicated in ASA unresponsiveness [5]. Recent reports suggested that patients with coronary artery disease (CAD) associated with high serum concentrations of cholesterol, triglyceride and C-reactive protein (CRP) had reduced response to ASA measured by platelet aggregation and urinary 11-dehydro thromboxane B2 (11dhTxB2) [6]. Compared to asymptomatic patients, those with CAD had significantly higher levels of urinary

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doi:10.1016/j.thromres.2012.03.025

11dhTxB2 following ASA ingestion. The HOPE [7] and the CHARISMA [8] studies showed that urinary 11dhTxB2 levels in ASA treated patients predict the future risk of stroke, myocardial infarction and cardiovascular death. These findings raise the possibility that elevated urinary 11dhTxB2 excretion identifies patients who while on ASA treatment are at elevated risk of adverse events and may benefit from additional anti-platelet agents or treatment modification.

ASA is widely recommended for the primary prevention of cardiovascular events in DM but the evidence supporting its efficacy is surprisingly scarce and controversial [9]. High on-ASA platelet reactivity (HAPR) has been proposed as a much more appropriate term than aspirin resistance to describe a high platelet reactivity status despite ASA therapy in an individual patient [10]. HAPR has been associated with atherothrombotic events following major vascular procedures and may identify patients at high risk for re-occlusion following percutaneous intervention (PCI) with stenting [11]. Recent observations concluded that healthy subjects with poor aspirin response not only manifest an incomplete inhibition of COX-1, but also a pro-inflammatory milieu and enhanced oxidative stress [12]. The same study also showed that diet-induced weight loss in subjects with central obesity reduces platelet activation and restores platelet sensitivity to nitric oxide, prostacyclin, and physiologic anti-aggregating agents [13]. Considering that platelets are not the only factor contributing to thrombus formation, it is not surprising that a single anti-platelet agent such as aspirin does not prevent all adverse events. To further investigate this topic we assessed the effect of short term ASA treatment on various oxidative and inflammatory biomarkers in DM patients and healthy subjects.

Material and Methods

Patients and Protocol

This study was as an open label, 1:1 randomized assignment ASA 100 mg daily, ASA 325 mg daily in consecutive DM patients (and controls) attending the endocrinology and diabetes outpatient clinic at the Hospital General de Occidente, Zapopan, Mexico, from January to August 2009. The diagnosis of type 2 DM was based on the presence of abnormal fasting glucose (normal range 70–110 mg/dl) abnormal glucose tolerance test, chronic hyperglycemia and metabolic disturbances of lipid, carbohydrate and protein metabolism due to defects in insulin production and/or activity. Exclusion criteria were macro-albuminuria or symptomatic nephropathy, cardiovascular disease (myocardial infarction, angina, peripheral arterial disease and stroke), concomitant systemic acute or chronic inflammatory diseases (bacterial or viral infections), concomitant acute or chronic autoimmune disorders, pregnancy, aspirin use within 30 days of study recruitment or intolerance to ASA. DM patients with hypertension, obesity, dyslipidaemia and micro-albuminuria were accepted within the study: hypertension was defined as a systolic blood pressure >130 mm Hg and/or diastolic blood pressure >85 mm Hg and obesity as a Body Mass Index (BMI) >27 for the Mexican population. Seventy-five type 2 DM patients (58 F, 17 M, mean age 53 ± 14 years, mean disease duration 9.6 ± 7.6 years) and 86 age and sex matched healthy individuals (52 F, 34 M, mean age 46 ± 10 years) recruited from hospital personnel (physicians, nurses, laboratory, etc.). Fasting blood and urine samples were obtained from all participants (DM and controls) at study entry before the ingestion of ASA (baseline sample) and at the end of the 7 days of daily ingestion of 100 or 325 mg of ASA (post-ASA sample). Patients were instructed not to modify other regular medications throughout the study. Physicians involved in the care of the patients were not involved in data analysis or interpretation. The study (#153/07) was approved by the ethics committee of the Hospital General de Occidente, Zapopan (Mexico), and all participants signed an informed consent before entering the study in accordance with the declaration of Helsinki.

Measurement of urinary 11dhTxB2 and 8-isoprostane (8-isoPGF2α)

Urinary 11dhTxB2 was measured on spot urine samples collected from each study subject before and after ASA ingestion using the AspirinWorks® 11dhTxB_2 ELISA (Corgenix, Inc. Broomfield, Colorado). Urinary 8-iso-prostaglandin-F2α (8-isoPGF2) was measured on the same samples using the 8-Isoprostane EIA kit (Cayman Chemical Co, Ann Arbor, Michigan, Catalog # 516351) according to manufacturers' instructions. All urine samples were stored frozen at −70 °C until tested. Urinary creatinine was also measured to normalize 11dhTxB2 or 8-isoPGF2α as a ratio to creatinine. The results were expressed in picograms of 11dhTxB2 (or 8-isoPGF2α) per milligram of creatinine. The urinary 11dhTxB2 cut-off value of 1,500 pg/mg creatinine established by the manufacturer was used to classify an abnormal (poor) ASA response (>1,500) from a normal (good) ASA response (<1,500) as compared to an ostensible healthy population.

Measurement of Human Soluble P-Selectin

sP-Selectin was measured on serum collected from study subjects before and after ASA ingestion by the Human sP-Seletin/CD62P Immunoassay (R&D Systems, Inc. Minneapolis, Minnesota), according to manufacturers' instructions. Results were expressed in ng/mL. All serum samples were stored frozen at −70 °C until tested.

Measurement of Nitrite and Nitrate

Nitrite (NO_2^-) and nitrate (NO_3^-) were determined in serum by a modified Griess reaction, following the reduction of nitrate to nitrite using nitrate reductase and nicotinamide adenine dinucleotide phosphate (NADPH). The assay was performed in a standard flat-bottomed 96-well microtitre plate divided into halves: one half served for the measurement of NO_2^- and the other half for the sum of NO_2^- and NO_3^- concentrations. The concentration of NO_3^- was obtained by subtracting NO_2^- from the sum of NO_2^- and NO_3^-. To each well was added 50 μL of standard or diluted sample and analyzed in duplicate. The assay was blanked against phosphate buffer. In half plate, 4 μL of nitrate reductase (Sigma-Aldrich) and 10 μL of NADPH (Sigma-Aldrich) were added to each well at final concentrations of 6.3 U/L and 550 μmol/L respectively. The plate was incubated at room temperature for 2 h. The Griess reaction was initiated by the addition to each well of equal volumes of 2% sulfanilamide (Sigma-Aldrich) in H3PO4 5% and 0.2% N-(1-naphthyl)-ethylenediamine dihydrochloride (Sigma-Aldrich) in water, mixed immediately before use. The absorbance of the reaction mixture was measured at 540 nm after 10 min at room temperature and the concentrations of the metabolites expressed as μM.

Measurement of Paraoxonase 1 Activity

Serum paraoxonase 1 activity (PON1) was measured in serum as follows: paraoxon (1 mM) (Sigma-Aldrich) freshly prepared in 50 mM glycine buffer containing 1 mM calcium chloride (pH 10.5) was incubated at 37 °C with patients serum for 10 min in 96 well plates (PolySorp) and p-nitrophenol formation monitored at 412 nm. Enzyme activity was calculated with a molar extinction coefficient of 18.290 Mcm^{-1} and expressed as U/L, which is defined as 1 μmol of p-nitrophenol generated per minute per liter under assay conditions.

Statistical Analysis

For categorical variables, Chi-squared test or Fisher's exact test were used; for continuous variables and to analyze the effect of ASA ingestion on study variables, paired *t*-test and Wilcoxon Signed Rank Sum test (when applicable) were used. Association between variables was assessed by univariate analysis (Pearson's rho) and the assumptions of

Table 1
Demographics characteristics of the study cohort.

	Type 2 DM (n = 75)	Healthy controls (n = 86)
Mean age (years)	53.7 ± 14.1	46.8 ± 10.5
Gender (F/M)	58/17	52/34
Mean disease duration (years)	9.6 ± 7.6	n/a
Mean BMI (Kg/m^2)	28.9 ± 4.4	n/a
Mean fasting glucose (mg/dL)	154.5 ± 78.7	n/a
Mean HbA1c (%)	8.1 ± 2.1	n/a
Hypertension (%)	56.2	n/a
Nephropathy (%)	7.1	n/a
Retinopathy (%)	11.9	n/a
Insulin (%)	25.3	n/a
Oral anti-diabetics (%)	70.7	n/a
Other medications: blood pressure, statins, etc. (%)	19.2	n/a

n/a = not available or applicable.

the univariate analysis were tested by multiple regression. Statistical analysis was performed the JMP 8.0.1 program from SAS Institute Inc. North Carolina, USA.

Results

Correlation of Baseline Variables in DM and Controls

Demographics and clinical characteristics of the study participants are summarized in Table 1. Correlation of baseline biomarkers in DM patients showed only a weak negative association between serum sP-Selectin and NO_3^- ($r = -0.293$, $p = 0.06$). No baseline correlations were identified in the control group.

Comparison of Baseline and Post-ASA Markers Between DM and Controls

A preliminary analysis (not shown) did not reveal any significant differences in urinary 11dhTxB2 levels at baseline or post-ASA between subjects ingesting 100 mg or 325 mg of ASA in either the DM or control groups, therefore these data were pooled.

The results of the study are summarized in Table 2. At baseline the mean urinary 11dhTxB2 concentration of DM patients was 49.6% higher than that of healthy controls ($p = 0.001$) and was 59.6% higher post-ASA ingestion ($p < 0.0001$). ASA treatment inhibited urinary 11dhTxB2 excretion by 71.5% in DM patients and by 75.1% in controls. As many as 14.8% of DM patients had post-ASA levels above the response level cut-off of 1500 pg 11dhTxB2/mg creatinine compared to 8.4% of controls though this difference was not statistically significant ($p = 0.37$). These patients were subsequently defined as poor ASA responders.

Age had no effect on 11dhTxB2 levels whereas gender demonstrated minor effects. Within the DM group, urinary 11dhTxB2 excretion was greater in females than males (3152 vs 2836 pg/mg creatinine) and the same trend occurred in the control group (2329 vs 2136 pg/mg creatinine) though differences were not significant ($p = 0.148$). Amongst the following (independent) variables: age, gender, disease duration, hypertension, nephropathy, retinopathy, HbA1c, fasting glucose, BMI and ASA ingestion, multiple regression analysis with 11dhTxB2 as the dependent variable, revealed that ASA ingestion (100 mg) and BMI were the only predictors of urinary 11dhTxB2 excretion ($t = 2.55$, $p = 0.0141$ and $t = 2.17$, $p = 0.0347$, respectively).

Baseline mean urinary 8-isoPGF2α concentration of DM patients was 44.4% higher than that of healthy controls ($p < 0.0001$). ASA ingestion did not inhibit urinary 8-isoPGF2α levels and the mean post-ASA 8-isoPGF2α level of DM (1509 ± 529) remained 50% higher than the mean post-ASA level of controls ($p < 0.0001$).

The mean baseline sP-Selectin level of DM patients was 29.9% higher than that of healthy controls ($p = 0.019$). ASA ingestion inhibited sP-Selectin levels of DM by 17.8% ($p = 0.11$), but demonstrated no effect on controls.

The mean baseline serum NO_2^- level of DM patients was 145.8% higher than that of healthy controls ($p < 0.0001$). Neither DM patients nor controls expressed any change of serum nitrite NO_2^- levels post-ASA ingestion. The mean baseline serum NO_3^- level of DM patients was 141.1% higher than that of healthy controls ($p < 0.0001$). ASA induced a 20.4% decrease of serum NO_3^- level in DM patients but had no effect on controls. The mean baseline PON1 activity of DM patients was similar to that of healthy controls and ASA ingestion affected neither DM patients nor controls.

Comparison of Biomarkers Between Normal and Poor ASA Responders in DM Patients

According to the post-ASA cut-off level of 1500 pg 11dhTxB2/mg creatinine to define normal response to ASA ingestion, we identified 15 poor responders amongst DM patients (ASA 100 mg/day n = 8, ASA 325 mg/day n = 7) and 8 poor responders amongst controls (ASA 100 mg/day n = 7, ASA 325 mg/day n = 1). Amongst the biomarkers tested, only baseline urinary 8-iso PGF2α excretion ($p < 0.009$) discriminated between normal and poor thromboxane response to ASA ingestion amongst subjects in either group (Table 3).

Table 2
Baseline and post-ASA ingestion effect on platelet, oxidative and inflammatory biomakers.

Marker	Sample	Controls mean ± SD	DM mean ± SD	Δ % Con vs DM	P value Con vs DM
11dhTxB2 (pg/mg creatinine)	Baseline	2450 ± 1572	3665 ± 2465	+49.6	0.0017
	Post ASA	624 ± 509	996 ± 845	+59.6	<0.0001
	ASA inhibition	*75.1% (8.4% PR)	*71.5% (14.8% PR)		
8-iso PGF2α (pg/mg creatinine)	Baseline	1009 ± 412	1457 ± 543	+44.4	<0.0001
	Post ASA	1005 ± 364	1509 ± 529	+50.1	<0.0001
	ASA inhibition	0.4%	0%		
P-Selectin (ng/mL)	Baseline	93.0 ± 26.1	120.8 ± 56.7	+29.9	0.0195
	Post ASA	90.3 ± 26.9	99.3 ± 36.4	+9.9	0.1456
	ASA inhibition	2.9%	17.8% (p = 0.1197)		
NO_2^- (μmol/L)	Baseline	4.8 ± 5.3	11.8 ± 7.3	+145.8	<0.0001
	Post ASA	8.4 ± 5.8	14.6 ± 6.5	+73.8	<0.0001
	ASA inhibition	0%	0%		
NO_3^- (μmol/L)	Baseline	20.9 ± 16.7	50.4 ± 39.2	+141.1	<0.0001
	Post ASA	20.6 ± 16.1	40.1 ± 28.6	+94.6	<0.0001
	ASA inhibition	1.4%	20.4% (p = 0.1425)		
PON-1 activity(U/L)	Baseline	282.6 ± 70.2	289.4 ± 61.9	+2.4%	NS
	Post ASA	285.9 ± 66.2	288.6 ± 66.0	+0.9%	NS
	ASA inhibition	0%	0%		

* PR = Poor Responders to aspirin (diabetes PR versus control PR p = 0.374).

Table 3
Comparison of biomarker levels at baseline and after ASA treatment (Post ASA) between normal (11-dhTxB2 < 1,500) and poor ASA responders (11-dhTxB2 > 1,500) in DM patients.

	Baseline			Post ASA		
	Normal ASA responders (n = 55)	Poor ASA responders (n = 9)	P value	Normal ASA responders (n = 55)	Poor ASA responders (n = 9)	p value
8-isoPGF2α (pg/mg creatinine)	1135 ± 565	1459 ± 194 *	0.009	1205 ± 547	1611 ± 590	0.065
P-Selectin (ng/mL)	104 ± 42	122 ± 31	0.103	94 ± 32	105 ± 33	0.427
NO_2^- (μmol/L)	8.4 ± 7.1	11.1 ± 9.9	0.607	11.5 ± 6.9	13.6 ± 7.3	0.544
NO_3^- (μmol/L)	35.0 ± 33.8	43.6 ± 19.4	0.145	31.3 ± 25.9	27.8 ± 21.1	0.856
PON1 activity (U/L)	285 ± 67	285 ± 54.8	0.775	286 ± 67	293 ± 50	0.616

Discussion

Our DM patients presented with a 50% higher baseline and post-ASA urinary 11dhTxB2 excretion than healthy controls in keeping with suspected platelet hyper-activation and/or alternative sources of thrmboxane generation that may contribute to the development of athero-thrombotic sequelae [14]. Moreover, DM is characterized by enhanced lipid peroxidation [15]: consistent with the urinary 8-isoPGF2α excretion, a sensitive marker of lipid peroxidation or oxidative stress, noted in DM compared to controls both at baseline and post-ASA treatment. A similar association was noted for the nitric oxide metabolites NO_2^- and NO_3^-, also consistent with the knowledge that whole body nitric oxide synthesis is increased in diabetes [16]. However, ASA treatment had no effect on urinary 8-isoPGF2α excretion, NO_2^-, NO_3^- and inflammatory sP-Selectin, the latter elevated only at baseline. P-selectin is involved in the initial recruitment of leukocytes to the site of injury during inflammation and because of this is considered a pro-inflammatory and platelet activation marker [17,18]. The interpretation of our sP-selectin results should take into consideration that ex vivo blood clotting during sample processing may activate platelets and artifactually influence serum sP-selectin levels. As our patients were devoid of nephropathy and retinopathy, and allowing for the relation between 11dhTxB2 and BMI, the elevated sP-selectin and the overproduction of urinary 11dhTxB2 and 8-isoPGF2α may be viewed as a reflection of low grade oxidative inflammation occurring in DM [19].

In fact, urinary excretion of 8-isoPGF2α was increased in the 14.8% DM patients identified as poor responders to ASA in whom urinary 11dhTxB2 excretion remained above the cut-off after ASA treatment compared to normal responders. These results suggest that incomplete inhibition of urinary 11dhTxB2 levels after ASA ingestion may contribute to cardiovascular disease in DM, though it may merely be an effect indicating an underlying cause of disease. Apart from possible incomplete inhibition of a classic platelet activation pathway, the high post-ASA 11dhTxB2 excretion may derive from extra-platelet sources: indeed endothelial cells, monocytes and megakaryocytes may contribute to whole body thromboxane production [20]. Early studies in healthy individuals showed a significant decrease of 2,3-dinor-thromboxane B2 after a wide range of aspirin doses [20], whereas more recent data reveal that low-dose aspirin may induce a uniform and persistent suppression of urinary 11dhTxB2 in healthy individuals based on radioimmunoassay testing [21]. A DM study witnessed a reduction in urinary 11dhTxB2 excretion of 72% with only 5% of patients reaching over 95% inhibition of urinary thromboxane [14]. Finally, different degrees of ASA insensitivity occur at different ASA dosages as measured by different laboratory tests [22].

Of greatest importance is the confirmation that DM patients who are identified by systemic thromboxane generation as poor ASA responders are in a state of enhanced oxidative stress [23] that may play an important role in aspirin response [24]. Platelet 8-iso-PGF2α originates from the activation of the catalytic subunit of NADPH oxidase and allows platelet recruitment via the expression of gpIIb/IIIa receptor complex [25,26].

In clinical practice ASA is widely prescribed under the general assumption that it completely inhibits platelet (COX-1) derived thromboxane; however, the measurement of post-ASA systemic thromboxane levels may identify DM patients who remain exposed to cardiovascular risk due to an underlying clinically active oxidative process. Urinary 11dhTxB2 allows for the identification of non-platelet sources of thromboxane production that may be associated with inflammation. A future consideration would be the incorporation of a COX-1 specific platelet function test, most appropriately serum thromboxane, in combination with the urinary 11dhTxB2 in order to better identify the source of the thromboxane generation. ASA doses employed (100 and 325 mg) were most likely insufficient to inhibit COX-2 mediated production of thromboxane. Whether different ASA doses (including twice daily) would improve outcomes will require additional studies. More importantly, abnormal thromboxane generation while ingesting ASA is associated with enhanced oxidative stress that may be quenched by specific antioxidant measures, whether dietary or pharmacological. These issues should be taken into account in further trials.

Conflict of Interest Statement

LRL and IJM are employees of Corgenix. KG has received consulting fees from Corgenix.

Acknowledgements

Supported by Senit Foundation, Scotland, UK.

References

[1] Patrono C, Garcia-Rodriguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med 2005;353:2373–83.
[2] Maree AO, Fitzgerald DJ. Variable platelet response to aspirin and clopidogrel in atherothrombotic disease. Circulation 2007;115:2196–207.
[3] Reilly IA, FitzGerald GA. Inhibition of thromboxane formation in vivo and ex vivo: implications for therapy with antiplatelet inhibitory drugs. Blood 1987;69:180–6.
[4] Hankle GJ, Eikelboom JW. Aspirin resistance. Lancet 2006;367:606–17.
[5] Zimmermann N, Hohlfeld T. Clinical implications of aspirin resistance. Thromb Haemost 2008;100:379–90.
[6] Friend M, Vucenik I, Miller M. Research pointers: Platelet responsiveness to aspirin in patients with hyperlipidemia. BMJ 2003;326:82–3.
[7] Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation 2002;105:1650–5.
[8] Eikelboom JW, Hankey GJ, Thom J, Bhatt DL, Steg G, Montalescot G, et al. Incomplete inhibition of thromboxane biosynthesis by acetylsalicylic acid. Determinants and effects on cardiovascular risk. Circulation 2008;118:1705–12.
[9] Butalia S, Leung AA, Ghali WA, Rabi DM. Aspirin effect on the incidence of major adverse cardiovascular events in patients with diabetes mellitus: a systematic review and meta-analysis. Cardiovasc Diabetol 2011;10:25.
[10] Breet NJ, Van Werkum JW, Bouman HJ, Kelder JC, Ten Berg JM, Hackeng CM. High on-aspirin platelet reactivity as measured with aggregation-based cycloxygenase-1 inhibition sensitive platelet function tests is associated with the occurrence of atherthombotic events. J Thromb Haemost 2010;8:2140–8.
[11] Rajagopalan S, Ford I, Bachoo P, Hillis GS, Croal B, Greaves M, et al. Platelet activation, myocardial ischemic events and postoperative non-response to aspirin in patients undergoing major vascular surgery. J Thromb Haemost 2007;5:2028–35.

[12] Russo I, Frascaroli C, Mattiello L, Viretto M, Barale C, Doronzo G, et al. Aspirin resistance subjects present a pro-inflammatory milieu with an increased oxidative stress: in these conditions, high glucose fails to influence platelet response to agoinists. Diabetologia 2011;54(Suppl. 1):S36.
[13] Russo I, Traversa M, Bonomo K, DeSalve A, Mattiello L, DelMese P, et al. In central obesity, weight loss restores platelet sensitivity to nitric oxide and prostacyclin. Obesity 2010;18:788–97.
[14] Gonçalves LH, Dusse LM, Fernandes AP, Gomes KB, Sóter MO, Alves MT, et al. Urinary 11-dehydro thromboxane B levels in type 2 diabetic patients before and during aspirin intake. Clin Chim Acta 2011;412:1366–70.
[15] Gopaul NK, Anggård EE, Mallet AI, Betteridge DJ, Wolff SP, Nourooz-Zadeh J. Plasma 8-epi-PGF2 alpha levels are elevated in individuals with non-insulin dependent diabetes mellitus. FEBS Lett 1995;368:225–9.
[16] O'Byrne S, Forte P, Roberts II LJ, Morrow JD, Johnston A, Anggård E, et al. Nitric oxide synthesis and isoprostane production in subjects with type 1 diabetes and normal urinary albumin excretion. Diabetes 2000;49:857–62.
[17] Nurden AT. Platelets, inflammation and tissue regeneration. Thromb Haemost 2011;105(Suppl. 1):S13–33.
[18] Woollard KJ, Chin-Dusting J. P-selectin antagonism in inflammatory disease. Curr Pharm Des 2010;16:4113–8.
[19] El-Mesallamy H, Hamdy N, Suwailem S, Mostafa S. Oxidative stress and platelet activation: markers of myocardial infarction in type 2 diabetes mellitus. Angiology 2010;61:14–8.
[20] FitzGerald GA, Oates JA, Hawiger J, Maas RL, Roberts II LJ, Lawson JA, et al. Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. J Clin Invest 1983;71:678–88.
[21] Santilli F, Rocca B, De Cristofaro R, Lattanzio S, Pietrangelo L, Habib A, et al. Platelet cyclooxygenase inhibition by low-dose aspirin is not reflected consistently by platelet function assays: implications for aspirin "resistance". J Am Coll Cardiol 2009;53:667–77.
[22] DiChiara J, Bliden KP, Tantry US, Hamed MS, Antonino MJ, Suarez TA, et al. The effect of aspirin dosing on platelet function in diabetic and nondiabetic patients: an analysis from the aspirin-induced platelet effect (ASPECT) study. Diabetes 2007;56:3014–9.
[23] Csiszar A, Stef G, Pacher P, Ungvari Z. Oxidative stress-induced isoprostane formation may contribute to aspirin resistance in platelets. Prostaglandins Leukot Essent Fatty Acids 2002;66:557–8.
[24] Schwedhelm E, Bierend A, Maas R, Trinks R, Kom GD, Tsikas D, et al. Redox-generated isoprostanes are associated with residual platelet activity in aspirin-treated patients with stable coronary heart disease. J Thromb Haemost 2010;8:2662–70.
[25] Ting HJ, Khasawneh FT. Platelet function and Isoprostane biology. Should isoprostanes be the newest member of the orphan-ligand family? J Biomed Sci 2010;17:24.
[26] Pignatelli P, Carnevale R, Di Santo S, Bartimoccia S, Sanguigni V, Lenti L, et al. Inherited human gp91phox deficiency is associated with impaired isoprostane formation and platelet dysfunction. Arterioscler Thromb Vasc Biol 2011;31: 423–34.