Effect of ASA dose doubling versus switching to clopidogrel on plasma inflammatory markers concentration in patients with type 2 diabetes and high platelet reactivity: The AVOCADO study

Marek Rosiak[1*], Marek Postula[2*], Agnieszka Kaplon-Cieslicka[1],
Agnieszka Kondracka[3], Ewa Trzepla[1], Andrzej Czlonkowski[2],
Piotr K. Janicki[4], Krzysztof J. Filipiak[1], Grzegorz Opolski[1]

[1]Department of Cardiology, Medical University of Warsaw, Poland
[2]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Poland
[3]Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Poland
[4]Department of Anesthesiology, Penn State College of Medicine, Hershey, Pennsylvania, USA

Abstract

Background: *The aim of the study was to compare the effects of 2 strategies of antiplatelet treatment (i.e., 150 mg ASA vs. 75 mg clpoidogrel) on plasma level of inflammatory markers in type 2 diabetes mellitus (T2DM) patients with high platelet reactivity (HPR).*

Methods: *Study cohort consisted of 304 T2DM patients on chronic ASA therapy (75 mg per day) participating in the Aspirin Versus/Or Clopidogrel in Aspirin-resistant Diabetics inflammation Outcomes (AVOCADO) study. Patients with HPR defined as Platelet Function Analyzer (PFA)-100 collagene/epinephrine closure time (CEPI-CT) < 193 s (n = 80) were randomized to 150 mg of ASA or 75 mg of clopidogrel in 2:3 ratio, respectively. Concentrations of the selected inflammatory markers, including tumor necrosis factor (TNF)-α, interleukin (IL)-6, soluble CD40 ligand (sCD40L), and high sensitivity C-reactive protein (hsCRP), were measured and compared in both treatment groups before and after 8 weeks of treatment in both groups.*

Results: *Out of 234 patients included into final analysis, the total of 34.2% (n = 80) patients displayed HPR, of which 14.1% (n = 33) were randomized into 150 mg of ASA group and 20.1% (n = 47) into 75 mg of clopidogrel group. Treatment with clopidogrel was a positive predictor (stepwise multiple regression analysis) of reduction of sCD40L concentration (odds ratio [OR] 4.15; p = 0.013), while treatment with 150 mg ASA was a positive predictor of reduction of IL-6 concentration (OR 4.38; p = 0.033). There was no statistically significant differences between clopidogrel and ASA 150 mg treatment in respect to predictive value for decreased hsCRP concentrations or increased TNF-α concentrations.*

Conclusions: *Increasing the dose of ASA from 75 mg to 150 mg daily or switching ASA 75 mg to clopidogrel 75 mg daily may reduce concentrations of some inflammatory markers (in particular hsCRP, IL-6 and CD40L) in T2DM patients with HPR treated previously with 75 mg of ASA.* (Cardiol J 2013; 20, 5: 545–551)

Key words: platelets, antiplatelet, inflammation, cardiovascular

Introduction

Patients with type 2 diabetes (T2DM) have a 2- to 4-fold higher risk for cardiovascular (CV) disease as compared with non-diabetic individuals [1]. It is caused partly by hypercoagulable state as evidenced by high platelet reactivity (HPR) [2]. Etiology of HPR is complex, and seems to be a result of hyperglycemia, metabolic disorders, oxidative stress and inflammation [3]. In particular, T2DM patients have higher concentrations of inflammatory markers, platelet activation and coagulation markers than healthy subjects [4–6]. Moreover, higher concentrations of inflammatory markers correlate with increased CV risk [7, 8]. There have been very few studies that evaluated the anti-inflammatory effects of antiplatelet therapy in T2DM patients [9, 10]. According to the American Diabetes Association guidelines, high--risk T2DM patients should be treated with low doses of acetylsalicylic acid (ASA) in primary or secondary prevention of myocardial infarction (MI) [11]. However, some of T2DM patients experience CV events despite treatment with ASA and that might be, at least in part, related to HPR, despite treatment with low-doses of ASA [12, 13].

In spite of recent advances in antiplatelet therapy the optimal treatment strategy is yet to be established in patients with HPR despite treatment with low dose of ASA (i.e. 75 mg per day). Previous studies demonstrated that T2DM patients treated with 162 mg ASA had lower incidence of HPR than those receiving 81 mg, however increasing the dose to 325 mg had not resulted in further improvement in terms of platelet reactivity [14, 15]. Also in the AVOCADO study switching to higher dose of ASA reduced platelet reactivity in T2DM patients with HPR initially treated with 75 mg of ASA per day [16].

It was hypothesized that dose-related inhibition of platelet aggregation may be due to effects of ASA beyond inhibition of its primary target cyclooxygenase (COX-1) by acetylation and was termed a non-COX-1 effect [15]. Thus, in our study shear dependent platelet functions were measured with less COX-1 inhibition dependent device — Platelet Function Analyzer (PFA)-100.

The aim of our prospective and randomized study was to compare the effects of 2 strategies of antiplatelet treatment (150 mg ASA vs. 75 mg clopidogrel) on the plasma level of selected inflammatory mediators (hsCRP, IL-6, TNF-α, sCD40L) in T2DM patients with established HPR, (diagnosed with PFA-100 collagen/epinephrine closure time (CEPI-CT) assay during previous treatment with 75 mg of ASA).

Methods

The study subjects were recruited consecutively from patients with T2DM (participating in a multi-center, prospective, randomized, and open-label Aspirin Versus/Or Clopidogrel in Aspirin-resistant Diabetics inflammation Outcomes (AVOCADO) study presenting to the outpatient clinic of the Central Teaching Hospital of the Medical University of Warsaw. The full characterization of the study population, including the inclusion and exclusion criteria were published previously [17]. Briefly, the Caucasian subjects with T2DM were recruited who, at the time of enrollment, had been taking ASA tablets at the dose of 75 mg per day for at least 3 months for primary or secondary prevention of myocardial infarction (MI). No clopidogrel or antiplatelet drugs other than ASA were used in any of the investigated patients. All patients had been taking oral antidiabetic agents and/or insulin for at least 6 months; diet-controlled diabetic patients were not included. Compliance to ASA therapy at the study entry was determined based upon the patient's own statement and serum thromboxane B_2 (sTXB$_2$) level measurement.

Blood sample and assay procedures

Blood samples were taken in the morning 2–3 h after the last ASA dose. Regular laboratory testing was performed using standard laboratory techniques. The concentration of functional epitope of the von Willebrand factor (vWF) molecule (vWF:Ag) was measured in citrate plasma samples using an enzyme immunoassay kit according to the manufacturer's instructions (vWF Activity Kit, American Diagnostica Inc., USA). Serum TXB$_2$ concentrations were measured also with an enzyme immunoassay (EIA) kit according to the manufacturer's instructions (EIA kits, Cayman Chemicals, Ann Arbor, MI, USA). Each lot of TXB$_2$ EIA kit was tested for the impact of interferences. The correlation of results in three dilutions of 5 random samples was assessed, as was proposed in kit protocol. The decision to use the assay without purification was taken after analysis of results, as differences of results did not exceed 20%. Samples with results outside the standard curve were re-assayed with appropriate dilutions. The compliance with ASA treatment was defined by the sTXB$_2$ levels below 7.2 ng/mL [13].

ELISAs were used to determine concentrations of tumor necrosis factor (TNF)-α (Quanti-

kine® HS ELISA Human TNF-α Immunoassay), interleukin (IL)-6 (Quantikine® HS ELISA Human IL-6 Immunoassay; both R&D Systems, Inc., Minneapolis, USA) and soluble CD40 ligand (sCD40L; Human soluble CD40 Ligand Immunoassay, R&D Systems, Inc., NE, USA), and high sensitivity C-reactive protein (hsCRP) concentrations were assessed using Cobas Integra 800 (Roche, Basel, Switzerland).

Analysis of platelet functions

Platelet reactivity was measured with PFA-100 assay (Dade-Behring International, Inc., Newark, DE, USA). These assays were performed as described in detail previously [13]. In current study, normal platelet reactivity on ASA therapy with a PFA-100 was defined as CEPI-CT $\geq$ 193 s (the manufacturer's lower limit of the normal range for aspirin-free healthy controls). Patients with HPR defined as CEPI-CT < 193 s were randomized to double dose (150 mg) of ASA or 75 mg of clopidogrel in 2:3 ratio, respectively. After 8 weeks of follow-up period inflammatory markers concentrations were re-assayed.

Statistical analysis

Normally distributed continuous variables were presented as means ± SD, whereas variables with a highly skewed distribution were presented as medians (interquartile ranges). Categorical variables were presented as frequencies (percentages). Normality of distribution was assessed using graphical methods. Differences between groups were analyzed using Student's t-test, the Mann-Whitney U-test, the χ^2 or Cochran-Mantel-Haenszel test, as appropriate. Selected variables were checked for associations with changes in inflammatory markers concentrations using a univariate and multivariate stepwise logistic regression models. The results were presented as odds ratios (OR) with their 95% confidence intervals (CI). A 2-sided p-value of < 0.05 was considered significant.

Power analysis

We were planning a prospective study of 2 independent experimental groups (clopidogrel 75 mg vs. ASA 150 mg), with 3 patients treated with clopidogrel 75 mg treated patients per 2 patients treated with ASA 150 mg (ratio 3:2). Prior data indicate that the response rate (defined as percentage of patients in the groups with decreased or increased of any measured inflammatory marker) among clopidogrel treated patients was 0.5. If the true response rate for ASA-treated subjects differs

by 0.35, we will need to study at least 28 ASA 150 mg treated patients and at least 39 clopidogrel 75 mg treated subjects (total number of subjects at least 77) to be able to reject the null hypothesis that the response rates for subjects in both treated group are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. We used an Fisher's exact test to evaluate this null hypothesis.

Results

From the initially enrolled 304 patients, complete clinical data and blood samples finally became available for 260 patients. Subsequently, 8 patients were eliminated from the analysis based on suspected ASA non-compliance (sTXB$_2$ concentrations > 7.2 ng/mL). Baseline characteristics are presented in Table 1. Twenty patients were excluded from the analysis due to elevated concentrations of hsCRP (> 10 mg/L) indicating potential subclinical infection. Out of 234 patients included into final analysis 34.2% (n = 80) patients had HPR of which 14.1% (n = 33) were randomized to 150 mg of ASA group and 20.1% (n = 47) to 75 mg of clopidogrel group.

After 8 weeks of treatment with either medication we observed a significant reduction of hsCRP: Δ (change in concentrations of marker after 8 weeks of follow-up) = –0.4 (–1.3 – 0.2); p = 0.006 and IL-6: Δ = –0.714 (–1.435 – 0.150); p < 0.001, as well an increase of TNF-α: Δ = 0.552 (0.064 – 0.931); p < 0.001 in group receiving 150 mg of ASA. We also observed a significant reduction of hsCRP: Δ = –0.3 (–1.0 – 0.1); p = 0.008, no change for Il-6: Δ = 0.045 (–1.247 – 0.779); p = 0,587 and an increase of TNF-α: Δ = 0.778 (0.129 – 1.670); p < 0.001 in group receiving clopidogrel. The calculated response rate for hsCRP and TNF-α in both experimental groups was similar and did not differ significantly (p > 0.05) between 150 mg ASA and 75 mg clopidogrel group by the Fisher's exact test analysis. In a Mann-Whitney test analysis we found a significant difference in the magnitude of change for IL-6 concentrations between groups (–0.714 in 150 mg of ASA group vs. 0.024 in clopidogrel group; p = 0.034). There was also significantly higher percentage of patients with IL-6 reduction in group receiving 150 mg of ASA than in group receiving clopidogrel (72.7% vs. 48.9%, respectively; p = 0.033 by Fisher's exact test).

There was no difference in the change of concentrations of sCD40L between studied groups, but there was a significantly higher percentage

Table 1. Demographic and clinical characteristics of the study patients (n = 254).

Demographics	
Male	136 (53.5%)
Age [years]	67.1 ± 8.5
SBP [mm Hg]	141.3 ± 19.3
DBP [mm Hg]	79.8 ± 11.6
BMI [kg/m^2]	30.7 ± 4.9
WHR	0.96 ± 0.08
Comorbidities	
Hypertension	235 (92.5%)
Coronary artery disease	137 (53.9%)
Prior MI	77 (30.3%)
Dyslipidemia	214 (84.3%)
Chronic heart failure	94 (37.2%)
Prior stroke and/or TIA	28 (11.0%)
History of smoking	149 (58.7%)
Current smoking	26 (10.2%)
Concurrent medications	
Metformin	162 (63.8%)
Insulin	83 (32.7%)
Beta-blockers	183 (72.0%)
ACE inhibitors	168 (66.1%)
Statins	184 (72.4%)
Proton pump inhibitors	31 (12.2%)
Biochemical parameters	
WBC [10^3/μL]	7.1 ± 2.0
HGB [g/dL]	13.9 ± 1.3
HTC [%]	41.6 ± 3.7
PLT [10^3/μL]	228.8 ± 61.3
MPV [fl]	9.9 ± 1.2
eGFR (MDRD) [mLmin/ /1.73 m^2]	70.9 ± 20.4
HbA1c [%]	7.0 ± 1.3
Total cholesterol [mg/dL]	164.9 ± 40.0
HDL [mg/dL]	48.7 ± 13.9
LDL [mg/dL]	88.9 ± 33.5
Triglycerides [mg/dL]	136.7 ± 68.3
vWF [%]	144.7 ± 57.4
CEPI-CT [s]	257 (172–300)
sTXB$_2$ [pg/mL]	153.2 (45.7–546.3)
Inflammatory markers (n = 234)*	
hsCRP [mg/L]	2.3 (1.4–4.0)
sCD40L [ng/mL]	0.582 (0.349–0.909)
TNF-α [pg/mL]	1.753 (1.244–2.292)
IL-6 [pg/mL]	2.193 (1.504–3.672)

*Inflammatory markers concentrations after excluding patients with hsCRP > 10 mg/L; SBP — systolic blood pressure; DBP — diastolic blood pressure; BMI — body mass index; WHR — waist-to-hip ratio; MI — myocardial infarction; TIA — transient ischemic attack; ACE — angiotensin converting enzyme; WBC — white blood cells count; HGB — hemoglobin; HTC — hematocrit; PLT — platelet count; MPV — mean platelet volume; eGFR — estimated glomerular filtration rate; HbA1c — glycosylated hemoglobin; HDL — high density lipoproteins; LDL — low density lipoproteins; vWF — von Willebrand factor; CEPI-CT — collagen/epinephrine closure time; sTXB$_2$ — serum thromboxane B$_2$ concentration; hsCRP — high-sensitivity C-reactive protein; sCD40L — soluble CD40 ligand; TNF-α — tumor necrosis factor-alpha; IL-6 — interleukin-6
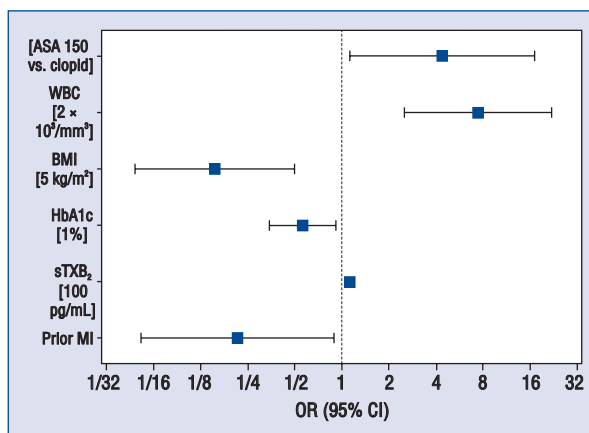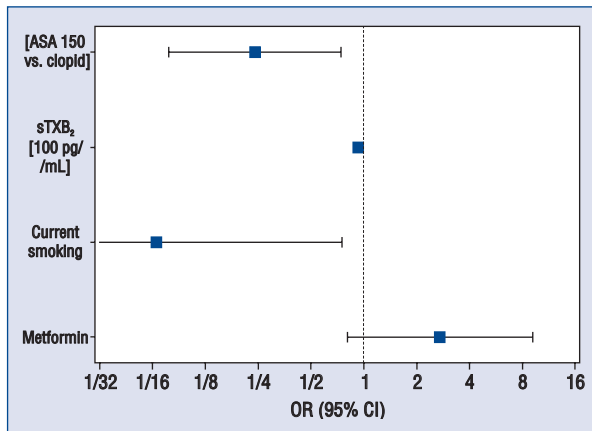

Figure 1. Factors influencing reduction of interleukin-6 (IL-6) concentrations in multivariate analysis; ASA 150 — patients randomized to receiving 150 mg of acetylsalicylic acid; clopid — patients randomized to receiving 75 mg of clopidogrel; WBC — white blood cells count; BMI — body mass index; HbA1c — glycosylated hemoglobin; sTXB$_2$ — serum thromboxane-B$_2$; MI — myocardial infarction; OR — odds ratio; CI — confidence interval.

of patients in clopidogrel group with sCD40L reduction than in group taking 150 mg of ASA (61.7% vs. 33.3%, respectively; p = 0.012, by Fisher's exact test).

In stepwise multiple logistic regression analysis adjusted to age and gender, treatment with 150 mg ASA (OR 4.38; 95% CI 1.13 – 17.02; p = 0.033) and higher white blood count (WBC) (for each 2 × × 10^3/mm^3: OR 7.43; 95% CI 2.51 – 21.97; p < 0.001) were positive predictors of reduction of IL-6 concentration, while higher glycosylated hemoglobin (HbA1c) (for each 1%: OR 0.56; 95% CI 0.34 – 0.91; p = 0.02) and body mass index (BMI) (for each 5 kg/m^2: OR 0.15; 95% CI 0.05 – 0.50; p = 0.002) and history of MI (OR 0.21; 95% CI 0.05 – 0.88; p = 0.033) were negative predictors of reduction of IL-6 concentration (Fig. 1). Patients treated with statins had higher chance of reduction of hsCRP concentration (OR 3.72; 95% CI 1.05 – 13.2; p = 0.042), while patients with history of MI had lower chance of reduction of this parameter (OR 0.25; 95% CI 0.08 – 0.81; p = 0.021). Only higher HbA1c was a negative predictor of reduction of TNF-α concentration (OR for reduction of TNF-α: 0.287; 95% CI 0.09 – 0.87; p = 0.027). There was no difference in change of hsCRP and TNF-α concentrations between studied groups.

Treatment with clopidogrel was a positive predictor of reduction of sCD40L concentration

Figure 2. Factors influencing reduction of soluble CD40 ligand (sCD40L) concentrations in multivariate analysis; ASA 150 — patients randomized to receiving 150 mg of acetylsalicylic acid; clopid — patients randomized to receiving 75 mg of clopidogrel; sTXB$_2$ — serum thromboxane-B$_2$; OR — odds ratio; CI — confidence interval.

(OR 4.15; 95% CI 1.34 – 12.82; p = 0.013) while current smoking (OR 0.93; 95% CI 0.87 – 0.99; p = 0.028) and higher sTXB$_2$ concentrations (OR 0.93; 0.87 – 0.99; p = 0.024) were negative predictors of reduction of this parameter (Fig. 2).

Discussion

The present study demonstrate that treatment with either 150 mg of ASA or 75 mg of clopidogrel result in significant reduction of hsCRP, sCD40L, IL-6 (only in and ASA treated patients) and in increase of TNF-α inflammatory markers concentration in T2DM patients with stable coronary artery disease (CAD) or multiple risk factors for CAD. No statistically significant differences were observed between the treatment groups in respect to the magnitude of changes (defined as the fraction of patients treated with ASA or clopidogrel with decreased concentrations of hsCRP or increased concentration of TNF-α). Treatment with ASA vs. clopidogrel caused however different effect on response rate related to Il-6 and sCD40L inflammatory markers (i.e., significant increase in the response rate for decreased IL-6 in only ASA 150 mg group, and significant increase in the response for decreased sCD40L only in clopdogrel group). Moreover, the results of this study show that both antiplatelet therapies were less effective in terms of their anti-inflammatory activity in patients with higher BMI, currently smoking and with a history of MI.

Several previous studies demonstrated anti-inflammatory effect of low doses of ASA and clopidogrel, mainly in patients with CAD [18–25]. The results of subanalysis of Physician's Health Study suggested that cardioprotective effect of ASA correlated with its anti-inflammatory effect [26]. There are very few studies assessing the effect of different doses of ASA on inflammatory state in patients with T2DM. Hovens et al. [9] demonstrated that there was no difference in hsCRP and IL-6 concentrations in patients with T2DM treated with either 100 mg or 300 mg of ASA, or placebo.

In our study doubling the dose of ASA in patients with HPR resulted in a significant reduction of hsCRP and IL-6 concentrations with more than 4-fold higher chance of IL-6 reduction than in a group treated with clopidogrel. Herder et al. [27] observed that high IL-6 levels in patients with diabetes were associated with increased risk for primary CV events (MI, stroke and CV death). Thus, doubling the dose of ASA by reducing IL-6 and hsCRP concentrations could be more effective in reducing the CV risk of patients with diabetes and HPR during treatment with 75 mg of ASA, but this hypothesis should be confirmed in further larger studies with well defined clinical end-points.

In the Diabetes Heart Study higher hsCRP concentration was a predictor of mortality in patients with T2DM [28]. In our study both antiplatelet treatment strategies resulted in significant reduction in hsCRP concentrations. In multivariate analysis the history of MI was a negative predictor of reduction of hsCRP concentrations (OR 0.25; 95% CI 0.08 – 0.80; p = 0.021). According to the results of recent meta-analysis, treatment with ASA in primary prevention of CV diseases remains of uncertain value in diabetic population [29]. Patients with a history of MI tend to have higher concentrations of hsCRP and treatment with ASA results in more reduction of MI risk in patients with higher levels of hsCRP than in patients with lower concentrations of hsCPR [26, 30, 31]. However, in our study patients with T2DM and with a history of MI which were chronically treated with 75 mg of ASA had lower baseline hsCRP concentrations than patients treated with ASA in primary prevention of MI, although due to small number of subjects the difference was not statistically significant. Moreover, treatment with higher dose of ASA or switching from ASA to clopidogrel resulted in additional reduction of hsCRP concentrations especially in patients without history of MI. This may indicate that patients treated with 75 mg of ASA in primary prevention of MI might require

modification of antiplatelet therapy, higher doses of ASA or switching to clopidogrel, which may cause additional benefit in terms of reducing hsCRP levels in this subgroup of patients.

In our study the median of sCD40L concentrations after 8 weeks of treatment with clopidogrel increased, but we observed a decrease in sCD40L concentrations in almost twice as many patients in this group as in group treated with 150 mg of ASA. There was also more than 4-fold higher chance of reduction in sCD40L concentration in patients treated with clopidogrel than in patients treated with 150 mg of ASA. Several studies have indicated that levels of circulating sCD40L are significantly higher in patients with acute MI or unstable angina and that sCD40L may be the marker of high risk population among patients with acute coronary syndrome [32, 33]. Therefore, it was suggested that sCD40L may be a therapeutic target and the reduction of its concentrations may lead to improved clinical outcome [34]. Our study is the first showing that clopidogrel is much more effective than ASA in reducing sCD40L concentrations in diabetic population, but it seems to be less effective in smokers than in non-smoking population. These results may indicate, that switching to clopidogrel, along with smoking cessation, could be an optimal therapy in patients with T2DM and HPR during treatment with 75 mg of ASA in terms of reduction of inflammatory markers concentrations.

Limitations of the study

The inherent limitations of open-label design apply to this study. Another limitation is related to the choice of the test for measurement of platelet reactivity for diagnosis of HPR. Light transmission aggregometry (LTA) is considered to be the gold standard platelet function test but is poorly standardized, requires a specialist laboratory and is unlikely to be used widely in routine clinical practice [35]. Moreover LTA measures more specifically effects of COX-1 dependent pathway of platelet activation, while PFA-100 by using different platelet agonists, measures more global platelet reactivity [36].

Conclusions

Increasing the dose of ASA from 75 mg to 150 mg daily or switching ASA 75 mg to clopidogrel 75 mg daily reduce concentrations of hsCRP, IL-6 and sCD40L inflammatory markers in T2DM patients with HPR and treated previously with 75 mg of ASA. Doubling the dose of ASA was more successful in reducing IL-6, while clopidogrel was better in reducing sCD40L. Anti-inflammatory effects of these 2 strategies were smaller in currently smoking patients with a history of MI, poorer long-term glycemic control and higher BMI.

Conflict of interest: none declared

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