Aspirin Resistance in Subjects with Premature Coronary Artery Disease in Relation to Abdominal Fat and Adipocytokines

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Citation: Yaturu S, Moussa S (2017) Aspirin Resistance in Subjects with Premature Coronary Artery Disease in Relation to Abdominal Fat and Adipocytokines. J Heart Cardiovasc Res. Vol. 2 No. 1: 106.

Abstract

Objective: The objective of this study was to evaluate aspirin resistance in young men with atherosclerotic cardiovascular disease (ASCVD) and in relation to abdominal fat by body composition and adipocyte hormones.

Methods: Forty subjects with coronary artery diseases (CAD), scheduled for left heart for coronary vascular evaluation were enrolled in this observational study from the outpatient clinic of the Department of Cardiology at Overton Brooks Veterans health Administration Medical Center between 2007 and 2008. All study subjects were on aspirin at a dose of 81mg daily and had history of coronary artery disease. Aspirin response was measured by the levels of urinary 11-dehydro-thromboxane beta (11DhTx2), using an enzyme immunoassay kit.

Results: Approximately 53% of the patients were resistant to aspirin. Levels of 11DhTx2/cre correlated with insulin levels (r=0.51; p=0.0008), insulin resistance (r=0.46; p=0.0036), HbA1C(r=0.50; p<0.01), visfatin levels (r=0.30; p=0.05), levels of IL-6(r=0.43; p=0.005) and with percentage of abdominal fat (r=0.41; p<0.01).

Conclusion: Aspirin resistance is common on an 81 mg daily dose and is to related abdominal fat, insulin resistance and IL-6. Subjects with abdominal fat may need to be evaluated for adequacy of aspirin response.

Keywords:
Abdominal fat; Anti-inflammatory drugs; Adipocyte hormones; Coronary artery disease; Insulin resistance

Introduction

Long term use of aspirin was shown to decrease the vascular occlusive events of up to nonfatal myocardial infarction (MI), nonfatal stroke and all-cause mortality up to 34%, 25% and 18% respectively [1]. An update from the U.S. Preventive Services Task Force (USPSTF) in 2009 states that aspirin reduces the risk of myocardial infarctions in men and strokes in women. The risk of serious bleeding is increased with aspirin use. [2]. Platelets play a major role in initiation of thrombosis and thrombotic complication. Hence low dose aspirin is recommended for prevention of cardiovascular events.

Aspirin inhibits the COx-1enzyme irreversibly and decrease the generation of thromboxane- A2 (TxA2), which is a potent mediator of platelet aggregation and activation. This mechanism explains how aspirin use benefits in subjects with high risk vascular disease [3]. Aspirin resistance or poor response to response is used when aspirin is ineffective and is defined when it fails to suppress the thromboxane generation and hence increase the risk of cardiovascular events in a high-risk population [4].

The most common causes for aspirin resistance reported include polymorphisms in the COX-1 gene [4-6] or concurrent use of nonsteroidal anti-inflammatory drugs that may compete with aspirin at the COX-1 receptor site, [5] poor body weight and conditions associated with a high platelet turnover [7-10]. In this study we evaluated aspirin responsiveness in relation to abdominal fat, insulin resistance and adipocyte hormones, including the inflammatory cytokines.

Materials and Methods

This was a single-center open-label observational clinical study. In this prospective observational study, urine and blood samples were collected after the informed consent at Overton Brooks VA Medical Center at Shreveport, Louisiana, after the approval of the protocol and consent from the Institutional

Review Board at LSUHSC, Shreveport, as well as the Research and Development Committee at VAMC. The urine and serum samples for this clinical study were collected at Overton Brooks VAMC, Shreveport, LA between 2006 and 2009. Some of the assays were carried out at Shreveport, LA and supplemental analyses of the samples were carried out at Stratton VAMC in Albany, NY, and the Pharmaceutical Research Institute at the College of Pharmacy and Health Sciences, in Rensselaer, NY, after IRB approval at Albany, NY. Forty subjects with coronary artery disease (CAD), scheduled for left heart catheterization for coronary vascular evaluation were enrolled in this observational study from the outpatient clinic of the Department of Cardiology at Overton Brooks Veterans Health Administration Medical Center between 2007 and 2008.

The inclusion criteria included men aged 30-59 years of all ethnic groups with history of coronary artery disease and positive nuclear imaging study and normal kidney function, (evaluated by creatinine <1.4 mg/dL and eGFR >70 mL/min) and were under treatment of 81 mg aspirin daily.

Aspirin resistance was measured by urinary 11-DH Tx B2 concentrations with an enzyme immunoassay kit. Clinical parameters collected include duration of diabetes, history of hypertension, and history of premature coronary artery disease, weight, and height and waist circumference of the subjects. Basic labs collected include fasting lipids, HbA1C, and serum creatinine. Body composition analysis was carried out by DXA.

Laboratory measurements

Venous blood samples were drawn from the subjects as they awaited cardiac catheterization. Plasma from the samples was stored at -80°C until analysis.

Insulin and adipocytokines

Levels of insulin, C-reactive protein (CRP), tumor necrosis factor α (TNF- α), interleukin-6 (IL-6), total adiponectin, monocyte chemo attractant protein-1(MCP-1) and visfatin were measured in duplicate using commercially available kits (ALPCO diagnostics, USA). The sensitivity of the insulin assay is 1 μIU /mL. Inter- and intra-assay coefficients of variation were 2.6% - 3.6% and 2.8% - 4%, respectively. The insulin resistance index calculation was done using the formula of homeostatic model assessment (HOMA-R). HOMA-R is equal to (plasma glucose level x plasma insulin level/22.5). The sensitivity of the CRP assay was 0.124 ng/mL. Inter- and intra-assay coefficients of variation were 5.5–6% and 11– 13%, respectively. The sensitivity of the TNF-α assay was 4.8 pg/mL. Inter- and intra-assay coefficients of variation were ± 10.8% and 4–8.3%, respectively.

The sensitivity of the adiponectin assay was 0.234 ng/mL (range of 0.375–12 ng/mL). Inter- and intra-assay coefficients of variation were 2.8–5.5% and 2.97–3.84%, respectively. Sensitivity of MCP-1 is 2.3 pg/mL. Inter and intra-assay coefficients of variation were 8.7% and 4.7% respectively. The sensitivity of the IL-6 is 0.01ng/mL. Inter- and intra-assay coefficients of variation were 2.2% and 1.7%.respectively.

Urinary 11-dehydro-thromboxane beta-2 (11DhTx2) levels were measured using an enzyme immunoassay kit from Cayman chemicals. The sensitivity of the assay is 120 pg/ml and specificity of 100%; an intra- and inter assay variability of 5-7%. Levels of 11DhTx2 >1500 pg/mg of creatinine were considered indicative of aspirin resistance. Aspirin: Bioassay of aspirin in the serum was carried out by Liquid Chromatography Mass Spectrometry (LC/MS/MS) in MRM mode Aspirin levels are expressed as μg/mL and are the sum of salicylic acid, which were eluted at different times as various glucuronides possess different retention times.

Statistical analyses

Statistical analyses were carried out using Microsoft Excel 2007. All data are presented as means ± S.D. A p value of <0.05 was considered statistically significant. The clinical and biochemical parameters of the subjects with 11DhTx2 /cre levels qualifying aspirin resistance were compared with and those with normal levels. The association of 1DhTx2 /cre levels with other clinical and biochemical parameters was calculated.

Results

Participants included 40 men with a mean age of 53 years with coronary artery disease. Most of them were Caucasian. Baseline characteristics of the cohort are shown in **Table 1**. Biochemical parameters and adipocytokines levels of our study subjects are shown in **Table 2**. Levels of 11DhTx2/cre levels >1500 is considered as aspirin resistance or poor response to aspirin. Approximately 53% of subjects had aspirin resistance. There were no significant differences in the clinical parameters such as age, history of hypertension, BMI, or waist-to-hip ratio between the subjects with 11DhTx2/cre levels <1500 and those with 11DhTx2/cre levels >1500. There were no significant differences in the biochemical parameters such as creatinine and thyroid function tests between the two groups. There was no correlation of levels of 11DhTx2/cre with age. Levels of 11DhTx2/cre correlated with insulin levels (r=0.51; p=0.0008), insulin resistance (r=0.46; p=0.0036), HbA1C (r=0.50; p<0.01), visfatin levels (r=0.30; p=0.05), levels of IL-6 (r=0.43; p=0.005) and percentage of abdominal fat (r=0.41; p<0.01). Levels of 11DhTx2/cre did not have an association with the levels of CRP, TNF-α or MCP-1 levels **(Table 3)**.

Table 1 Clinical characteristics of subjects with premature CAD.

	Mean
Age	53 ± 3
BMI	31 ± 6.4
Creatinine	1.05 ± 0.16
GFR	81.5 ± 15
HTN (%)	85%
DM (%)	40%
PAD	5%

Current smoker	21%
% Abdominal fat	34.7 ± 1.8

DM: diabetes; BMI: body mass index; GFR: glomerular filtration rate; HTN: hypertension; PAD: peripheral arterial disease; CAD: Coronary artery disease

Table 2 Biochemical and adipocytokines parameters in subjects with premature CAD.

	Mean ± SD
Insulin	13.5 ± 14
MCP-1	184 ± 99
CRP	4.5 ± 0.76
IL-6	7.6 ± 1.7
TNF-α	14.8 ± 2.3
Visfatin	2.09 ±0.55

MCP-1: Monocyte chemo attractant protein-1; CRP: C-reactive protein; IL-6: interleukin-6; TNF- α: tumor necrosis factor α; CAD: Coronary artery disease

Table 3 Relationship between 11DhTxB2 levels and inflammatory markers, insulin and insulin resistance.

	Correlation	p-value
Insulin	0.51853	0.0008
IR	0.45526	0.0036
MCP-1	-0.0815	NS
CRP	-0.0414	NS
IL-6	0.43105	0.005
TNF	-0.0163	NS

MCP-1: Monocyte chemo attractant protein-1; CRP: C-reactive protein; IL-6: interleukin-6; TNF- α: tumor necrosis factor α; CAD: Coronary artery disease

Discussion

We noted 53% of subjects had aspirin resistance (poor response to aspirin). In preparation for the catheterization, they had fasted for 12 hours. Similar to our data, Henry and associates reported from a study of 24-hour time-dependent aspirin efficacy in patients with stable coronary artery disease, aspirin resistance in 24.7% at 24 h (p<0.0001) [11]. They concluded that aspirin resistance at 24 h after ingestion was related to biological inflammatory markers, current smoking and diabetes, and the authors concluded that once daily aspirin does not provide stable 24-h antiplatelet protection in a significant proportion of CAD patients [11].

Because the platelet pool was replenished at a rate of 10–15% per day and 20–30% of uninhibited platelets are required to ensure normal haemostasis [12], administration of a low dose of aspirin once a day was considered normally sufficient to overcome replenishing of the platelet pool by normal platelet turnover [13]. From these we may consider that a reasonable treatment strategy to mitigate the effect of enhanced platelet turnover would be to increase aspirin dose.

In a study to characterize the kinetics and determinants of platelet cyclooxygenase-1 recovery in aspirin-treated diabetic and non-diabetic patients by Rocca and associates noted Platelet TXB(2) production was profoundly suppressed at 12 hours and serum TXB(2) recovered linearly, with a large inter individual variability and diabetic patients in the third tertile of recovery slopes (≥ 0.10 ng/ml hr (-1) showed significantly higher mean platelet volume, body mass index, and younger age. Higher body weight was the only independent predictor of a faster recovery in non-diabetics. Aspirin 100 mg twice daily completely reversed the abnormal TXB2 recovery in both diabetics and non-diabetics [14]. Based on this it may be a reasonable treatment strategy to mitigate the effect of enhanced platelet turnover to increase aspirin dosing frequency or add some other agent to improve aspirin resistance [15,16].

Interleukin-6 (IL-6) is a pleiotropic cytokine with central roles in immune and inflammatory reactions. Interleukin-6 (IL-6) is a well-established, independent marker of multiple distinct types of atherosclerosis. IL-6 is known to increase platelet activity both *in vivo* and *in vitro* studies. IL-6 is shown to activate platelets *in vitro* via a mechanism involving arachidonic acid metabolism [17]. Platelet function in patients receiving interleukin-6 as cytokine therapy said to have enhanced *ex vivo* agonist-induced platelet maximum aggregation (MA) and that paralleled an increase in plasma levels of TXB2 suggesting that IL-6 alters platelet function *in vivo* [18].

In our study the 11DhTx2 correlated with abdominal fat, IL-6 levels but not TNF- α. Similar to our data, Cartier and associates noted in a study of obesity, hyperinsulinemia is related to elevated IL-6 and TNF-alfa levels. They concluded that Il-6 may be one of the mediators of hyperinsulinemia. In their study they noted strong association of TNF- α to total adiposity whereas Levels of IL-6 to visceral adiposity. Based on their results they concluded that hyperinsulinemic state was related to visceral adiposity based on their observation of strong association of IL-6 levels to visceral adiposity than TNF- α. [15]. Since we noted the association of aspirin resistance with insulin and insulin resistance, possibly it all be related to IL-6.

The association of aspirin resistance with abdominal fat, IL-6 and insulin levels can be explained by elevated IL-6 and abdominal fat. In a study to investigate the relationship between aspirin resistance, ischaemic stroke subtype, stroke severity, and inflammatory cytokines, Englyst and associates that IL-6 was independently associated with aspirin resistance and also with severity of stroke [16]. Since aspirin resistance is associated with IL-6, therapies that lower IL-6 and improve aspirin resistance may be beneficial.

Conclusion

Aspirin resistance is high on 81 mg daily dose and is to related abdominal fat, insulin resistance and IL-6. Subjects with

increased abdominal fat may need to be evaluated for adequacy of aspirin response.

Acknowledgements

Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs or the United States government.

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