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Clinical Determinants of Aspirin Resistance in Diabetes

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Abstract

Recent studies indicate that not all diabetic subjects benefit from aspirin therapy. Our objective is to characterize diabetic subjects with aspirin resistance using urine thromboxane, and VerifyNow measures. Our results suggest that cardiovascular disease, microalbuminuria, poor diabetes control, and increased waist circumference help identify aspirin resistance in diabetes.

Keywords

Platelets; Diabetes; Insulin Resistance; Aspirin Resistance; Thrombosis

Introduction

Aspirin (ASA) reduces mortality in secondary prevention of cardiovascular disease (CVD), but an important question is how to identify subjects that may not benefit from ASA therapy. ASA Resistance (ASA-R) refers to the lack of an anticipated effect of aspirin on a laboratory measure of its anti platelet effect that is associated with a higher rate of future cardiovascular events [1]. In this study, we define the characteristics of subjects with diabetes and two clinically validated measures of ASAR: VerifyNow (VN) and urine thromboxane (11-dh-TxB2).

Research Design and Methods

We studied 55 subjects with diabetes all taking aspirin (81 mg, or 325) for at least 2 weeks including the morning of testing. Subjects reported fasting to the Clinical and Translational Research Center. The study was approved by our IRB and all patients provided written informed consent prior to testing. Clinical cardiovascular disease was defined by prior coronary

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Conflicts of Interest

The authors declare that they have no conflict of interest.

artery bypass surgery, or thrombotic stroke. The study excluded subjects if they met any of the following criteria: bleeding diathesis or a history of gastrointestinal bleeding, illicit drug or alcohol abuse, coagulopathy, major surgery within 6 weeks prior to study, platelet count $<100,000/\text{mm}^3$, hematocrit $<25\%$, creatinine $>4\text{mg/dL}$, or current use of non-steroidal anti-inflammatory drugs, anticoagulants, or antiplatelet drugs other than aspirin.

VerifyNow™ Aspirin Assay

VerifyNow™ (Accumetrics, CA) is a turbidimetric optical detection assay designed to measure platelet aggregation that is based upon the ability of activated platelets to bind to fibrinogen [2]. The fibrinogen-coated beads aggregate in whole blood in proportion to the number of unblocked platelet GPIIb/IIIa receptors. The instrument reports aggregation as aspirin resistance units (ARU). ASA-R is defined by previously reported criteria: ≥ 550 ARU by VerifyNow™ [3]. The coefficient of variance was 2.5% on repeated measures within patients. The between-patient coefficient of variance was 12.5%.

Urinary 11-dh-TxB2

AspirinWorks® (Corgenix, Co) is an enzyme-linked immunosorbent assay (ELISA). In brief, assay buffer and urine was incubated with a monoclonal antibody followed by the addition of an 11-dh-TxB2-alkaline phosphate tracer. Urinary 11-dh-TxB2 concentrations was determined by spectrophotometry at 405nm and expressed as pg/mg creatinine [4,5]. ASA-R is defined as ≥ 1500 pg 11-dh-TxB2/mg creatinine during treatment with 81mg or 325 mg of aspirin [5]. The coefficient of variance was 4% on repeated measures within patients. The between-patient coefficient of variance was 14%.

Biochemical Measures

We calculated HOMA-IR based on the following formula (fasting insulin X fasting glucose/22.5, with fasting insulin expressed in IU/ml and fasting glucose expressed in mmol/l), as previously described [6]. We measured weight, height, waist circumference, fasting lipids, HbA1c, uric acid, serum creatinine and urine microalbumin in all subjects.

Statistical Analysis

Variables are presented as mean $\pm$ SE. Unpaired T-test was used to compare levels of platelet function between and ASA-R and ASA-S patients. $p < 0.05$ was considered statistically significant. Non-parametric data were log transformed. Logistic regression was used to explain the changes in our variables. We used SPSS software (Chicago, IL).

Results

Our study subjects were mostly Caucasian, middle-aged adults (Age: 59 ± 1.4 years) with 51% males. Subjects were obese (BMI: $37 \pm 3 \text{ Kg/m}^2$). 51% of the subjects were on statin therapy, 36% on angiotensin converting enzyme or blocking receptor, and 43% on insulin therapy. 47 subjects were on 81 mg and 8 subjects were on 325 mg of ASA. Study subjects were insulin resistant as per Adult Treatment Panel (ATP) III definitions of the metabolic syndrome with triglycerides: $209 \pm 27 \text{ mg/dl}$, waist circumference: $111 \pm 2 \text{ cm}$, HDL: $47 \pm 2 \text{ mg/dl}$, uric acid: 5.9 ± 0.3 and blood pressure (systolic/diastolic) of $130 \pm 2 / 78 \pm 1.2 \text{ mm Hg}$. Diabetes control was poor with HbA1c: $8.8 \pm 0.3\%$, and fasting glucose: $178 \pm 11 \text{ mg/dl}$. The average duration of diabetes was 10.5 ± 1 years. Six subjects had prior CVD, and 16 had microalbuminuria ($> 30 \text{ mg of albumin/ mg creatinine}$). Measures of ASA-R were: 11-dh-TxB2: $1465 \pm 142 \text{ pg/mg creatinine}$, and VN: $455 \pm 22 \text{ ARU}$. 45 % of diabetic subjects were ASA-R per 11-dh-TxB2 criteria and 22 % per the VN criteria.

We classified diabetic subjects into ASA-R and ASA-S cohorts (table1). Prior CVD, microalbuminuria, increased HbA1c, and HOMA-IR characterized ASA-R subjects. We performed a logistic regression with ASA-R as the dependent variable using the co-variables listed in table 1. Microalbuminuria predicted 16% ($p=0.08$), and HOMA-IR predicted 27% ($p=0.04$) of the variance in 11-dh-TxB2 measures. Increased waist circumference predicted 22% ($p=0.01$) of the variance of VN measures. There was no significant correlation between sex, age, smoking, medication use and ASA-R measures. Measures of ASA-R were not different in subjects on either 325 or 81 mg of ASA. The two measures of ASA-R (11-dh-TxB2 and VN) were modestly correlated ($R^2=0.1$, $P=0.03$).

Conclusion

This is the first study to suggest that microalbuminuria, increased waist circumference, and poor diabetes control help identify ASA-R. Possible mechanism(s) for ASA-R in diabetes may be related to aspirin-mediated nonspecific acetylation competing with nonenzymatic modifications as protein glycosylation [7]. Diabetic patients are also more likely to have an increased risk of oxidative damage due to the production of free radicals resulting in ASA-insensitive 11-dh-TxB2 biosynthesis [8].

The discordance between 11-dh-TxB2 and VN measures is evident from several studies [9]. Urinary 11-dh-TxB2 is considered a marker of inflammation and oxidative stress [10], as well as a global index of thromboxane synthesis. It originates from other blood elements such as erythrocytes and monocytes and from renal biosynthesis [11]. This may explain the higher percentage of subjects who were ASA-R using 11-dh-TxB2 measures but not using VN. The main limitation of the study is the small and heterogeneous sample. In small sample sizes, HOMA-IR in subjects on pharmacological therapy can be misleading. However, the overall correlation of both increased waist circumference and poor diabetes control to ASA-R makes HOMA-IR correlation plausible.

The American Diabetes Association recommends that all diabetic subjects at high risk of CVD be on ASA therapy [12]. In two major studies of type 2 diabetes, ASA use did not decrease cardiovascular events or improve mortality [13,14]. Based on our findings, testing for ASA resistance in diabetes and clinical trials for alternative therapy (as clopidogrel, or higher doses of ASA) are needed.

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Abbreviations

ASA	Aspirin
ASA-R	Aspirin Resistance
ASA-S	Aspirin Sensitive
VN	VerifyNow
11-dh-TxB2	11 dehydro thromboxane B2
CVD	Cardiovascular Disease

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Characteristics of Aspirin Sensitive and Resistant Cohorts

Table 1

	11-dh-TxB2			VerifyNow		
	ASA-S (n=30)	ASA-R (n=25)	P value	ASA-S (n=43)	ASA-R (n=12)	P value
CVD (n)	1	5	<0.001	1	4	<0.001
Microalbuminuria (n)	4	11	<0.001	8	4	0.1
HbA1C (%)	8.1 ± 0.4	8.9 ± 0.6	0.2	8.1 ± 0.4	9.9 ± 0.9	0.05
WC (cm)	108 ± 2.7	113 ± 3.9	0.3	109 ± 3	124 ± 7	0.02
HOMA-IR*	5.6 ± 0.7	10.3 ± 2.9	0.04	7.2 ± 1	8.0 ± 2	0.7

ASA-R is defined by: ≥ 550 ARU by VerifyNow, and ≥ 1500 pg 11-dh-TxB2/mg creatinine during treatment with 81mg or 325 mg of aspirin
ASA-R: Aspirin Resistant, ASA-S: Aspirin Sensitive, CVD: Cardiovascular disease, HbA1C: Glycated Hemoglobin, WC: Waist Circumference
* HOMA-IR (fasting insulin X fasting glucose/22.5, with fasting insulin expressed in IU/ml and fasting glucose expressed in mmol/l)
Values are presented in Means $\pm$ SE.