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Endothelial dysfunction in young healthy men is associated with aspirin resistance

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ABSTRACT

The aim of this study was to investigate the relation between endothelial dysfunction and aspirin response in a young healthy population (102 men aged 18–40). Initial concentrations of the NO pathway metabolites (ADMA, L-arginine, SDMA), cardiovascular risk markers, oxidative stress markers (MDA, thiol index), sICAM1, sVCAM1, PAI-1, sE-selectin, sP-selectin, VEGF, thromboxane B2, 6-keto-$PGF_{1\alpha}$ and arachidonate-induced platelet aggregation (to separate aspirin resistant from sensitive group) were measured. Flow-mediated-vasodilation (FMD) was measured before and after intravenous infusion of 16.0 g of L-arginine. Measurements were repeated following aspirin administration (75 mg/24 h) for 4 days. Both groups were homogenous regarding demographic and biochemical characteristics reflecting cardiovascular risk. Aspirin resistant subjects were characterized by lower baseline FMD and higher FMD following aspirin and L-arginine treatment, as compared to aspirin sensitive control. MDA and nitrotyrosine were greater, whereas thiol index was lower in aspirin resistant men. The sICAM1, sVCAM1, PAI-1, sE-selectin, sP-selectin and VEGF levels were similar in the analyzed groups. Thromboxane in aspirin resistant subjects was greater both at baseline and following aspirin therapy. However, a significant decrease following aspirin treatment was present in both groups. Aspirin resistance in young men is associated with endothelial dysfunction, which could be due to oxidative stress resulting from lipid peroxidation.

1. Introduction

The endothelium modulates vascular smooth muscle tone by numerous factors which are released in response to a broad variety of physiological and pharmacological stimuli [1]. Endothelial dysfunction (ED) and decreased nitric oxide (NO) bioavailability have demonstrated playing a pivotal role in the pathogenesis of atherosclerosis [2–4]. Oxidative stress and ED are observed in patients with cardiovascular disease and are considered to be important risk factors for developing their adverse outcomes. Aspirin is a potent antiplatelet and antioxidant agent and reduction in vascular production of superoxide following treatment with aspirin has already been demonstrated [5,6]. Nonetheless, a growing body of evidence suggests that increased oxidative stress may also play a causative role in the pathophysiology of aspirin resistance (AR) [7,8]. Numerous populations were investigated for the incidence and frequency of AR and related risk factors whose prevalence depending on the subpopulation and diagnostic criteria is estimated to be 8–45% [9]. Nevertheless, the relation between AR and ED in young asymptomatic and drug naïve men has not been reported yet.

Since oxidative stress induces ED [10] and increases the level of aspirin-insensitive thromboxane biosynthesis [8], it can be postulated that oxidative stress may trigger AR as well. Both in young healthy humans and in hypertensive ones cyclooxygenase (COX) inhibition with a non-selective inhibitor indomethacin has improved flow mediated vasodilation (FMD) [11]. The beneficial effects of aspirin in atherosclerosis are generally attributed to its antiplatelet activities [12], but the effects of aspirin on endothelial function as well as the effect of ED on aspirin response require further investigation. Not only might AR be associated with insufficient inhibiting of platelet function, but it is also related to impaired aspirin action directly on the endothelium. On the other hand, the poor aspirin response of platelets could be triggered by pre-existing ED and increased oxidative stress. Hence, the aim of this study was to analyze whether in young and

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clinically healthy men (asymptomatic and drug naïve), a newly diagnosed ED could be associated with AR.

2. Materials and methods

2.1. Ethics statement

All experiments were conducted and approved in accordance with the guidelines of the Bioethics Committee at Wroclaw Medical University and adhered to the principles of the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects (revised November 13, 2001, effective December 13, 2001). All participants provided their written consent to participate in the study. The written consent form had been approved by the ethics committee.

2.2. Material

Healthy male volunteers (n = 102, mean age 18–40 years) from the university community in Wroclaw, Poland, were recruited to participate in this study from leaflets distributed in the campus area in Wroclaw. The recruited subjects were healthy, normotensive individuals. Exclusion criteria for the study were: hypertension (HTN), coexisting diabetes mellitus, chronic inflammatory diseases, infections, smoking, malignancies, mental disorders and any pharmacological treatment during 1 month before enrollment to the study. Subjects with unknown histories of HTN underwent a 24-hour ambulatory blood pressure monitoring using a Welch Allyn ABPM 6100S (Skaneateles Falls, NY, USA) in order to exclude hypertension. "White coat" hypertensive subjects were not excluded from this study.

2.3. Study protocol

A scheme of the study protocol is shown in Fig. 1. Each subject was given a medical examination. At 7:30–8.30 AM on the day of the medical exam, fasting blood was drawn from the antecubital vein. In order to avoid platelet activation, blood was drawn without venous stasis. The following parameters were measured:

1. Whole blood aggregation analysis
2. Metabolites of the NO pathway (ADMA, SDMA and L-arginine)
3. Prostanoids levels (TxB_2 and 6-keto-$PGF_{1\alpha}$)
4. Markers of endothelial activation (sICAM1, sVCAM1, sE-selectin, sP-selectin, VEGF, and PAI-1)
5. Markers of oxidative stress [malonyl dialdehyde (MDA), thiol index (GSH/GSSG) and nitrotyrosine].
6. Concentrations of total cholesterol (TCh), LDL and HDL cholesterol, triglycerides, serum creatinine, glucose, hsCRP, K^+ and uric acid.

After blood had been drawn, the vasodilatory reactivity of the brachial artery following reversible ischemia (FMD) was measured to diagnose ED. The cut-off point for ED was established as <8% change in the diameter of the brachial artery in response to reactive hyperemia compared with the baseline value [13]. Subsequently to the baseline FMD testing, the subjects had 16.0 g of L-arginine (Fresenius®, Bad Homburg vor der Höhe, Hessen, Germany) administered intravenously, followed, after 25 min, by a second FMD measurement [11].

After being tested, all subjects were given 75 mg of aspirin orally (Polocard 75 mg, Polpharma Co., Starogard Gdanski, Poland) daily for 4 days, after which all the procedures were repeated. Afterwards, subjects were assigned to one of two subgroups according to the aspirin response (Fig. 2):

1) aspirin sensitive subjects
2) aspirin resistant subjects.

Patients with persisting aggregation greater than 30% of baseline aggregation values following treatment with aspirin were considered as aspirin resistant [14–16].

2.4. Measurements of whole blood platelet aggregation

Whole blood aggregation was measured using an impedance aggregometer (Multiplate® analyzer, Dynabyte Medical, Munich,

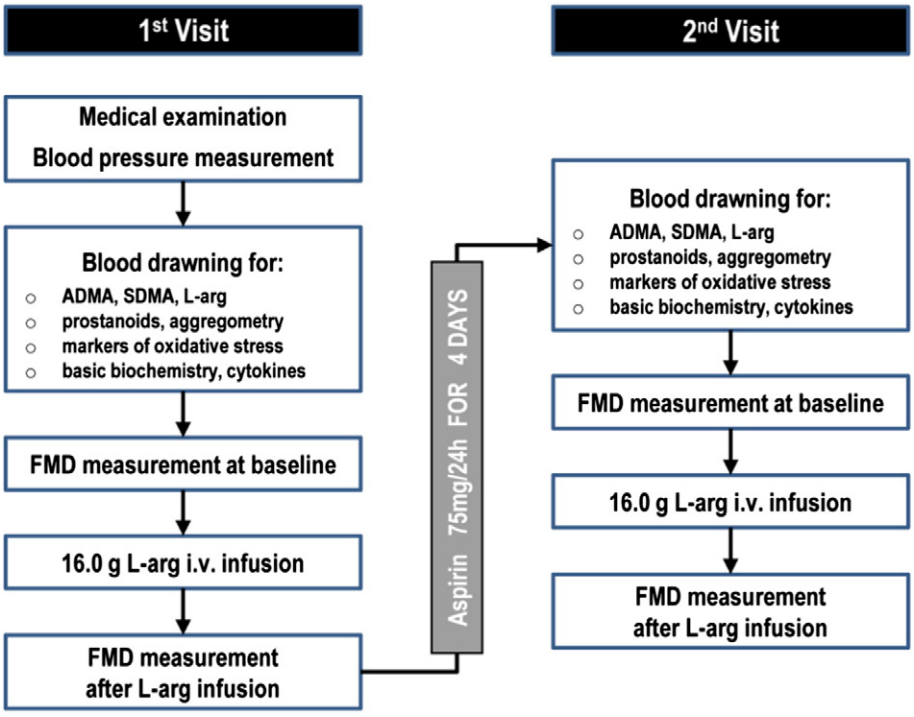

Fig. 1. Scheme of the study protocol. First, a medical examination of each subject was performed, followed by a blood pressure measurement. Next, blood samples for subsequent analyses were collected, and a baseline FMD measurement was performed. Subsequently, 16.0 g of L-arginine was administered intravenously, followed by a second ultrasound FMD measurement. Then each subject was told to take an oral aspirin tablet (75 mg/day at 8:00 PM for 4 days). On the fourth day, the protocol was repeated.