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Impairment of the extrusion transporter for asymmetric dimethyl-L-arginine: A novel mechanism underlying vasospastic angina

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ABSTRACT

A 37-year old male patient presented with frequent angina attacks (up to 40/day) largely resistant to classical vasodilator therapy. The patient showed severe coronary and peripheral endothelial dysfunction, increased platelet aggregation and increased platelet-derived superoxide production. The endothelial nitric oxide synthase (eNOS)-inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) reduced superoxide formation in platelets identifying "uncoupled" eNOS as a superoxide source. Oral L-arginine normalized coronary and peripheral endothelial dysfunction and reduced platelet aggregation and eNOS-derived superoxide production. Plasma concentrations of the endogenous NOS inhibitor asymmetric dimethyl-L-arginine (ADMA), representing an independent risk factor for cardiovascular disease, were normal in the patient. However, immediately after oral administration of cationic amino acid (CAA), plasma ADMA levels rose markedly, demonstrating increased ADMA efflux from intracellular stores. ADMA efflux from mononuclear cells of the patient was accelerated by CAA, but not neutral amino acids (NAA) demonstrating impairment of y^+LAT (whose expression was found reduced in these cells). These data suggest that impairment of y^+LAT may cause intracellular (endothelial) ADMA accumulation leading to systemic endothelial dysfunction. This may represent a novel mechanism underlying vasospastic angina and vascular dysfunction in general. Moreover, these new findings contribute to the understanding of the L-arginine paradox, the improvement of eNOS activity by oral L-arginine despite sufficient cellular L-arginine levels to ensure proper function of this enzyme.

1. Introduction

Vasospastic angina describes a form of angina pectoris caused by coronary artery spasm, which is brought about by a sudden occlusive vasoconstriction of a segment of the epicardial artery, resulting in a dramatic reduction of coronary blood flow [1]. In the most classical form, vasospasms occur at rest (Prinzmetal's variant angina) but may be also triggered by exercise [1]. Importantly, spasms may occur in coronary arteries with various degrees of stenosis but also in angiographic normal arteries. Vasospastic angina represents about 2.0% of hospital admissions due to unstable angina. Smoking is the only recognized risk factor and there is a 5:1 male/female ratio. The pathogenesis may involve either endothelial dysfunction due to decreased vascular NO bioavailability (for review see [2]) and/or hyperreactivity of the vascular smooth muscle to vasoconstrictors [3,4]. Interestingly, several studies have demonstrated simultaneous occurrence of endothelial dysfunction in brachial and coronary arteries pointing to a systemic vascular disease rather than a phenomenon restricted to coronary arteries [5,6]. Previous studies have shown that endothelial dysfunction in patients with vasospastic angina is associated with decreased basal NO production [7,8].

More recent reports demonstrated that concentrations of the eNOS inhibitor asymmetric dimethyl-L-arginine (ADMA) in the coronary circulation are higher in patients with vasospastic angina than in controls [9]. However, the plasma concentrations in the coronary circulation are not high enough to explain eNOS uncoupling.

Abbreviations: AA, amino acid; CAA, cationic AA; NAA, neutral AA; ADMA, asymmetric dimethyl-L-arginine; DDAH, dimethylarginine dimethylaminohydrolase; (h)CAT, (human) cationic amino acid transporter; FMD, flow mediated dilation; LAD, left anterior descending artery; L-NAME, N (G)-nitro-L-arginine methyl ester; LPI, lysinuric protein intolerance; (e)NOS, (endothelial) NO synthase; NTG, nitroglycerin; PDBU, phorbolester dibutyrate; PRMT, protein arginine methyltransferase; qRT-PCR, quantitative reverse transcription and PCR; ROS, reactive oxygen species; y^+LAT, system y^+L amino acid transporter.

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Whether ADMA accumulates in endothelial cells under pathophysiological conditions remains an open question. ADMA is formed in cells by degradation of proteins containing arginine residues that have previously been methylated by S-adenosylmethionine-dependent methyltransferases (PRMTs). There are two major routes of ADMA elimination: renal excretion and enzymatic degradation by dimethylarginine dimethylaminohydrolases (DDAH-1 and -2) [10]. Because DDAH is mainly present in liver and kidney, both elimination routes require export from the ADMA-generating (endothelial) cell to the plasma. We hypothesized that ADMA transport is mediated by the same carrier proteins as transport of other cationic amino acids (CAA). The major CAA transporters in non-epithelial cells belong to two related protein families: the CAA transporters (CAT) and the system y^+L AA transporters (y^+LAT) [11]. However, a physiological role of CAA export from non-epithelial cells has not been demonstrated so far.

Our present study identifies system y^+L as the major export route for ADMA under physiological conditions. It further indicates that a reduction in system y^+L-mediated ADMA efflux from endothelial cells may lead to intracellular ADMA accumulation and eNOS uncoupling and may account – at least in part – for systemic endothelial dysfunction and for the variant angina symptoms observed in the patient in this case report.

2. Materials and methods

For detailed protocols see online Supplement.

3. Results

3.1. Patient characteristics

The 37 year old patient without any cardiovascular risk factor experienced up to 40 attacks of angina pectoris per day despite medical treatment with isosorbide dinitrate (40 mg b.i.d.), amlodipin (5 mg b.i.d.), aspirin (100 mg q.d.), clopidogrel (75 mg q.d.) and cholesterol-lowering therapy with statins. The attacks responded to short term treatment with nitroglycerin spray only. In addition, the patient required treatment with antidepressants. There were no other concomitant diseases. All laboratory values including triglycerides and cholesterol were within normal limits. All previous diagnostic procedures including resting ECG, bicycle ergometry, echocardiogram, stress echocardiogram and diagnostic catheterization revealed no abnormalities at all. The patient was admitted to our hospital with the working diagnosis of vasospastic angina pectoris for further diagnostic evaluation and optimization of treatment.

3.2. Coronary and peripheral endothelial function before and after L-arginine treatment

Since vasospastic angina has been demonstrated to be associated with – or secondary to – endothelial dysfunction (see Section 4), we determined coronary endothelial function by an intracoronary infusion of the endothelium-dependent vasodilator acetylcholine. At an estimated intracoronary concentration of $10^{-7.3}$ M, acetylcholine caused a complete occlusion of the left anterior descending artery (LAD), which responded nicely to intracoronary nitroglycerin (NTG, 0.25 mg) (Fig. 1A–E). After L-arginine treatment for 3 mo (6 g q.d.), the patient tolerated all intra-coronary acetylcholine concentrations (from $10^{-7.3}$ M to $10^{-5.6}$ M) and even responded with a dilation of the LAD indicating good endothelial function.

In the patient, we also established severe peripheral endothelial dysfunction. He achieved only 3% flow-mediated dilation (FMD) of the brachial artery (Fig. 1F) compared to 8–12% in age-matched controls (data not shown). Upon chronic L-arginine treatment (6 g t.i.d.), the FMD of the peripheral artery increased from 3% to 13% (Fig. 1F). The FMD decreased to 8% after a 4 mo pause in the L-arginine treatment, but came back to 13% in response to a single L-arginine dose (9 g). These data demonstrate that L-arginine was very efficient in restoring and maintaining endothelial function in this patient. In contrast, endothelium-independent dilatation in response to NTG (0.8 mg, sublingual) was unchanged by the arginine treatment (Fig. 1F).

3.3. Effects of oral L-arginine treatment on platelet superoxide production and aggregatory response

Reactive oxygen species (ROS) formation measured in isolated washed platelets from the patient was significantly higher than in platelets from a healthy control subject (Fig. 1G). The NOS inhibitor L-NAME (that blocks both, eNOS-dependent NO and ROS formation) increased the superoxide signal in control platelets (due to inhibtion of NO production). In the patient's platelets it strongly decreased the signal. These observations are compatible with eNOS uncoupling. Chronic treatment with L-arginine (6 g t.i.d.) decreased oxidative stress almost to control levels. L-NAME had only a marginal effect on ROS formation in these platelets. In contrast, when the L-arginine treatment was paused, ROS formation by the patient's platelets was again very high and could be inhibited by L-NAME. This situation was reversed by a single dose of L-arginine (9 g).

In addition to ROS formation, platelet aggregation in response to the protein kinase C stimulator phorbolester dibutyrate (PDBU, 10 μM) was clearly enhanced in the patient as compared to the control subject. Treatment with L-arginine (6 g q.d. for 3 mo) markedly inhibited PDBU-induced platelet aggregation in the patient whereas platelet aggregatory responses remained unchanged in the control subject (Fig. 1H).

3.4. L-arginine handling

Initial amino acid determinations revealed reduced L-arginine and elevated L-ornithine plasma concentrations in the patient (18 and 117 μM, respectively). The good clinical and endothelial function response to acute and chronic L-arginine treatment suggested that an L-arginine deficiency might underlie the clinical symptoms of the patient. In order to monitor L-arginine resorption and metabolism, we compared the level of L-arginine and its metabolites in plasma and urine of the patient and control subjects after a single oral dose of 9 g L-arginine. The increase in plasma L-arginine was similar in both, patient and control subject, reaching a peak of 400–500 μM between 30 min and 2 h and slightly decreasing thereafter (Fig. 2A). Note that at this point, the patient had normal basic L-arginine plasma levels due to a long term L-arginine treatment (6 g t.i.d.) that was suspended for only 10 d prior to the measurement. However, after longer pauses in the L-arginine treatment, plasma L-arginine levels dropped again to 20–50 μM (data not shown). The L-arginine, L-ornithine, L-proline, L-glutamine and L-glutamate content of urine collected during three hours after L-arginine intake did not differ significantly between patient and controls (data not shown). In addition, the patient showed normal urea-derived nitrogen in plasma and urine. However, after L-arginine intake, plasma L-ornithine levels increased strikingly more then in control subjects (data not shown). In addition, the patient exhibited elevated L-glutamine concentrations in the plasma that were further increased upon L-arginine intake (data not shown). Taken together these data show normal L-arginine resorption and metabolism in the patient under L-arginine treatment, but a somehow reduced metabolism of L-ornithine and its metabolite L-glutamine.