Local and systemic 20S proteasome release in patients with stenting of stenotic saphenous vein bypass grafts – a pilot study

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Abstract

Introduction: The ubiquitin-proteasome system is involved in the development and progression of atherosclerosis. We tested the release of local and systemic proteasome in patients with advanced coronary artery disease.
Material and methods: Thirty-nine symptomatic male patients (68 ±1 years) with a flow-limiting stenosis (63 ±1% diameter reduction) in their saphenous vein aortocoronary bypass graft (SVG) underwent stent implantation under protection with a distal balloon device, which permits aspiration of the plaque-derived particulate debris and fluid; the 20S proteasome concentration was measured in the venous plasma of 28 healthy controls and of these 39 patients as well as in the soluble and particulate fraction of the aspirate before and after stenting, using an enzyme-linked immunosorbent assay.
Results: The peripheral venous 20S proteasome plasma concentration was higher in patients undergoing SVG stenting (1327 ±102 ng/ml) than in healthy subjects (447 ±29 ng/ml, $p < 0.0001$) and related to angiographic baseline diameter stenosis ($r = 0.3596$, $p = 0.0179$, $n = 43$ stenoses) and the development of restenosis (circulating 20S proteasome concentrations > median of 1335 ng/ml: 40 ±9% diameter reduction within 6.0 ±0.4 months, $n = 20$ stenoses vs. ≤ 1335 ng/ml: 17 ±3% diameter reduction within 6.4 ±0.5 months, $n = 21$ stenoses, $p = 0.0059$). 20S proteasome concentration in the particulate debris fraction averaged 7010 ±890 ng/ml. However, the plaque-derived 20S proteasome concentration during intervention was less in patients with high (> 1335 ng/ml) venous 20S proteasome concentration (5846 ±993 ng/ml, $n = 21$ stenoses) than in those with lower (≤ 1335 ng/ml) concentration (8538 ±1548 ng/ml, $n = 22$ stenoses, $p = 0.0308$).
Conclusions: We propose that release of 20S proteasome from an atherosclerotic lesion may reflect progression of coronary atherosclerosis and could possibly serve as a biomarker.

Key words: atherosclerosis, percutaneous coronary intervention, protection device.

Introduction

The ubiquitin-proteasome system is the major pathway of non-lysosomal degradation of intracellular proteins and is central to processes such as apoptosis, inflammation and proliferation, which are all decisive elements of atherosclerosis [1]. Apart from the more established 26S

proteasome, there is also a 20S proteasome which is functional in the extracellular space; its plasma concentration is increased in a variety of notably inflammatory states [2]. Increased activity of the ubiquitin system is associated with unstable coronary atherosclerotic plaques and acute coronary syndromes [3]. Of note, decreased vascular 20S proteasome activity increases ubiquitin levels and contributes to atherosclerosis progression in human carotid plaques [4]. Diabetic patients have greater 20S proteasome activity in their carotid plaques, and rosiglitazone attenuates the inflammation, including 20S proteasome activity, in the atherosclerotic lesion [5].

In the present study we therefore investigated systemic 20S proteasome concentrations in the plasma of patients with a severe stenosis in their saphenous vein aortocoronary bypass graft (SVG) and related them to disease progression. To obtain information on the proteasome concentration in coronary atherosclerotic plaques, 20S proteasome concentrations were also determined in the aspirate sampled during stent implantation under protection of an occlusion/aspiration device [6].

Table I. Clinical and laboratory data, current medication, graft age, target vessel and stent type

Arterial hypertension[1]	37 (95)
Obesity	20 (51)
Hyperuricaemia	5 (13)
Diabetes mellitus	14 (36)
Renal failure[2]	13 (33)
Ex-smoker[3]/smoker	8 (21)
Total cholesterol [mg/dl]	169 ±6
HDL cholesterol [mg/dl]	47 ±2
LDL cholesterol [mg/dl]	90 ±5
Triglycerides [mg/dl]	177 ±25
Apolipoprotein A1 [mg/dl]	123 ±3
Apolipoprotein B [mg/dl]	83 ±4
Lipoprotein (a) [mg/dl]	62 ±10
Serum creatinine [mg/dl]	1.4 ±0.1
Aspirin	36 (92)
Clopidogrel	35 (90)
Vitamin K antagonists	2 (5)
Heparin	2 (5)
β-Adrenoceptor antagonists	36 (92)
Statins	33 (85)
ACE inhibitors	26 (67)
AT1 receptor antagonists	10 (26)
Diuretics	39 (100)
Anti-diabetics	14 (36)
Graft age [years]	12 ±1
– LAD	15**
– LCX	13*
– RCA	11*
BMS	19[a]
DES (Cypher™/Taxus™)	20[a] (3/17)

LAD – left anterior descending coronary artery, LCX – left circumflex coronary artery, RCA – right coronary artery, sirolimus-eluting Cypher™ stent [Johnson&Johnson, New Brunswick, NJ, USA], paclitaxel-eluting TAXUS™ stent [Boston Scientific, Natic, MA, USA], mean ± SEM (n, %)
[1]Hypertension defined as blood pressure > 140/90 mm Hg and/or current anti-hypertensive medication on admission, [2]Calculated glomerular filtration rate < 60 ml/min, [3]Abstention from smoking for more than 6 months
**represents one patient who received two stents, [a]represents two patients each with two stents*

Material and methods

Patients

Thirty-nine symptomatic male patients (68 ±1 years, 82 ±2 kg body weight, Canadian Cardiovascular Society (CCS) class [7] 2.4 ±0.1; NYHA class 1.9 ±0.1) with a flow-limiting stenosis in their SVG were studied; 4 patients were treated for 2 stenoses each such that a total of 43 stenoses were studied. Clinical and laboratory data, current medication, graft age, target vessel and stent type are presented in Table I. Twenty-eight apparently healthy subjects (44 ±19 years) served as controls. Full informed consent was obtained from all subjects and patients before participating in the study following approval by the local ethics committee of the Medical Faculty, University of Duisburg-Essen, and conforming with the principles outlined in the revised Declaration of Helsinki.

Intervention and follow-up

The interventional procedure, using a distal balloon occlusion device (TriAktiv®, Kensey Nash, Exton, PA, USA) during stenting, and quantitative angiography prior to and after stent implantation were performed as described before [6-8]. Six months after stent implantation quantitative coronary angiography was performed to determine the degree of restenosis.

Blood and aspirate samples

Venous blood (VB; 10 ml; potassium-EDTA S-Monovette; Sarstedt, Germany) was obtained via the cubital vein in controls and via the femoral vein in patients prior to intervention. Twenty ml of coronary arterial blood was aspirated distal to the lesion via the flushing catheter prior to (AB) and

after stent implantation (ASP); the latter was diluted approximately two-fold with saline and filtered through a 40 μm mesh filter. In each instance, visible particulate plaque-derived debris was retained on the filter. VB, AB and filtered ASP were immediately centrifuged (800 g for 10 min at 4°C), and the plasma was removed, quickly frozen in liquid nitrogen and stored at –80°C until further analysis.

Filters were washed with 100 μl cell lysis buffer (PromoCell GmbH, Heidelberg, DE) and controlled microscopically to ensure complete removal of the plaque-derived particulate debris. The particulate debris was dissolved by sonification (3 × 30 s with 1 min intervals on ice). The samples were incubated on ice for a further 15 min and then centrifuged for 10 min at 13 000 g and 4°C. The supernatants were removed and stored at –80°C until further analysis.

The concentration of 20S proteasome present within VB, AB and ASP plasma and the plaque-derived particulate debris extracts was measured using an enzyme-linked immunosorbent assay as described recently [9, 10].

Statistic analysis

Data are presented as means (± SEM) and compared between groups with the unpaired two-sided Student *t*-test; linear regression was performed; statistics were done using dedicated software (Graph-Pad Software, San Diego, CA); *p* values < 0.05 were considered significant. The 20S proteasome data were normally distributed in the observed ranges.

Results

Prior to stent implantation, the stenosis amounted to 63 ±1% diameter reduction, and minimal lumen diameter averaged 1.4 ±0.1 mm. Immediately after stent implantation, the diameter stenosis was reduced to 6 ±1%, and minimal lumen diameter was increased to 3.6 ±0.1 mm. Following stent implantation diameter re-stenosis was 28 ±5% after 6 ±0.3 months.

20S proteasome in plasma and aspirate

The 20S proteasome concentration in venous plasma was significantly lower in controls (447 ±29 ng/ml) than in patients undergoing SVG stenting (1327 ±102 ng/ml, Figure 1). In patients, similar 20S proteasome concentrations were obtained in the soluble aspirate fraction before (AB, 1001 ±128 ng/ml) and after stenting (ASP, 934 ±171 ng/ml).

The 20S proteasome concentration in the particulate debris-derived extracts averaged 7010 ±890 ng/ml and was thus 5- to 7-fold higher than in venous or coronary arterial plasma, respectively. The 20S proteasome venous plasma concentration

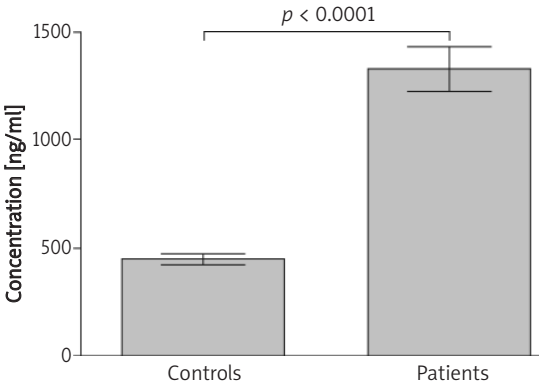

Figure 1. Systemic 20S proteasome plasma concentrations in healthy controls and patients with a severe stenosis in their saphenous vein aortocoronary bypass graft

was related to diameter stenosis at baseline (r = 0.3596, p = 0.0179, n = 43 stenoses, Figure 2).

When grouping patients according to their median venous 20S proteasome concentration of 1335 ng/ml (794 ±65 ng/ml, n = 22 stenoses vs. 1836 ±106 ng/ml, n = 21 stenoses, Figure 2), restenosis was more pronounced (40 ±9% within 6.0 ±0.4 months, n = 20 stenoses) in those with higher 20S proteasome concentration than in those with a lower concentration (17 ±3% within 6.4 ±0.5 months, n = 21 stenoses, p = 0.0059, Figure 2). In those with higher venous 20S proteasome concentration, the 20S proteasome concentration in the plaque-derived particular debris extract was lower (5846 ±993 ng/ml, n = 21stenoses) than in those with lower venous 20S proteasome concentration (8538 ±1548 ng/ml, n = 22 stenoses, p = 0.0308, Figure 2).

Discussion

To date, almost no information exists about the origin or biological role of the extracellular 20S proteasome in health and disease. In human coronary artery plaques high amounts of 20S proteasome have been found. They most likely represent clots of inactivated 20S proteasomes [11] which have become insufficient to clear the cell from damaged proteins, leading to an accumulation of oxidized and ubiquitinated protein aggregates [3] and possibly contributing to apoptosis, plaque instability and acute complications [12, 13]. We have previously shown that plaque rupture during stent implantation induces local release of serotonin and thromboxane A_2 [6] and TNF-α [8] and is associated with peri-interventional coronary microembolization [14-16] with consequent microinfarction [17-20] and reduced coronary reserve [17, 19]. We can only speculate that the containment of 20S proteasome in the particulate plaque debris in our present study

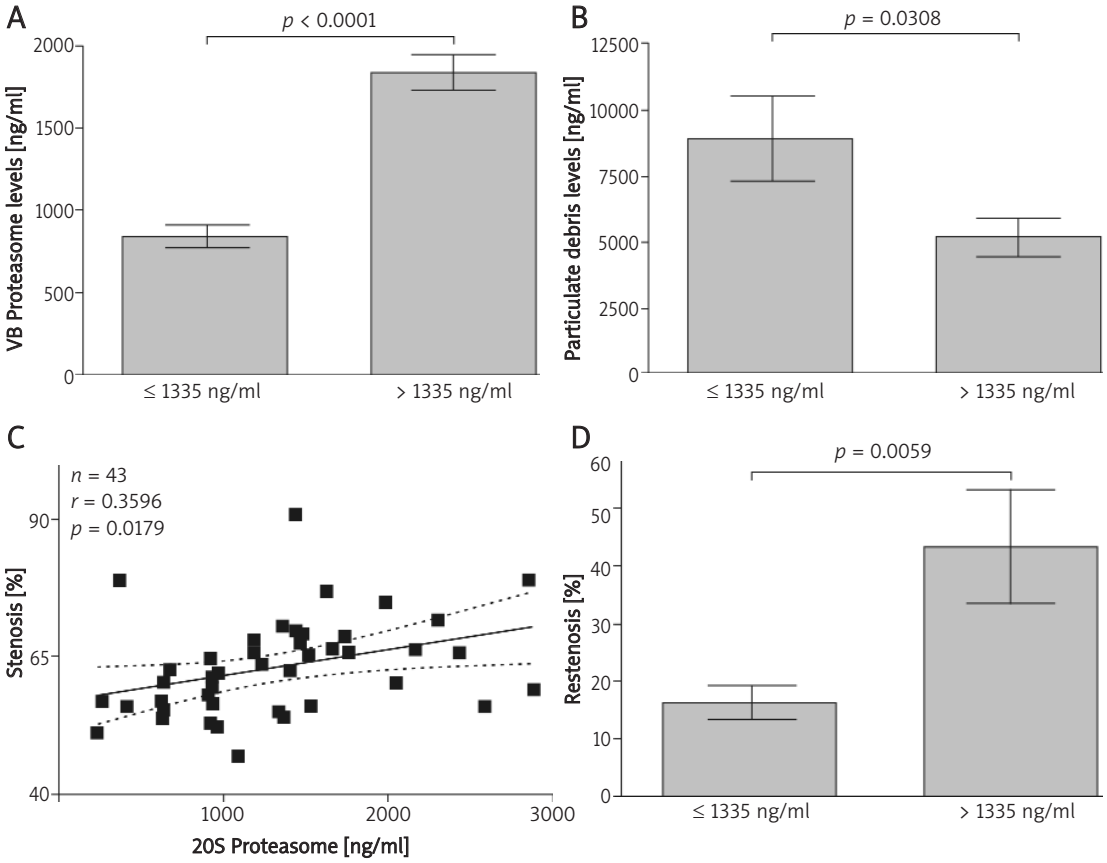

Figure 2. Systemic 20S proteasome plasma concentrations (**A**), local plaque-derived 20S proteasome concentrations (**B**), angiographic diameter reduction of saphenous vein aortocoronary bypass stenosis at baseline (**C**), and diameter reduction of restenosis six months after stent implantation (**D**) in patients grouped with respect to their median systemic plasma 20S proteasome concentration.

represents a more innocuous storage compartment, whereas the circulating systemic 20S proteasome reflects release from the unstable plaque and is therefore more closely related to complications such as restenosis. Such release from the unstable plaque would suggest the use of systemic 20S proteasome as a potential biomarker of disease progression.

The current study is a pilot study with several limitations: the number of patients is small and without age-, sex-, or BMI-matched controls, and the results must be confirmed in future, prospective, larger scale studies. Of note, the present study was done in saphenous vein bypass grafts, and arterial grafts may have a different profile. Aspirin was present in 92% of our study population, and aspirin reduces 20S proteasome activity in atherosclerotic rabbits [21]; it will be difficult to recruit aspirin-naïve patients undergoing PCI. Also, the use of statins in 85% of our study population may have impacted on our results, since statins largely reduce periprocedural coronary microembolization and its inflammatory consequences [22]; again, statin-naïve patients are increasingly rare.

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