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Influence of elastin-derived peptides on metalloprotease production in endothelial cells

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Abstract. Matrix metalloproteases (MMPs) are a family of zinc-dependent endopeptidases that degrade extracellular matrix proteins. MMP-1 and MMP-2 are produced by endothelial cells and are involved in specific vascular pathologies, including atherosclerosis and aortal aneurysm. One of the most important differences between these two metalloproteases is the possibility of hydrolysis of elastin and collagen type IV by MMP-2, but not by MMP-1. Elastin-derived peptides are generated as a result of the degradation of elastin fibers. The aim of our study was to compare the production of MMP-1 and MMP-2 in cultured human arterial endothelial cells derived from vascular pathologies localized at three different sites, the coronary artery, iliac artery and aorta, measured as their concentration in cell culture medium. The second aim was to evaluate the influence of κ-elastin (at concentrations 0.1, 0.4, 1.0, 2.5 or 5.0 μg/ml) on the production of the evaluated metalloproteases in three endothelial cell lines. The production of MMP-1 was statistically significantly greater in endothelial cells derived from the aorta compared to that in the endothelium obtained from the coronary and iliac arteries. There were no statistically significant differences in the production of MMP-2 among the endothelial cell lines tested. The addition of κ-elastin at all evaluated concentrations did not statistically significantly influence the concentration of MMP-1 in the cultured coronary artery endothelium. Furthermore, no statistically significant differences were observed in the cultured iliac artery endothelium. In the cultured endothelium derived from the aorta, κ-elastin at concentrations of 0.1 and 0.4 μg/ml significantly increased the amount of MMP-1.

Introduction

Matrix metalloproteases (MMPs), also termed matrixins, are a family of zinc-dependent endopeptidases that degrade proteins of the extracellular matrix (ECM). The timely breakdown of ECM is essential for a variety of processes, including embryonic development, morphogenesis, angiogenesis, reproduction, osteogenesis, tissue resorption and vascular remodeling.

MMP-1 (collagenase 1) hydrolyzes collagen types I, II, III, VII, VIII, X and XI, as well as gelatin, fibronectin, vitronectin, laminin, tenascin and aggrecan, and links protein, myelin basic protein and versican. MMP-2 (gellatinase) degrades collagen types I, II, III, IV, V, VII, X and XI, gelatin, elastin, fibronectin, vitronectin, laminin, entactin, tenascin, SPARC and aggrecan, and links protein, galectin-3, versican, decanin and myelin basic protein (1,2). One of the most important differences between theses two metalloproteases is the possibility of the hydrolysis of elastin and collagen type IV by MMP-2, but not by MMP-1.

The endothelium, a single layer of cells constituting the inner surface of blood vessels, was long considered to be merely a barrier between blood and smooth muscle cells of the vessel wall. Since then, the ability of the endothelium to synthesize and release various substances with multidirectorial biological functions has been elucidated, and it is now considered the biggest endocrine gland of the human body. The endothelium plays a crucial role in the regulation of vasomotorics and haemostasis. Substances produced by endothelial cells are also involved in angiogenesis and inflammation processes.

Atherosclerosis is a systemic multifocal disease leading to various clinical events, depending on the vascular site where it is most pronounced: coronary arteries, cerebral arteries or iliac and lower limb arteries. Endothelial dysfunction is considered a key factor preceding atherosclerotic lesions. The theory of unified response to injury, formulated by Ross (3), postulates a stereotypic vascular wall reaction generated by various factors, leading, not only to endothelial damage, but also to endothelial dysfunction.

Metalloproteases are produced by endothelial cells and are involved in various vascular pathologies, including atherosclerosis and aortal aneurysm. The latter is presently considered the equivalent of coronary artery disease (CAD), placing such patients in a group of secondary CAD prevention, independently of the presence or absence of coronary heart disease itself (4-9).

Elastin-derived peptides (EDPs) are generated as a result of a degradation of elastin fibres. Elastin has a slow metabo-

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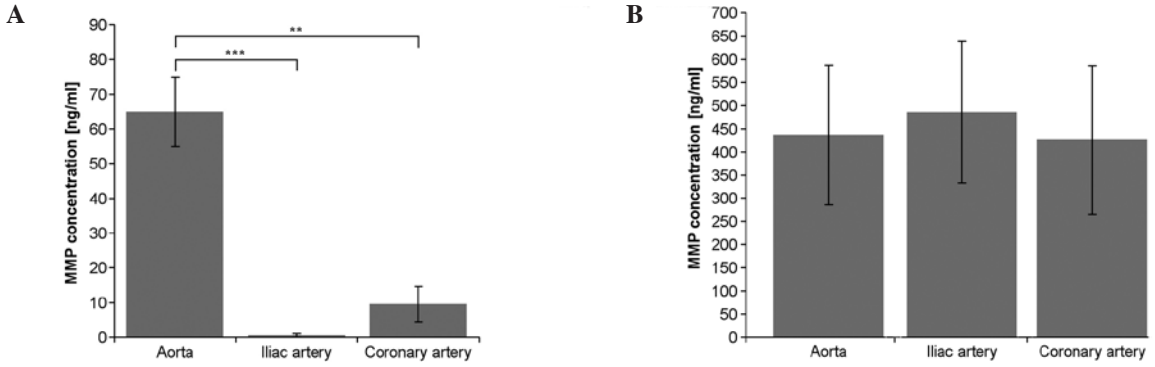

Figure 1. Concentration of MMP-1 (A) and MMP-2 (B) in various types of endothelial cells (controls). Values are presented as the mean ± SD. Significantly different at $^{**}p<0.01$ and $^{***}p<0.001$, respectively.

Table I. Concentration of MMP-1 (ng/ml) in the various types of endothelial cell lines.

Cell line	Control	E1	E2	E3	E4	E5
Aorta	64.8±10.0	79.8±13.7	87.4±13.3	73.4±14.4	66.8±10.0	69.3±7.50
Iliac artery	0.50±0.40	0.30±0.30	1.10±0.80	1.70±1.70	2.30±2.60	1.50±1.40
Coronary artery	9.50±5.10	9.60±4.30	11.1±5.40	11.5±4.40	10.5±3.30	10.5±2.20

E1, E2, E3, E4 and E5: κ-elastin concentrations of 0.1, 0.4, 1.0, 2.5 or 5.0 μg/ml, respectively. Data represent the mean ± SD (n=8).

lism, which is accelerated in atherosclerosis, lung emphysema, neoplasms or arthritis (10). Oligopeptide sequences VCVAPG are detected in both insoluble elastin and EDPs. These sequences activate the elastin receptor and exert a multitude of biological effects. EDPs stimulate the synthesis and release of metalloproteases. In experimental studies, rabbits receiving injections of EDPs developed atherosclerosis (11). Studies indicate that disturbances of elastin metabolism leading to increased serum levels of EDPs are one of the risk factors of atherosclerosis. Since κ-elastin is an acknowledged EDP, numerous experiments have evaluated its influence on aorta and endothelial cells (12-14).

The aim of our study was to compare the production of MMP-1 and MMP-2 in cultured human arterial endothelial cells derived from vascular pathologies localized at three different sites, the coronary artery, iliac artery and aorta, measured as their concentration in cell culture medium. The second aim was to evaluate the influence of κ-elastin on the production of the evaluated metalloproteases in the three studied endothelial cell lines.

Materials and methods

Cell culture. Human endothelial cells isolated from the coronary artery, iliac artery or aorta were purchased from Lonza. The cells were subcultured according to the manufacturer's recommendations. Briefly, the cells were maintained in EBM-2 medium with 5% FBS and endothelial cell-specific supplements (IGF, VEGF and heparin) in a 95% CO_2 atmosphere at 37˚C. Cells were used in experiments on 3-4 split. After trypsinization, the cells were grown to confluence on 24-well plates. Subsequently, they were incubated with κ-elastin at concentrations of 0.1, 0.4, 1.0, 2.5 or 5.0 μg/ml, respectively, for 24 h. Next, the cell culture medium was removed, centrifuged for 15 min at 3,000 rpm and stored at -70˚C for subsequent analyses.

ELISA. MMP-1 and MMP-2 concentrations were determined using commercially available kits (GE Healthcare). The antibodies used for detection were specific for active MMP forms only. Due to the high concentration of MMP-2 in the cell lysates, analytes were diluted twenty times just before determination. Resulting optical densities were plotted against standards, and the absolute concentration in ng/ml was obtained and used for the statistical analyses.

Statistical analysis. Data were expressed as the mean ± standard deviation (SD). Statistical significance was calculated using the parametric one-way ANOVA test for normal distributions, assuming the homogeneity and heterogeneity of variances, followed by the Tukey HSD test. The Kruskal-Wallis rank test was applied in the case of non-normality of distributions, followed by the Steel-Dwass test. Dunnett's test or Steel's test were used to compare each group to the control. Prior to the parametrical analyses, the normal distribution was verified with the Shapiro-Wilk test. The homogeneity of variance was analyzed using the Levene test. The accepted level of statistical significance was $p<0.05$.

Results

The production of MMP-1 was statistically significantly greater in endothelial cells derived from the aorta compared

Table II. Concentration of MMP-2 (ng/ml) in various endothelium cell lines.

Cell line	Control	E1	E2	E3	E4	E5
Aorta	436.7±150.7	365.4±52.90	422.4±102.1	430.5±106.0	406.5±93.90	416.6±72.0
Iliac artery	485.4±152.3	481.1±209.0	435.5±72.40	434.1±79.70	423.6±123.3	426.5±65.8
Coronary artery	425.8±160.6	386.1±79.00	356.8±96.90	332.2±89.40	326.0±95.40	331.4±43.7

E1, E2, E3, E4 and E5: κ-elastin concentrations of 0.1, 0.4, 1.0, 2.5 or 5.0 μg/ml, respectively. Data represent the mean ± SD (n=8).

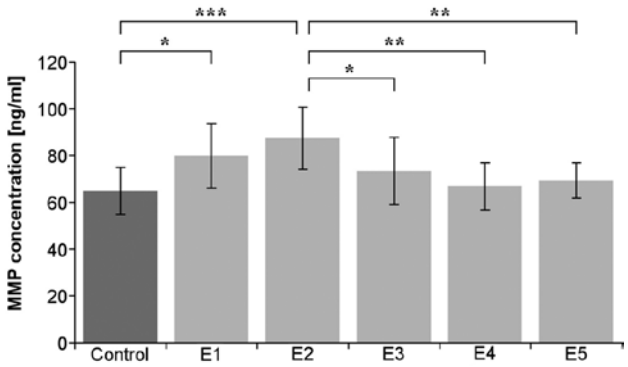

Figure 2. Effects of various concentrations of κ-elastin on MMP-1 in human aortic endothelial cells. E1, E2, E3, E4 and E5: κ-elastin concentrations of 0.1, 0.4, 1.0, 2.5 or 5.0 μg/ml, respectively. Values are presented as the mean ± SD. Significantly different at $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$, respectively.

to the production of MMP-1 in endothelium from the coronary and iliac arteries (Fig. 1A). There were no statistically significant differences in the production of MMP-2 among the studied endothelium cell lines (Fig. 1B).

The addition of κ-elastin at all evaluated concentrations did not statistically significantly influence the concentration of MMP-1 in the cultured coronary artery endothelium. Additionally, no statistically significant differences were observed in the cultured iliac artery endothelium (Table I). In the cultured endothelium derived from aorta, κ-elastin at concentrations of 0.1 and 0.4 μg/ml statistically significantly increased the amount of MMP-1 (Fig. 2).

None of the concentrations of κ-elastin used in our study influenced the levels of MMP-2 in the three endothelial cell lines with statistical significance (Table II).

Discussion

The endothelium represents an extremely biologically active region of the blood vessel. Human umbilical vein endothelial cells (HUVECs) are the most commonly used source of endothelium for cell cultures. Various properties of endothelial cells depend on the vascular location. Jackson *et al* (15) observed that HUVECs produced substantially higher levels of both MMP-1 and MMP-2 compared to levels in endothelium derived from neonatal foreskin. As atherosclerosis is the process affecting arteries, it is likely that experiments performed on arterial endothelial cell lines would elucidate the pathology of this process more precisely than studies carried out on HUVECs. Basu *et al* (16) postulated that various levels of blood flow in different arteries cause structural and functional heterogeneity in vascular remodeling, making specific arteries prone to atherosclerosis. In their study, significantly higher expression of MMP-9 and MMP-13, but not MMP-2, was noted in the endothelium from the carotic artery. Burridge and Freidman (17) compared endothelium from porcine atheroprone coronary artery with atheroresistant iliac artery, and observed different gene expression profiles. The differences observed in their study did not include the metalloprotease genes evaluated in our study. The experimental model of cell culture chosen for the present study allowed for the elimination of the influence of blood flow and sheer stress. As all endothelial cell lines treated under at the same conditions, the obtained results were determined solely in regards to genetic factors. Aboyans *et al* (18) indicated that, although atherosclerotic lesions first occur predominately in large vessels, the more distal arteries may also be affected by aging. In order to avoid this potential bias, the endothelial cell lines used in our experiment were derived only from large arteries.

The most significant clinical manifestation of atherosclerosis is myocardial infarct, which is caused mainly by the rupture of unstable atherosclerotic plaque. A previous study by Galis *et al* (19) revealed increased expression of both MMP-1 and MMP-2 in a vulnerable region of the shoulder of an atherosclerotic plaque. Restenosis occurring after percutaneous angiovascular procedures, including stent implantation or balloon angioplasty, is a major clinical problem. Experimental animal studies revealed that endovascular procedures led to an increased and sustained expression of MMP-2 in injured arteries, detected 7-60 days after the procedure (20). Tummers *et al* (21) evaluated the serum levels of MMP-2 in rats undergoing balloon angioplasty of the carotid artery. An elevation was detected between 7 and 14 days after the procedure. Tummers *et al* postulated that an early and persistent increase in the serum level of MMP-2 may be a useful marker of vascular basement membrane remodeling and the presence of intimal hyperplasia. In the present study, the level of active MMP-2 in endothelial cell culture was evaluated. This concentration depended not only on the level of MMP-2 gene expression, but also on posttranslational processes involving the activation of proenzyme into active metalloprotease. The results of our experiment indicate that the place of origin of endothelial cells does not influence MMP-2 production. Elastin-derived peptides, which may be increased in various pathologies, also do not influence MMP-2 levels. The experiments of Feldman *et al* (20) and Tummers *et al* (21) were performed on animals, whereas our experiment was carried

out on human arterial endothelial cells. Our results support the hypothesis of Tummers *et al*, and eliminate the potential bias caused by the influence of different sites of vascular pathology and EDP on MMP-2 levels.

The serum level of MMP-2 may be a marker of a broader spectrum of processes affecting the cardiovascular system. Yasmin *et al* (22) observed increased serum levels of MMP-2 in patients with systolic hypertension and arterial stiffening. Friese *et al* (23) reported that elevation of the serum concentration of MMP-2 occurs when hypertension is accompanied by end-stage renal disease. In the arterial wall, metalloproteases are produced not only by endothelial cells, but also by smooth muscle cells and inflammatory cells (4,26). When the endothelium is not damaged, vascular smooth muscle cells have no contact with the blood stream, and the serum level of metalloproteases reflects their production by the endothelium. MMP-2 is also involved in aorta calcification, as well as the calcification of atherosclerotic plaques in coronary arteries (24,25).

The aorta is the largest arterial vessel in the body. It is the site of several vascular pathologies, including atherosclerotic plaques, calcification and aneurysms. Abdominal aortic aneurysm (AAA) is a complex and multifactorial disease (5), and several metalloproteases are involved in its pathogenesis. Annabi *et al* (8) observed an increased activity of MMP-1 in AAA. Nishimura *et al* (6) found that MMP-2 activity was higher in small AAAs of a diameter between 30 and 45 mm. In another study, MMP-1 was detected in the endothelium of AAA, whereas MMP-2 was present in endothelial cells of matured neovessels within AAAs (27).

MMP-1 and MMP-2 produced by endothelial cells participate in various (both physiological and pathological) processes. Our results indicate that the production of MMP-2, in contrast to MMP-1, is similar in endothelial cells derived from various parts of the arterial vascular system. We also demonstrated that elastin-derived peptides, which can be released in various pathologies, influence the concentration of MMP-1, but not MMP-2. Until measurement of the serum level of MMP-2 as a marker of certain vascular pathologies is introduced, the influence of other cardiovascular risk factors on its production in various endothelial cells must be evaluated.

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