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Original Article

Values of osteoprotegerin in aortic valve tissue in patients with significant aortic stenosis depend on the existence of concomitant coronary artery disease

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ABSTRACT

Introduction: Calcific aortic valve stenosis (CAVS) is a serious clinical problem. The strongest predictor of CAVS progression is the amount of calcium in the aortic valve. The pathogenesis of CAVS is largely consistent with the pathogenesis of atherosclerosis; however, about 50% of patients with CAVS do not exhibit significant atherosclerosis. Cardiovascular calcification is currently considered an actively regulated process, in which the important role is attributed to the RANKL/RANK/OPG (receptor activator of nuclear factor κB ligand/RANK/osteoprotegerin) axis. We measured OPG levels in the tissue of calcified, stenotic aortic valves in relation to the presence or absence of coronary artery disease (CAD).
Materials and methods: Aortic valve samples were collected from 105 patients with calcified, mainly severe aortic stenosis, who were divided into two groups according to the presence of CAD. In Group A ($n=44$), there were normal coronary artery findings, while in Group B ($n=61$), there was angiographically demonstrated >50% stenosis of at least one coronary artery. The control Group C ($n=21$) consisted of patients without aortic stenosis and with normal angiographic findings on coronary arteries.
Results: The highest tissue concentrations of OPG [median (pmol/L), 25th–75th percentile] were found in Group A [6.95, 3.96–18.37], which was significantly different compared to the other two groups ($P=.026$ and .001, respectively). The levels of OPG in Group B [4.15, 2.47–9.16] and in Group C [2.25, 1.01–5.08] did not differ significantly ($P=.078$); however, the lowest concentrations of OPG were found in Group C. Neither age nor gender in our study had effect on tissue levels of OPG ($P=.994$ for gender; $P=.848$ for age).
Conclusion: Calcified and narrowed aortic valves, compared to the normal valves, were accompanied by a change in tissue concentrations of OPG, which is, in addition, dependent on the presence or absence of CAD. The highest tissue concentrations of OPG in our work were found in patients with significant aortic stenosis without concomitant CAD.

1. Introduction

Calcific aortic valve stenosis (CAVS) is a serious clinical problem. Of those over 65 years, some degree of aortic stenosis can be found in 2–3% of individuals and up to 25%, in the same age group, who present with nonobstructive aortic valve disease [1–4]. The strongest predictor of CAVS progression is the amount of calcium in the aortic valve [5]. Calcification of the aortic valve is only one form of cardiovascular calcification, which also includes calcification of the intimal layer of arteries in the case of classical atherosclerosis or calcification of the medial layer of arteries in Mönckeberg arteriosclerosis [6]. The pathogenesis of CAVS is largely consistent with the pathogenesis of atherosclerosis; however, about 50% of patients with CAVS do not exhibit significant atherosclerosis [7,8]. Cardiovascular calcification is currently considered an actively regulated process, which resembles bone tissue remodeling and involves a wide range of cells, cytokines, and signal molecules [9,10]. Among them, the important role is now being attributed to RANKL/RANK/OPG (receptor activator of nuclear factor κB ligand/RANK/osteoprotegerin) axis, which was first described in the process

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of bone remodeling. Measuring serum levels of RANKL and OPG were the subject of our previous work dealing with calcium deposition in stenotic aortic valves in relation to the presence or absence of coronary artery disease (CAD) [11]. Based on previous results, this work is devoted to measuring OPG levels in the tissue of calcified stenotic aortic valves in relation to the presence or absence of CAD.

2. Materials and methods

2.1. Patient groups

Aortic valve samples were collected during cardiac surgery from 105 patients with calcified, mainly severe aortic stenosis. The patients were divided into two groups according to the presence of CAD. In Group A ($n=44$), there were normal coronary artery findings, while in Group B ($n=61$), there was angiographically demonstrated >50% stenosis of at least one coronary artery. The control Group C ($n=21$) consisted of patients without aortic stenosis and with normal angiographic coronary artery findings who were heart transplant candidates (mainly due to dilated cardiomyopathy). Patients with bicuspid aortic valve and chronic kidney disease were excluded. Moreover, each patient was tested for renal function and glomerular filtration rate <60 ml/min/1.73 m^2 was also an exclusion criterion. All patients provided a signed informed consent and the study was approved by the ethics committee.

2.2. Tissue samples processing

Aortic valve samples were collected in the operating room immediately after surgical removal. The tissue samples were excised from aortic valve leaflets and deep frozen (−80°C) immediately after withdrawal. The frozen tissue was cut into small pieces and powdered by grinding with a prechilled abrasive material, with the occasional addition of liquid N_2 to prevent thawing. Once the tissue was ground into a fine powder, the extraction solution (1% TRITON-X 100, 1% IGEPAL, 0.03% aminocaproic acid, and 100 mM Tris pH 7.4) was added and the mixture was incubated at room temperature for 1 h. The mixture was then centrifuged at 10,000*g* and 4°C for 10 min and supernatant was immediately analyzed. The concentration of total protein was measured using the BCA method (Sigma-Aldrich) and the concentration of OPG was related to the concentration of total protein in the extract of homogenized tissue. OPG was determined by a commercial Human Osteoprotegerin ELISA kit from Biovendor — Laboratorni Medicina (Brno, Czech Republic) [12,13]. The kit has been validated and consequently used in more than 70 scientific publications. The assay measures total OPG (either free or bound to sRANKL) concentration. The antibodies used in this ELISA are specific for human OPG with no detectable cross-reactivities to human sRANKL and TRAIL (tumor necrosis factor-related apoptosis inducing ligand) at 120 pmol/L. Approximately 1% cross-reactivity with recombinant mouse OPG and less than 0.06% with recombinant human CD40 and recombinant human sTNF RI and sTNF RII have been observed. Determination of OPG does not interfere with hemoglobin (1.0 mg/ml), bilirubin (170 μmol/L), and triglycerides (5.0 mmol/L).

2.3. Statistical analysis

Data are expressed as mean ± standard deviation for continuous variables and as a percentage for categorical variables. OPG values are expressed as median with interquartile range (25th and 75th percentile). The Kruskal–Wallis test was used to compare the values of OPG between all groups (A, B, and control Group C) and the Mann–Whitney test was used to compare Group A and Group B. The analysis of variance applied to logarithmic data of OPG was used when comparing the values of OPG adjusted for other variables (age and gender).

3. Results

3.1. Baseline characteristics

Baseline characteristics of both groups (A and B) including distribution of age and gender in control Group C are summarized in Table 1. From the table, it is apparent that patients in Group B were significantly older with a higher incidence of hypertension and diabetes mellitus. Group A had a conversely higher proportion of patients with severe aortic stenosis (although the difference was not statistically significant), which corresponds to higher flow rates and pressure gradients on aortic valves. It should be noted that all patients in Group A and Group B had at least moderately severe aortic stenosis. In control Group C, patients were significantly younger with a higher proportion of men.

3.2. OPG levels

Levels of OPG in each group are shown in Fig. 1. The highest tissue concentrations of OPG [median (pmol/L), 25th–75th percentile] were found in Group A (6.95, 3.96–18.37). When using the Mann–Whitney test, the levels of OPG in Group B were significantly lower (4.15, 2.47–9.16, $P=.026$), even after adjustment for age and sex ($P=.025$). The lowest tissue concentrations of OPG were achieved in control Group C (2.25, 1.01–5.08), which according to the Kruskal–Wallis test was significantly different from Group A ($P=.001$); however, when compared to Group B, the difference did not reach statistical significance ($P=.078$). The tissue concentrations of OPG in the respective groups with regard to gender and age of the studied individuals are documented in Figs. 2 and 3. In our study, neither age nor gender had effect on tissue levels of OPG ($P=.994$ for gender; $P=.848$ for age).

4. Discussion

OPG is a member of the superfamily of tumor necrosis factors and is produced by various cells (endothelial cells, vascular smooth muscle cells, osteoblasts, etc.) and in different organs [14–16]. OPG acts as a decoy receptor for RANKL, thus inhibiting its interaction with RANK, a transmembrane receptor on the cell surface of cells in the monocyte–

Table 1
Baseline patient characteristics (Group A, Group B, and Group C)

	Group A ($n=44$)	Group B ($n=61$)	Group C ($n=21$)	*P* (A vs. B)
Age (years)	67.3 ± 10.7	71.4 ± 8.4	48.0 ± 13.6	.04
Sex (%, female)	43.2	36.1	19.0	n.s.
BMI (kg/m^2)	27.6	30.0		.02
Cardiovascular risk factors (%)				
Hyperlipidemia	50.0	34.4		n.s.
Hypertension	54.5	77.0		.02
Diabetes mellitus	25.0	45.9		.03
Cardiovascular conditions (%)				
Peripheral vascular disease	4.5	13.1		n.s.
Previous stroke/TIA	6.8	14.8		n.s.
Aortic stenosis severity				
Severe AS (%)	93.2	85.2		n.s.
AS peak velocity (m/s)	4.4 ± 0.8	3.8 ± 0.8		<.01
AS PGmax/mean (mmHg)	77.3 ± 32.7/ 51.3 ± 19.7	60.5 ± 23.6/ 37.5 ± 15.4		<.01
CAD (%)				
Normal angiogram	45.5			
Nonobstructive CAD	54.5			
1-Vessel disease		31.2		
2-Vessel disease		34.4		
3-Vessel disease		26.2		
Left main disease		8.2		

BMI, body mass index; TIA, transient ischemic attack; AS, aortic stenosis; PGmax/mean, peak pressure gradient/mean pressure gradient.