PLATELET ACTIVATION PLAYS AN IMPORTANT ROLE IN REGULATION OF AORTIC VALVE CALCIFICATION IN CALCIFIC AORTIC VALVE DISEASE

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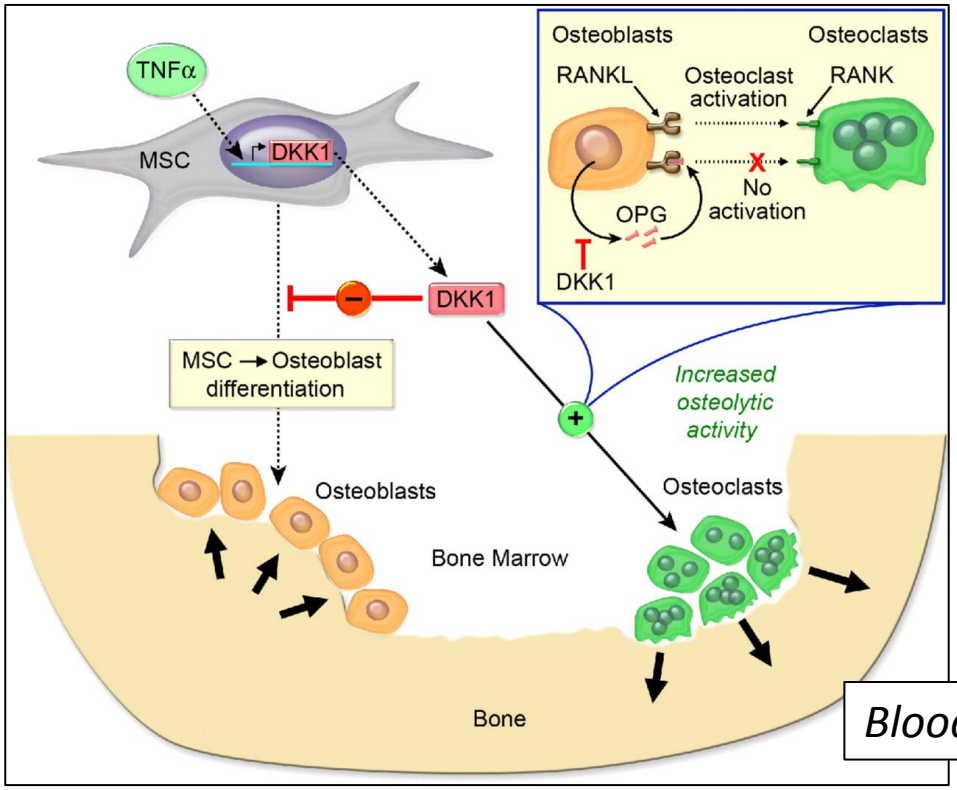
BACKGROUND I

- Calcified aortic valve disease is a progressive mineralization of aortic valve.
- Every 50th of individuals ≥ 65 years old has calcified aortic stenosis (CAS), with 80% progressing to symptoms requiring a intervention.
- The most significant predictor of clinical progression of calcified aortic valve disease is the load of calcium in aortic valve.

BACKGROUND II

- The process of calcium deposition in aortic valve is a multi-factorial event where several pathways interact and influence disease progression.

- OPG (Osteoprotegerin) / RANKL (Receptor Activator Of Nuclear Factor Kappa B Ligand) / RANK cytokine axis and Dickkopf-1 (Dkk-1) signaling might be along the causal pathway in regulation of valvular calcification in CAS.



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- Dickkopf-1 (DKK-1) might be along the causal pathway in regulation of valvular calcification.
- The effects of DKK-1 are mediated by inhibition of Wnt signaling, which directly limited osteoprotegerin (OPG) expression.
- It was suggested, that a significant amount of DKK-1 is produced by activated platelets.

PURPOSE

- The study focused on serum levels, aortic valve tissue concentrations and mRNA expression of DKK-1 and OPG.

METHODS

Serum DKK-1 and OPG were measured in

- Patients with ***symptomatic calcific aortic stenosis*** (CAS); N=313, mean age 74.3 (9.7) years, 56.2% men)
- ***Control group*** without CAS; N=100, mean age 66.6 (12.1) years, 60% men.

Tissue samples were collected

- ***during aortic valve replacement*** (AVR) (N=172, mean age 70.8 (9.5) years, 58.1% men
- ***from explanted hearts*** during transplantation (N=116, mean age 54.4 (13.1) years,83.6% men).

METHODS

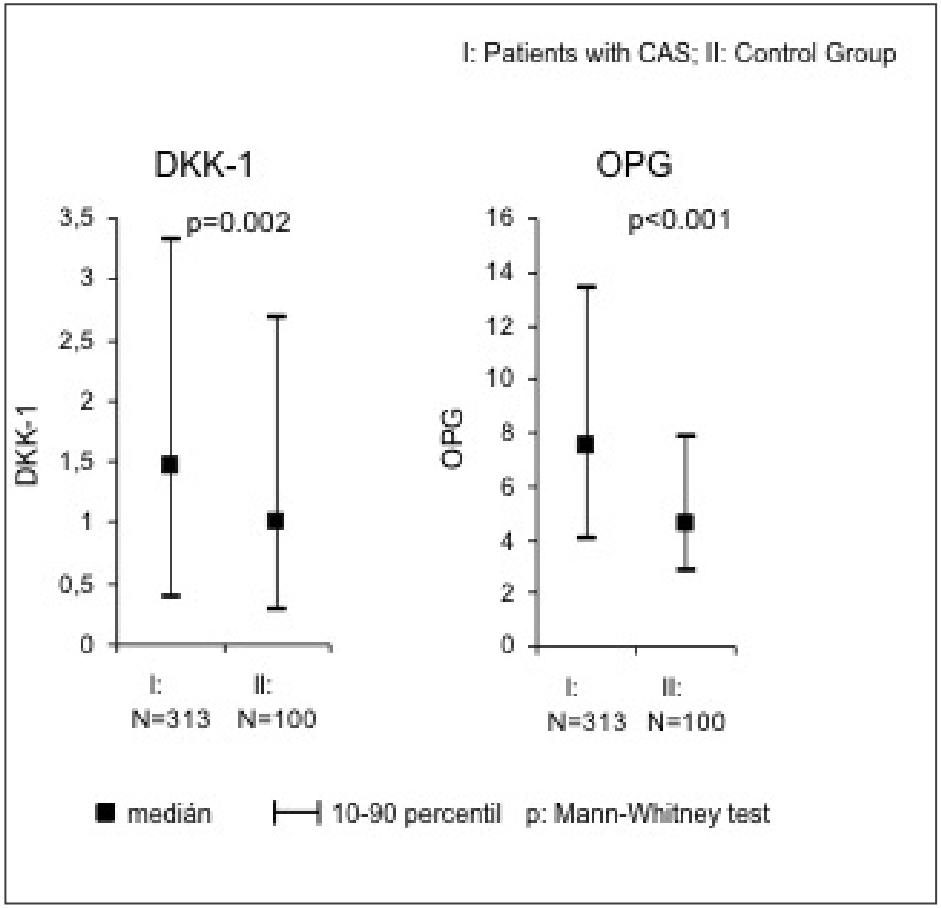
- Serum samples were stored at −70 °C until assayed. Samples were assayed using a DKK-1 and OPG ELISA kits (Biovendor, Laboratorni Medicina); according to the manufacturer's protocol.
- The tissue samples were excised from aortic valve leaflets and deep frozen (−80°C) immediately after withdrawal.

- **tissue concentrations** of DKK-1 and OPG were determined by a commercial Human ELISA kits

The frozen tissue was cut into small pieces and powdered by grinding with a prechilled abrasive material, with the occasional addition of liquid N2 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 100mMTris pH7.4) was added and the mixture was incubated at room temperature for 1 h. The mixture was then centrifuged at 10,000g and 4°C for 10min and supernatant was immediately analyzed. The concentration of total protein was measured using the BCA method (Sigma-Aldrich) and the concentrations of DKK-1 and OPG were related to the concentration of total protein in the extract of homogenized tissue.

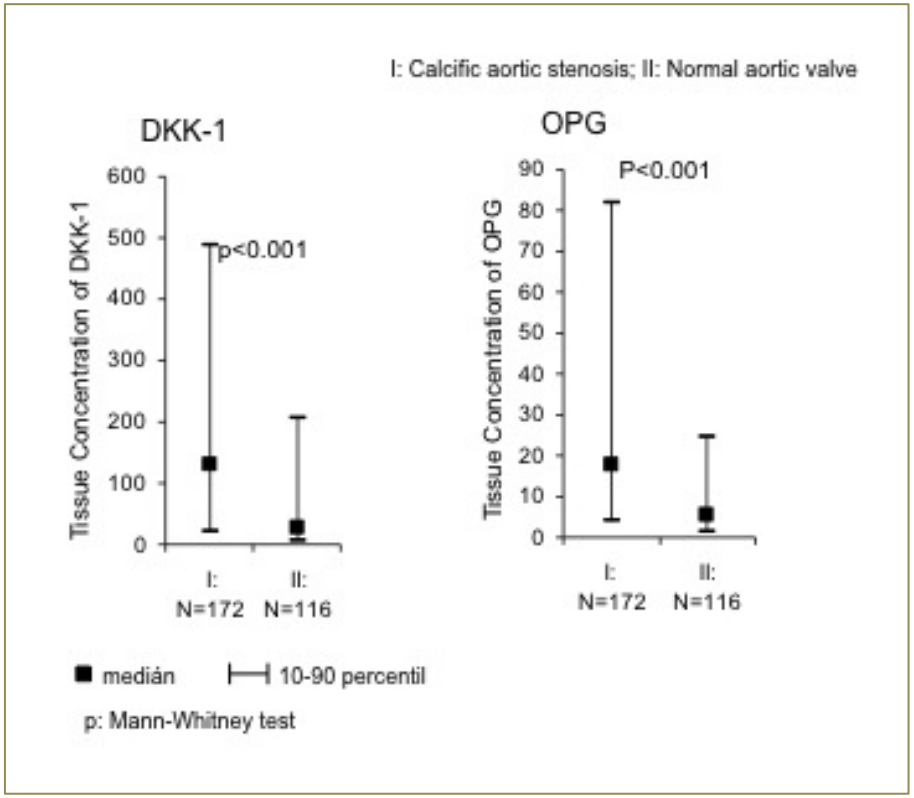
- **mRNA expression** of DKK-1 and OPG was performed.

RESULTS I - SERUM



Serum levels of DKK-1 and OPG were significantly higher in CAS in comparison to control group; DKK [Median (5th; 95th percentile): 1.5 (0.3; 3.8) vs. 1.0 (0.2; 3.4) ng/ml; p=0.002] and OPG [7.5 (3.5; 16.0) vs. 4.6 (2.6; 10.4) pmol/l; p<0.001].

RESULTS II - TISSUE



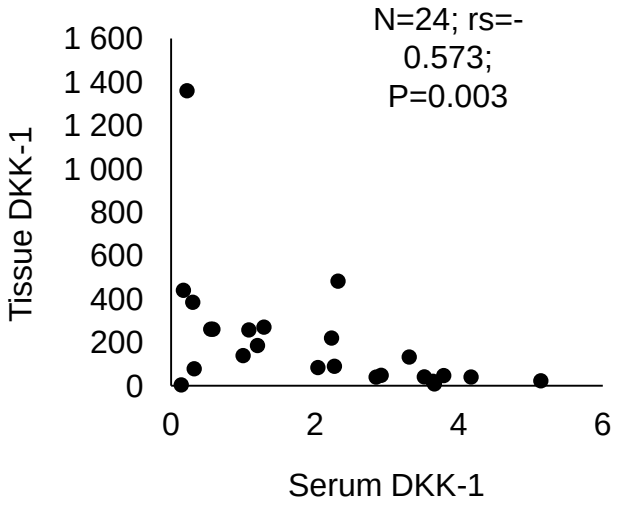
Concentrations of DKK-1 were significantly higher in tissue from CAS [131.5 (11.2; 889.7) pg/ml] in comparison with normal valves [27.3 (5.2; 764.2) pg/ml; p<0.001]. **Results for OPG were analogical**; OPG in CAS [17.9 (3.4; 122.3) pmol/l] and normal valves [5.3 (1.0;48.5) pmol/l; p<0.001].

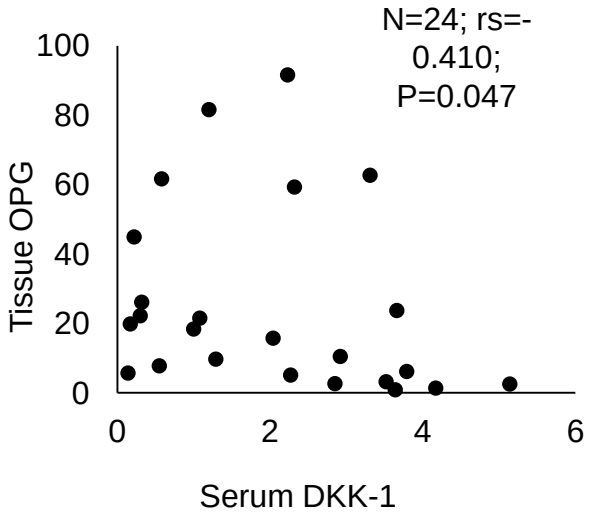
RESULTS II - TISSUE

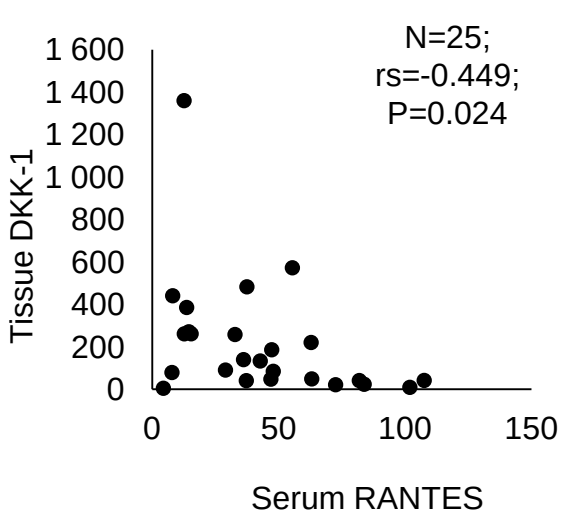
mRNA expression

Of OPG (hTNFESF11B, gen ID/NCBI 4982) was significantly lower in tissue from stenotic valves [1.16 (1.11;1.22)] in comparison to expression in tissue from normal valves [1.21(1.14;1.42); $p<0.001$].

DKK-1 (gen ID/NCBI 22943) protein expression was not detected in aortic valve tissue (irrespective of diseased or normal valves).







In CAS, significant correlations were found between

- **circulating and tissue DKK-1** (rs=-0.573;p=0.003),
- **circulating DKK-1 and tissue OPG** (rs = -0.410;p=0.047).
- Circulating and tissue **DKK-1** also **correlated with circulating levels of platelet derived RANTES** (rs=0.449;p=0.024) and **Platelet factor 4** (rs=0.697;p<0.0001).

CONCLUSIONS

- Circulating platelet-derived DKK-1 is significantly higher in CAS in comparison to normal aortic valves.
- Significant negative correlation was observed between serum DKK-1 and tissue concentration of OPG.
- mRNA expression of OPG was significantly lower in stenotic valves in comparison to normal aortic valve.

CLINICAL IMPLICATION

Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo

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- Antiplatelets in CAS?
- DKK-1 antagonist as a causal therapy of CAS?

Dickkopf-1 (I ed bone and
Wnt signalin atous SCID-
eloma (MM) c K1 is physio-
bone disease ator of bone
tion of osteol nclude that
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metabolism and tumor growth in a SCID-rab system. SCID-rab mice were engrafted with primary MM cells expressing varying levels of DKK1 from 11 patients and treated with control and DKK1-neutralizing antibodies for 4 to 6 weeks. Whereas bone mineral density (BMD) of

of anti-DKK1–treated mice had increased numbers of osteocalcin-expressing osteoblasts and reduced number of multinucleated TRAP-expressing osteoclasts. The bone anabolic effect of anti-DKK1 was associated with reduced MM burden ($P < .04$). Anti-DKK1 also significantly in-

and that blocking DKK1 activity in myelomatous bones reduces osteolytic bone resorption, increases bone formation, and helps control MM growth. (Blood. 2007; 109:2106-2111)