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Pro-inflammatory endothelial dysfunction and systemic inflammatory response in context of modeled comorbid conditions

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Motivation and Aim: Calciprotein particles (CPPs) are indispensable scavengers of excessive Ca^{2+} and PO_4^{3-} ions in blood, being internalised and recycled by liver and spleen macrophages, monocytes, and endothelial cells (ECs) [1, 2]. Earlier, it was shown that supraphysiological concentrations of CPPs (25 μg/mL), which have been detected in patients with end-stage renal disease, cause endothelial cell dysfunction by inducing release of pro-inflammatory cytokines (IL-6, IL-8, and MCP-1/CCL2) [3–11]. Here we evaluated whether physiological levels of CPPs (10 μg/mL), which correspond to 10 % increase in serum ionised calcium, and similar amounts of calcium incorporated into calciprotein monomers (CPM) are capable of causing endothelial activation.
Methods and Algorithms: CPM and CPPs were synthesised using supersaturation of saline solution with Ca^{2+} and PO_4^{3-} ions and albumin as a single mineral chaperone. Before the experiments, we calibrate the dose of Ca^{2+} ions, albumin-centric CPM, and albumin-centric CPPs to the physiological values observed in a human population (10 % increase in ionised calcium in a human population). Upon the addition of physiological calcium levels (10 μg/mL), delivered as ionised calcium (using $CaCl_2$ as Ca^{2+} donor), albumin-centric CPM, or albumin-centric CPPs, to human coronary artery endothelial cells (HCAEC) and human internal thoracic artery endothelial cells (HITAEC) for 24 hours, we measured gene expression by RT-qPCR and pro-inflammatory cytokines by dot blotting and ELISA. To model calcium stress in a living organism, we have intravenously administered $CaCl_2$, albumin-centric CPM, or albumin-centric CPPs (10 μg/mL) to Wistar rats.
Results: Mineral stress modeling revealed the physiological pattern of calcium distribution (free Ca^{2+} ions: 50 %; calciprotein monomers: 20 %; calciprotein particles: 30 %, unbound to bound calcium ratio of 1:1), confirming the pathophysiological relevance of CPM and CPP synthesis setting and indicating the role of CPM and CPP as primary and secondary depot of circulating Ca^{2+} ions. Addition of 10 μg/mL calcium elevated Ca^{2+} level in serum-free cell culture medium and in the rat serum by 10 % (1.1-fold) that corresponds to the difference between the highest and lowest Ca^{2+} quartiles in the human population. Hence, addition of 10 μg/mL calcium was considered as the relevant strategy to simulate the physiological increase of ionised calcium and to analyse the pathogenic effects of circulating calcium sources (Ca^{2+}, CPM, and CPP). Albumin-centric CPPs and CPM were internalised by HCAEC and HITAEC after 1-hour incubation in the pulsatile flow system. In contrast to Ca^{2+} ions and albumin-centric

CPM, albumin-centric CPPs (10 μg/mL) caused a significant increase in production of interleukin (IL)-6, IL-8, and monocyte chemoattractant protein (MCP-1/CCL2) into the milieu and elevated expression of *VCAM1*, *ICAM1*, *SELE*, *IL6*, *CXCL8*, and *CXCL1* genes by HCAEC and HITAEC after 24 hours of incubation. Further, incubation of HCAEC and HITAEC with albumin-centric CPPs induced excessive release of plasminogen activator inhibitor 1 (PAI-1/serpin E1), MCP-1/CCL2, IL-8, urokinase-type plasminogen activator receptor (uPAR), and macrophage inflammatory protein-3 alpha (MIP-3α). Co-incubation of monocytes with albumin-centric CPPs in the pulsatile flow system induce increased release of PAI-1, CXCL1 and CXCL5 chemokines, adiponectin, lipocalin-2, IL-6, IL-8, MIP-1α/1β, uPAR, MIP-3α, and matrix metalloproteinase 9 (MMP-9), whilst Ca^{2+} ions and albumin-centric CPM did not cause such consequences. However, intravenous administration of Ca^{2+} ions, albumin-centric CPM and albumin-centric CPPs (10 μg/mL calcium) to Wistar rats triggered a pronounced cytokine response documented as an elevation of 12, 17, and 14 cytokines respectively. Among these cytokines were granulocyte-macrophage colony-stimulating factor (GM-CSF), CX3CL1 (fractalkine), MCP-1/CCL2, CXCL7, CCL11, CCL17, PAI-1, MMP-2, MMP-3, hepatokines (hepassocin, fetuin A, hepatocyte growth factor (HGF), fibroblast growth factor 21 (FGF-21), growth/differentiation factor 15 (GDF-15)), receptor for advanced glycation end-products (RAGE), adiponectin, fibulin-3, galectin-1, and endostatin. Hence.

Conclusion: Among three distinct mechanisms of calcium delivery, albumin-centric CPPs were the only which provoked pro-inflammatory endothelial cell dysfunction and pro-inflammatory monocyte activation if added at physiological levels (10 μg/mL), whilst Ca^{2+} ions and CPM did not cause significant alterations. *In vivo*, calcium stress induced systemic inflammatory response and hepatic acute phase response irrespectively of the calcium source.

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References

1. Smith E.R., Hewitson T.D., Jahnen-Dechent W. Calciprotein particles: mineral behaving badly? *Curr Opin Nephrol Hypertens*. 2020;29(4):378-386. doi 10.1097/MNH.0000000000000609
2. Jahnen-Dechent W., Pasch A. Solving the insoluble: calciprotein particles mediate bulk mineral transport. *Kidney Int.* 2023;103(4):663-665. doi 10.1016/j.kint.2023.01.011
3. Shishkova D., Lobov A., Repkin E. et al. Calciprotein Particles Induce Cellular Compartment-Specific Proteome Alterations in Human Arterial Endothelial Cells. *J Cardiovasc Dev Dis*. 2023;11(1):5. doi 10.3390/jcdd11010005
4. Feenstra L., Kutikhin A.G., Shishkova D.K. et al. Calciprotein Particles Induce Endothelial Dysfunction by Impairing Endothelial Nitric Oxide Metabolism. *Arterioscler Thromb Vasc Biol*. 2023;43(3):443-455. doi 10.1161/ATVBAHA.122.318420
5. Shishkova D., Lobov A., Zainullina B. et al. Calciprotein Particles Cause Physiologically Significant Pro-Inflammatory Response in Endothelial Cells and Systemic Circulation. *Int J Mol Sci*. 2022;23(23):14941. doi 10.3390/ijms232314941
6. Bogdanov L., Shishkova D., Mukhamadiyarov R. et al. Excessive Adventitial and Perivascular Vascularisation Correlates with Vascular Inflammation and Intimal Hyperplasia. *Int J Mol Sci*. 2022;23(20):12156. doi 10.3390/ijms232012156
7. Shishkova D.K., Velikanova E.A., Bogdanov L.A. et al. Calciprotein Particles Link Disturbed Mineral Homeostasis with Cardiovascular Disease by Causing Endothelial Dysfunction and Vascular Inflammation. *Int J Mol Sci*. 2021;22(22):12458. doi 10.3390/ijms222212458

8. Kutikhin A.G., Feenstra L., Kostyunin A.E., Yuzhalin A.E., Hillebrands J.L., Krenning G. Calciprotein Particles: Balancing Mineral Homeostasis and Vascular Pathology. *Arterioscler Thromb Vasc Biol*. 2021;41(5):1607-1624. doi 10.1161/ATVBAHA.120.315697
9. Shishkova D., Markova V., Sinitsky M., Tsepokina A., Velikanova E., Bogdanov L., Glushkova T., Kutikhin A. Calciprotein Particles Cause Endothelial Dysfunction under Flow. *Int J Mol Sci*. 2020;21(22):8802. doi 10.3390/ijms21228802
10. Shishkova D., Velikanova E., Sinitsky M., Tsepokina A., Gruzdeva O., Bogdanov L., Kutikhin A. Calcium Phosphate Bions Cause Intimal Hyperplasia in Intact Aortas of Normolipidemic Rats through Endothelial Injury. *Int J Mol Sci*. 2019;20(22):5728. doi 10.3390/ijms20225728
11. Kutikhin A.G., Velikanova E.A., Mukhamadiyarov R.A. et al. Apoptosis-mediated endothelial toxicity but not direct calcification or functional changes in anti-calcification proteins defines pathogenic effects of calcium phosphate bions. *Sci Rep*. 2016;6:27255. doi 10.1038/srep27255