Nuclear abundance of NF-κB subunits p50 and p65 were significantly lower in megakaryocytes derived from kl/kl mice than in megakaryocytes from klotho wild-type mice, an effect again found reversed in megakaryocytes from kl/kl mice treated with LVD. Transfection of MEG-01 cells with p50/p65 significantly increased STIM1 and Orai1 expression on mRNA as well as on protein level.
Conclusions: In conclusion, klotho deficiency leads to blunted SOCE in platelets resulting in impaired thrombus formation at least in part due to impairment of NF-κB-dependent STIM1 and Orai1 expression in megakaryocytes by excessive 1,25(OH)2 vitamin D levels.

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Effects of NO on the expression and function of bradykinin type 2 receptors. Role for angioedema?

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Purpose: The pathophysiology of angioedema induced by angiotensin-converting enzyme inhibitors (ACEi) is not well understood. While activation of bradykinin (BK) type 2 receptors (B2) has been established, the triggering events remain obscure. In this study we investigated effect of NO as one product of B2 stimulation.
Methods: Brain (bEND3), human umbilical vein (HUVEC) and porcine aortic endothelial cells (PAEC) were incubated with the NO-donors DEA/NO (10μM), DETA/NO (100μM) and SNAP (1-100μM) for 3, 6 and 12 h. B2 expression was assessed by RT-PCR and westernblot using monoclonal B2 antibodies staining either glycosylated or unglycosylated B2. Function of B2 was determined by monitoring iCa2+ upon activation with BK. Likewise, B2 protein levels were evaluated in aorta, heart and lung of (1) two different eNOS-/- strains, (2) mice with endothelial-specific overexpression of eNOS (eNOS-tg), (3) in C57Bl/6 mice treated with NOS inhibitor L-nitroarginine (L-NA). Here, B2 function was evaluated in aortic rings.
Results: After 3h incubation with DEA/NO of bEND3, B2-mRNA was unchanged (160±32%, n=6, P>0.05). Likewise, B2 protein levels remained stable (98±13.6%, n=8, P>0.05). Similar results were obtained in PAECs and HUVECs and after extended incubation time and increased concentration. There was also a similar rise of iCa2+ in response to 0.01-100 μM BK in bEND3 treated with DEA/NO or vehicle as demonstrated by almost identical flourescence signals. These results obtained in cell lines and primary cultured PAEC were confirmed using transgenic mice with different levels of vascular NO-bioavailability. In aorta, neither the complete lack of endothelial NO in eNOS-/- (93±26%, n=5, P>0.05), nor overexpression in eNOS-tg (112±13.7%, n=10, P>0.05) changed B2 protein expression and a similar observation was made in C56Bl/6 treated with L-NA (110±19.4%, n=5, P>0.05). Aortic constrictor responses to 10 μM BK (related to maximal constriction to KCl) were identical in C57Bl/6 (24.3±2.7%, n=7) and eNOS-tg (30±11.8%, n=4, P>0.05). However, B2 signaling involved generation of NO as constrictor responses were significantly increased following L-NA in both C57BL/6 (64±18.3%, n=4) and eNOS-tg (67±3.6%, n=4) and a similar constriction was observed in eNOS-/- without (61±10.9%, n=7) and with L-NA (61±14.6%, n=6). None of these responses differed significantly.
Conclusion: These data demonstrate that NO does not regulate vascular B2 gene expression and function suggesting that changes of B2 expression by this product of B2 activation are unlikely involved in triggering ACEi-induced angioedema.

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Anti-inflammatory and antioxidant effects of cerium oxide nanoparticles in human endothelial cells

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Purpose: The vascular endothelium, by virtue of its strategic location and important role in cardiovascular disease, represents an important target for drug or gene therapy. Studies have focused on targeting the endothelium with nanoparticles, as therapeutic agents. Cerium oxide nanoparticles (nanoceria) have attracted much attention due to their wide range of beneficial effects. We evaluated the cytocompatibility and the anti-inflammatory and anti-oxidant effects of nanoceria on endothelial umbilical vein endothelail cells (HUVECs).
Methods and results: HUVECs were incubated for 24 and 48h with nanoceria in cell culture medium at 50 μg/mL. Cell viability was assayed by WST-1 assay, vascular adhesion molecule (VCAM-1) expression by surface enzyme immunoassay, intracellular ROS production by the fluorescent probe 6-carboxy-2'-7'-dichlorofluoresceine, cytokine production by ELISA assay and interaction nanoparticles/cells evaluated by transmission electron microscopy (TEM). Nanoceria did not affect cell growth and vitality in HUVECs at any of the incubation time. Nanoceria reduced the H2O2 (25 μmol/L)-induced ROS production in a time-dependent manner (-40%±10%, -26%±8%, p<0.05, at 48 and 24 h, respectively) and VCAM-1 surface exposure induced by TNF-α (25 ng/mL). (-35%± 6% at 24 h, p<0.01). Moreover, nanoceria significantly reduced IL-6 (38±1.9 pg/mL and 34±1.5 pg/mL vs TNF-α 45±2.25 pg/mL, at 24 and 48h respectively, p<0.05) and Il-8 (633±31 pg/mL and 807±40 pg/mL vs TNF-α 885±27 pg/mL, at 24 and 48h respectively, p<0.05) release in cell culture medium after stimulation with TNF-α. TEM analysis revealed that nanoceria was uptake by HUVECs and after 24h its localization found mostly in the cytoplasm without a preferential subcellular site.
Conclusions: These findings highlight the antioxidant and antiinflammatory activity of nanoceria in the endothelium, that combined with lack of toxicity, makes it an extremely promising therapeutic tool.

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Predictors of carotid artery wall inflammation in patients with coronary artery disease. The role of a new non-invasive method for detection of plaque inflammation

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Purpose: The role of inflammation in the progression and destabilization of atherosclerotic plaques is well recognized. Efforts have been made to use noninvasive methods to quantify the inflammatory status in the vessel wall. Microwave radiometry (MR), a new non-invasive method, allows in vivo measuring of internal temperature of tissues, reflecting inflammatory activation. The aim of this study was to evaluate in patients with coronary artery disease, which factors predict the presence of carotid artery wall inflammation, as determined by microwave radiometry.
Methods: We included 200 patients with significant coronary artery disease (≥50% stenosis in at least one major epicardial vessel). All patients underwent carotid artery examination by basic ultrasound imaging and MR. During ultrasound study, plaque texture, surface and echogenicity were analyzed. Thermal heterogeneity (ΔT) by MR was assigned as maximal temperature along the carotid artery minus minimum. ΔT ≥0.90°C was assigned as high ΔT. Vessel- and patient-based multivariate logistic regression analysis was performed to determine those factors, which independently predict the unilateral or bilateral presence of high local inflammation as measured by ΔT.
Results: Mean ΔT of all carotid arteries (n=400) was 0.80±0.49°C. Mean IMT of all carotid arteries was 2.04±1.09 mm. In 40 (20%) patients bilateral high ΔT was observed, in 61 (30.5%) patients high ΔT was detected only in one carotid artery and 99 (49.5%) patients had low ΔT bilaterally. In multivariate vessel-based analysis, plaque texture (p=0.03, OR: 2.27, 95% CI 1.11-4.67), plaque surface (p<0.001, OR: 0.12, 95% CI: 0.06-0.25), plaque echogenicity (p=0.01, OR: 0.22, 95% CI 0.07-0.69) and hypertension (p=0.01, OR: 2.60, 95% CI 1.24-5.47) were independent predictors for carotid high ΔT. In multivariate patient-based analysis, male sex (p=0.02, OR: 0.35, 95% CI 0.15-0.83), the presence of carotid plaques bilaterally (p=0.02, OR:3.18, 95% CI 1.18-8.27), diabetes mellitus (p=0.04, OR:2.16, 95% CI 1.02-4.59) and hypertension (p=0.05, OR=2.92, 95% CI 1.02-8.41) were independent predictors for bilateral high ΔT.
Conclusions: Systemic factors, including arterial hypertension and diabetes mellitus, seem to play an important role in the presence of bilateral high local inflammatory status of human carotid artery atheromas.

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PF-4var/CXCL4L1 predicts outcome in stable coronary artery disease patients with preserved left ventricular function

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Purpose: To investigate the determinants and prognostic value of the CXC chemokine PF-4var/CXCL4L1, a nonallelic variant of PF-4/CXCL4, in patients with stable coronary artery disease (CAD) and preserved left ventricular (LV) function.
Methods and results: We evaluated 205 consecutive patients with stable CAD and preserved LV function (LV ejection fraction ≥ 50%). Blood samples for PF-4var/CXCL4L1 as well as NT-proBNP were taken at inclusion. Patients were followed (median follow-up 2.5 years) for the combined endpoint of cardiac death, non-fatal acute myocardial infarction, stroke or hospitalisation for heart failure. Median PF-4var/CXCL4L1 was 10 ng/ml (interquartile range 8-16 ng/ml). Independent determinants of PF-4var/CXCL4L1 levels were age, gender and circulating platelet number. Patients who experienced cardiac events (n=20) during follow-up showed lower levels of PF-4var/CXCL4L1 (8.5 [5.3-10] ng/ml versus 12 [8-16] ng/ml, p=0.033). ROC analysis for events showed an area under the curve (AUC) of 0.82 (95% CI 0.73-0.90, p<0.001) for higher NT-proBNP levels and an AUC of 0.32 (95% CI 0.19-0.45, p=0.009) for lower PF-4var/CXCL4L1 levels. Cox proportional hazard analysis showed that PF-4var/CXCL4L1 has an independent prognostic value on top of NT-proBNP.

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