Abstract: P534

**It takes two to tango. Concurrent induction of calcification and senescence in vascular smooth muscle cells**

**Authors:**
J Herrmann¹, M Toelle¹, W Zidek¹, M Van Der Giet¹, M Schuchardt¹, ¹Charite - Campus Benjamin Franklin - Berlin - Germany,

**Topic(s):**
Stem Cells, Cell Cycle, Cell Senescence, Cell Death

**Citation:**
Cardiovascular Research (2018) 114 (Supplement 1), S131

Background: Cardiovascular disease is the leading cause of death worldwide. An important aspect of the pathogenesis of cardiovascular disease is the mineralization of the vessel wall, resulting in vessel stiffness. Important triggers of vascular calcification are oxidative stress and cellular senescence, yet the interplay between oxidative stress, cellular senescence and vascular calcification is only incompletely understood.

Purpose: The effects of doxorubicin (DOX) on cellular senescence and vascular calcification are examined in order to implement DOX as a model substance for researching underlying pathophysiologic pathways.

Methods: Vascular smooth muscle cells of rats (rVSMC) were used for in vitro experiments and aortic rings of rats for ex vivo experiments. Calcium content was quantified photometrical via o-cresolphthalein method and visualized with Alizarin Red staining. ALP activity was quantified using the p-nitro-phenol assay. Gene expression of alkaline phosphate (alp), osteoprotegerin (opg), core binding factor a (cbfa1), p53 and p21 was measured via real-time PCR. Gene expression of p21 and cbfa1 was visualized with in situ hybridization technique. The formation of reactive oxygen species (ROS) was assessed with DHE. Apoptosis is quantified with a luminogenic DEVD-peptide substrate. Senescence associated heterochromatin foci (SAHF) are detected with immunofluorescence staining of p-histone H2A.

Results: Treatments of rVSMC with DOX increase cell mineralization both in vitro and ex vivo. Ex vivo experiments with aortic rings from rats show that aortic mineralization is located in the media. DOX stimulation dose dependently induces ALP enzyme activity. Gene expression of markers of osteoblastic transdifferentiation (alp, opg and cbfa1) dose and time dependently increase upon DOX stimulation. Furthermore stimulation with DOX dose and time dependently induces gene expression of senescence markers p21 and p53. In situ hybridization of rVSMC after stimulation with DOX visualizes colocalization of p21 and cbfa1 on single cell level. DOX stimulation induces ROS production, apoptosis and the formation of SAHF in rVSMC.

Conclusion: Dox is an effective inducer of vascular calcification both in vitro and ex vivo and an effective inducer of cellular senescence. DOX can thus serve as a promising model substance for understanding the underlying pathways that link calcification and senescence.