Initial phase of anthracycline cardiotoxicity involves metabolic switch and activation of cardiac fibroblasts

K. Urbanek¹, M. Donniacuo², D. Cappetta², M. Telesca², M. Riemma², G. Belloccio², L. Berrino², F. Rossi², G. Castaldo¹, A. De Angelis²

¹University of Naples Federico II, Naples, Italy
²University of Campania “Luigi Vanvitelli”, Department of Experimental Medicine, Naples, Italy

Funding Acknowledgements: Type of funding sources: Public grant(s) – National budget only. Main funding source(s): MUR

Background: Doxorubicin (DOX) is an effective anticancer drug, but its application is hampered by cardiotoxicity with asymptomatic diastolic dysfunction as the earliest manifestation. Interstitial fibrosis leads to impaired relaxation, but the timing of fibrotic process and the mechanisms that operate shortly after DOX exposure are not clear. While changes in energy metabolism by DOX is recognized in cardiomyocytes, the effects of DOX on cardiac fibroblasts (CFs) metabolism are unknown. Thus, in a model of anthracycline cardiomyopathy, we asked whether the activation of CFs anticipates the onset of DOX-induced early diastolic dysfunction and evaluated the effects of DOX on the activation and metabolism of CFs.

Methods: Fischer 344 rats were exposed to 6 injections of 2.5 mg/kg of DOX over 2 weeks. Heart function was assessed by echocardiography and left ventricular catheterization. To test the very early in vivo effects of DOX on CFs, primary CFs were isolated from hearts of DOX-treated rats after the first injection of DOX. In another set of experiments, CFs were exposed to DOX in vitro. Cell metabolism was measured by a Seahorse-XF Real-Time ATP assay and cell phenotype was determined by immunocytochemistry and molecular biology.

Results: Early effects of DOX consisted of unchanged ejection fraction and the evidence of diastolic dysfunction. Markers of pro-fibrotic remodeling and histological evidence of CFs transformation were present in the heart immediately after treatment completion. Importantly, at the very beginning of the DOXO exposure, when cardiac function was normal and there was no histological evidence of increased fibrosis, the conversion of CFs to myofibroblasts, evidenced by SMA, was detected. Oxygen consumption rate and extracellular acidification, revealed the increased metabolic activity of CFs after DOX exposure and the switch to the glycolytic energy production. These effects were consistent in CFs isolated from the hearts of DOX-treated animals and in naive CFs exposed to DOX in vitro. The metabolic switch was paralleled with the phenotype change of CFs towards extracellular matrix-producing cells. Upon DOX, CFs upregulated markers of myofibroblast differentiation and the activation of pro-fibrotic signaling (aSMA, TGFβ and phospho-SMAD). CFs maintained their functional properties in scratch assay. CFs upregulated also NOX2, indicating these cells as an intramyocardial source of reactive oxygen in the initial phase of the disease.

Conclusion: The metabolic switch and activation of CFs anticipate the onset of DOX-induced early diastolic dysfunction. The effect of DOX on the metabolic profile in CFs represents a novel and potentially targetable component of the very early phase of the pathogenesis of anthracycline cardiomyopathy.