New therapeutic strategies for doxorubicin-induced dilated cardiomyopathy

G. Esposito[1], A. De Angelis[1], E. Piegari[1], R. Russo[1], D. Cappetta[1], C. Frati[2], F. Quaini[2], K. Urbanek[1], L. Berrino[1], F. Rossi[1]

[1]Dept. of Experimental Medicine, Division of Pharmacology, Second University of Naples, Italy
[2]Dept. of Medicine and Pathology, University of Parma, Italy

The improvement of antineoplastic therapies has resulted in a growing population of cancer patients with a long-term survival. However, the subjects that underwent chemotherapy with anthracyclines, including doxorubicin (DOX) have a high risk of heart failure (Singal et al., 1998). Studies in animals have proposed the cardiac progenitor cells (CPCs) as the cellular target responsible for DOX-induced cardiomyopathy (De Angelis et al., 2010). Moreover, recent study has documented that in myocardium of patients with anthracycline cardiomyopathy, a fraction of senescent $p16^{INK4a}$-positive human CPCs (hCPCs) was 5-fold higher than in controls. The large part of senescent hCPCs in the failing hearts of patients treated with DOX suggests that permanent arrest of hCPC growth affects the homeostasis of the heart and can promote the development of heart failure (Piegari et al., 2013).

The objectives of the present study were to evaluate the regenerative potential of hCPCs exposed to DOX in an experimental model of DOX-induced dilated cardiomyopathy and to determine whether an activator of Sirt-1 resveratrol (RES), an anti-oxidant and anti-inflammatory molecule with anti-aptototic and cardioprotective effects (Hung et al., 2000), can counteract the negative effects of DOX on hCPCs.

For this purpose, 3 months old rats received 6 i.p. injections of 2.5mg/kg of DOX over a period of 2 weeks to reach a cumulative dose of 15mg/kg. The DOX-treated rats were then randomly divided into three groups: 1) DOX-hCPCs: receiving intramyocardial injection of 50,000 hCPCs, isolated from fragments of human myocardium; 2) DOX-D-hCPCs: receiving an equal number of hCPCs exposed to DOX (0.5μM for 24h); 3) DOX: injected with saline. Three weeks after treatment, the cardiac function was assessed by echocardiography and the cardiac tissue was subjected to histological analysis.

For in vitro studies, hCPCs were treated for 24 and 48h with 0.5 and 1μM of DOX. The treatment with RES (0.5μM) was carried out together with DOX. Cell proliferation, oxidative stress, apoptosis and the expression of proteins involved in stress response or resistance to apoptosis were evaluated.

The mortality in DOX-D-hCPCs and DOX groups was significantly higher compared to DOX-hCPCs group. An improvement in anatomy and cardiac function and a reduction of ascites was observed only in DOX-hCPCs group. Tissue analysis showed extensive areas of regeneration in the left ventricle of DOX-hCPCs rats; these areas were significantly smaller in DOX-D-hCPCs group. The presence of human cells within the rat hearts was confirmed by PCR for *Alu* sequences, specifically present in humans. These data support the hypothesis that the exposure of hCPCs to DOX deteriorates their regenerative potential.

The treatment of DOX-exposed cells with RES resulted in an increase of hCPCs viability and a decrease of oxidative stress and apoptosis, counteracting the negative effects of DOX. The positive effect of the RES on hCPCs was associated with the increased expression of Sirt-1, Sirt-2 and Sirt-3 together with the augmented expression of anti-oxidant enzymes Cu/Zn-SOD, Mn-SOD and catalase.

In conclusion, the cellular damage caused by DOX reduces the regenerative potential of hCPCs. The results with RES indicate that the administration of Sirt-1 activator may be able to reduce the loss of hCPCs and prevent cell senescence, preserving the regenerative capacity of hCPCs. Importantly, a pool of functionally competent hCPCs that is still present within the heart even in severe depression of ventricular function, suggests that a small fraction of hCPCs could be isolated from DOX-treated patient, amplified and then used to treat the same patient.

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