CARDIOTOXIC EFFECTS OF SHORT-TERM DOXORUBICIN ADMINISTRATION: INVOLVEMENT OF CONNEXIN 43 IN CALCIUM IMPAIRMENT.

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The use of Doxorubicin (DOXO), a potent and broad-spectrum antineoplastic agent, is limited by the development of cardiotoxicity (Wallace 2003). The precise mechanism underlying DOXO-induced cardiotoxicity is still not completely elucidated; it is multifactorial, although alterations in calcium homeostasis, which is important for electrical impulse propagation and cell death, might be involved (Zhang et al., 2014). Since even the Cx43 is involved in these phenomena, in this study we have analysed the effects of DOXO on Cx43 expression and localization and on calcium homeostasis. Because damage caused by anthracyclines on cardiomyocytes is immediate after each injection (Pecoraro et al., 2015), in the present study we established a short-time model of DOXO-induced cardiomyopathy. C57BL/6j female mice were randomly divided in groups and injected with DOXO (2 or 10 mg/Kg) for 1-3 or 7 days once every other day. Cardiac function was assessed by echocardiography. SERCAII and phospholamban (PLB) expression were assessed by Western blot analysis, intracellular [Ca2+] were detected spectrofluorometrically by means of FURA-2AM, and Cx43 expression and localization was analysed by Western blot and confirmed by Immunofluorescence analysis. Doses and times chosen in our experimental model are able to induce cardiac dysfunction. As demonstrated by echocardiography, in fact, DOXO-treated mice exhibited decreased cardiac systolic function as measured by the % Ejection Fraction (EF) and % Fraction Shortening (FS) and increased Left Ventricular end Diastolic Diameter (LVEDD) and Left Ventricular end Systolic Diameter (LVESD). DOXO induces impairment in Ca2+ homeostasis, already evident after a single administration. In fact, we observed higher levels of basal Ca2+ concentration in primary cardiomyocytes of DOXO-treated mice and a significant reduction of SERCAII expression with a contemporary increased expression of PLB, a protein with inhibitory effects on SERCAII activity. Furthermore, DOXO administration affects Cx43 expression and localization, since our data demonstrate an increase of Cx43 expression on mitochondria (mCx43) in DOXO-treated mice in our experimental model. It has been postulated that mCx43 is part of multiprotein complex that somehow controls mitochondrial homeostasis and it could also form hemichannels that serve as a conduit for ion flux (Decrock et al., 2011) like Ca2+. In agreement with this hypothesis, in our experimental model, we found that increased mCx43 was associated with a reduced accumulation of Ca2+ in the mitochondria. In this work we have shown that even a short-term administration of DOXO induces significant changes in calcium homeostasis. Furthermore, a tight connection between the state/function of connexin and Ca2+ signalling in cardiomyocytes has been proved.

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