DOXORUBICIN-INDUCED HEARTH FAILURE IS DEPENDENT ON BOTH OXIDATIVE- AND NITROSATIVE STRESS.

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INTRODUCTION**.** Due to increasing numbers of young cancer survivors, the understanding of the biological mechanisms underlying the chemotherapy side effects, is highly relevant. Doxorubicin is a chemotherapeutic agent whose clinical use is hampered by the serious dose-dependent cardiotoxicity which ultimately results in left ventricular dysfunction, and, in the worst cases, congestive heart failure. Doxorubicin-induced cardiomyopathy is a lethal disease and, when congestive heart failure develops, mortality is approximately 50%. Unfortunately, no effective treatment is at present available. There is a large evidence that oxidative stress and inflammation are implicated in the pathogenesis of congestive heart failure. The sustained inflammatory/oxidative environment leads to cell damage becoming stuck in a vicious circle of impaired pathways.
The accumulation of Reactive Oxygen Species is widely accepted as a key factor of cardiotoxic effects, but evidence indicates that also nitrosative stress is involved.
Oxidative and nitrosative stress are strictly linked in Doxorubicin-induced heart failure. Mitochondrial Connexin 43 conferred cardioprotection by reducing mitochondrial ROS production in Doxorubicin-induced cardiotoxicity. This study aimed to evaluate the involvement of Mitochondrial Connexin 43 in Doxorubicin-induced nitrosative stress and heart failure. In fact, Doxorubicin stimulates mitochondrial superoxide over production and consequently the generation of other ROS able to trigger the activation of NF-*κ*B, thus leading to enhanced iNOS expression and NO production. It is reported that an increase in NO production in the myocardium may cause nitration of actin and other cytoskeletal proteins altering their structure and causing harmful effects on the contractile function of myofilaments.

MATERIALS AND METHODS**.** Rat cardiomyocytes H9c2 were treated with Doxorubicin and in absence or in presence of Radicicol, an inhibitor of Connexin 43 translocation to mitochondria. Fluorescence-activated cell sorting (FACS) analysis and quantitative Real Time-PCR showed that Doxorubicin increased SOD and CAT *gene* and protein expression. Moreover, the expression of molecules involved in apoptosis and NF-kB pathway was analysed by FACS and Western blot. It is known that increased expression of iNOS results in NO overproduction that quickly reacts with hydrogen peroxide or superoxide, generated by mitochondria, forming highly reactive and harmful peroxynitrite.

RESULTS: In H9c2 cells, FACS analysis showed that co-treatment with Doxorubicin and Radicicol increased SOD and CAT expression and the apoptotic response, as shown by hypodiploid nuclei. In H9c2 cells low *iNOS gene* expression levels have been observed in both untreated and treated cells, while it increased after 6 hours of Doxorubicin-Radicicol co-treatment. Western blot analysis showed a significant increase of iNOS expression in Doxorubicin-treated H9c2 cells and a further increase in Radicicol-pretreated cells after 6 hours of treatment. Since in presence of high levels of O_2^-, NO quickly reacted to form peroxynitrite thus inducing nitration of the aromatic side-chains of tyrosine in proteins, we evaluated the levels of nytrotirosine in H9c2 cells treated as described. The cytofluorimetric analysis showed a significant increase of nitrotyrosine levels in Doxorubicin-treated cells. Inhibition of mitochondrial Connexin43 translocation by Radicicol further enhanced nitrotyrosine levels in particular after 6 hours of treatment.

The induction of apoptosis was confirmed by Western blot analysis that showed how the combined treatment with Doxorubicin and Radicicol increased caspase 9 expression and reduced

procaspase 3 levels. Moreover, after 3 hours of Doxorubicin treatment a significant increase in IKKα expression was observed, more evident after 6 hours of co-treatment with Doxorubicin and Radicicol. These results suggested a rapid activation of pathway involved in inflammatory response mostly in the presence of Connexin 43 inhibitor.

CONCLUSIONS: In our models of rat cardiomyocytes, the understanding of the mechanisms underlying the cellular insult induced by Doxorubicin will result in developing new applications for preventing cardiotoxicity and consequently the heart failure. Antioxidant enzymes are mostly increased in presence of Radicicol. Therefore, our findings help to strengthen the role of Connexin 43 in cardioprotection mechanisms. Moreover, this study was aimed to explore the molecular basis of cardiomyocytes damage for tailoring medical treatment to the specific Doxorubicin-induced intracellular alterations not only to prevent heart failure, but also in an attempt to stop the progression of end-stage heart failure.