



UNIVERSITÀ DEGLI STUDI DI SALERNO



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Dipartimento di Farmacia

PhD Program

in **Drug Discovery and Development**

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PhD Thesis in

***Molecular basis of
cardiomiopathy***

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ABSTRACT

The normal heart rhythm is guaranteed by an important intercellular junctions system, named Gap Junction (GJs). Each GJs consists of two units called connexons formed by six specific trans-membrane proteins named connexins (Cx). Recent reports suggest the presence of Cx43 in the inner mitochondrial membrane where it plays an important cardioprotection mechanism. Alterations in Cx43 expression and distribution were observed in several myocardium disease; i.e. in hypertrophic cardiomyopathy, heart failure and ischemia. Thus, in this doctoral study, we investigated the role of Cx43, and in particular of mitochondrial Cx43, in different cardiomyopathies models.

At first, we have investigated the involvement of mitochondrial Cx43 in an *in vitro* model of chemical hypoxia. Hypoxia was induced by adding Cobalt Chloride (CoCl_2) on H9c2 cardiomyoblast cell line, both in absence and in presence of Radicol, an Hsp 90 inhibitor that blocks Cx43 translocation to the mitochondria. Our results showed that CoCl_2 reduces the expression of Cx43 on the cell membrane and, moreover, it increases Cx43 expression at mitochondrial level, where it is involved in the regulation of reactive oxygen species production,

calcium storage and mitochondrial membrane depolarization. Furthermore, in an *in vivo* Doxorubicin (DOXO)-induced cardiotoxicity in a short-term mouse model we have studied the modulation of Cx43 expression/activity and its dysregulation. Our results showed that DOXO is able to induce significant changes in calcium homeostasis and alterations in Cx43 expression and localization. These effects are evident even in the heart of mice that received a single DOXO-administration. Finally, we have investigated if the pretreatment with Diazoxide (DZX), an opener of mitochondrial K_{ATP} -channels, attenuates DOXO-induced cardiotoxicity in a short-term mouse model. Our results demonstrate that DZX represents a promising protective intervention against DOXO-induced cardiotoxicity, by reducing the calcium homeostasis alteration and trying to restore the major cardiac parameters altered by DOXO treatment. This is in agreement with our hypothesis that mitochondrial Cx43 and DZX are involved in the cardioprotection mechanism.