CardioPaTh
Cardiovascular Pathophysiology & Therapeutics

Identification of novel molecular mechanisms and therapeutic strategy in cardiovascular diseases (CVD)

PhD thesis

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"A man is but the product of his thoughts; what he thinks, he becomes"

Gandhi

Contents

CHAPTER 1

General introduction and outline of the thesis

Cardiovascular diseases (CVD) are a critical health issue being the major cause of death. Research studies in this field allowed the identification of several molecular mechanisms that regulate cardiac function and novel therapeutic drugs. In this context, inflammation plays a key role in the development and progression of cardiovascular diseases as demonstrated by its involvement in atherosclerotic processes and its association with increased risk for cardiovascular events. Furthermore, several clinical trials demonstrate the effectiveness of anti-inflammatory treatment in the management of cardiovascular diseases (1). It has been suggested that the transcription factor NFkappaB could represent a functional bridge between inflammation and cardiac pathologies. Indeed, the release of cytokines from activated macrophages which infiltrate the cardiac tissue induces the activation of NFkappaB also in the cardiomyocyte leading to hypertrophic gene expression and cardiac damage (2). Several regulators of NFkappaB signaling have been identified and, among them, G Protein-coupled receptor kinases seem to play a key role. These kinases are part of a family of serine/threonine kinases that phosphorylate and desensitize GPCR (such as adrenergic and muscarinic receptors). GRK2,in particular, is the most abundant in the heart and is a critical regulator of cardiac function both in physiological and pathological conditions (3). Indeed, the ablation of the GRK2 gene in myocytes affects cardiac phenotypes in adulthood leading to a prevalent eccentric remodeling after chronic exposure to β adrenergic stimulation (4). Moreover, the total deletion of the kinase is lethal by preventing the correct development of the cardiovascular system in prenatal life (5). This developmental importance of GRK2 also concerns the endothelium, which exerts a crucial role in maintaining vascular integrity by achieving whole-organ metabolic homeostasis via the production of factors associated with vasoconstriction or vasorelaxation (6, 7).The deletion of GRK2 in endothelial cells resulted in alteration of vascular phenotype and integrity, due to an increase of inflammation and oxidative stress (8).Several findings endorse the proof of concept that GRKs also regulate intracellular signaling independently from GPCR by affecting non-GPCR receptors or by direct interaction with target molecules (9). In this context, GRK2 displays a very complex “interactome”(9). Several findings confirmed that GRK2 localizes into mitochondria (7, 10-12) , acting as a key mediator of production and expenditure of energy (7).Considering that mitochondrial

dysfunction contributes to developing cardiovascular diseases and heart failure (13), GRK2 seems to be critical to preserve mitochondrial integrity and favor cardiac cell survival (14). Several other contributors to CVD have been identified, such as air pollution. Clinical and epidemiological studies demonstrate that short- and long-term exposure to air pollution increases mortality due to respiratory and cardiovascular diseases. Also, anthracyclines, the most effective antineoplastic drugs, exert a dose-dependent cardiotoxic effect leading to irreversible heart failure.

During my Ph.D. fellowship, I participated in several studies which contributed to increase the knowledge in these fields.

Outline of the thesis

The thesis is divided into five parts:

Part I: The role of GRK2 in cardiovascular disease (CVD) and its inhibition as a potential therapeutic strategy

In the first part of the thesis, I report results in the field of cardiovascular diseases, focusing on the role of GRK2 in CVD and its effects on energy metabolism.

Part II: Molecular mechanisms of endothelial dysfunction

In this section, I report my contribution in the field of the endothelial function allowing the identification of novel regulators.

Part III: Risk factors for the development of CVD: air pollution, inflammation, and doxorubicin treatment

In this part of the thesis, I collected my studies on novel cardiovascular risk factors focusing on the effects of air pollution and inflammation on cardiovascular diseases and the cardiotoxic effects of anti-cancer drugs.

Part IV: Cancer and inflammation

In this section, I reported my contribution in the oncology field. I participated in a study that proposes a novel small molecule for the treatment of cancer.

Part V: Discussion and conclusions.

The last section of the thesis is a broad discussion of the addressed topics with the conclusions.

Part I

THE ROLE OF GRK2 IN CARDIOVASCULAR DISEASE AND ITS INHIBITION AS POTENTIAL THERAPEUTIC STRATEGY

CHAPTER 2

Functional Role of Mitochondria in Arrhythmogenesis

Growing evidence indicates that mitochondria play a functional role in arrhythmogenesis. We report here the molecular mechanisms underlying the action of these highly dynamic organelles in the regulation of cell metabolism, action potential, and, overall, heart excitability. In particular, we examine the role of cardiac mitochondria in linking metabolism and cell excitability. Several studies have shown the influence of mitochondrial energetic status on the sarcolemmal action potential and heart excitability. The first experimental evidence derives from the simultaneous detection of ΔΨm and AP. ΔΨm oscillations, induced by photo-oxidation, coincide with AP oscillations: in particular, during ΔΨm collapse also AP collapses until a cell non-excitable state, while ΔΨm recovery is mirrored by AP recovery. The impact of mitochondria on cellular excitability is mainly mediated by a class of ion channels abundant in cardiac tissue: energy sensing, ATP sensitive K+ channels on the sarcolemmal membrane (sarcKATP). These channels are heteromultimeric inhibited by intracellular ATP and activated by ADP, Pi, Mg2+ ,and extremely sensitive to oxidation. Therefore, sarcKATP channels represent a crucial link between electrical function and metabolism, a dynamic relationship that has been confirmed by independent investigators (15, 16) , (Fig.1).

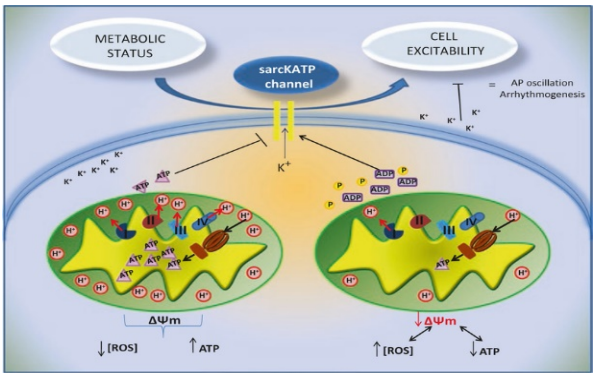

Fig 1: Role of sarcKATP channels in linking cell metabolic status and cell excitability. Mitochondrial dysfunction lead to increase of ROS and reduction of mitochondrial potential with consequent decrease of ATP production. ATP decrease, ATP and Pi activate sarcKATP channel, with K dissipating current and reduction of AP that predispose to arrhythmia.

Ca2+ homeostasis in mitochondria is mainly ensured by Ca2+ influx through Mitochondrial Ca^{2+} Uniporter (MCU), and Ca2+ efflux through mitochondrial Na+/Ca2+ exchanger (17). The administration of MCU blockers reduces the incidence of ventricular fibrillation in anesthetized rats (18). Most likely, the inhibition of MCU opening plays a protective role in keeping mitochondrial Ca2+ concentration low. Indeed, mitochondrial Ca2+ overload can induce mitochondrial dysfunction with an increase of open probability of mPTP(18). Mitochondrial Ca2+ overload might trigger the opening of the mPTP, causing uncoupling of oxidative phosphorylation, swelling of the mitochondria, and rupture of the mitochondrial outer membrane. It is not easy to define precisely the role of MCU in arrhythmogenesis given the non-specific effects of the compounds used as blockers (ruthenium red and Ru360); however, mitochondrial dysfunction as a result of Ca2+ overload suggests that these organelles can contribute also indirectly to arrhythmogenesis, through ROS production and ΔΨm collapse in response to high Ca2+ influx. In cardiacmyocytes, mitochondria and SR are structurally and functionally related, co-localizing in the so-called mitochondrial microdomain; mitochondrial Ca2+ influx through MCU is tightly linked to SR Ca2+ release (19). Alterations of RyR2 on SR, and the consequent increase of Ca2+ leak in the cytosol, are among the causes of cardiac arrhythmias (20). Mitochondria, while not representing the primary cause, can contribute strongly to the arrhythmic phenotype, being a key component of the following vicious cycle (Fig. 2):RyR alterations → increased Ca^{2+} leak → mitochondrial Ca^{2+} overload → mitochondrial dysfunction → ROS production → RyR2 oxidation → further increase of Ca^{2+} leak.

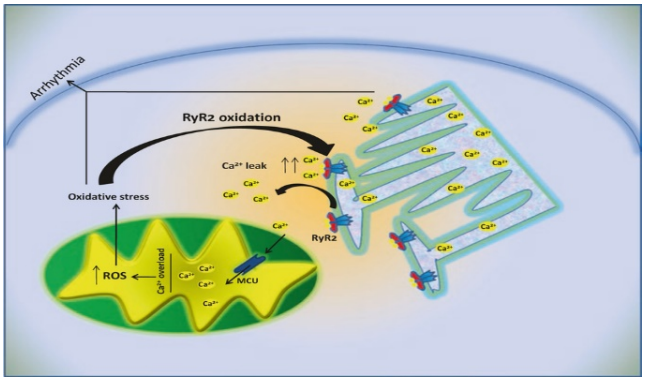

Fig 2: Ca2+ dependent pathways and mitochondrial function in arrhythmogenesis. Alterations of Ca2+ release from sarcoplasmic reticulum can induce mitochondrial dysfunction as consequence of mitochondrial Ca2+ overload. Mitochondrial dysfunction in turn, through means of oxidative stress, can mediate further alterations with increase of Ca2+ leak from SR.

As mentioned above, the role of mitochondria in cardiac arrhythmia is the result of a complex network between mitochondrial environment and extramitochondrial environment (cytosol, SR, plasmatic membrane). In particular, these dynamic organelles can contribute to electrical alterations in different circumstances, both when excitability dysregulation is a consequence of metabolic alterations, and when electrical dysfunction is the result of altered Ca2+ homeostasis.

CHAPTER 3

Mechanistic Role of Kinases in the Regulation of Mitochondrial Fitness

Mounting evidence indicates that mitochondria contain multiple phosphorylation substrates and that protein kinase translocate into mitochondria, suggesting that protein phosphorylation in this organelle could be fundamental for the regulation of its function. In particular, PINK1 is a kinase associated with mitochondria: the loss of this kinase expression causes mitochondrial dysfunction and mitophagy (15). Parkin is an E3 ubiquitin ligase suggested to be downstream of PINK1 to regulate the removal of damaged mitochondria. Indeed, PINK1 is activated by mitochondria membrane potential depolarization and is imported into mitochondria to activate Parkin (16). This latter causes proteasomal degradation of outer mitochondrial membrane proteins (21) and selective autophagy of damaged mitochondria(22), suggesting that PINK1 and Parkin mediate a mitochondrial quality control pathway. The removal of damaged mitochondria was thought to be mainly attributable to the activation of PINK1-Parkin-Ubiquitin cascade: PINK1 directly phosphorylates Parkin at Ser65 which on turn activates Ubiquitin (23). Recent findings changed this view: PINK1 has been shown to recruit Parkin to mitochondria also in presence of mutation of Ser65 to Alanine suggesting the ability of PINK1 to regulate Parkin in a phosphorylation independent manner. Moreover, PINK1 directly phosphorylates Ubiquitin at Ser65 which in turn activates Parkin (23). Thus, PINK1 phosphorylates at Ser65 both Parkin and Ubiquitin to induce the full activation of Parkin (Fig 3).

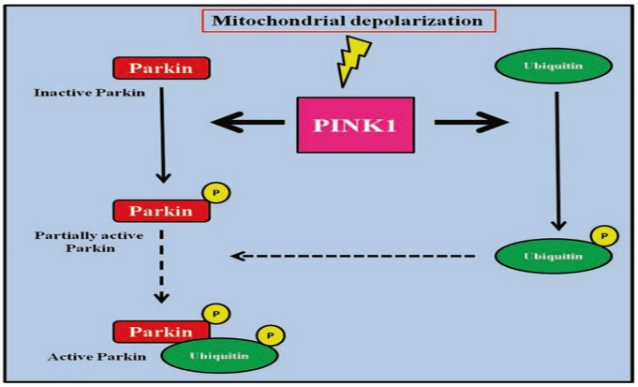

Fig 3: The full activation of Parkin is dependent on PINK1-mediated phosphorylation of both Parkin and ubiquitin at Ser65

CHAPTER 4

GRKs and β-Arrestins: "Gatekeepers" of Mitochondrial Function in the Failing Heart

Mitochondrial regulation of energy production, calcium homeostasis, and cell death are critical for cardiac function. Accordingly, the structural and functional abnormalities of these organelles (mitochondrial dysfunction) contribute to developing cardiovascular diseases and heart failure. Therefore the preservation of mitochondrial integrity is essential for cardiac cell survival. Mitochondrial function is regulated by several proteins, including GRK2 and β-arrestins which act in a GPCR independent manner to orchestrate intracellular signaling associated with key mitochondrial processes. It is now ascertained that GRK2 can recover mitochondrial function in response to insults (Fig 4).

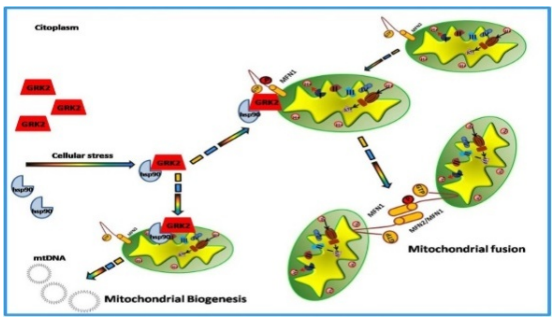

Figure 4. GRK2 activities in mitochondria.

β-arrestins affect several intracellular signaling pathways within the cell which in turn are involved in the regulation of mitochondrial function, but a direct regulation of mitochondria needs further investigations. In this review, we discuss the recent acquisitions on the role of GRK2 and β-arrestins in the regulation of mitochondrial function (Fig 5).

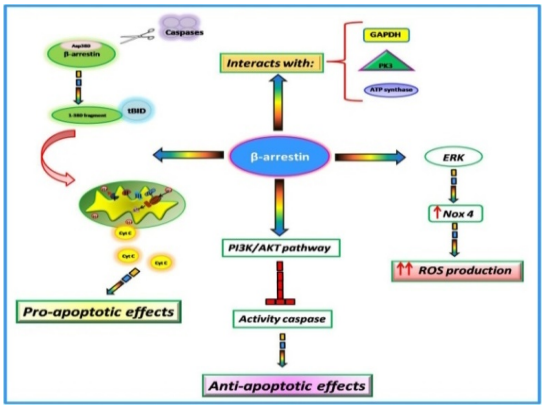

Figure 5. Non-GPCR activities of β-arrestins.

Several reports support the proof of concept that GRK2 and β-arrestins can regulate intracellular signaling in a GPCR independent manner. These activities affect several compartments within the cell, including mitochondria. The involvement of GRK2 in the regulation of mitochondrial function has been recently identified showing its ability to regulate ATP content, ROS production, mitochondrial dynamics, and apoptosis. However, the exact role of the kinase (detrimental or protective) remains to be elucidated given the opposing results from reports on this issue. Overall, we tried to reconcile these opposing findings pointing to a protective role of GRK2 in mitochondria through its binding to HSP90. Such an effect has important implications in the onset of cardiovascular disease, which are characterized by an impaired mitochondrial function. In this context, β-arrestins are novel identified targets whose activities in mitochondria are not completely clear yet. Few studies are available on β-arrestins dependent regulation of mitochondrial functions thus further investigations are needed. However, the available ones strongly suggest the involvement of β-arrestins in the regulation of mitochondrial ROS production and mitochondrial respiration. A better understanding of the role of these proteins in mitochondria could have important implications in providing the basis for new therapeutic approaches to treat mitochondrial dysfunction in cardiovascular diseases.

CHAPTER 5

Pharmacological inhibition of GRK2 improves cardiac metabolism and function in experimental heart failure

The effects of GRK2 inhibition on myocardial metabolism in heart failure (HF) are unchartered. In this work, we evaluated the impact of pharmacological inhibition of GRK2 by a cyclic peptide, C7, on metabolic, biochemical, and functional phenotypes in experimental HF. C7 was initially tested on adult mice ventricular myocyte from wild type and GRK2 myocardial deficient mice (GRK2-cKO), to assess the selectivity on GRK2 inhibition. Then, chronic infusion of 2 mg/kg/day of C7 was performed in HF mice with cryogenic myocardial infarction. Cardiac function in vivo was assessed by echocardiography and cardiac catheterization. Histological, biochemical, and metabolic studies were performed on heart samples at time points. C7 induces a significant increase of contractility in wild type but not in adult ventricle myocytes from GRK2-cKO mice, thus confirming C7 selectivity for GRK2. In HF mice, 4 weeks of treatment with C7 improved metabolic features, including mitochondrial organization and function, and restored the biochemical and contractile responses. Based on the pharmacological data collected in healthy mice, we evaluated the effects of C7 in mice with post-MI HF at the dose of 2 mg/kg/day (GRK2 inh). As shown in Figure 6A and 6B MI of the left ventricle anterior wall induces in 6 weeks induces statistically significant left ventricle dilation with the deterioration of systolic cardiac function. At this time point, we started continuous delivery of the C7 or vehicle for 4 weeks by intraperitoneal implantation of osmotic mini-pumps. Higher HR at baseline characterizes post-MI HF with reduced chronotropic reserve after ISO (Figure 6C). C7 reduces resting HR and restores chronotropic reserve in response to ISO (Figure 6C). C7-treated but not sham-treated post-MI HF group shows a significant reduction of left ventricle dimension, amelioration of EF, and +dP/dT both at baseline and after stimulation with ISO (Figure 6A, 6B, and 6D).Thus our findings suggest that GRK2 is a critical molecule in the physiological regulation of cardiac metabolism. Its alterations in the failing

heart can be pharmacologically targeted, leading to the correction of metabolic and functional abnormalities observed in HF.

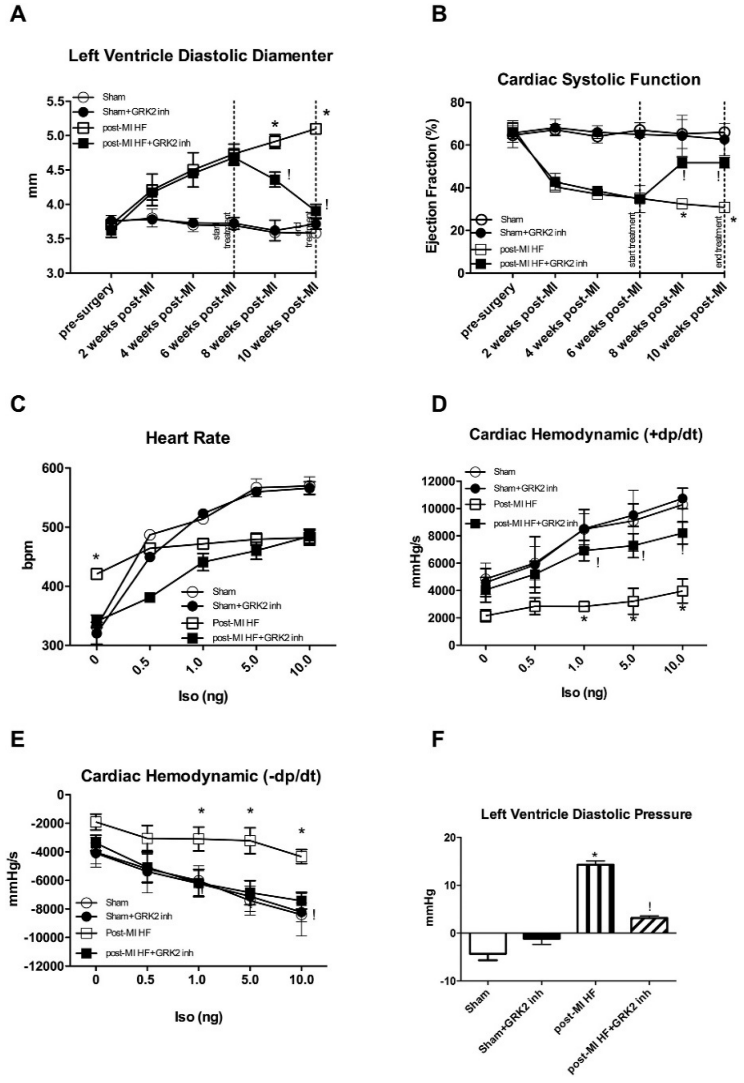

Fig 6: Reduced cardiac dilation and improved function in post-MI HF mice treated with GRK2 inhibitor. (A, B) Left ventricle diastolic diameter (LVDd) and EF measured by serial echocardiography (n = 12 per group). Induced MI led to a progressive increase of LVDd and

reduced EF, becoming significant at 6 weeks post surgery (vs. sham, P < 0.05). GRK2 inhibitor treatment arrests cardiac dilation and promotes amelioration of systolic cardiac function in HF (! vs. post-MI HF, P < 0.05). (c–f) Functional response to acute iso infusion in vivo, illustrating heart rate, and ±dp/dt max response to graded concentrations of the catecholamine (n = 6, per group). HR is higher in post-MI HF mice respect to sham at baseline (* vs. sham, P < 0.05). No significant differences are evident between sham and HF groups treated with GRK2 inh at 2 mg/kg/day. +dp/dt values are reduced upon iso iv infusion with depressed diastolic function and increased left ventricle diastolic pressure in post-MI HF (* vs. sham, P < 0.05). GRK2 inhibitor treatment improved cardiac contractility in mice with HF along with improved diastolic function and reduced left ventricle pressure (! vs. post-MI HF). EF, ejection fraction; HF, heart failure; Iso, isoproterenol; LVDd, left ventricle diastolic diameter; MI, myocardial Infarction; other abbreviations as in previous figures.*

CHAPTER 6

Antidiabetic and Cardioprotective Effects of Pharmacological Inhibition of GRK2 in db/db mice

Despite the availability of several therapies for the management of blood glucose in diabetic patients, most of the treatments do not show benefits on diabetic cardiomyopathy, while others even favor the progression of the disease. New pharmacological targets are needed that might help the management of diabetes and its cardiovascular complications at the same time. GRK2 appears a promising target, given its established role in insulin resistance and systolic heart failure. Using a custom peptide inhibitor of GRK2, we assessed in vitro in L6 myoblasts the effects of GRK2 inhibition on glucose extraction and insulin signaling. Afterward, we treated diabetic male mice (db/db) for 2 weeks. Glucose tolerance (IGTT) and insulin sensitivity (ITT) were ameliorated, as was skeletal muscle glucose uptake and insulin signaling. In the heart, at the same time, the GRK2 inhibitor ameliorated inflammatory and cytokine responses reduced oxidative stress and corrected patterns of fetal gene expression, typical of diabetic cardiomyopathy. GRK2 inhibition represents a promising therapeutic target for diabetes and its cardiovascular complications. We, therefore, examined the effects of KRXc7 administration on insulin signaling in skeletal muscle. Immunoblot analyses showed that the expression of insulin-signaling relevant proteins, such as IRS1 (Fig 7A), AKT (Fig 7B), ERK1/2 (Fig 7C), and AMPK (Fig 7D), was not modified by KRX-C7; the inhibitor, on the contrary, enhanced insulin-mediated tyrosine phosphorylation of IRS1 (Fig 7A), Serine 473 phosphorylation of AKT (Fig 7B), phosphorylation of ERK (Fig 7C), and AMPK phosphorylation (Fig 7D). These data demonstrate that GRK2 plays an essential role in diabetic muscles to regulate insulin signaling and insulin-mediated glucose homeostasis and its inhibition can restore insulin sensitivity in metabolically active tissues.

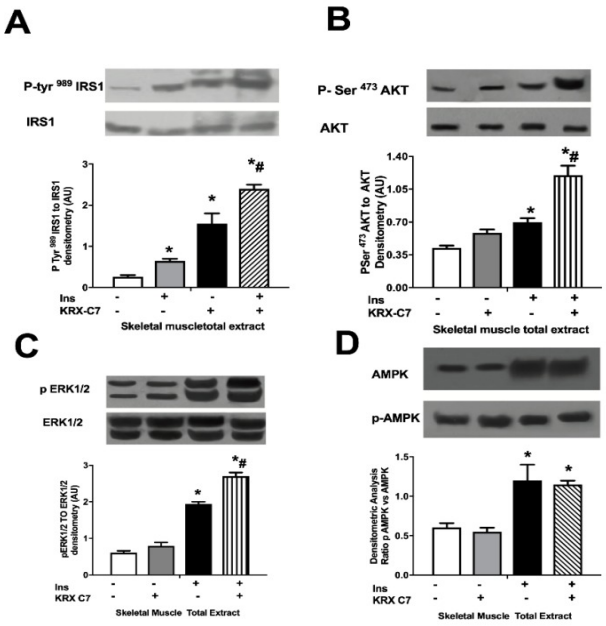

*Fig 7: KRX-C7 improve insulin signaling in db/db mice. (A) Effects of KRX-C7 chronic infusion in the femoral quadriceps of db/db mice on insulin induced phospho-IRS1. (B) Effects of KRX-C7 chronic infusion in the femoral quadriceps of db/db mice on insulin induced Phospho-Ser473AKT. (C) Effects of KRX-C7 chronic infusion in the femoral quadriceps of db/db mice on insulin induced Phosphotyrosine 42/44 ERK. (D) Effects of KRX-C7 chronic infusion in the femoral quadriceps of db/db mice on insulin induced Phospho-Ser272-AMPK. For all graphs, densitometric analysis is expressed after normalization for appropriate control protein. Data points represent the mean ± SEM of three independent experiments. * p < 0.05 vs. Ins−; # p < 0.05 vs. Ins+.*

It is known that db/db mice recapitulate diabetes-induced cardiomyopathy (DCM) phenotype, including increased myocardial proinflammatory factors, such as NF-κB, and oxidative damage. To evaluate the effects of KRX-C7 on DCM, we assessed the activation of NF-κB in the heart. The levels of phosphorylation of the p65 subunit were reduced in KRX-C7 treated db/db mice concerning the vehicle-treated group (Fig 8 A). Accordingly, the expression of related proinflammatory cytokines and chemokines, such as IL-6, MCP-2, and MMP9, was similarly reduced (Fig 8B). We also evaluated protein oxidation levels in the myocardium as a marker of oxidative damage and, interestingly, found it reduced as well (Fig 8C). Moreover, the treatment with KRX-C7 reduced Atrial Natriuretic Factor (ANF) and Sarco/Endoplasmic reticulum Calcium ATPase (SerCa) transcriptional levels

(Fig 8D) in the myocardium, confirming the beneficial effects of this drug on DCM.

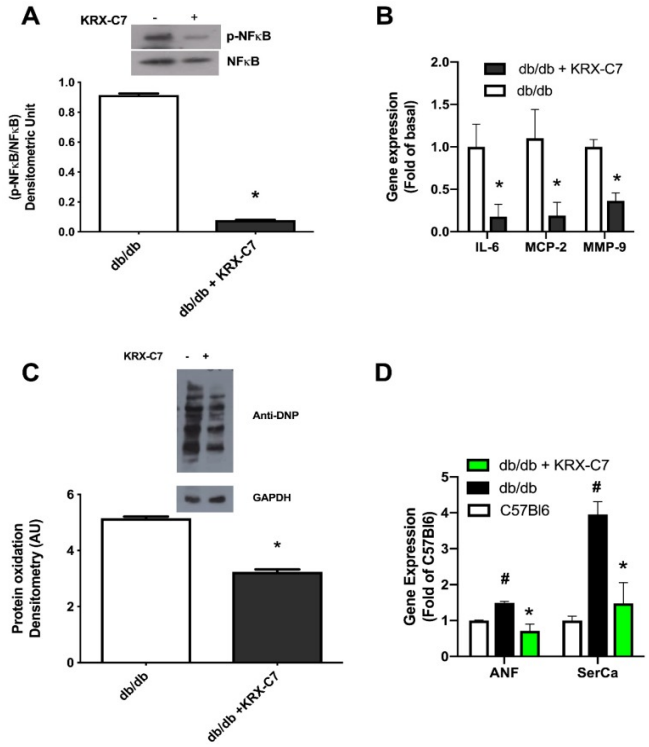

Fig 8: GRK2 inhibition reduces oxidative and hypertrophy responses in the diabetic heart. (A) Western blot analysis of phospho-NF-κB and total NF-κB levels on whole protein lysate from the cardiac tissue of KRX-C7 treated and control db/db mice (p < 0.05). (B) Gene expression analysis of proinflammatory factors, IL-6, MCP-2, and MMP9, by real-time PCR on total RNA extract from the myocardium of KRX-C7 treated and control db/db mice expressed as fold change of control (* p < 0.05). (C) Levels of protein oxidation in cardiac tissue from KRX-C7 treated and control db/db mice shown as levels of 2,4-dinitrophenylhydrazine (DNP) incorporated into proteins. GAPDH was used as the loading control. The image is representative of three independent experiments. * p < 0.05. (D) Gene expression levels of ANF and SERCa2A, performed by real-time PCR on total RNA extract from the myocardium of C57Bl6 mice, db/db mice, and KRX-C7 treated db/db mice, expressed as fold of C57Bl6 (# p < 0.05 vs. C57Bl6, * p < 0.05 vs. db/db).*

Part II

MOLECULAR MECHANISMS OF ENDOTHELIAL DYSFUNCTION

CHAPTER 7

Parathyroid hormone causes endothelial dysfunction by inducing mitochondrial ROS and specific oxidative signal transduction modifications.

Vitamin D deficiency contributes to cardiovascular risk (CVR), with hyperparathyroidism advocated as a putative mechanism. Indeed, mounting evidence supports the hypothesis that parathyroid hormone (PTH) impairs endothelial function, even though mechanisms are not fully elucidated. The present study was designed to verify in vitro the ability of sustained exposure to PTH to cause endothelial dysfunction, exploring the underlying mechanisms. In bovine aortic endothelial cells (BAECs), we evaluated the effects of PTH exposure (0.1 nM–24 hours) on both endothelial response to vasodilators, such as bradykinin (Bk (30 nM)) and acetylcholine (Ach (1 μM)), and angiogenic competence. Pretreatment with PTH impaired endothelial response to Bk but not to Ach, in terms of cytosolic calcium fluxes and NO production. To explore the underlying mechanisms, we assessed the production of total and mitochondrial ROS (tROS and mROS, respectively) in response to PTH (at 1 and 3 hours). PTH increased ROS generation, to an extent high enough to determine oxidation of Bk receptor B2. Conversely, the oxidation levels of M1 and M3 Ach receptors were not affected by PTH. A mROS selective scavenger (MitoTEMPO (5 μM)) restored the endothelial responsiveness to Bk while the well-known antioxidant properties of vitamin D (100 nM) failed to counteract PTH-mediated oxidative stress. PTH determined mitochondrial calcium fluxes ([Ca2+]mt) and the mitochondrial calcium uniporter inhibitor Ru360 (10 μM) reduced mROS production and prevented the PTH-mediated endothelial dysfunction. Angiogenic competence was evaluated as tubular formations in the endothelial Matrigel assay and showed a significant impairment in PTH-pretreated cells (0.1 nM–24 hours), despite the increase in VEGF transcriptional levels. VEGFR2 oxidation occurred in response to PTH, suggesting that even the impairment of angiogenesis was due to the ROS surge.These results indicate that PTH affects endothelial function through ROS production, driven by mitochondrial calcium overload. PTH-

induced oxidative stress might act as signaling modifiers, altering specific pathways (Bk and VEGF) and preserving others (Ach) (Fig 9).

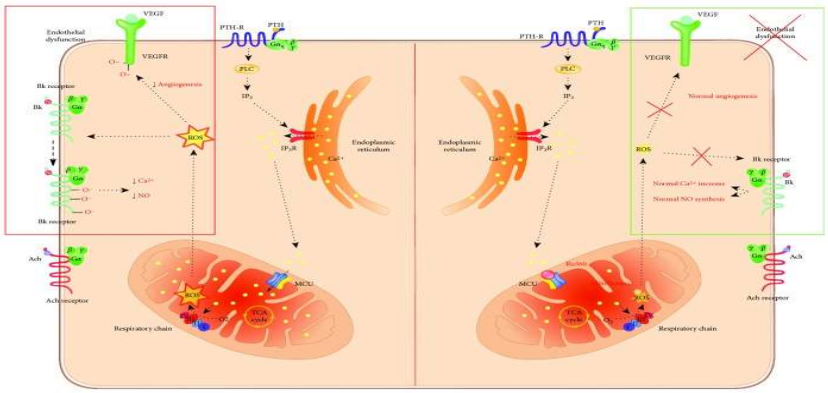

Fig 9: PTH-dependent endothelial dysfunction: hypothesis of molecular mechanisms involved. PTH, through IP3 production, induces increase of cytosolic calcium with relative increase in mitochondrial calcium uptake by MCU. The resultant mitochondrial calcium overload determines an increase of ROS production which in turn mediates the alteration of certain signaling (Bk, VEGFR) rather than other ones (Ach) by protein oxidation thus perturbing the endothelial functionality. Ru360 and MitoTEMPO, respectively, by inhibiting MCU and scavenging mitochondrial ROS, prevent perturbations in key signaling molecules for endothelial homeostasis.

CHAPTER 8

Vitamin-D concentrations, cardiovascular risk, and events - a review of epidemiological evidence

Vitamin D has long been established as an elemental factor of bone physiology. Beyond mineral metabolism, the expression of the vitamin D receptor has been identified throughout the cardiovascular (CV) system. Experimental studies showed beneficial effects of vitamin D on heart and vessels, but vitamin D intoxication in animals also led to hypercalcemia and vascular calcification. Our knowledge has been extended by epidemiological studies that showed that 25-hydroxyvitamin D (25(OH)D) levels are inversely associated with an increased CV risk itself (Fig 10), but also with established CV risk factors, such as arterial hypertension, endothelial dysfunction, and atherosclerosis. Conversely, randomized controlled trials could not document significant and consistent effects of vitamin D supplementation on CV risk or events. Potential explanations may lie in differences in reference ranges or the possibility that low vitamin D in CV disease is only an epiphenomenon. In the latter case, the key question is why low 25(OH)D levels are such a strong predictor of health. While we wait for new data, the current conclusion is that vitamin D is a strong risk marker for CV risk factors and for CV diseases itself.

Severe vitamin D deficiency is a major risk factor for osteomalacia and fractures, but might also adversely affect the cardiovascular system. It is not completely clear whether and at which concentrations vitamin D, as it is present in community-dwelling adults, is relevant for cardiovascular outcomes. In general, observational studies reported strong associations between low 25(OH)D concentrations and increased cardiovascular risk, arterial hypertension, dyslipidemia and endothelial dysfunction.

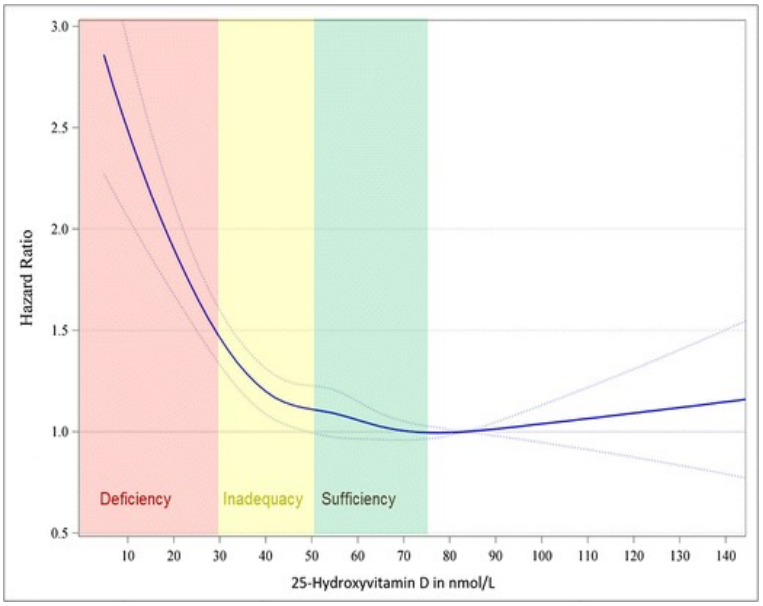

Fig 10: Dose-response trend of hazard ratios of death from all causes by standardized 25-hydroxyvitamin D concentrations

CHAPTER 9

Autocrine Bradykininin Release Promotes Ischemic Preconditioning-Induced Cytoprotection in Bovine Aortic Endothelial Cells

This study aimed to assess whether ischemic preconditioning (PC) induces bradykinin (Bk) synthesis in bovine aortic endothelial cells (bAECs) and if so, to explore the molecular mechanisms by which this peptide provides cytoprotection against hypoxia. PC was induced by exposing bAECs to three cycles of 15 min of hypoxia followed by 15 min of reoxygenation. Bk synthesis peaked in correspondence to the early and late phases of PC (10−12 M and 10−11 M, respectively) and was abolished by a selective tissue kallikrein inhibitor, aprotinin (Fig 11). Stimulation with exogenous Bk at concentrations of 10−12 M and 10−11 M reduced the cell death induced by 12 h of hypoxia by 50%. Pretreatment with HOE−140, a Bk receptor 2 (BKR2) inhibitor, in bAECs exposed to 12 h of hypoxia, abrogated the cytoprotective effect of early and late PC, whereas des-Arg-HOE-140, a Bk receptor 1 (BKR1) inhibitor, affected only the late PC. Also, we found that PC evoked endocytosis and the recycling of BKR2 during both the early and late phases, and that inhibition of these pathways affected PC-mediated cytoprotection. Finally, we evaluated the activation of PKA and Akt in the presence or absence of BKR2 inhibitor. HOE-140 abrogated PKA and Akt activation during both early and late PC. Consistently, BKR2 inhibition abolished cross-talk between PKA and Akt in PC.

In bAECs, Bk-synthesis evoked by PC mediates the protection against both apoptotic and necrotic hypoxia-induced cell death in an autocrine manner, by both BKR2- and BKR1-dependent mechanisms.

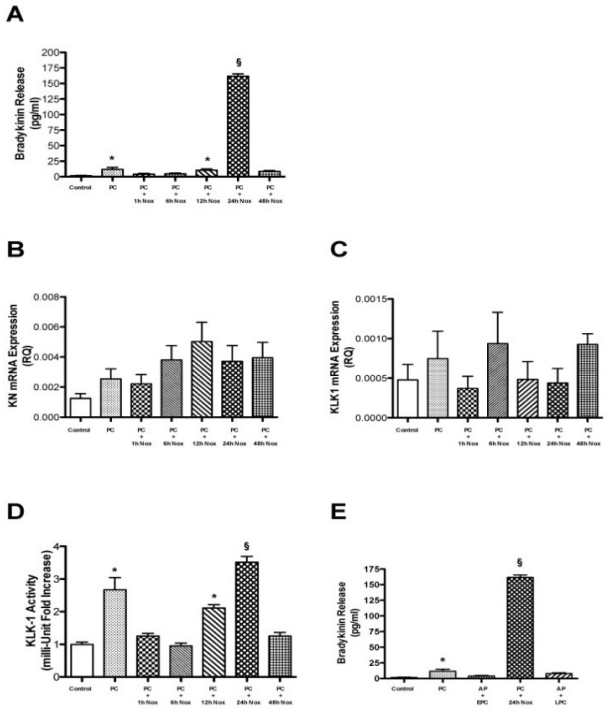

*Fig 11: Cells were subjected to preconditioning (PC). (A) Bradykinin (Bk) production was assessed at different times following PC (Nox: normoxia). The bar graph shows the concentration (mean ± SEM) of Bk, representative of four independent experiments. The transcription of the mRNAs coding for (B) kininogen (Kn) and (C) tissue kallikrein (KLK1) was assessed at different times following PC. The bar graph represents the mean ± SEM, expressed as the RQ value, of four independent experiments. (D) KLK1 activity was assessed at different times following PC. The bar graph shows the mean ± SEM, expressed as the fold increase in KLK1 activity over that in control cells, of five independent experiments. (E) Bk synthesis was measured in early and late preconditioned cells, in the presence and absence of a KLK1 selective inhibitor, aprotinin (AP). The bar graph shows the concentration (mean ± SEM) of Bk, representative of three independent experiments. Nox: normoxia. * $p < 0.001$ vs. control; § $p < 0.001$ vs. control and PC, by one-way ANOVA with a post hoc test of HSD.*

Part III

RISK FACTORS FOR THE DEVELOPMENT OF CVD: AIR POLLUTION, INFLAMMATION AND DOXORUBICIN TREATMENT

CHAPTER 10

The mechanisms of air pollution and particulate matter in cardiovascular diseases

Clinical and epidemiological studies demonstrate that short- and long-term exposure to air pollution increases mortality due to respiratory and cardiovascular diseases (Fig 12). Given the increased industrialization and the increased sources of pollutants (i.e., cars exhaust emissions, cigarette smoke, industry emissions, burning of fossil fuels, incineration of garbage), air pollution has become a key public health issue to solve. Among pollutants, the particulate matter (PM) is a mixture of solid and liquid particles that differently affects human health depending on their size (i.e., PM10 with a diameter<10 μm reach the lung and PM2.5 with a diameter <2.5 μm penetrate deeper into the lung). In particular, acute exposure to PM10 and PM2.5 increases the rate of cardiovascular deaths. Thus, appropriate interventions to reduce air pollution may promote great benefits to public health by reducing the risk of cardiovascular diseases. Several biological mechanisms have been identified to date which could be responsible for PM dependent adverse cardiovascular outcomes. Indeed, the exposure to PM10 and PM2.5 induces sustained oxidative stress and inflammation. PM2.5 is also able to increase autonomic nervous system activation. Some potential therapeutic approaches have been tested both in pre-clinical and clinical studies, based on the intake of antioxidants from dietary or by pharmacological administration. Studies are still in progress to increase the knowledge of PM activation of intracellular pathways and propose new strategies of intervention.

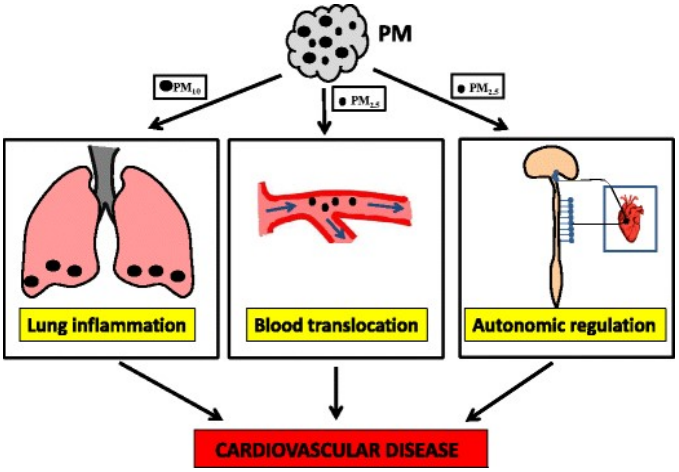

Fig 12: PM target tissues. PM can affect cardiovascular system by different potential mechanisms: direct or indirect lung inflammation, direct blood translocation, and autonomic regulation.

CHAPTER 11

NFkappaB is a Key Player in the Crosstalk between Inflammation and Cardiovascular Diseases

Inflammation is a key mechanism of cardiovascular diseases. It is an essential component of atherosclerosis and a significant risk factor for the development of cardiovascular events. In the crosstalk between inflammation and cardiovascular diseases, the transcription factor NFκB seems to be a key player since it is involved in the development and progression of both inflammation and cardiac and vascular damage (Fig 13).

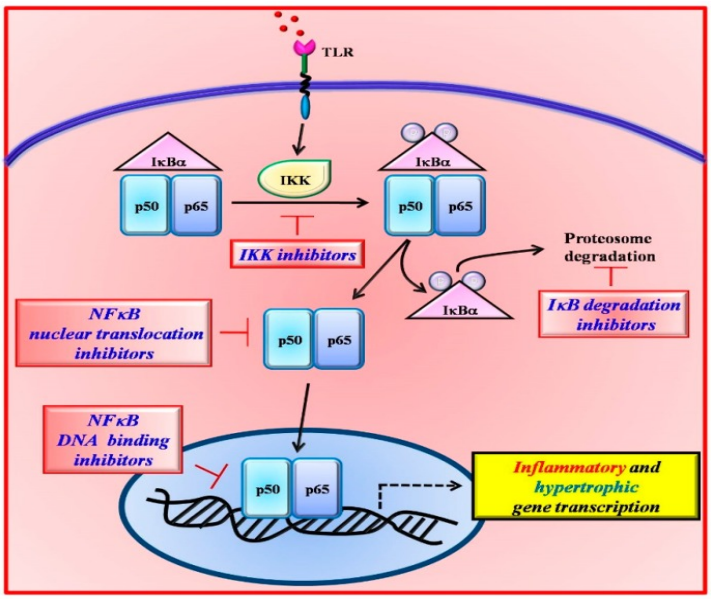

Fig 13: The inhibition of NFκB signaling can occur at different steps of its activation pathway. Several inhibitors have been designed which act on IκBα degradation (TP-110), IKK activity (EF-24), NFκB nuclear translocation (SN-50), and DNA binding (tacrolimus).

Given the involvement of GRKs in both inflammatory and cardiac diseases and their ability to regulate NFκB, several inhibitors have been designed on the GRKs sequence (24). Among them, two specific compounds resulted to

be effective inhibitors of NFκB in the heart: a peptide which reproduces the RH domain of GRK5 (TAT-RH) and the inhibitor of GRK2 kinase activity (Ant-124) (Figure 14).

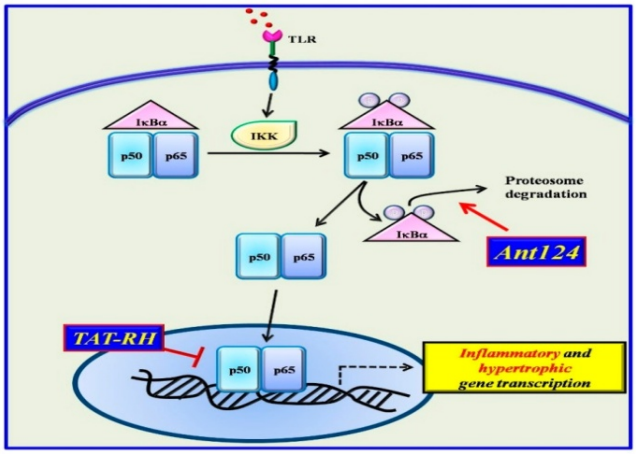

Fig 14: Two novel inhibitors have been designed based on the structure of GRKs. Ant124, which reproduces the HJ loop of GRK2, inhibits NFkB activation by blocking IκBα degradation. TAT-RH, which reproduces the RH domain of GRK5, binds IκBα and blocks the complex IκBα/NFκB in the nucleus thus preventing NFκB activation.

Inflammation plays a key role in the development and progression of cardiovascular diseases as demonstrated by its involvement in atherosclerotic processes and its association with increased risk for cardiovascular events. Furthermore, several clinical trials demonstrate the effectiveness of anti-inflammatory treatment in the management of cardiovascular diseases. In this context, the transcription factor NFκB could represent a functional bridge between inflammation and cardiac pathologies. Indeed, the release of cytokines from activated macrophages that infiltrate the cardiac tissue induces the activation of NFκB also in the cardiomyocyte leading to hypertrophic gene expression and cardiac damage. The effectiveness of GRK-dependent inhibition of NFκB suggests that it could represent a potential therapeutic target for the treatment of cardiac dysfunction and the associated inflammatory phenotype. However, it should be considered that the systemic delivery of NFκB inhibitors could induce side effects by affecting other non-target organs. Thus, in the future,

strategies for tissue-specific targeting of such inhibitors (i.e., the use of nanotechnology) should be developed to avoid potential side effects.

CHAPTER 12

The novel butyrate derivative phenylalanine-butyramide protects from doxorubicin-induced cardiotoxicity

Butyric acid (BUT), a short-chain fatty acid produced daily by the gut microbiota, has proven beneficial in models of cardiovascular diseases. With advancements in cancer survival, an increasing number of patients are at risk of anticancer drug cardiotoxicity. Here we assess whether the novel BUT derivative phenylalanine-butyramide (FBA) protects from doxorubicin (DOXO) cardiotoxicity, by decreasing oxidative stress and improving mitochondrial function. In C57BL6 mice, DOXO produced left ventricular dilatation assessed by echocardiography. FBA prevented left ventricular dilatation, fibrosis, and cardiomyocyte apoptosis when co-administered with DOXO (Fig 17). DOXO increased atrial natriuretic peptide, brain natriuretic peptide, connective tissue growth factor, and matrix metalloproteinase-2 mRNAs, which were not elevated on co-treatment with FBA. DOXO, but not FBA + DOXO mice, also showed higher nitrotyrosine levels and increased inducible nitric oxide synthase expression. Accordingly, DOXO hearts showed lower levels of intracellular catalase vs. sham, while pre-treatment with FBA prevented this decrease. We then assessed for reactive oxygen species (ROS) emission: DOXO induced increased activity of mitochondrial superoxide dismutase and higher production of H2O2, which were blunted by FBA pre-treatment. FBA also ameliorated mitochondrial state 3 and state 4 respiration rates that were compromised by DOXO. Furthermore, in DOXO animals, the mitochondrial degree of coupling was significantly increased vs. sham, while FBA was able to prevent such increase, contributing to limit ROS production, Finally, FBA reduced DOXO damage in human cellular models, and increased the tumor-killing action of DOXO. Phenylalanine-butyramide protects against experimental doxorubicin cardiotoxicity. Such protection is accompanied by a reduction in oxidative stress and amelioration of mitochondrial function.

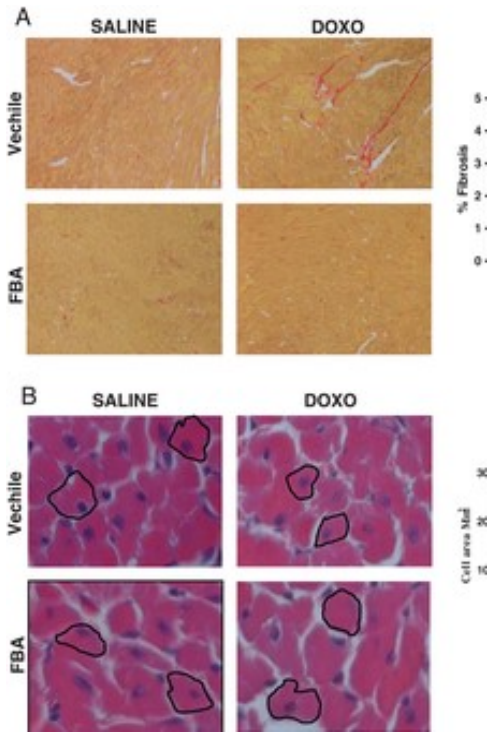

*Fig 17: Phenylalanine-butyramide (FBA) protects heart from remodelling induced by doxorubicin (DOXO). (A) FBA reduces interstitial fibrosis provoked by DOXO in the heart. (B) FBA protects the heart from reduction of cardiomyocyte size induced by DOXO. (C) Cell damage and DNA fragmentation is prevented by FBA administration in comparison with DOXO. **P < 0.005 vs. sham; ***P < 0.0005 vs. sham; ###P < 0.0005 vs. DOXO by one-way ANOVA analysis followed by Bonferroni post-hoc test.*

CHAPTER 13

Diazoxide Improves Mitochondrial Connexin 43 Expression in a Mouse Model of Doxorubicin-Induced Cardiotoxicity

Doxorubicin (DOXO) administration induces alterations in Connexin 43 (Cx43) expression and localization, thus, inducing alterations in chemical and electrical signal transmission between cardiomyocytes and in intracellular calcium homeostasis even evident after a single administration. This study was designed to evaluate if Diazoxide (DZX), a specific opener of mitochondrial KATP channels widely used for its cardioprotective effects, can fight DOXO-induced cardiotoxicity in a short-time mouse model. DZX (20 mg/kg i.p.) was administered 30 min before DOXO (10 mg/kg i.p.) in C57BL/6j female mice for 1–3 or seven days once every other day. A recovery of cardiac parameters, evaluated by Echocardiography, was observed in DZX+DOXO co-treated mice. Western blot analysis performed on heart lysates showed an increase in Sarco/endoplasmic reticulum Ca2+-ATPase (SERCAII) and a reduction in phospholamban (PLB) amounts in DZX+DOXO co-treated mice (Fig 18). A contemporary recovery of intracellular Ca2+-signal, detected spectrofluorometrically using FURA-2AM, was observed in these mice. Cx43 expression and localization, analyzed by Western blot and confirmed by immunofluorescence analysis, showed that DZX co-treatment increases Cx43 amount both on the sarcoplasmic membrane and on mitochondria. In conclusion, these data demonstrate that, in a short-time mouse model of DOXO-induced cardiotoxicity, DZX exerts its cardioprotective effects also by enhancing the amount Cx43.

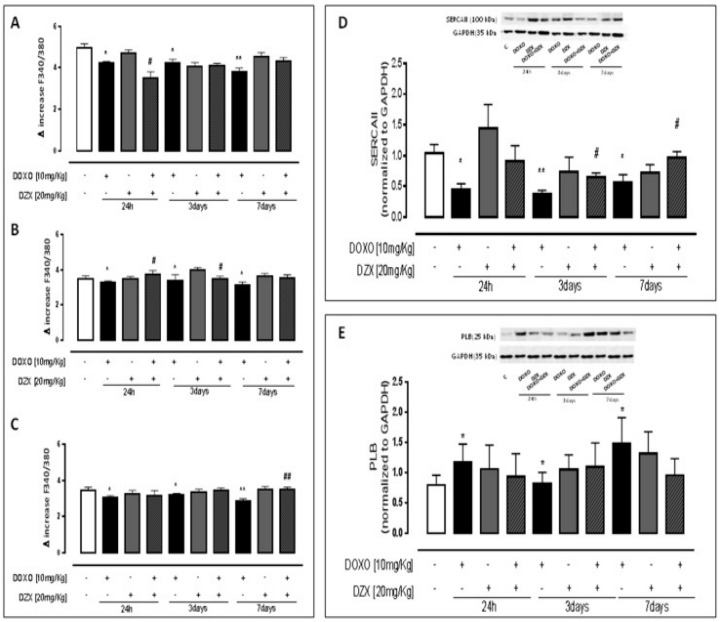

*Fig 18: Effect of DOXO (10 mg/kg; i.p.) or DZX (20 mg/kg; i.p.) or combined DZX+DOXO treatment on calcium homeostasis. Mice received a single administration (1th group), two administrations (2nd group), or three administrations (3rd group) of DOXO (10 mg/kg; i.p.) or DZX (20 mg/kg; i.p.) or combined DZX+DOXO treatment and primary cardiomyocytes were isolated by enzymatic digestion. Intracellular calcium content in cell suspensions was evaluated by using ionomycin (1 µM) (**A**); reticulum calcium content was evaluated by means of thapsygargin (100 nM) (**B**) and mitochondrial calcium content was evaluated by using FCCP (50 nM) (**C**). Results were expressed as mean ± S.E.M. of delta (δ) increase of FURA-2 AM ratio fluorescence (340/380 nm) from at least three independent experiments each performed in duplicate. Data were analyzed by Student's* t-test. * p *< 0.05, and ** p < 0.005 vs. control; # p < 0.05 and ## p < 0.005 DZX+DOXO vs. DOXO. Effect of DOXO or DZX or combined DZX+DOXO treatment on SERCA II (**D**) and PLB (**E**) expression. Mice received a single administration (1th group), two administrations (2nd group), or three administrations (3rd group) of DOXO (10 mg/kg; i.p.) or DZX (20 mg/kg; i.p.) or combined DZX+DOXO treatment and SERCA II and PLB amount was detected by Western blot analysis into tissue homogenates from mice; GAPDH amount was used as loading control. Values were expressed as mean ± S.E.M. from at least three independent experiments each performed in duplicate. Data were analyzed by Student's* t-test. * p *< 0.05 and ** p < 0.005 vs. control; # p < 0.05 DZX+DOXO vs. DOXO.*

Part IV

CANCER AND INFLAMMATION

CHAPTER 14

A Novel Small Peptide Inhibitor of NFκB, RH10, Blocks Oxidative Stress-Dependent Phenotypes in Cancer

The RH domain of GRK5 is an effective modulator of cancer growth through the inhibition of NFκB activity. This study aimed to identify the minimum effective sequence of RH that is still able to inhibit tumor growth and could be used as a peptide-based drug for therapy. Starting from the RH sequence, small peptides were cloned and tested in KAT-4 cells. The effects on NFκB signaling and its dependent phenotypes were evaluated by Western blot, TUNEL assay, proliferation assay, and angiogenesis in vitro. In vivo experiments were performed in KAT-4 xenografts in Balb/c nude mice.

A minimum RH ten amino acids long sequence (RH10) was able to interact with IκB, to increase IκB levels, to induce apoptosis, to inhibit KAT4-cell proliferation, NFκB activation, ROS production, and angiogenesis in vitro (Fig 15).

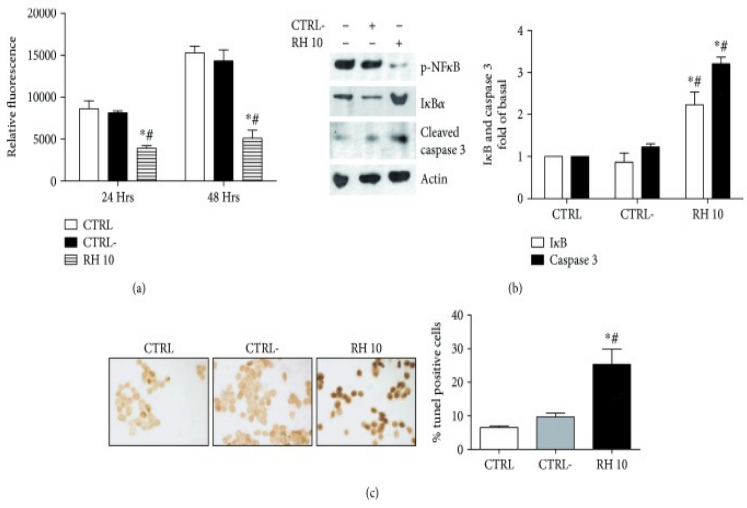

*Fig 15: RH10 inhibits cancer cell proliferation by the modulation of NFκB activity. (a) KAT-4 were treated with RH10 or CTRL−. A proliferation assay was performed 24 and 48 hours after treatment. RH10 significantly inhibits cell proliferation at both 24 and 48 hours after treatment (*p < 0.05 vs. CTRL, #p < 0.05 vs. CTRL−). Data are reported as mean ± SD. (b) The effects of RH10 on NFκB signaling and apoptosis were evaluated by Western blot using*

*specific antibodies. RH10 reduces NFκB activation and increases IκB and caspase 3 levels. The inset shows a representative blot from three independent experiments. Data are reported as mean ± SD (*p < 0.05 vs. CTRL; #p < 0.05 vs. CTRL−). (c) Apoptosis was evaluated by TUNEL assay. Cells were treated with RH10 or CTRL−. The assay was performed according to the manufacturers' instructions. The images are representative of the results from three independent experiments. Data are reported as mean ± SD (*p < 0.05 vs. CTRL; #p < 0.05 vs. CTRL−).*

In vivo, the peptide inhibited tumor growth in a dose-dependent manner (Fig 16). We also tested its effects in combination with chemotherapeutic drugs and radiotherapy. RH10 ameliorated the antitumor responses to cisplatin, doxorubicin, and ionizing radiation. Our data propose RH10 as a potential peptide-based drug to use for cancer treatment both alone or in combination with anticancer therapies.

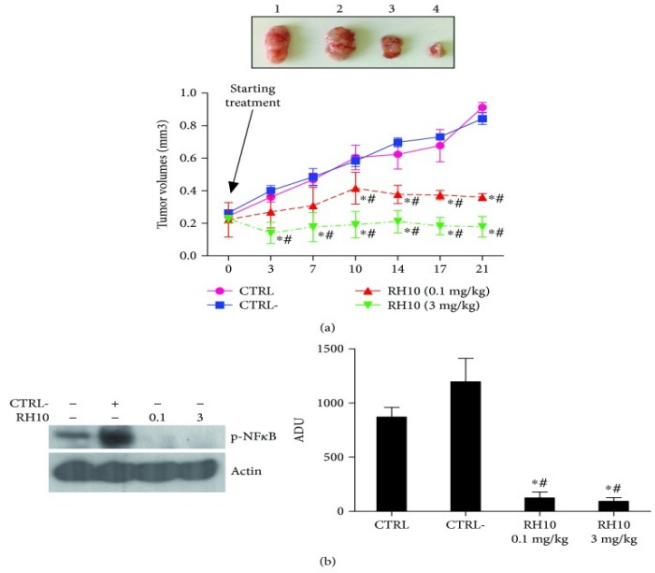

*Fig 16: RH10 inhibits tumor growth in a dose-dependent manner. (a) Tumor cell lines were inoculated in nude mice, and tumors were treated with 3 mg/kg CTRL−, 0.1 mg/kg RH10, and 3 mg/kg RH10 twice a week for three weeks. Results are reported in the graph as mean ± SD, and a representative image is shown (*p < 0.05 vs. CTRL, # p < 0.05 vs. CTRL−; ANOVA and Bonferroni post hoc test). (b) Phospho-NFκB levels were evaluated by Western blot in lysates from untreated and treated tumors. RH10 reduced p-NFκB levels at all dosages compared with CTRL and CTRL−. The image is the representative of the results from the three independent experiments in different tumor samples. Data are reported as mean ± SD (*p < 0.05 vs. CTRL, #p < 0.05 vs. CTRL−). ADU = arbitrary densitometric units.*

Part V

DISCUSSION AND CONCLUSIONS

Discussion

Part I. The role of GRK2 in cardiovascular disease (CVD) and its inhibition as a potential therapeutic strategy

The mitochondrial-dependent regulation of energy production, calcium homeostasis, and cell death is critical for cardiac function. Therefore, it is not surprising that alterations of mitochondrial functions lead to the development of cardiac pathologies or susceptibility to injury. Indeed, mitochondrial dysfunction has been identified as the cause of several heart diseases. It was identified the involvement of G protein-coupled receptor kinase 2 (GRK2) in the regulation of mitochondrial function showing its ability to regulate ATP content, ROS production, mitochondrial dynamics, and apoptosis. The upregulation of GRK2 has important effects on the onset of cardiovascular disease, which are characterized by an impaired mitochondrial function. The understanding of the role of this kinase in mitochondria provides the basis for new therapeutic approaches to treat mitochondrial dysfunction in cardiovascular diseases. Recently, we have identified KRX-C7, a novel GRK2 inhibitor, obtained through side-chain cyclization of the linear peptide derived from fragment 383–390 of the HJ loop of GRK2 (25, 26), which enhanced GRK2 inhibition properties, such as stability and selectivity, compared to parent linear molecules. In particular, GRK2 inhibition ameliorates mitochondrial biogenesis and cardiac ATP content with the restoration of cardiovascular function. On the other hand, in recent years, several reports have demonstrated a key role for GRK2 also in the modulation of insulin sensitivity in physiological and pathological conditions (27). Indeed, GRK2 accumulation leads to the shut-off of insulin signaling and inhibits glucose extraction. So in diabetes, GRK2 can be an innovative therapeutic target with double-positive effects on diabetes and its associated cardiac phenotype. RX-C7 improves insulin sensitivity and glucose homeostasis in vitro and in vivo, in an animal model of T2DM.Therefore, the inhibition of GRK2 represents possible therapeutic strategies to reduce morbidity and mortality in metabolic diseases, such as type 2 diabetes mellitus (T2DM) with the improvement of structure and function cardiac.

Part II. Molecular mechanisms of endothelial dysfunction

The endothelium is ubiquitous in the human body. Therefore, each molecule synthesized by endothelium might affect all organs. In detail, the endothelium has a pivotal role in the modulation of heart cell function (28). Indeed, the impairment of endothelium-dependent relaxation represents a key feature of endothelial dysfunction found in many cardiovascular diseases, such as hypertension and heart failure (29). During heart failure, a calcium deprivation occurs inducing PTH release, and the increased levels of the hormone could contribute to maladaptive responses(30, 31)bearing important clinical and therapeutic implications. Cardiac tissue and the vascular system express the PTH receptor, moreover, this hormone causes endothelial dysfunction in a ROS-dependent manner, through a surge of mitochondrial Ca2+. The relationship between PTH and endothelial dysfunction, as a mechanism of CVR, provide a possible explanation of the discordant observations regarding the increased CVR in vitamin D–deprived populations. Indeed, severe vitamin D deficiency is a major risk factor for cardiovascular system. Among molecules released by the endothelium, we have recently shown that in endothelial cells the oxidative stress induces bradykinin (Bk) release that, through an autocrine mechanism, promotes ischemic preconditioning-induced cytoprotection against prolonged hypoxia. Thus, these data may have important clinical relevance to identify drugs that improve resistance to hypoxia in ischemic organs, like the heart.

Part III. Risk factors for the development of CVD: air pollution, inflammation, and doxorubicin treatment

Inflammation is associated with increased risk for cardiovascular events. Furthermore, several clinical trials demonstrate the effectiveness of anti-inflammatory treatment in the management of cardiovascular diseases. Several factors can induce an inflammatory condition. In this context, the transcription factor NFκB represents a functional bridge between inflammation and cardiac pathologies. Indeed, the inhibition of NFκB suggests that it could represent a potential therapeutic target for the treatment of cardiac dysfunction and the associated inflammatory

phenotype. However, also air pollution contributes to the development of cardiovascular events by inducing a systemic inflammatory condition. Therefore, appropriate interventions to reduce air pollution may promote great benefits to public health by reducing the inflammatory response and the risk of associated CV diseases. Furthermore, several anti-cancer treatments could increase cardiovascular risk due to their cardiotoxic effects. Doxorubicin is the best example (32). Mechanisms of DOXO-induced cardiotoxicity are very complex and remain elusive. Several strategies have been developed to protect the heart from DOXO cardiotoxicity, including limitation of cumulative dose and the development of molecules aimed at lowering oxidative stress, such as the novel butyrate derivative phenylalanine-butyramide. Exogenous administration of phenylalanine-butyramide (FBA), a synthetic derivative of butyric acid, protects from DOXO-induced cardiotoxicity by decreasing oxidative stress and improving mitochondrial function. Also, diazoxide (DZX), a specific opener of mitochondrial KATP channels widely used for its cardioprotective effects, itcan reduce the damage induced by DOXO. Indeed, DZX improves Cx43 expression and mitoCx43, thus enhancing cardiac function and Calcium homeostasis.

Part IV. Cancer and inflammation

The inhibition of NFκB signaling is an attractive goal for cancer research since this transcription factor is strictly associated with cancer development and progression favoring tumor growth and inflammatory responses and inhibiting apoptotic events (33, 34). In particular, RH10 is a specific inhibitor of NFκB that affects all NFκB-dependent phenotypes which are responsible for tumor progression (apoptosis, cell proliferation, angiogenesis, and oxidative stress). As a consequence, RH10 can significantly reduce tumor growth and be an adjuvant in common therapies sensitizing cells to better respond to some chemotherapeutic drugs or ionizing radiation. Our results suggest RH10 as a potential small molecule to use for anti-cancer treatment.

Conclusions

In this long-lasting research journey, we investigated: a) the functional role of mitochondria in CVD; b)the role of GRK2 in the development of CVD and the beneficial effects of its inhibition in CVD, also associated with other pathologies such as diabetes; c) novel mechanisms in the regulation of endothelial function; d) the involvement of inflammation, air pollution and drug cardiotoxicity in the development and progression of CVD; e) the crosstalk between cancer and inflammation. Besides this latter topic, cardiovascular diseases were the main topic of my research studies. All my studies significantly contributed to increasing the knowledge in their specific field and allowed the identification of novel potential targets or the proposal of novel treatments. Further studies will be needed to better explore these issues but these data have great translational potentiality with important clinical implications.

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Curriculum Vitae

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EDUCATION AND TRAINING

2014-

Master Degree in Medical Biotechnology

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- Rating: 110/110
- Title thesis: GRK2 regulates inflammation by inducing mitochondrial biogenesis

2012-

Bachelor of Science Degree in Biotechnology

Federico II University of Naples

- Rating: 106/110
- Title thesis: Modification of endothelial gene expression in transgenic animalsTie2CRE / GRK2 flox / flox, by Real Time PCR.

2009-

Scientific High School Diploma

ITAS - Liceo Scientifico "Elena di Savoia", Largo San Marcellino 15, 80138 Naples – Italy

- Rating: 100/100

WORK EXPERIENCE

Research activity as student

2016- Contract of technologist for participation in the project of training related to the Project of Research and Development called "Development and Evaluation Preclinical and Clinical Phase 0 and Phase 1 of Molecules in Action Nutraceutical, Cosmeceutical, Pharmaceutical and / or Therapeutic Indications for new Molecules already approved ", at the University of Salerno

Sector: Molecular biology

2015- Contract of self-employment provision, for the following activities: "Evaluation of the contractility of cardiomyocytes isolated from mouse hearts in a model of chronic hypertrophy", at the Department of Neuroscience, Reproductive Sciences and Dentistry, University of Naples Federico II

Sector: Molecular biology

TECHNICAL SKILLS AND COMPETENCES

Analysis of chemicalclinical laboratory, enzyme assay and bacteriological, using modern equipment and materials

Methods in cellular and molecular biology, biochemistry clinical, including Extraction of DNA / RNA, PCR, Electrophoresis, western blot, Real-time PCR, immuno-preciptation, ubiquitination test

Management of transgenic animal lines, job with animal model, vascular reactivity Technique, Cardiomyocytes isolation from mice

Single Channel Analysis, determination of calcium flux (cytosolic calcium, and mithocondrial calcium) on cardiomyocytes and cell line

ADDITIONAL INFORMATION

Conferences

ESC Working Group Myocardial Function and Cellular Biology of the Heart 2019
The involvement of GRK2 in stress response to radiation during cardiovascular invasive procedure
A. Fiordelisi 2, *G. Di Gioia1, I. Colaiori2, A. Katbeh1, J. Gambardella1, A. Pagliuso1 ,E. Barbato1, J. Bartunek2, D. Sorriento1, G. Iaccarino1* (1 Napoli, 2 Belgio)

SIIA Società Italiana dell'Ipertensione Arteriosa-Lega Italiana Contro l'Ipertensione Arteriosa 2019
Coinvolgimento di GRK2 nella risposta allo stress da radiazioni durante le procedure invasive cardiovascolari
A. Fiordelisi 2, *G. Di Gioia1, I. Colaiori2, A. Katbeh1, J. Gambardella1, A. Pagliuso1 ,E. Barbato1, J. Bartunek2, D. Sorriento1, G. Iaccarino1* (1 Napoli, 2 Belgio)

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GRK2 is a novel early marker of cardiotoxicity in response to doxorubicin
A. Fiordelisi 2, J. Gambardella 1, M. Ciccarelli 1, P. Campiglia 1, B. Trimarco 2, G. Iaccarino 1, D. Sorriento 2 (1 Salerno, 2 Napoli)

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Ruolo di GRK5 nella cardiomiopatia indotta da stress catecolaminergico
M. De Rosa 1, L. Napolitano 1, J. Gambardella 2, **A. Fiordelisi** 1, D. Sorriento 1, B. Trimarco 1, G. Iaccarino 2, M. Ciccarelli 2 (1 Napoli, 2 Salerno)

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J. Gambardella 1, M. Ciccarelli 1, **A. Fiordelisi** 2, P. Campiglia 1, B. Trimarco 2, G. Iaccarino 1, D. Sorriento 2 (1 Salerno, 2 Napoli)

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GRK2 induce ipertrofia cardiaca mediante regolazione dell'attività di NFKAPPAB
A. Carillo 1 , D. Sorriento 1 , G. Santulli 1 , A. Franco 1 , **A. Fiordelisi** 1 , B. Trimarco 1 , G. Iaccarino 2 (1 Napoli, 2 Baronissi)

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Un nuovo composto, sm13, inibisce la crescita cellulare, inducendo apoptosi p53-dipendente
D. Sorriento 1, C. Del Giudice 1 , A. Bertamino 2, J. Gambardella 1 , **A. Fiordelisi** 1 , P. Campiglia 2, G. Iaccarino 2, B. Trimarco 1 (1 Napoli, 2 Salerno)

Mother tongue	Italian				
Other language	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
English	B1	B1	B1	B1	B1

Levels: A1 and A2: Basic user - B1 and B2: Independent user - C1 and C2: Proficient user

List of all publications

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