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Targeting oxidative stress for improving cardiovascular performance in the elderly

La modulazione dello stress ossidativo: un nuovo target per migliorare le prestazioni cardiovascolari nell'anziano

G. CORBI[1], V. CONTI[2], A. FILIPPELLI[2]

[1] Dept. of Medicine and Health Sciences "V. Tiberio", University of Molise, Campobasso, Italy; [2] Dept. of Medicine and Surgery, University of Salerno, Italy

Lo stress ossidativo è implicato nella fisiopatologia di diverse malattie cardiovascolari, come dimostra la correlazione tra i marcatori di stress ossidativo e d'insufficienza cardiaca (HF).
Le specie reattive dell'ossigeno (ROS) giocano un ruolo importante nei processi di signaling, ma la loro sovrapproduzione genera stress ossidativo. Studi precedenti hanno dimostrato che l'HF è associato a deficit di antiossidanti ed aumento dello stress ossidativo. Inoltre, tali cambiamenti si correlano alla funzione emodinamica, suggerendo il loro ruolo nella patogenesi della disfunzione cardiaca. Un meccanismo importante coinvolto nella risposta cellulare cardiovascolare è rappresentato dalle sirtuine. L'inibizione di SIRT1 determina la soppressione dei geni attivati dall'esposizione ad uno shock termico, mentre la sua attivazione ne migliora la risposta. Quindi, la capacità di SIRT1 di modulare la resistenza allo stress è multiforme e non solo legata allo stress ossidativo, ma anche ad altri stimoli stressanti. Recentemente diversi studi hanno dimostrato la capacità dell'attività fisica di indurre attivazione di SIRT1 che, a sua volta, ha la capacità di mediare gli effetti antiossidanti favorevoli dell'allenamento. Anche altri agenti in grado di attivare SIRT1 hanno dimostrato effetti sulla funzione cardiaca. La supplementazione con resveratrolo ha dimostrato di ridurre i fattori di rischio cardiovascolare, ed il suo effetto positivo sulla risposta all'esercizio e sulla capacità aerobica nei ratti sembra essere mediato da SIRT1. Il futuro della ricerca deve essere indirizzato a chiarire il possibile ruolo della terapia antiossidante negli studi clinici, in particolare alla definizione di una standardizzazione delle procedure, delle dosi e della durata del trattamento.

***Parole chiave*: Stress ossidativo, Sirtuine, Resveratrolo, Curcumina, Antiossidanti**

In the last decades the worldwide population has exhibited an increasing life expectancy with a consequent raise in elderlies, with resultant impact on the prevalence of age-related diseases, with the cardiovascular conditions as the most prevalent illnesses [1].
Increasing evidence suggests that chronic systemic inflammation and accumulating oxidative stress are related to the aging process and play a role in developing many chronic diseases such as atherosclerosis, hypertension, COPD [2-8]. At the same time metabolic changes show high influence in to prevent or to reduce the severity of age-related pathologies, suggesting that management of these factors could have an effect on the progression of the diseases [9-11].

OXIDATIVE STRESS AND CARDIOVASCULAR DISEASES

Oxidative stress is implicated in the pathophysiology of several cardiovascular diseases, such as heart failure (HF), hypertension and myocardial

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■ Corrispondenza: Graziamaria Corbi, Dept. of Medicine and Health Sciences "V. Tiberio", University of Molise, via De Santis snc, 86100 Campobasso, Italy - Tel. +39 0874 404771 - Fax +39 0874 404778 - E-mail: graziamaria.corbi@unimol.it

infarction, as evidenced by a correlation between oxidative stress markers and HF in human and animal studies [12] [13] and by direct molecular evidence for an etiological role of reactive oxygen species (ROS) [14]. During life, cardiovascular system is constantly exposed to oxidative stress, which occurs when ROS are produced in excess of the endogenous antioxidants [15]. The balance between the production of ROS and the activation of the antioxidant defence system is crucial for the human physiology and the control of cellular homeostasis. ROS play an important role in signalling processes, but their overproduction generates oxidative stress. In fact, ROS can regulate cellular functions; in turn their overproduction causes damage to cellular constituents, including DNA, proteins, and lipids, especially when occurs with insufficient antioxidant enzyme activity.

Several in vitro and in vivo studies have demonstrated ROS activation in the cardiovascular system in response to various stressors, and animal studies have also suggested that antioxidants and ROS defence pathways can ameliorate ROS-mediated cardiac abnormalities [16].

The importance of oxidative stress, as well as adrenergic nervous system hyperactivity [17-34] is increasingly emerging with respect to a pathophysiological mechanism responsible of cardiac hypertrophy, cardiomyocyte apoptosis, development and progression of HF [35]. Moreover, an increased production of ROS in the vascular wall and a reduction in nitric oxide bioavailability lead to endothelial dysfunction in atherogenesis [35].

Specifically, ROS can directly impair contractile function by modifying proteins central to excitation-contraction coupling, by activation of hypertrophy signalling kinases and transcription factors and mediating apoptosis. They also stimulate cardiac fibroblast proliferation and activate the matrix metalloproteinases, leading to the extracellular matrix remodelling. These cellular events are involved in the development and progression of maladaptive myocardial remodelling and failure [36] [37].

ROS overproduction also occurs in response to several stressors, including chemicals, drugs, pollutants, high-caloric diets, and exercise. Physical exercise can increase oxidative stress, eventually causing a perturbation of homeostasis that is dependent on training specificity [38-42] and workload [43], but in turn it is also able to counterbalance the deleterious effects of ROS by activation of several antioxidant systems, such as Super Oxide Dismutases (SODs), Heat Shock Proteins (HSPs) and catalase [43-46]. The mechanisms by which ROS mediate these different biologic responses are not fully understood, but in many cases involve activation of specific redox-sensitive signalling molecules [45].

SYSTEMS INVOLVED IN CARDIOVASCULAR ANTIOXIDANT RESPONSE

Previous studies demonstrated that HF subsequent to myocardial infarction was associated with antioxidant deficit as well as increased oxidative stress. Furthermore, these changes correlated with the hemodynamic function, suggesting their role in the pathogenesis of cardiac dysfunction.

The main protective systems involved in antioxidant cellular defence are represented by Glutathione peroxidase (GSHPx), superoxide dismutases (SOD), and catalase.

GSHPx catalyses the reduction of H2O2 and hydroperoxides, which results in prevention of the more toxic radicals formation. It has been demonstrated that overexpression of the GSHPx gene attenuated myocardial remodelling and preserved diastolic function in diabetic heart [47], suggesting that therapies designed to interfere with oxidative stress by using GSHPx could be beneficial to prevent myocardial remodelling and failure [48].

The SOD catalyse the dismutation of superoxide into oxygen and hydrogen peroxide during physiological and pathological conditions. Manganese superoxide dismutase (MnSOD) is the primary mitochondrial antioxidant enzyme and is essential for maintaining normal cell development and function. Overexpression of the MnSOD gene has been shown to be beneficial in various animal models of cardiac diseases [49]. Recently, Shen et al. [50] demonstrated that protection of cardiac mitochondria by overexpression of the MnSOD gene reduced the severity of diabetic cardiomyopathy, and completely normalized contractility in diabetic myocytes. Results from this study and from other authors [51] showed that elevating levels of MnSOD provided extensive protection to diabetic mitochondria and provided overall protection to the diabetic heart, as well as to the brain. Interestingly, MnSOD gene overexpression also elevated levels of myocyte catalase and mitochondrial GSHPx, which might also act together with MnSOD against oxidative stress. On the contrary, Nojiri

Tab. I. Classes of Sirtuins, their localization and prevalent activity.

	Localization	Activity
SIRT1	Nuclear and cytoplasmic	NAD-dependent Deacetylase
SIRT2	Cytoplasmic and nuclear	NAD-dependent Deacetylase
SIRT3	Mitochondrial, nuclear and cytoplasmic	NAD-dependent Deacetylase
SIRT4	Mitochondrial	ADP-ribosyl transferase
SIRT5	Mitochondrial	NAD-dependent Deacetylase, Desuccinylase, Demalonylase
SIRT6	Nuclear	NAD-dependent Deacetylase, ADP-ribosyl transferase, defatty-acylase
SIRT7	Nucleolar	NAD-dependent Deacetylase

et al. [52] reported that heart/muscle-specific Mn-SOD-deficient mice developed progressive HF with specific molecular defects in mitochondrial respiration in association with excess formation of superoxide and transcriptional alterations of genes associated with HF. Catalase, which reduces hydrogen peroxide to water, represents a primary safeguard of the antioxidant system and some recent studies have suggested that this enzyme might play an important role in the pathophysiology of HF [53]. Recently Kumar et al. [54] showed that subjects with ischaemic heart disease, myocardial infarction and unstable angina had increased TBARS levels and reduced SOD, catalase, and ascorbic acid levels. These findings are in accord to meta-analyses that identified strong and statistically significant inverse associations of GSH-Px, SOD, and catalase activities with coronary heart disease outcomes [55]. This supports a role of oxidative stress in the pathogenesis of coronary artery disease and in the outcome of patients undergone to coronary revascularization procedures [56-61].

REGULATOR OF CARDIOVASCULAR ANTIOXIDANT RESPONSE

An important mechanism involved in cellular cardiovascular response is represented by family of sirtuins (Tab. I, Fig. 1), a cluster of proteins composed by seven homologues regulating cellular biology and metabolism through deacetylation of histones and other cellular factors such as NFkB, HSF1, p53, FOXOs, and PGC- 1. Sirtuins are found in different subcellular locations, including the nucleus, cytosol, and mitochondria. The regulation of mammalian lifespan by sirtuins has important therapeutic implications for age-related diseases.

SIRT1, the best characterized member of the family, is involved in many functions of human physiology, including DNA repair, cell cycle regulation, apoptosis, gene expression, and aging [62]. SIRT1 can modulate the cellular stress response directly deacetylating some proteins and regulating their expression. Furthermore, this enzyme modulates the threshold of cell death in the setting of exogenous stress, including oxidative damage, and regulation of other targets linked to cell death. Then the ability of SIRT1 to modulate stress resistance is multifaceted and it is not only linked to oxidative stress, but also to other stressful stimuli. It also seems to be implicated in processes of morphological

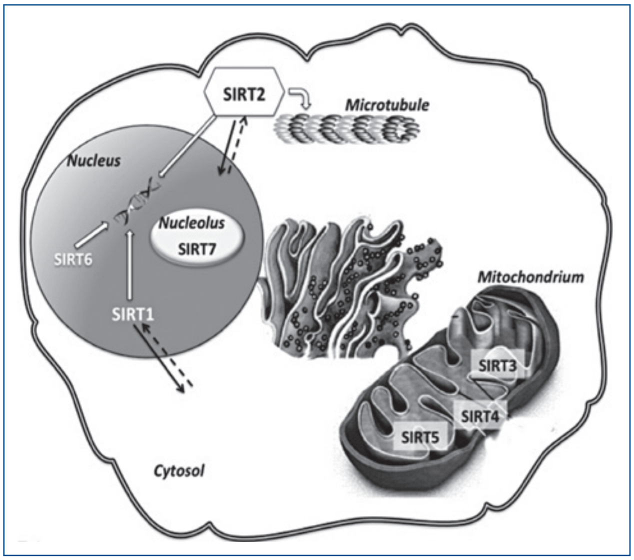

Fig. 1. Sirtuins localization in the cell. In the figure the main localization of sirtuins is showed. The solid line indicates the shift from the primary to the secondary location; the broken line demonstrates the shift from the secondary to the primary location.

heart development. In fact, the full-body SIRT1 knockout mouse displays ventricular adult heart abnormalities [63], but a severe developmental phenotype, together with high neonatal mortality rates make use of it difficult to study the physiological role of SIRT1 in the adult heart. Interestingly, high levels of SIRT1 expression (> 9-fold) in the heart cause hypertrophy, loss of cardiac function, and elevated apoptosis [63]. On the other hand, moderate overexpression (2.5 to 7-fold) of SIRT1 in transgenic mouse hearts protects against oxidative stress, and results in increased expression of antioxidants [64].

SIRT3 modulates mitochondrial gene expression and function via a general deacetylation of mitochondrial proteins involved in oxidative phosphorylation [65], or ROS handling [66].

TARGETING ANTIOXIDANT REGULATORS

Recently it has been demonstrated that by acting on this mechanism other factors could influence cardiac performance.

In particular, physical activity has been demonstrated able to reduce generation of oxidants during ischemia-reperfusion damage and to have a calcium-protective role via activation of the ROS scavenger, MnSOD. This better oxidative status consequent to a correct program of physical activity is partially responsible for some benefits (such as decreased arterial stiffness, improved endothelial function and metabolic and clotting setting, and reduced body weight) [67-71]. In part, it seems that positive effects of the physical exercise on heart in terms of antioxidant activity could be ascribable to a greater expression and activity of SOD and HSPs.

An 8-week training period has been shown to reduce body weight and cardiac abnormalities characteristic of senescence heart, such as structural changes, ventricular systolic pressure, left ventricle weight/body weight ratio, and heart rate [71], suggesting that exercise training antioxidant effects might be also mediated by mechanisms involving metabolic pathways.

In fact, both exercise training and caloric restriction improve the antioxidant system, and this could also explain the analogy between benefits derived by these tools [72].

In another study the exercise training increased SIRT3 and MnSOD levels in quadriceps muscle of wild-type, but not AMPK $\alpha 2$ kinase dead mice, suggesting an important role for AMPK in regulating mitochondrial function and ROS handling in skeletal muscle in response to exercise training [73]. Then the possibility to act on this mechanism has induced several researcher to test the efficacy of sirtuins' activator other then physical activity.

Resveratrol is a polyphenol found in the skin of grapes, berries and peanuts that can activate AMPK and sirtuins. In rodents resveratrol supplementation has been shown to decrease cardiovascular risk factors, including blood lipids [74] and VCAM-1 [75], to improve cardiovascular function and physical capacity, and to decrease inflammation in the vasculature of aged animals leading to improved vascular function [76]. Specifically, the positive effect of resveratrol on training response and aerobic capacity in rats has been shown to be mediated via SIRT1 [77 78].

Recently Meng et al. [79] found that resveratrol administration had improved the enzymatic and non-enzymatic antioxidant system against the atherogenic diet as well as in normal condition. These effects were similar in both heart tissue and haemolysate which is consistent with the previous study, treatment of apoE knockout mice with resveratrol for 7 days results in the upregulation of superoxide dismutase, glutathione peroxidase, and catalase in heart tissue [79]. The authors hypothesized that resveratrol activated the SIRT1, eNOS and regulated the phosphorylation of AMPK against the atherogenic diet [80]. SIRT1 binds directly to eNOS and has been shown to target eNOS for deacetylation, thereby stimulating nitric oxide production and promoting vascular relaxation [80].

In another study a Resveratrol treatment protected rats against diet induced Insulin Resistance, increased SIRT1 and SIRT3 expressions, mtDNA, and mitochondrial biogenesis. Moreover, the activities of mitochondrial antioxidant enzymes were increased, suggesting that Resveratrol ameliorates insulin sensitivity consistent with improved SIRT3 expressions and rebalance between subsarcolemmal mitochondrial oxidative stress and antioxidant competence in high-fat diet rats [81 82].

In another interesting study, Gliemann et al. [83] tested the hypothesis that oral resveratrol supplementation enhances the positive cardiovascular adaptations to exercise training in aged subjects by increasing SIRT1-mediated signalling and by promoting the endogenous antioxidant system. The authors showed that high-intensity exercise training potently improves a number of parameters related to vascular function and cardiovascular health in aged men, but that concomitant oral resveratrol supplementation blunts several of these positive effects of exercise training. Specifically, resveratrol had adverse effects on improvements in maximal oxygen uptake, on blood pressure reduction and on the lowering of blood lipids induced by exercise training, rejecting the hypothesis that resveratrol improves cardiovascular health by enhanced SIRT1-dependent signalling and improved antioxidant defence. Furthermore, the authors concluded that training enhances the capacity for ROS formation via increased levels of NOX and that removal of ROS via resveratrol treatment may limit training-induced adaptations [83].

On the other hand, other studies showed as a regular ingestion of a different antioxidant, as curcumin, significantly increased carotid arterial compliance in postmenopausal women. In the study by Akazawa et al. [84], the magnitude of improvement by curcumin was similar to that of exercise training alone. Moreover, the combination of exercise training and curcumin ingestion led to a greater improvement in arterial compliance compared to that achieved with either treatment alone. These results suggested that a combination of exercise and curcumin could have a strong positive effect on arterial compliance [84].

Other antioxidants (e.g. vitamin E, vitamin C, coenzyme Q10, etc.) have been tested in several experimental and clinical models with mixed success.

Lane et al. conducted a population-based study to examine the association between consumption of certain nutrients and prevalence of peripheral arterial disease, and they found that increased consumption of antioxidants, vitamin E and C was associated with reduced odds of peripheral arterial disease [85]. Other studies demonstrated the importance of vitamin E for protection against cardiac ischemia-reperfusion injury using vitamin E deficient animal models. These observations indicate that the modulation of oxidative stress by antioxidants appears to have a positive outcome in the prevention of CVDs. Despite this, the protective effects of vitamin E remain controversial, because it requires prolonged and very high levels of oral treatment to achieve cardiac concentrations that are protective from reperfusion injury.

Recently Takahashi et al. demonstrated that a 12-week supervised walking program improved oxidative stress status in postmenopausal women, but combining the exercise program and vitamin E supplementation showed no additive effects on the improvement of oxidative stress status [86].

On the large differences in results it should probably be considered that in these studies antioxidant agents might have been tested at different doses, or durations, or that the wrong drug or combination of drugs has been used. Therefore, regardless of these controversial data from clinical studies, oxidative stress still remains a potential attractive target for CVDs prevention and therapy. Possible future therapies aimed at decreasing mitochondrial either than nuclear oxidative damage should also be considered [87].

Recently Klishadi et al. [88] showed that SIRT3 decreased levels induced by ischemia reperfusion can be reverted by Losartan at non-hypotensive dose which exerts anti-ischemic effects in part by normalizing the SIRT3 protein level and upregulating the survival factors encoding genes transcription in ischemic tissue of the heart.

CONCLUSIONS

Surely the oxidative stress represents one of the most important and intriguing mechanism involved in the genesis, development and progression of several cardiovascular diseases, and still many pathways involving oxidant and antioxidant response should be better clarify. The future research should be addressed to better clarify the possible role of the antioxidants therapy in clinical studies, in particular defining a standardization of procedures, doses and duration of treatment in order to make comparable the various data and to understand the real effectiveness of antioxidants in the prevention and treatment of cardiovascular diseases.

Oxidative stress is implicated in the pathophysiology of several cardiovascular diseases, as evidenced by correlation between oxidative stress markers and Heart Failure (HF) and by direct molecular evidence for an etiological role of reactive oxygen species (ROS).
ROS play an important role in signalling processes, but their overproduction generates oxidative stress. Previous studies demonstrated that HF was associated with antioxidant deficit as well as increased oxidative stress. Furthermore, these changes correlated with the hemodynamic function, suggesting their role in the pathogenesis of cardiac dysfunction. An important mechanism involved in cellular cardiovascular response is represented by sirtuins. SIRT1 inhibition determines suppression of genes ac-

tivated by exposure to heat shock, on the contrary, SIRT1 activation enhances the heat shock response. Then the ability of SIRT1 to modulate stress resistance is multifaceted and not only linked to oxidative stress, but also to other stressful stimuli. Recently several studies have demonstrated the capability of physical activity to induce SIRT1 activity and, in turn, the ability of this enzyme to mediate the favourable antioxidant effects of the exercise training. Other agents able to induce SIRT1 activity have demonstrated some effects on cardiac function. Resveratrol supplementation has been shown to decrease cardiovascular risk factors, and the positive effect of resveratrol on training response and aerobic capacity in rats to be mediated via SIRT1. The future research should be addressed to better clarify the possible role of the antioxidants therapy in clinical studies, in particular defining a standardization of procedures, doses and duration of treatment.

***Key words*: Oxidative stress, Sirtuins, Resveratrol, Curcumin, Antioxidants**

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