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N-acetylcysteine reduces myocardial injury progression in experimental models

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ARTICLE INFORMATION

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Abbreviations
OE: oxidative stress
NAC: N-acetylcysteine

ABSTRACT

N-acetylcysteine is known in a number of medical specialties and its ability to decrease the impact of reperfusion injury in acute myocardial infarction has boosted its use in cardiology over the past decades. N-acetylcysteine has a far-reaching range of effects since it functions as a protective agent against oxygen radicals through sulfhydryl groups in important regions of the cell membrane that interfere and affect endothelial functioning and complex adhesion processes as side effects; as well as other phenomena of the extravascular compartment. These processes are closely related to the cardiovascular system.
Keywords: Myocardial injury, Reperfusion injury, Oxidative stress, N-acetylcysteine

La N-acetilcisteína reduce el progreso de daño cardíaco en modelos experimentales

RESUMEN

La N-acetilcisteína es conocida en varias especialidades médicas. Su empleo en cardiología se ha incrementado desde hace décadas, por su potencial para disminuir el impacto del daño por reperfusión en el infarto miocárdico agudo. Pero el espectro de sus efectos es aún mayor, tiene acciones sobre los radicales de oxígeno, con un papel protector, por la vía de los grupos sulfhidrilos de regiones importantes de la membrana celular, los cuales interfieren y tienen efecto en la función endotelial y en los procesos complejos de adhesión como efectos secundarios; así como otros fenómenos del compartimento extravascular. Estos procesos están estrechamente relacionados con el aparato cardiovascular.
Palabras clave: Lesión miocárdica, Daño por reperfusión, Estrés oxidativo, N-acetilcisteína

INTRODUCTION

Heart failure is visibly increasing internationally among the older population coupled with a steady upturn in the prevalence of obesity, diabetes mellitus and high blood pressure. However, its pathogenesis is complex and multifactorial. There is conclusive clinical and experimental data suggesting the central role played by oxidative stress (OS) in the pathogenesis of heart failure[1].

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Reactive oxygen species are formed from incomplete reduction of this gas during normal respiration in all aerobic organisms. These species are highly reactive and include free radicals containing one or more unpaired electrons, such as superoxide ($O_2^{\bullet -}$) and hydroxyl radical ($^{\bullet}OH$), and nonradicals such as hydrogen peroxide (H_2O_2). It is estimated that between 0.2-2.0% of molecular oxygen consumed by the mitochondria in vitro may be converted to superoxide anion by the electron transport chain, but the amount of superoxide anion produced in vivo may be far less. In addition to mitochondrial respiration, superoxide anion is generated by nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, uncoupled nitric oxide synthase, xanthine oxidase, lipoxygenases, myeloperoxidase, and cytochrome P450 isozymes. Because free radicals' production is inherent to normal physiology, cells have evolved both (enzymatic and nonenzymatic) antioxidant defense mechanisms to maintain redox balance. A shift in redox homeostasis to an imbalance between reactive oxygen species generation and endogenous antioxidant mechanisms results in OS, which is implicated in the pathogenesis of various diseases including those of the cardiovascular system[2].

OXIDATIVE STRESS

Oxidative stress can contribute to the development of cardiac remodeling and fibrosis[3,4], and these cardiac adaptations may ultimately lead to failing of the myocardium[5]. For example, oxidative stress has been demonstrated to increase the expression of cardiac collagen types I and IV and fibronectin and impair cardiac contractility in diabetic rats[6]. The role played by oxidative stress in the development of angiotensin II-dependent cardiac fibrosis in rats[7] and mice[8] has also been reported. There is evidence that left ventricular glutathione content (L-gamma-Glutamyl-L-cysteinyl-glycine) is reduced in heart failure[9], and a particular interest in restoring glutathione content via oral supplementation of its precursor, N-acetylcysteine (NAC), which reduces OS, and restores heart failure-related cardiac damage and function in rats[9] is currently emerging. Besides, NAC treatment reduces the expression of the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) whose receptors are expressed in these rats. Therefore, NAC treatment reduces serum levels of TNF-α, matrix metalloproteinase 9 (MMP-9) and metalloproteinase 2 (MMP-2) in patients with acute myocardial infarction[10]. These cytokines and enzymes play an important role in the development of cardiac fibrosis, remodeling, and subsequent cardiac dysfunction.

The N-acetylcysteine molecule is widely used as a mucolytic agent; that is, it is targeted at softening mucus in the respiratory tract. It can be available as effervescent tablets or as an additive to flu medications[11].

Little is known about NAC's antioxidant action and it may be one of the most potent antioxidant molecules, to which a beneficial therapeutic application could be ascribed[12].

A number of dual-action antioxidant molecules have been developed to date that are capable of preventing oxidation by metal cation chelation and trapping free radicals. These include penicillamine, for treating rheumatoid arthritis; mesna (sodium;2-sulfanylethanesulfonate), employed as a uroprotector in cyclophosphamide-associated chemotherapy, to prevent hemorrhagic cystitis; and N-acetylcysteine[11,12].

The latter is a drug containing a free mercapto (-SH) group, responsible for its therapeutic action. It acts as an antioxidant by neutralizing free radicals before they react with any structure in the body. In this way, damage to cellular structures and spread of radical genesis, so frequent after generation, is avoided. N-acetylcysteine is in turn transformed into a far more stable (-S) radical (unreactive) when these radicals have reacted, because: a) the large size of the sulphur atom allows it to retain the electronic charge and b) it is easily inactivated when forming dimers by means of disulphide bridges[12].

It is likewise a preventive antioxidant as mercapto and carboxylic groups are capable of combining with metal cations that would act as chelating agents and prevent rapid formation of new free radicals[12]. Furthermore, it should be noted that NAC resembles glutathione, the main detoxifying antioxidant in the cell. This way, it will be involved –among other things– in phase II metabolic reactions, which makes it easier for toxins and different types of substances to be excreted due to their nucleophilic nature, as they hold the mercapto (-SH) group[11].

CLINICAL AND EXPERIMENTAL STUDIES

Several researchers have attempted to re-evaluate the mechanism by which NAC acts as a precursor to glutathione synthesis in the context of its antioxidant

activity. Hence, some results from recent studies have been reviewed to establish all necessary requirements and assess NAC-induced antioxidant activity.

Change to compensate for left ventricular hypertrophy during heart failure is a critical event in patients with sustained high blood pressure, as occurs in systemic hypertension and aortic stenosis[13]. There are quite a few mechanisms implicated in the development of heart failure, but its pathophysiology is not yet fully understood[14,15]. Included among these is the OS, that plays a role in pathological cardiac remodeling and transition to heart failure[2,16]. But the importance of OS in inducing myocardial injury and antioxidant therapy remains a controversial issue when treating heart failure[17,18].

Glutathione is an endogenous non-protein tripeptide made up of three amino acids (cysteine, glutamate and glycine) that plays a key role in cellular defense against oxidative stress[19]. It is synthesized and maintained at high concentrations in cells[20]. Reduced state glutathione changes take place during heart failure and its total concentration decreases within the myocardium[21,22]. This is why NAC −a molecule with antioxidant properties containing sulfhydryl groups− has the ability to act as a cysteine precursor for glutathione synthesis and has been shown to restore glutathione levels and reduce OS in rats with myocardial infarction after administration[21].

Moreover, NAC has been evaluated in experimental models of cardiac injury, particularly hypertrophic cardiomyopathy in which NAC reduced left ventricular dysfunction, interstitial fibrosis, and arrhythmogenic propensity[22,23]. Yet, the effects of NAC during transition to compensate for left ventricular hypertrophy in clinical heart failure have not been totally established. A University of São Paulo, Brazil, therefore conducted research aimed at demonstrating the benefits of NAC in reducing myocardial fibrosis to compensate for left ventricular hypertrophy during heart failure in an experimental model of induced supravalvular aortic stenosis in rats[24]. Animal models were allocated into three treatment groups and different variables were assessed by echocardiography and morphological/histological analysis of the left ventricle and other organs. Some laboratory techniques, including high-performance liquid chromatography, were also applied to eventually conclude that treatment with NAC fully restored glutathione in the myocardium, reduced systemic and myocardial OS, induced changes in mitogen-activated protein kinases signaling cascade and reduced myocardial fibrosis.

There are other scientific studies in experimental models, such as that of Giam *et al*[25], which have primarily focused on demonstrating that NAC is capable of reducing OS, remodeling and fibrosis in heart failure. Hypertrophic cardiomyopathy often progresses to heart failure with preserved ejection fraction due to gradual alteration of diastolic function with or without systolic function impairment. Causes remain elusive, but several risk factors within the genetically predisposed variety of the disease have been related to it, mirroring what occurs in familial hypertrophic cardiomyopathy; among them are high blood pressure and hyperlipidemia, related diagnoses that are likely to be the most associated in the future. Hence the growing interest in various treatments aimed at relieving the diastolic dysfunction observed in this disease[25].

There is no research linking the strong implications of redox modifications in hypertrophic cardiomyopathy due to mutations in the sarcomeric proteins, and the potential role of reactive oxygen species in the myofilament proteins to induce exacerbation of the phenotype in this cardiomyopathy. Previous studies, however, indicate that the association of these reactive oxygen species with modifications of sarcomeric proteins is commonly the major mechanism responsible for impairment of diastolic function in familial hypertrophic cardiomyopathy.

Tsai *et al*[26], reported a significant increase in myofilament response to Ca^{2+}, and diastolic dysfunction, which is −probably− the cause of cardiac remodeling. Meanwhile, Alves *et al*[27] and Wilder *et al*[28], in similar trials, assessed the redox state and ventricular function of a transgenic mouse model of familial hypertrophic cardiomyopathy expressing a mutation in the tropomyosin gene, where glutamic acid at residue 180 had been exchanged for a glycine (Tm-E180G). All of the (Tm-E180G)-mice developed severe diastolic dysfunction, along with further hypertrophy and left atrial dilation at two weeks of age; besides displaying early signs of oxidative stress in the form of increased oxidative modifications of myosin filaments and activation of the mitogen-activated protein kinases signaling cascade. The significance of such studies entails the fact of reversibility of left atrial hypertrophy and dilatation. They also provide evidence of basic mechanisms capable of reversing diastolic dysfunction of hypertrophic cardiomyopathy within the myofilaments.

These previously shown results underpin the

hypothesis that treatment with NAC, a glutathione precursor, can reverse OS in experimental models, as well as progression to hypertrophy and diastolic dysfunction[26,28].

REFERENCES

1. Seddon M, Looi YH, Shah AM. Oxidative stress and redox signalling in cardiac hypertrophy and heart failure. Heart. 2007;93(8):903-7.
2. Madamanchi NR, Runge MS. Redox signaling in cardiovascular health and disease. Free Radic Biol Med. 2013;61:473-501.
3. Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. Am J Physiol Heart Circ Physiol. 2011;301(6):H2181-90.
4. Tham YK, Bernardo BC, Ooi JY, Weeks KL, McMullen JR. Pathophysiology of cardiac hypertrophy and heart failure: signaling pathways and novel therapeutic targets. Arch Toxicol. 2015; 89(9):1401-38.
5. Aragno M, Mastrocola R, Alloatti G, Vercellinatto I, Bardini P, Geuna S, *et al*. Oxidative stress triggers cardiac fibrosis in the heart of diabetic rats. Endocrinology. 2008;149(1):380-8.
6. Zhao W, Zhao T, Chen Y, Ahokas RA, Sun Y. Oxidative stress mediates cardiac fibrosis by enhancing transforming growth factor-beta1 in hypertensive rats. Mol Cell Biochem. 2008;317(1-2):43-50.
7. Worou ME, Belmokhtar K, Bonnet P, Vourc'h P, Machet MC, Khamis G, *et al*. Hemin decreases cardiac oxidative stress and fibrosis in a rat model of systemic hypertension via PI3K/Aktsignalling. Cardiovasc Res. 2011;91(2):320-9.
8. Li YQ, Li XB, Guo SJ, Chu SL, Gao PJ, Zhu DL, *et al*. Apocynin attenuates oxidative stress and cardiac fibrosis in angiotensin II-induced cardiac diastolic dysfunction in mice. Acta Pharmacol Sin. 2013;34(3):352-9.
9. Talasaz AH, Khalili H, Jenab Y, Salarifar M, Broumand MA, Darabi F. N-Acetylcysteine effects on transforming growth factor-β and tumor necrosis factor-α serum levels as pro-fibrotic and inflammatory biomarkers in patients following ST-segment elevation myocardial infarction. Drugs R D. 2013;13(3):199-205.
10. Lehrer SS, Geeves MA. The myosin-activated thin filament regulatory state, M^{-}-open: a link to hypertrophic cardiomyopathy (HCM). J Muscle Res Cell Motil. 2014;35(2):153-60.
11. Cazzola M, Calzetta L, Page C, Jardim J, Chuchalin AG, Rogliani P, *et al*. Influence of N-acetylcysteine on chronic bronchitis or COPD exacerbations: a meta-analysis. Eur Respir Rev. 2015;24(137):451-61.
12. Lasram MM, Lamine AJ, Dhouib IB, Bouzid K, Annabi A, Belhadjhmida N, *et al*. Antioxidant and anti-inflammatory effects of N-acetylcysteine against malathion-induced liver damages and immunotoxicity in rats. Life Sci. 2014;107(1-2):50-8.
13. Drazner MH. The progression of hypertensive heart disease. Circulation. 2011;123(3):327-34.
14. Crozatier B, Ventura-Clapier R. Inhibition of hypertrophy, per se, may not be a good therapeutic strategy in ventricular pressure overload: other approaches could be more beneficial. Circulation. 2015;131(16):1448-57.
15. Zwadlo C, Schmidtmann E, Szaroszyk M, Kattih B, Froese N, Hinz H, *et al*. Antiandrogenic therapy with finasteride attenuates cardiac hypertrophy and left ventricular dysfunction. Circulation. 2015; 131(12):1071-81.
16. Sag CM, Santos CX, Shah AM. Redox regulation of cardiac hypertrophy. J Mol Cell Cardiol. 2014;73: 103-11.
17. Münzel T, Gori T, Keaney JF, Maack C, Daiber A. Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications. Eur Heart J. 2015;36(38):2555-64.
18. Altenhöfer S, Radermacher KA, Kleikers PW, Wingler K, Schmidt HH. Evolution of NADPH oxidase inhibitors: Selectivity and mechanisms for target engagement. Antioxid Redox Signal. 2015; 23(5):406-27.
19. Fratelli M, Goodwin LO, Ørom UA, Lombardi S, Tonelli R, Mengozzi M, *et al*. Gene expression profiling reveals a signaling role of glutathione in redox regulation. Proc Natl Acad Sci U S A. 2005; 102(39):13998-4003.
20. Rushworth GF, Megson IL. Existing and potential therapeutic uses for N-acetylcysteine: the need for conversion to intracellular glutathione for antioxidant benefits. Pharmacol Ther. 2014;141(2): 150-9.
21. Adamy C, Mulder P, Khouzami L, Andrieu-abadie N, Defer N, Candiani G, *et al*. Neutral sphingomyelinase inhibition participates to the benefits of N-acetylcysteine treatment in post-myocardial infarction failing heart rats. J Mol Cell Cardiol. 2007; 43(3):344-53.
22. Lombardi R, Rodriguez G, Chen SN, Ripplinger CM, Li W, Chen J, *et al*. Resolution of established cardiac hypertrophy and fibrosis and prevention

of systolic dysfunction in a transgenic rabbit model of human cardiomyopathy through thiol-sensitive mechanisms. Circulation. 2009;119(10): 1398-407.

23. Foltz WU, Wagner M, Rudakova E, Volk T. N-acetylcysteine prevents electrical remodeling and attenuates cellular hypertrophy in epicardial myocytes of rats with ascending aortic stenosis. Basic Res Cardiol [Internet]. 2012 [cited 11 Feb 2019]; 107(5):290. Available at: http://doi.org/10.1007/s00395-012-0290-4
24. Reyes DR, Gomes MJ, Rosa CM, Pagan LU, Damatto FC, Damatto RL, *et al*. N-Acetylcysteine influence on oxidative stress and cardiac remodeling in rats during transition from compensated left ventricular hypertrophy to heart failure. Cell Physiol Biochem. 2017;44(6):2310-21.
25. Giam B, Chu PY, Kuruppu S, Smith AI, Horlock D, Kiriazis H, *et al*. N-acetylcysteine attenuates the development of cardiac fibrosis and remodeling in a mouse model of heart failure. Physiol Rep [Internet]. 2016 [cited 12 Feb 2020];4(7):e12757. Available at: http://doi.org/10.14814/phy2.12757
26. Tsai CT, Wu CK, Lee JK, Chang SN, Kuo YM, Wang YC, *et al*. TNF-α down-regulates sarcoplasmic reticulum Ca^{2+} ATPase expression and leads to left ventricular diastolic dysfunction through binding of NF-κB to promoter response element. Cardiovasc Res. 2015;105(3):318-29.
27. Alves ML, Dias FA, Gaffin RD, Simon JN, Montminy EM, Biesiadecki BJ, *et al*. Desensitization of myofilaments to Ca as a therapeutic target for hypertrophic cardiomyopathy with mutations in thin filamento proteins. Circ Cardiovasc Genet. 2014;7(2):132-43.
28. Wilder T, Ryba DM, Wieczorek DF, Wolska BM, Solaro RJ. N-acetylcysteine reverses diastolic dysfunction and hypertrophy in familial hypertrophic cardiomyopathy. Am J Physiol Heart Circ Physiol. 2015;309(10):H1720-30.