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ROLE OF N-ACETYLCYSTEINE IN RESCUE OF MICE HEART

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ABSTRACT: An imbalance between oxidants and antioxidants generates the oxidative stress. Oxidative stress is the major cause of many diseases like cardiovascular, respiratory disease, renal diseases, neurological diseases, cancer, and aging. Cardiovascular complications are the leading cause of mortality in patients so the purpose of this review is to evaluate the effectiveness of N-acetylcysteine (NAC) in the treatment of cardiovascular diseases. N-acetylcysteine is an antioxidant which confers its protective effect by minimizing oxidative stress and oxidative stress associated problems. A search using the Pub Med database was conducted using the keywords N-acetylcysteine and mice heart. All research articles related to the use of N-acetylcysteine in protection of mice heart were discussed. A total of 93 articles were identified during this broad search while 30 articles were included for review recommending the N-acetylcysteine for protection of heart. This review proved that N-acetylcysteine has a positive role in management of multiple heart diseases.

INTRODUCTION: Under cellular stress mitochondria starts production of free reactive oxygen species (ROS). The imbalance between the ROS and antioxidant defense system induce oxidative stress causing cell apoptosis and organ injury [1].

Accumulating evidence has indicated that ROS generated in heart in different circumstances further contributed to a broad range of pathological conditions. Different cardiovascular diseases in which ROS is generated include: cardiomyopathy, cardiac inflammation, ischemic reperfusion injury, drug induced cardiotoxicity, myocardial infarction and aging heart.

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N-acetyl cysteine (NAC) is a free radical scavenger, acts as a precursor of antioxidant glutathione and also helps in its synthesis. The thiol (sulfhydryl) group of NAC confers antioxidant effects [2] and thus helps to inhibit the accumulation of ROS. NAC also reverses cardiac myocyte dysfunction induced by oxidative stress [3].

Pubmed data with respect to the use of NAC in treatment of heart diseases was summed up in four categories.

1. **NAC and cardiac inflammation:** After cardiac surgery in neonatal mice complication arises due to activation of inflammatory system [4]. Two main reasons for cardiac inflammation depicted in database are as follows:

   - Lipopolysaccharide (LPS) induced inflammation
   - Macrophage induced inflammatory responses

Gram-negative bacteria release endotoxin LPS [4] that initiates the sepsis [5]. Sepsis is defined as systematic inflammatory process due to oxidative stress which causes heart failure [5, 6].

A study on mice showed that LPS induced cytokines activation and altered the cardiac function which was attenuated by NAC [4, 7]. NAC reversed LPS-elicited contractile function of cardiomyocytes [6]. LPS also induced autophagy in cardiomyocytes [6, 7] which can be minimized by NAC [7].

LPS induced production of pro-inflammatory cytokines e.g., tumor necrosis factor-alpha (TNF-alpha) which is responsible for myocardial depression and sepsis shock [7]. Induction of TNF-alpha was inhibited by NAC [8]. LPS also induced cardiomyocyte contractile dysfunction which was prevented by the antioxidant NAC [4-7].

Other reason of cardiac inflammation is macrophage induced inflammatory response which was due to arginase-II (Arg-II). Arg-II elevated the ROS production from mitochondria, which further promoted macrophage pro-inflammatory responses contributing to generate insulin resistance and atherosclerosis. NAC was reported to prevent the Arg-II-mediated inflammatory responses by scavenging ROS [9].

2. **NAC and cardiomyopathy:** Cardiomyopathy is typically divided into several sub-types: HIV cardiomyopathy, pressure overload hypertrophy, dilated cardiomyopathy, diabetic cardiomyopathy, alcoholic cardiomyopathy and drug induced cardiomyopathy.

    - **HIV cardiomyopathy:** HIV infected individual have to face many complications like immune system abnormality, tumour, pulmonary, gastrointestinal, neurological and psychiatric complications. A study showed that in these patients HIV cardiomyopathy prevalence remains high. Myocardial dysfunction, decrease in glutathione (GSH) levels and GSH/GSSG ratio, and increase in H_2O_2 level were found in HIV cardiomyopathy in mice heart. Chen F et al showed that NAC increased GSH and GSH/GSSG and also reversed H_2O_2 level. NAC was also found to reverses cardiac myocyte dysfunction and markers of oxidative stress [3].

    - **Pressure overload hypertrophy:** Pressure overload hypertrophy is associated with substrate switch from fatty acid to glucose oxidation. In cardiac hypertrophy fatty acid utilization increases ROS production. Hexokinase-II (HKII) plays important in regulation of cardiac hypertrophy by binding to mitochondrial membrane and also by decreasing ROS. Wu et al showed that when HKII dissociated from mitochondrial membrane it induced cardiac hypertrophy. But this situation was abrogated upon NAC treatment [10].

Characteristic features of HCM are interstitial fibrosis and contractile dysfunction [11, 12]. Grieve DJ *et al*., [11] reported that nicotinamide adenosine dinucleotide phosphate oxidase isoform (Nox-2) oxidase was liable to the development of pressure-overload LVH by contributing cardiac contractile dysfunction and interstitial fibrosis. NAC treatment reduced fibrosis and improved the contractile function [11]. Another study analyzed that NAC pretreatment reduced oxidative stress marker (malondialdehyde and 4-hydroxy-2(E)-nonenal) and collagen fraction in heart thus preserved cardiac function [12].

- **Dilated cardiomyopathy:** It was found that mutations in the mitochondrial thioredoxin reductase gene cause dilated cardiomyopathy (DCM) in patients. Thioredoxins are proteins that act as antioxidants and paly important role in cardiac defense by reducing oxidative stress. Horstkotte *et al*., [13] reported that thioredoxin reductase deficiency induce loss of mitochondrial integrity and function, which was fixed on pretreatment with NAC [13].

- **Diabetic cardiomyopathy:** Diabetes mellitus (DM) contributes to development of cardiovascular diseases by promoting oxidative stress [14]. In diabetic condition enzyme and antioxidant levels reduce.

Oxidative stress reduces the expression of many essential enzyme in heart e.g., aldose reductase (AR). AR has been associated in the pathogenesis of various diabetic complications. Study was on murine diabetic mice model showed that daily administration of NAC reduced oxidative stress and increased GSH in heart [15].

Accumulation of triacylglycerol in non-adipose tissue leads to development of diabetes and cardiomyopathy [2].

The key player of diabetic cardiomyopathy induction includes obesity and insulin resistance. Both of these factors are associated with enhanced fatty acid utilization due to which free radicals are accumulated and alter calcium transient in heart. This alteration is prevented by NAC [16].

- **Alcoholic cardiomyopathy**: In mice it was determined that the ethanol exposure causes myocardial injury through, induction of oxidative stress and stimulates alcoholic cardiomyopathy. In alcoholic cardiomyopathy mitochondrial damage occurs which decreases mitochondrial GSH content. The study in mice showed that if NAC administration before exposure to alcohol then the chances of alcohol-induced myocardial injuries and oxidative stress was significantly inhibited [17].

- **Drug induced cardiomyopathy:** Anticancer drugs also known to induce cardiotoxicity for example adriamycin (ADR) [18] and doxorubicin (Dox) [19, 20]. These drugs cause cardiomyopathy by promoting oxidative stress. Results of mice model of cardiotoxicity showed that the NAC pretreatment diminished oxidative stress [19]. Another study showed that the Dox induce cardiotoxicity reduce metallothionein (MT) in mice. NAC and GSH significantly rescued MT [20].

Certain drugs when metabolized in the body release aldehydes as by product. Aldehydes also produced in body as a result of lipid peroxidation. These are known to induce cardiac impairment. Acrolein is an aldehyde releases as a result of oxidation of unsaturated fatty acids and by metabolism of drugs. It contributes to myocardial dysfunction by facilitating oxidative stress. NAC pretreatment attenuated the myocardial dysfunction [21].

3. **NAC and ischemic reperfusion/heart failure:** Ischemic heart disease (IHD) is the leading cause of death in population. Many potential reasons exist e.g., mitochondrial dysfunction and oxidative stress. Myocardial ischemia/reperfusion (I/R) leads to generation of excess amount of ROS from mitochondria [13]. This ROS is able to open the mitochondrial permeability transition pore and relocalized the Phosphatase and tensin homologs deleted on chromosome 10 (PTEN) to mitochondria [22]. This event further contributes to tissue injury and impairment of heart functions [13].

   In obstructive sleep apnea syndrome (OSAS) patient's chronic intermittent hypoxia (CIH) is considered as important cause of cardiovascular diseases. In these patients repeated hypoxia and reoxygenation cycle induce oxidative stress which producing hypoxia-reperfusion injury. The CIH induced the apoptosis in cardiomyocytes [22, 23].

   NAC protect the heart by decreasing the mitochondrial PTEN protein levels [22] and inhibiting oxidative stress in CIH [23].

   Oxidative stress, adrenergic stress and endoplasmic reticulum (ER) stress contribute in the pathogenesis of heart [24-26]. An increase in ROS is associated with the development of heart failure and cardiac arrhythmias [24, 26].

   Oxidative stress contributes to the skeletal muscles damage by the deficiency of the cytoskeletal protein dystrophin in duchenne muscular dystrophy (DMD). Chance of heart failure is high in DMD patients. NAC treatment reduces oxidative damage and is favorable therapy to DMD patients with heart failure [26].

   Ischemic injury enhances the susceptibility of heart for aging. Increasing age in patients leads to oxidative stress based age related myocarditis. In the pathogenesis of aging redox regulating mechanisms get impaired. An example of it was thioredoxin (TRX) which induced age related myocarditis.

A study reported that 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative stress was positive in aged mice showing that long-term treatment of NAC suppressed the progression of spontaneous myocarditis [27].

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4. **NAC and cell signaling in heart cells:** ROS induced metabolic alteration in heart which contributes to the development of cardiovascular complication for which heart has limited ability to repair.

Ca^{2+} signaling is important for proper heart functioning [16, 24]. The sympathetic adrenergic system is involved in Ca^{2+} handling and contractility of cardiomyocyte. ROS causes disturbance in this system and ultimately affects Ca^{2+} load this can be reversed by NAC administration [24]. Similarly accumulation of saturated fatty acid e.g., palmitate decreased the cytosolic Ca^{2+} transient which was prevented by NAC treatment.

ROS also induced collagen deposition in heart which further results in delayed heart function. In fibrotic conditions stress-responsive signaling kinases are activated like malondialdehyde and 4-hydroxy-2(E)-nonenal can be reduced by NAC [12].

ROS generation develops the trans mural pressure which ultimately increases the expression of NADPH oxidase inducing constriction in arteries. NAC has been found to abolish arteries constriction by inhibiting NADPH oxidase [28].

ROS also activates the transcriptional factors called nuclear factor erythroid 2-related factor-2 (Nrf2). It regulates the expression of antioxidant genes and ROS detoxifying genes. Activation of Nrf2/ARE pathway confers endothelial dysfunction which is reported to be prevented by pretreatment with NAC and other antioxidants [14].

Endothelial dysfunction leads to cardiac remolding and heart failure. For maintaining cardiac performance β-adrenoceptor plays important role [8, 29]. Abnormality in the function of β-adrenoceptor activates adverse signalling pathways e.g., nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and ROS pathway [29]. NADPH oxidase isoform Nox2 also gets activated which in turns induces the hypertrophy [11].

In LPS induced sepsis gp91phox-containing NADH oxidase is activated which induces the TNF-alpha expression. gp91phox-containing NADH oxidase further activates the ERK1/2 and p38 MAPK pathway which causes myocardial dysfunction. NAC can revert this by reducing oxidative stress [8]. Oxidative stress activates the interleukin 6 (IL-6)-type cytokines blocking Janus kinase (JAK) pathway in ischemia-reperfusion and heart failure [30].

CONCLUSION: We concluded that the NAC may be an effective therapeutic agent in the management of heart disease. This review highlights the importance of NAC as antioxidant agent which ameliorates the oxidative stress in different heart diseases.

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REFERENCES:

1. Kuznetsov AV, Kehrer I, Kozlov AV, Haller M, Redl H, Hermann M, Grimm M and Troppmair J: Mitochondrial ROS production under cellular stress: comparison of different detection methods. Anal Bioanal Chem 2011; 400(8):2383-90.
2. Kuramoto K, Okamura T, Yamaguchi T, Nakamura TY, Wakabayashi S, Morinaga H, Nomura M, Yanase T, Otsu K, Usuda N, Matsumura S, Inoue K, Fushiki T, Kojima Y, Hashimoto T, Sakai F, Hirose F and Osumi T: Perilipin 5, a lipid droplet-binding protein, protects heart from oxidative burden by sequestering fatty acid from excessive oxidation. J Biol Chem 2012; 287(28):23852-63.

3. Chen F, Lewis W, Hollander JM, Baseler W and Finkel MS: N-acetylcysteine reverses cardiac myocyte dysfunction in HIV-Tat proteinopathy. J Appl Physiol (1985) 2012; 113(1):105-13.
4. Mukherjee R, McQuinn TC, Dugan MA, Saul JP and Spinale FG: Cardiac function and circulating cytokines after endotoxin exposure in neonatal mice. Pediatr Res 2010; 68(5):381-6.
5. Turdi S, Han X, Huff AF, Roe ND, Hu N, Gao F and Ren J: Cardiac-specific overexpression of catalase attenuates lipopolysaccharide-induced myocardial contractile dysfunction: role of autophagy. Free Radic Biol Med 2012; 53(6):1327-38.
6. Ceylan-Isik AF, Zhao P, Zhang B, Xiao X, Su G and Ren J: Cardiac overexpression of metallothionein rescues cardiac contractile dysfunction and endoplasmic reticulum stress but not autophagy in sepsis. J Mol Cell Cardiol 2010; 48(2):367-78.
7. Yuan H, Perry CN, Huang C, Iwai-Kanai E, Carreira RS, Glembotski CC and Gottlieb RA: LPS-induced autophagy is mediated by oxidative signaling in cardiomyocytes and is associated with cytoprotection. Am J Physiol Heart Circ Physiol 2009; 296(2):H470-9.
8. Peng T, Lu X and Feng Q: Pivotal role of gp91phox-containing NADH oxidase in lipopolysaccharide-induced tumor necrosis factor-alpha expression and myocardial depression. Circulation 2005; 111(13):1637-44.
9. Ming XF, Rajapakse AG, Yepuri G, Xiong Y, Carvas JM, Ruffieux J, Scerri I, Wu Z, Popp K, Li J, Sartori C, Scherrer U, Kwak BR, Montani JP and Yang Z: Arginase II Promotes Macrophage Inflammatory Responses Through Mitochondrial Reactive Oxygen Species, Contributing to Insulin Resistance and Atherogenesis. J Am Heart Assoc 2012; 1(4):e000992.
10. Wu R, Wyatt E, Chawla K, Tran M, Ghanefar M, Laakso M, Epting CL and Ardehali H: Hexokinase II knockdown results in exaggerated cardiac hypertrophy via increased ROS production. EMBO Mol Med 2012 ; 4(7):633-46
11. Grieve DJ, Byrne JA, Siva A, Layland J, Johar S, Cave AC and Shah AM: Involvement of the nicotinamide adenosine dinucleotide phosphate oxidase isoform Nox2 in cardiac contractile dysfunction occurring in response to pressure overload. J Am Coll Cardiol 2006; 47(4):817-26.
12. Marian AJ, Senthil V, Chen SN and Lombardi R: Antifibrotic effects of antioxidant N-acetylcysteine in a mouse model of human hypertrophic cardiomyopathy mutation. J Am Coll Cardiol 2006 ;47(4):827-34.
13. Horstkotte J, Perisic T, Schneider M, Lange P, Schroeder M, Kiermayer C, Hinkel R, Ziegler T, Mandal PK, David R, Schulz S, Schmitt S, Widder J, Sinowatz F, Becker BF, Bauersachs J, Naebauer M, Franz WM, Jeremias I, Brielmeier M, Zischka H, Conrad M and Kupatt C: Mitochondrial thioredoxin reductase is essential for early postischemic myocardial protection. Circulation 2011; 124(25):2892-902.
14. Ungvari Z, Bailey-Downs L, Gautam T, Jimenez R, Losonczy G, Zhang C, Ballabh P, Recchia FA, Wilkerson DC, Sonntag WE, Pearson K, de Cabo R and Csiszar A: Adaptive induction of NF-E2-related factor-2-driven antioxidant genes in endothelial cells in response to hyperglycemia. Am J Physiol Heart Circ Physiol 2011; 300(4):H1133-40.
15. Iwata K, Nishinaka T, Matsuno K, Kakehi T, Katsuyama M, Ibi M and Yabe-Nishimura C: The activity of aldose reductase is elevated in diabetic mouse heart. J Pharmacol Sci 2007; 103(4):408-16.
16. Fauconnier J, Andersson DC, Zhang SJ, Lanner JT, Wibom R, Katz A, Bruton JD and Westerblad H: Effects of palmitate on Ca(2+) handling in adult control and ob/ob cardiomyocytes: impact of mitochondrial reactive oxygen species. Diabetes 2007; 56(4):1136-42.
17. Kannan M, Wang L and Kang YJ: Myocardial oxidative stress and toxicity induced by acute ethanol exposure in mice. Exp Biol Med (Maywood) 2004; 229(6):553-9.
18. Schmitt-Gräff A and Scheulen ME: Prevention of adriamycin cardiotoxicity by niacin, isocitrate or N-acetyl-cysteine in mice. A morphological study. Pathol Res Pract 1986; 181(2):168-74.
19. Doroshow JH, Locker GY, Ifrim I and Myers CE: Prevention of doxorubicin cardiac toxicity in the mouse by N-acetylcysteine. J Clin Invest 1981 Oct; 68(4):1053-64.
20. Fu Z, Guo J, Jing L, Li R, Zhang T and Peng S: Enhanced toxicity and ROS generation by doxorubicin in primary cultures of cardiomyocytes from neonatal metallothionein-I/II null mice. Toxicol In Vitro 2010; 24(6):1584-91.
21. Luo J, Hill BG, Gu Y, Cai J, Srivastava S, Bhatnagar A and Prabhu SD: Mechanisms of acrolein-induced myocardial dysfunction: implications for environmental and endogenous aldehyde exposure. Am J Physiol Heart Circ Physiol 2007; 293(6):H3673-84.
22. Zu L, Zheng X, Wang B, Parajuli N, Steenbergen C, Becker LC and Cai ZP: Ischemic preconditioning attenuates mitochondrial localization of PTEN induced by ischemia-reperfusion. Am J Physiol Heart Circ Physiol 2011; 300(6):H2177-86.
23. Liu JN, Zhang JX, Lu G, Qiu Y, Yang D, Yin GY and Zhang XL: The effect of oxidative stress in myocardial cell injury in mice exposed to chronic intermittent hypoxia. Chin Med J (Engl) 2010; 123(1):74-8.
24. Andersson DC, Fauconnier J, Yamada T, Lacampagne A, Zhang SJ, Katz A and Westerblad H: Mitochondrial production of reactive oxygen species contributes to the β-adrenergic stimulation of mouse cardiomyocytes. J Physiol 2011; 589(Pt 7):1791-801.
25. Guo R, Ma H, Gao F, Zhong L and Ren J: Metallothionein alleviates oxidative stress-induced endoplasmic reticulum stress and myocardial dysfunction. J Mol Cell Cardiol 2009; 47(2):228-37.
26. Williams IA and Allen DG: The role of reactive oxygen species in the hearts of dystrophin-deficient mdx mice. Am J Physiol Heart Circ Physiol 2007; 293(3):H1969-77.
27. Miyamoto M, Kishimoto C, Nimata M, Nakamura H and Yodoi J: Thioredoxin, a redox-regulating protein, is expressed in spontaneous myocarditis in inbred strains of mice. Int J Cardiol 2004; 95(2-3):315-9.
28. Nowicki PT, Flavahan S, Hassanain H, Mitra S, Holland S, Goldschmidt-Clermont PJ and Flavahan NA: Redox signaling of the arteriolar myogenic response. Circ Res 2001; 89(2):114-6.
29. Xu Q, Dalic A, Fang L, Kiriazis H, Ritchie RH, Sim K, Gao XM, Drummond G, Sarwar M, Zhang YY, Dart AM, Du XJ. Myocardial oxidative stress contributes to transgenic β_2-adrenoceptor activation-induced cardiomyopathy and heart failure. Br J Pharmacol 2011; 162(5):1012-28.
30. Kurdi M, Sivakumaran V, Duhé RJ, Aon MA, Paolocci N and Booz GW: Depletion of cellular glutathione modulates LIF-induced JAK1-STAT3 signaling in cardiac myocytes. Br J Pharmacol 2011; 162(5):1012-28.

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