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N-Acetylcysteine in Acute Cardiology: 10 Years Later

What Do We Know and What Would We Like to Know?!

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N-acetylcysteine (NAC) is known in a variety of branches of medicine. This paper addresses in detail the action of NAC as it is emerging from research and clinical trials over the past decade in cardiology, giving rise to new concepts. The result is a process resembling creation of a mosaic from individual pieces. Also, the role of NAC in acute cardiology, during acute reperfusion in particular, is defined. (J Am Coll Cardiol 2002;39:1422–8) © 2002 by the American College of Cardiology Foundation

N-acetylcysteine (NAC) is known in a variety of branches of medicine. In cardiology, it has been increasingly used over the past decade, with its main potential being the minimization of the impact of reperfusion injury on the treatment of acute myocardial infarction. Still, the spectrum of its effects is much broader: it has an effect on oxygen radicals; it may protect, via sulfhydryl groups, the key regions of the cellular membrane; it exerts an effect on endothelial function and a complex of adhesive processes and, as a secondary effect, also processes in the extravascular compartment. Additionally, it possesses a variety of other actions to be discussed. Moreover, it is related to the whole cardiovascular system.

OXIDATIVE STRESS

Problem description. Throughout its life, the cell is exposed to the action of molecular oxygen in a host of redox processes. Oxygen radicals are produced even under resting conditions. It is estimated that mitochondria turn about 2% of the total amount of oxygen processed into superoxide ions and not into the normal end product, which is water (1). The superoxide ion is subsequently converted—with the action of metalloproteins, most often lactoferrin containing iron from neutrophil granules (Fenton's reaction)—via hydrogen peroxide as an intermediary product into a hydroxyl radical. Alternatively, by direct reaction with hydrogen peroxide, again giving rise to a hydroxyl radical and oxygen singlet (Haber-Weiss reaction). Oxygen radicals are responsible for a variety of other processes and so-called oxidative stress in particular, with the latter referring to a complex of various abnormal processes affecting the isolated cell, the tissue, or the whole body (2–4). The importance of these processes is particularly evident when the cell is unable to defend itself against the radicals, such as when it is exposed to excessively high concentrations of free oxygen radicals of exogenous origin in a phase when it is injured and loses defensive mechanisms or when it excessively produces free radicals. Oxidative stress may result in a temporary or permanent change in the properties of proteins and, possibly, in a change in nucleic acids (5–7). The process of aging is generally believed to be simply a consequence of long-term exposure to oxygen radicals (8–10).

Reperfusion as a deleterious process. Reperfusion is based on the reintroduction of fully oxygenated blood into the targeted areas of myocardium previously modified by ischemia. This is associated with exacerbation of various stages of cellular injury as assessed by morphological, physiological and biochemical criteria. The typical sequels of reperfusion injury are release of intracellular enzymes, altered calcium ion homeostasis with their intracellular influx, and sarcolemmal phospholipid breakdown terminated by cell membrane disruption. This ultimate stage is called lethal reperfusion injury and is synonymous with cell and tissue necrosis as a measurable parameter. Under normal conditions, existing cardiomyocyte homeostasis often undergoes two combined processes: functional changes and structural changes. Functional changes involve electrical instability with a broad range of ventricular arrhythmias and depressed ventricular contractility (stunning). Structural alterations consist of microvascular lesions (slow-flow, no-reflow phenomenon), hemorrhagic intramyocardial foci and non-apoptotic cellular death in the end stage. Myocardial reperfusion injury involves the reperfusion-induced conversion of reversible injured myocardial and endothelial cells, as well as interstitial tissue, into irreversibly altered cells. It implies that cells in potentially viable conditions just before reperfusion underwent lethal injury during the perireperfusion period.

Elementary findings. In cardiology, the target cell—the cardiomyocyte—possesses both structural and functional properties as volume regulation, conduction and contraction. Some 10 years ago, the concept of oxidative stress was fairly mysterious. Currently, there is a trend toward a renaissance of previously recognized findings. In 1935, Tennant and Wiggers (11) described ventricular fibrillation after release of coronary artery ligation. The accelerated idioventricular rhythm was reported by Goldberg et al. (12) after the introduction of reperfusion therapy into clinical practice. In 1975, Heyndrickx et al. (13) gave an account of post-ischemic contractile myocardial dysfunction after

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Abbreviations and Acronyms

GISSI = Gruppo Italiano per lo Studio della Streptochinasi nell' Infarto Miocardico study
GUSTO = Global Utilization of Streptokinase and T-PA for Occluded Coronary Artery trial
ISIS = International Study of Infarct Survival
ISLAND = Infarct Size Limitation: Acute N-acetylcysteine Defense trial
MPG = mercaptopropionyl glycine
NAC = N-acetylcysteine
-SH = sulfhydryl groups

reperfusion. Since 1982, when Braunwald and Kloner (14) renamed the condition as "stunned myocardium," it has become a generally recognized phenomenon after all types of acute reperfusion. This refers to the functional consequences of reperfusion on the myocardium. The primary causes may be processes leading to sulfhydryl group (-SH) oxidation (15). Oxidative stress occurs after reperfusion therapy for acute myocardial infarction (MI), irrespective of the method of reperfusion (i.e., medical or mechanical therapy) (16,17). Recently, a rise in oxygen radicals during treatment of acute MI using percutaneous transluminal coronary angioplasty was reported (18).

Current concepts. Essentially, these processes involve an action of free oxygen radicals on unsaturated fatty acyl chains in cell phospholipids and their peroxidation (the process affects both the cellular membrane and lysosomes) (19–21). This results in damage of lysophosphatidyl acyltransferase, i.e., an -SH sensitive enzyme with a subsequent impact on further changes in the glutathione system (responsible for redox elimination of oxygen radicals) (22). If prolonged, oxidative stress may result in structural alterations. Of the three possibilities—hemorrhage, microvascular damage and cell necrosis—the latter is, in the case of reperfusion, called lethal reperfusion injury. This occurs mostly in situations when the myocyte is significantly damaged by previous prolonged ischemia. A piece of evidence indicating these processes are very topical, especially today when the mainstay of therapy is one of the techniques of reperfusion, is supplied by principles of cellular defense that have been identified. Today, the elementary features of gene expression have been defined for so-called ARE (antioxidant reactive elements), after an ischemia-reperfusion experiment lasting no more than dozens of minutes (23). Theoretically, and philosophically as well, the first episode of oxidative stress is birth, when the lungs of the neonate are filled with oxygen. Nature would certainly not fail to include this event in the evolutionary process. In 1989, the potential for pharmacological protection of the myocardium against reperfusion injury by thiol-containing agents (i.e., those containing -SH groups) at the time of release of the coronary artery ligature (i.e., in the reperfusion phase of the experiment) was first described, which was, until then, regarded as non-feasible (24).

In addition to this common situation (in cardiology), oxidative disorders are associated with a number of chronic conditions. Chronic heart failure (HF) is a case in point, as the condition has been clearly associated with -SH group depletion and an increased incidence of apoptosis. Like -SH group oxidation in acute conditions (ischemic-reperfusion processes), apoptosis in chronic conditions has been explained by DNA oxidation (the implication being it is of importance with chronic HF of any etiology) (15). Undesirable oxidative damage to myocardial and vascular cells in general occurs also with nitrate tolerance (this is also explained by -SH group depletion, increased production of oxygen radicals with an effect on cyclic guanosine monophosphate and loss of cell relaxation). Diastolic HF is possibly caused by similar processes.

Free oxygen radicals and their role. The free oxygen radical hypothesis seems to be the most widely recognized aspect of reperfusion injury because of both the direct and indirect evidence supporting it. In fact, free oxygen radicals are involved in various pathways in the form of calcium overload of cardiomyocytes and the spectrum of sarcolemmal phospholipid changes that are present in the initial phase of membrane injury. Measurable changes include hydroxyl radical detection and lipid oxidation (lipid endoperoxides, lipid hydroperoxides and conjugated dienes formation). The transient role of superoxides, with their ultrashort half-life, is also well defined. These processes are also enhanced by conversion of xanthine dehydrogenase into xanthine oxygenase. On the other hand, many substances such as antioxidants and/or free-radical scavengers have been shown to minimize reperfusion injury and indirectly support the key role played by oxygen radicals in reperfusion injury.

Of special importance are processes involved in the so-called endogenous antiradical defense. These include, in particular, binding to albumin and hemoglobin, reactions with various lipids with no essential function, and reaction with the urate system. Additionally, there is evidence on external enhancement of these processes, for example, superoxide dismutase (targeted on the superoxide anion), catalase (inactivating primarily hydrogen peroxide), allopurinol and ascorbic acid. The role of alpha-tocopherol is currently being re-examined. Compared with free radicals, lipid peroxides are relatively more stable and less sensitive to the effect of natural defense; thus, they can be used as a measurable index of reperfusion injury (method using phenyl-tert-butylnitron).

Today, it seems that the theory of oxidative stress ceases to be mere theory; and accumulating evidence in the fields of molecular cardiology, biochemistry and gene expression/re-expression may result in new therapeutic approaches.

SULFHYDRYLS, MEMBRANE INJURY, REGIONAL REPERFUSION AND N-ACETYLCYSTEINE (NAC) OVER THE PAST 10 YEARS

Experimental model. We have been studying intensively the issue of regional reperfusion in acute MI since 1984 (i.e.,

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at the time when thrombolytic therapy was relatively well established). The experience gained led to an effort to minimize the extent of myocardial injury. In 1990, we performed an experimental study in dogs with 2 h of ischemia and 2 h reperfusion: NAC was administered at a total dose of 100 mg/kg b.w., so most of the dose coincided with the reperfusion phase of the experiment. This resulted in reduction of structural injury (smaller extent of necrosis) compared with a control group of animals. Similarly, a significant reduction in the incidence of ventricular arrhythmias as a functional consequence was seen in the reperfusion phase in the treated group (25). Because of the surprising results, the study was repeated, and the results were confirmed (26). At that time, NAC had been selected on the basis of a thorough study of the substance showing a unique spectrum of effects; and, most importantly, it was readily available.

Biochemical view. A chemically similar substance, aminoethanethiol, was first used in 1960. At that time, this substance was intended to confer protection against the effects of radiation (27). Seen from today's perspective, aminoethanethiol is referred to as cysteamine, and radiation-induced alterations at the cellular level are not dissimilar to the ischemia-reperfusion insult. Another property of cysteamine that seemed to be an advantage was its ability to act at a low pH, i.e., under acidotic conditions, which exist as a rule in the ischemic phase of experiments. Needless to say, the element most affected by the ischemic and reperfusion burden is the cellular membrane. An accompanying process is a decrease in thiols and -SH (28,29). Substances containing -SH groups may confer a protective effect. Dimethylthiolurea was such a successful candidate, improving left ventricular (LV) contractility, most likely via hydroxyl radical elimination (30). A most important finding was that -SH-containing substances may be effective even when administered at the time of reperfusion; initially, it was believed that protective agents should be administered exclusively in the ischemic phase of experiments. Mercaptopropionyl glycine (MPG) works in an exactly identical manner as when administered before coronary artery occlusion (24). The effects of MPG were known from an earlier pharmacological study (31). Mercaptopropionyl glycine is a direct free-radical scavenger. Sulfhydryl groups are oxidizable structures, thus explaining the elimination of free oxygen radicals. The importance of the cysteine-cystine redox system is well known in terms of its biochemistry. Moreover, -SH groups can easily bind with free -SH groups of structural and functional membrane proteins and prevent—at least temporarily—their damage. It is by this particular mechanism that reactive enzyme groups are protected reversibly against the effects of peroxides and, possibly, other radicals. This is the assumed result of mixed disulfides and the main role of disulfide bonds. Cysteine is clearly a metabolite of NAC in vivo (32). There is also an important relationship between cysteine and/or NAC and glutathione: cysteine can be clearly regarded as a glutathione precursor (33,34). Glutathione, and/or the ratio between its reduced and oxidized phases, is a directly measurable marker whose dynamics in an ischemic-reperfusion insult are related to cardiac function (35,36). Glutathione peroxidase is an important enzyme in the degradation cascade of reactive free oxygen radicals. In this respect, NAC may help replenish the depleted glutathione stores (37). Early pharmacologic studies reported an effect of NAC in a model of ischemia-reperfusion (38). N-acetylcysteine is most likely also related to the modulation of damaged endothelium. The endothelium, in the phase of cellular swelling, no-reflow phenomenon and, possibly, the more substantial changes, cannot produce the endothelium-derived relaxing factor (nitric oxide). Hypothetically, the role can be taken over by the positive action of the -SH groups of NAC and routinely administered nitroglycerin replacing the missing nitric oxide. This action, however, does not involve direct elimination of free oxygen radicals. We will not address the issue of nitrate tolerance. Still, in the context of oxygen radical generation and no-reflow phenomenon, mention should be made of the interaction of neutrophils, endothelia and intercellular adhesion molecule-1. The oxidase system of neutrophils and free oxygen radicals may substantially affect the MAC-1 neutrophil receptors (CD11b/CD18), thus modulating thrombocyte behavior, vascular permeability and release of agents from mast cells and, by this mechanism, affect the vascular and extravascular compartment interface (39,40). At least a decrease in circulating polymorphonuclear neutrophils and hydrogen peroxide levels after NAC administration has been documented (41). In general, cysteine derivatives show a certain anticoagulation activity that is beneficial in coronary thrombosis. A report was published as early as 1952 describing a decrease in prothrombin activity (prothrombin time prolongation) several hours after cystamine administration (42). Finally, NAC may also be an angiotensin-converting enzyme inhibitor: NAC significantly reduces (by 50%) angiotensin II levels in humans and angiotensin-convertase activity by 31% in the plasma of rats (43). Basal NAC pharmacokinetics in man has been investigated in detail (44).

Pharmacology of NAC. N-acetylcysteine is a well-documented substance in medicine. Its defined volume of distribution is 0.33 l/kg, its renal clearance is 0.21 l/h per kg, and its elimination half-life is 2.27 h (44). Originally, NAC has been used to liquefy mucus in bronchi; NAC is also the antidote for paracetamol poisoning. It should also be mentioned that cysteine derivatives have been intensively investigated in military medicine because of their protective effects against radiation and that NAC has been investigated as a modulator of radiographic contrast agent–induced nephrotoxicity (45–47).

From the point of cardiology, it is important that NAC is readily hydrolyzed to cysteine and can arise from essential methionine (an amino acid containing an atom of sulfur). Cysteine is a precursor of glutathione. By itself, cysteine has

no significant hemodynamic effects; but, when combined with nitrates (the indication being either enhancement of the nitrate mechanism or elimination of nitrate tolerance through -SH donation), it may cause a decrease in blood pressure and, possibly, headache via intracranial blood bed dilation. These findings were made, for example, in a study addressing the management of unstable angina pectoris and comparing the effects of intravenous nitroglycerin versus a combination of intravenous nitroglycerin and NAC (48). However, the most important finding of this study was a reduction in the incidence of MI. Similarly, another randomized study involving the same category of patients compared transdermal nitroglycerin with nitroglycerine/NAC: although all the outcomes of unstable angina pectoris were reduced significantly by combination therapy, a relatively high incidence of induced headache was demonstrated (49). Induced vasodilation can be used even in the treatment of lung edema or congestive HF. A randomized study confirmed an equal effect of conventional therapy for acute cardiogenic pulmonary edema with furosemide and morphine versus the tested combination of nitroglycerine and NAC (50). Another randomized study conducted in patients with chronic HF demonstrated that a combination of isosorbide dinitrate and NAC results in a more significant effect on preload than isosorbide dinitrate alone. Moreover, the former combination significantly reduced the plasma levels of atrial natriuretic peptide (51). This has clear implications for prevention of pulmonary congestion. Its effect on platelets is yet another, by no means negligible, action of this molecule. A significant effect of the combination of NAC and nitroglycerin on inhibiting platelet thrombus formation has been reported, whereas the dose of nitroglycerin, when used alone, had no effect whatsoever (52). Intervention regarding low-density lipoprotein oxidation to oppose atherogenesis seems to be another promising line of research.

Despite this, new combinations of NAC action under pathophysiologic conditions, particularly in ischemia-reperfusion situations, continue to emerge. The role of NAC in various pharmacologic and clinical interventions is shown in Tables 1 and 2.

Clinical baseline. These unique properties of NAC, its demonstrated role in clinical practice from other indications, and results of early experimental studies imitating ischemia-reperfusion conditions led to clinical trials with NAC in acute cardiology. The first clinical observation in 1991 documented normalization of LV function after a previous infarction (53). This served as a stimulus to launch a pilot study referred to as ISLAND (Infarct Size Limitation: Acute N-acetylcysteine Defense trial) (54). This was followed by a detailed analysis of the data obtained and waiting for results from other centers (55–58). Clinical studies regarding the role of NAC in modulating ischemic-reperfusion injury in acute MI and conducted to date are very small, yet they have yielded positive results. The first study, published in 1995, reported data obtained from 29 consecutive patients: of this number, only 27 were eligible. A total of 20 patients were assigned to combination therapy, including streptokinase, NAC and nitroglycerin. The remaining seven patients were treated without NAC (56). The ISLAND study, published in 1996, was already a randomized, angiography- and echocardiography-controlled study. Of the total 57 consecutive patients, 45 were randomized: 21 patients were treated with streptokinase and 19 with a combination of streptokinase and NAC. Five patients were ineligible for analysis. Angiography divided the whole series of patients into 14 patients with reperfusion induced by combination therapy, 15 patients with reperfusion induced by streptokinase alone and 11 patients with failed reperfusion. The main analysis focused on the 14 patients receiving combination therapy with angiography-documented reperfusion; this group was compared with the other groups. The first group differed clearly from other groups by a reduction in infarct size and improved LV function as shown by electrocardiogram. Similarly, the whole group of 19 patients receiving combi-

Table 1. Major Clinical Roles for NAC

Historically	Relation to cysteamine—a radioprotective agent (military use)
Noncardiac uses	Bronchosecretolytic
	Antidote in paracetamol poisoning
	Antinephrotoxic effect (prevention of radiographic contrast agent-induced renal function alteration)
General uses in cardiology	Vasodilation and induced left ventricular effect
	Donation of -SH groups
	Nitrate enhancement
	Vascular/platelet effect
	Application: antianginal effect
	heart-failure modulation
Other effects	Mainly in research: stabilization of atherosclerotic process
	Modulation of cell death protection

NAC = N-acetylcysteine; -SH = sulfhydryl groups.

Table 2. Postulated Roles for NAC in Acute Cardiology

Target: Ischemic-Reperfusion Effect	
Factors involved:	(a) *direct protection against effects of FOR*
	(aa) redox mechanism cysteine-cystine
	(ab) relation to glutathione
	(b) indirect protection against FOR, *formation of mixed disulfides with -SH groups of membrane peptide/enzymes*
	(c) effect on modulating processes and factors
	(ca) effect on platelet function
	(cb) action under low-pH (ischemic) conditions
	(cc) interaction with neutrophils
	(cd) NAC = ACE inhibition
	(ce) modulation of coagulation
	(cf) effect on adhesion processes (via adhesion molecules)
	(cg) arrhythmogenesis modulation

NAC action outside the area of acute cardiology is outside the scope of this article, which is why they are mentioned only briefly.

ACE = angiotensin-converting enzyme; FOR = free-oxygen radicals; NAC = N-acetylcysteine; -SH = sulfhydryl groups.

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Table 3. Current Independent Pilot Clinical Studies With NAC in Acute Myocardial Infarction

Study	Result
Arstall et al. (53)	Decreased level of oxidative stress
n = 27 (20 with combination therapy)	More rapid reperfusion
	Better left ventricular preservation
ISLAND (55)	Reduced infarct size
n = 45 (19 with combination therapy, 14 with proven reperfusion)	Better preservation of global and regional left ventricular function
	Modification of QRS complex morphology in ECG

ECG = electrocardiogram; ISLAND = Infarct Size Limitation: Acute N-acetylcysteine Defense trial; NAC = N-acetylcysteine.

nation therapy differed, regardless of the result of coronary angiography, from the group of 21 patients treated exclusively with streptokinase (58). A summary of data is shown in Table 3.

An important finding was that there is an electrocardiographic marker indicating an additional beneficial functional effect: a rudimentary R wave or R wave recovery in the left precordial leads (55,59). For the purpose of more accurate quantification of risk region and final infarct size, there are methods available, such as nuclear imaging, echocardiography and myocardial blush grading. At the same time, basic research provided evidence of an association between free oxygen radicals and arrhythmogenesis in the modulation of calcium-sensitive nonselective cation current irrespective of changes in intracellular calcium ion levels. Most likely, there is a mechanism that modulates the membrane channels by -SH: dithiothreitol inhibits nonselective cation current activation (60). This finding in pigs is fully consistent with our previous finding made in dogs at a time when the role of transmembrane current and modified channels was not yet known (25).

WAITING FOR ADDITIONAL EVIDENCE

NAC today. N-acetylcysteine has been selected for use in ischemia-reperfusion injury more or less empirically. Today, it seems to have a place in cardiology—particularly in acute cardiology—as an agent specifically modulating reperfusion injury (61). The number of papers indicating the importance of NAC in basic research, experimental and clinical cardiology, cardiac surgery, as well as in other branches of medicine, has been growing. A list of references from all fields is outside the scope of this communication; however, representative papers underlining the comprehensive nature of the effect of NAC are included. This is logical because this perspective clearly shows the interplay of individual pathophysiological processes. Today, NAC is perceived as a drug applicable in acute coronary syndromes (62). N-acetylcysteine directly eliminates hydroxyl radicals and increases the nitric-oxidase system-dependent coronary flow (63). This is an advantage when using NAC as part of cardioplegic solutions (64). Evidence is also available that NAC acts during cardiopulmonary bypass as an oxygen radical scavenger and also stabilizes neutrophils in relation to their oxidative response to the bypass (65). In this context, the interaction of effects on the intravascular and extravascular compartment interface is also of importance. Through its action on vascular cell adhesion molecules-1 and action of endothelium-mediated adhesiveness via nuclear factor-kappa B, NAC may reduce the impact of oxidative stress (66,67). Furthermore, NAC improves endothelium-dependent vasodilation both on peripheral and coronary arteries (68). Not irrelevant in this context is the finding that NAC decreases plasma homocysteine levels (69). The reduction may be as high as 60% or more (70). The complex of NAC effects is complemented by a series of findings revealing that the substance reduces oxidative lesions in target tissue, allows adequate oxygen utilization and improves overall oxygenation, with a potential reduction in mechanical ventilation both in lung injury and in hyperoxemic situations or during hyperbaroxia (71–73). Finally, there is a report wherein NAC modulated the course of severe systolic dysfunction in patients with viral myocarditis (74).

WHAT WE KNOW AND WHAT WE WANT TO KNOW

Present status. First, a large body of evidence has been accumulated from basic research, experimental physiology and cardiology and also from other clinical subspecialties (a basic review is provided in this communication).

Second, we are aware of several experimental studies not confirming a beneficial effect of NAC; however, no study has demonstrated deleterious effects of NAC, the number of papers reporting a beneficial effect of NAC is incomparably higher than those not confirming a beneficial effect, and there is a lack of comparability in the methods used.

Third, we know that small clinical studies demonstrating an effect of NAC have been conducted in acute and reperfusion cardiology.

Fourth, we know that the past 10 years have not diminished—but have not boosted—the importance of NAC.

Question formulation. It is therefore logical to formulate what we want to learn: Is NAC clearly a beneficial agent reducing reperfusion injury in the treatment of acute MI as practiced today? To be able to answer this question, a large randomized multicentric study of the scope of GISSI, ISIS or GUSTO would be required.

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