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(54) Title: N-ACETYL COMPOSITIONS AS ADJUNCT THERAPY FOR TREATMENT AND PREVENTION OF CYSTEINE/GLUTATHIONE DEFICIENCY

(57) Abstract: Life-threatening hepatotoxicity in the setting of acetaminophen overdose is due to depletion of glutathione (GSH), a vital cysteine-containing tripeptide that protects cells and organs against oxidant injury. Rapid administration of N-acetylcysteine (NAC), which provides the cysteine necessary to replenish the depleted GSH, is the standard of care for preventing injury in acetaminophen overdose. Beneficial effects of NAC treatment have also been demonstrated in respiratory, cardiovascular, endocrine and infectious and other diseases. In fact, over fifty randomized placebo-controlled trials conducted in diverse clinical settings document positive responses to NAC treatment. The present invention relates to cysteine/glutathione (GSH) deficiency as a previously unrecognized clinical entity that can complicate the course of commonly encountered diseases and methods of treatment of this generalized deficiency involving administering N-acetylcysteine (NAC) or a pharmaceutically acceptable salt or derivative to a subject in need thereof and monitoring the subjects appropriate glutathione blood levels as needed.



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N-ACETYL COMPOSITIONS AS ADJUNCT THERAPY FOR TREATMENT AND PREVENTION OF CYSTEINE/GLUTATHIONE DEFICIENCY

This application claims the benefit of the filing date of provisional applications
5 SN 60/496,119 and 60/496,127, both of which were filed August 19, 2003.

FIELD OF THE INVENTION

The present invention relates to cysteine/glutathione deficiency as a previously
unrecognized clinical entity that can complicate the course of commonly encountered
10 diseases and methods of treatment of this generalized deficiency comprising administering
N-acetylcysteine or a pharmaceutically acceptable salt or derivative to patients in need
thereof and monitoring the appropriate glutathione blood levels as needed.

SUMMARY OF THE INVENTION

The present invention relates to cysteine/glutathione (GSH) deficiency as a
15 previously unrecognized clinical entity that can complicate the course of commonly
encountered diseases and methods of treatment of this generalized deficiency comprising
administering N-acetylcysteine (NAC) or a pharmaceutically acceptable salt or derivative
and monitoring the appropriate glutathione blood levels as needed.

According to one embodiment of the invention, a method of treatment to prevent
20 development of glutathione deficiency as a consequence of disease, a treatment, or a
condition comprises administering to a subject at risk of glutathione deficiency as a
consequence of disease, a treatment, or a condition a therapeutic amount of NAC or a
pharmaceutically acceptable salt or derivative sufficient to increase intracellular
glutathione levels or decrease oxidative stress and measuring and monitoring the level of
25 glutathione in the blood of patients as needed. According to another embodiment of the
invention, a method of treatment to restore glutathione levels comprises administering to
subjects in need of glutathione level restoration, as determined by measurement or by a

physician, a therapeutic amount of NAC or a pharmaceutically acceptable salt or derivative thereof sufficient to increase intracellular glutathione levels or decrease oxidative stress and monitoring restoration by measuring the level of glutathione in blood as needed. According to another embodiment of the invention, a method of treatment to decrease oxidized glutathione levels elevated as a consequence of disease, a treatment, or a condition comprises administering to a subject suffering from oxidative stress a therapeutic amount of NAC or a pharmaceutically acceptable salt or derivative sufficient to decrease oxidized glutathione levels elevated as a consequence of disease and monitoring the level of oxidized glutathione in blood as needed.

10

BACKGROUND OF THE INVENTION

GSH is a central component of the oxidative-reductive (redox) apparatus of every cell. One of its key functions is to combine with, and thereby inactivate (detoxify), reactive oxidative intermediates (ROI), other oxidative molecules, and certain drugs, exogenous chemicals and toxins. Because GSH is depleted in these reactions, it must continually be replenished to maintain cell and organ viability and to support normal cellular functions. Drug intoxications resulting in severe GSH depletion, notably acetaminophen overdose, cause extensive hepatic injury if treatment to replenish GSH is not initiated before GSH stores are depleted to below critical protective levels.

Synthesis of GSH requires cysteine, a conditionally essential amino acid that must be obtained from dietary sources or by conversion of dietary methionine via the cystathionase pathway. If the supply of cysteine is adequate, normal GSH levels are maintained. In contrast, if supplies of cysteine are inadequate to maintain GSH homeostasis in the face of increased GSH consumption, GSH depletion occurs.

GSH depletion impacts a wide variety of cellular processes, ranging from DNA synthesis and gene expression to sugar metabolism and lactate production. The

pleiotropic activity of this key intracellular molecule, which arose very early in evolution, derives from its participation in the energy economy and the synthetic and catabolic activities of virtually all cells. In higher animals, it also participates in regulating the expression or activity of extracellular molecules, including many of the cytokines and adhesion molecules implicated in inflammatory reactions and other disease processes.

Acute GSH depletion causes severe — often fatal — oxidative and/or alkylation injury. This injury can be prevented (in acetaminophen overdose, for example) by rapid treatment with N-acetylcysteine (NAC), an efficient non-toxic source of cysteine, which is necessary to replenish hepatocellular GSH. Chronic or slowly arising GSH deficiency due to administration of GSH-depleting drugs, or to diseases and conditions that deplete GSH, can be similarly debilitating.

Systematic review of a series of randomized placebo-controlled trials conducted over the last 25 years demonstrates that treatment with NAC provides beneficial effects in a number of respiratory, cardiovascular, endocrine and infectious and other disease settings. Rapid administration of NAC is the standard of care for preventing hepatic injury in acetaminophen overdose. It provides the cysteine necessary to replenish a crucial intracellular tripeptide — glutamylcysteinylglycine, commonly known as GSH, which is depleted during detoxification of excessive amounts of acetaminophen. Since orally-administered NAC is rapidly converted by first-pass metabolism to cysteine, it results in replenishment of GSH as well as supplying cysteine for additional metabolic and protein synthetic processes. Thus, the beneficial effects observed following NAC treatment in the trials reviewed here suggest that cysteine/GSH deficiency contributes to the pathophysiology of a wide range of diseases and that avoidance of this deficiency may be important in treating these diseases.

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GSH-depleting drugs, or to diseases and conditions that deplete GSH, can be similarly debilitating[1].

The following discussion provides evidence for the development of cysteine/GSH deficiency in a variety of disease settings and considers the biochemical mechanisms through which this deficiency, and its correction, can impact disease processes.

Search and inclusion criteria

The following studies were selected by searching the PubMed database using the Endnote interface. Publications included here describe randomized placebo-controlled NAC trials testing N-acetylcysteine (NAC) for efficacy in a variety of disease settings, were located by a search strategy using the following keywords: placebo AND N-acetylcysteine AND NOT Animal. Abstracts were excluded, as were publications that were not written in the English language. In addition, studies with fewer than 10 patients and/or unclear endpoints were excluded. All other publications that met the search criteria are included. The included studies are discussed below and summarized in table 1, in which we classify the findings according to whether they reports that NAC treatment results in 1) significant clinical benefit, 2) significant findings whose clinical relevance is unclear, or 3) no significant difference between the control and treatment groups.

Publications describing NAC trials that were not placebo-controlled, or describing observational NAC studies conducted with NAC, were located using the keyword N-acetylcysteine in combination with keywords identifying individual diseases. These trials and studies are not exhaustively reviewed here. Many, but not all, are listed by citation in table 2; some are discussed in later sections. We also discuss, but do not exhaustively

review, findings from animal studies and from biochemical and other studies that provide information relevant to disease and GSH depletion mechanisms.

GSH deficiency and disease

5 A role for GSH deficiency in the clinical manifestations of a broad spectrum of diseases and conditions is suggested either by the direct documentation of low GSH levels in these conditions or by the demonstration of significant improvement in patient condition following NAC administration. Over fifty randomized placebo-controlled trials demonstrate beneficial effects of NAC treatment (table 1)[2-62] in diseases and
10 conditions that include systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome (ARDS), chronic lung disease (CLD), chronic obstructive pulmonary disease (COPD), neurodegenerative disease, cardiovascular disease, alcoholism, infectious disease (e.g., HIV infection, chronic HCV hepatitis), hepatic and renal failure, diabetes, malnutrition and certain autoimmune diseases.

15 The mechanisms that underlie the development of GSH deficiency in disease are reasonably well understood, at least in some instances. A wide variety of inflammatory and metabolic stimuli common during active disease increase the production of intracellular oxidants. In addition, neutrophils and other cells present at sites of inflammation release oxidants (reactive oxygen and nitrogen intermediates) that enter
20 other cells and add to the internal oxidant burden. GSH provides the main defense against toxic oxidative intermediates by reducing and thereby inactivating them. However, in so doing, GSH is oxidized to GSH disulfide (GSSG). GSSG is then either rapidly reduced to GSH by GSSG-reductase and NADPH or is excreted from the cell and only in part recovered from the circulation.

Factors that may contribute to GSH deficiency include GSH losses that occur when GSH is enzymatically conjugated to exogenous chemicals (drugs, dietary components and toxins) and excreted from the cell as GSH or acetylcysteine mercapturates (conjugates). In addition, disease processes may decrease the cellular uptake or synthesis of cysteine or cystine, increase GSH efflux[63], or increase the loss of cysteine/GSH sulfur due to accelerated oxidation to the final oxidized forms (sulfate and taurine)[64, 65]. Because a balance between cysteine supply and GSH utilization must be maintained, if oxidant production or levels of substrate for GSH conjugation are high and cysteine supplies for GSH replenishment become limiting, severe GSH deficiency may occur.

Importantly, there are significant potential iatrogenic contributions to GSH depletion. Inadvertent treatment with higher doses of acetaminophen than patients can tolerate is perhaps the most common. This can be particularly dangerous for patients with conditions in which GSH depletion tends to occur as a consequence of the disease process or following treatment with drugs that are detoxified by GSH. In addition, long-term maintenance on parenteral nutrition may result in GSH depletion since parenteral nutrition formulations are not necessarily designed to provide adequate cysteine equivalents to meet the metabolic needs of diseased patients. In the absence of adequate attention to maintenance of adequate cysteine supplies, physicians and other caregivers can inadvertently contribute to GSH deficiency.

Patient behavior may also result in the development of GSH deficiency. Chronic over-consumption of alcohol is well known to deplete GSH in certain tissues, particularly the liver, and thus to render patients susceptible to acetaminophen toxicity at doses well below those that cause toxicity in healthy individuals. Indeed, the FDA has issued a

warning to this effect (<http://www.fda.gov/ohrms/dockets/ac/02/briefing/3882b1.htm>).

However, chronic consumption of acetaminophen or other GSH-depleting drugs, even well below toxic dose levels, can gradually deplete GSH to the point where these drugs elicit toxicity. Such practices become more dangerous if patients are malnourished or are
5 GSH deficient for other reasons.

In summary, GSH deficiency occurs more frequently than previously suspected. GSH is readily replenished by *de novo* synthesis as long as sufficient supplies of cysteine are available, either directly from dietary sources or indirectly by conversion of dietary methionine. However, failure to obtain sufficient dietary cysteine to replace that lost
10 when GSH is oxidized or conjugated to drugs or exogenous chemicals results in cysteine, and hence GSH, deficiency (referred to here as cysteine/GSH deficiency) that may necessitate pharmacological intervention.

Dietary sources of cysteine

15 Cysteine utilized in the body is derived from dietary cysteine and methionine, sulfur-containing amino acids that are largely obtained from digested protein. Since mammals obtain cysteine both directly from the diet and by degradation of dietary methionine, the normal cysteine requirement can be satisfied from dietary sources. However, as indicated above, an additional source of cysteine may be required when
20 cysteine loss (e.g., via GSH loss) outstrips the usual dietary supply.

Requirements for sulfur-containing amino acids in humans are based upon nitrogen and sulfur amino acid balance studies conducted with healthy individuals. The average American diet contains about 100 g of protein daily, greater than half of which is animal protein with a relatively high content of sulfur-containing amino acids. The
25 recommended daily allowance (RDA) for sulfur amino acids (SAA) for an adult male is

about 1g (200 mg of methionine and an additional 810 mg methionine that can be replaced by an equivalent amount of cysteine[66]). A healthy, well-fed person will often consume greater than twice the sulfur amino acid RDA. However, poor appetite and/or a tendency to select fresh food with low sulfur amino acid content or bioavailability[67] or
5 processed food depleted of sulfur amino acids[68-70] can result in cysteine deficiency even in otherwise healthy people. Furthermore, as evidence here indicates, the need for sulfur amino acids can be substantially increased in many disease states.

The limited ability of the body to store amino acids is an additional problem. The human liver does contain a reservoir of cysteine (about 1 g) that is largely present in
10 GSH. Since this amount approximates the daily sulfur amino acid requirement, it provides only a short-term source to maintain a stable cysteine supply despite intermittent methionine and cysteine consumption. Under conditions of excessive cysteine requirements or deficient cysteine/methionine consumption, GSH is also released from skeletal muscle and other tissues to supply cysteine. This results in decreased antioxidant
15 and detoxification functions throughout the body. Consequently, even short term inadequate intake of sulfur amino acids can pose a risk to individuals who may consume adequate amounts most of the time[71, 72].

Mechanisms that may mediate the clinical effects of cysteine/GSH deficiency

20 GSH has multiple roles in cells, ranging from neutralization of (ROI) to acting as a co-enzyme in a variety of metabolic processes. The widespread participation of GSH in biochemical reactions of importance to cell growth, differentiation and function offers mechanistic insights into how interfering with GSH homeostasis could influence the course of varied disease processes. A full discussion of the preclinical data bearing on
25 these issues is beyond the present scope. However, to provide a mechanistic context for

the clinical findings we discuss, we have summarized some of the key processes regulated by GSH:

Oxidative reactions. In its most well-known role, GSH participates in enzyme mediated reactions to neutralize ROI and thus prevents the accumulation of ROI damage to DNA, proteins and lipids. Glutathione peroxidases play a key role in this process by catalyzing the reaction of GSH with peroxides, including hydrogen peroxide and lipid peroxides. Thus, decreasing GSH can sharply augment oxidative damage and result in cell death or loss of function.

DNA synthesis. Low GSH availability can impair DNA synthesis since GSH acts (via glutaredoxin) as a coenzyme for ribonucleotide reductase, an enzyme required for the synthesis of deoxyribonucleotides[73-75].

Gene expression and signal transduction. GSH has been shown to regulate or influence the expression of several genes, notably inflammatory genes under the control of transcription factors nuclear factor kappa B (NF- κ B) and activator protein 1 (AP-1), even in settings where there is no marked overproduction of ROI. In addition, GSH has been shown to regulate T cell signaling by controlling phosphorylation of phospholipase C (PLC) γ 1, which is required to stimulate the calcium flux that occurs early in the T cell receptor signaling cascade[76, 77]. Importantly, GSH has also been shown to regulate the expression of vascular cell adhesion molecule-1 (VCAM-1) on vascular endothelial cells, one of the early features in the pathogenesis of atherosclerosis and other inflammatory diseases[21, 78-81].

Enzymes and protein functions. GSH regulates the activity of enzymes and other intracellular molecules by post-translational modifications (glutathionylations) that control the oxidation state of protein-SH groups. When intracellular GSH is at its normal

level for a particular cell type in a healthy individual, most of the free protein thiol groups are reduced, i.e., are present as protein-SH. In contrast, when GSH levels are low and/or GSSG levels are increased, GSH is reversibly coupled to many free thiols to create mixed disulfides (protein-S-S-G)[82]. These S-glutathionylated proteins, which may be
5 functionally altered, then persist as such until GSH levels return to normal.

By controlling the activities of a series of enzymes and other intracellular proteins, glutathionylation can rapidly and reversibly alter the metabolic status of cells in response to changes in the redox environment. For example, glutathionylation has been shown to regulate actin polymerization[83], to inhibit the activity of several key enzymes
10 (including glyceraldehyde-3-phosphate dehydrogenase, carbonic anhydrase and protein tyrosine phosphatase) and to activates or stabilize other enzymes (including HIV-1 protease and the NF- κ B transcription factor[84]). Nitrosylation of protein thiols has similarly been shown to increase under oxidative conditions[85-88] and to alter functions of key enzymes[89] and other molecules[90-92]. Thus, both glutathionylation and
15 nitrosylation are of central importance to mechanisms through which cysteine/GSH deficiency may impact cell, and hence organ, function.

As indicated above, these types of post-translational modifications are highly sensitive to shifts in the intracellular redox balance. They are rapidly initiated when GSH is depleted and rapidly reversed when GSH is replenished. As such, they provide the
20 kind of flexible response to oxidative stress necessary for organisms living in an oxidative environment. However, at the extreme, they may underlie some of the pathologic changes that occur when chronic cysteine/GSH deficiency occurs in disease.

Glutaredoxin and thioredoxin. Glutaredoxin (Grx) and thioredoxin (Trx)

belong to the two major disulfide reductase enzyme families, which take electrons from GSH and NADPH via Grx reductase and Trx reductase, respectively[73-75, 93-95]

Trx and Grx interact with proteins to regulate functional activity, both directly and
5 via glutathionylation. Intracellular GSH and GSSG levels play a major role in this regulation. The activity of Grx is directly regulated by the amount of intracellular GSH and GSSG, which controls the status of the Grx active site. The active sites in Trx (Cys-Gly-Pro-Cys) and Grx (Cys-Pro-Tyr-Cys) contain a dithiol that can be oxidized when GSH levels are low (or GSSG levels increase) to form an internal disulfide between the
10 two cysteine residues or a mixed disulfide in which GSH is bound to one or both cysteine residues in the active site. Formation of the Grx mixed disulfide[82, 93, 96] represents a special case of protein glutathionylation since it arms the Grx for glutathionylation of other proteins. Although Trx can also be glutathionylated[96], current data indicate that glutathionylation is mainly mediated by the Grx mixed disulfide[97-99].

15 Oxidation of Grx and Trx active sites can also regulate Trx or Grx functions mediated by direct binding to key intracellular proteins. For example, under reducing conditions, Trx and Grx protect cells from apoptosis by binding to and inactivating the apoptosis-signaling kinase I[99, 100] whereas this binding is blocked and apoptosis induction proceeds at low GSH levels (and/or high GSSG levels)[74, 75].

20 **Selenoenzymes.** Decreasing GSH increases the intracellular redox potential of the GSH/GSSG couple and puts an additional burden on the thioredoxin-thioredoxin reductase system. This may be quite important in patients who have low selenium levels, since human thioredoxin reductases are selenoenzymes with an essential selenocysteine residue in the active site[75, 101-105]. Cysteine/GSH deficiency in these patients, in

whom thioredoxin reductase activity is compromised, may make them particularly susceptible to cell damage under oxidative stress.

Thus, cysteine/GSH deficiency can impact cell and organ function through multiple pathways operating at the same or different sites, depending on the underlying mechanisms responsible for depleting GSH. This potential for affecting different pathways in different diseases perhaps explains why the effects of cysteine/GSH deficiency have not been readily recognizable as a single clinical entity.

N-acetylcysteine (NAC) treatment to relieve cysteine/GSH deficiency

Clinical experience in the treatment of acetaminophen toxicity has established that rapid administration of NAC, an essentially non-toxic cysteine source, restores normal GSH levels in solid tissues and the systemic circulation and thus prevents the potentially lethal consequences of severe cysteine/GSH deficiency induced by acetaminophen overdose. In addition to this well-known role for NAC, however, NAC treatment has been shown to be clinically beneficial in a wide variety of diseases and conditions. In fact, over fifty randomized placebo-controlled trials (table 1) have reported beneficial effects of NAC treatment. Collectively, these studies demonstrate that cysteine/GSH deficiency is an important emerging clinical entity and that NAC administration offers an effective method for treating this deficiency.

Although various forms of cysteine and its precursors have been used as nutritional and therapeutic sources of cysteine, NAC is the most widely used and extensively studied*. NAC is about 10 times more stable than cysteine and much more soluble than the stable cysteine disulfide, cystine. L-2-oxothiazolidine-4-carboxylate (procysteine/OTC) has also been used effectively in some studies[106] as have

glutathione and glutathione monoethyl ester[107]. In addition, dietary methionine is an effective source of cysteine, as is S-adenosylmethionine (referred to either as SAM or SAM-e)[108]. We focus on NAC in this review because NAC is the cysteine source for used correcting cysteine/GSH deficiency in most studies and because NAC is already
5 approved for therapeutic use for treatment of acetaminophen overdose and as a mucolytic agent in cystic fibrosis.

Surprisingly, given the diverse roles that GSH plays in cellular physiology and regulation of enzyme activity and protein function (see above), GSH deficiency has mainly been discussed from a clinical perspective in terms of the loss of intracellular
10 protection against oxidative stress. Similarly, NAC is principally considered to be an antioxidant rather than a source of cysteine for GSH replenishment. However, while antioxidants such as vitamin E and vitamin C can spare GSH under conditions of oxidative stress, GSH loss due to oxidative or detoxifying reactions can only be offset by GSH resynthesis, which requires a cysteine source.

15 **Cysteine/GSH deficiency and NAC therapy in disease settings**

In the sections that follow, we discuss reported outcomes of NAC therapy in various clinical settings. We largely focus on findings from the randomized placebo-controlled studies (table 1), but also discuss selected findings from observational studies that further illuminate clinical aspects of cysteine/GSH deficiency. Citations for these and other observational NAC treatment
20 studies are presented in table 2, which provides a partial survey of the literature in this area.

Acetaminophen toxicity.

The toxicity of acetaminophen is due to depletion of GSH in hepatocytes^{g-g}[109-114]. Acute dose levels of acetaminophen likely to cause severe liver toxicity are well established for healthy individuals[110]. However, under conditions in which GSH levels are compromised, doses of

acetaminophen that are within the usual prescribed range can also cause hepatic injury and failure[71, 110]. Thus, acetaminophen usage, and the usage of other GSH-depleting drugs, may be quite important to overall pathology in diseases and conditions such as those discussed in the section that follow, where GSH deficiency is known to occur.

5 Hepatic and gastrointestinal disease.

Hepatic failure (Table 1a). Acetaminophen overdose is a well-known cause of fulminant hepatic failure. In fact, a recent study indicates that acetaminophen overdose and idiosyncratic drug reactions have now replaced viral hepatitis as the most frequent causes of acute liver failure in the United States[115]. N-acetylcysteine (NAC), which
10 provides the cysteine necessary to replenish GSH depleted by toxic agents, is extremely effective in preventing liver damage due to acetaminophen toxicity. Although placebo-controlled studies demonstrating this point are scarce (one report), NAC administered promptly and at a sufficient dose^{e.g.}[2, 71, 109, 110, 113, 116-159] has been the standard of care for treatment of acetaminophen poisoning for many years[110]. In addition, NAC
15 has been suggested as treatment for *Amanita phalloides* poisoning[160] and for other exposures to toxic agents^{e.g.}[161-163].

The importance of administering NAC within the first twenty-four hours of acetaminophen overdose is clear. However, later administration of NAC has also been shown to be effective. In a placebo-controlled study of patients with acetaminophen-
20 induced fulminant hepatic failure who had not previously received NAC, both trial arms showed similar rates of deterioration and recovery of liver function (table 1a). However, subjects in the NAC arm showed a significant increase in survival and a corresponding decrease in the incidence of cerebral edema and hypotension requiring inotropic support [2].

Consistent with these findings, an observational study with one hundred patients who have already developed fulminant hepatic failure due to untreated acetaminophen overdose showed significantly increased survival in the NAC-treated group[138]. A subsequent study, also observational, showed that NAC treatment significantly improves
5 cardiac index, mean oxygen delivery, mean arterial pressure, oxygen consumption and oxygen extraction ratio in patients with fulminant hepatic failure due either to untreated acetaminophen overdose or to other causes[164].

The chronic consumption of alcohol poses a special risk with respect to acetaminophen overdose[139, 147] because alcoholic patients often have lower GSH
10 levels. In such patients, doses of acetaminophen below those usually considered toxic can be expected to deplete GSH below the critical threshold for hepatocellular necrosis[165]. Thus, a recent study suggests that due to an increased risk of developing hepatotoxicity, patients with chronic alcoholism and suspected acetaminophen poisoning should be treated with NAC regardless of risk estimation[166-168].

15 The US Food and Drug Administration (FDA) recognized the importance of alcohol consumption in predisposing to acetaminophen toxicity by ruling (in 1998) that acetaminophen package labels must include the following warning: "If you consume three or more alcoholic drinks every day, ask your doctor whether you should take acetaminophen or other pain relievers/reducers. Acetaminophen may cause liver
20 damage." The reasons underlying this ruling are reviewed in a report accessible at http://www.fda.gov/ohrms/dockets/ac/02/briefing/3882b1_01_E-Final%20Rule.pdf.

Gastrointestinal disease. Two randomized, placebo-controlled studies showed beneficial effects of NAC treatment on GI-related pathophysiology (see table 1a). In one

study conducted as part of an effort to develop chemoprevention for carcinogenesis of the large bowel, NAC treatment was found to lower the proliferative index in the colonic crypt epithelium of subjects who previously had adenomatous polyps [55]. In the second study, NAC treatment in the presence of hyperoxic ventilation better preserved end-organ
5 oxygen utilization and was associated with improved gastric intra-mucosal pH.

A published abstract of a placebo-controlled trial indicated that NAC treatment decreased the sensitivity and specificity of the *Helicobacter pylori* stool antigen test. However, we were unable to obtain the full text article for evaluation of this conclusion.

Protein-Energy Malnutrition (PEM). Several studies have demonstrated GSH
10 depletion in children with the edematous syndromes of PEM, kwashiorkor and marasmic-kwashiorkor[54, 169-171]. Children with edematous PEM also have elevated levels of biomarkers of cellular oxidant damage[172, 173], indicating that a greater sensitivity to the effects of free radicals on cellular components. The observation that levels of biomarkers of oxidant damage normalize as soon as clinical signs and symptoms
15 resolve[172] suggests that oxidant damage plays an important role in the pathogenesis of the disease.

In a study of children with edematous PEM, Jahoor and colleagues showed that erythrocyte GSH depletion is due to a slower rate of synthesis secondary to inadequate cysteine availability[171]. In another more recent study of a similar group of children
20 with edematous PEM, Jahoor and colleagues demonstrated that GSH synthesis rate and concentration can be restored during the early phase of nutritional rehabilitation if diets are supplemented with NAC[54]. The observation that edema is lost at a faster rate by the group whose GSH pools were restored early with NAC suggests that early restoration of GSH homeostasis in children with edematous PEM accelerates recovery. This

possibility is supported by another study showing that increases in GSH levels in children with kwashiorkor are associated with recovery[173].

These findings also raise the question of whether the modest malnutrition common in elderly people, who also frequently have low GSH levels[174], puts the elderly at risk for developing clinically significant cysteine/GSH deficiency and hence at increased risk of hepatic and other tissue injury associated with consumption of GSH-depleting pharmaceuticals such as acetaminophen.

Cardiovascular disease

Nine placebo-controlled studies showed beneficial effects of NAC treatment in cardiovascular disease (see table 1b). NAC treatment enhanced the coronary vasodilator effects of nitroglycerin in patients with unstable angina, yielding significant clinical benefit[13, 14, 159, 175-178]. In addition, several placebo-controlled studies demonstrated that treatment with NAC decreased development of nitrate tolerance in patients with stable angina[13, 14, 159, 177]. Nevertheless, the use of NAC in combination with nitroglycerin is limited because it is frequently associated with severe headache[177], most likely due to enhancement of the vasodilator effect.

NAC treatment was also shown to be effective as preventative cardiac measure. A placebo-controlled study evaluating NAC pretreatment in cardiac risk patients examined during periods of hyperoxia showed that the pretreatment attenuated tissue oxygenation impairment and preserved myocardial performance better pretreatment with placebo [11]. NAC treatment in a randomized, placebo-controlled study of 134 chronic hemodialysis patients who are at high risk for cardiovascular events also shows that NAC treatment is associated with a significant decrease (RR 0.60, $p=0.03$) in composite cardiovascular outcome, including occurrence of myocardial infarction, need for coronary angioplasty or

coronary artery bypass surgery, ischemic stroke, or peripheral vascular disease[17]. The NAC-treated group in this study also had much lower serum oxidized LDL values at the end of the study.

In an observational trial with patients undergoing thrombolytic therapy for myocardial ischemia, 24-hour intravenous infusion of GSH significantly decreased the oxidative stress of reperfusion injury as demonstrated by decreased plasma malondialdehyde (MDA) [179]. The GSH-treated patients also had fewer episodes of arrhythmia.

Renal disease.

Acute renal failure. Nephrotoxicity from *acute* acetaminophen overdose in the absence of hepatic toxicity has only rarely been described[148, 180]. However, analgesic nephropathy associated with chronic use of compounds containing phenacetin (which is metabolized to acetaminophen) and other non-narcotic analgesic products has been recognized as a cause of renal failure for years[181, 182]. The percentage of incident cases of end-stage kidney failure due to analgesic nephropathy as the principal cause varies from 1% to over 10%, depending on the country considered. In addition, chronic use of these analgesics may contribute to the risk of kidney failure in individuals with chronic kidney injury from other causes. Several large studies have estimated the relative risk of development of kidney failure as a function of analgesic consumption[183, 184]. Most recently, Fored and colleagues reported a dose-dependent increase in the risk of chronic renal failure associated with chronic exposure to acetaminophen in the absence of aspirin use. These investigators showed that the relative risk of chronic renal failure was 5.3 for regular users of acetaminophen consuming ≥ 1.4 g/day on average[184].

Contrast-induced nephropathy (CIN). Six of twelve placebo-controlled studies showed beneficial effects of NAC treatment in CIN (see table 1c). Tepel and colleagues have reported evidence from a placebo-controlled study implicating GSH deficiency in the pathogenesis of contrast nephropathy, a form of acute renal failure[3-5]. These

5 investigators compared NAC to placebo in a study of 83 patients with baseline renal impairment undergoing computed tomography with a nonionic low-osmolality contrast agent. They demonstrated a 90% reduction in the incidence of acute renal failure in subjects treated with NAC, suggesting that GSH replenishment is protective in this clinical setting.

10 A total of twelve placebo-controlled studies with similar design have been reported[3, 6-10, 185-189]. Five of these[6-10] found about the same level of protection (RR^* 0.13 to 0.32) reported by Tepel[3]. Another study, with 183 patients, demonstrated a significant reduction in the incidence of contrast nephropathy (from 8.5% to 0%, $p=0.02$) only in the subset of patients receiving lower volumes (<140 mL) of contrast agent[190],

15 and yet another[189] showed borderline statistically significant protection for the subgroup of patients with the lowest pre-contrast renal function (creatinine clearances: 30-59 mL/min/1.73 m²). One study [9] addressed the mechanisms by which NAC treatment may protect kidney function and reported increased renal nitric oxide (NO) production in the NAC treated subjects. However, four studies failed to show any

20 protective effect of NAC in preventing contrast nephropathy[185-188]. An additional study using an abbreviated intravenous NAC protocol showed a beneficial effect[191].

The striking differences in outcomes (considerable decreases in relative risk vs absence of any protection) in these large, well-designed studies is puzzling, inasmuch as

most of the study designs were similar with respect to the degree of renal impairment, percentage of patients with diabetes mellitus, use of saline loading and of nonionic, low-osmolality contrast agents, and the doses of NAC used. The incidence of contrast-induced nephropathy in the placebo-treated groups of the studies that showed protection
5 tended to be higher than in those that did not ($23\pm14\%$ vs $12\pm6\%$, $P = 0.11$) although the difference did not achieve statistical significance, suggesting that the difference in outcomes is probably not due predominantly to differences in host factors.

In some negative studies, the follow-up time may have been insufficient to demonstrate the full effects of NAC treatment [189]. In addition, since the source of the
10 NAC used was not stated in most of the studies, it is difficult to exclude differences in NAC potency (related to different sources, vide infra) or the presence of contaminants as a possible factor in the outcomes. Thus, there is currently no obvious explanation for the different findings in these studies. Even so, the majority of the published studies support a strong protective effect of NAC in preventing contrast-induced nephropathy.

15 Three metaanalyses have recently been published in this highly active area. All found significant heterogeneity among the studies reviewed. Two interpreted the studies to show evidence for reduction in the risk of contrast-induced nephropathy with use of NAC[192, 193]. One concluded that the heterogeneity in the studies precluded aggregation of the data as needed to complete the metaanalysis[194]. Two of the studies
20 recommended initiation of a large randomized placebo-controlled trial to address the efficacy of NAC in this setting[193, 194].

Kidney transplantation. Delayed graft function (DGF) after kidney transplantation is probably in large part caused by production of ROI and other reactive

* Relative Risk

species following reperfusion of the transplant organ after a period of warm and cold ischemia. In general, these reactive molecules are detoxified by GSH-dependent mechanisms, including conjugation to GSH by a family of GSH-S-transferase (GST) enzymes, some of which are expressed in large quantity in the proximal tubule of the kidney[195]. In an observational study of 229 kidney transplant recipients, donor (but not recipient) GST M1B polymorphism was associated with significantly lower rates (RR 0.33) of DGF after transplantation[196]. Lack of association with other GST alleles in this study complicates the interpretation of the specific role(s) GST may play in reducing DGF risk. However, since the association was only observed when the protective allele was carried by the transplanted organ, there is reason to suspect that a particular GST (or a closely linked protein) may be protective in this setting.

Endocrine disease (Insulin sensitivity)

Three randomized placebo-controlled trials demonstrate beneficial effects of NAC treatment in insulin-related disease (see table 1d). One study demonstrates that oral administration of NAC to patients with non-insulin dependent diabetes mellitus (NIDDM) reverses the elevation of soluble vascular cell adhesion molecule-1 (VCAM-1) [21], a substance which promotes accumulation of macrophages and T-lymphocytes at sites of inflammation and increases progression of vascular damage[79, 80]. A second placebo-controlled study by the same group shows that intravenous GSH infusion significantly increases both intraerythrocytic GSH/GSSG ratio and total glucose uptake in NIDDM patients and suggests that abnormal intracellular GSH redox status in these patients plays an important role in reducing insulin sensitivity[22]. Consistent with these findings, in an ongoing study in type 2 diabetics, Jahoor and colleagues have demonstrated that two weeks of dietary supplementation with NAC elicited significant increases in both

erythrocyte GSH concentration and the rate of its synthesis, suggesting that positive clinical effects of NAC are mediated through improved GSH availability[197].

Finally, a study of patients with hyperinsulism secondary to polycystic ovary disease demonstrates that NAC treatment significantly decreases serum insulin levels and insulin sensitivity without altering fasting glucose levels [20]. The authors conclude that NAC may be a new treatment for the improvement of circulating insulin levels and insulin sensitivity in these patients.

Metabolic and genetic disease.

Genetic defects that impair GSH synthesis or homeostasis are well known (reviewed by Ristoff[198]). The most common defect affects GSH synthetase (GS) and has a wide range of disease manifestations, including hemolytic anemia, progressive neurological symptoms, metabolic acidosis and, in the most severe form, death during the neonatal period. Data from a small observational study suggests that early supplementation with Vitamins C and E may improve long-term outcome in these patients[199].

Cystic fibrosis. Two placebo-controlled studies report beneficial effects of NAC treatment on lung function in cystic fibrosis (table 1e). A third study reports improvement in measures of lung function, but saw no significant clinical differences between NAC and placebo arm subjects. A very short fourth study (2 weeks) failed to find any significant difference between the trial arms.

Sickle cell disease. In a placebo-controlled trial examining NAC treatment in sickle cell disease, NAC was found to inhibit dense cell formation and decrease the number of episodes of vaso-occlusive disease. These improvements in patient condition were associated with increase in glutathione levels.

Homocysteine metabolism. Two of three NAC randomized, placebo-controlled trials showed beneficial effects of NAC treatment in patients with elevated homocysteine (see table 1e). NAC significantly increased homocysteine removal by hemodialysis. This reduction in plasma homocysteine was significantly correlated with reduction in pulse pressure and improved endothelial function. In a second trial, NAC decreased plasma homocysteine levels in patients with elevated plasma lipoprotein A.

Pulmonary disease

Chronic lung disease/Chronic Obstructive Pulmonary Disease (CLD/COPD) (see table 1f). In twelve of eighteen randomized, placebo-controlled trials, NAC treated patients showed beneficial effects for patients with forms of chronic lung disease. Patients to whom NAC was administered orally demonstrated decreased days of illness, improved response to steroids, decreased disease exacerbation rates and a general increase in well-being[23-34]. One study showed a benefit whose clinical relevance is unclear and seven failed to find significant benefit[200-204]. Metaanalyses of the bronchitis studies showed that NAC treatment significantly decreases the costs of hospitalizations, emergency room visits, medications, and work time lost [205-210].

Beneficial effects of NAC treatment have been demonstrated in COPD patients, who showed significant improvements in Forced Expiratory Volume during the first second (FEV1) and Maximum Expiratory Flow (MEF50)[210]. Mucociliary clearance in COPD subjects and in otherwise healthy smokers also showed improvement[36] as did modulation of cancer-associated biomarkers in specific organs in smokers[35];

Systemic Inflammatory Response Syndrome (SIRS) and Acute Lung Injury (ALI); multiple organ system failure (MOSF)(see tables 1f-h). Five of seven randomized placebo-controlled studies showed beneficial effects of NAC as adjunct therapy acute

lung injury and end-organ failure. Results of these studies indicate that oxidative stress and cysteine/GSH depletion play a major role in inflammation leading to capillary leak syndromes and end-organ failure[44, 46, 211]. These conclusion is supported and partially explained by evidence showing that 1) NAC decreases the cytotoxic effects of 5 TNF- α and other inflammatory cytokines[212]; 2) NAC decreases neutrophil elastase production in acute lung injury[213-217] and, 3) NAC increases neutrophil protection and decreases mortality in cecal ligation and puncture septic shock[218].

In ALI, results from randomized, placebo controlled trials demonstrate significant antioxidant effects and improved outcome[11, 42, 44, 211, 215, 219-223]. Data from 10 these trials show that NAC treatment decreases the level of respiratory distress, the work of breathing and number of days of mechanical ventilation. In addition, data from the trials show that NAC treatment improves static lung compliance, oxygenation measured either as oxygen index or partial pressure of arterial oxygen (PaO₂), and sustained diaphragmatic muscle activity[211, 219, 222, 223]

15 Three studies failed to demonstrate significant benefit of NAC treatment in ALI[45, 219, 224], most likely because of the timing of NAC administration. In the studies in which NAC was found to be beneficial [11, 42, 44, 211, 215, 219-223], treatment was initiated 24-48 hours and maintained for 3-10 days after the inciting event. In contrast, in the studies in which no benefit was associated with NAC treatment[45, 20 219, 224], the treatment was either terminated before day 3 of illness or begun after chronic lung disease with ventilator dependence was well established.

Septic shock and infectious disease.

Septic shock. Three of four randomized placebo-controlled trials demonstrate beneficial effects of NAC treatment in septic shock, as demonstrated by improved tissue

oxygenation, decreased veno-arterial PCO₂, decreased IL-8 production, increased cardiac index and overall increased survival. One study, however, demonstrated progressive decrease in mean arterial pressure, cardiac index and stroke work (see table 1g). In this latter study, which is listed as adverse in table 1g, NAC was administered earlier than the preceding studies (two hours after hemodynamic stabilization), which may have confounded the endpoint. In any event, findings from this study suggest that the timing of NAC administration requires further investigation.

HIV disease. A broad series of studies clearly demonstrates GSH levels in erythrocytes, lymphocytes and other peripheral blood mononuclear cells progressively decrease as HIV disease advances[47-49, 225-228]. In addition, careful pharmacokinetic studies demonstrate that the low GSH in HIV-infected individuals is due to limited availability of sufficient cysteine to maintain cellular GSH homeostasis[229, 230]. In fact, a massive peripheral tissue catabolism of sulfur-containing peptides and amino acids has been observed in HIV patients[64, 65].

Five of six randomized placebo-controlled studies show beneficial effects of NAC treatment in HIV infection. Several trials collectively demonstrated that NAC administration to HIV-infected subjects with low GSH levels replenishes lymphocyte and erythrocyte GSH (see table 1g) [48, 50]. Importantly, one of these studies demonstrates that NAC treatment significantly improves T cell function[50]. This finding supports the idea that cysteine/GSH deficiency contributes to the immunodeficiency in HIV-infected individuals and plays an important *and reversible* role in the functional impairment of those T cells that are still present at later stages of HIV disease.

Cysteine/GSH deficiency may also contribute to the failure of the innate immune system and the development of opportunistic infections in the final stages of HIV disease.

Observational studies have shown that HIV-infected individuals with low CD4 T cell counts and low cellular and systemic GSH levels frequently have elevated blood levels of thioredoxin (Trx), which is an effective chemokine[231]. In mice, circulating Trx (like other chemokines) blocks neutrophil migration to infection sites and hence interferes with innate defense against invading pathogens[218]. Similar interference may occur in HIV infection, since the survival of infected individuals with Trx levels above the normal range is significantly decreased compared to survival of subjects with Trx levels in the normal range[82]. Since NAC treatment lowers Trx levels[94, 232] this may contribute to the observed association between NAC ingestion and prolonged survival in HIV disease[47, 48, 227, 233].

The improvement in T cell function observed in HIV-infected subjects treated with NAC[50] suggests that NAC treatment may be a useful adjunct in HIV vaccination. In addition, this improvement provides a rationale for the strong associations observed between low GSH levels and decreased survival in HIV infection[49] and between NAC administration and improved survival in an open-label NAC study[234].

Influenza. One randomized placebo-controlled trial demonstrated that long-term therapy with oral NAC during “cold” season significantly attenuated the frequency and severity of influenza episodes in elderly subjects and in patients suffering from chronic non-respiratory diseases.

Malaria. NAC (300 mg/kg given intravenously over 20 hours) was tested as adjunctive therapy for severe malaria in two double-blind, placebo-controlled studies[51],[52]. Results from one study (30 subjects[51]) showed that elevated serum lactate levels, an indication of disease severity in potentially life-threatening malaria[235-237], returned to normal twice as quickly in the patients who received NAC than in those

who received placebo. Summary of results from a second study (108 subjects [52]) indicate strong positive findings, leading the authors to a call for a large double blind trial of NAC as an adjunctive therapy for severe malaria. (Unfortunately, we were unable to obtain the full text article describing this latter study.)

5 The mechanism(s) through which NAC produced beneficial effects in these studies were not defined. However, several key processes in *falciparum* malaria are potential NAC targets. For example, NAC is known to decrease the activity of TNF- α , which has been shown to mediate cerebral dysfunction in murine cerebral malaria[238] and has been implicated in the pathogenesis of severe human malaria[239]. In addition,
10 NAC may act by preventing adherence of *Plasmodium falciparum*-infected red blood cells to CD36 on postcapillary venular endothelium, an important step in the pathogenesis of severe malaria[240]. Finally, NAC may play a direct role in decreasing serum lactate production by replenishing GSH and thereby reversing the loss of glyceraldehyde phosphate dehydrogenase activity and the shift to glycolytic metabolism likely to occur
15 when GSH is depleted[82].

Multiorgan failure (table 1h).

NAC treatment for SIRS and MOSF has not been very well studied. One randomized, placebo-controlled study demonstrated that NAC improves *ex vivo* phagocytosis activity.[220] Three additional studies show a NAC-associated increase in
20 cardiac index and decrease in systemic vascular resistance but have conflicting results with respect to oxygen delivery, oxygen extraction and serum lactate levels [44, 45, 222]. None of these studies report a significant difference in mortality between the NAC and control groups. However, all of the studies were relatively small and had heterogenous SIRS etiologies, suggesting that larger, better-controlled studies are required before

conclusions can be drawn with respect to the clinical benefits of NAC treatment in SIRS and MOSF.

The only side effect reported for NAC treatment for inflammatory conditions is mild to moderate anticoagulation[106]. This may be attributable to antioxidant-mediated release of nitric oxide (NO) from sequestered peroxynitrate. It was not found to be clinically significant.

Neurologic and musculoskeletal disease (table 1i)

Evidence implicating GSH deficiency in a series of neurodegenerative diseases is summarized in a recent review[241] by Schulz and colleagues, who also discuss the use of NAC and other drugs as potential therapeutic approaches. Few randomized placebo-controlled trials have been conducted in this area (table 1i). One, testing NAC treatment in Alzheimer's disease, reported significant benefits for some outcome measures and favorable trends for others[60]. Another, testing NAC treatment in amyotrophic lateral sclerosis, found no significant benefit[242].

NAC treatment was also shown to be beneficial for frail geriatric patients responding to exercise in that it significantly enhanced knee extensor strength and increased the sum of all strength parameters.

Otolaryngologic and ophthalmologic disease.

Otic disease. Data from a randomized placebo-controlled trial demonstrate that NAC improves response to treatment in otitis media with effusion (OME), a sustained non-specific inflammation of the middle ear mucosa accompanied by secretory transformation of the epithelium and accumulation of fluid in the middle ear space[53] (see table 1j). Instillation of NAC in the middle ear in children who were undergoing their first bilateral insertion of ventilation tubes (VT) due to OME, followed by NAC

instillation at day 3 and 7 after VT insertion, significantly decreased OME recurrence, increased the time until VT extrusion ($p < 0.0167$) and decreased VT re-insertions ($p < 0.025$). In addition, the number of episodes of ear problems and visits at the ENT clinic were significantly decreased in the NAC-treated children ($p < 0.0383$).

5 Pre-clinical studies also point to the importance of oxidative stress and GSH depletion in the genesis of noise and toxin-induced hearing loss[243, 244]. Medications with inner ear toxicity such as aminoglycoside antibiotics and the chemotherapy agent cisplatin have been shown to damage the cochlea through the generation of oxygen free radicals. Hearing loss and cochlear damage associated with administration of these
10 compounds has been shown, in animal models, to be greatly reduced by administration of both NAC and methionine[245-247]. Similarly, studies with animal models show that permanent cochlear damage due to acute acoustic overexposure, which induces ischemia reperfusion, glutamate excitotoxicity, free radical generation and GSH depletion[243, 244, 246, 248], can be almost completely prevented by systemic administration of NAC
15 or methionine[246, 248]. Thus, the stage is now set for clinical trials using NAC or similar agents to prevent or reverse the acute cochlear injury associated with noise or a variety of ototoxins.

Ocular and oral disease. Beneficial effects have been demonstrated following NAC treatment in placebo-controlled studies in chronic blepharitis and in Sjogren's
20 syndrome, where improvements in ocular soreness, ocular irritability, halitosis and daytime thirst were noted. In addition, NAC treatment has been shown to significantly decrease dental plaque formation (table 1j).

Dermatologic disease (table 1k).

A placebo-controlled trial examining NAC treatment in progressive systemic sclerosis failed to find significant effects of NAC treatment.

Therapeutic dosing with NAC

NAC is the clinically accepted cysteine source used to treat GSH deficiency due to acetaminophen overdose. It can be administered by intravenous, enteral, and rectal routes. Oral NAC dosages for acetaminophen overdose start with a loading dose of 140 mg/kg body weight followed by doses of 70 mg/kg body weight administered every four hours over a period of three days[129]. Smaller dosages (600 mg – 8 g daily) have been administered for substantially longer periods in clinical trials for other conditions (table 1).

Although NAC has been administered orally at quite high dosages, little if any toxicity has been associated with NAC ingestion. The highest reported long-term NAC dosage (an average of 6.9g/day administered in 3-4 divided doses) was administered to 60 HIV-infected subjects for eight weeks during a placebo-controlled trial and to over 50 subjects for up to six months during the open-label continuation trial[49]. No adverse events requiring physician intervention were observed during either trial segment. During the placebo-controlled segment of the trial, 14/60 subjects reported gastric distress similar to that reported elsewhere[48, 49]. However, half of these subjects (7/14) were in the placebo arm, suggesting that the distress was related to ingestion of the excipient which may have contained significant amounts of lactose.

There are several reports of anaphylactoid and allergic responses in response to intravenous NAC administration[163, 249-256]. These adverse events may in part be explained by findings from preclinical studies demonstrating inflammatory-type responses in animals treated with the oxidized (dimeric) form of NAC[257] which can

contaminate NAC preparations that have not been protected against oxidation (see below). In any event, the anaphylactoid reactions to intravenous NAC are easily treated and do not interfere with completion of NAC administration[254].

Finally, caution may be indicated concerning the routine consumption of NAC and other sulfur amino acid precursors in the absence of diseases or conditions leading to cysteine/GSH deficiency, particularly in American and other populations in which the intake animal protein tends to be high and individuals may be ingesting 2-3 times the RDA for sulfur amino acids on a daily basis. We have shown that long-term administration of roughly 6g/day of NAC to HIV-infected individuals is safe[48, 49]. However, recent observational studies showing that colonic hydrogen sulfide production increases in proportion to consumption of animal protein raises questions[258] as to whether increasing sulfur amino acid intake might predispose toward ulcerative colitis. While the role of hydrogen sulfide in ulcerative colitis is still controversial[259-261], it is important to bear in mind that the long-term risks of routinely increasing sulfur amino acid intake in healthy individuals have not yet been evaluated. Current evidence suggests that 600-900 mg/day, the common daily dosage in Europe for cough and cold relief, may be a reasonable maximum dose for healthy individuals who wish to routinely take NAC.

NAC formulations

The best known NAC formulation in the US, MucomystTM (or the generic version thereof), is available as a 10% or 20% solution of NAC sodium salt that is typically administered orally for treatment of acetaminophen overdose. Since Mucomyst has a strong, disagreeable flavor, it is usually mixed with fruit juice or a soft drink before consumption. Still, as many physicians can attest, patients commonly find it very difficult to tolerate orally, thereby requiring administration via nasogastric tube.

Mucomyst is also administered intravenously in some settings, particularly when patients are unconscious or unable to retain the orally administered drug.

To overcome problems with oral administration, European manufacturers produce NAC in pill and capsule formulations. It is also produced and packaged in a variety of effervescent formulations (“fizzy tabs”) that can be dissolved in water, juice or carbonated drinks to create a pleasant tasting, readily tolerated beverage. These formulations are produced under European Good Manufacturing Practice (GMP) standards designed to minimize NAC oxidation to its dimeric form (“di-NAC”), which is pharmacologically active at very low concentrations with immunologic effects opposite to those of NAC[257]. In general, di-NAC constitutes less than 0.1% of the European GMP NAC formulations, which are intended for oral administration and have qualified for health insurance reimbursement[207].

Several US nutraceutical dealers manufacture and sell unbuffered (acidic) NAC. Since the production and packaging of nutraceutical products in the US is not regulated by the FDA, neither the content nor the purity of the NAC formulations currently produced and marketed in the US can be reliably judged. Manufacturing methods for these NAC preparations may not prevent formation of NAC by-products (e.g., di-NAC) and may not have been validated for stability during storage.

We (LAH, JPA, SCD) have recently suggested[262, 263] that NAC be administered, and perhaps co-formulated, with acetaminophen. Animal studies suggest that administration of roughly equimolar amounts of NAC and acetaminophen might be sufficient to accomplish this goal[264]. Co-administration of NAC with a GSH-depleting drug such as acetaminophen could decrease the toxicity of the drug, particular in settings where cysteine/GSH deficiency is likely. Thus, there is strong reason to believe that co-

administered NAC would more safely allow administration of acetaminophen or other GSH-depleting drugs when clinically warranted.

Conclusion: Cysteine/GSH deficiency - a new clinical entity

5 The evidence reviewed here reveals cysteine/GSH deficiency as an emerging clinical entity. The manifestations of this deficiency may vary in different disease settings, as may the biochemical mechanisms that mediate its effects. However, they are united by a common positive response to NAC therapy in over fifty randomized placebo-controlled trials (see table 1). Thus, the studies we have reviewed collectively argue for consideration of cysteine/GSH deficiency as a significant and treatable clinical entity.

10 Surprisingly, given the diverse roles that GSH plays in cellular physiology and regulation of enzyme activity and protein function, the consequences of low GSH levels have mainly been discussed from a clinical perspective in terms of the loss of protection against intracellular oxidative stress. However, while antioxidants such as vitamin E and vitamin C can spare GSH under conditions of oxidative stress, GSH loss can only be
15 offset by GSH resynthesis, indicating a central role for this molecule over and above its ability to counteract the effects of intracellular oxidants.

 Similarly, although NAC is a well-known source of cysteine for GSH replenishment in acetaminophen toxicity, it is principally cast as an antioxidant in other settings. By and large, physicians and the public in general tend to equate NAC with
20 vitamin C, vitamin E and other antioxidants. Indeed, like GSH, NAC can itself serve as an antioxidant. However, while other antioxidants can replace NAC and GSH in this role, only NAC or another cysteine source can provide the raw material necessary to replenish GSH and to enable GSH-dependent biochemical reactions.

We have pointed out that physicians may find NAC administration useful as adjunct therapy for diseases and conditions in which cysteine/GSH deficiency is likely to play a role. The positive findings in the placebo-controlled studies we have discussed support this argument. However, the absence of large multicenter trials testing NAC in various settings leaves this evidence still open to question. Hopefully, the recognition that cysteine/GSH deficiency is an important clinical entity will encourage support for such trials.

In the meantime, a conservative approach to the findings we have discussed suggests that patients with diseases or conditions in which cysteine/GSH deficiency has been demonstrated may be well advised to avoid unnecessary behaviors and exposures to medications that may exacerbate GSH depletion. In fact, when advising such patients, it seems reasonable for physicians to emphasize that alcohol usage be kept at modest levels and that acetaminophen usage should be kept strictly within the recommended dosing (or perhaps be accompanied by NAC administration).

The availability of over-the-counter NAC, and the low toxicity of this cysteine prodrug in situations where it has been tested, opens the possibility of patient or physician initiated therapy. However, if such therapy is elected, we suggest that the NAC preparation(s) used be prepared under GMP conditions and packaged to prevent oxidation of the product.

DETAILED DESCRIPTION OF THE INVENTION

The invention described herein provides a method of treatment to prevent development of glutathione deficiency as a consequence of disease, a treatment, or a condition comprising administering to a subject at risk of glutathione deficiency as a consequence of disease, a treatment or a condition a therapeutic amount of NAC or a

pharmaceutically acceptable salt or derivative sufficient to increase intracellular glutathione levels or decrease oxidative stress and measuring and monitoring the level of glutathione in blood in patients as needed. It further provides a method of treatment to restore glutathione levels comprising administering to subjects in need of glutathione level restoration, as determined by measurement or by a physician, a therapeutic amount of NAC or a pharmaceutically acceptable salt or derivative thereof sufficient to increase intracellular glutathione levels or decrease oxidative stress and monitoring restoration by measuring the level of glutathione in blood as needed. It further provides a method of treatment to decrease oxidized glutathione levels elevated as a consequence of disease, a treatment or a condition comprising administering to a subject suffering from oxidative stress a therapeutic amount of NAC or a pharmaceutically acceptable salt or derivative sufficient to decrease oxidized glutathione levels elevated as a consequence of disease and monitoring the level of oxidized glutathione in blood as needed.

The term "pharmaceutical composition" is used herein to denote a composition that may be administered to a mammalian host, *e.g.*, orally, topically, parenterally, by inhalation spray, or rectally, in unit dosage formulations containing conventional non-toxic carriers, diluents, adjuvants, vehicles and the like. The term "parenteral" as used herein, includes subcutaneous injections, intravenous, intramuscular, intracisternal injection, or by infusion techniques.

The term "therapeutically effective amount" is used herein to denote that amount of a drug or pharmaceutical agent that will elicit the therapeutic response of an animal or human that is being sought.

The pharmaceutical compositions containing NAC may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous, or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use may be prepared according to any known method, and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents, and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets may contain NAC

5 in admixture with non-toxic pharmaceutically-acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example corn starch or alginic acid; binding agents, for example, starch, gelatin or acacia; and lubricating agents, for example

10 magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the techniques described in U.S. Patent Nos. 4,356,108;

15 4,166,452; and 4,265,874, to form osmotic therapeutic tablets for controlled release.

Formulations for oral use may also be presented as hard gelatin capsules where NAC is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or a soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

20 Aqueous suspensions may contain the active compounds in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide

25 such as lecithin, or condensation products of an alkylene oxide with fatty acids, for

example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example, heptadecaethyl-eneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with
5 partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending NAC in a vegetable oil, for
10 example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as a liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

15 Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide NAC in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, sweetening, flavoring, and coloring agents may also
20 be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example a liquid paraffin, or a mixture thereof. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum
25 tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or

partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

5 Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectible aqueous or oleaginous suspension. This suspension may be formulated according to the known methods using suitable
10 dispersing or wetting agents and suspending agents described above. The sterile injectible preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils
15 are conveniently employed as solvent or suspending medium. For this purpose, any bland fixed oil may be employed using synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

 The compositions may also be in the form of suppositories for rectal administration of the compounds of the invention. These compositions can be prepared
20 by mixing NAC alone or in combination with a therapeutically effective amount of a therapeutic agent with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will thus melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols, for example.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compounds of the invention are contemplated. For the purpose of this application, topical applications shall include mouth washes and gargles.

The NAC alone or in combination with a therapeutically effective amount of a
5 therapeutic agent may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes may be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

Pharmaceutically-acceptable salts of NAC alone or in combination with a
10 therapeutically effective amount of a therapeutic agent, where a basic or acidic group is present in the structure, are also included within the scope of the invention. The term "pharmaceutically acceptable salts" refers to non-toxic salts of NAC alone or in combination with a therapeutically effective amount of a therapeutic agent, which are generally prepared by reacting the free base with a suitable organic or inorganic acid or
15 by reacting the acid with a suitable organic or inorganic base. Representative salts include the following salts: Acetate, Benzenesulfonate, Benzoate, Bicarbonate, Bisulfate, Bitartrate, Borate, Bromide, Calcium Edetate, Camsylate, Carbonate, Chloride, Clavulanate, Citrate, Dihydrochloride, Edetate, Edisylate, Estolate, Esylate, Fumarate, Gluceptate, Gluconate, Glutamate, Glycolylarsanilate, Hexylresorcinate, Hydrabamine,
20 Hydrobromide, Hydrochloride, Hydroxynaphthoate, Iodide, Isethionate, Lactate, Lactobionate, Laurate, Malate, Maleate, Mandelate, Methanesulfonate, Methylbromide, Methylnitrate, Methylsulfate, Monopotassium Maleate, Mucate, Napsylate, Nitrate, N-methylglucamine, Oxalate, Pamoate (Embonate), Palmitate, Pantothenate, Phosphate/diphosphate, Polygalacturonate, Potassium, Salicylate, Sodium, Stearate,
25 Subacetate, Succinate, Tannate, Tartrate, Teoclate, Tosylate, Triethiodide,

Trimethylammonium and Valerate. When an acidic substituent is present, such as—
COOH, there can be formed the ammonium, morpholinium, sodium, potassium, barium,
calcium salt, and the like, for use as the dosage form. When a basic group is present, such
as amino or a basic heteroaryl radical, such as pyridyl, an acidic salt, such as

5 hydrochloride, hydrobromide, phosphate, sulfate, trifluoroacetate, trichloroacetate,
acetate, oxlate, maleate, pyruvate, malonate, succinate, citrate, tartarate, fumarate,
mandelate, benzoate, cinnamate, methanesulfonate, ethanesulfonate, picrate and the like,
and include acids related to the pharmaceutically-acceptable salts listed in the Journal of
Pharmaceutical Science, 66, 2 (1977) p. 1-19.

10 Other salts which are not pharmaceutically acceptable may be useful in the
preparation of NAC alone or in combination with a therapeutically effective amount of a
therapeutic agent and these form a further aspect of the invention.

Thus, in another aspect of the present invention, there is provided a
pharmaceutical composition comprising NAC alone or in combination with a
15 therapeutically effective amount of a therapeutic agent, or a pharmaceutically acceptable
salt, solvate, or prodrug thereof, and one or more pharmaceutically acceptable carriers,
excipients, or diluents.

In another aspect of the present invention, there is provided a pharmaceutical
composition comprising a therapeutically effective amount of NAC alone or in
20 combination with a therapeutically effective amount of a therapeutic agent, or a
pharmaceutically acceptable salt, solvate, or prodrug thereof, and one or more
pharmaceutically acceptable carriers, excipients, or diluents, wherein said therapeutically
effective amount comprises a sufficient amount NAC for at least partial amelioration of
cysteine/glutathione deficiency in a disease or condition. In an embodiment of the
25 pharmaceutical composition, the disease comprises chronic obstructive pulmonary

disease (COPD). In another embodiment of the pharmaceutical composition, the disease comprises acute renal failure. In another embodiment of the pharmaceutical composition, the disease comprises sickle cell anemia. In another embodiment of the pharmaceutical composition, the disease comprises diabetes mellitus. In
5 another embodiment of the pharmaceutical composition, the disease comprises inflammatory diseases. In another embodiment of the pharmaceutical composition, the disease comprises human immunodeficiency virus mediated disease. In another embodiment of the pharmaceutical composition, the disease comprises malaria. In another embodiment of the pharmaceutical composition, the disease comprises protein-
10 energy malnutrition. In another embodiment of the pharmaceutical composition, the disease comprises otic disease. In another embodiment of the pharmaceutical composition, the disease comprises neurodegenerative disease. In another embodiment of the pharmaceutical composition, the disease comprises cardiovascular disease.

In another aspect, the present invention provides a pharmaceutical composition
15 comprising a therapeutically effective amount of NAC alone or in combination with a therapeutically effective amount of a therapeutic agent, and one or more pharmaceutically acceptable carriers, excipients, or diluents, wherein the pharmaceutical composition is used to replace or supplement compounds that restore cysteine/glutathione levels.

In another aspect, the present invention provides a method for
20 improvement of cysteine/glutathione deficiency in a disease or condition comprising administering to a subject in need thereof a NAC alone or in combination with a therapeutically effective amount of a therapeutic agent, wherein NAC alone or in combination with a therapeutically effective amount of a therapeutic agent is administered to the subject as a pharmaceutical composition comprising a therapeutically
25 effective amount of NAC and one or more pharmaceutically acceptable carriers,

excipients, or diluents, wherein said therapeutically effective amount of NAC comprises a sufficient amount of NAC for treatment or prevention of cysteine-glutathione deficiency in the disease or condition.

5 The term "treatment" as used herein, refers to the full spectrum of treatments for a given disorder from which the patient is suffering, including alleviation of one, most of all symptoms resulting from that disorder, to an outright cure for the particular disorder or prevention of the onset of the disorder.

Generally speaking, the amount of NAC per dosage unit is preferably from 1 mg to 25000 mg, preferably at least 3 mg to 2000 mg per dosage unit for oral administration, 10 and 20-20000 mg for parenteral administration. The unit dose of NAC alone or in combination with a therapeutically effective amount of a therapeutic agent, will usually comprise at least about 1.5 mg/kg to a maximum amount of 70 mg/kg (for pediatric doses), usually at least about 500 mg (for adult doses), and usually not more than about 2000 mg at the physician's discretion. Patients on therapy known to deplete 15 cysteine/glutathione or produce oxidative stress may benefit from higher amounts of NAC. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage will vary depending upon the host treated and the particular mode of administration.

This dosage has to be individualized by the clinician based on the specific clinical 20 condition of the subject being treated. Thus, it will be understood that the specific dosage level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

While the invention has been described and illustrated with reference to certain preferred embodiments thereof, those skilled in the art will appreciate that various changes, modifications and substitutions can be made therein without departing from the spirit and scope of the invention. For example, effective dosages other than the preferred dosages
5 as set forth herein may be applicable as a consequence of variations in the responsiveness of the mammal being treated for cysteine/glutathione deficiency in a disease or condition. Likewise, the specific pharmacological responses observed may vary according to and depending on the particular active compound selected or whether there are present pharmaceutical carriers, as well as the type of formulation and mode of administration
10 employed, and such expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention.

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Table 1

NAC treatment in randomized placebo-controlled studies

- Tables entries all refer to randomized placebo-controlled studies in which NAC, or in some cases another GSH-replenishing drug, were administered. Dosage shown is for NAC unless otherwise indicated. The treatment effect is scored as “Beneficial” when reported outcome(s) differs significantly from the placebo group ($p < 0.05$) for a clinical parameter of importance to patient well-being in the disease under study. If a significant difference between the NAC and placebo group was observed by the clinical relevance of the finding is unclear, the treatment effect is scored as “Significant (? Clin rel)”. Failure to find a significant difference is scored as “Not significant” while significant negative effects of NAC treatment are scored as “Adverse”.

Table 1a: Hepatic and GI pathophysiology

NAC use in hepatic and GI pathophysiology	n	Study length	Dosage	Treatment effect
Acetaminophen Toxicity* . IV NAC administration to subjects with acetaminophen-induced fulminant hepatic failure who had not previously received NAC (late presentation) increased survival and decreased both the incidence of cerebral edema and the frequency of hypotension requiring inotropic support. Rates of deterioration and recovery of liver function were not affected. Keays, 1991[2]	50	21 days	150 mg/kg for 15min; 50mg/kg for 4h; 100mg/kg over 16h.	Beneficial
Hepatic transplant patients. NAC induces mild vasodilation, improves oxygen delivery and consumption, and reduces base deficit. Bromley 1995[19]	50	5 hours	150mg/kg for 15min; 12.5mg/kg for 4h.	Beneficial

* Note: Although NAC is the accepted treatment for acetaminophen overdose, there do not appear to be any placebo-controlled trials that supported its initial acceptance. A series of studies that are not placebo-controlled, e.g., Smilkstein et al¹²⁷, have served this purpose (see the citations for acetaminophen under Toxic Agents in Table 2).

NAC use in hepatic and GI pathophysiology	n	Study length	Dosage	Treatment effect
Hepatitis C. Treatment with NAC in addition to conventional therapy with interferon--□ did not result in significant improvement of the treatment group. Grant 2000 [265]	147	1 year	1800mg NAC daily with Interferon-□	not significant
Colon cancer. NAC lowered the proliferative index in the colonic crypt epithelium of human volunteers who previously had adenomatous polyps. Estensen, 1999 [55]	64	12 weeks	800mg NAC daily, po	Beneficial
NAC safety. Gastroduodenoscopic examination following oral NAC administration disclosed no lesions. Histological examination of biopsy specimens from the antrum and corpus of the stomach disclosed no pathological changes. Marini, 1980 [62]	20	7 days	200mg 3X daily	Safe (no effect)
Hyperoxic ventilation. NAC preserved whole body oxygen uptake, oxygen extraction ratio, and gastric intramucosal pH during brief hyperoxic ventilation. Reinhart, 1995 [266]	38	72 hours	150ml/kg IV over a period of 15 min	Beneficial
Gastric mucosa. The systemic effect of NAC on gastric mucus was not powerful enough to improve the barium coating of the gastric mucosa. Kinnunen, 1989 [267]	100	3 days prior to treatment	600mg daily	not significant
Helicobacter Pylori. NAC decreased the sensitivity and specificity of the Helicobacter pylori stool antigen test. Demiturk, 2003 [268]	107	5 ml of 4% NAC TID for 3 days	107	Interferes with traditional clinical assay

NAC use in hepatic and GI pathophysiology	n	Study length	Dosage	Treatment effect
Protein-energy malnutrition. Malnourished children admitted for treatment of infection were treated with NAC or placebo (alanine). NAC restored GSH synthesis rate and concentration when administered during the early phase of treatment Badaloo, 2002[54]	32	50 days	0.5 mmol per kg per day	Beneficial

Table 1b: NAC use in cardiovascular pathophysiology

	n	Study length	Dosage	Beneficial effects
Hyperoxic ventilation in cardiac risk patients. NAC helped preserve whole-body oxygen consumption (VO ₂), oxygen delivery, cardiac index, left ventricular stroke work index and venoarterial carbon dioxide gradient during brief hyperoxia. Clinical signs of myocardial ischemia such as ST-depression did not occur if patients were prophylactically treated with NAC. Spies, 1996[11]	30	30 min	150mg/kg, IV	Beneficial
Stable angina and nitrate tolerance. None of the patients had developed nitrate tolerance at inclusion. NAC in combination with the long-acting nitrate, isosorbide-5-mononitrate (5-ISMN) significantly prolonged the total exercise time as compared with placebo/5-ISMN. No further effect was obtained after additional NTG doses. Svendsen, 1989[12]	10	24 hours	4800mg, po	Beneficial
Stable angina and nitrate tolerance. The reduction in ST segment depression was significantly more pronounced during isosorbide dinitrate (ISDN) + NAC as compared with ISDN + placebo. Exercise time and time to angina pectoris were unaffected. NAC augmented the antischemic effects of ISDN as assessed by ECG. Boesgaard, 1991[13]	7	8 days	2400mg daily, po	Beneficial
Stable angina and nitrate tolerance. Infusion of high doses of NAC in combination with isosorbide dinitrate (ISDN) affects and partially prevents the development of tolerance to antianginal effects normally observed during infusion with ISDN. Boesgaard, 1992[14]	10	30 hours	2g IV bolus followed by 5g/kg/hr IV	Beneficial

	n	Study length	Dosage	Beneficial effects
Unstable angina. Combined administration of nitroglycerin (NTG) and NAC in patients with unstable angina pectoris augments the clinical efficacy of NTG, largely by reducing the incidence of acute myocardial infarction. However, the high incidence of severe hypotension with NTG/NAC suggests that this regimen should be used with some caution. Horowitz, 1988 [175, 176]	46	6 hours	5g, IV	Beneficial
Unstable angina. The combination of nitroglycerin and NAC, associated with conventional medical therapy reduces the occurrence of outcome events. The high incidence of side effects (mainly intolerable headaches) limits the clinical applicability of this therapeutic strategy at least at the dosage used in the present study. Ardissino, 1997 [177]	200	4 months	600mg, po	Beneficial
Unstable angina and nitrate tolerance. Blood pressure and the response to the glyceryl trinitrate bolus we note significantly affected by the co-administration of intravenous NAC with intravenous isosorbide trinitrate (ISDN). Weber, 1992 [269]	19	72 hours	10g q 24h, IV	not significant
Atherosclerosis. Oral L-2-oxothiazolidine-4-carboxylic acid (OTC) improves brachial artery flow-mediated dilation and endothelium-derived nitric oxide action in human atherosclerosis. Vita, 1998 [15]	48	4 hours	4.5g OTC, po	Beneficial
Cardiac bypass. The oxidative burst response of peripheral neutrophils in the patients receiving NAC was significantly lower at all times during bypass. Andersen 1995 [16]	12	24 hours	100mg/kg bolus; then of 20 mg/kg continuous IV	Beneficial

	n	Study length	Dosage	Beneficial effects
Cardiac Bypass. Data shows that cardioplegic arrest initiates the apoptosis signal cascade in human left ventricular cardiac myocytes. This apoptosis induction can effectively be prevented by NAC. Fischer, 2004. [270]	36	36 hours ?	100mg/kg followed by infusion 20mg/kg.	NEED Paper
Myocardial ischemia. 24-hour intravenous infusion of GSH in addition to thrombolytic therapy significantly decreased plasma MDA, an oxidative stress indicator that is significantly elevated by thrombolytic treatment for acute myocardial infarction. Altomare, 1996[179]	96	24 hours	GSH, 1800mg IV bolus; 20mg/kg per min for 24h.	Beneficial

Table 1c: NAC use in renal pathophysiology

NAC use in renal pathophysiology	n	Study length	Dosage	Beneficial effects
Contrast-induced nephropathy (CIN). Prophylactic oral administration of NAC along with hydration in patients with chronic renal insufficiency prevents the reduction in renal function induced by iopromide, a nonionic low-osmolality contrast agent (relative risk, RR 0.10) Tepel, 2000, 2001 and 2003[3-5]	83	2 days	600mg PO bid	Beneficial
CIN. NAC protects patients with moderate chronic renal insufficiency from contrast-induced deterioration in renal function after coronary angiographic procedures, with minimal adverse effects (RR 0.32). Kay, 2003[6]	200	3 days	600mg PO bid	Beneficial
CIN. NAC decreased the incidence of post-catheterization nephropathy (RR 0.21) in patients with stable chronic renal insufficiency undergoing elective cardiac catheterization. Diaz-Sandoval, 2002[7]	54	2 days	600mg PO bid	Beneficial
CIN. NAC decreased the incidence of contrast-induced nephropathy (RR 0.13) in patients with stable chronic renal insufficiency undergoing coronary angiography. Shyu, 2002[8]	121	7 days	400mg PO bid for 2 days	Beneficial
CIN. In patients with serum creatinine > 1.2 mg/dL (106 μ mol/L) undergoing elective coronary angiography, NAC was associated with preservation of creatinine clearance at 24 and 96 hours, which decreased significantly in placebo-treated patients (21% at 24 hours and 18% at 96 hours, both $P < 0.005$). Efarti 2003. [9]	49	4 days	1000mg daily	Beneficial
CIN. NAC treatment prevented the development of CIN by 72 hours in patients with moderate renal insufficiency (serum creatinine ≥ 1.5 mg/dL) undergoing elective cardiac catheterization (4.8% vs 32.2%, $P = 0.046$). MacNeil 2003. [10]	43	3 days	600 mg 5 doses	Beneficial

NAC use in renal pathophysiology	n	Study length	Dosage	Beneficial effects
CIN: NAC is strongly associated with suppression of oxidative stress-mediated proximal tubular injury. Drager, 2004. [271]	24	4 days	1200mg daily	Beneficial
CIN. NAC failed to prevent CIN at 24 hours following coronary angiography in the group as a whole. The overall incidence was low (4.8%). Kefer et al. [189]	108	1 day	1200 mg	not significant
CIN. NAC failed to prevent CIN by 48 hours in patients with moderate chronic renal insufficiency (serum creatinine > 1.2 mg/dL and creatinine clearance < 50 mL/min) undergoing elective coronary angiography. The incidence of CIN in both groups was low (NAC 8.2%, control 6.4%). Oldemeyer 2003 [188]	96	2 days	1500 mg for 4 doses	not significant
CIN. In patients with reduced renal function undergoing angiography and/or angioplasty, the amount of contrast agent, but not the administration of prophylactic NAC, was a predictor of renal function deterioration. Brigouri, 2000 [190]	183	2 days	600mg PO bid	not significant
CIN. NAC failed to protect against radiocontrast-induced nephropathy in patients with baseline renal dysfunction (serum creatinine > 1.6 mg/dL or creatinine clearance < 60 mL/min) undergoing "cardiovascular interventions" using low-osmolality nonionic contrast media. Allaqaband et al, 2002 [186]	123	2 days	600mg PO bid	not significant
CIN. NAC failed to protect against radiocontrast-induced nephropathy in patients with baseline renal dysfunction (serum creatinine > 1.7 mg/dL) undergoing cardiac catheterization using low-osmolality nonionic contrast media. Durham, 2002 [185]	79	8	1200 mg 1 hr prior and 3 hrs after procedure	not significant

NAC use in renal pathophysiology	n	Study length	Dosage	Beneficial effects
CIN. NAC administration had no influence on the incidence of CIN at 48 hours (NAC, 13%; control 12%) after elective cardiac catheterization in patients with a serum creatinine > 1.2 mg/dL or a creatinine clearance < 50 mL/min. Boccalandro 2003. [187]	179	2 days	600mg 4 times daily	not significant
CIN. NAC administration had no significant effect vs. placebo. Goldenberg 2004. [272]	80	1 day	600mg PO tid	not significant
Hemodialysis. Acetylcysteine-dependant increase of homocysteine removal during a hemodialysis session improves plasma homocysteine concentration, pulse pressure and endothelial function in patients with end-stage renal failure. Scholze, 2004. [273]	20		5g in 5% glucose and 150mg/kg for nephroph y.	Beneficial
Hemodialysis. Treatment of patients with NAC reduces composite cardiovascular end points. Median treatment time was 14.5 months. Tepel 2003 [17]	134	1-24 months	600mg bid	Beneficial

Table 1d: NAC use in endocrine pathophysiology

NAC use in endocrine pathophysiology	n	Study length	Dosage	Beneficial effects
Polycystic Ovary Syndrome. NAC decreases circulating insulin levels and decreases insulin sensitivity in patients with polycystic ovary syndrome. Fulghesu 2002[20]	33	6 weeks	1.8 – 3g daily depending on weight	Beneficial
Diabetes. Oral NAC treatment increased erythrocyte GSH concentrations and GSH:GSSG ratio. NAC also decreased plasma soluble vascular cell adhesion molecule (VCAM-1), a measure of vascular damage in non-insulin dependent diabetes. De Mattia, 1998[21]	15	1 month M	1200 mg/day, po	Beneficial
Diabetes. IV administration of GSH increased both intra-erythrocytic GSH/GSSG ratio and total glucose uptake in non-insulin dependent diabetic (NIDDM) patients. Significant correlations were found between GSH/GSSG ratio. De Mattia, 1998[22]	20	2 hours	GSH, 1.35 gm ² min ⁻¹ IV	Beneficial

Table 1e NAC use in metabolic and genetic pathophysiology

NAC use in metabolic and genetic pathophysiology	n	Study length	Dosage	Beneficial effects
Sickle Cell Disease/Vaso-occlusive disease. NAC inhibited dense cell formation, restored glutathione levels toward normal, and decreased vaso-occlusive episodes (0.03 to 0.006 episodes per person-days). Pace 2003[18]	21	12 Months	2,400mg daily	Beneficial
Cystic Fibrosis (CF) and primary ciliary dyskinesia (PCD). No effect was seen in PCD patients. However, but lung function improved in NAC-treated CF patients in the period when the patients suffer most from lower airway infections. Stafanger,1988[40]	54	3 months	600mg <30kg or 800mg >30kg perday, po	Beneficial
CF. NAC treatment of patients with chronic pulmonary Pseudomonas aeruginosa infection did not significantly improve lung function in the overall treatment group. In patients with peak expiratory flow rate (PEFR) below 70% of predicted normal values, NAC significantly increased PEFR, forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) during the treatment period. Stafanger, 1989[41]	52	3 months	600mg <30kg or 800mg >30kg per day, po	Beneficial to a subgroup
CF. NAC and Ambroxol improved air trapping and FEV1. No other clinical differences were observed between the three groups. Ratjen, 1985[274]	36	12 weeks	NAC or Ambroxol 200mg 3 times daily	Beneficial
CF. Although the forced expiratory volume in one second (FEV1) improved in a number of NAC-trteated patients, the mean increase was within the range of the intra-individual variability. Gotz, 1980[275]	21	14 days ^M	9.5mg/kg/day po (average)	not significant

NAC use in metabolic and genetic pathophysiology	n	Study length	Dosage	Beneficial effects
Homocysteine in hemodialysis. NAC significantly increased the removal of homocysteine by hemodialysis. Plasma homocysteine levels decreased to 12+/-7% (mean+/-SD) of predialysis level in the treatment group compared with 58+/-22% in the control group (P<0.01). The reduction of plasma homocysteine concentration was significantly correlated with a reduction of pulse pressure and improved in endothelial function. Scholze 2004[273]	20	1 session	Intravenous 5g in 5% glucose solution	Beneficial
Homocysteine in hemodialysis. Plasma homocysteine levels decreased with chronic oral NAC therapy (-19%) but did not significantly differ from placebo-treated subjects (-8%, P = 0.07). Friedman, 2003 [276]	38	4 weeks	1200mg BID	not significant
High plasma homocysteine. In a double blind cross over trial in patients with high plasma Lp(a) (> 0.3 g/l), NAC treatment had no effect on plasma lipoprotein(a) levels but significantly reduced plasma homocysteine. Wiklund, 1996[277]	11	14 days	4000 mg daily, po	Beneficial

Table 1f NAC use in Pulmonary pathophysiology

NAC use in Pulmonary pathophysiology	n	Study length	Dosage	Beneficial effects
Asthma. NAC medication had no effect on any spirometric, lung mechanic or gas exchange variable, nor on the frequency of pulmonary symptoms. Bylin 1987[23]	25	9 weeks	600 mg	Not significant
Bronchitis. The frequency of exacerbations was significantly lower in the NAC-treated group. Grassi and Morandini, 1976[24]	69	6 months	600mg/day, 3days/wk, po	Beneficial
Bronchitis. Oral NAC changes the consistency of sputum with resultant ease of expectoration and the expectoration of increased volumes of sputum; peak expiratory flow rate was increased. Aylward, 1980[25]	29	4 weeks	600mg/day, po	Beneficial
Bronchitis. NAC was effective in decreasing sputum volume and viscosity in patients with acute and chronic bronchitis. Brocard, 1980[26]	215	10 days	600mg/day, po	Beneficial
Bronchitis. Oral NAC was associated with decreased thickness of sputum, improved ease of expectoration, and decreased incidence of exacerbations. Grassi, 1980, Ferrari, 1980[27, 28]	744	6 months	400mg/day, po	Beneficial
Bronchitis. The exacerbation rate was significantly lower in the NAC-treated group. Boman, 1983[29]	259	6 months	400mg/day, po	Beneficial
Bronchitis. Although improvement in subjective symptoms (sputum viscosity and character, difficulty in expectoration and cough severity) occurred in both treatment groups, improvements in difficulty in expectoration and cough severity were greater in patients receiving NAC. Jackson, 1984[30]	155	3 months	600mg/day, po	Beneficial

NAC use in Pulmonary pathophysiology	n	Study length	Dosage	Beneficial effects
Bronchitis. The number of days on which subjects in the NAC group were incapacitated was significantly decreased. There was no statistically significant difference in the number of exacerbations between the NAC and placebo groups although there was a slight trend towards improvement in the NAC group during the first 3 months. Parr and Huitson, 1987[31]	526	6 months	600mg/day, po	Beneficial
Bronchitis. NAC-treated group had reduced number of sick-leave days caused by exacerbations of chronic bronchitis. Rasmussen and Glennow, 1988[32]	116	6 months	600mg/day, po	Beneficial
Bronchitis. Pre-treatment with NAC resulted in a significantly greater increase in evening peak flows. Evald, 1989[33]	37	22 weeks	1200 mg/day, po	Beneficial
Bronchitis. NAC was significantly superior to placebo in terms of a favourable effect on General Health Questionnaire score, which measures general well-being in patients with mild chronic bronchitis.. Hansen, 1994[34]	153	22 weeks	1200 mg/day, po	Beneficial
Bronchitis. No significant result was found with respect to the frequency of coughs, dyspnea, sleep disorder, expectorate, morning and afternoon PEF, and PEF, FEV. Heinig, 1985[200]	59	2 years	600mg daily	not significant
Bronchitis. There was a trend toward significantly reduced number of exacerbations in the NAC-treated group but differences did not reach conventional levels of statistical significance. McGavin, 1985[159]	181	5 months	600mg/day, po	not significant
Bronchitis. No significant differences were found in lung function, mucociliary clearance curves or sputum viscosity following treatment with NAC compared to control or placebo measurements. Millar, 1985[201]	9	4 weeks	600mg/day, po	not significant

NAC use in Pulmonary pathophysiology	n	Study length	Dosage	Beneficial effects
Bronchitis. Differences between the NAC and placebo groups did not reach conventional levels of statistical significance for the number of exacerbations, total days taking an antibiotic; total days spent in bed, number of withdrawals, incidence of side effects, drug compliance, and the patients' assessment of the treatment. McGavin, 1985[202]	161	5 months	600mg/day, po	not significant
Bronchitis. NAC delivered by metered dose inhalers did not have any significant effect on patients' feeling of well-being, sensation of dyspnea, intensity of coughing, mucus production, or expectoration or lung function. NAC effect on exacerbations could not be estimated because the number of reported exacerbations was too low. Dueholm, 1992[204]	65	16 weeks	4mg twice daily, inhaled	not significant
COPD. Long-term oral administration of NAC attenuates hydrogen peroxide formation in the airways of COPD subjects. Kasielski, 2001[210]	44	12 months	600mg fizzy	Beneficial
COPD. The NAC group had significantly improved mucus viscosity, effective cough and clinical symptoms. Verstraeten, 1979[39]	60	10 days	600mg	Beneficial
COPD. The major findings of this study was that short-term, High-dose NAC treatment improved quadriceps endurance, reduced the disturbance in the prooxidant system and prevented exercise induced oxidative stress. Koechlin, 2004. [278]	9	15	1800mg for 4 days and 600mg the day of the test	Beneficial
Smoking. Improved mucus clearance in subjects on NAC when compared to placebo. Olivieri, 1985[36]	12	30 days	600mg/day	Beneficial

NAC use in Pulmonary pathophysiology	n	Study length	Dosage	Beneficial effects
Smoking. Observed an inhibitory effect of NAC toward the formation of lipophilic-DNA adducts, which can modulate certain cancer-associated biomarkers. VanShooten 2002[35]	41	6 months	1200mg/day, po	Beneficial
Bronchopulmonary dysplasia. Intratracheal NAC administration neither improved clinical condition nor hastened recovery in premature infants with chronic lung disease. Importantly, NAC administration in this form led to increased total airway resistance and cyanotic spells. Bibi, 1992[203]	22	24 hours	5% by tracheal administration	Adverse
Bronchopulmonary dysplasia (BPD) NAC did not prevent BPD or death in infants with extremely low birthweight. Ahola, 2003. [279]	391	6 Days	16 – 32 mg/kg/d	not significant
Diaphragm dysfunction. Preadministration of IV NAC attenuates the development of diaphragm fatigue in normal subjects breathing against high inspiratory loads. (Relevant to treatment of patients with ventilatory failure secondary to respiratory muscle fatigue.) Travaline, 1997[37]	4	3 weeks	150 mg/kg, IV	Beneficial
Mucociliary clearance rates. NAC has a 35% improvement in mucosalary clearance in subjects classified as slow clearers and protects against lung aggressors. Todisco, 1985[38]	161	60 days	.6g oral daily	Beneficial
Elective pulmonary surgery. No benefit was demonstrated either for postoperative pulmonary function or in the frequency of atelectasis. Jepsen, 1989[280]	38	5 days	600mg/day, po	not significant

NAC use in Pulmonary pathophysiology	n	Study length	Dosage	Beneficial effects
Elective upper laparotomy. In patients subjected to elective upper laparotomy given NAC prophylactically, no significant differences with respect to postoperative pulmonary function or in the frequency of atelectasis were detected between the NAC and placebo groups. Jepsen, 1989[280]	131	6 days	1200mg pre-surgery, po; 600 mg/day post-surgery, po	not significant
ARDS. Parenteral NAC treatment started within 8 h of diagnosis increases the intracellular GSH in the granulocytes of ARDS patients without decreasing spontaneous oxidant production by these cells. Laurent, 1996[215]	16	3 days	190mg/kg/day, IV	Significant (? Clin rel)
ARDS. No improvement could be demonstrated in the PaO ₂ /FiO ₂ ratio in the study group as compared with the control group on any day. Findings indicate that NAC acts as an anticoagulant and perhaps decreases pulmonary fibrin uptake during ARDS. Jepsen, 1992[223]	66	6 days	150mg/kg bolus; then 20mg/kg/hr, IV	not significant
ALI. Mechanical ventilatory support. IV NAC treatment improved systemic oxygenation and reduced the need for ventilatory support in patients presenting with mild-to-moderate acute lung injury. Development of ARDS and mortality were not significantly reduced. Suter, 1994[211]	61	3 days	40 mg/kg/day, IV	Beneficial
ALI. Pretreatment with NAC prevents lung injury by diminishing elastase activity in ALI. The release of mediators, especially myeloperoxidase, is not affected. De Backer, 1996[214]	18	12 hours	72mg/kg bolus, 72mg/kg over 12 hours, IV	Beneficial
ALI. NAC and OTZ both repleted RBC GSH. The number of days of acute lung injury was decreased and there was also a significant increase in cardiac index in both treatment groups. There was no difference in mortality among groups. Bernard, 1997[222]	48	10 days	NAC, 70mg/kg q8hr, IV; OTZ, 63mg/kg q8hr, IV	Beneficial

Table 1g NAC use in septic shock and infectious disease

NAC use in septic shock and infectious disease	n	Study length	Dosage	Beneficial effects
Septic shock. NAC provided a transient improvement in tissue oxygenation in about half of the septic shock patients, as indicated by the increase in V02 and gastric intramucosal pH and decrease in veno-arterial PC ⁰² . The NAC responders had a higher survival rate. Spies, 1994[42]	58	2 hrs	150 mg/kg for 15 min, then 12.5 mg/hr over 90 min, IV	Beneficial
Septic shock. Short-term IV infusion of NAC was well-tolerated, improved respiratory function and shortened ICU stay in survivors. Production of IL-8, a potential mediator of septic lung injury, was attenuated. Spapen, 1998[43]	22	24 hours	150 mg/kg bolus; then 50 mg/kg over 4 hr, IV	Beneficial
Septic shock. After NAC treatment, hepatosplanchnic flow and function improved. The increase of liver blood flow index was not caused by redistribution to the hepatosplanchnic area, but by an increase of cardiac index. Rank, 2000[44]	60	2 hours	150mg/kg over 15 min before 12.5mg/kg/hr for 90 min	Beneficial
Cardiac performance in septic shock. Early administration of NAC (within the first 2 hours of hemodynamic stabilization) resulted in a progressive decrease in MAP, cardiac index, and left ventricular stroke work index relative both to subject time-zero measurement and to values for the placebo group ($p < .01$, repeated-measures analysis of variance). Peake, 1996[281]	20	48 hours	150 mg/kg over 15min; 50 mg/kg over next 4hrs; 100 mg/kg over next 24hrs	Adverse
Influenza. Long term treatment with oral NAC during the cold-season appears to significantly attenuate the frequency and severity of influenza or influenza-like episodes in elderly subjects and/or patients suffering from chronic non-respiratory diseases. De Flora, 1997[46]	262	6 months	1200mg/day, po	Beneficial

NAC use in septic shock and infectious disease	n	Study length	Dosage	Beneficial effects
HIV. In subjects with CD4 counts >200/ul, NAC treatment resulted in normalization of plasma cysteine levels and a lower rate of decline in the CD4 count. Akerlund, 1996 [47]	45	4 months	800 mg/day, po	Beneficial
HIV. Placebo-controlled study established the long-term safety of NAC administration and demonstrated that NAC treatment replenishes whole blood GSH and T cell GSH in HIV infected individuals. Viral load was not affected. Replenishment was associated with increased survival in the open-label portion of the study. De Rosa, 2000 and Herzenberg, 1997 [48, 49]	81	8 weeks	3,200 – 8,000 mg/day, po	Beneficial
HIV. NAC treatment but resulted in a marked improvement in immune function. It did not consistently alter viral load. Subjects with and without antiretroviral therapy (n=40, n=29, respectively) were included. Breitkreutz, 2000 [50] (Also reviewed in Droge 2000[282])	69	7 months	0.6 - 3.6g depending on GSH levels, po	Beneficial
HIV. A significant increase in whole blood GSH was seen after oral administration of L-2-oxothiazolidine-4-carboxylic acid (OTC), a cysteine pro-drug, in the 1,500mg and the 3,000mg dose groups. Barditch-Crovo, 1998 [283]	37	4 weeks	OTC, 1500-3000mg/day, po	Beneficial
HIV. Individuals at the beginning of antiretroviral therapy were supplemented with NAC or placebo. In NAC-treated patients, hematocrit remained stable and an increase in CD4 cell count took place earlier than that in the control group. Spada, 2002 [227]	20	180 days	600mg/day	Beneficial

NAC use in septic shock and infectious disease	n	Study length	Dosage	Beneficial effects
HIV. In subjects with CD4 counts <200/ul, NAC did not replenish either cysteine or GSH levels in plasma. NAC did not significantly decrease the risk of adverse reactions to trimethoprim-sulphamethoxazole (TMP-SMX) in this study. Akerlund, 1997 [284]	50	10 weeks	800 mg/day po	Not significant
Malaria. In severe encephalopathy secondary to quinine-treated malaria, serum lactate levels normalized twice as quickly after NAC as after placebo. The NAC-treated patients could be switched from intravenous to oral therapy earlier than individuals who received placebo, but the difference was not significant. Watt, 2002 [51]	30	20 hours	300mg/kg, IV	Beneficial

Table 1h NAC use in multiorgan failure

NAC use in multiorgan failure	n	Study length	Dosage	Beneficial effects
Hemodynamic effects. NAC treatment increased cardiovascular index and vasodilation but did not affect oxygen extraction or lactate concentration. Agusti, 1997. [45]	10	45 min	150 mg/kg	Significant (? Clin rel)
Intensive care. There was no statistically significant difference between the two groups regarding outcome as indicated by mortality and the required days of inotropic support, mechanical ventilation, and intensive care. Molnar, 1998[285]	50	3 to 5 days	Intravenous admin of 150mg/kg followed by 12/mg/kg	not significant

Table 1i NAC use in neurologic and musculoskeletal pathophysiology

NAC use in neurologic and musculoskeletal pathophysiology	n	Study length	Dosage	Beneficial effects
Alzheimer's Disease. Comparison of interval change favored NAC treatment as measured by MMSE, the ADL (Activity of Daily Life) scale and other psychometric tests. Adair, 2001[60]	47	24 weeks	50 mg/kg/day	Beneficial
Amyotrophic Lateral Sclerosis. Treatment of subjects with ALS did not result in a major increase in 12 month survival or reduction in disease progression. Louwerse, 1995[242]	110	1 year	50mg/kg daily	not significant
Exercise. NAC attenuated the decrease in reduced GSH and the increase in oxidized GSH resulting from intense, intermittent exercise. Time to fatigue was unchanged. Medved, 2003[58]	8		125 mg/kg/hr for 15 min, then 25 mg/kg/hr	Beneficial
Exercise. This is the first demonstration that NAC infusion during prolonged submaximal exercise substantially enhanced performance in well trained individuals. Medved, 2004. [286]			125 mg/kg/hr for 15 min, then 25 mg/kg/20 min	Beneficial
Exercise. In frail geriatric patients responding to physical exercise, NAC enhanced knee extensor strength, increased the sum of all strength parameters and decreased plasma TNF- α . Hauer, 2003[59]	44	6 weeks	1800mg daily	Beneficial

Table 1j NAC use in otolaryngologic, ophthalmologic and dental pathophysiology

NAC use in otolaryngologic, ophthalmologic and dental pathophysiology	n	Study length	Dosage	Beneficial effects
Otitis Media. Instillation of NAC into one ear at the time of bilateral insertion of ventilation tubes (VTs) and on days 3 and 7 afterwards decreased recurrence of otitis media with effusion, decreased re-insertion of VTs, and increased time until VT extrusion. The number of episodes of ear problems and visits to the ENT clinic were decreased. Ovesen, 2000[53]	75	39 months	0.05ml Mucomyst solution at 1, 3 and 7 days	Beneficial
Sjogren's syndrome. NAC treatment improved ocular soreness ($p = 0.004$), ocular irritability ($p = 0.006$), halitosis ($p = 0.033$) and daytime thirst ($p = 0.033$). Walters, 1986[56]	26	6 weeks	200mg daily	Beneficial
Chronic blepharitis. NAC improves tear film quality as detected by fluorescein break-up time (FBUT) and mucous fern pattern. Yalcin, 2002[57]	40	4 months	300mg/day, po	Beneficial
Prevention of plaque formation. Comparison of plaque indices for control and test periods showed a statistically significant ($p < .001$) 25.56% reduction on plaque attributable to NAC. Bowles, 1985[61]	28	2 weeks	10% aqueous solution of NAC buffered to pH 6.5 with flavoring agents added	Beneficial

Table 1k NAC use in dermatologic pathophysiology

NAC use in dermatologic pathophysiology	n	Study length	Dosage	Beneficial effects
Progressive Systemic Sclerosis. Several simple measures of function and skin distensibility were tested. No significant differences between the NAC and placebo groups were observed over the year of the study. Furst, 1979[287]	22	I year	1000 – 2000mg daily	not significant

Table 2

NAC treatment: reports of observational studies

Classification	Disease	Clinical findings	GSH measures	Clinical findings plus GSH measures
Hepatic Function	Acetaminophen Toxicity	[2, 71, 110, 122, 124, 126, 131, 135, 137, 138, 140, 141, 146-148, 152, 153, 288]		[128, 133, 134, 136, 159, 289]
	Alcohol	[290]	[168]	[291, 292]
	Hepatitis	[161, 293, 294]		[295-299]
Renal Function	Chronic Kidney Failure	[300]		[301]
	Dialysis	[302]		
	Nephrotoxicity	[3, 190]		
	□-Amanitin	[160]		
Cardiovascular	Angina			
	Arteriosclerosis/Cardiac Risk	[303-307]	[308]	[307, 309]
	Myocardial Infarction			[310]
	Cardiomyopathy			[311]
Endocrine	Diabetes	[312-314]		[106, 315-323]
Pulmonary	Reviews	[324-326]	[327, 328]	[329]
	Bronchopulmonary	[330, 331]		
	ARDS		[332]	[333, 334]
	Fibrosing Aveolitis			[335, 336]
	Chronic Asthma	[23, 337]		

Classification	Disease	Clinical findings	GSH measures	Clinical findings plus GSH measures
	Chronic Bronchitis/COPD	[338-340]		
	Cystic Fibrosis	[341]		[178, 342-346]
	Pulmonary Fibrosis		[347, 348]	[349-352]
	Smoking	[35]		[36, 353-356]
	Lung Cancer	[357]		
Critical Care	Intensive Care			[358-360]
	Sepsis/Septic Shock	[42, 361-363]		[43, 364, 365]
	Malnutrition		[173, 366]	[54, 170, 171]
Infection	HIV	[367-369]	[370-378]	[379-388]
	Helicobacter pylori		[389]	[390]
	Influenza		[391]	
	Malaria			
	Epilepsy		[392, 393]	[394, 395]
Gastrointestinal	Inflammatory Bowel Disease		[396]	[397-399]
	Barrett's Esophagus			[400]
	Liver Disease	[401-405]	[143]	[406, 407]
	Liver transplantation			[81]
	Colon Cancer			[408-411]
Optic	Blepharitis			
	Cataract	[412-414]		
	Eale's Disease			[415]
Skin	Psoriasis	[416]		[417]
	Photodermatitis			[418]

Classification	Disease	Clinical findings	GSH measures	Clinical findings plus GSH measures
Immune System	Rheumatoid Arthritis			[419-421]
	Comm. Variable Immunodeficiency			[422]
Urogenital	Prostate	[423-425]		[426, 427] ³
	Urinary			
Aging		[428-430]		[431-436]
Toxic Agents	Arsenic Poisoning	[437]		
	Other Chemicals and Medications	[438]	[439-441]	
Perinatal	Preeclampsia	[442, 443]		
	Neonates			[444-446]
Metabolism	Phenylketonuria			[447]
Misc. Reviews		[448-451]		[241, 452-454]

³ Cancer Treatment Review

We claim:

1. A method of treatment to prevent development of glutathione deficiency as a consequence of disease, a treatment or a condition comprising administering to a subject at risk of glutathione deficiency as a consequence of disease, or a treatment, or a condition, a therapeutic amount of N-acetylcysteine or a pharmaceutically acceptable salt or derivative thereof sufficient to increase intracellular glutathione levels or decrease oxidative stress and measuring and monitoring the level of glutathione in blood in patients as needed.
2. A method of treatment to restore glutathione levels comprising administering to subjects in need of glutathione level restoration as determined by measurement or by physician a therapeutic amount of N-acetylcysteine or a pharmaceutically acceptable salt or derivative thereof sufficient to increase intracellular glutathione levels or decrease oxidative stress and monitoring restoration by measuring the level of glutathione in blood as needed.
3. A method of treatment to decrease oxidized glutathione levels elevated as a consequence of disease, a treatment or a condition, comprising administering to a subject suffering from oxidative stress a therapeutic amount of N-acetylcysteine or a pharmaceutically acceptable salt or derivative thereof sufficient to decrease oxidized glutathione levels elevated as a consequence of disease, a treatment, or a condition and monitoring the level of oxidized glutathione in blood as needed.