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N-Acetylcysteine for Lung Cancer Prevention*

Nico van Zandwijk, MD, PhD, FCCP

Lung cancer arises as a focal transformation of chronically injured epithelium with cigarette smoke as one of its well recognized causes. Apart from oxidants, cigarette smoke contains several precarcinogens, and it is surprising that not every heavy smoker becomes a victim of malignant disease. This points to the interindividual variability in susceptibility to carcinogens and there are several lines of evidence that metabolic factors are involved in such variability. Metabolism of carcinogens and also the subsequent multisteps of carcinogenesis are affected by host factors and governed by the balance between opposite forces, such as metabolic activation and detoxification, formation, and scavenging of radicals and DNA damage and repair. This implies that carcinogenic compounds can initiate tumor growth only in amounts saturating detoxification mechanisms. In this context it is well known that glutathione plays a crucial role in the detoxification of xenobiotics. N-acetylcysteine (NAC), an aminothiol and precursor of intracellular cysteine and glutathione, has been shown not only to be an efficient antidote in acetaminophen poisoning but also to possess important chemopreventive properties. In this article, sites and mechanisms of the therapeutic action of NAC are reviewed with special reference to its chemopreventive characteristics.

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Key words: antidote; antioxidant; chemoprevention; mucolytic agent; NAC
cancer chemopreventive agent and is listed under the category of sulfur compounds.2

In this article, an overview of sites and mechanisms of therapeutic actions of NAC is given with special reference to its chemopreventive characteristics.

HISTORY OF NAC

In 1962, NAC was first mentioned as a mucolytic agent.3 At that time, cysteine and its derivatives had been found to lower the viscosity of mucus. The efficacy of NAC applied directly to the airways by nebulization or direct instillation is well established. The mechanism of action of NAC administered by this route was proposed to be mediated by the free sulfhydryl group in NAC, cleaving disulfide bonds in the glycoprotein macromolecules of mucus/sputum, thereby forming smaller molecular weight mixed disulfides of NAC and glycoprotein subunits.4,5 The topical mucolytic action was not specific to NAC but is shared by other compounds having sulfhydryl groups able to reduce disulfide bonds in mucus glycoproteins.6

When taken orally, NAC is rapidly absorbed, deacetylated, and incorporated in the intracellular and extracellular glutathione stores.7 NAC can be regarded as one of the cysteine derivatives that combines the least toxicity with the best ability to be a precursor of glutathione.8 Another cysteine derivative, S-carboxymethyl-cysteine, specifically devel-

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oped for systemic use, showed a low efficiency as precursor of intracellular cysteine since most of the administered compound was excreted unchanged into the urine.9

A different mechanism of action must have been responsible for the mucoregulatory effect of systemic NAC that has been reported in several studies.10,11 Interestingly, large randomized studies not only show a reduction in the frequency of exacerbations of chronic bronchitis by NAC, but also of seasonal viral symptoms.12,13

There is a rapidly growing body of evidence that points to the antioxidant properties of thiols like NAC and glutathione preventing the progression of pulmonary injury in patients with chronic obstructive pulmonary disease (COPD) by blocking free radical reactions.14

In the late 1970s, NAC found wide application as an antidote in acute acetaminophen (paracetamol) poisoning. The hepatorenal toxicity of acetaminophen is mediated by a reactive metabolite normally detoxified by reduced glutathione.15 If glutathione is depleted, covalent binding to macromolecules, oxidation of sulfhydryl groups in enzymes, or both can lead to cell death. Oral NAC mitigates acetaminophen-induced hepatorenal damage if given within 10 h.16 NAC turned out to be a safe agent, even when doses as large as 30 g/d × 3 were given to adults who had overdosed themselves with acetaminophen.17 NAC was well tolerated even in the presence of underlying pathophysiologic conditions caused by the overdose and emergency procedures. After repeated high doses, nausea/vomiting and diarrhea were reported in up to 50% and 35% of patients, respectively. In clinical practice, NAC turned out to be an important adjunct to prevent toxic reactions of chemotherapeutic agents such as ifosfamide.18 Other areas of clinical toxicology where protective effects of NAC have been documented are listed in Table 1. In all these cases, NAC is supposed to block reactive metabolites/molecules and free radical reactions.

### Table 1—Possible Applications of NAC in Clinical Toxicology

<table>
<thead>
<tr>
<th>Poisons</th>
<th>Mechanism of Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylonitrile</td>
<td>Reactive metabolite</td>
</tr>
<tr>
<td>Bromobenzene</td>
<td>Reactive metabolite</td>
</tr>
<tr>
<td>Acrolein</td>
<td>Reactive molecule</td>
</tr>
<tr>
<td>Naphtalene</td>
<td>Reactive metabolites</td>
</tr>
<tr>
<td>Dichlorodiyel sulfide</td>
<td>Reactive molecule</td>
</tr>
<tr>
<td>(mustard gas)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Mechanism of Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>Reactive metabolite</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Reactive metabolite</td>
</tr>
<tr>
<td>Ifosfamide</td>
<td>Reactive metabolite</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Free radical damage</td>
</tr>
</tbody>
</table>

**Chemoprevention**

It is generally accepted that the pathogenesis of lung cancer occurs in several steps. This traditional view on carcinogenesis is derived primarily from studies of animal models, but more recent insight relies on the molecular analysis of cancer-related genes and growth factors. The well-known clinical observation that the latent period between exposure to a cancer-causing agent and the development of clinical cancer can be as long as several decades, eg, the period between onset of cigarette smoking and the first signs of lung cancer, has led to the assumption that not one but several pathogenic events must have taken place during this time.19,20

Classically four major discrete and distinct phases have been identified: (1) initiation; (2) promotion; (3) conversion; and (4) progression. The initiation phase is described as an early, rapid, and largely irreversible change in a permanently altered cell. Tumor initiation begins through mutation of genetic material from exposure to carcinogens. Promotion of carcinogenesis is a more gradual process during which an initiated cell acquires more and more malignant characteristics. This complex phase of carcinogenesis, involving a variety of cellular changes, is thought to last decades in humans.

The two final stages of carcinogenesis are conversion and progression. In contrast to initiation and promotion, which have been studied extensively in experimental animals, much less is known about tumor conversion and tumor progression. A key feature of these stages is continued growth or replication of the abnormal cells, which is necessary, not only for their expansion, but also for the generation of new, potentially “more malignant” properties, by the acquisition of additional inheritable changes in the developing clone(s). The multistep nature of the carcinogenic process raises the possibility of intervention at different stages.21 Intervention at the initiation stage involves approaches such as elimination of carcinogens or interference with the metabolic activation of (pre)carcinogens to reactive intermediates. It has to be stressed that it is not easy to eliminate risk factors, even if they are known. This is especially true for risk factors resulting from our life-style, considering that millions of smokers still constitute the largest body of volunteers in the epidemiology of lung cancer. The discussion on abandoning smoking is often clouded by the argument that most heavy smokers will never acquire lung cancer. This also points to the interindividual variability in susceptibility to carcinogens, and there are several lines of evidence that metabolic factors are involved in such variability. Metabolism of carcinogens, and also the
subsequent steps of carcinogenesis, are affected by host factors and governed by a balance between opposite forces, such as metabolic activation and detoxification (of carcinogens) and consequent formation and scavenging of reactive metabolites (radicals) and damage and repair of DNA.\textsuperscript{22,23}

**NAC as a Chemopreventive Agent**

NAC and reduced glutathione are typical examples of compounds that are expected to provide chemopreventive effects by multiple mechanisms and thus to protect against a broad range of mutagens and carcinogens. Glutathione itself has been used as a protective agent.\textsuperscript{24} However, as this tripeptide does not readily cross cell membranes, its effectiveness in clinical practice is very limited.

Cysteine \textit{per se} is essential for intracellular glutathione synthesis, but its use in humans has been hampered by toxicity problems. Therefore, cysteine conjugates have been synthesized to provide the precursor of glutathione avoiding toxicity. One of them is NAC.

Most laboratory investigations to assess these antimutagenic and anticarcinogenic activities of NAC and glutathione have been carried out by de Flora and coworkers.\textsuperscript{25,26} They were among the first to recognize the inhibition of mutagenicity by NAC of several direct-acting mutagens and reactive oxygen species (oxidants).\textsuperscript{25,26} Oxidants in tobacco smoke exist in sufficient concentrations to suggest that they play a major role in injuring cells of the lower respiratory tract. It has been estimated that each puff of smoke contains 10\textsuperscript{16} oxidant molecules, which include aldehydes, epoxides, peroxides, and free radicals.\textsuperscript{27} Furthermore, smokers have increased quantities of neutrophils and macrophages in the lower respiratory tract\textsuperscript{28} that apart from leukotrienes and cytokines may release additional oxidants capable of causing cell injury.\textsuperscript{29} Tobacco smoke oxidants severely deplete intracellular antioxidants in lung cells \textit{in vitro} by a mechanism that is related to increased oxidant stress.\textsuperscript{30} In this context, it is of interest to note that K-ras oncogene DNA has been shown to be quickly transformed (activated) after exposure to oxidation.\textsuperscript{31}

NAC is able to detoxify reactive electrophiles and free radicals either through conjugation or reduction reactions. First, it reduces reactive oxygen species to less reactive ones.\textsuperscript{30} Second, NAC is deacetylated in many tissues and cells to form cysteine, supporting glutathione biosynthesis that serves directly as an antioxidant or as a substrate in the glutathione redox cycle.\textsuperscript{7,32}

Interestingly, different effects of different doses of NAC on potent carcinogens such as benzo(a)pyrene, 2-aminofluorene and aflatoxin B\textsubscript{1} have been recognized.\textsuperscript{33} Intermediate doses of NAC often stimulated their activation to mutagenic metabolites, whereas high doses inhibited their mutagenic response. The observed modulation patterns suggest that NAC induces the conversion of promutagens into electrophilic metabolites, which are then blocked by the thiol itself.\textsuperscript{34} Experimentally, it has been shown that NAC, as a precursor of intracellular glutathione, is also capable of stimulating (phase 2) enzymes in the glutathione cycle (GSH peroxidase, GSSG reductase, GSH S-transferase).\textsuperscript{2}

Repair of DNA damage has also been found to be stimulated by thiols like NAC and glutathione in experiments with cultured hepatocytes exposed to roentgen irradiation and carcinogens.\textsuperscript{35,36} In a rat hepatocarcinogenesis model, NAC administered by gavage was able to inhibit the formation of carcinogen-DNA adducts,\textsuperscript{37} which is regarded as one of the first steps of carcinogenesis. The laboratory observations above were confirmed in several different experimental tumor models (Table 2).

**EUROSCAN**

Patients successfully treated for lung or head and neck cancer remain at significant risk for the development of a second primary tumor. In Euroscan, the European Chemoprevention Study under auspices of the European Organization for Research and Treatment of Cancer (EORTC), patients following potentially curative treatment of lung and head and neck cancer are randomized to one of the following schemes: NAC (600 mg daily), retinol palmitate, both drugs, or no drugs, for a 2-year period.\textsuperscript{38} To date, more than 2,600 individuals are included. An interim analysis showed that NAC is very well tolerated. Only a small proportion (8\%) of the persons receiving NAC in a dose, reported to increase plasma glutathione,\textsuperscript{39} suffered from side effects. The toxicity of NAC consisted mainly of mild gastrointestinal symptoms, \textit{ie}, dyspepsia. Side effects invariably resolved when the intervention was interrupted and as to date have not led to any major complication. The side effects of retinol palmitate used in the same study elicited were more expressed.\textsuperscript{38}

**Conclusion**

Since the first publication on potential chemopreventive activity of NAC in 1984, independent groups of investigators have confirmed this observation (Table 2). It has become apparent from preclinical studies that NAC exerts its chemopreventive effects by multiple mechanisms and thus may provide protection against different mutagens and carcinogens in different stages of carcinogenesis.

The relative ease by which NAC has reached the phase 3 trial stage in chemoprevention in Europe is
Table 2—Protective Effects of NAC in Animal Studies

<table>
<thead>
<tr>
<th>Species</th>
<th>Carcinogen</th>
<th>Target Organ</th>
<th>End Point Neoplastic/Preneoplastic</th>
<th>Observation</th>
<th>Source, yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Urethane</td>
<td>Lung</td>
<td>Adenoma</td>
<td>Prevention of adenoma induction by NAC in diet</td>
<td>De Flora et al, 1986</td>
</tr>
<tr>
<td></td>
<td>DMBA* + TPA†</td>
<td>Skin</td>
<td>Papilloma</td>
<td>Inhibition of tumor promotion by NAC in diet</td>
<td>Rotstein and Slaga, 1988</td>
</tr>
<tr>
<td>Rat</td>
<td>1,2-dimethylhydrazine</td>
<td>Colon</td>
<td>Carcinoma</td>
<td>Reduction of multiplicity of carcinomas by NAC in drinking water</td>
<td>Wilpert et al, 1986</td>
</tr>
<tr>
<td></td>
<td>N-acetyl-2-aminoazophenol</td>
<td>Liver</td>
<td>DNA structure and metabolic activity</td>
<td>Protection against carcinogen-induced DNA damage (NAC in diet)</td>
<td>Cesarone et al, 1987</td>
</tr>
<tr>
<td></td>
<td>N-methyl-N-nitro-N-nitosoguanidine</td>
<td>Liver</td>
<td>DNA</td>
<td>Protection against carcinogen-induced DNA damage (ip† NAC and NAC in diet)</td>
<td>Chan et al, 1986</td>
</tr>
<tr>
<td></td>
<td>N-methyl-N-nitrosourea</td>
<td>Mammary gland</td>
<td>Adenocarcinoma</td>
<td>Reduction of multiplicity of tumor by NAC in diet</td>
<td>Boone et al, 1992</td>
</tr>
<tr>
<td></td>
<td>Cigarette smoke</td>
<td>Lung</td>
<td>Hyperplasia and metaplasia</td>
<td>Prevention of cytologic and cytogenetic damage by NAC in diet</td>
<td>Balansky et al, 1992</td>
</tr>
<tr>
<td>Hamster</td>
<td>N-methyl-N-nitrosourea</td>
<td>Lung/trachea</td>
<td>Squamous cell carcinoma in trachea</td>
<td>Reduction of carcinoma formation by NAC in diet</td>
<td>Boone et al, 1992</td>
</tr>
</tbody>
</table>

*7,12-dimethylbenz[a]anthracene.
†12-O-tetradecanoyl 1-phorbol-13-acetate.
†ip=intraperitoneal.


In the clinical setting, NAC has been used to prevent the hepatorenal failure after acetaminophen intoxication.

In Euroscan, NAC is also well tolerated when taken continuously in a dose of 600 mg daily. Only minor side effects, mainly consisting of dyspepsia, have been recorded in a small group of the test population.

If NAC holds its promise and turns out to be effective in preventing second primary tumors, this drug is certainly a candidate to be used on a wider scale for chemopreventive purposes.

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