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N-Acetylcysteine (NAC) and Glutathione (GSH): Antioxidant and Chemopreventive Properties, With Special Reference to Lung Cancer

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Abstract Lung cancer arises as a focal transformation of chronically injured epithelium with cigarette smoke as one of its well-recognized causes. Apart from oxidants (free radicals), cigarette smoke contains such a multitude of (pre)carcinogens that it is astonishing that not every heavy smoker becomes a victim of malignancy. This points to the interindividual variability in susceptibility to carcinogens; several lines of evidence suggest that metabolic factors are involved in such variability. Metabolism of carcinogens as well as the subsequent (multi)steps of carcinogenesis are affected by host factors and governed by the balance between opposing forces, such as metabolic activation and detoxification, formation and scavenging of radicals, and DNA damage and repair, which seem to imply that carcinogenic compounds can initiate tumor growth only in amounts saturating detoxification mechanisms. In this context it is well known that glutathione (GSH) plays a crucial role in the detoxification of xenobiotics. *N*-Acetylcysteine (NAC), an aminothiol and synthetic precursor of intracellular cysteine and GSH, has been used for many years in Europe as a mucolytic drug. Clinically, it is a safe agent without major side effects and has been considered to have a place in cancer prevention, too. The antimutagenic and anticarcinogenic properties of NAC could be ascribed to multiple protective mechanisms, such as NAC nucleophilicity, antioxidant activity, its ability to act as a precursor of intracellular reduced GSH, modulation of detoxification, and DNA repair processes. On these grounds, NAC has emerged as a most promising cancer chemopreventive agent. Since 1988, NAC has been tested in a large European chemoprevention study (EUROSCAN) involving high-risk individuals to prevent the occurrence of a second primary (lung) cancer. Toxicity data of this and other studies confirm that a long-term daily usage of NAC in a 600 mg dose is safe, and may be recommended for clinical chemopreventive research.

Key words: *N*-Acetylcysteine, antioxidant, chemoprevention, lung cancer

The treatment and prevention of cancer have traditionally been viewed as two different disciplines. Although this has, in large part, led to the relegation of responsibility for cancer prevention to public health and epidemiology experts, rapidly increasing understanding of the process of carcinogenesis, as well as the identification of patient groups with an elevated risk for a (second) cancer, has in recent years led to an increasing involvement of clinicians in cancer prevention. The identification of retinoids in the early 1990s as clinical cancer chemopreventive agents, *i.e.*, agents that prevent or delay the occurrence of cancer, must be regarded as a major breakthrough in this area [1]. Today, disease-specific preventive interventions are receiving more and more attention.

This article focuses on *N*-acetylcysteine (NAC) and glutathione (GSH) and chemoprevention of lung cancer. It contains toxicity data derived

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from EUROSCAN, a European-wide chemoprevention study where NAC is one of the potential chemopreventive agents studied.

LUNG CANCER

Lung cancer remains an overwhelming oncological problem because of its high incidence, low surgical curability, and failure of conventional systemic treatment to cure it. Even in the presence of major reductions in tobacco consumption, the incidence of lung cancer is not expected to significantly decrease during the next 20 years because of the large cohort of people already affected by long-term tobacco exposure [2]. So far, screening programs have not significantly improved lung cancer-related mortality. Obviously, smoking cessation is still the single most important means of prevention and the use of chemopreventive measures should not be an excuse for not carrying out maximum efforts to obtain a smoke-free society. However, the reality is that complete elimination of carcinogens from tobacco smoke is not likely to be achieved. Thus, additional preventive measures, such as chemoprevention, must be sought.

Cancer of the lung arises as a focal transformation of chronically injured epithelium with cigarette smoke as one of its well-recognized causes [3]. Apart from oxidants, cigarette smoke contains an abundance of (pre)carcinogens (Table I); it is in fact surprising that not every heavy smoker becomes a victim of malignant disease. This points to the interindividual variability in susceptibility to carcinogens. There is ample epidemiologic and genetic evidence that dietary and metabolic factors play an important role in such variability [4–7].

Metabolism of carcinogens and the subsequent (multi)steps of carcinogenesis are affected by the balance between opposing forces, such as metabolic activation and detoxification, formation and scavenging of free radicals, and DNA damage and repair [8]. This probably means that carcinogenic compounds can initiate tumor growth only when amounts saturate detoxification mechanisms. In this context it is well known that GSH plays a crucial role in the detoxification of reactive carcinogen species [9].

It is not surprising that in smokers, GSH levels have been exhausted as a consequence of inhaling cigarette smoke [10]. It has been estimated that each puff of smoke contains 10^{16} oxidant molecules, including aldehydes, epoxides, peroxides and free radicals [11]. The oxidants alone in tobacco smoke thus exist in sufficient concentrations to induce epithelial injury. Furthermore, smokers have increased quantities of neutrophils and macrophages in the lower respiratory tract [12] that, apart from leukotrienes and cytokines, may release additional oxidants capable of causing cell injury [13]. *In vitro,* tobacco smoke oxidants severely deplete intracellular antioxidants in lung cells. It is of interest to note that K-*ras* oncogene DNA has been shown to be quickly transformed (activated) after exposure to oxidation [14]. Blocking several of these different mutagenic stimuli has been shown to exert a clear preventive effect.

TABLE I. Examples of Toxic and Carcinogenic Substances in Cigarette Smoke*

Acetone	Cyanide
Acrolein	Free radicals
Benzene	Hydrazine
Benzo(*a*)pyrene	Methane
Butane	Nicotine
Crotonaldehyde	Nitrous oxide

* Contains ±4000 chemicals including ±40 known carcinogens

NAC: MUCOLYTIC, ANTIDOTE, ANTIOXIDANT AND CHEMOPREVENTIVE AGENT

Within in the last decade, NAC has increasingly been recognized as an antioxidant and potential chemopreventive agent; however, this aminothiol and precursor of reduced GSH has been in clinical practice for more than 30 years. NAC was first mentioned as a mucolytic agent in 1962 [15]. 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 installation 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 [16,17]. The topical mucolytic action is not specific to NAC but is shared by other compounds having sulfhydryl groups able to reduce disulfide bonds in mucus glycoproteins [18].

When taken orally NAC is rapidly absorbed, deacetylated, and incorporated into the intra- and extracellular GSH stores [19]. NAC may regarded as one of the cysteine derivatives that combine the least toxicity with the best ability to be a precursor of GSH [20]. Another cysteine derivative, *S*-carboxymethylcysteine, specifically developed for systemic use, showed a low efficiency as a precursor of intracellular cysteine since most of the administered compound was excreted unchanged into the urine [21].

A different mechanism of action must be responsible for the mucoregulatory effect of systemic NAC that has been reported in several studies [22,23]. 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 [24, 25]. There is a rapidly growing body of evidence that points to the antioxidant properties of thiols like NAC and GSH in preventing the progression of pulmonary injury in chronic obstructive pulmonary disease patients by blocking free radical reactions [26].

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 GSH [27]. If GSH is depleted, covalent binding to macromolecules and/or oxidation of sulfhydryl groups in enzymes can lead to cell death. Oral NAC mitigates acetamino-phen-induced hepatorenal damage if given within 10 hours [28]. NAC turned out to be a safe agent, even when doses as large as 30 gm/day x 3 days were given to adults, who had overdosed with acetaminophen [29]. 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 in the prevention of toxicities caused by chemotherapeutic agents such as ifosfamide [30]. Other areas of clinical toxicology where protective effects of NAC have been documented are

TABLE II. Possible Protective Applications of NAC in Clinical Toxicology

Poisons	Mechanism of Toxicity
Acrylonitrile	Reactive metabolite
Bromobenzene	Reactive metabolite
Acrolein	Reactive molecule
Naphthalene	Reactive metabolites
Dichlorodiethyl sulfide (mustard gas)	Reactive molecule
Drugs	
Acetaminophen	Reactive metabolite
Cyclophosphamide	Reactive metabolite
Iphosphamide	Reactive metabolite
Doxorubicin	Free radical damage

listed in Table II. In all these cases, NAC is supposed to block reactive metabolites/molecules and free radical reactions.

NAC and reduced GSH 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. GSH itself has been used as a protective agent [31]. However, as this tripeptide does not readily cross cell membranes; its effectiveness in clinical practice is very limited. Cysteine *per se* is essential for intracellular GSH synthesis but its use in humans has been hampered by toxicity problems.

NAC, which is a cysteine conjugate, has been synthesized to provide a precursor of GSH, avoiding the toxicity of cysteine. The majority of laboratory investigations to assess these antimutagenic and anticarcinogenic activities of NAC and GSH have been carried out by De Flora and co-workers [9,32], who were some of the first to recognize the inhibition of mutagenicity by NAC of several direct-acting mutagens and reactive oxygen species (oxidants).

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 [33]. Secondly, NAC is deacetylated in many tissues and cells to form cysteine, supporting GSH biosynthesis which serves directly as an antioxidant or as a substrate in the GSH redox cycle [19,34].

Interestingly, different effects of different doses of NAC on potent carcinogens such as benzo(*a*)pyrene, 2-aminofluorene, and aflatoxin B_1 have been recognized [35]. Intermediate doses of NAC often stimulated activation to mutagenic metabolites, whereas high doses inhibited mutagenic responses. The observed modulation patterns suggest that NAC induces the conversion of promutagens to electrophilic metabolites, which are then blocked by the thiol itself [36]. Experimentally it has been shown that NAC, as a precursor of intracellular GSH, is also capable of stimulating phase II enzymes in the GSH cycle (GSH peroxidase, GSSH reductase, GSH-*S*-transferase) [37].

Repair of DNA damage is stimulated by thiols like NAC and GSH in experiments with cultured hepatocytes exposed to Roentgen irradiation and carcinogens [38,39]. In a rat hepatocarcinogenesis model, NAC administered by gavage was able to inhibit the formation of carcinogen-DNA adducts [40] regarded as one of the first steps of carcinogenesis. The laboratory observations above were confirmed in several different experimental tumor models (Table III). On this basis NAC has emerged as one of the most promising cancer chemopreventive agents to such an extent that it is one of the few compounds attaining the stage of evaluation in humans. In the USA, NAC has entered Phase II clinical trials and since 1987, NAC has been tested in Europe, leading to Phase II and Phase III studies.

CHEMOPREVENTION STUDIES WITH *N*-ACETYLCYSTEINE

EUROSCAN, the large European-wide prevention study in patients previously treated for lung or head and neck cancer who are at elevated risk for a second primary (lung) cancer, started in 1988 under the auspices of the European Organization for Research and Treatment of Cancer (EORTC). An economic 2x2 factorial design was chosen to investigate the possible chemopreventive effect of NAC and natural vitamin A. On the basis of previous studies in animals and humans, a daily dose of 600 mg for NAC was chosen. In contrast to previous studies, only favorable stage patients were eligible for this study [48].

With toxicity data from 2,129 patients available, NAC, as reported earlier, continues to be tolerated very well (Table IV). Eighty-six percent of patients did not experience any side effects during the two-year intervention period, and in the 14% of patients who experienced toxicity, side effects seem rather well-tolerated. The most common side effect noted was dyspepsia (Table V). In general, a compliance of approximately 75% was seen during the two-year intervention period.

We found that at least 94% of the EUROSCAN participants were significantly exposed to tobacco smoke, one of the important etiological factors, for many years before the diagnosis of their index tumor. After treatment (of the index tumor) and during intervention, around 15% of the patients continue to smoke.

In a dose-finding study in 30 healthy volunteers at the Netherlands Cancer Institute, we found that higher daily doses of NAC elicited significantly more toxicity. With a dose of 600 mg tid, 60% of the test population experi-

TABLE III. Protective Effects of *N*-Acetylcysteine (NAC) in Animal Studies.

Species	Carcinogen	Target Organ	Endpoint: Neoplastic/ Preneoplastic	Observation	Reference
Mouse	Urethane	Lung	Adenoma	Prevention of adenoma induction by NAC in diet	[41]
	DMBA* + TPA**	Skin	Papilloma	Inhibition of tumor promotion by NAC in diet	[42]
Rat	1,2-Dimethylhydrazine	Colon	Carcinoma	Reduction of carcinoma multiplicity by NAC in drinking water	[43]
	N-Acetyl-2-aminofluorene	Zymbal gland	Squamous cell carcinoma	Prevention of sebaceous carcinoma formation	[44]
	N-Acetyl-2-aminofluorene	Liver	DNA structure and metabolic activity	Protection against carcinogen-induced DNA damage (NAC in diet)	[44]
	N-Methyl-*N*-nitro-*N*-nitrosoguanidine	Liver	DNA	Protection against carcinogen-induced DNA damage (ip NAC and NAC in diet)	[45]
	N-Methyl-*N*-nitrosurea	Mammary glands	Adenocarcinoma	Reduction of tumor multiplicity by NAC in diet	[46]
	Cigarette Smoke	Lung	Hyper- and metaplasia	Prevention of cytological and cytogenetic damage by NAC in diet	[47]
Hamster	*N*-Methyl-*N*-nitrosurea	Lung/trachea	Squamous cell carcinoma in trachea	Reduction of carcinoma formation by NAC in diet	[46]

* 7,12-dimethylbenz(*a*)anthracene; ** 12-*O*-tetradecanoyl-1-phorbol-13-acetate

TABLE IV. EUROSCAN Interim Results*

Number of patients with and without side effects by treatment group			
	No side effects n (%)	Side effects n (%)	Total n (%)
Retinol[1] + NAC[2]	411 (74.6)	140 (25.4)	551 (100)
Retinol	426 (76.4)	130 (23.4)	556 (100)
NAC	472 (86.4)	74 (13.6)	546 (100)
No drugs	562 (97.8)	12 (2.2)	538 (100)
Total	1,835 (83.8)	356 (16.2)	2,191 (100)

* (includes 2,191 patients with at least one follow-up);
[1] Retinol = 300,000 IU, NAC = 600 mg

TABLE V. EUROSCAN Interim Results[1]

Type of side effects by treatment group					
	Retinol + NAC	Retinol	NAC	No drugs	Total
Dryness	5	6	4	0	15
Desquamation	7	13	2	0	22
Itching	13	16	5	1	35
Headache	9	3	2	0	14
Dyspepsia	12	9	19	2	42
Bleeding	1	2	0	0	3
Hair loss	5	3	2	0	10
Other	26	16	12	2	56
Total	78	68	46	5	197
Missing	62	62	28	7	159
No. of side effects[2]	140	130	74	12	356

[1] (includes 2,191 patients with at least one follow-up); [2] well-tolerated, poorly tolerated, or unbearable side effects.

TABLE VI. Dose-finding Studies with NAC

30 healthy volunteers, 14 males, 16 females, age 23–51 years.	
2 x 600 mg NAC for 4 weeks (n = 12)	
3 volunteers	experienced toxicity
2 volunteers	flatulence, spastic abdominal pain
1 volunteer	exacerbation of erythema
3 x 600 mg NAC for 4 weeks (n = 18)	
11 volunteers	experienced toxicity (100% gastrointestinal: nausea, flatulence, spastic abdominal pain)
6 volunteers	WHO I
1 volunteer	WHO II
4 volunteers	WHO III

enced gastrointestinal toxicity which frequently caused interruption of the study. With a dosage of 600 mg bid, side effects were less prominent, but caused interruption in 25% (Table VI) of the participants. Thus a dose of 600 mg daily seems to be a reasonable choice for a large population awaiting chemopreventive measures.

CONCLUSION

Since the first publication on the potential chemopreventive activity of NAC in 1984, independent groups of investigators have confirmed this observation. 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 during different stages of carcinogenesis.

The relative ease by which NAC has reached the Phase III trial stage in chemoprevention in Europe has obviously been facilitated by the fact that this agent has been in clinical practice for more than 30 years. In large groups of patients with chronic obstructive lung disease, NAC turned out to be a safe agent with minor side effects, even when prescribed for prolonged periods of time. The clinical safety has been underscored by the observation that NAC is prescribed in very high doses to prevent hepatorenal failure after acetaminophen intoxication.

In EUROSCAN, NAC continues to be very well tolerated when taken for prolonged periods in doses of 600 mg daily. Only minor side effects, mainly dyspepsia, have been recorded in a small group of the test population. Doubling or tripling this dose is accompanied by significant toxicity in healthy volunteers.

If NAC holds its promise and turns out to be effective in preventing second primary tumors, it may certainly be a candidate for wide-scale use as a chemopreventive agent.

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