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The chemistry and biological activities of N-acetylcysteine

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Abstract

Background: N-acetylcysteine (NAC) has been in clinical practice for several decades. It has been used as a mucolytic agent and for the treatment of numerous disorders including paracetamol indotoxication, doxorubicin cardiotoxicity, ischemia-reperfusion cardiac injury, acute respiratory distress syndrome, bronchitis, chemotherapy-induced toxicity, HIV/AIDS, heavy metal toxicity and psychiatric disorders.

Scope of Review: The mechanisms underlying the therapeutic and clinical applications of NAC are complex and still unclear. The present review is focused on the chemistry of NAC and its interactions and functions at the organ, tissue and cellular levels in an attempt to bridge the gap between its recognized biological activities and chemistry.

Major Conclusions: The antioxidative activity of NAC as of other thiols can be attributed to its fast reactions with $^{\cdot}OH$, $^{\cdot}NO_2$, $CO_3^{\cdot-}$ and thiyl radicals as well as to restitution of impaired targets in vital cellular components. NAC reacts relatively slowly with superoxide, hydrogen-peroxide and peroxynitrite, which cast some doubt on the importance of these reactions under physiological conditions. The uniqueness of NAC is most probably due to efficient reduction of disulfide bonds in proteins thus altering their structures and disrupting their ligand bonding, competition with larger reducing molecules in sterically less accessible spaces, and serving as a precursor of cysteine for GSH synthesis.

General Significance: The outlined reactions only partially explain the diverse biological effects of NAC, and further studies are required for determining its ability to cross the cell membrane and the blood-brain barrier as well as elucidating its reactions with components of cells signaling pathways.

Key Words: NAC, antioxidant, GSH precursor, redox potential, disulfide bond, cell-permeability

Abbreviations: BBB, blood-brain barrier; CD, cluster of differentiation; $CO_3^{\cdot-}$, carbon trioxide ion radical; ERK, extracellular signal regulated kinase; GSH, glutathione; HNO, nitroxyl; HOCl, hypocholorous acid; HOSCN, hypothiocyanous acid; Ig, immunoglobulin; I-κB, inhibitor of nuclear factor kappa B; IKK, Inhibitor of nuclear factor kappa B kinase; IL, interleukin; INF-γ, interferon; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; mTOR ,mammalian target of rapamycin; NAC, N-acetylcysteine; $^{\cdot}N_3$, azide radical; NAPQI, N-acetyl-p-benzoquinone imine; NF-κB, nuclear factor kappa B; NO, nitric oxide; $^{\cdot}NO_2$, nitrogen dioxide radical; $O_2^{\cdot-}$, superoxide ion radical; $^{\cdot}OH$, hydroxyl radical; PMN, polymorphonuclear leukocytes; RNS, reactive nitrogen species; ROS, reactive oxygen species; $RS^{\cdot}$, thiyl radical; RS^-, thiolate; RSH, thiol; RSOH, sulfenic acid; SOD, superoxide dismutase; TNF, tumor necrosis factor

1. INTRODUCTION

N-acetylcysteine (also known as N-acetyl cysteine, N-acetyl-L-cysteine or NAC) has been in clinical practice for several decades. NAC has been used as a mucolytic agent and for the treatment of numerous disorders such as acetaminophen (paracetamol) intoxication, doxorubicin-induced cardiotoxicity, stable angina pectoris, ischemia-reperfusion cardiac injury, acute respiratory distress syndrome, bronchitis, chemotherapy-induced toxicity, HIV/AIDS, radio-contrast-induced nephropathy, heavy metal toxicity and psychiatric disorders including schizophrenia, bipolar disorder and addiction ([1-12] for reviews).

NAC, the acetylated precursor of the amino acid L-cysteine, is pharmaceutically available either intravenously, orally, or by inhalation. NAC has relatively low toxicity and is associated with mild side effects such as nausea, vomiting, rhinorreah, pruritus and tachycardia [4]. The terminal half-life of NAC after a single intravenous administration is 5.6 hours where 30% of the drug is cleared by renal excretion [13]. The relatively low bioavailability of NAC (below 5% [13-15]) is thought to be associated with its N-deacetylation in the intestinal mucosa and first pass metabolism in the liver. The plasma is a rather pro-oxidizing milieu and, therefore, redox exchange reactions between NAC, cystine and cysteine proteins in the plasma produce NAC-cysteine, NAC-NAC and cysteine [16, 17]. The latter can cross the epithelial cell membrane and sustain the synthesis of glutathione (GSH), which is the ubiquitous source of the thiol pool in the body and an important antioxidant involved in numerous physiological processes [18-20]. These include detoxification of electrophilic xenobiotics, modulation of redox regulated signal transduction, regulation of immune response, prostaglandin and leukotriene metabolism, antioxidant defense, neurotransmitter signaling and modulation of cell proliferation ([19] for a review). The synthesis of GSH is tightly regulated at various levels and is kept at the mM concentration range [21]. Hence, the notion that the physiologic functions and therapeutic effects of NAC are largely associated with maintaining the levels of intracellular GSH is reasonable, and it is often difficult to discern the direct effect of NAC from those related to GSH.

The present review is focused on the chemistry of NAC and its interactions and functions at the organ, tissue and cellular levels in an attempt to bridge the gap between its chemical features and recognized biological activities. For simplicity and practicality the various proposed mechanisms underlying NAC effects, which are presented here in their respective context, are not necessarily mutually exclusive but might operate concurrently.

2. The chemistry of NAC

NAC is a derivative of cysteine with an acetyl group attached to its nitrogen atom and like most thiols (RSH) can be oxidized by a large variety of radicals and also serve as a nucleophile (electron pair donor). The reactivity of thiolate anions (RS^-) towards nitrogen dioxide ($^{\cdot}NO_2$), carbon trioxide ion ($CO_3^{\cdot-}$), azide ($^{\cdot}N_3$) or superoxide exceeds that of RSH with the exception of hydroxyl radical ($^{\cdot}OH$), which efficiently abstracts H-atom from RSH [22]. RS^- reactivity towards non-radical oxidants, such as hydrogen peroxide (H_2O_2) [23], peroxynitrite [24-26] and hypochloric acid (HOCl) [27, 28] also exceeds that of RSH. RS^- reactions may proceed *via* one-electron oxidation or two-electron oxidation to generate as the initial products thiyl radical ($RS^{\cdot}$) (e.g., reaction 1) or sulfenic acid (RSOH) (e.g., reaction 2), respectively.

$$RS^- + CO_3^{\cdot-} \rightarrow RS^{\cdot} + CO_3^{2-} \quad (1)$$

$$RS^- + H_2O_2 \xrightarrow{H^+} RSOH + H_2O \quad (2)$$

One-electron oxidation of thiols yields the respective thiyl radicals ($E^o(RS^{\cdot}/RS^-) = 0.8$ V [29]), which readily oxidize other biomolecules or participate in a chain reaction yielding superoxide (reactions 3 and 4) and/or forming the respective peroxyl radical (reaction 5), which can oxidize further RS^- (reaction 6).

$$RS^{\cdot} + RS^- \rightarrow RSSR^{\cdot-} \quad (3)$$

$$RSSR^{-} + O_2 \rightarrow RSSR + O_2^{\cdot -} \quad (4)$$

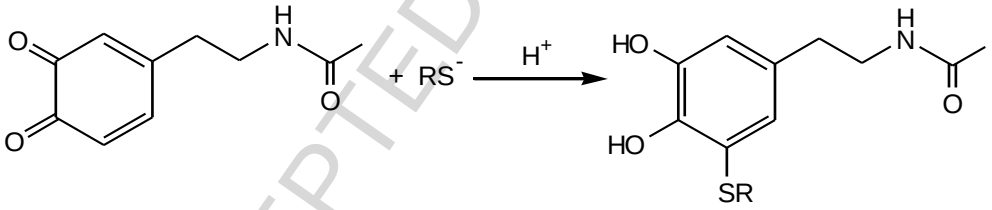
$$RS^{\cdot} + O_2 \rightarrow RSOO^{\cdot} \quad (5)$$

$$RSOO^{\cdot} + RS^{-} \xrightarrow{H^{+}} RSOOH + RS^{\cdot} \quad (6)$$

The two-electron oxidation of RS^{-} yields sulfenic acid, which produces the thiol-disulfide *via* reaction 7.

$$RSOH + RS^{-} \rightarrow RSSR + OH^{-} \quad (7)$$

The nucleophilic addition of thiols (Michael addition) also proceeds through RS^{-} as demonstrated for the reaction of NAC with N-acyldopamine quinone (reaction 8) [30].



(8)

Hence, the effective rate constants of thiols with various substrates at physiological pH increase with the decrease in their respective pK_a(-SH) values. The pK_a of NAC is relatively high (9.51 (Ionic strength (I) = 1 M), 9.87 (I = 0.02 M) [31, 32] compared to other common thiols such as GSH (8.7 [33]), cysteine (8.18 (I = 0.1 M [33]) and cysteamine (8.3 (I = 0.2 M [34]), and therefore its reactivity towards most oxidants and electrophiles is lower than that of other thiols.

The rate constants of the reactions of NAC with various substrates under different experimental conditions, which have been determined using various techniques, and are summarized in Table 1. NAC reacts rapidly with $^{\cdot}OH$, $^{\cdot}NO_2$, $CO_3^{\cdot -}$ and thiyl radicals, which eventually lead to the formation of $O_2^{\cdot -}$. NAC reacts also with nitroxyl (HNO), the reduced and protonated form of nitric oxide (NO) (pK_a(HNO) = 11.4 [46, 47]), which has been demonstrated as a unique species with potentially important pharmacological activities [48, 49]. The reactivity of

thiols towards HNO is relatively high [41], and the reaction proceeds *via* addition of RSH to HNO (reaction 9).

$$RSH + HNO \rightarrow RSNHOH \quad (9)$$

The adduct can react with another RSH to generate the thiol-disulfide and hydroxylamine (reaction 10) or it can form a sulfinamide *via* the formation of a sulfiminium intermediate (eq. 11) [50].

$$RSNHOH + RSH \rightarrow RSSR + NH_2OH \quad (10)$$

$$RSNHOH \rightarrow \rightarrow \rightarrow RS(O)NH_2 \quad (11)$$

There is no direct reaction between thiols and NO [51-54], and nitrosation of thiols by NO takes place via the intermediates formed during autoxidation of NO (reactions 12 – 14).

$$2\,NO + O_2 \rightarrow 2\,{}^{\cdot}NO_2 \quad (12)$$

$$NO_2 + NO \rightleftharpoons N_2O_3 \quad (13)$$

$$N_2O_3 + H_2O \rightarrow 2\,NO_2^- + 2H^+ \quad (14)$$

The nitrosation is initiated by ${}^{\cdot}NO_2$ (reaction 15) followed by the fast reaction of $RS^{\cdot}$ with NO (reaction 16) [55].

$${}^{\cdot}NO_2 + RS^- \rightarrow NO_2^- + RS^{\cdot} \quad (15)$$

$$RS^{\cdot} + NO \rightarrow RSNO \quad (16)$$

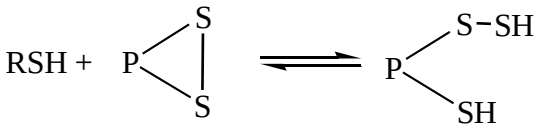
If NO competes efficiently with RS^- for ${}^{\cdot}NO_2$, nitrosation may take place *via* reaction 17 since N_2O_3 is capable of nitrosating directly the thiols [54].

$$RS^- + N_2O_3 \rightarrow RSNO + NO_2^- \quad (17)$$

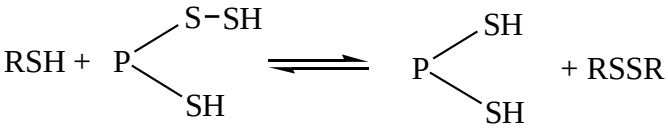
The rate constant of NAC reaction with peroxynitrite has been determined to be 415 $M^{-1}s^{-1}$ at pH 7.4 and 37°C [26], and relatively high concentrations of NAC are required (> 1 mM) to successfully compete with the self-decomposition of peroxynitrite ($\tau_{1/2}$ = 1.9 s at pH 7.4 and 37°C [56]), which produces $^{\cdot}OH$ and $^{\cdot}NO_2$ radicals [57]. Furthermore, peroxynitrite readily reacts with CO_2 (k = 5.8 x 10^4 $M^{-1}s^{-1}$ at pH 7.4 and 37°C [58]) to generate $^{\cdot}NO_2$ and $CO_3^{\cdot-}$ [57]. Since the concentration of CO_2 under physiological conditions is relatively high (1 – 2 mM), NAC cannot compete with CO_2 for peroxynitrite at concentrations below 0.1 M.

The toxicity of most quinones is attributed to their reduction to the corresponding semiquinone radicals, which are readily oxidized by oxygen forming $O_2^{\cdot-}$ and/or to their reaction with GSH leading to GSH depletion [59]. Hence, the effect of NAC on the detoxification of paraquat (methyl viologen) [60-62], doxorubicin [63-65] and acetaminophen [66, 67] might be attributed to NAC addition to doxorubicin and N-acetyl-p-benzoquinone imine (NAPQI) thus replacing GSH, to the reduction of the various semiquinone radicals to their corresponding hydroquinones and/or to an enhancement of GSH synthesis. The experimental results with doxorubicin and acetaminophen are in agreement with the suggestion that NAC helps to maintain GSH intracellular levels [65-67], although NAC was also shown to reduce *in vivo* the semi-iminoquinone back to acetaminophen [66], and to decrease paraquat-induced yield of $O_2^{\cdot-}$ [62]. Recently, the rate constant of NAPQI reaction with NAC was estimated to be 9-folds higher than that with GSH (Table 1) where NAPQI is reduced back to acetaminophen and the thiol is oxidized to RSSR [44]. Thus, it has been concluded that NAPQI participates in a catalytic reaction with GSH and NAC, and that addition of these thiols to NAPQI does not take place [44].

Thiol/disulfide interchange takes place spontaneously and may also be catalyzed by thiol transferase (e.g., eqs 18 and 19) [68].



(18)



(19)

The distribution of intracellular thiols among their thiol, disulfide and mixed disulfide forms depends, among other factors, on the redox potential of the RSH/RSSR pair at the intracellular pH. The observation that there is a linear correlation between the thiolate basicities (logK_a) and the redox potential of the RSH/RSSR pairs [69] implies that NAC is a stronger reducing agent than GSH, cysteine and cysteamine, e.g., the redox potential of NAC thiol/disulfide pair is higher by 63 mV and 106 mV than those of GSH/GSSG and cysteine/cystine redox pairs, respectively [32]. The adjacent *N*-acetyl and carboxylate groups (instead of the respective $-NH_3^+$ and −CONH− moieties in GSH and peptides) both stabilize the high electron density and the concomitant high basicity and strong reducing power of the thiolate site in NAC. Hence, NAC can reduce disulfide bonds in proteins thus disrupting their ligand bonding and altering their structures. The latter can rationalize the mucolytic activity of NAC, which can reduce the disulfide bonds in cross-linked mucous proteins. Other examples associated with protein modification induced by NAC include: decrease in the angiotensin II receptor binding in vascular smooth muscle cells [70]; blocking tumor necrosis factor (TNF)-induced signaling by lowering the cytokine affinity to the receptor [71]; reducing ligand binding capacity of betaglycan [72]; increasing c-Src cysteine reduced thiol content in cells, which primed the shift of the enzyme from the membrane into perinuclear endolysosomes [73]; modifying the redox state of functional membrane proteins with exofacial SH critical for their activity [74]. The thiolate basicity in GSH is approximately the same as that of typical thiolates in peptides and proteins. Consequently, a strong disulfide-reducing and concomitant mucolytic activity

of glutathione is not anticipated. Interestingly, some of the reducing processes take place also with GSH itself [74, 75].

NAC is a metal binding compound, as is the case with other thiols, having two potential coordination sites at the thiol and carboxyl groups where the latter is deprotonated at neutral pH. NAC is capable of binding transition metal ions, such as Cu(II) and Fe(III) [76], and heavy metal ions such as Cd(II) [77], Hg(II) [78] and Pb(II) [79] primarily through its thiol side chain. Thus, by chelating toxic metal ions NAC forms complex structures, which are readily excreted from the body removing them from intracellular or extracellular spaces. For example, NAC enhances the renal excretion of Cr(IV) and Pb(IV) in rats exposed to potassium dichromate and lead tetraacetate [80]; attenuates copper overload-induced oxidative injury in brain of rat [81]; decreases the concentration of Hg(II), which induced renal damage [82]; protects against Cd(II)-induced damage in rat liver cells [83]. On the other hand, NAC/Cu(II) significantly alters growth and induces apoptosis in human cancer lines whereas NAC/Fe(III) and NAC/Zn(II) do not [76].

2.1. NAC as an antioxidant

Reactive oxygen species (ROS), which oxidize lipids, proteins and DNA causing cellular damage and subsequent cell death, have been implicated in the pathophysiology of many disorders including neurodegenerative diseases. Endogenous antioxidant defense mechanisms include scavenging of ROS and reactive nitrogen species (RNS) or their precursors, binding of redox-active metal ions involved in the catalysis of ROS and RNS generation, and up-regulation of endogenous antioxidant defenses. Additionally, exogenous antioxidants could be very effective in diminishing the cumulative effects of oxidative stress.

Does NAC operate as an efficient antioxidant? NAC reacts neither with O_2 nor with NO. The rate constants of the reactions of NAC with $O_2^{\cdot-}$, H_2O_2 and peroxynitrite are relatively low (Table 1), which make the importance of these reactions under physiological conditions doubtful. In contrast, NAC readily reacts with highly oxidizing radicals such as $^{\cdot}OH$, $^{\cdot}NO_2$ and $CO_3^{\cdot-}$ and can

also bind redox-active metal ions. Thiols can also afford radio-protection through the donation of reducing equivalents, i.e., the carbon-centered radicals formed on DNA backbone or proteins by $^{\cdot}$OH attack can be restituted via hydrogen donation from RSH (sometimes called "repair reaction"). Such process is most likely effective under hypoxic conditions where thiols compete with oxygen for the carbon-centered radicals. While GSH is not a major intracellular radio-protector under normoxia [84], other thiols or reducing systems may be useful in the radiation response [85]. Interestingly, NAC does not protect against ionizing radiation-induced cell killing [85-88], possibly due to poor cell permeability (see Section 2.2.).

2.2. Does NAC cross cell membrane and blood-brain barrier (BBB)?

The therapeutic use of antioxidants depends also on their ability to cross the cell membrane and those designed as neuroprotective treatment in acute or chronic neurological disorders should readily cross the BBB. Figure 1 shows some of the characteristics of the BBB including the endothelial cell membrane. Cellular membranes are only permeable to lipid-soluble molecules, but allow selective intra-cellular passage of water and other substances via numerous channels and transporters.

Having a -COOH group (pK_a = 3.31 [32]) and a –SH group (pK_a = 9.87 [32]), NAC at pH 7.4 is negatively charged (Fig. 2). Its neutral, membrane permeating form, constitutes as little as 0.001% of the total NAC. Indeed, the partition coefficient of NAC in heptane/0.1 M phosphate buffer (pH 7.4) is $P = 4 \times 10^{-4}$ (log$P$ = -3.4) [89], and its distribution coefficient in octanol/0.1 M phosphate buffer (pH 7.4) is $D = 4 \times 10^{-6}$ (log$D$ = -5.4) while log$D$ = 0.85 for NAC ethyl ester, which is a neutral molecule at pH 7.4 [15]. The neutral form of NAC becomes predominant at pH < 3.3, allowing membrane penetration from the gastric fluid (pH 1.5 – 3.3) by passive diffusion. Once NAC enters the systemic circulation by the gastric or by other intravenous routes, it can only leave the blood vessels after N-deacetylation or by a carrier-mediated active transport, which has not yet been reported for NAC. Similar to NAC, GSH (pK_{a1}(-COOH) = 1.9, pK_{a2}(-COOH) = 3.5,

pK_a(-SH) = 8.7, pK_a(-NH_2) = 10.1 [33]) is in its ionic form at pH 7.4 and does not cross the cell membrane and BBB [90, 91], but its precursor cysteine (pK_a(-COOH) = 1.9, pK_a(-SH) = 8.18, pK_a(-NH_2) = 10.36 [33]) is a neutral species at 1.9 < pH < 8.2 that does cross the cell membrane and BBB, and is also transported by a ubiquitous more effective alanine-serine-cysteine sodium-dependent transport system [92] or by a less efficient hetero-exchange with glutamate as cystine in astroglial cells [93].

Some papers refer to NAC as a membrane-permeable cysteine precursor [18, 94-97], others assume that NAC operates inside the cells [2]. Cotgreave *et al*. [98] reported that isolated intestinal epithelial cells of rats rapidly metabolize ^{14}C-NAC (cysteine moiety) to ^{14}C-cysteine when a dose of NAC was inserted into the isolated intestinal segment, and neither free NAC nor disulfide-bound NAC could be detected intracellularly. In other experiments, NAC was not detected, free or bound in disulfides, in either of the bronchoalveolar lavage components of volunteers/patients receiving the drug orally [99, 100]. Giustarini et al. [15] have shown that when rats were intravenously injected with NAC, the concentrations of NAC and cysteine in RBC were very small, but increased dramatically when NAC was replaced with NAC ethyl ester. Mazor *et al*. [101] reported that NAC treatment of red blood cells (RBC) exposed to oxidizing agents, as well as of control cells, enhanced cellular thiol levels and concluded that NAC penetrates the cells easily although such an enhancement can be attributed to penetration of cysteine formed outside the cells via N-deacetylation.

The published reports on the ability of NAC to cross the BBB are also contradictory. Sheffner *et al*. [102] have demonstrated that 2 h following oral administration of ^{35}S-NAC to rats, an appreciable radioactivity was observed in all tissues tested. The highest concentration of ^{35}S was in kidney and liver, followed in descending order by adrenal, lung, spleen, blood, muscle and brain. McLellan *et al*. [103] reported that the intraperitoneal or tail vein injection of ^{14}C-NAC to mice resulted in its uptake into most tissues tested, except for the brain and spinal cord. Similarly, Arfsten *et al*. [104] reported that ^{14}C-NAC and/or its metabolite cysteine rapidly distributed to most

tissues, excluding the brain, after intra-oral administration of the drug in rats. Erickson *et al*. [105] measured low level of ^{14}C-NAC uptake by the brain following intraperitoneal administration to mice, and reported that the BBB permeability of NAC increased following intraperitoneal administration of lipopolysaccharide (LPS). Offen *et al*. [106] have shown that oral or intraperitoneal administration to mice of NAC or NAC amide, which is a neutral molecule at pH 7.4, resulted in the appearance of NAC amide but not of NAC in brain extract. When NAC was administrated intravenously to rats, only low levels of cysteine were measured [15]. When NAC was replaced with NAC ethyl ester, there was a dramatic increase in the levels of NAC and cysteine due to rapid hydrolysis of NAC ethyl ester in the brain [15]. By contrast, Neuwelt *et al*. [107] reported that ^{14}C-NAC crossed the BBB extremely well when given intra-arterial into the carotid artery of rats, and Farr et al. [108] have demonstrated that the majority of ^{14}C-NAC crossed the BBB when mice were administered with the drug by injection into the jugular vein. A plausible explanation is that NAC can enter the cell when the membrane is impaired under oxidative stress, i.e., formation of aqueous pores (leaks), permeable to both non-electrolytes and ions [109-111]. Indeed, Erickson *et al*. [105] used ^{14}C-NAC and showed that LPS increases the BBB permeability of NAC, but this observation does not explain in their LPS model the protective effect of NAC in the serum, but not in the brain.

The assay of NAC in biological systems is complex because as a typical thiol, it might be oxidize to disulfide species or undergo transhydrogenation reactions with other thiol redox couples, resulting in the potential introduction of artifacts. An alternate experimental approach, which has not been previously tested, would be to label the carbon on the acetyl rather than on the cysteine moiety coupled with measurements of intracellular thiol levels.

3. Biological activities of NAC

NAC has been shown to interact with various metabolic pathways including, but not limited to, regulation of cell cycle and apoptosis; carcinogenesis and tumor progression; mutagenesis; gene

expression and signal transduction; immune-modulation; cytoskeleton and trafficking; and mitochondrial functions [2]. As presented herein, the GSH-independent mechanisms underlying NAC activity are only partially understood. Furthermore, since the reactions of NAC with various ROS as well as reactive nitrogen species (RNS) are kinetically unfavorable, the elucidation of such mechanism(s) is not straightforward. It is not attempted to cover the entire literature but rather to present different aspects of NAC biologic activities and cite various examples.

3.1. NAC and regulation of cell cycle and apoptosis

Various effects of NAC on regulation of cell cycle and apoptosis have been reported, including the inhibition of proliferation of mammalian, normal human cells [112-114], and also of transformed cells [115]. The authors of these studies found that NAC modulates the levels of various target genes and/or proteins. For example, the NAC-induced inhibition of proliferation of keratonocytes, and colon and ovary carcinoma cells was associated with up-regulation of p53, small heat shock protein 27, N-myc downstream-regulated gene-1, E-cadherin, and with suppression of microtubules aggregation and of c-Src tyrosine kinase [115]. More importantly, studies have clearly shown that NAC can affect cell cycle regulation and inhibit induction of cyclin D and DNA synthesis, which led to a G1 arrest of phorbol ester-induced NIH 3T3 cells *in vitro* [116]. NAC also induced cyclin-dependent kinase inhibitors such as p16 and p21, independent of p53, which resulted in G1 arrest [117]. An additional effect of NAC on regulation of cell cycle was seen upon studying pheochromocytoma PC12 cells, commonly used for the study of cellular signaling system. NAC also activated Ras-extracellular signal regulated kinase (ERK), inducing immediate early genes such as *c-fos* and *c-jun*, and inhibiting DNA synthesis and proliferation [118]. Similarly, treatment of hepatic stellate cells with NAC resulted in sustained activation of ERK, Sp1 phosphrylation, induction of p21 expression and G1-growth arrest [119]. Apparently, this effect on mitogen-activated protein kinase signaling pathways was shown to depend on the redox-state of the cells [120]. Inhibition of angiotensin II-ERK mitogenic activation by NAC was also seen for cardiac

fibroblasts [121]. Interestingly, NAC inhibited phosphorylation of the angiotensin-II epidermal growth factor receptor, but not the receptor's stimulated response. The inhibition of the trans-activation of the receptor indicates that NAC affected the cross-talk between a G-protein linked receptor and a tyrosine kinase receptor [122]. Numerous studies conducted using both *in vivo* and *in vitro* experimental models have also demonstrated that NAC can modulate apoptosis [123]. For example, NAC was shown to prevent apoptosis of serum-deprived neuronal cells [124], glutamate-induced apoptosis of oligodendrocytes, and TNF-α-induced apoptosis of fibroblasts [125] and of human U937 neurons [126]. Similar protective effect of NAC was also shown against $O_2^{\cdot-}$-mediated apoptosis of selenite-treated HepG2 cells. The apoptotic pathway initiated by elevation of $O_2^{\cdot-}$ flux was characterized by the release of cytochrome c, alteration of mitochondrial membrane potential, caspase-3 activation and DNA fragmentation. Treatment with NAC significantly reduced the level of $O_2^{\cdot-}$ and inhibited the apoptotic pathway [127]. NAC was also shown to protect against peroxynitrite-induced apoptosis by modulating levels of $O_2^{\cdot-}$ and H_2O_2 [128], and to afford protection against cocaine-induced apoptosis by up-regulating anti-oxidative enzymes such as manganese superoxide dismutase (Mn-SOD), Cu/Zn-SOD, glutathione peroxidase [129] and catalase [130]. The anti-apoptotic effect of NAC is reportedly associated with changes in various genes/proteins such as an increase in *c-jun* and *c-fos* expression in TGF-β-treated human ovarian adenocarcinoma cell line [131]. In particular, the anti-apoptotic effect of NAC was associated with modulation of the levels of cell cycle proteins such as p53, retinoblastoma, and cyclin-dependent kinase inhibitor p21. However, evidence has shown that the modulation of apoptosis afforded by NAC depends on both cell-type and stimuli-specific and is thus very complex [132]. Underscoring this complexity are several reports demonstrating pro-apoptotic effect of NAC as well [112]. NAC enhanced hypoxia-induced caspase-3 activation and apoptosis in murine embryonic fibroblasts, and human pancreatic, melanoma and lung carcinoma cells. NAC inhibited hypoxia-induced nuclear factor kappa B (NF-κB) binding to DNA and NF-κB-dependent gene expression [133, 134]. Thus, the conclusion that NAC is solely an anti-apoptotic agent is probably an over-generalization.

3.2. NAC, signal transduction and gene expression

The effects of NAC are most commonly attributed to its capability to scavenge ROS and elevate cellular GSH levels [35, 60, 135-142], although it has also been shown that thiols supplementation (oral or intra-peritoneal) can be associated with an increase of cysteine level without a concomitant rise in GSH synthesis [143]. This is especially true when GSH pools are normal [144]. Regardless of its origin, the redox state of thiol proteins is widely considered to be a principal mechanism by which ROS and RNS are integrated into cellular signal transduction pathways [19, 145], and it is not surprising that NAC affects redox-sensitive signal transduction and gene expression both *in vitro* and *in vivo*. For practical reasons, the following discussion is focused on the effects of NAC on NF-κB, which is central to the regulation and expression of stress response genes under inflammatory and oxidative challenges [146]. Nevertheless, NAC affects also other signal transduction pathways and the expression of various genes [123], and directly modulating the activity of common transcription factors both *in vitro* and *in vivo*. NF-κB represents a family of proteins sharing the Rel homology domain, which bind to DNA as homo- or hetero-dimers (p50/p65) and activate a multitude of cellular stress-related and early response genes such as genes for cytokines, growth factors, adhesion molecules, and acute-phase proteins. While oxidative stress is an effective inducer of NF-κB, treatment of cultured cells *in vitro* or clinical sepsis with NAC suppressed NF-κB activation and subsequent cytokine production [147, 148], possibly reflecting redox-regulation of transcription factor expression. NF-κB is naturally bound to an inhibitor of NF-κB (I-κB) that prevents its nuclear translocation. Dissociation of I-κB following its phosphorylation by specific kinase of NF-κB (IKK) allows its poly-ubiquitination and degradation by the 26S proteasome, and the transport of NF-κB to the nucleus. Administration of NAC suppressed the 19S regulatory, but not the 20S catalytic subunit of 26S proteasome activity, thereby inhibiting NF-κB activation [149]. Furthermore, NAC also inhibited the IKK themselves [150]. In contrast, NAC was shown to activate NF-κB and elevate at least one of its target genes, Mn-SOD in

human microvascular endothelial and lung adenocarcinoma (A549) cells. As other reducing agents activate NF-κB, it has been suggested that an oxidized form of NF-κB, which is not in complex with I-κB, exists in the cytosolic fraction and must be reduced (reduction of a disulfide in the p50 or p65 subunits) to exert its DNA binding activity. Indeed, NF-κB has been shown to be activated in the absence of I-κB degradation through an iron-mediated mechanism [151, 152]. Modifications of p65, such as phosphorylation at serine 536, required for optimal function were also induced by NAC through activation of phosphatidylinositol 3-kinase [153]. It is possible that these seemingly contradicting results could actually converge in a single signaling event for a specific gene such as Mn-SOD [154]. Interestingly, N-acetyl-D-cysteine, which cannot be de-acetylated or participate in the biosynthesis of GSH, activates NF-κB [155].

3.3. NAC, cytoskeleton and trafficking

NAC has been shown to modulate the levels of various adhesion molecules [156, 157] and to affect cytoskeleton structure and trafficking. *In vitro* studies have demonstrated that treatment of human epidermoid carcinoma cells with NAC protected against menadione (2-methyl-1,4-naphtoquinone) induced oxidation stress. The effect of NAC was attributed to improved cell adhesion properties. It was suggested that NAC modulated the kinetics of focal points development rather than changing the expression of receptors for extracellular matrix molecules [158]. These findings that NAC can modulate cytoskeleton-dependent processes such as cell-cell interaction have been corroborated also using non-adherent cells [159, 160]. Intracellular transport of NF-κB was also affected by NAC and the cellular redox state. Oxidative modification of tubulin by disulfide links between cysteine-containing subunits was shown to affect its assembly into microtubules. Addition of NAC to cultured neurons and developing fetal rat brain restored tubulin dynamics and improved the nuclear transport of NF-κB [161]. NAC was also shown to modulate the levels of cluster of differentiation 11b (CD11b), a surface-integrin that bridges cytoskeleton and cell membranes. CD11b, which acts as a binding protein for intracellular adhesion molecule-1

undergoes ROS-mediated up-regulation in activated microglial cells in various neurodegenerative diseases. In contrast, addition of NAC was shown to down-regulate the levels of CD11b *via* an NO-guanylate cyclase cGMP pathway [162]. NAC was reported to affect trafficking of intracellular proteins.

Cytochrome P450 proteins, which are known for their metabolic role in detoxification of drugs, are also responsible for generation of deleterious ROS. Studies of transiently transduced HepG2 cells expressing endoplasmic reticular cytochrome P450 3A4 have shown that treatment with NAC not only reduced the levels of ROS, but also and more importantly suppressed the secretion of proteins such as intracellular adhesion molecule-1 (ICAM-1), metalloproteinase-2 (MMP-2), platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). Thus, NAC was shown to alter both the autocrine and paracrine signaling [163].

3.4. NAC and immuno-modulation

Overwhelming data supports the immuno-modulatory activity of NAC. Clinically, NAC improved the ocular symptoms of subjects with Sjogren's syndrome [164], enhanced natural killer and T-cell function, and delayed the reduction in CD4+ levels in HIV patients [165, 166]. Administration of NAC to post-menopausal women improved immune functions as exhibited by enhanced phagocytic capacity, leukocytes chemotaxis, natural killer function, and decreased TNF-α and interleukin-8 (IL-8) levels [167]. NAC was also proven beneficial in patients with the autoimmune disorder systematic lupus erythematosus (SLE). In these patients, the mechanism underlying NAC activity was ascribed to a blockade of the mammalian target of rapamycin (mTOR) in T lymphocytes. Activation of mTOR occurs upon GSH depletion or after exposure to NO, which causes mitochondrial hyperpolarization and can lead to down-regulation of the transcription factor forkhead box P3 and subsequent decline in CD4+ CD25+ T cell population. NAC blocked the activation of mTOR and increased the number of T lymphocytes [168]. Similar *in vitro* enhancement of T-cell growth and function (production of IL-2) was demonstrated when

peripheral blood T-cell were treated with NAC [169]. NAC was reported to affect both cellular and humoral immunity by inhibiting the production polyclonal immunoglobulins (Ig) from B cells as it down-regulating the expression of B cell co-stimulatory surface molecules (CD40 and CD27), and IL-4 production [170]. NAC also enhanced the phagocytosis of IgG-opsonzied yeast particles by human polymorphonuclear leukocytes (PMN) [171], and the antibody-dependent cellular cytotoxicity of PMN from HIV+ patients [172]. In fact, NAC reversed the T1 helper cells/T2 helper cells cytokine balance in activated macrophages [173]. Similarly, NAC impaired chemotaxis of PMN and monocytes [174], and phorbol-stimulated aggregation of PMN [175], while concomitantly lowering H_2O_2 levels [176]. Additional changes in the levels of ROS/RNS were also reported for NAC-associated immuno-modulatory effects. NAC inhibited NF-κB-mediated LPS, IL-1β, or interferon (INF-γ)-induced NO production by macrophages, glial cells and astrocytes [177]. These findings are in agreement with the inhibition of inducible NO synthase by NAC *in vivo*. [178]. NAC also decreased lipid peroxidation and generation of $O_2^{\cdot -}$ by activated PMN in a calcium-independent manner [179].

3.5. NAC and mitochondria

Unsurprisingly, studies have demonstrated that NAC can affect mitochondrial processes, especially those associated with oxidative phosphorylation. Animal studies have shown that long term treatment with NAC can improve both heart- and brain-mitochondrial activity in rats [180], and to protect against age-related decline in specific activities of complex I, IV and V in hepatic mitochondria of mice [181]. Similar protective effect was also seen in rats subjected to traumatic brain injury. NAC not only restored the mitochondrial electron transfer but also improved calcium uptake activity [182]. *In vitro* studies corroborated these findings and showed that NAC can protect hepatic mitochondrial cytochrome c oxidase, complex I, IV and V activities, preserve ATP levels [183, 184], and mitochondrial potential [185]. The restoration of the electron chain transfer process by NAC was attributed at least in part to the redox-state of the thiols groups in the mitochondrial

complex [186, 187]. In another animal study, NAC was shown to protect against INF-γ induced xanthine oxidase mediated suppression of hepatic cytochrome P450. The protective effect was attributed to the scavenging of superoxide by NAC rather than to its non-heme iron chelation properties, although the latter does occur [188]. Other studies have shown that NAC mildly stimulated detoxifying phase II enzymes but had little influence on phase I enzymes [142, 189]

3.6. NAC, mutagenesis, carcinogenesis and tumor progression

NAC demonstrated anti-mutagenic and anti-neoplastic activities, which include blocking of electrophilic metabolites and of direct-acting compounds, either of endogenous or exogenous source, attenuation of several xenobiotic-metabolizing pathways, and protection of DNA-dependent nuclear enzymes mutations [142, 190]. The modulation of genotoxic, oncogenic, and tumor progression processes by NAC was studied extensively in biochemical, cellular and whole animal models [123]. For example, NAC inhibited hydroxyl-generated adduct of isolated DNA [191], and NO-induced single-strand DNA breaks [192]. NAC was also shown to protect endothelial, lymphoid and epithelial cells against genotoxic insults *in vitro* [193, 194]. Similarly, NAC also attenuated cytogenetic alterations in animals exposed to cigarette smoke [195, 196]. The anti-proliferative and anti-apoptotic effects of NAC and some of its interaction with various signal transduction pathways were described in previous paragraphs. NAC was reported to modulate tumor progression both *in vitro* and *in vivo*. It was shown to inhibit angiogenesis (e.g. inhibition of production of vascular endothelial growth factor) [113, 197, 198] and to decrease tumor invasiveness. This chemopreventive feature was attributed to inhibition of extracellular matrix degrading enzymes. For example, NAC was shown to suppress type IV collagenease and to prevent invasion and metastasis in murine models [199]. It was also shown to inhibit MMP-2 and MMP-9 in human cancer cells, which could alter tumor progression and metastasis [200, 201]. At least in the case of MMP-9, the inhibition was attributed to S-nitrosylation of the pro-metaloproteinase.

Computational molecular modeling demonstrated the feasibility of NAC docking at the MMP-9's nearest the active-site zinc [202].

3.7. NAC and heart disease

The possible therapeutic effects of NAC in heart disease were addressed in several studies. Equivocal effects of NAC on the levels of homocysteine and lipoprotein in plasma have been reported [203-205]. Still, NAC was shown to suppress the severity of experimental atherosclerosis in apoliproteinE-deficient mice by decreasing $O_2^{\cdot-}$ levels and macrophage aggregation [206]. Using the same animal model NAC was shown to inhibit NF-κB, MMP-2 and MMP-9, and to suppress the deleterious atherosclerotic plaque destabilization process [157]. NAC was also suggested as a therapeutic in ischemia-reperfusion injury, where ROS play an important role, by affording protection against ischemia-reperfusion injury in the Langendorff isolated heart model [207]. In this model the effect of NAC was ascribed to a direct scavenging of hydroxyl radicals and to improvement of the coronary microvasculature. The latter could result from the formation of S-nitrosothiols and inhibition of angiotensin converting enzyme [208]. Interestingly, NAC was shown to improve cardiac function without modulating the levels of GSH [209]. [209]. Clinically, administration of NAC with nitroglycerin and streptokinase resulted in reduction of oxidative damage and improved left ventricular function in patients suffering from myocardial infarction [210, 211]. The cardio-protective effects of NAC were also associated with changes in platelet aggregation [212] and with macro-vascular dilation [213]. Similarly, NAC was shown to improve vascular dilation and to restore cerebrovascular responsiveness in animals subjected to experimental brain injury [214]. NAC was also reported to affect microvasculature through inhibition of the mitogen-and stress-activated protein kinase endothelin-1 pathway *in vitro*. NAC suppressed expression of endothlin-1, a potent vasoconstrictor produced by endothelial cells, by inhibiting p65 Ser276-MSK phosphorylation of NF-κB. This is in contrast to previous reports, which described NAC-mediated inhibition of NF-κB activation induced by TNF-α as a general phenomenon, the

drug had no effect on I-κB degradation, p65 translocation, or phosphorylation of Ser536, indicating that such activity is cell-type specific [215].

3.8. NAC and psychiatric disorders

The field of neuropsychiatry provides an excellent opportunity to illustrate the mechanistic complexity of NAC. This is mainly because many neuropsychiatric disorders have a multi-factorial etiology that involves inflammatory pathways, glutamatergic transmission, oxidative stress, GSH metabolism, mitochondrial function, neurotrophins and apoptosis [12]. Since NAC is known to interact with most of these pathways it has been studied for its possible use for treatment of various neuropsychiatric disorders. Indeed, in recent years more than twenty clinical trials (randomized or otherwise) have employed NAC as an adjunctive treatment in various disorders. These include methamphetamine [216] and cannabis dependence [217, 218], nicotine [219, 220] and cocaine addiction [221-223], pathological gambling [224], obsessive-compulsive disorder [225], trichotillomania, nail biting and skin picking [226], schizophrenia [227, 228], bipolar disorder [228, 229], autism [230], and Alzheimer's disease [231, 232]. Interestingly, in most of these studies NAC was proven beneficial as it improved clinical outcome. Most of the plausible mechanisms presented herein are not exclusive to neuropsychiatric disorders but rather pertain to a broader scope of patho-physiological processes. This is also evidenced by similar efficacy in neurological conditions such as Alzheimer's disease.

As stated above, NAC was proven an effective immuno-modulator. Similarly, it was used to modulate peripheral and central nervous system inflammatory pathways and cytokine levels in neuropsychiatric disorders. NAC reduced the levels of pro-inflammatory cytokines TNF-α, IL-1β and of NF-κB in rodents subjected to traumatic brain injury or focal cerebral ischemia [233, 234], and decreased the levels of pro-inflammatory cytokines IL-6 and IL-10 in LPS-treated rat fetal brain [235]. In particular, NAC suppressed microglia activation [236], which are known to promote

neurotoxicity [237], and to inhibit NF-κB-mediated LPS, IL-1β, or INF-γ-induced NO production by macrophages, glial cells and astrocytes [177].

NAC also affects neurotransmission. It can modulate the levels of excessive extracellular glutamate, which cause excitotoxic damage in models of schizophrenia and addiction. For example, NAC normalized the levels of glutamate in the nucleus accumbens of cocaine-treated rats [238]. NAC appears to modulate intracellular calcium, which is germane to the dysregulation of receptor mediated calcium release, documented in a number of psychiatric disorders [239-241]. NAC can also drive the cystine/glutamate antiporter to decrease the levels of glutamate and suppress the activation of metabotropic glutamate receptors (mGluR2/3), which ultimately reduce the synaptic release of glutamate [12]. It has been suggested that NAC-induced changes in GSH levels could modulate the N-methyl-D-aspartate activity. Dysregulation of the neurotransmitter dopamine is also considered a contributing factor in neuro-toxicity. Additionally, dopamine can undergo auto-oxidation with molecular oxygen to produce superoxide and semiubiquinone, which can participate in deleterious processes. It has been demonstrated that NAC blocked amphetamine-triggered dopaminergic response *in vivo* [242] and prevented the down-regulation of dopamine transporter [243]. Expectedly, it has also been suggested that NAC can modulate dopamine release *via* modulation of the cellular GSH levels and redox status. Alterations in GSH and ROS levels and dysregulation of mitochondrial function are highly associated with neuropsychiatric disorders [12]. Treatment with NAC inhibited lipid peroxidation and increased the activity of glutathione reductase in brain tissue of animals [244], restored the mitochondrial membrane potential in astroglial cells [245], and replenished GSH levels in brain tissue of animals and improved their function [246, 247]. Similarly, treatment with NAC was associated with protection of mitochondrial Complex I and IV activities both *in vivo* and *in vitro* [248]. Loss of neuronal cells has been implicated in neuro-degenerative disorders such as Alzheimer's and Parkinson's diseases. NAC enhanced the survival of cultured neurons [249], and inhibited the 6-hydroxydopamine-induced dopaminergic neuron loss both *in vitro* and *in vivo* [250, 251]. NAC inhibited apoptosis associated with trophic

factor deprivation [125] *via* regulation of cell cycle [124, 252] . Its anti-apoptotic effect was associated with an increase in the levels of phosphorylated ERK and MAPK [253]. Treatment of EAAC1-deficient (excitatory amino acid transporter) mice with NAC reduced ROS levels, increased GSH levels, protected against dopaminergic neurons cell loss, and enhanced motor function [254]. Using similar animal model, NAC reversed cognitive impairment [255], although this finding was not replicated clinically [256].

4. Clinical caveats implied by the effects of NAC

Although NAC is traditionally considered as an antioxidant with proven benefits in various clinical conditions and experimental models, it is also implicated in some deleterious processes both *in vitro* and *in vivo*. Autoxidation of thiols in the presence of redox-active transition metals can lead to biological damage via the thiol oxidation by the metal ion (reaction 20) followed by the generation of superoxide (reactions 3 – 4, 21), H_2O_2 (reaction 22) and $^{\cdot}OH$ (reaction 23) [257].

$$RS^- + M^{n+} \rightarrow RS^{\cdot} + M^{(n-1)+} \quad (20)$$

$$M^{(n-1)+} + O_2 \rightarrow M^{n+} + O_2^{\cdot -} \quad (21)$$

$$2O_2^{\cdot -} + 2H^+ \rightarrow O_2 + H_2O_2 \quad (22)$$

$$M^{(n-1)+} + H_2O_2 \rightarrow M^{n+} + {}^{\cdot}OH + OH^- \quad (23)$$

Indeed, it has been demonstrated that NAC increased $^{\cdot}OH$ generation in a system with Fe(III)-citrate and H_2O_2 by reducing ferric iron to its catalytic, active Fe^{2+} [258]. NAC also induced DNA damage in the presence of Cu(II), and bathocuproine, a specific Cu(I) chelator, and catalase inhibited the DNA damage [259]. The role of metal ions has been demonstrated *in vivo* when NAC plus deferoxamine (an efficient iron chelator) protected rats against oxidative stress and improved [260] and improved the oxidative parameters in ill patients with prolonged hypotension [261].

Since NAC has the potential to act as a pro-oxidant, it has been suggested to avoid administering it in the absence of a significant oxidative stress. NAC showed no benefit and in fact was noted to be harmful if given 24 h after admission to the intensive care unit in patients with multi-system organ failure [262]. Interestingly, administration of NAC to healthy individuals decreased their GSH/GSSG ratio [263]. The construct of hormesis refers to a biphasic dose response to an agent where a low dose stimulation or beneficial effect is contrasted by a high dose inhibitory or toxic effect. It is an adaptive signaling response of cells and organisms to a moderate stimulus [264]. As an exemplar, low grade oxidative stress upregulates superoxide generation to trigger changes of gene expression that attenuate aging effects, a pathways that is blocked by antioxidants such as NAC and vitamin C [265]. The clinical implications of this theoretical effect remain to be confirmed.

5. Concluding remarks

The molecular mechanisms by which NAC exerts its diverse effects are complex and still unclear. NAC has been shown to interact with numerous biochemical pathways. Its main mechanism involves serving as a precursor of cysteine and replenishing cellular GSH levels. Additional mechanisms include scavenging of $^{\cdot}OH$, $^{\cdot}NO_2$, $CO_3^{\cdot-}$ and thiyl radicals as well as detoxification of semiquinones, HOCl, HNO and heavy metals. Importantly, under physiological conditions NAC does not react with NO, superoxide, H_2O_2 and peroxynitrite. Possible chemical and biochemical routes involving NAC are summarized in Fig. 3.

What differentiates NAC from other thiols? NAC is a small molecule and its pK_a(-SH) is higher than most natural thiols and their derivatives, which can participate in all the reactions outlined in Table 1 more efficiently than NAC at physiological pH. However, the relatively high pK_a of NAC implies that the redox potential of the NAC thiol/disulfide pair is higher than that of other thiols, and that NAC can efficiently reduce disulfide bonds in proteins thus disrupting their ligand bonding and altering their structures as in the case of mucous proteins. In addition, NAC is a

small molecule and might compete with larger reducing molecules in sterically less accessible spaces. It is very likely that the pathways described in Fig. 3 only partially explain the divergent biological effects of NAC, and further studies are required for determining its ability to cross the cell membrane and the blood-brain barrier as well as elucidating its reactions with components of cells signaling pathways.

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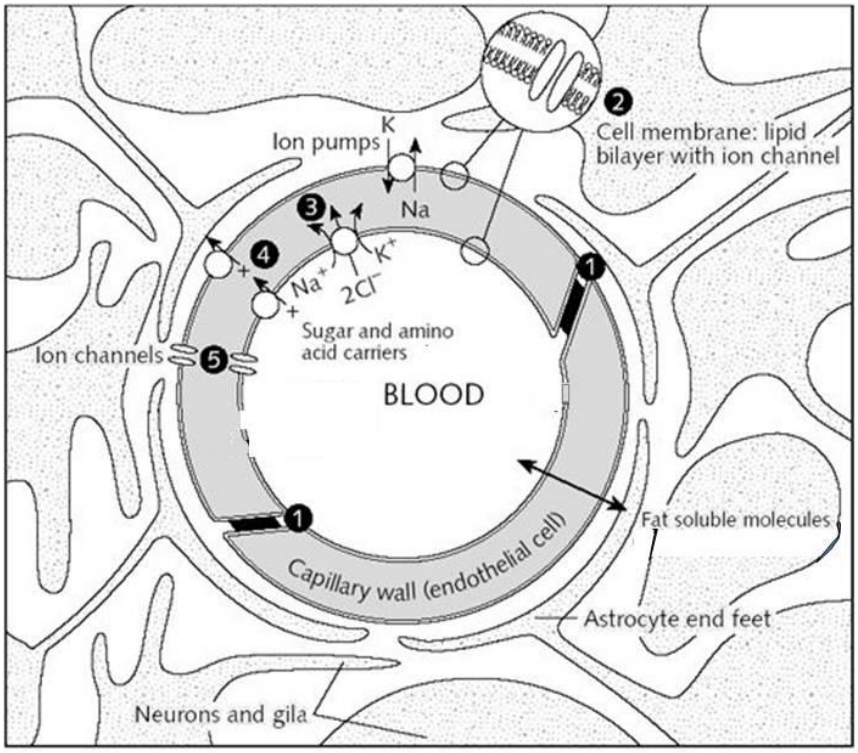
Figures Legends

Figure 1. Characteristics of the BBB are: (1) tight junctions that seal the pathway between the endothelial cells; (2) lipid nature of the cell membranes of the capillary wall, which makes it a barrier to water-soluble molecules; (3), (4), and (5) represent some of the carriers and ion channels. The figure is a modification of the one in http://www.answers.com/topic/blood-brain-barrier.

Figure 2. Distribution of the various protonated forms of NAC as a function of pH using pK_a(-COOH) = 3.31 and pK_a(-SH) = 9.87 at I = 0.02 M [32].

Figure 3. Plausible routes for the biological activities of NAC (red color - major routes, blue color - plausible routes, black color - insignificant routes under physiological conditions).



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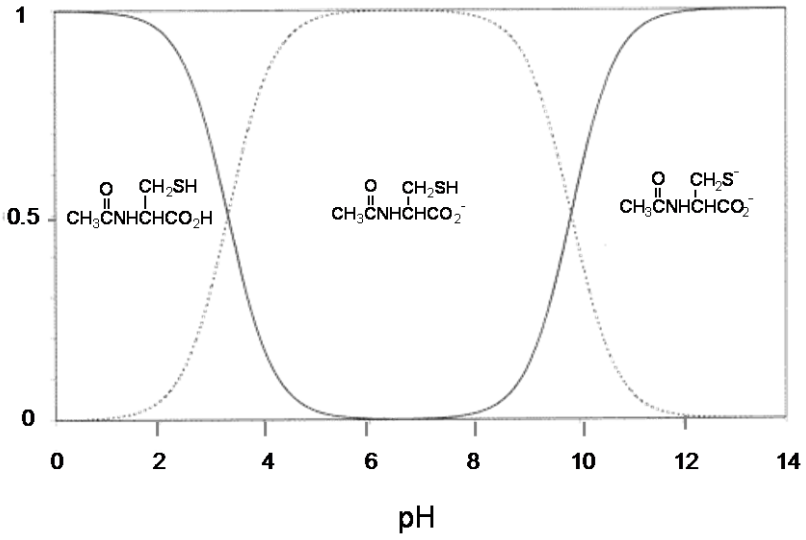

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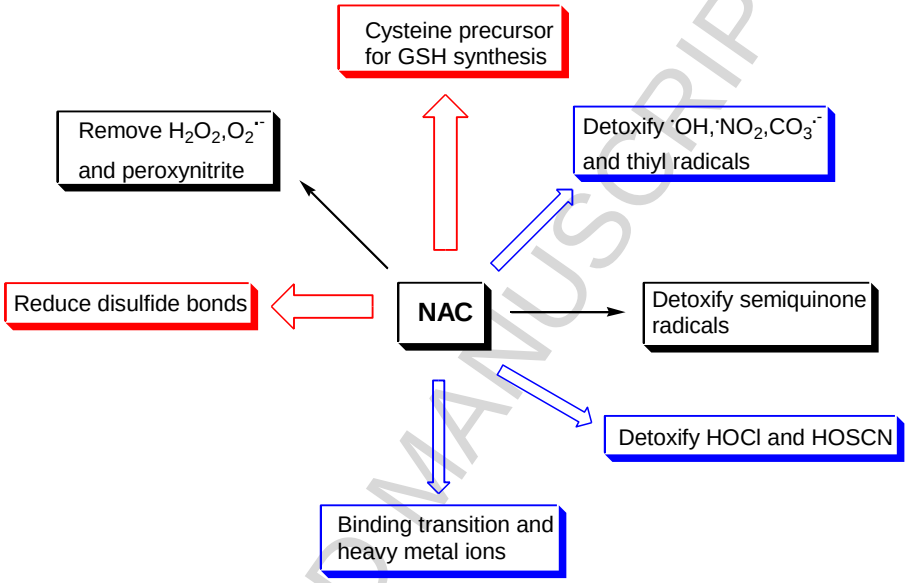

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Table 1. Rate constants of NAC reactions with various compounds

Compound	Rate constant ($M^{-1}s^{-1}$)	Exp. conditions	Ref.
$^{\cdot}OH$	1.36×10^{10}	pH 7, RT	[35]
$CO_3^{\cdot-}$	$\approx 1.0 \times 10^{7}$	pH 7, RT	[36]
	1.8×10^{8}	pH 12, RT	[36]
$O_2^{\cdot-}$	68 ± 6	pH 7, RT	[37]
	$< 10^{3}$	pH 7.4, 25°C	[35]
H_2O_2	0.16 ± 0.01	pH 7.4, 37°C	[23]
	0.85 ± 0.09	pH 7.4, 25°C	[35]
$^{\cdot}NO_2$	$\approx 2.4 \times 10^{8}$ (a)	pH > pK_a, RT	[38]
	$\approx 1.0 \times 10^{7}$ (b)	pH 7.4, RT	[39]
NACysS$^{\cdot}$	1.1×10^{9}	pH = 11.2, RT	[40]
	7×10^{8}	pH = 8.5, RT	[40]
HNO	5×10^{5}	pH 7.4, 37°C	[41]
HOSCN	7.7×10^{3}	pH 7.4, 22°C	[42]
HOCl	$> 10^{7}$ $M^{-1}s^{-1}$ (c)	pH ≈ 7, 21- 24°C	[27, 43]
N-chlorotaurine	46 ± 7	pH 7.4, 24°C	[43]
peroxynitrite	415 ± 10	pH 7.4, 37°C	[26]
NAPQI	$(1.36 \pm 0.2) \times 10^{4}$	pH 7, 25°C	[44]
e_{aq}^{-}	5×10^{9}	pH 7.1, RT	[40]
R′R″C$^{\cdot}$-OR‴	$10^{7} - 10^{8}$ (d)	pH 5, RT	[45]
5,5′-dithiobis-(2-nitrobenzoic)	$(1.77 \pm 0.21) \times 10^{5}$	pH 7, RT	[15]

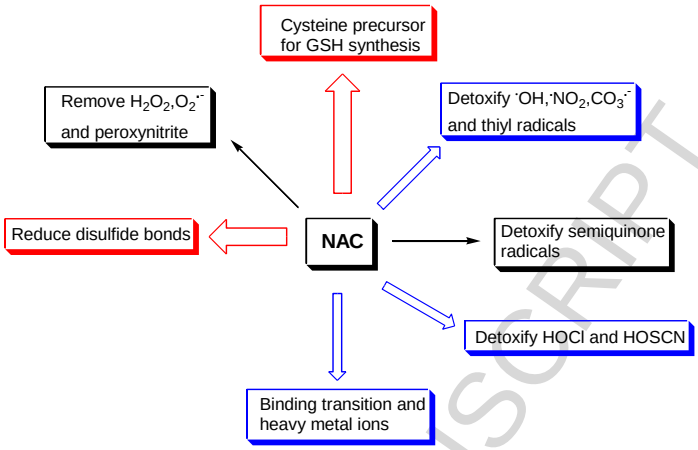
RT – room temperature

a – Estimated using the rate constant determined for cysteine at pH 9.2 [38].

b – Estimated using the rate constants 2×10^{7} and 5×10^{7} $M^{-1}s^{-1}$ determined at pH 7.4 for cysteine and GSH, respectively [39].

c – Estimated using the lower limit of the rate constant for HOCl reaction with GSH at pH 7 and 21°C (> 10^7 $M^{-1}s^{-1}$) [27] and the ratio 0.5 between the rate constants of HOCl reactions with cysteine and GSH at pH 7.4 [43].
d – The rate constant of the "repair reaction" has been determined using GSH and penicillamine with radicals derived from methanol, ethanol, propan-1-ol, propan-2-ol, ethylene glycol, tetrahydrofuran and 1,4-dioxane with the abstracted hydrogen being located α to the hydroxy or alkoxy function (R′R″C˙-OR‴) . H-abstraction from RH by thiyl radicals (reverse process) occurred with rate constants of the order of 10^3-10^4 $M^{-1}s^{-1}$.

Graphical abstract



Highlights

- The chemistry of N-acetylcyteine (NAC) is reviewed.
- NAC can detoxify oxidizing radicals and bind redox-active metal ions.
- NAC is a precursor of cysteine thus maintaining GSH intracellular levels.
- NAC can efficiently reduce disulfide bonds in proteins thus altering their structures.
- Not all mechanisms underlying the biological activities of NAC are already clear.

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Title:
The chemistry and biological activities of N-acetylcysteine

Date:
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