Review paper

N-acetylcysteine as an anti-oxidant and anti-inflammatory drug and its some clinical applications

DOROTA MAGDALENA RADOMSKA-LEŚNIEWSKA[1], PIOTR SKOPIŃSKI[1,2]

[1]Department of Histology and Embryology, Center of Biostructure, Warsaw Medical University, Warsaw, Poland
[2]Department of Ophthalmology, Second Faculty of Medicine, Warsaw Medical University, Warsaw, Poland

Abstract

N-acetylcysteine (NAC) a derivative of amino acid L-cysteine is currently used mainly as an antioxidant. Sulfhydryl group (–SH) within its molecule makes possible to directly scavenge reactive oxygen species (ROS). Indirect antioxidant action of NAC rely on augmentation of the level of reduced glutathione (GSH) – a major body's antioxidant. Since oxidative stress takes a part in pathogenesis of a broad spectrum of diseases, NAC could became a valuable supplement to conventional treatment. N-acetylcysteine has been used as an antidote for aminophen intoxication. N-acetylcysteine seems to be promising in some pulmonary diseases and gynecological disorders. Supplementation of this antioxidant caused also positive results in some eye disorders: diabetic retinopathy, age macular degeneration, cataract and dry eye syndrome. It was also found to have some preventative capabilities in cancer, influenza and in contrast-induced nephropathy.

Key words: *N-acetylcysteine, oxidative stress, pulmonary disease, polycystic ovary syndrome, endometriosis, eye disorders, cancer, contrast-induced nephropathy.*

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Introduction

N-acetylcysteine (NAC) a sulfhydryl substance is a derivative of amino acid L-cysteine. Mucolytic activity of NAC was used for the first time in the treatment of some respiratory diseases (e.g. chronic bronchitis) over 40 years ago [1]. Detoxifying properties of NAC were discovered in the 1970s and since then NAC was being used as an antidote in aminophen intoxication [2]. Currently it is known mainly as an antioxidant displaying direct and indirect activities [3]. Oxidative stress – the imbalance between reactive oxygen species (ROS) and actions of the antioxidant network – takes part in pathogenesis of a broad spectrum of diseases including cancer, cardiovascular, arthritis, diabetes, influenza-like symptomatology as well as some lung disturbances namely pulmonary oxygen toxicity, adult respiratory distress syndrome, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis [4] and cystic fibrosis. Increasing number of publications confirm efficacy of using NAC in the above mention diseases [3-6].

Mechanism of action

Antioxidant properties of NAC come from its specific structure. *N*-acetylcysteine contains amino acid *L*-cysteine plus an acetyl ($CO\text{-}CH_3$) group attached to the amino (NH_2) group. All amino acids including *L*-cysteine with sulphur group are characterized by antioxidant properties. The possible pathways through which NAC modulates an oxidative stress and inflammation, is presented in Fig. 1. Since *L*-cysteine is a precursor of reduced glutathione (GSH), synthesis of NAC contributes to augmentation of the level of this major intracellular antioxidant [3]. Depleted pool of GSH is often caused by oxidative stress and inflammation. *N*-acetylcysteine can therefore normalize disturbed redox status of the cells and thus influence redox – sensitive cell signaling and transcription pathways. A number of publications confirm inhibition by NAC activation of c-Jun *N*-terminal kinase, p38 MAP kinase, SAPK/INK, c-Fos pathways as well as nuclear factor κB (NF-κB) which regulates a numerous of proinflammatory and antiapoptotic genes [3, 5, 6].

Correspondence: Dorota Magdalena Radomska-Leśniewska, Department of Histology and Embriology, Center of Biostructure, Warsaw Medical University, Chałubińskiego 5, 02-004 Warsaw, Poland, phone +48 22 629 56 75, fax +48 22 629 52 82, e-mail: mradomska@ib.amwaw.edu.pl

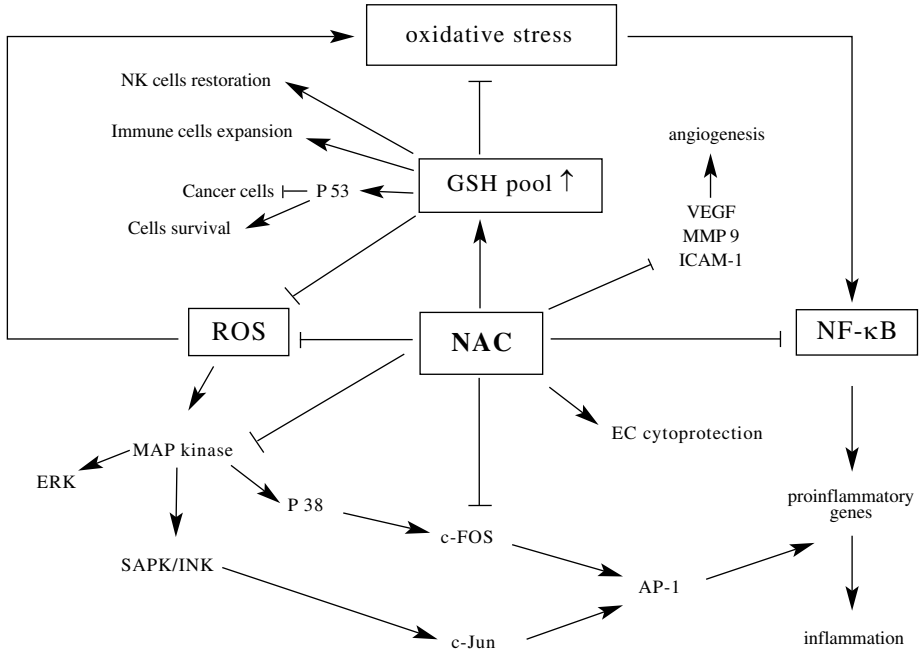

Fig. 1. Mechanism of action of *N*-acetylcysteine. The activations steps by NAC are indicated by arrows, and inhibition by NAC is depicted by the symbol ⊥. Abbreviations are GSH, reduced glutatione; ROS – reactive oxygen species; NF-κB – nuclear factor κB; MAP kinase – mitogen activated kinase; SAPK – stress activated protein kinase; INK – c-Jun N-terminal kinase; ERK – extracellular signal-regulated kinase; EC – endothelial cells; AP-1 – activator protein 1; VEGF – vascular endothelial growth factor; MMP9 – metalloproteinase 9; ICAM-1 – intercellular adhesion molecule-1

Sulfhydryl group (–SH) in the NAC molecule make possible also to directly scavenge reactive oxygen species (ROS) such as superoxide radical (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radical OH [3, 6]. These species primarily are produced by the mitochondria in the cells as by-products of cellular metabolic pathways. In physiological level ROS play a significant role in proper functioning of many mechanisms that control cell division. They serve as critical second messengers in a variety of intracellular signaling pathways mediating a lot of important processes e.g. activation of transcriptions factors (NF-κB, AP-1), regulation of protein phosphorylation and regulation of calcium level inside the cells as well as phagocytosis process. However they possess highly reactive and toxic properties [7].

Surprisingly, NAC can exert also pro-oxidant capabilities (auto-oxidation process) under certain conditions, inducing generation of H_2O_2 in the presence of O_2 [8] and leading to cell damage.

Antiinflammatory action of NAC manifests by inhibition of many proinflammatory cytokines activity including interleukin 8 (IL-8), IL-6, tumour necrosis factor α (TNF-α) [5, 9, 10]. Fibroblast proliferation and collagen synthesis is also down-regulated by this drug [11]. These kinds of NAC activities can be a result of modulation of transcriptional activities through several pathways involving c-Fos/c-Jun, NF-κB, STAT, and cyclin inhibitors [6].

Since disturbed angiogenesis is associated with a number of diseases, therapeutic efficiency of NAC may also be correlated with its antiangiogenic activity. *N*-acetylcysteine was described as inhibitor of vascular endothelial growth factor (VEGF) – induced angiogenesis [12], endothelial cell invasion as well as angiogenesis *in vitro*, presumably because of the inhibition of metalloproteinase activities (MMP) [13]. In fact, our group study confirmed MMP9 and proangiogenic intercellular adhesion molecule-1 (ICAM-1) inhibition by this drug [5, 10]. Vascular endothelial growth factor, the most powerful stimulator of angiogenesis, was also downregulated by NAC [14]. This antioxidant drug reduced VEGF expression in ras-transformed tumor cells [15]. Aluigi *et al.* [16] demonstrated direct cytoprotective and anti-genotoxic effects mediated by NAC on endothelial cells.

Diversity of applying NAC is the source of broad spectrum of used dosage and routes of administrations. Oral administration (tablet or inhalations) can range from 250 to 1800 mg/day and is used mainly in lung diseases [4]. In intravenous infusion, applied mostly in treatment of aminophen overdose, NAC is usually used at the doses

of 150 mg/kg and results after 15 minutes in a plasma peak of 554 mg/l (3.4 mM) [17]. Toxicological data shows that intakes of 60-80 g NAC per day orally could be consumed without causing significant adverse effects [18].

Cancer

Severe and long lasting oxidative stress can be very toxic for the cells, because it can lead to stable changes in the structure of biologically significant macromolecules such as DNA, proteins, carbohydrates, and others. Oxidative modification of DNA as well as oxidative protein accumulation are the factors responsible for tumor transformation of the cells and enhancing their malignancy potential [7]. That is why antioxidant drugs such NAC are suggested to be important in prevention of cancer diseases.

Reliene and Schiestl [19] observed after NAC administration a decrease of incidence and multiplicity of lymphoma in ataxia telangiectasia mutated (ATM) deficient mice during 2 years study. The number of tumors in NAC treated ATM mice was diminished from 4.6 to 2.8 per mouse. Moreover NAC significantly increased the lifespan, from 50 to 68 weeks, in these animals.

Studies in athymic nude mice model of tumor angiogenesis demonstrated NAC as an effective chemoprevention agent. Additionally NAC action was found to be synergistic with doxorubicin in blocking experimental tumorigenesis [20, 21]. Application of NAC (7 mg/ml) diminished UV induced ROS in melanocyte cell line and protects these cells from UV – induced oxidative damage. N-acetylcysteine reduced formation of 8-oxoguanine in mice skin protecting melanocytes from UV induced melanoma [22]. N-acetylcysteine inhibited VEGF production in the human melanoma cell line [23]. Cavalieri *et al.* [24] described a reduction of estrogen-DNA adducts formation by NAC and resveratrol in *in vitro* models. Their effects were additive in human breast epithelial cell line. In Estensen *et al*. [25] study NAC was given to 34 patients (800 mg/day, 12 weeks) with previous adenomatous colonic polyps, which are at high risk for colon cancer development. They observed proliferative index reduction within colonic crypts.

Many animal experimental models revealed anticancer and antiproliferative NAC activity. Eight week NAC therapy was shown to inhibited MDA-MB-435 breast carcinoma cell proliferation and metastasis in athymic nude mice tumorigenic model [26]. Endothelial cell apoptosis, reduction in microvascular density and angiostatin accumulation in the tumor core were the result of NAC treatment. As *in vitro* study showed, antiangiogenic activity of NAC was mediated by angiostatin production followed by endothelial cells apoptosis and vascular degradation within the tumor.

Androgen-independent prostate carcinoma PC-3 cells proliferation was inhibited by 1 mM NAC in a dose- and time-depended manner [8]. Nuclear factor κB induced by IKK-β was also inhibited by NAC in these studies. *N*-acetylcysteine activated Act and Erk1/2 signaling pathway increasing CYR 61 protein level, probably as a part of antiproliferative mechanism in PC-3 cells. Study of Lambert *et al*. [27] revealed that NAC (0-2 mM) intensified inhibitory anticancer activity of polyphenol epigallocatechin-3-gallate (EGCG) in murine (CL13) and human (H1299) lung cancer cells. Enhanced (8.8 fold) apoptosis was observed after combined application of NAC and EGCG when compared to treatment with either agent alone. Authors suggest that EGCG and NAC form adduct EGCG-2-NAC which can participate in EGCG-mediated cell killing.

A large trial "Euroscan" performed for estimating efficacy of NAC and vitamin A (in retinyl palmitate) in prevention of tumors relapse or appearing a second primary tumors in 2592 patients with head, neck or lung cancer, revealed no significant differences between studied and control groups [28].

Can *N*-acetylcysteine disturb anti-cancer therapy?

Some reports indicated that antioxidant properties of NAC can be dangerous if applied during anticancer therapy. Most of anticancer therapies (including radiation therapy, most chemotherapies, natural therapies) are based on growth of ROS production in cancer cells leading to their apoptosis [29, 30]. Whereas increasing glutathione level NAC inhibits the level of ROS in malignant (and normal) cells and prevent them from apoptosis. Han *et al.* [30] presented that anticancer drug, MG 132, increased ROS production in lung cancer cells slowing down the growth of these cells. Treatment by NAC resulted in stop of this therapeutic effect. Synthetic selenium compound methylselenic acid (MSA) generated oxidative stress and apoptosis in L9981 and 95-D lung cancer cells. Pretreatment with NAC inhibited the antiproliferative effect of MSA [29]. Similar mechanism was also seen for natural treatment of cancer, such as curcumin [31], ginseng [32], berberine [33], selenite sodium [34], melatonin [35], EGCF [36] where NAC abolished anticancer effect of the natural drugs.

N-acetylcysteine reduces toxicity caused by chemotherapy

Analysis of 33 clinical trial reviews performed between 1966 and 2007 by Block *et al*. [37] lead to conclude that in most cases antioxidants including NAC, vitamin A, and E, selenium, Co-Q10, ellagic acid and *L*-carnitine diminished toxicities caused by anticancer therapy. This effect was seen in 24 studies, while 9 studies exerted no difference. Increased toxicities was noted by only 1 (vitamin A) reported study [38, 39].

N-acetylcysteine protected against radiation-mediated genotoxicity during radiation therapy in humans (lymphoblastoids cells) and mice (peripheral blood), however it did not protect against cell death [40].

The efficiency of vitamin E and *N*-acetylcysteine as an antioxidant adjuvant therapy was shown in chemotherapy/radiotherapy course during acute lymphoblastic leukemia (ALL) in 40 children study. Toxicity of chemo- and radiotherapy measured as a diminished level of malondialdehyde, as well as increased level of glutathione peroxidase and decreased occurrence of toxic hepatitis was significantly reduced [41].

As the cardiovascular and cancer diseases affect mostly aged people in developed countries both of the diseases can coexist in one patient. Since cancer treatment (e.g. chemotherapeutic agents, radiotherapy or their combination) usually influence the cardiovascular system, oncologist should cooperate with cardiologist to minimalize toxicity of cancer therapy. Pathomechanism of cardiotoxicity in the course of cancer treatment seems to involve endothelial cells damage [42]. Cai [13] and Aluigi [16] demonstrated that application of NAC resulted in higher endothelium resistance to apoptosis induced by external stimuli. However, random control trials that tested cardioprotective activity of NAC in cancer patients receiving antracyclines, have shown methodological limitation [43].

Pulmonary diseases

Chronic obstructive pulmonary disease

There are discrepancies between reports concerning effects of NAC therapy on chronic obstructive pulmonary disease (COPD) patients. A group of 169 patients with moderate to severe COPD were treated orally for 6 month by 600 mg/day of NAC added to standard therapy (β2-agonists, anticholinergics, theophylline and inhaled and/or oral corticosteroids). A drop in exacerbations number and small but significant improvement in forced expiratory volume in 1 second (FEV_1) and maximum expiratory flow rate at 50% of vital capacity (MEF50) were observed [44]. Van Overveld *et al*. [45] studied a therapeutic effect of NAC applied for 10 weeks at the dose of 600 mg/day in a 20 COPD patients with moderate airflow obstruction. No positive effect on lung function parameters after NAC administration was observed in this study. Three years multicenter study "BRONCUS" included 523 patients with COPD receiving 600 mg NAC daily or placebo have not met primary end points of exacerbation rate and deterioration of FEV_1 [46]. Similarly Black study [47] with COPD patients receiving 600 mg/day/7 days did not exert any improvement on lung function, breathlessness and hospitalization period. The same doses of NAC were administered in Schermer [48] three years study of 286 COPD or chronic bronchitis in comparison to fluticasone or placebo group. Unfortunately exacerbation rate as well as annual post-bronchodilator FEV_1 decline has not improved.

Some last year's report focus on high (> 600 mg/day) doses of NAC in COPD therapy. Zuin *et al*. [49] compared therapeutic effect of NAC, applied at the doses 600 and 1200 mg/day for 10 day to 123 COPD patients with disease exacerbations, with placebo. Lung function (FEV_1) and other clinical outcomes (e.g. cough and lung auscultation) were ameliorated by administration of both doses of NAC. However 1200 mg/day was more effective in CRP level normalization, reduction of IL-8 level and difficulty of expectoration. Two months of oral NAC therapy (600 mg b.i.d.) in 55 clinically stable patients with COPD did not influence lung function parameters [50]. In another study 24 moderate to severe COPD patients were treated with 1200 mg/day of NAC for 6 weeks. The FEV_1 and inspiratory capacity (IC) – a marker of dynamic hyperinflation – were higher at rest and especially after exercise in a NAC group comparing to placebo [51]. These positive results of NAC therapy were caused probably due to reduction in airtrapping. Meta-analysis by Grandjean [52] and Stay [53] of the double blind placebo-controlled studies on NAC effectiveness in COPD has revealed similar positive results. *N*-acetylcysteine applicated from 3 to 6 months (400-1200 mg per day) induced an improvement of symptoms and diminished multiplicity of acute exacerbation in safety way (no increasing adverse effects) in COPD patients.

Cystic fibrosis

Cystic fibrosis (CF) is caused by an autosomal recessive mutation of CF transmembrane conductance regulator (CFTR) protein, which has been shown to function as a GSH transporter [54]. The disease is characterized by neutrophilic airway inflammation. Neutrophils – high oxidant producers – are involved in generating systemic redox imbalance in the course of the disease. Recurrent pulmonary infections and inflammation occurring in these patients can lead to respiratory failure and death. *N*-acetylcysteine as a mucoactive, anti-inflammatory and antioxidant agent was expected to have a benefit therapeutic effect in CS patients. In 4 weeks phase I trial [55] NAC was administered orally to 18 CF patients in a high doses (600-1000 mg) three times daily. Elastase activity and neutrophil burden as well as IL-8 level, measured in airway fluid, obtained by sputum induction, came down significantly. Moreover a consequence of NAC therapy was restoration of circulating neutrophils and GSH deficiency as well as a drop in a number of airway neutrophils.

A phase II randomized, placebo controlled clinical study [54] examined the influence of low (700 g) or high (2800 mg) doses of NAC in 12 week treatment of 21 patients with CS. High NAC doses were well tolerated but had no effect on clinical or inflammatory parameters except extracellular glutathione level, which slightly increased. Further studies are needed to learn if NAC can indeed be useful in CS therapy.

Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic disease with progressive fibrosis of the lung parenchyma, caused

by repeated lung injury leading to respiratory failure [56]. The etiology of this poor prognosis disease remains unknown. Oxidative stress which is elevated in IPF patients play a crucial role in disease pathophysiology [5]. Current therapy based on corticosteroids and immunosuppressive agents results in limited benefit.

IFIGENIA randomized placebo-controlled 1 year studies were conducted in 182 patients, with high doses (1800 mg/day) of NAC added to azothioprine and prednisone therapy. Disease progression, measured by vital capacity (+9%) and diffusing capacity (Dl_{co}; +24%) slowed down significantly [56, 57]. Similar effects were also achieved by Bennet [58] as a result of small randomized trial based on high NAC doses as an addition to standard therapy. Conversely, other study has not shown beneficial effect of NAC [59, 60].

Infective diseases

Results of placebo-controlled randomized trial, conducted on 262 older adults and nonrespiratory chronic degenerative disease patients, for 6 months during influenza season, have shown that NAC at the dose of 600 mg twice daily, was able to attenuate influenza virus [61]. Although seroconversion rates to A/H1N1 virus have been similar in both group ($p = 0.0006$) only 29% of patients have had clinically expressed influenza in NAC group (vs. 51% in placebo). Moreover the severity of illness in NAC group was much weaker than in placebo group.

HIV positive patients apart from immune deficiency exert a low level of cysteine and glutathione. They are considered to live in chronic oxidative stress [62]. Glutathione deficiency can be dangerous, as it can cause muscular atrophy and cachexia. Furthermore progressive decrease of GSH levels in CD4 T lymphocytes and erythrocytes is associated with HIV disease progression and highly correlated with decreased survival [63]. Indeed, as several placebo controlled randomized trials reported, NAC administered orally to GSH deficiency HIV patients restored level of GSH in CD4 T cells and erythrocytes as well as improves T cells function [18].

Gynaecological disorders

Endometriosis

Endometriosis is known as a common gynaecological disorder affecting about 10% of women in a reproductive age. It is characterized by presence of endometrial tissue outside of the uterine cavity, resulting in pelvic pain, infertility and dysmenorhea [64]. Development of the disease is caused by implantation of endometrial cells in the peritoneal cavity and their proliferation leading to invade peritoneum and disease progression. Pathophysiology of endometriosis includes chronic inflammation within oxidative stress and pathological angiogenesis [65, 66].

Foyouzi *et al.* [67] studied the effect of NAC used at the concentration of 10-30 mmol/l on proliferation of cultured stromal cells from women with endometriosis. They obtained the dose depended inhibition ranging from 52 to 85% respectively. Similar inhibitory effect (63%) was obtained by Wu and Guo [68], after 10 mmol/l of NAC used in the culture of stromal endometriotic cells.

The study of Ngo [64] included the effect of NAC on endometriotic cells derived from patients with endometriosis and mouse model of endometriosis. They have found the correlation between increase level of ROS production, alteration of ROS detoxification pathways, decrease of catalase level in endometriotic cells, cellular proliferation and activation of MAP kinase ERK1/2. Proinflammatory cytokines and growth factors as well as markers of oxidative stress were elevated in peritoneal fluid of endometriosis patients [19, 64, 69]. Examined cells were incubated with increasing concentration of NAC resulting in dose depended inhibition of intracellular concentration of H_2O_2 and proliferation rate. Activated RAF/MEK/ERK signaling pathway present in oxidative stress tissue was elevated in endometrial and stromal cells of patients with endometriosis and in the endometriotic cells. Two hours incubation with NAC at the concentration of 10 mmol/l caused decrease of pERK level to the level of control cells (stromal and epithelial cells from healthy women).

It is known that in endometriotic tissue, the level of angiogenesis and proangiogenic factors like VEGF are significantly elevated [65, 70, 71]. Thalidomide, an antiangiogenic factor, administered 6 month with goserelin (GnRH analog) to woman with ovarian endometriosis in a pilot study, resulted in remission of pain and resolution of ovarian cyst [65, 72]. Another antiangiogenic factors such as anti-VEGF antibodies, TNP 470 and endostatin were found to have angiostatic therapeutic action in animal model of endometriosis [65].

N-acetylcysteine exerted antiangiogenic action [12-16] seems to be promising in endometriosis treatment as it can reduce oxidative stress, chronic inflammation and elevated angiogenesis level.

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) affecting up to 10% women of reproductive age is characterized by hyperandrogenism, enlarged cystic ovaries, chronic anovulation. 30-60% of PCOS women have obesity and more than 50% have hyperinsulinemia [73].

N-acetylcysteine was found to exert a benefit effect in PCOS as have shown last year trials. This antioxidant at the dose of 1800 mg/day was administered to hyperinsulinemic PCOS women as an adjunct therapy to clominphene ameliorated their insulin sensitivity and the level of circulating insulin [63]. Similar effect was achieved by Masha *et al*. study [74]. *N*-acetylcysteine (1200 mg/day) plus nitric oxide precursor – *L*-arginine (1600 mg/day) therapy restored gonadal function in PCOS women via improvement of insulin sensitivity. Results of other investigations indicated

that NAC applied in clominphene citrate (CC)-resistant women with PCOS exerted clinically and statistically significant increase in ovulation and pregnancy [75, 76] NAC therapy was also used as an adjunct therapy of CC-resistant women with PCOS [77] following unilateral laparoscopic ovarian drilling (LOD). Women received 1200 mg/day of NAC or placebo for 5 days during 12 consecutive cycles after LOD. A significant increase in ovulation and pregnancy rates was noted in NAC group. Furthermore in NAC group significantly higher live birth rates and lower miscarriage rates was noted. A randomized controlled trial, reported by Hashim *et al.* [78], with CC-resistant PCOS women, compared a therapy by NAC plus CC with metformin plus CC. Three months metformin plus CC administration resulted in significantly higher ovulation and pregnancy rates, higher level of serum estrogens, endometrial thickness and serum progesterone level on 21-23 cycles days.

N-acetylcysteine at the dose 1200-1800 mg/day has benefit effect in women with POCS although metformin therapy seems to be more effective.

Contrast-induced nephropathy

Ten percent of all hospital-acquired renal failure composes contrast-induced acute kidney injury (CI-AKI). Pathogenesis of this disorder included hypoxia of renal medulla caused by hemodynamic changes of renal blood flow and toxic influence of contrast media on renal cells [79]. Contrast-induced acute kidney injury is associated with renal dialysis risk and increased mortality and morbidity.

A lot of last 10 year trials reporting NAC usefulness in nephrotoxicity of contrast – induced media resulted in discrepant findings. Most of meta-analysis, that did not consider the great heterogeneity between the trials, concluded a beneficial protecting effect of NAC on contrast-induced nephropathy (CIN) in which NAC reduced a risk of CIN up to 50% [80]. In the largest, Kelly *et al.* [81] meta-analysis included 41 studies, NAC, as compared with saline alone, significantly diminished the risk for nephrotoxicity. Buguori *et al.* [79] reported that the influence of NAC on CI-AKI was dose depended.

Eye disorders

The role of oxidative mechanisms in diabetes, AMD (age macular degeneration), dry eye syndrome and cataract seems to be essential for development of the pathological changes in ophthalmic tissues. The eye is at high risk to be damaged by oxidative stress. Molecular oxygen is able to directly destruct or lead to the generation of secondary reactions which can initiate oxidative processes [82].

Diabetes and diabetic retinopathy

Diabetes is characterized by poor glycemic control and increased oxidative stress in tissues [83]. Hyperglycemia induces metabolic disorders (ischemia and hypoxia) leading to development of retinal capillary lesions and diabetic retinopathy [84].

Reactive oxygen species are involved in the destruction of pancreatic β cells and the development of insulin-dependent diabetes mellitus (IDDM). In experimental model of alloxan induced IDDM Ho *et al.* [85] had shown that NAC (500 mg/kg) supplementation inhibited alloxan-induced NF-κB activation and reduced hyperglycemia in mice. Results of other investigations [86] suggest that NAC reduces thrombotic propensity in type 2 diabetes patients by increasing platelet antioxidant status and GSH synthesis, thereby lowering platelet-derived ROS. *N*-acetylcysteine might represent an alternative or additional therapy to aspirin that could reduce thrombotic risk in type 2 diabetes.

Macrophage/microglia activation, pericyte loss and endothelial/perivascular cell changes occur early in the pathogenesis of diabetic retinopathy (DR). Tsai *et al*. [87] shows that these changes are associated with an increase in plasma markers of oxidative stress and inflammation and can be minimized by treatment with NAC. As streptozotocin induced diabetes (STZ) model revealed therapies reducing free radicals would help to minimize the early events in diabetic retinopathy. Diabetes patients are at an elevated risk for developing corneal complications and delayed wound healing. Xu *et al.* [88] studied the effects (*in vitro*) of high glucose on epidermal growth factor receptor (EGFR) signaling and on epithelial wound healing in the cornea. They observed that high glucose, likely through ROS, impairs the EGFR-phosphatidylinositol 3-kinase/Akt pathway, resulting in delayed corneal epithelial wound healing. In conclusions they noted that antioxidants in combination with EGFR ligands may be promising potential therapeutics for diabetic keratopathy.

Age macular degeneration

Early steps of age macular degeneration (AMD) involve accumulation of waste material in the space between the Bruch's membrane and the pigmented epithelial layer, cause formation of drusen and lead to damage of retinal pigmented epithelial cells (RPE) layer. Dysfunction or death of these cells is crucial to AMD pathogenesis. This layer serves as the blood retinal barrier and regulates the transport of oxygen, substrates and waste products between the choroidal vasculature and the retina. Chronic oxidative stress and inflammation are linked to RPE senescence and the pathogenesis of AMD [89].

The inflammatory response in AMD is characterized by mononuclear leukocyte infiltration of the outer blood-retina barrier formed by the RPE. Yang *et al*. [89] have found that NAC significantly reduced apoptosis of RPE cells induced by MCP-1 activated monocytes and inhibited ROS production involved in this process.

Products of dietary carotenoids (CDA) could be genotoxic in retinal pigment epithelial cells (ARPE-19) [90].

Lutein (LBP)-induced genotoxic effects could worsen oxidative stress, DNA damage and cell death incurred by LBP. *N*-acetylcysteine was shown to attenuate genotoxic influence of β-apo-8-carotenol (BA8C). Cytotoxicity of CDA in ARPE-19 cells was also significantly ameliorated by NAC [91]. The general suggestion is that high carotenoid supplementation for treatment of AMD should be used cautiously.

The final stages of AMD involved choroidal neovascularisation (CNV). The role of NAC in the development of CNV was investigated by Hara *et al.* [92]. *N*-acetylcysteine inhibited indicators of oxidative stress and the activation of NF-κB induced by laser injury, as well as suppressed macrophage and neutrophil infiltration and the development of CNV.

Dry eye syndrome

Acetylcysteine eye drops are an effective alternative in treating people who have problems with sticky, viscous mucus on the eye (filamentary keratitis). In a double blind, placebo controlled crossover trial of 26 patients with Sjögren's syndrome, application of NAC (200 mg three times per day) improved eye-related symptoms. The supplement also showed some promise for mouth-related symptoms, but the effects were less clear-cut [93]. Latest clinical investigations [94] show that topical administration of NAC is very effective in patients with meibomian gland dysfunction. Significant improvement for the symptoms of itching, burning and increase of average of Shiermer test was noted.

Cataracts

A cataract is a cloudiness or opacity in the normally transparent crystalline lens of the eye. This cloudiness can cause a decrease in vision acuity and sometimes may lead to hand movements behind the eye or blindness. The leading risk factor of cataract is aging. The Framingham Eye Study found that nine out of ten people older than 75 have cataracts [95].

As glutathione is an important antioxidant in the lens it has been suggested that increasing GSH level NAC supplementation could be used to reduce cataract risk [96].

Diabetes elevates the risk of cataract formation. This form of cataract can result from sorbitol accumulation in the lens. Pathophysiology of early cataract development and the potential benefit of supplementation with vitamin B_6 and NAC among the diabetic population were studied by Jain *et al.* [97]. High-glucose concentrations can cause the oxidation and modification of proteins in the lens. Vitamin B_6 (pyridoxine) and NAC supplementation may be helpful in slowing the oxidation of lens proteins. The study of Liebermann [98] raised the possibility that administration of NAC may reverse early cataracts. Zhang *et al*. [99] evaluated the effect of NAC and glutathione ethyl ester (GSH-EE) eye drops on the progression of diabetic cataract formation in rats induced by STZ. Author concluded that NAC and GSH-EE can slightly inhibit the progression of the diabetic cataract at the earlier stage.

N-acetylcysteine, used as an ophthalmic drug is promising in the treatment of a range of ophthalmic disorders with oxidative stress component involved in pathogenesis including cataract, glaucoma, dry eye syndrome, vitreous floaters, inflammatory disorders, corneal, retinal and systemic diseases and its ophthalmic complications.

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