Principles behind SLE treatment with N-acetylcysteine

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Abstract
Systemic lupus erythematosus (SLE) is a multisystem chronic autoimmune disease in which disrupted molecular pathways lead to multiple clinical manifestations. Currently approved treatments include hydroxychloroquine, some immunosuppressive medications, and some biologics. They all come with a range of side effects. N-acetylcysteine (NAC) is an antioxidant that has shown potential benefits in SLE patients without having major side effects. The following review highlights the molecular mechanisms behind the therapeutic effect of NAC in SLE patients. A higher-than-normal mitochondrial transmembrane potential or mitochondrial hyperpolarization (MHP) was found in lymphocytes from SLE patients. MHP is attributed to the blocked electron transport, and it is associated with the depletion of ATP and glutathione and the accumulation of oxidative stress-generating mitochondria due to diminished mitophagy. Comprehensive metabolome analyses identified the accumulation of kynurenine as the most predictive metabolic biomarker of lupus over matched healthy subjects. Cysteine is the rate-limiting constituent in the production of reduced glutathione, and it can be replaced by its precursor NAC. Kynurenine accumulation has been reversed by treatment with NAC but not placebo in the setting of a double-blind placebo-controlled clinical trial of 3-month duration. Mitochondrial oxidative stress and its responsiveness to NAC have been linked to systemic inflammation, gut microbiome changes, and organ damage in lupus-prone mice. Given the unique safety of NAC and chronicity of SLE, the clinical trial of longer duration is being pursued.

Keywords: systemic lupus erythematosus, N-acetylcysteine, mechanistic target of rapamycin, mitochondrial hyperpolarization

Systemic lupus erythematosus (SLE) is a multisystem chronic autoimmune disease in which every organ and tissue might be affected. A complex interplay of disrupted molecular pathways leads to the multiple clinical manifestations reflecting the diversity of organ involvement in this disease. A multitude of genetic, environmental and hormonal factors pave the way for its development. One of this disease’s hallmarks is the production of several autoantibodies. A lack of understanding of the complete pathogenesis of SLE has led to the lack of great advancements in its treatment. Currently, hydroxychloroquine remains the cornerstone in therapy due to the established benefits and accepted safety profile. Other alternatives include immunosuppressive medications (glucocorticoids, mycophenolate, cyclophosphamide, cyclosporine) and biologic agents (eg, rituximab, belimumab). However, these carry their own side effects. Hence came the need to study potential non-immunosuppressive drugs like N-acetylcysteine (NAC).

A commonly agreed upon the fact in the pathogenesis of SLE is the dysfunction of T cells, B cells, dendritic cells, macrophages, and neutrophils, likely triggered by genes and/or the environment. In fact, even though autoantibodies are produced by B cells, it is well known now that T cells critically participate in the pathogenesis of SLE because of their ability to guide B cells in autoantibody production. Hence, studies have been focusing on understanding of the intrinsic abnormalities in T cells that lead to B-cell dysfunction. One of those findings is the abnormal activation and processing of cell death signals by the immune system which in turn lead to necrosis of the cells and stimulate the production of antinuclear antibodies in the process. In a study done by Gergely et al, it was found that mitochondria which are the organelles that control the signal processing of T cells are dysfunctional in lupus patients, exhibit a higher-than-normal mitochondrial transmembrane potential (ΔΨm) or mitochondrial hyperpolarization and are ATP (adenosine triphosphate) depleted which predispose to cell death by necrosis instead of apoptosis. The process of necrosis activates macrophages and dendritic cells and enhances their capacity to produce nitric oxide and interferon alpha in SLE. It was also found in this study that reactive oxygen intermediates (ROIs) are higher in lupus patients compared with healthy controls. Mitochondrial ROI production and the increase in membrane potential or mitochondrial hyperpolarization (MHP) are early checkpoints in Fas- and H2O2-induced cell death. In addition to that, reduced levels of glutathione were found in freshly isolated peripheral blood lymphocytes (PBLs) from lupus patients, which was consistent with ongoing oxidative stress in vivo. The deficiency of glutathione predisposes cells for ROI-induced cell death. Protection against ROI-mediated cell death is dependent on the availability of reduced glutathione.

A study done on murine Lupus models by Chen et al showed that the NAD+–modulating ectoenzyme CD38 regulates mitochondrial fitness in SLE CD8+ T cells and negatively affects their function and ability to combat infection. CD38 reduces cellular NAD+ levels, and therefore suppresses mitophagy by inhibiting the recruitment of damaged mitochondria to the phagophore through the SIRT1-PINK1-Parkin pathway. These events result in the accumulation of damaged depolarized mitochondria in CD38hiCD8+ T cells and reduce their ability to clear viruses effectively. These data argue strongly that mitochondria are dysfunctional in SLE T cells.

In another study, it was found that T cells of SLE patients show robust activation of the mechanistic target of rapamycin
Cysteine is the rate-limiting constituent in the production of reduced glutathione, and it can be replaced by its cell permeable precursor, N-acetylcysteine [13]. Commercially available since long time, NAC is relatively safe and inexpensive medication. This drug is not naturally found, but cysteine is present in some foods such as chicken, garlic, and yogurt. When ingested, NAC activates the biosynthesis of glutathione which helps in detoxification and elimination of free radicals due to its powerful antioxidant properties [14].

In a study done by Suwannaroj et al. [15], it was found that SLE mice treated with NAC had a significantly lower anti-DNA antibody production at 24 weeks compared to control mice and had a modest improvement in mortality [15]. Shi et al. linked F-actin over-polymerization to increased ROI production, apoptosis, and aberrant migration of bone marrow mesenchymal stem cells (MSCs) from SLE patients [16]. NAC treatment made F-actin more orderly and migration of SLE MSCs in vitro. Of note, oral administration of NAC also reversed MSC abnormalities and markedly reduced serum autoantibody levels and ameliorated lupus nephritis (LN) in MRL/lpr mice (Table 1).

Trichloroethene (TCE) exposure has been implicated in the development of autoimmunity, including autoimmune hepatitis (AIH) and SLE. TCE-induced antinuclear antibodies (ANA) and the formation 4-hydroxynonenal (HNE)-modified immune complexes in the bloodstream of lupus-prone MRL mice [17]. Moreover, TCE triggers prominent lobular inflammation and hepatocellular proliferation in the liver of MRL mice, which were abrogated by treatment with NAC. Furthermore, TCE dramatically increased lymphocytic infiltration by T<sub>1</sub> and B cells and triggered a profound loss of regulatory T cells (Tregs) in the liver. Of note, TCE-mediated skewing of hepatic and splenic immune lineage development was effectively reversed by NAC (Table 1). Remarkably, NAC also blocked microbiome changes along with systemic autoimmunity in lupus-prone MRL/lpr mice [18], suggesting that gut dysbiosis is driven by oxidative stress on the organismal levels (Table 1).

Based on GSH depletion in patients with SLE [6], a 3-month phase I-phase II double-blind placebo-controlled randomized pilot study of NAC in 36 subjects was done to look for its immunological and therapeutic impact (Table 2). The study found the drug to be safe and effective at doses of 2.4 and 4.8 g/day in reversing the depletion of glutathione and in improving disease activity and the fatigue level. This dose of NAC reduced the SLEDAI (SLE Disease Activity Index) score and the BILAG (British Isles Lupus Assessment Group) score and profoundly reduced mTOR activity in T lymphocytes [19]. Specifically, the double negative T cells are the main cells affected by the blockade of mTOR by NAC [19]. In fact, kynurenine's accumulation plays a role in the activation of mTOR in SLE [19]. Kynurenine is a metabolite of the pentose phosphate pathway which serves as a metabolic checkpoint in the pathogenesis of SLE in double negative T cells which are a source of interleukin 4, interleukin 17 and necrotic debris. Treatment with NAC increased the abundance of NADPH which in turn resulted in increased catabolism by NADPH-dependent kynurenine hydroxylase leading to lower levels of kynurenine which subsequently inhibited the mTOR pathway in those T cells [19].

Direct blockade of mTOR with sirolimus also exerts promising clinical efficacy and moderated mitochondrial oxidative stress in patients [23] and mice with SLE [24,25]. These findings implicated a pro-inflammatory, positive feedback loop between mTOR activation and mitochondrial dysfunction in SLE.

Another study by Doherty et al. [22] was done also on the finding that SLE patients' PBL show mitochondrial dysfunction and oxidative stress. The aim of this study was to determine the electrochemical basis of mitochondrial dysfunction by measuring the electron transport chain (ETC) activity and its regulation by NAC. Seven SLE patients, 11 healthy donors, and 10 non-lupus inflammatory arthritis donors constituted the study population. The result of this study showed that lupus PBLs have increased oxygen consumption through mitochondrial ETC complex I, which is the main source of oxidative stress in SLE, and this complex is inhibited by NAC [22].

One study done by Garcia et al. [21] used the Attention-Deficit and Hyperactivity Disorder (ADHD) Self-Reported Scale (ASRS) to assess 49 patients with SLE and 46 matched healthy control subjects. Twenty-four of the patients with lupus were randomized to be given either NAC at a dose of 2.4 g/day, or NAC at a dose of 4.8 g/day or just placebo. It was found that ASRS scores were increased in patients with SLE compared with control subjects, and that this score was reduced in SLE patients treated with NAC [21].

The effect of NAC and atorvastatin on the endothelial dysfunction in patients with SLE was assessed using 32 SLE patients

### Table 1

| Study Type                                      | Year of Publication | Study Model                  | Study Purpose                                      | Study Population       | Results                                                                 | Reference |
|------------------------------------------------|---------------------|-------------------------------|---------------------------------------------------|------------------------|-------------------------------------------------------------------------|-----------|
| Antioxidants suppress mortality in female NZB × NZW F1 mouse model of systemic lupus erythematosus (parallel study) | 2001 | Mice | Examine immunomodulatory effects of NAC and cysteamine on autoimmune disease, glomerulonephritis, and mortality in the female SLE mice | Female NZB×NZW F1 (B/W) SLE mice | NAC significantly suppressed anti-DNA antibody levels and improved mortality in the female mouse model of SLE | [10] |
| NAC effects in mice in vivo and human cells in vitro | 2014 | Mice | Examine NAC effect on ROI production and F-actin polymerization in SLE | MRL/lpr mice | NAC reduced autoantibody production and nephritis | [11] |
| NAC effects on lupus in MRL mice                | 2019 | Mice | Examine NAC effect on liver injury | MRL       | NAC reduced hepatitis | [12] |
| NAC effects on gut microbiome in SLE mice       | 2021 | Mice | Examine NAC effect on oxidative stress | MRL/lpr  | NAC corrected microbiome skewing and blocked autoimmunity | [13] |

NAC, N-acetylcysteine; ROI, reactive oxygen intermediate; SLE, systemic lupus erythematosus.
along with 10 healthy control subjects who were age and sex-matched. The dose of NAC used was 600 mg 3 times a day for 2 weeks. Results showed a reduction in the stiffness index and in the reflection index in the NAC-treated group suggesting improvement in endothelial dysfunction, which is associated with decreased incidence of cardiovascular and cerebrovascular accidents [20]. Figure 1 below summarizes the above findings.

Regarding reported side effects with NAC, there is inadequate measuring and reporting of the side effects of NAC in trials. Although unclear, there has been a trend of increasing side effects with increasing dosages of NAC [24]. Some of the side effects encountered with the use of oral NAC are nausea, bloating, and bad taste [9]. In addition to this, another limitation of the use of NAC is the oral drug bioavailability: oral administration is reported to lead to NAC concentrations below 15 μM in the circulation whereas intravenous injections of NAC achieve serum concentrations between 100 and 1500 μM [21]. To improve lipophilicity of NAC, an amide derivative, N-acetylcysteine reduces disease activity by blocking mammalian target of rapamycin in T cells from systemic lupus erythematosus patients (randomized, double-blind, placebo-controlled trial) [8].

In conclusion, the mechanism of NAC in the treatment of SLE seems to be associated with its role in the oxidative stress. A recently published 36-week open-label clinical trial was conducted in 8 older adults (OAs) and 8 young adults (YAs). OAs were studied after GlyNAC (combination of glycine and NAC) supplementation for 24 weeks, and GlyNAC withdrawal for 12 weeks. GlyNAC supplementation for 24 weeks in OAs corrected RBC-GSH (glutathione) deficiency, oxidative stress, and mitochondrial dysfunction; and improved inflammation, endothelial dysfunction, insulin-resistance, genomic-damage, cognition, strength, gait-speed, and exercise capacity; and lowered body-fat and waist-circumference [11].

The above-mentioned SLE-related studies have shown promising results with NAC use especially considering its relative safety compared to other existing, biological or conventional, immunosuppressive therapies, which invariably predispose to infections, a leading cause of death in patients with SLE. Currently, a phase II trial is under way to evaluate the tolerance and effect of NAC in SLE patients, and assess SLEDAI, BILAG, Fatigue Assessment Scale, Patient Reported Outcomes Measurement Information System, ASRS, prednisone use,
liver and bone marrow function as secondary outcomes. Similar to rapamycin [32], via blockade of mitochondrial oxidative stress and mTOR [10,33], NAC may have the premise to expand antiviral CD8 T cells and reverse infections [8] and thus potentially expand life-span in SLE [34]. We are currently conducting a double-blind placebo-controlled trial of 12-month duration which should provide further information on safety and efficacy of NAC in SLE (ClinicalTrials.gov Identifier: NCT00775476).

Conflicts of interest
The authors declare that they have no conflicts of interest.

Funding
This work was supported in part by grants R01AI072648, R01AI122176, and U01AR076092 from the National Institutes of Health, the Phillips Lupus and Autoimmunity Center of Excellence, and the Central New York Community Foundation.

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How to cite this article: Nasr S, Perl A. Principles behind SLE treatment with N-acetylcysteine. *Immunometabolism.* 2022;4(4):e00010. doi: 10.1097/IN9.0000000000000010.