Original Article

Niclosamid elegantramine attenuates systemic lupus erythematosus and lupus nephritis in MRL/lpr mice

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Abstract: Systemic lupus erythematosus (SLE) is an autoimmune disease with multiple organ involvement. Lupus nephritis (LN) is a severe manifestation of the disease and the most common cause of mortality in SLE patients. The etiology of LN is multifactorial and accumulating evidence suggests that mitochondrial dysfunction contributes to LN initiation and progression. Mild mitochondrial uncoupler niclosamide ethanolamine salt (NEN) has recently been shown to be efficacious in the treatment of both diabetic kidney disease and non-diabetic adriamycin nephropathy. However, its role in autoimmune kidney disease has not been explored. Here, we report for the first time that NEN attenuated SLE and lupus nephritis in MRL/lpr mice. NEN treatment reduced urinary protein excretion and attenuated glomerular lesions in this model. NEN treatment also decreased urinary excretion of tubular injury biomarkers NGAL and Kim-1, restored renal tubule phenotypic alterations, inhibited tubular proliferation, and suppressed renal interstitial inflammation and fibrosis. In addition, NEN diet supplementation restored redox imbalance, promoted mitochondrial biogenesis, and improved energy dysregulation in the kidney. Importantly, NEN prevented the enlargement of lymph nodes and the spleen, and decreased serum anti-dsDNA antibody levels in the MRL/lpr mice. Therefore, our data suggest that this mild mitochondrial uncoupling agent has great potential for translational application as a novel therapy for autoimmune disease.

Keywords: Niclosamide ethanolamine salt, systemic lupus erythematosus, lupus nephritis, mitochondrial dysfunction

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder that is characterized by loss of tolerance to nuclear self-antigens and sustained overproduction of pathogenic autoantibodies. The clinical presentation of SLE is heterogeneous and multiple organs are involved [1]. As a severe manifestation of the disease, lupus nephritis (LN) is the most common cause of mortality in SLE patients. Despite advancement in diagnosis and treatment over the past decades, LN is still a challenge for clinicians [2]. Therefore, it is imperative to further study the pathogenesis of LN, with the intent of developing effective therapeutics for this pathology.

Although the etiology of LN is multifactorial and remains largely unknown, previous studies indicate that autoantibody production, immune complex deposition, complement activation, inflammation, fibrogenesis, aberrant cell proliferation, and apoptosis are all pathophysiological processes involved in LN [3]. In addition, metabolic pathways are increasingly considered to play a key role in autoimmune disease [4]. In patients with SLE and in lupus-prone mice, immune cells and target organs have metabolic abnormalities [5, 6].

Mitochondria are the major site of cellular energy metabolism and participate in many cellular signal transduction pathways [7]. The kidney is abundant in mitochondria and accumulating evidence suggests that mitochondrial dysfunction contributes to LN initiation and progression [8]. Mitochondria are also the main source of reactive oxygen species (ROS). Studies indicate
that a redox imbalance correlates with disease activity and mediates target organ damage in patients with SLE and lupus-prone mice [9]. Therefore, mitochondria are potential targets for the treatment of SLE and LN [10].

Niclosamide ethanolamine salt (NEN) is a mild mitochondrial uncoupler and is widely used as an anthelmintic drug [11]. Mitochondrial uncoupling is a key mechanism that regulates superoxide formation and mitochondrial biogenesis [12, 13]. Previous studies demonstrate that NEN is efficacious in treating diabetic kidney disease and non-diabetic adriamycin nephropathy [14-16]. However, its role in autoimmune kidney disease has not been investigated to date. Therefore, this study seeks to determine the effects of NEN treatment in lupus-prone MRL/lpr mice, and explore the mechanisms involved.

Materials and methods

Animal experiments

Female MRL/lpr mice (3-4 weeks old) were purchased from Shanghai SLAC Laboratory Animal Company (Shanghai, China). Age matched female C57BL/6J mice were purchased from Guangdong Medical Laboratory Animal Center. All mice were housed in the Central Animal Facility at Shenzhen Graduate School of Peking University. At 12 weeks old, the MRL/lpr mice were allocated randomly into either the MRL/lpr group (SLE group, n = 6) or the MRL/lpr+NEN group (SLE+NEN, n = 6). Female C57BL/6J mice served as the control group (n = 6). The control and SLE groups received a standard diet, and the SLE+NEN group received a standard diet supplemented with 10 g/kg NEN (Hubei ShengTian HengChuang Biotech Co., Ltd, Wuhan, China) for 8 weeks. All animal procedures were approved by the Guangzhou University of Chinese Medicine Institutional Animal Care and Use Committee. All experiments were performed in accordance with relevant guidelines and regulations.

Tissue preparation

Mice were sacrificed following the 8 weeks of NEN supplementation. The spleen and lymph nodes (submaxillary, parotid, axillary, mesenteric, and inguinal) were isolated, weighed, and imaged. The kidneys were dissected and rinsed in phosphate buffered saline. A portion of the kidney was fixed in 10% formalin for pathological examination and immunohistochemical staining. The renal cortex (1 mm³) was fixed in 2.5% glutaraldehyde and then post-fixed in 1% osmic acid for transmission electron microscopy (TEM). The remaining renal tissues were immediately snap-frozen in liquid nitrogen and stored at -80°C for later analyses.

Light microscopy

Paraffin-embedded renal sections (4 μm) were stained with periodic acid-Schiff (PAS), hematoxylin and eosin (HE), and Masson trichrome. Using PAS staining, thirty glomeruli in each section were randomly selected under 40× magnification. Glomerular tuft area, number of nuclei, glomerular injury, and the degree of glomerulosclerosis were measured and evaluated. Glomerular injury was scored on a semiquantitative scale: 0 = normal [35 to 40 cells/glomerular cross section (GCS)]; 1 = mild [glomeruli with mild hypercellularity (41 to 50 cells/GCS)]; 2 = moderate [glomeruli with moderate hypercellularity (51 to 60 cells/GCS) and/or segmental or global sclerosis and/or, necrosis and crescent formation]. The glomerulosclerosis index was scored on a scale of 0 to 4 based on the percentage of PAS staining positive area within the glomerulus (0, <5%; 1, 5%-25%; 2, 25%-50%; 3, 50%-75%; 4, >75%). The PAS staining was also used to evaluate tubular injury that was defined by the presence of tubular dilation, atrophy, intraluminal casts, loss of proximal tubular brush border, and tubular cell vacuolization and detachment. Twenty tubular areas in each section were randomly selected under 20× magnification and scored on a scale of 0 to 4 on the percentage of PAS staining positive area within the tubule (0, <5%; 1, 5%-25%; 2, 25%-50%; 3, 50%-75%; 4, >75%). HE staining was used to evaluate the renal inflammatory infiltration index. Twenty inflammation regions under 40× magnification were randomly selected. The inflammatory infiltration index was determined according to the number of cell layers and graded on a scale of 0 to 3 (score: 0 = none; 1 = <5 cell layers; 2 = 5 to 10 cell layers; 3 = >10 cell layers). Masson trichrome staining was used to evaluate interstitial fibrosis. Fifteen interstitial fields in renal cortex were randomly selected.
under 20× magnification. Interstitial fibrosis was defined as the area occupied by positive interstitium and graded on a scale of 0 to 4 (0, <5%; 1, 5%-25%; 2, 25%-50%; 3, 50%-75%; 4, >75%).

**Immunohistochemical staining**

Immunohistochemical staining was performed on 4 μm thick renal sections. After antigen retrieval with citrate buffer, sections were incubated with primary antibodies against WT-1, p-E-cadherin (S838+S840), Ki-67, catalase, and superoxide dismutase 2 (SOD2) respectively. The sections were then washed and incubated with HRP-polymer conjugated anti-Mouse/Rabbit IgG complex. Staining was detected using a diaminobenzidine tetrahydrochloride solution and counterstain with hematoxylin. Twenty glomeruli and renal tubular areas were randomly selected to count WT-1 and Ki-67 positive cells.

**Immunofluorescence staining**

Frozen kidney sections (6 μm) were fixed with acetone for seconds. After rinsing, the sections were incubated with FITC conjugated primary antibodies against IgG and C3 respectively. Images were visualized and captured on a confocal microscope (LSM710, Carl Zeiss, Oberkochen, Germany). Ten to twenty images were randomly selected in each section to calculate the positive area ratio of IgG and C3 in glomerulus.

**Transmission electron microscopy**

TEM images were photographed by JEM-1400 (JEOL, Tokyo, Japan). Eight images per sample were analyzed using Image J software (National Institutes of Health, Bethesda, MD, USA) and the average podocyte foot process width (FPW) was calculated according to a previously described method [17]. Glomerular immune complex deposition in mesangial, subendothelial, and subepithelial regions was observed and photographed.

**ELISA**

Urinary neutrophil gelatinase-associated lipocalin (NGAL) and Kim-1 (R&D Systems, Minneapolis, MN, USA) levels were measured by ELISA according to the manufacturer’s instructions. The serum level of anti-dsDNA antibodies was also measured by ELISA, as previously reported [18]. Briefly, 96-well culture plates were pre-coated with 5 μg/ml calf thymus dsDNA (Sigma-Aldrich, USA) overnight. A mouse anti-dsDNA monoclonal antibody (EMD Millipore, Temecula, CA, USA) was used to prepare a reference standard curve. For analysis, absorbance was measured at 450 nm.

**Urinary protein and H2O2 assay**

Urine was collected using metabolic cages (Techniplast, Buguggiate, Italy). Urinary protein was detected using metabolic cages (Techniplast, Buguggiate, Italy). Urinary protein was detected by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Urinary H2O2 level was determined using Amplex UltraRed reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions.

**Immunoblotting analysis**

Renal tissues were lysed and prepared in sample loading buffer (Bio-Rad). The lysates were subjected to immunoblotting analysis according to previously described method [17]. Antibodies against p-E-cadherin (S838+S840) and E-cadherin were purchased from Abcam (Cambridge, UK). Antibodies against Vimentin, proliferating cell nuclear antigen (PCNA), p-4E-BP1 (Ser65), 4E-BP1, p-S6RP (Ser235/236), S6RP, p-Erk1/2 (Thr202/Tyr204), Erk1/2, p-p38 MAPK (Thr180/Tyr182), p38 MAPK, catalase, superoxide dismutase 2 (SOD2), VDAC, TOM20, COX IV, p-AMPK (Thr172), and AMPK were purchased from Cell Signaling Technology (Danvers, MA, USA). An antibody against Tfam was from Novus Biologicals (Littleton, CO, USA). β-actin antibody was from Sigma Aldrich (St. Louis, MO, USA). β-actin was used as the loading control.

**mRNA analysis**

Total RNA was extracted from renal tissues using the TRIzol Plus RNA purification kit (Invitrogen). First-strand cDNA was generated and quantitative real-time PCR (qPCR) was subsequently carried out in a QuantStudio 5 real-time PCR systems (Applied Biosystems, Foster City, CA, USA) as previously described [16]. Primers were synthesized by Sangon Biotechnology Company (Shanghai, China) and sequences are listed in Table 1. The amplification conditions were as follows: 95°C for 5 min fol-
lowed by 45 cycles of 95°C for 15 s, 55°C for 15 s, and 72°C for 20 s. The relative mRNA expression was calculated by $2^{\Delta\Delta CT}$ and normalized against the housekeeping gene β-actin.

### Results

**NEN treatment reduced urinary protein excretion and attenuated glomerular lesions**

Proteinuria is an important clinical manifestation of various renal diseases and glomerular lesions are the typical characteristics of LN. So we measured urinary protein excretion and evaluated glomerular pathological changes in our mouse model of SLE. Compared to control mice, urinary protein excretion significantly increased in the SLE group, as expected (Figure 1A). This increase was significantly reduced by 8 weeks of NEN treatment (Figure 1A). The SLE group presented with abnormally enlarged glomerular tuft area, and exhibited glomerular injury and glomerulosclerosis (Figure 1B-D and 1H). NEN treatment significantly attenuated these pathological changes (Figure 1B-D and 1H).

In the SLE group, the total number of nuclei per glomerulus increased compared to controls, while the number of podocytes (WT-1 positive cells) decreased significantly. These pathologi-
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A

B

C

D

E

F

G

H

I

J

PAS

WT-1 immunostaining

EM

control

SLE

SLE+NEN
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Figure 1. NEN reduced urinary protein excretion and attenuated glomerular lesions. (A) Determination of urinary protein excretion in each group. n = 6 per group. Measurement and evaluation of (B) glomerular tuft area, (C) glomerular injury index, and (D) glomerulosclerosis index in each group. n = 6 per group. (E, F) Total nuclei count and WT-1 positive cells per glomerulus in each group. n = 6 per group. (G) Measurement of FPW in different groups. n = 3 per group. (H, I) Representative images of PAS staining and WT-1 immunostaining in each group. Scale bar, 20 μm. (J) Representative TEM images showing pathological alterations of foot processes. Scale bar, 500 nm. **P<0.01 and ***P<0.001 vs. control group. *P<0.05, **P<0.01 and ***P<0.001 vs. SLE group.
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Figure 2. NEN ameliorated glomerular immune complex deposition. (A, B) Bar graphs indicating the analysis of IgG and C3 deposition in glomeruli from each group. (C, D) Representative immunofluorescence staining images of IgG and C3 in different groups. (E) Representative TEM images displaying the electron dense deposits in glomeruli of SLE and SLE+NEN groups. (a, b, e, f) Mesangial region; (c) Subendothelial region; (d) Subepithelial region; (g, h) Subendothelial region. Scale bar for (a and e), 2 μm; Scale bar for (b-d and f-h), 1 μm. n = 6 per group. ***P<0.001 vs. control group. ##P<0.01 vs. SLE group.

Figure 3. Effects of NEN treatment on renal tubular injury. A. Evaluation of renal tubular injury index in each group. B, C. Measurement of urinary NGAL and Ki-67 excretion in various groups. D. Representative images of tubule PAS staining. scale bar, 50 μm. n = 6 per group. ***P<0.001 vs. control group. ##P<0.01 and ###P<0.001 vs. SLE group.

group (Figure 4A-D). NEN treatment significantly upregulated p-E-cadherin expression and restored the ratio of vimentin to p-E-cadherin (Figure 4A, 4B and 4D).

TGF-β1 is a key mediator regulating cell phenotype alteration and proliferation. Compared to control mice, renal TGF-β1 mRNA level increased significantly in SLE mice, and this was significantly downregulated by NEN treatment (Figure 5A). Ki-67 and PCNA are markers to evaluate cell proliferative activity. In the SLE group, the number of Ki-67 positive tubular cells, evaluated by immunohistochemistry, and PCNA level in renal tissue, detected by immunoblotting, both increased remarkably compared to controls. These were both significantly reduced by NEN treatment (Figure 5B-E).
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To determine the signaling pathways involved in renal tubular proliferation, we performed immunoblotting assays to detect p-4E-BP1 (Ser65), 4E-BP1, p-S6RP (Ser235/236), S6RP, p-Erk1/2 (Thr202/Tyr204), Erk1/2, p-p38 MAPK (Thr180/Tyr182), and p38 MAPK. Increased p-4E-BP1 (Ser65), 4E-BP1, and p-S6RP (Ser235/236) and decreased p-p38 MAPK (Thr180/Tyr182) expression was seen in SLE mice when compared to controls (Figure 5D, 5F-H and 5J). NEN supplementation significantly reduced p-4E-BP1 (Ser65) and 4E-BP1 but did not have a significant effect on the other signaling pathway proteins (Figure 5D and 5F-M).

**NEN suppressed renal interstitial inflammation and fibrosis**

Interstitial inflammation is a prominent pathological feature of LN and predicts deterioration of renal function [22]. Compared to control mice, the renal inflammatory infiltration index increased significantly in the SLE mice (Figure 6A and 6J). Consistent with inflammation, the mRNA expression levels of monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-1β, tumor necrosis factor α (TNF-α), and IL-6 were obviously upregulated (Figure 6C-F). NEN treatment inhibited the inflammatory infiltration and downregulated mRNA expression levels of
Figure 5. NEN inhibited renal tubular proliferation. A. Determination of renal TGF-β1 mRNA expression level in each group. n = 6 per group. B. Counting of Ki-67 positive cells in renal tubules of different groups. n = 6 per group. C. Representative images of Ki-67 immunostaining in each group. Scale bar, 20 μm. D-M. Immunoblotting bands and quantitative analysis of p-4E-BP1 (Ser65), 4E-BP1, p-S6RP (Ser235/236), S6RP, p-Erk1/2 (Thr202/Tyr204), Erk1/2, p-p38 MAPK (Thr180/Tyr182), and p38 MAPK in each group. n = 4 per group. *P<0.05, **P<0.01 and ***P<0.001 vs. control group. *P<0.05, **P<0.01 and ***P<0.001 vs. SLE group.
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MCP-1 and IL-1β (Figure 6A, 6J, 6C and 6D). However, the mRNA levels of TNF-α and IL-6 were not significantly reduced by NEN (Figure 6E and 6F).

Renal interstitial fibrosis is associated with poor prognosis in various kidney diseases. Therefore, we evaluated the interstitial fibrosis index and measured fibrosis related mRNA expression, including fibronectin (FN), collagen (COL) III, and COL I. In agreement with an increased renal interstitial fibrosis index, the mRNA levels of FN, COL III, and COL I were also significantly increased in the SLE mice (Figure 6B, 6K and 6G-I). NEN treatment significantly reduced the interstitial fibrosis index and down-regulated the mRNA levels of FN and COL III (Figure 6B, 6K, 6G and 6H). However, the mRNA level of COL I was not significantly affected by NEN (Figure 6I).

NEN treatment restored redox imbalance in the kidney

Catalase and SOD2 are important enzymes that regulate the intracellular redox state. Compared to control mice, the protein levels of renal catalase and SOD2 decreased and urinary \( \text{H}_2\text{O}_2 \) excretion increased remarkably in SLE mice (Figure 7A-D). NEN treatment restored the renal redox imbalance in lupus mice (Figure 7A-D). Immunohistochemical staining revealed that catalase and SOD2 were primarily expressed in renal tubules and scarcely in glomeruli (Figure 7E and 7F). This suggests that the renal tubules might be the site of oxidative stress in this disease.

NEN promoted mitochondrial biogenesis and improved energy dysregulation in the kidney

The structural integrity of mitochondria is essential for maintaining their physiological function. Tfam is a transcription factor and plays a critical role in mitochondrial biogenesis [23]. In SLE mice, Tfam levels and mitochondrial components, including voltage-dependent anion channel (VDAC), transporter of outer mitochondrial membrane (TOM20), and cytochrome oxidase (COX) IV, were decreased (Figure 8A-E). As the guardian of metabolism...
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Figure 7. NEN recovered redox imbalance in kidney. A-C. Immunoblotting bands and quantitative analysis of catalase and SOD2 in each group. n = 4 per group. D. Bar graph showing the fold change of urinary H_2O_2 excretion in various groups. n = 6 per group. E, F. Representative immunohistochemistry images showing the expression of catalase and SOD2 in the kidney. Scale bar, 20 μm. **P<0.01 and ***P<0.001 vs. control group. ##P<0.01 and ###P<0.001 vs. SLE group.

and mitochondrial homeostasis, AMP-activated protein kinase (AMPK) was activated in response to mitochondrial deficiency in the kidneys of SLE mice (Figure 8A, 8F and 8G). NEN treatment significantly increased the expression of Tfam and mitochondrial components, improving energy dysregulation in the kidneys of these mice (Figure 8A-G).

NEN treatment prevented the enlargement of lymph nodes and the spleen, and decreased serum anti-dsDNA antibody levels

The lymph nodes and spleen are important organs in the regulation and initiation of immune responses. In the SLE group, abnormally enlarged spleens and lymph nodes were
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Figure 8. NEN promoted mitochondrial biogenesis and improved energy dysregulation in the kidney. A. Immunoblotting images of Tfam, VDAC, TOM20, COX IV, p-AMPK (Thr172), and AMPK in renal tissues of various groups. B-G. Bar graphs showing the quantitative analysis of these proteins. n = 4 per group. **\(P<0.01\) and ***\(P<0.001\) vs. control group. #\(P<0.05\), ##\(P<0.01\) and ###\(P<0.001\) vs. SLE group.

observed, which was notably prevented by NEN treatment (Figure 9A-D). Consistent with these changes in the spleen and lymph nodes, increased serum anti-dsDNA antibody levels in SLE group were significantly decreased by NEN (Figure 9E).

Discussion

In this study, we report for the first time that NEN treatment is efficacious in attenuating SLE and LN. Potential mechanisms involved in its protective action include regulation of redox state, promotion of mitochondrial biogenesis, and modulation of the immune response.

Glomerular injury is the basis for pathologic classification in LN [24]. In the current study, SLE mice had increased total nuclei in their glomeruli. Considering the loss of podocytes observed, we hypothesized that this increase may be due to proliferation of mesangial or endothelial cells or infiltrating inflammatory cells. NEN treatment not only prevented an increase in cell nuclei counts in the glomeruli but also attenuated podocyte loss. Therefore,
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Figure 9. NEN prevented the enlargement of lymph nodes and spleen, and decreased serum anti-dsDNA antibody level. A, B. Representative images of lymph nodes from SLE and SLE+NEN group mice, and bar graphs showing the results of weight statistics. C, D. Images of the spleen and the weight statistics in various groups mice. E. Measurement of serum anti-dsDNA antibody levels in SLE and SLE+NEN group mice. n = 6 per group. ***P<0.001 vs. control group. ##P<0.01 and ###P<0.001 vs. SLE group.

NEN may exert different effects on different cell types.

Although glomerular lesions are the focus of LN research, prominent tubular injury also impacts renal outcome [25]. In the normal kidney, renal tubular cells exhibit a characteristic phenotype and proliferate at a relatively slow rate [26]. In the stressed kidney of lupus mice, renal tubules undergo phenotypic alterations and cells are hyperproliferative. TGF-β1 is known to mediate tubular cell phenotypic alteration and proliferation [27]. Therefore, an upregulation in TGF-β1 mRNA level may explain the pathological changes in our model. We also explored cell signaling pathways that may participate in tubular cell growth and proliferation. The data demonstrated that multiple pathways are involved and the translation repressor protein, 4E-BP1, may be a potential therapeutic target of NEN.

In addition to glomerular and tubular injury, interstitial inflammation also plays an important role in LN progression. In SLE mice, inflammatory cell infiltration was observed in the renal interstitium. Their distribution was predominantly surrounding the blood vessels and the glomeruli, particularly at the vascular pole. Consistent with this increase in inflammatory cells, the mRNA levels of inflammation related cytokines including MCP-1, IL-1β, TNF-α, and IL-6, were upregulated. NEN treatment significantly downregulated the expression of MCP-1 and IL-1β, suggesting that NEN has inhibitory effects on inflammation. However, the mechanisms behind this require further studies.

Interstitial fibrosis is a common feature of various chronic kidney diseases. It is a sign of chronicity in LN progression and a predictor of poor
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prognosis. A previous study indicated that renal fibrosis is associated with defective fatty acid oxidation in renal tubular cells and that targeting the metabolic defect may prevent renal fibrosis [28]. It is well known that mitochondrial uncoupling increases the oxidation of fatty acids. So the preventing effects of NEN on interstitial fibrosis could be at least partly attributed to its uncoupling action on mitochondria in renal tubular cells.

Oxidative stress is implicated in LN progression. Consistent with previous studies [29], the kidneys in SLE mice had a redox imbalance, demonstrated by decreased catalase and SOD2 expression, and increased urinary H₂O₂ excretion. Mitochondrial uncouplers are able to dissipate protonmotive forces and greatly reduce ROS production [12]. Therefore, we hypothesized that the protective effect of NEN on redox imbalance might be associated with its uncoupling function and ability to upregulate catalase. In addition, Tfam loss, mitochondrial components deficiency, and energy homeostasis dysregulation also occurred in the kidneys of SLE mice. Mice with tubule-specific deletion of Tfam developed severe mitochondrial loss and energy deficit, progressing to renal fibrosis and failure [30]. NEN’s upregulation of Tfam may explain its positive role in mitochondrial biogenesis and regulation of energy metabolism. This also strongly suggests that mitochondrial integrity is crucial in maintaining function. However, the underlying molecular mechanisms remain unclear.

The spleen and lymph nodes are involved in B and T cells mediated immune responses. Splenomegaly and lymphadenopathy are the manifestations of immune disorders in SLE mice [31]. In this study, we found that NEN treatment notably reduced the enlarged spleen and lymph nodes. So we postulated that NEN may exert immunomodulatory effects on B cell activation. As expected, the increased serum anti-dsDNA antibody level in SLE mice was significantly reduced by NEN treatment. In line with the level of serum anti-dsDNA antibodies, glomerular immune complex deposits in SLE mice were also attenuated by NEN. Taking these results into account, we conclude that the renal improvement induced by NEN treatment is associated with its immunomodulatory effects.

In summary, we demonstrate here the efficacy of NEN treatment in SLE and LN. These findings suggest that this mild mitochondrial uncoupling agent has great potential for translational application as a novel therapy for autoimmune disease.

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Disclosure of conflict of interest

None.

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