Individualizing Therapy in Lupus Nephritis

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The ideal therapeutic approach for lupus nephritis (LN) is to quickly achieve a complete remission and maintain that response long-term while minimizing drug toxicity, and prevent tissue damage and death. The combination therapy consisting of multiple medications is aimed at incorporating drugs with complementary actions at reduced doses to achieve additive or synergistic therapeutic effects while minimizing toxicity. Here, we review the available evidence using combination therapies (triple therapy) and how such strategies can improve therapeutic efficacy in LN, which will mainly focus on the combination of high-dose corticosteroids with mycophenolate mofetil (MMF) and a calcineurin inhibitor (CNI) at low dose. We discuss the rationale, efficacy, and safety of the therapy, as well as its molecular mechanisms. We also discuss the questions raised from the trials and briefly describe emerging approaches developed on the basis of combination therapy, and these advances that promise to improve on the standard-of-care treatments and toward individual therapy in LN.

Renal involvement still significantly affects the survival and quality of life of patients with systemic lupus erythematosus (SLE). Although prognosis of LN has improved dramatically over the past decades,[1,2] long-term outcome remains unsatisfactory not only because of progression toward end-stage kidney disease in a significant subset of patients, but also because of disease or treatment-related comorbidities.[3–6] Thus, questions remain regarding the choice and timing of drug administration and tapering until withdrawal, which both can affect the balance between the control of disease activity and damage of the organs related to long-standing immunosuppression.[7–9] Exploring the potential of combination therapies using currently available drugs may provide opportunities to make better choice of treatment individualization for LN. In this review, we discuss the available therapeutic evidences in LN using combination therapies (triple therapy) and how such strategies can improve therapeutic efficacy in LN, which will mainly focus on the combination of high-dose corticosteroids with MMF and a CNI at low dose.

Rationale of Combination Therapy

SLE is an autoimmune disease that affects multiple organs and tissues, renal involvement is the most important predictor of morbidity and mortality. The immune dysregulation is fundamental to the pathogenesis of LN, with B cells, T cells, and complement activation involved in the development of the disease. Current widely accepted treatment regimens for LN incorporate high-dose corticosteroids for rapid control of inflammation and immunosuppression to control inflammation and autoimmunity. However, the incidence of complete remission with these regimens remains low, and adverse events are still a major concern. Therefore, new therapeutic approaches for LN are needed.

MMF is known to be a selective lymphocyte antiproliferative agent and reversibly inhibits the de novo pathway of purine synthesis in the proliferation of B and T lymphocytes.[10] The Aspreva Lupus Management Study firmly established the use of MMF as an alternative initial treatment for LN.[11] Corticosteroids combined with MMF is one of the current standard-of-care induction treatment regimens for active severe LN.[12,13] CNIs block T-cell activation through suppressing the calcium and calcimodulin-dependent phosphatase calcineurin. They are attractive therapeutic options for LN. Their effects attributed both to their immunosuppressive efficacy and the action of these agents on podocyte biology leading to more rapid proteinuria suppression and a higher complete response rate.[14]

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Tacrolimus (TAC) is a potent CNI used for prevention of rejection in organ transplant recipients.[15–17] Results from a randomized clinical trial, which compared TAC against intravenous cyclophosphamide (IVCY) in active proliferative LN, showed comparable efficacy of TAC (complete response rates were 52.4% in the TAC group and 38.5% in the IVCY group).[18] Combination therapy consisting of multiple medications is aimed at incorporating drugs with complementary actions at reduced doses to achieve additive or synergistic therapeutic effects while minimizing toxicity. In fact, combination therapy with steroids, TAC, and MMF has been used for many years as an antirejection therapy in transplant patients.[15–17]

We conducted a pilot study to evaluate the combination therapy of high-dose steroids with MMF and TAC at low dose in patients with concurrent class IV and V LN, which constitutes an important fraction of severe LN and is often refractory to conventional treatment.[19] The combination therapy demonstrated a higher incidence of complete remission and overall response in patients with LN with significant membranous features in the kidney biopsy compared with IVCY and steroids. In addition, the combination therapy group experienced fewer adverse events than the IVCY group.[19]

Efficacy and Safety of Combination Therapy

To further assess the efficacy and safety of the combination therapy, a prospective, multicenter, randomized controlled trial was conducted in China.[20] A total of 368 patients with biopsy-proven LN with class III, IV, V, III+V, and IV+V lesions were randomly assigned to the combination regimen or IVCY group. Both groups received i.v. methylprednisolone pulse therapy (0.5 g/d) for 3 days, followed by oral prednisone. The combination group received TAC (2 mg twice daily) and MMF (0.5 g twice daily), whereas the IVCY group received an initiating dose of 0.75 (adjusted to 0.5 to 1.0) g/m^2 of body surface area every 4 weeks. After 6 months of therapy, significantly more patients in the combination group than in the IVCY group achieved complete remission (45.9% vs. 25.6%, $P < 0.001$). The cumulative probability of complete remission was also higher in the combination group than the IVCY group (45.8% vs. 26.8%, $P < 0.001$). The overall response incidence was significantly higher in the combination group compared with the IVCY group (83.5% vs. 63.0%, $P < 0.001$). Noteworthy, the combination therapy is associated with more rapid proteinuria reduction and thus a higher early response rate. The median time to overall response was shorter (8.9 weeks vs. 13.0 weeks) in the combination group. In addition to markedly decreased proteinuria, the combination group also accompanied with significant changes in SLE–disease activity index score and serum C3 levels at the same time.[20] These observations, together with the finding of transcriptional profile of renal biopsy tissue from patients with LN and in a mouse model of lupus-like nephritis, which will be introduced later on, implicate that the immune mechanism of the combination therapy plays a role in treatment of LN.[21]

The disease manifestations and outcomes in LN are heterogeneous, and renal histopathology findings have an important role in informing treatment decisions and prognosis prediction. Subgroup analysis was performed according to the pathologic classification. It was shown that the incidence of complete remission rate was higher in the combination group than the IVCY group among patients with class IV LN (51.5% vs. 29.9%), class V LN (33.1% vs. 7.8%), and class IV+V LN (45.2% vs. 26.5%).[22] These findings suggested that combination therapy may be a valuable treatment approach in patients with LN not only with proliferative lesions (class IV) but with membranous (class V) lesions that usually do not respond well to conventional treatment. Patients with class V (with or without concurrent class IV or III) may need to consider choosing the combination therapy as an alternative therapy, including young women to avoid ovarian toxicity from cyclophosphamide therapy. The combination therapy needs to be used cautiously in patients to avoid nephrotoxicity and metabolic side effects of CNI.

To observe resolution of renal tissue injury after treatment, repeat renal biopsies were done in some patients after treatment. It was revealed that glomerular mesangial and subendothelial immune deposits were significantly reduced, the "wire loops" and thrombi disappeared with remaining mild to moderate mesangial expansion and occasional endothelial cell proliferation. The intensity of staining for glomerular IgG deposition also decreased. Although the activity index markedly decreased in both treatment groups, with numerically more pronounced changes in the combination group (Figure 1). These observations indicated that clinical remission accompanied histologic remission in the kidney tissue after the treatment.

Most patients in the combination group tolerate the therapy well, with a similar incidence of adverse events (50.3% vs. 52.5%) to IVCY during the induction phase.[20] Compared with IVCY, the combination therapy was less likely to cause ovarian failure, as well as gastrointestinal symptoms, leukopenia, and liver dysfunction.[20,22] However, despite no statistical significance, the incidence of serious adverse events was numerically higher in the combination group (7.2% vs. 2.8%), mostly due to infection, including pneumonia, varicella zoster virus, and upper respiratory tract
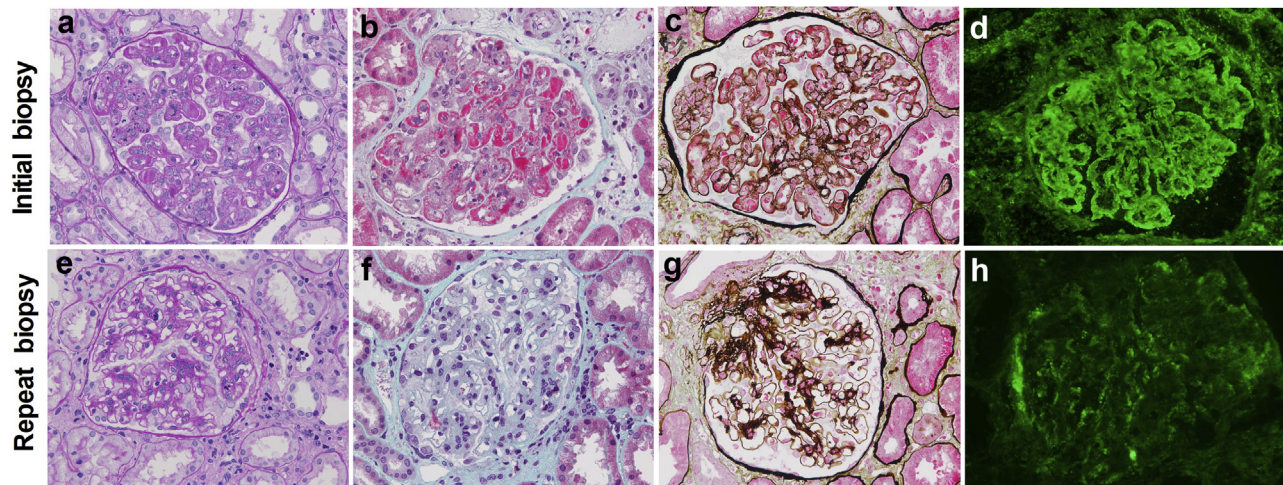
Figure 1. Histologic changes in a patient who achieved complete remission after induction therapy with combination therapy. The initial kidney biopsy revealed that the glomeruli showed diffuse and massive immune complex deposits in the mesangial and subendothelial areas, with thrombi in the capillary lumens. (a) Periodic acid–Schiff, original magnification ×400. (b) Masson trichrome, original magnification ×400. (c) Periodic acid–Schiff methenamine silver Masson, original magnification ×400. (d) Immunofluorescent labeling of IgG, original magnification ×400. A repeated biopsy indicated that glomerular mesangial and subendothelial deposits were significantly decreased and that "wire loops" and thrombi disappeared with remaining mild-to-moderate mesangial expansion and occasional endothelial cell proliferation. The intensity of staining for IgG also decreased. (e) Periodic acid–Schiff, original magnification ×400. (f) Masson trichrome, original magnification ×400. (g) Periodic acid–Schiff methenamine silver Masson, original magnification ×400. (h) Immunofluorescent labeling of IgG, original magnification ×400.

infection.[20] The adverse events should be monitored cautiously during treatment. Therapeutic drug monitoring is a useful tool to minimize drug-related toxicities. In this trial, the mean blood trough concentration of TAC was approximately 5.5 ng/ml and mycophenolic acid area under the concentration-versus-time curve was approximately 30 mg.h/l during the induction phase. The target blood trough concentrations of TAC were 5 to 10 ng/ml according to the protocol. The dosage of TAC was reduced in those who had a blood concentration that continued to be >10 ng/ml, with or without signs of toxicity or changes of serum creatinine. Mycophenolic acid area under the concentration-versus-time curve between 35 and 45 mg.h/l was suggested during initial therapy.[23] In those who had obvious gastrointestinal symptoms or leukopenia, the dosage of MMF was reduced. Because the pharmacokinetics of TAC and mycophenolic acid has a high interindividual variability, the blood levels should be carefully monitored and adjusted during treatment.

To assess the efficacy and safety of combination therapy for maintenance treatment in LN, we continued to treat patients for an additional 18 months and compared them with those of azathioprine (AZA) treatment in 206 patients with LN.[24] Patients who achieved a complete or partial remission in the induction trial were recruited for an additional 18 months of treatment. The combination group continued to receive reduced doses of TAC (2–3 mg/d) and MMF (0.5–0.75 mg/d), whereas the IVCY group was switched to AZA

(2 mg/kg body weight per day). Oral prednisone was administered to all participants at a dosage of 10 mg/d. There were no significant differences in cumulative renal relapse rates (5.47% vs. 7.62%, $P = 0.74$) between groups. The adverse events were significantly higher in the AZA group, and the combination group had a lower withdrawal rate. The percentages of patients in the combination therapy group who maintained their complete remission status at 12 and 18 months were 72.5% and 78.3%, respectively, and the corresponding percentages in the AZA group were 67.6% and 78.0%; the differences were not significant between the 2 groups.[24] Accumulated evidence suggests that earlier achievement of remission is associated with a better long-term outcome, patients with early remission spent significantly less of their follow-up time with active disease, had lower annual relapse rates, and lower cumulative steroid dosage, lower average scores for disease activity (SLE–disease activity index) than those with persistent activity.[25–27] As we discussed previously, the complete remission observed in the combination group not only showed decreased proteinuria, but also was accompanied with significant changes in SLE–disease activity index score and serum C3 levels. Repeat renal biopsy revealed a solution of immune injury in kidney tissue. This evidence indicated that combination therapy preserved the kidney from active tissue injury much more rapidly and effectively than IVCY. In addition, combination therapy was associated with a decreased incidence of

adverse events compared with AZA, especially in leukopenia and liver dysfunction. This may be related to the lower dosages required during the maintenance phase. Therefore, combination therapy is more effective than IVCY, and long-term and extended studies are needed to evaluate the advantage of combination therapy in LN. In addition, we may need to consider using combination therapy for induction therapy in active LN to quickly achieve a complete remission, and then sequentially switch to the regimen of standard-of-care treatment for maintenance therapy.

The approach of this kind of combination therapy was tested in subsequent clinical trials.[28–31] Meta-analysis showed that the combination therapy is more effective for inducing remission compared with IVCY, MMF, and CNIs.[22, 32,33] Besides initial treatment, it can also help refractory or relapsing patients achieve a renal response and reduce the use of steroids.[28–31] Not only tacrolimus but also cyclosporine combined with MMF can induce complete remission in LN and was well tolerated.[30,31] Recently, the addition of low-dose voclosporin, a new CNI, showed a superior renal response compared with background MMF and corticosteroids alone in the Aurinia Urinary Protein Reduction Active-Lupus With Voclosporin trial.[34] The complete renal remission rate was significantly higher with low-dose voclosporin than with placebo at week 24 (32.6% vs. 19.3%) and week 48 (49.4% vs. 23.9%). Complete renal remission was achieved more rapidly in the voclosporin plus MMF group than in the MMF-alone group.[34] Importantly, it also demonstrated the efficacy of the combination therapy in a global cohort, suggesting that such combination therapy may be applicable to multi-ethnic patients with LN. In the Aurinia Urinary Protein Reduction Active-Lupus With Voclosporin study, however, there were more serious adverse events, including deaths, in the combination therapy group.[34] The incidence of adverse events was disproportionately higher in low-dose voclosporin group even when compared with the high-dose group, suggesting mortality may not be directly linked to drug exposure but rather to other factors, which is expected to be addressed in future studies.[35]

Other triple regiments included various drug combinations of corticosteroids, CNIs, cyclophosphamide, or mizoribine.[36–38] Observational studies, most of which were performed in Japan, reported short-term efficacy of these regimens. But the results were limited by small sample size, insufficient observation period, and absence of controls.

Recently, several novel approaches to treatment that have more specific effects on the immune system have been studied in LN.[7] B cells play an important role in the pathogenesis of LN and are therefore attractive therapeutic targets. B-cell depletion by rituximab results in enhanced expression of B lymphocyte-stimulator, also known as B-cell–activating factor. The B lymphocyte-stimulator stimulates B-cell reconstitution and may facilitate development of autoreactive B cells, negating the effect of anti-CD20 therapy.[39] Using belimumab (a humanized monoclonal antibody inhibitor of soluble B-cell–activating factor) may theoretically decrease relapses induced by higher B-cell–activating factor levels post- rituximab. Recently, reports illustrated a promising value of adding belimumab after rituximab treatment in LN.[40–45] The Immune Tolerance Network CALI-BRATE study will further test this hypothesis by sequential administration of cyclophosphamide plus rituximab followed by belimumab in a multicenter randomized clinical trial (identifier: NCT02260934).

Molecular Basis of Combination Therapy

To explore the underlying molecular and cellular mechanisms of increased efficacy of the combination therapy regimen, especially to reveal whether there are any additive or synergistic effects from this kind of combination, we used a mouse model of LN, MRL/lpr mice, and treated them with monotherapies of prednisone, MMF, or tacrolimus, or with their combination.[21] Transcriptome profile of kidney tissue from the combination therapy group was most similar to that of the healthy control, indicating that the combination therapy effectively restored the expression of genes altered in the LN kidney to a normal phenotype. Pathway enrichment analysis revealed that several key molecules or pathways involved in LN were regulated uniquely in the combination therapy group. Compared with monotherapies, the top downregulated differentially expressed genes were involved in both T- and B-cell receptors and in type II interferon signaling pathways in the combination group.[21] In addition, the study demonstrated that the combination therapy led to better stabilization of the podocyte actin cytoskeleton through the reciprocal regulation of RhoA and Rac1 activities. The beneficial effects of the combination therapy may be due to the addictive influence on immune or nonimmune pathways in the kidney.[21]

Interestingly, the combination group showed enhanced suppression in the activity of toll-like receptor (TLR) 7 and the expression of interleukin (IL)-6/Stat3 pathway, which are known to be deeply involved in the pathogenesis of LN.[21] These findings were further validated in renal biopsy samples from patients with LN before and after treatments with MMF, TAC, or combination therapy. Plenty of data implied an important role for TLR7 in both

murine and human SLE and LN. Unregulated TLR7 induces distinct effector B cells and contributes to pathogenic responses in lupus.[46] Enhanced responsiveness to TLR7 may adversely affect B-cell tolerance even at the early transitional stage and facilitate expansion of autoreactive B cells.[47,48] Pharmacological TLR7 activation stimulates a B-cell– and dendritic cell–dependent systemic immune response and aggravates LN.[49,50] IL-6 is a pleiotropic cytokine with a wide range of biological activities in immune regulation and inflammation.[51] IL-6 levels are elevated in both human and murine lupus and blocking IL-6 or its receptor had a beneficial effect in models of lupus.[52] However, phase II clinical trials of anti–IL-6 monoclonal antibody failed to demonstrate an anticipated efficacy in patients with SLE or LN.[53,54] Because many components of the immune system are simultaneously involved in the generation of systemic and renal autoimmunity in LN, it may be insufficient to intervene in a single pathway to treat LN. In our validation study in patients with LN, circulating levels of IL-6 were significantly suppressed in patients who had shown complete remission response to combination therapy, when compared with those who had not.[21] Whether serum IL-6 level could serve as an informative biomarker for treatment choice and a disease activity monitor in patients with LN may need to be determined in further study.

Conclusion

LN is an aggressive inflammatory disease; the ideal therapeutic approach is to quickly achieve a complete remission and maintain that response long-term while minimizing drug toxicity. Knowledge of pathogenesis of LN is growing quickly, and such new advances need to be translated into clinical practice. Combination therapy consisting of multiple medications is aimed at incorporating drugs with complementary actions at reduced doses to achieve additive or synergistic therapeutic effects while minimizing toxicity; such an approach could ideally be used to tailor treatment to the underlying molecular pathways. Combination therapies based on current and novel immunosuppressive and biological agents might hold particular promise for the development of innovative and highly individual therapies for LN.

DISCLOSURE

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