Low-Density Lipoprotein Apheresis Therapy for Steroid- and Cyclosporine-Resistant Idiopathic Membranous Nephropathy

Yoshinori Sato1,2, Shinichiro Tsunoda2,3, Tsuyoshi Nozue3, Qinya Pan3, Harue Wakasugi1 and Ashio Yoshimura1

Abstract

We herein report three cases of Nephrotic Syndrome (NS) with idiopathic membranous nephropathy (IMN) in which the patients were resistant to steroid (PSL) and cyclosporine (CyA) therapy. All three patients had a high risk of renal failure because of persistently high levels of proteinuria. We performed low-density lipoprotein apheresis (LDL-A) and achieved complete remission in two cases. The third patients developed NS relapse and a deteriorating renal function; however, withdrawal from dialysis therapy was achieved. There are no proven therapies for treating patients with PSL- and CyA-resistant IMN with acute deteriorating renal function or difficulties in maintaining fluid balance. We evaluated the treatment course and physiological mechanisms and reviewed similar cases in the pertinent literature.

Key words: LDL-apheresis, idiopathic membranous nephropathy(IMN), cyclosporine, nephrotic syndrome (NS)

(DOI: 10.2169/internalmedicine.51.7204)

Case Reports

Case 1

A 75-year-old woman was admitted to our hospital with tibial edema and severe proteinuria that had persisted for one year. She presented with hypoalbuminemia and hypoproteinemia. Her laboratory data were as follows: total protein (TP) 5.1 g/day, albumin (Alb) 2.5 g/dL, low-density lipoprotein cholesterol (LDL-Chol) 298 mg/dL and urinary protein (U-pro) 4.1 g/dL. A renal biopsy performed on day 2 revealed membranous nephropathy (Fig. 1a, b). Two courses of intravenous methylprednisolone (mPSL; 0.5 g/day) were administered over three consecutive days starting on days 19 and 53. Treatment with 50 mg (1 mg/kg) of prednisolone (PSL) was initiated after mPSL therapy on day 22. Seventy-five mg of cyclosporine (CyA) twice daily was added to the PSL therapy on day 37. The patient’s CyA trough level was 96 mg/dL. Despite treatment with two courses of mPSL therapy, PSL therapy and CyA therapy, the heavy proteinuria and hypoproteinemia persisted. LDL-A therapy was commenced on day 67 and repeated twice a week for three weeks. Gradually, the proteinuria decreased and the serum protein levels increased (Fig. 2). After day 201, the estimated urinary protein level was <0.3 g/day. Complete remission (CR) was maintained for almost two years after discharge without the need for immunosuppressive therapy.

Case 2

A 35-year-old man was admitted to our hospital with tibial edema and severe proteinuria. He had been previously hospitalized for thrombotic thrombocytopenic purpura (TTP) almost four years earlier. He had recovered completely even without steroid therapy and suffered no relapses until the present day. The tibial edema was noticed almost five months prior to admission. On admission, laboratory data...
Case 3

A 76-year-old man was admitted to our hospital with hyposarca and severe proteinuria. His past medical history included essential hypertension and advanced transverse colon cancer. The latter was treated with curable colectomy almost seven years prior to admission. The laboratory data were as follows: TP 4.8 g/day, Alb 2.47 g/dL, creatinine (Cre) 1.3 mg/dL, LDL-Chol 154 mg/dL and U-pro 4.4 g/dL. No findings corresponded to a relapse of colon cancer. A renal biopsy performed on day 1 revealed membranous nephropathy (Fig. 1e, f). Two courses of intravenous mPSL (0.5 g/day) were administered over three consecutive days on days 3 and 36. Treatment with 50 mg (1 mg/kg) of PSL was initiated after mPSL therapy on day 7. Treatment with 75 mg of CyA twice daily was added to the PSL therapy on day 16 and increased to 200 mg/day on day 61. The patient’s CyA trough levels were 68 mg/dL (for CyA 150 mg/day) and 98 mg/dL (for CyA 200 mg/day) (Fig. 2). The hyposarca, severe hypoalbuminemia and heavy proteinuria persisted despite this treatment. On day 42, LDL-A therapy once a week for five weeks was commenced. To manage fluid balance, low-efficiency hemodialysis three times a week for three weeks was initiated on day 64. Gradually, the urinary protein levels decreased almost one month after the cessation of LDL-A therapy. Due to an increase in the urinary volume, the patient was withdrawn from dialysis therapy.

Figure 1. Pathological analysis identified the criteria of membranous nephropathy (MN) in all cases. These findings were confirmed by light, immunoglobulin G (IgG) immunofluorescence, and electron microscopy. There were no secondary focal segmental glomerular sclerosis lesions or severe tubulo-interstitial changes (e.g., changes in inflammation or fibrosis). Case 1: (a) Granular IgG deposits in capillary membrane by immunofluorescence microscopy. (b) Electron-dense deposits in subepithelial. Stages 2 and 3 are heterogeneously evident. Case 2: (c) Granular IgG deposits in along numerous capillary membrane by immunofluorescence microscopy. (d) Electron-dense deposits in subepithelial. Stages 1 and 2 are heterogeneously evident. Case 3: (e) Granular IgG deposits in along capillary membrane by immunofluorescence microscopy. (f) Diffuse electron-dense deposits in subepithelial (stages 1 and 2 are heterogeneously evident). Intramembranous dense deposit are small numbered, but detected.
Unfortunately, emergency abdominal surgery to treat a strangulated inguinal hernia was performed on day 113. CyA and steroid therapy were discontinued, causing Nephrotic Syndrome (NS) relapse. Low-efficiency hemodialysis was recommenced on day 114 owing to a deterioration of renal function. The patient recovered enough to tolerate renewed low-dose PSL and CyA therapy on day 131 (Fig. 2). However, we could not achieve NS remission. Follow-up six months after discharge was not possible, as the patient was admitted to another hospital. The latest available laboratory data for this patient were as follows: TP 3.9 g/day, Alb 2.28 g/dL, Cre 2.28 mg/dL, LDL-Chol 126 mg/dL and U-pro 4.31 g/dL.

Methods and subjects

LDL-A therapy was performed in three cases (two men and one woman) with steroid- and cyclosporine-resistant biopsy-proven idiopathic membranous nephropathy. All three patients had persistent proteinuria for more than six months before starting LDL-A therapy (Table 1). LDL-A therapy was performed in our cases using hollow polysulfone fibers (Sulflux, Kanegafuchi Chemical Industrial Co., Ltd., Kaneda, Osaka, Japan) as plasma separators and a dextran sulphate cellulose column (Lipsorba 15 LA: Kaneka, Japan) as the LDL absorber. Plasma (3,000 mL) was treated for two hours in each LDL-A session.

Figure 2. Treatment courses in 3 cases of nephrotic syndrome with idiopathic membranous nephropathy.
**Table 1. Patient Data before Low-density Lipoprotein Apheresis (LDL-A)**

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (on admission)</th>
<th>S-cre (mg/dL)</th>
<th>LDL-Chol (mg/dL)</th>
<th>Total protein (g/dL)</th>
<th>Persistent proteinuria (months)</th>
<th>Average proteinuria (g/day; before LDL-A therapy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>F</td>
<td>75</td>
<td>0.9</td>
<td>236</td>
<td>3.4</td>
<td>12</td>
<td>4.06</td>
</tr>
<tr>
<td>No. 2</td>
<td>M</td>
<td>35</td>
<td>0.8</td>
<td>134</td>
<td>4.1</td>
<td>5</td>
<td>4.19</td>
</tr>
<tr>
<td>No. 3</td>
<td>M</td>
<td>76</td>
<td>1.3</td>
<td>100</td>
<td>4.0</td>
<td>8</td>
<td>4.26</td>
</tr>
</tbody>
</table>

**Case Global Sclerosis (Number, %)**

<table>
<thead>
<tr>
<th>Case</th>
<th>Segmental Sclerosis (Number, %)</th>
<th>Interstitial Change (%)</th>
<th>IF Findings (IgG/C3)</th>
<th>EM Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>None, 0%</td>
<td>&lt;1%</td>
<td>Fine Granular/ Mes deposition (Stage 2 and 3, heterogeneously)</td>
<td></td>
</tr>
<tr>
<td>No. 2</td>
<td>1/21, 4.8%</td>
<td>&lt;1%</td>
<td>Fine Granular/ Mes deposition (Stage 1 and 2, heterogeneously)</td>
<td></td>
</tr>
<tr>
<td>No. 3</td>
<td>1/21, 4.8%</td>
<td>10%</td>
<td>Fine Granular/ Mes deposition (Stage 1 and 2, heterogeneously) and intramembranous dense deposit</td>
<td></td>
</tr>
</tbody>
</table>

S-cre: serum creatinine, LDL-Chol: low-density lipoprotein cholesterol, Mes: Mesangium

**Discussion**

IMN is one of the most common causes of NS in adults (1). The difficulty of choosing a line of treatment for patients with IMN has been vigorously discussed, not only because of the possibility of inducing renal failure and/or end-stage renal disease, but also because spontaneous remission (SR) is more common than expected (2). However, we recognize that severe proteinuria and renal impairment lasting for an extended period of time are important factors leading to the deterioration of renal function or increasing mortality even after appropriate treatment intervention (3).

We administered LDL-A as additional therapy in patients with IMN for at least six months (Table 1). Research has found that if proteinuria >3.5 g/day persists for longer than six months, the possibility of SR declines to 75% (3-5). The efficacy of CyA treatment in patients with steroid-resistant membranous nephropathy (MN) has been previously reported (3). In our cases, initial proteinuria measurements were not available, as, in each case, the proteinuria had persisted for several months prior to admission. However, based on the results of spot urine tests and clinical data obtained on presentation, continuous massive proteinuria was evident in each case (Table 1). The definitive initial and follow-up periods for IMN are unclear, and although the duration of CyA and PSL therapy was relatively brief in our cases, failure to achieve remission and/or deterioration of renal function was highly expected (3). Moreover, fluid control and edema were not manageable in our cases. Particularly, case 3 required dialysis, from which a temporary withdrawal was achieved. Owing to LDL-A therapy, CR was achieved in cases 1 and 2.

Regarding LDL-A, the treatment procedures used in this study were similar to those mentioned in previous reports (6-8). In each case, following a rapid decrease in the LDL levels after initiating treatment with LDL-A, urinary protein excretion decreased dramatically after one month. The remarkable results observed in our cases are similar to those reported in other cases of LDL-A therapy in patients with PSL-resistant NS with focal segmental glomerular sclerosis (7). An *in vitro* study demonstrated that very low-density lipoproteins (VLDLs) induce a dose-dependent reduction in the number of specific binding sites for dexamethasone in cultured smooth muscle cells, which leads to improvements in hyperlipidemia. Therefore, LDL-A may upregulate the steroid binding of systemic cells for both high LDL and VLDL. LDL-A may also improve sensitivity to PSL, thus resulting in a good clinical course (7, 9).

In all of our cases, the CyA trough levels measured using monoclonal assays prior to the administration of LDL-A therapy were lower than those cited in previous reports. Catran et al. adjusted therapy in steroid-resistant patients with IMN to achieve a whole-blood 12-hour CyA trough level between 125-225 μg/L (3). In all our cases, after LDL-A therapy was completed, the CyA trough levels increased to >125 μg/dL. This implies that appropriate CyA dosage combined with LDL-A therapy improves clinical courses. However, this hypothesis remains controversial. Rapid CyA tapering in cases 1 and 2 caused no relapses. For case 3, maintaining appropriate CyA trough levels by re-establishing CyA therapy resulted in no improvements. LDL facilitates
the transport of CyA across the cell membrane by means of the LDL receptor. LDL is an important carrier of CyA in plasma (10). LDL-A treatment could thus improve the pharmacologic effectiveness of lymphocytes at low serum CyA levels. In addition, the C2 level has been utilized more often than the trough level as an indicator of immunosuppressive effects on lymphocytes, as stated in a recent report (11). Therefore, in our study, the increases in the CyA trough levels alone cannot explain the remission attained with LDL-A therapy.

Although LDL-A therapy contributed to the improved outcomes in our cases, the benefits of LDL-A therapy in patients with PSL- and CyA-resistant NS with IMN have not yet been clearly proven. Therefore, the possibility of SR could not be ruled out in our cases. A recent report states that SR is common when baseline proteinuria does not exceed 8 g/day with a persistent reduction >50% during the first year (2). The average level of proteinuria in our cases was approximately 4 g/day. Compared to our cases, it is possible that SR may have occurred after LDL-A therapy coincidentally in that study (2). However, the usefulness of LDL-A therapy was demonstrated in that report because only 37.1% of patients developed SR when baseline proteinuria was >3.5 g/day. Moreover, if SR in patients with NS with IMN depends on a >50% reduction in baseline proteinuria during the first year, the timeline was incomplete in our cases. Based on our patients’ clinical courses during hospitalization, it was unlikely that adequate reduction would have occurred without LDL-A therapy. In the report by Polanco et al. (2), immunosuppressive therapy was initiated in 78.5% of patients in whom SR had not occurred because of rapidly declining renal function and/or severe NS. In our cases, especially in case 3, fluid balance was not controlled and the administration of PSL and CyA was required. Regardless of the proteinuria levels, maintaining an optimal fluid balance may also be important for predicting SR. To date, SR is unlikely in patients with IMN and acute deteriorated renal function or difficulty in maintaining fluid balance. However, there are still no proven therapies for PSL- and CyA-resistant NS patients.

Compared to previous reports of LDL-A therapy for NS patients with IMN, superior clinical outcomes were achieved in our cases. Stenvinkel et al. (12) reported low efficacy of LDL-A therapy for patients with prolonged NS with IMN (Table 2). The most remarkable differences between our cases and those of Stenvinkel et al. are that we used combined therapy and the duration of NS was longer in our cases. Ideura et al. (13) reported good clinical results with LDL-A as an additional therapy in PSL- and CyA-resistant NS with IMN patients after renal allograft transplant. Because of the very short period of persistent NS, SR could not be completely ruled out in our cases; however, earlier intervention appears to be very effective in such cases.

Good clinical results may have been achieved in our study due to the relatively early timing of LDL-A intervention and appropriate comorbid immunosuppressive therapy. At this time, it would be premature to conclude why our patients achieved good clinical courses following LDL-A treatment. Generally, LDL-A therapy is reported to be effective in steroid resistant patients with focal segmental sclerosis (FSGS) (7, 14). Muso et al. concluded that among steroid refractory FSGS cases, early rapid reduction induced by LDL-A therapy results in good clinical outcomes (14). IMN with secondary FSGS lesions is occasionally seen on renal biopsy reviews. However, no FSGS-like lesions were observed in any of our cases (Table 1). Ueda et al. reported that LDL-A therapy can restore PSL and CyA sensitivity in steroid-resistant nephrotic syndrome by reducing the level of multi-drug resistant gene-1 (MDR-1) (15). In our patients, LDL-A therapy may have increased sensitivity to immunosuppressants via inhibitory effects on MDR-1 gene expression even at low serum PSL and CyA levels.

Table 2. Previous Reports and Reviews of Low-density Lipoprotein Apheresis (LDL-A) in Membranous Nephropathy (MN)

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Muso, et al. (6)</th>
<th>Stenvinkel, et al. (12)</th>
<th>Ideura, et al. (13)</th>
<th>Current report</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-A therapy</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Times</td>
<td>2 times</td>
<td>Once or twice a week (upto 13 times)</td>
<td>Twice a week (upto 12 times)</td>
<td>Once a week (upto 5–7 times)</td>
</tr>
<tr>
<td>Immunosuppressive therapy</td>
<td>PSL</td>
<td>None (3)</td>
<td>PSL + CyA</td>
<td>PSL + CyA</td>
</tr>
<tr>
<td>Average proteinuria or albuminuria (before LDL-A therapy)</td>
<td>NA</td>
<td>8 g/day (albuminuria)</td>
<td>3–9 g/day (proteinuria)</td>
<td>4.08 g/day (proteinuria)</td>
</tr>
<tr>
<td>Persistent proteinuria (months)</td>
<td>1.5</td>
<td>10–38</td>
<td>2</td>
<td>6–13</td>
</tr>
<tr>
<td>Conclusion</td>
<td>PR</td>
<td>Effectiveness (proteinuria had persisted after LDL-A)</td>
<td>CR</td>
<td>CR (2 cases)</td>
</tr>
<tr>
<td>PR (1 case)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PSL: prednisolone, CyA: cyclosporine, CR: complete remission, PR: partial remission
In conclusion, we herein reported the use of LDL-A as an additional therapeutic intervention for PSL- and CyA-resistant NS patients with IMN. CR was achieved in two cases and partial remission was achieved in one case. LDL-A therapy may improve sensitivity to PSL and CyA, resulting in a good clinical course. Based on the results of our cases, LDL-A therapy combined with immunosuppressive therapy for recalcitrant NS with IMN might be a promising treatment. Large-scale intervention trials are required to establish the definitive treatment protocol.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement
The authors would like to thank Nobuaki Yamanaka M.D. (Tokyo Kidney Research Institute, Tokyo, Japan) for reviewing the results of our renal biopsies. We would also like to thank Dr. Matsushita M.D. and Mr. Fukumura for their valuable help (Department of Pathology, Yokohama Sakae Kyosai Hospital, Yokohama, Japan).

References