Review

β2-Microglobulin-selective direct hemoperfusion column for the treatment of dialysis-related amyloidosis

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Received 16 June 2005; received in revised form 1 August 2005; accepted 1 August 2005

Available online 6 September 2005

Abstract

Lixelle is a direct hemoperfusion-type adsorption column that was developed to selectively eliminate β2-microglobulin (β2-m) from the circulating blood of patients with dialysis-related amyloidosis (DRA). The adsorbent in Lixelle comprises porous cellulose beads to which hydrophobic hexadecyl alkyl chain is covalently bound. One milliliter of wet Lixelle beads eliminates more than 1 mg of β2-m in vitro. In hemodialysis patients who were treated with Lixelle, Lixelle improved joint pain, nocturnal awakening, pinch strength, motor terminal latency, and their activity of daily living. The adsorbent adsorbs β2-m selectively but not specifically, as well as inflammatory cytokines such as interleukin-1β and IL-6 which are considered to be involved in the development of DRA. Lixelle treatments reduce the circulating levels of β2-m and inflammatory cytokines, thereby improving the symptoms of patients with DRA.

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Keywords: β2-microglobulin; Dialysis-related amyloidosis; Direct hemoperfusion; Adsorption column; Lixelle

1. Introduction

Dialysis-related amyloidosis (DRA) is one of the serious complications of long-term hemodialysis. The main symptoms in patients with DRA are joint pain which reduces their activity of daily living (ADL), reduced grip strength, carpal tunnel syndrome (CTS), destructive spondyloarthropathy (DSA), and bone cysts [1]. β2-microglobulin (β2-m) is the precursor of amyloid deposits in DRA, which was found by biochemical analysis by Gejyo et al. in 1985 [2]. DRA is caused by amyloid deposits on synovial membranes and bones [3] and eventually on systemic organs [4,5]. It is diagnosed by β2-m immunostaining of sediments of amyloid fibrils in biopsy samples of synovium or bone.

β2-m is a component of the major histocompatibility complex on the cell surface. β2-m is flaked away from the cell membrane and catabolized in renal tubules. β2-m accumulates in the blood as renal function decreases. Conventional hemodialysis does not efficiently remove β2-m from the blood, and the serum concentration of β2-m in hemodialysis patients increases to a level that is 50 times or more higher than that in normal subjects [6]. In hemodialysis patients, aggressive removal of β2-m from the blood is considered to be effective for preventing and treating DRA. The therapeutic column, Lixelle™ (Kaneka Corporation, Osaka, Japan), was developed to eliminate β2-m from the circulating blood by selective adsorption. This review discusses the specifications, adsorption spectra, clinical indications, results of clinical studies, and the safety of Lixelle treatments.

2. Specifications of Lixelle and its usage

Lixelle is a direct hemoperfusion-type adsorbent column containing porous cellulose beads with a mean diameter of 460 μm. The porous cellulose beads are covalently bound to hexadecyl groups that adsorb β2-m [7]. Lixelle was designed to selectively adsorb β2-m through the combination of hydrophobic interaction and appropriate pore size. With the highly hydrophobic hexadecyl group serving as a ligand, the Lixelle adsorbent binds mainly hydrophobic substances. Since most proteins have a hydrophobic moiety, albumin, and immunoglobulins may also interact with the ligand. The surface of Lixelle beads has numerous pores of small size that excludes albumin which has a molecular weight of 68,000 Da, and larger proteins, resulting in the selective adsorption of β2-m which has a molecular weight of 11,800 Da. The
mechanism by which Lixelle selectively adsorbs $\beta_2$-m is illustrated in Fig. 1.

Two types of Lixelle columns, S-35 and S-15, are commercially available (Fig. 2). Lixelle S-35 has a column volume of 350 ml with a priming volume of 177 ml, while Lixelle S-15 has a column volume of 150 ml with a priming volume of 65 ml. Lixelle is sterilized in an autoclave with the beads in a sodium citrate buffer, which serves as a filling solution. Before the Lixelle column is used, the column must be washed with heparinized saline for anticoagulation of the column. Lixelle is a single-use device, and is used in combination with a dialyzer which is placed downstream of Lixelle via an exclusive connection tube (Fig. 3). The treatment with Lixelle is carried out under similar conditions as those of patients on conventional hemodialysis, with blood rates of up to 250 ml/min under continuous heparinization of the blood. As an anticoagulant, high molecular weight heparin, whose molecular weights are approximately from 6000 to 12,000 Da, is used at a speed of 1000 to 1500 IU/h during Lixelle treatments in general.

3. Performance of Lixelle and the adsorption spectra

Lixelle beads adsorb a larger amount of $\beta_2$-m as the concentration of $\beta_2$-m increases, and the beads have an adsorption capacity of more than 1 mg of $\beta_2$-m per 1 ml of wet beads in vitro [8]. A correlation between the amount of $\beta_2$-m adsorbed by the Lixelle beads and the pretreatment serum $\beta_2$-m level was observed among patients who were being treated with Lixelle [9], and the total amount of $\beta_2$-m eliminated by Lixelle S-35 in combination with a dialyzer ranged from 200 mg to more than 300 mg. Lixelle S-35 reduced the serum $\beta_2$-m concentration by a mean of 74% during the first treatment, and the serum $\beta_2$-m concentration at the end of each Lixelle treatment was less than 10 mg/l [10].

The adsorption spectra in vitro showed that Lixelle beads adsorb peptides and proteins whose molecular weight ranges from 4,000 to 20,000 Da [11]. Lixelle beads adsorbed not only $\beta_2$-m but also lysozyme and retinol-binding protein, whose serum concentrations are elevated in hemodialysis patients compared with healthy subjects. Insulin was also found to be adsorbed on Lixelle beads in vitro, however, no clinical changes in the concentrations were observed during clinical treatments using Lixelle in combination with hemodialyser. The detailed adsorption spectra are shown in Fig. 4. Another in vitro study using Lixelle beads showed that inflammatory cytokines such as interleukin (IL)-1$\beta$ and IL-6, which are considered to be involved in the development of DRA, were also adsorbed to the beads [12]. It was also observed that the blood cell counts of patients who were being treated with Lixelle did not significantly change, and that Lixelle was sufficiently hemocompatible [13].

4. Intended use of Lixelle and clinical indications

Lixelle is intended to be used for the removal of $\beta_2$-m from the blood of hemodialysis patients. The clinical indications for Lixelle treatments that have been approved
by the Japanese Ministry of Health, Welfare and Labour are as follows:

Lixelle treatments can be administered to hemodialysis patients who have DRA accompanied by pain and who satisfy the following criteria:

(a) presence of amyloid deposits composed of $\beta_2$-m as demonstrated by staining of specimens obtained by surgery or biopsy;
(b) duration of hemodialysis of 10 years or longer with a past history of carpal tunnel syndrome; and
(c) presence of bone cyst as proven by diagnostic imaging.

5. Clinical evaluation of Lixelle treatments

The majority of clinical studies on Lixelle in Japan have been conducted on hemodialysis patients who satisfy these criteria. Lixelle treatments are usually conducted three times per week at the time of hemodialysis sessions.

5. Clinical evaluation of Lixelle treatments

The major clinical trials that have been conducted to confirm the effectiveness of treatments with Lixelle S-35 are listed in Tables 1 and 2. Among these, one was a prospective controlled study [10] and the other studies were open clinical studies without a control group. In the clinical evaluation of Lixelle, both subjective parameters such as joint pain, stiffness, and activity of daily living (ADL), and objective parameters such as number of times of nocturnal awakening, grasping power, pinch strength, motor nerve latency, area of bone cyst, and thickness of synovia, have been used, although the clinical endpoints in the evaluation of DRA have not been well established. It was found that Lixelle treatments for up to two years significantly improved or normalized most of these parameters.

6. Effect of Lixelle treatments on subjective parameters of DRA

Gejyo et al. [10] investigated the clinical efficacy of Lixelle treatments in patients with DRA in a prospective multicenter controlled trial in which patients received hemodialysis using a polysulfone membrane with or without Lixelle treatments for two years. In those patients who were treated with Lixelle, the scores for the subjective parameters of joint pain, limitation of ADL, and stiffness improved by fifty percent of the respective score at study entry within the first 3 months of Lixelle treatments, and the improved scores were maintained up through the end of the study. On the other hand, in the control group, the scores for ADL and stiffness tended to worsen. It was also found that among the patients who were treated with Lixelle, the level of serum $\beta_2$-m clearance by Lixelle was correlated with the changes in ADL and joint stiffness scores, suggesting that removal of serum $\beta_2$-m alleviates the symptoms of DRA.

7. Effect of Lixelle treatments on objective parameters of DRA

Abe et al. [14] showed that Lixelle treatments for one year significantly improved pinch strength and motor nerve latency, area of bone cyst, and thickness of synovia.
Table 3
Results of post-marketing surveillance of Lixelle S-35 treatments

<table>
<thead>
<tr>
<th>Duration of PMS</th>
<th>3 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of facilities</td>
<td>58 clinical facilities</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>183 patients</td>
</tr>
<tr>
<td>Total number of treatments</td>
<td>13,476 treatments</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adverse clinical events</th>
<th>Patients (%)</th>
<th>Episodes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypotension</td>
<td>26</td>
<td>14.2</td>
</tr>
<tr>
<td>Decrease in hematocrit</td>
<td>9</td>
<td>4.9</td>
</tr>
<tr>
<td>Hypovolemia</td>
<td>6</td>
<td>3.3</td>
</tr>
<tr>
<td>Vomiting/nausea</td>
<td>5</td>
<td>2.7</td>
</tr>
<tr>
<td>Anemia</td>
<td>4</td>
<td>2.2</td>
</tr>
<tr>
<td>Palpitation</td>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td>Fatigue/malaise</td>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td>Chill/shiver</td>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>Increase in pain</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Hypertension after dialysis</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Pharyngeal pain</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Chest discomfort</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Chest oppression</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>25.1</td>
</tr>
</tbody>
</table>

PMS: post-marketing surveillance.

Hypotension, and there was a good correlation between the degree of improvement of pinch strength and the release of pain in wrists or fingers caused by carpal tunnel syndrome. Homma et al. [15] reported that Lixelle treatments for 5 months significantly reduced the cystic areas on humeral heads as observed by X-ray, and that the cystic area started to increase after discontinuing Lixelle treatments. Gejyo et al. [10] found that hemodialysis patients who were treated with Lixelle did not develop additional bone cysts, whereas there was a significant increase in the number of bone cysts in hemodialysis patients who were not treated with Lixelle over a 2-year period. A long-term clinical study on the effects of Lixelle treatments on objective parameters, such as the need for surgery for carpal tunnel syndrome, is under way to further clarify the clinical efficacy of Lixelle treatments.

8. Post-marketing surveillance of Lixelle

In a 3-year post-marketing surveillance of Lixelle treatments at 58 centers in Japan, a total of 13,476 treatments with Lixelle S-35 in combination with a hemodialyzer that had been administered to 183 patients were surveyed. The three most frequently observed adverse events were temporary hypotension [156 episodes (1.2% of total number of treatments) in 26 patients (14% of total number of patients)], decrease in hematocrit [37 episodes (0.27%) in 9 patients (4.9%)], and hypovolemia [105 episodes (0.78%) in 6 patients (3.3%)], as shown in Table 3.

Most of these adverse events that occurred during Lixelle S-35 treatments were temporary and could be managed by the attending physician using measures that are taken during conventional hemodialysis treatments. Hypotension during a Lixelle S-35 treatment was observed more frequently among those patients with DRA complicated with heart failure, arteriosclerosis obliterans, or a tendency to develop hypotension even during conventional hemodialysis alone.

Patients who developed blood cloting in the column, dialyzer, or blood tubing showed a reduction in hematocrit of more than 20% of the initial level. Temporary hypotension may arise among patients receiving treatment with Lixelle S-35 since the extracorporeal blood volume in these patients is 177 ml greater than that in patients undergoing hemodialysis treatment alone. Therefore, a compact adsorption column, Lixelle S-15, was developed in which the extracorporeal blood volume was only 65 ml greater than that in patients undergoing hemodialysis treatment alone, while keeping the β2-m removal rate as high as possible. It is not known what causes the reduction in hematocrit and anemia among patients receiving Lixelle treatments; however, one possible cause is loss of blood due to blood clotting in the column, dialyzer or blood tubing. The amount of anticoagulant administered during Lixelle treatments should be optimized for each patient to prevent blood clotting.

It was found that the incidence of adverse events such as hypovolemia or reduction in hematocrit was lower among patients who were treated with Lixelle S-15 (8%) than among those who were treated with Lixelle S-35 (25%), and that treatment with Lixelle S-15 exhibited the same efficacy as treatment with Lixelle S-35 with regard to improving the clinical symptoms of DRA [16].

9. Future prospects

Since Lixelle adsorbs not only β2-m but also inflammatory cytokines, Tsuchida et al. [17] treated patients with systemic inflammatory response syndrome by direct hemoperfusion with a Lixelle column, and found that the adsorption rates of IL-1α, IL-1R antagonist, IL-6, IL-8, and TNF-α ranged between 35 and 75% at 5 min after the start of treatment. It was confirmed that the removal of cytokines by Lixelle was beneficial to the patients since there were increases in the systolic and diastolic blood pressures of the patients during the course of the Lixelle treatments [17].

Tsuchida et al. [18] also found in an in vitro study that Lixelle adsorbed microorganism components such as lipopolysaccharide endotoxin from contaminated water. This finding suggests that Lixelle can be used to treat endotoxemia.

10. Conclusions

Lixelle efficiently and directly removes β2-m from the blood of hemodialysis patients. Lixelle is indicated for patients with DRA who have undergone long-term hemodialysis therapy. Lixelle treatments improve subjective symptoms of patients and objective parameters of DRA; in particular, they attenuate joint pain and improve the ADL of patients. Lixelle improves the subjective and objective parameters of DRA by causing acute reductions in the serum levels of β2-m and inflammatory cytokines.
References


