Direct hemoperfusion by using Lixelle® column for the treatment of systemic inflammatory response syndrome

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Abstract. We have previously reported that Lixelle® which was used for β2-microglobulin (BMG) adsorption column could adsorb not only BMG but also inflammatory cytokines and microbial fragments such as endotoxin (ET) and peptidoglycan (PG). The aim of this study was to estimate that an adsorbent column was used in direct hemoperfusion in patients clinically having systemic inflammatory response syndrome (SIRS), and the relationship between a decrease in cytokines and clinical course was examined. Meanwhile, as regards in vivo rate of removing cytokine based on pre-treatment cytokine concentration versus pre-column concentration at the time of evaluation, it increased with lapse of time, and the removing rate was 40% with 4 h direct hemoperfusion by using the Lixelle column in some of the patients. As to in vivo rates of adsorbing IL-1β, IL-1Ra, IL-6, IL-8 and TNF-α before and after the use of column at the time of evaluation, the rates 5 min after the start were 31.4, 39.3, 36.4, 76.2 and 71.6% respectively. Clinically, it was possible to increase blood pressure and recover from shock status. With the use of this column, removal of inflammatory cytokine by adsorption can be expected. Thus, it can be applied to the treatment of hypercytokinemia.

Introduction

In systemic inflammatory response syndrome (SIRS) and multiple organ failure (MOF), hypercytokinemia occurs due to sepsis and postoperative infection (1). For its treatment, blood purification methods are being applied clinically, such as continuous hemodialysis (CHD), continuous hemofiltration (CHF), continuous hemodiafiltration (CHDF) and plasma exchange (PE) (2). In these treatments, substances of medium molecular weight and cytokines that are contained in the plasma of SIRS patients are filtered by or adsorbed to the blood purifying membrane. However, the amount that can be removed is small, and thus, effective removal is not attained (3).

In 1996, Kaneka Corporation started the marketing of a direct hemoperfusion column (Lixelle®) which selectively adsorbs β2-microglobulin (BMG) for the treatment of dialysis-related amyloidosis (DRA) in Japan (4). Clinical improvements are reported such as regression of bone cyst and improvement of pain (Furuyoshi S, et al, Blood Purif 9: abs. 9, 1991). The column contains porous cellulose beads, the surface of which having ligand of hexadecyl group. Thus, it has properties of adsorbing substances through hydrophobic interaction. The surface of the porous cellulose beads of this adsorbs substances through hydrophobic interaction, and it is reported that protein with molecular weight of 30,000 Da or less is mainly adsorbed (5). So various cytokines and microbial fragments such as endotoxin (ET) and peptidoglycan (PG) have being adsorbed by Lixelle column (6,7).

In our present study, we attempted to investigate clinical course and cytokine adsorption rate with the use of Lixelle column in direct hemoperfusion in patients with SIRS.

Patients and methods

Patients. Selected for this study were 5 patients who were admitted to the hospital of Osaka City University and who met SIRS diagnostic standards. Of these, 2 patients were undergoing hemodialysis (HD) who developed sepsis due to catheter infection, and 3 patients had infection after heptectomy, coronary bypass surgery and colectomy. Table I shows, for each patient, the age, underlying disease, complication, results of hematological examination, and blood purifying method. In each of the patients, the leukocyte count was ≥12,000/μl, and CRP showed a high value. All of them had fever of ≥38°C.

Cytokine adsorbing capability in vivo. In each of the patients, Lixelle column was used twice in direct hemoperfusion alone, or in combination with billirubin adsorption therapy. Each of the direct hemoperfusion and billirubin adsorption therapy was performed for 3 h with blood flow at 100 ml/min. To measure
**Table I. Patients covered.**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Primary disease</th>
<th>Complication</th>
<th>Body temperature</th>
<th>Blood pressure (mmHg)</th>
<th>WBC (jul)</th>
<th>CRP (mg/dl)</th>
<th>Treatment type</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.M</td>
<td>70</td>
<td>F</td>
<td>CRF</td>
<td>Catheter infection</td>
<td>39.0</td>
<td>99/51</td>
<td>12,100</td>
<td>11.3</td>
<td>DHP (Lixelle)</td>
</tr>
<tr>
<td>Y.T</td>
<td>47</td>
<td>F</td>
<td>CRF</td>
<td>Catheter infection</td>
<td>39.5</td>
<td>102/58</td>
<td>13,500</td>
<td>1.8</td>
<td>DHP (Lixelle)</td>
</tr>
<tr>
<td>T.K</td>
<td>66</td>
<td>M</td>
<td>Perforation of colon</td>
<td>Sepsis</td>
<td>38.5</td>
<td>90/40</td>
<td>15,200</td>
<td>10.4</td>
<td>Lixelle + Bil adsorption</td>
</tr>
<tr>
<td>M.M</td>
<td>69</td>
<td>M</td>
<td>HCC</td>
<td>Liver failure</td>
<td>38.8</td>
<td>121/62</td>
<td>12,100</td>
<td>7.3</td>
<td>DHP (Lixelle)</td>
</tr>
<tr>
<td>Y.N</td>
<td>57</td>
<td>M</td>
<td>CRF</td>
<td>AMI</td>
<td>40.0</td>
<td>92/53</td>
<td>22,300</td>
<td>30.6</td>
<td>Lixelle + Bil adsorption</td>
</tr>
</tbody>
</table>

CRF: chronic renal failure; HCC, hepatocellular carcinoma; AMI, acute myocardial infarction; WBC, white blood cell; CRP, C-reactive protein; DHP, direct hemoperfusion; Bil, bilirubin; Lixelle, BMG adsorbent column.

**Table II. In vivo rates of adsorbing various cytokines at 5 min and 3 h after start of the treatment.**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Pre-column (pg/ml)</th>
<th>Post-column (pg/ml)</th>
<th>Adsorbed rate (%)</th>
<th>Pre-column (pg/ml)</th>
<th>Post-column (pg/ml)</th>
<th>Adsorbed rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1ß</td>
<td>2.00±0.85</td>
<td>1.58±0.36</td>
<td>31.4</td>
<td>2.86±0.40</td>
<td>2.44±1.09</td>
<td>18.0</td>
</tr>
<tr>
<td>IL-1Ra</td>
<td>3.240±1,500</td>
<td>2.005±0.928</td>
<td>39.3</td>
<td>3.072±1,204</td>
<td>2.776±0.974</td>
<td>17.7</td>
</tr>
<tr>
<td>IL-6</td>
<td>121.1±34.1</td>
<td>81.8±25.4</td>
<td>36.4</td>
<td>149.5±37.3</td>
<td>133.7±34.9</td>
<td>12.9</td>
</tr>
<tr>
<td>IL-8</td>
<td>162.2±44.0</td>
<td>38.6±9.7</td>
<td>76.2</td>
<td>198.6±33.0</td>
<td>133.2±17.7</td>
<td>31.8</td>
</tr>
<tr>
<td>TNF-α</td>
<td>8.52±1,54</td>
<td>3.01±0.75</td>
<td>71.6</td>
<td>6.28±1.35</td>
<td>5.11±1.10</td>
<td>32.9</td>
</tr>
</tbody>
</table>

The data represent the values of ten subjects (mean ± SEM).

**Results**

**Cytokine adsorbing capability in vivo.** For IL-1ß, IL-1Ra, IL-6, IL-8, and TNF-α, the adsorbing rates in vivo before and after the use of Lixelle column tended to decrease with lapse of time. However, the reduction rates at 5 min after the start were 31.4, 39.3, 36.4, and 76.2%, respectively, and at 3 h after the start, the rates were 18.0, 17.7, 12.9, 31.8, and 32.9%, respectively (Table II). In regard to changes with time for IL-6 and IL-1Ra before and after the use of this column, the adsorbing effect was observed to decrease with lapse of time (Fig. 1). In evaluation of one patient before and after the treatment, the reduction rate before and after the use of column increased with lapse of time. In direct 4-h hemo-
perfusion, the rates of removing IL-1Ra, IL-6 and IL-8 were 44.1, 22.1 and 41.4% respectively (Fig. 2).

Clinical course. In clinical course, the systolic blood pressure at the start, after 1, 2 and 3 h, and after completion were 114.8±15.6, 120.1±23.1, 128.3±23.0, 150.6±16.2, and 154.9±15.4 mmHg, respectively. As compared with pre-treatment values, the values significantly increased at 3 h after start of the treatment. As for diastolic blood pressure, the values were 70.2±12.0, 69.3±8.9, 81.0±13.4, 102.0±32.4, and 105.1±29.3 mmHg respectively. The values significantly increased at 3 h after start of the treatment. Heart rates were 102±32.4, 105.1±29.3, 108.4±33.3, 106.7±27.6, and 96.9±28.8 beats/min, respectively. As compared with the values before the start, no significant changes were observed (Fig. 3).

Discussion

It is reported that in SIRS and MOF excessive cytokine is released into the blood due to sepsis or postoperative infection, bringing about various symptoms (1). Blood purifying methods, such as CHD, CHF, CHDF and PE, are being applied clinically for the purpose of removing cytokine. However, concerning actual cytokine removing effect, there has been no established opinion. For the treatment of SIRS, Hirasawa et al. reported the effectiveness of CHDF in particular (8). In this method, polymethylmethacrylate membrane is used for adsorbing ET and cytokine to the blood purifying membrane, with blood flow at 60 ml/min, flow of dialysate at 500 ml/h, and flow of filtration at 300 ml/h, and the treatment is given continuously for 72 h. By thus performing blood purification in a comparatively slow manner, it is intended to treat hypercytokinemia by filtering substances and by making use of adsorbing properties. The effect is reported with IL-6 as an indicator (8).

The Lixelle column was introduced for the treatment of DRA. The column selectively adsorbs BMG. It is reported that with the use of this column, improvement of clinical symptoms, such as regression of bone cyst and improvement of pain, was obtained (5). The BMG adsorbent column contains porous cellulose beads, the surface of which have ligands of the hexadecyl group. Thus, it has properties of adsorbing substances through hydrophobic interaction (Fig. 4).
As regards its adsorbing capability, it is reported that 1 ml of BMG adsorbent beads can adsorb more than 1 mg of BMG (4). The surface of the porous cellulose beads adsorbs substances through hydrophobic interaction, and it is reported that protein with molecular weight of \( \leq 30,000 \text{ Da} \) is mainly adsorbed (9). Thus, the removal of not only BMG but also these inflammatory cytokines and microbial fragments such as ET and PG can influence the clinical effect in treatment of DRA (6,7).

In the treatment of patients with SIRS, the adsorption rates for IL-1β, IL-1Ra, IL-6, IL-8, and TNF-α before and after the use of column were 30-75% at 5 min after start of the treatment, and 10-30% at 3 h after start of the treatment (Table II). Concerning changes with time in adsorption efficiency before and after the use of Lixelle column for IL-6 and IL-1Ra, the efficiency decreased with lapse of time. However, sufficient adsorbing capability was observed up to 3 h (Fig. 1). In a patient under 4 h treatment, the removing rate before and after the use of column in data before and after the treatment increased with lapse of time, and in 4 h direct hemoperfusion, the rates of removing IL-1Ra, IL-6, and IL-8 were 20-40% (Fig. 2). Clinical effect of cytokine removal by Lixelle column was confirmed by the increase of systolic blood pressure and diastolic blood pressure after start of the treatment with lapse of time (Fig. 3).

In case of septic shock, hypotension occurs following fever and tachycardia, and TNF-α, IL-1, IL-8, etc. in blood also increase. These cytokines act on vascular endothelial cells and vascular smooth muscle cells, thereby bringing about increased vascular permeability and vasodilation through production of PGI₂ and nitrogen monoxide (NO), resulting in secondary decrease in blood pressure (10-12). Therefore, it is considered that removal of inflammatory cytokines by adsorption secondarily acts on peripheral blood vessels, thereby causing an increase in blood pressure, leading to improved general condition. The effect of increased blood pressure obtained with the use of Lixelle column is the same as the effect obtained with ET adsorbing column (Toraymyxin®, Toray Industries, Inc., Tokyo, Japan) in direct hemoperfusion in patients having ET in blood (13). Thus, it could be possible that Lixelle not only adsorb cytokines but also constituent of microbes mainly associated with ET. Therefore, the column is expected to show a positive therapeutic effect when used alone or in combination with other blood purifying methods, not only for ET in blood resulting from gram negative bacteria, but also for sepsis and hypercytokinemia due to various causes.

It is necessary to make prospective studies hereafter concerning comparison with other blood purifying methods and the time of starting treatment. However, since Lixelle column can be used in direct hemoperfusion, it is possible to apply this column to various pathologic conditions.

References