Background: Protein-bound toxins such as indoxyl sulfate (IS) are not efficiently removed by conventional hemodialysis (HD).

Objectives: To improve the removal of IS, we performed an in vitro study to evaluate the effects of high dissolved hydrogen on the dissociation of IS from albumin using simulated HD.

Materials and Methods: Wasted dialysate from peritoneal dialysis was concentrated a hundred times using extracorporeal ultrafiltration method. Dialysate with high dissolved hydrogen was made by mixing concentrated dialysis solution and electrolyzed-reduced water. The amounts of free fractions of IS were determined by high performance liquid chromatography.

Results: IS was significantly dissociated from albumin using dialysate with high dissolved hydrogen compared with conventional dialysate (P < 0.05).

Conclusions: Effective removal of IS is expected using a dialysate with high dissolved hydrogen.

Keywords: Dialysis Solutions; Hydrogen; Indoxyl Sulfate; Albumins
high dissolved hydrogen using reverse osmosis (RO) water and high dissolved hydrogen solution, respectively and diluted 35 times with concentrated dialysate (KINDARY AF-2, FUSO, Japan). pH, partial pressure of carbon dioxide (PCO₂), oxygen (PO₂) and HCO₃ of conventional dialysate and high dissolved hydrogen dialysate were determined using i-STAT (300F, FUSO, Japan).

3.3. Examination of Indoxyl Sulfate Separated From Albumin Using Dialysate With High Dissolved Hydrogen

We obtained 100 L wasted dialysate after continuous ambulatory peritoneal dialysis (CAPD) in a patient. We concentrated CAPD solution 100 times by extracorporeal ultrafiltration method using FB-190E (CTA, low-flux, NIPRO, Japan). One hundred mL of this solution was mixed with 100 mL of conventional dialysate or dialysate with high dissolved hydrogen dialysate. Experiment was set at blood flow rate of 100 mL/min, filtration flow of 5 mL/min and dialyzer FB-70E (CTA, low-flux, NIPRO, Japan) as a filter to separate free IS and albumin-bound IS. We obtained 100 mL filtrated dialysate. The concentration of IS was determined by high performance liquid chromatography. Free fractions (FR) were calculated as below formula (4).

\[
FR = \frac{C_F}{C_B} \times 100
\]

CB: initial concentration of blood side
CF: concentration of filtration side

3.4. Statistical Analysis

Statistical analyses were conducted using Stat View (Version 5, SAS, USA). Continuous variables were described as mean ± standard deviation (SD) and compared with repeated measure ANOVA of Tukey-Kramer or Student’s t-test as appropriate. P value less than 0.05 or 0.01 was regarded as significant.

4. Results

Dissolved hydrogen concentration and ORP in the electrolyzed solution in the high dissolved hydrogen solution. Changes in dissolved hydrogen and ORP after making the solution are shown in Figure 1. Dissolved hydrogen decreased half values compared at baseline in 120 minutes. Time course of ORP gradually increased. These results suggested that the half-life of hydrogen water was 120 minutes; the experiment was performed using electrolyzed solution in the high dissolved hydrogen within 30 minutes after making.

Solute characteristics of both dialysate profiles are shown in Table 1. Dissolved hydrogen showed significantly higher than conventional dialysate (P < 0.01). Comparison between conventional dialysate and high dissolved hydrogen in the initial and filtrated concentrations of albumin IS and FR is shown in Table 2. FR of conventional dialysate showed 35.4 ± 4.0%. FR of high dissolved hydrogen showed 41.7 ± 2.6%. FR of high dissolved hydrogen was significantly higher than conventional dialysate (P < 0.05).

All data significantly changed (P < 0.01) compared with baseline. (n = 5, Mean ± SD).

### Table 1. Profile of Conventional Dialysate and High Dissolved Hydrogen Dialysate (n = 5) \(^a\)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Conventional Dialysate</th>
<th>High H₂ Dialysate</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.59 ± 0.18</td>
<td>7.60 ± 0.06</td>
<td>ns</td>
</tr>
<tr>
<td>PCO₂, mmHg</td>
<td>27.6 ± 8.92</td>
<td>27.3 ± 2.87</td>
<td>ns</td>
</tr>
<tr>
<td>PO₂, mmHg</td>
<td>144.6 ± 14.57</td>
<td>134 ± 20.22</td>
<td>ns</td>
</tr>
<tr>
<td>HCO₃, mmol/l.</td>
<td>25.4 ± 1.6</td>
<td>26.7 ± 1.0</td>
<td>ns</td>
</tr>
<tr>
<td>Dissolved H₂, ppb</td>
<td>0</td>
<td>699.8 ± 179.9</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

\(^a\) All of the values are presented as Mean ± SD.
5. Discussion

Protein-bound uremic toxins, especially IS, induce vascular inflammation, endothelial dysfunction and vascular calcification, which may explain the relatively poor prognosis of chronic kidney disease and patients under dialysis (8). Plasma IS was associated with first heart failure event in patients on HD (9). HD using a high-flux membrane cannot efficiently remove the protein-bound uremic toxins because of their high albumin-binding property. Especially, IS showed high protein-binding ratios (more than 95%) and low reduction rates by HD (< 35%). Removal of IS can be improved to some extent by increasing the diffusion of free forms with super-flux membrane HD and/or HDF (10). IS is largely albumin bound and inhibits drug protein binding (11-14). Furthermore, this compound accelerates the progression of glomerulosclerosis in the rat (15, 16). IF IS dissociates from albumin, it can easily remove by diffusion. Thus, IS removal is expected as new dialysis techniques. We focused on dialysate with high dissolved hydrogen and examined characteristics in this solution.

The level of dissolved hydrogen values was halved by two hours after obtaining electrolyzed water. These results suggested that the solution should be used immediately after preparation. Anti-oxidation has been confirmed using electrolyzed water by animal experiments (17-20). Furthermore, hydrogen gas has been reported to exhibit antioxidant properties. Electrolytic water containing hydrogen attracted more attention recently (21). Consumption of water with dissolved hydrogen produced by electrolysis by ad libitum drinking has the potential to ameliorate ischemia-induced cardio-renal injury in chronic kidney disease model rats (22). Using hydrogen-enriched solutions could ameliorate oxidative stress and albumin redox during HD (23).

Therefore, we focused on IS and developed a dialysate containing high dissolved hydrogen. We examined in vitro whether it is possible to separate IS from albumin. IS can be easily separated by dilution. This study demonstrated that hydrogen water promoted IS to dissociate from albumin during HD therapy. More beneficial effects would be expected in the combination of predilution mode on-line HDF with hydrogen water. More clinical studies are necessary on this issue. Dialysate with high dissolved hydrogen can significantly dissociate IS from albumin compared to conventional dialysate. Using this solution, high efficient IS removal was proved in vitro.

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Authors’ Contributions

Study concept and design: Yoshihiro Tange and Shigenori Yoshitake; Acquisition of data: Yoshihiro Tange; Analysis and interpretation of data: Yoshihiro Tange; Drafting of the manuscript: Yoshihiro Tange and Shigenori Yoshitake; Critical revision of the manuscript for important intellectual content: Yoshihiro Tange, Shingo Takesawa and Shigenori Yoshitake; Statistical analysis: Yoshihiro Tange; Administrative, technical and material supports: Shingo Takesawa and Shigenori Yoshitake; Study supervision: Shingo Takesawa and Shigenori Yoshitake.

References

8. Ito S, Yoshida M. Protein-bound uremic toxins: new culprits of...


