Prevention of the Febrile Reaction Occurring on Reinfusion of Cell-Free and Concentrated Autogenous Ascites

Shuji KATOH, Hirotaka TATSUKAWA, Morihiko KONDOH, Miho INOUE, Kazunori IDA and Fujio MIYAGAWA*

The febrile reaction that occurs on reinfusion of ascites was studied. Intravenous reinfusion of ascites was performed 213 times in 63 cases of ascites, which were refractory to treatment with various drugs including diuretics. In order to prevent fever on reinfusion of ascites, a screen filter and a depth filter were used; the results were more favorable with the screen filter. Fibrin was considered to be one of the substances removable by the screen filter. HPLC analysis of the filtered and concentrated ascites, after passage through the screen filter, revealed a fraction corresponding to albumin. Intravenous injection of this fraction into rabbits caused fever. Although the screen filter cannot completely prevent fever on reinfusion of ascites, it appears useful to prevent fever in some patients.

Key words: Malignant tumor, Reinfusion treatment with cell-free concentrated autogenous ascites, Screen filter, Febrile reaction

The intravenous reinfusion of autogenous ascites after filtration and concentration (referred to as reinfusion of ascites) performed for ascites in patients with cancer has been reported to be useful (1–8). However, fever frequently develops on reinfusion of ascites, which sometimes necessitates cessation of the procedure. Here, the prevention of the febrile reaction occurring on reinfusion of ascites was studied.

MATERIALS AND METHODS

We performed intravenous reinfusion of ascites 213 times in 63 cases (aged from 31 to 90 yr old, 38 males and 25 females) of ascites due to malignant tumor, which were refractory to treatment with various drugs including diuretics. The primary diseases of the patients were as follows: gastric cancer (17 cases), hepatoma (20 cases), colon cancer (6 cases), pancreatic cancer (6 cases), gallbladder cancer (5 cases), breast cancer (3 cases), duodenal cancer (1 case), malignant lymphoma (1 case), omental cancer (1 case), ovarian cancer (1 case), cholangioma (1 case), and unknown origin (1 case).

1. Use of a PVA and CELA filters

After peritoneal puncture, ascites was temporarily stored in a collection bag until filtration through a PVA filter (Kuraray Co., Ltd.; made of polyvinyl alcohol; inner diameter, 300 μ; membrane thickness; 100 μ, ultra fine caliber, less than 0.2 μ) to remove cellular components and bacteria. Then, it was concentrated by ultrafiltration (CELA filter, Kuraray; made of cellulose; inner diameter, 200 μ; membrane thickness, 8 μ), placed in a bag for intravenous infusion and then drip-infused for 2–4 h. For filtration and concentration, a double filtration monitor, Model KM8500 (Kuraray) was used.

2. Use of the SQ40S and SQ08 filters

Ascites from patients with malignant tumors was filtered and concentrated as described above, and then reinfused after passage through both an SQ40S
screen filter (Pall Biomedical Products Corp., Glen Cove, NY; contains 160 cm² of woven polyester pleated into a rigid housing, pore size, 40 μ; priming volume, 20 ml) and an SQ08 depth filter (Pall Biomedical Products Corp.; made of nylon; pore size, 0.8 μ; priming volume, 20 ml), to study the effect on the prevention of fever.

After explanation to the patients regarding the use of SQ40S or SQ08 for the prevention of febrile reaction. All patients consented to the use of the filters.

3. Analysis of filtrates on the membranes of SQ40S and SQ08

A scanning electron microscope (SEM) with energy dispersive X-ray micro-analysis equipment (EDX) was used, and the materials were prepared in accordance with the previous report (6).

4. Immunohistological study after reinfusion using an SQ40S screen filter

Egg white albumin was applied on a glass slide and then stamped with an SQ40S screen filter, followed by fixation in 95% ethanol and 30 min of drying time. The antifibrin antibody was added and then it was stored in a PBS buffer solution for 45 min. After staining, it was rinsed three times with the PBS buffer solution, mounted and then subjected to fluorescence microscopy. For the control study, an albumin preparation which was passed through an SQ40S was examined using the same procedure.

5. SDS polyacrylamide gel electrophoresis

The filtered and concentrated ascites, after passage through an SQ40S screen filter, was subjected to electrophoresis using an SDS-PAGE kit (Tefco: gel concentration, 12%; electrophoresis buffer, Tris-glycine, pH 8.3; electric current, 18 mA/gel; duration, 90 min), 2-mercaptoethanol was used to reduce the SS bonds. The staining solution was a 0.1% solution of CBD-R250 (mixture of acetic acid, 5%, and methanol, 40%).

6. Determination of endotoxin, serotonin, histamine and prostaglandin E

The filtered and concentrated ascites, after passage through an SQ40S filter, was examined. Endotoxin was determined by the endo-species method, serotonin by the fluorescence method, prostaglandin E by the RIA DCC method, and histamine by HPLC.

7. High performance liquid chromatography (HPLC)

The filtered and concentrated ascites, after passage through an SQ40S screen filter, was analyzed by HPLC. A TSK gel column (G3000SW_xl, 7.8 mm x 300 mmL) was used for fractional separation (injection volume, 150 μl; mobile phase, 0.1M NaH₂PO₄-NaOH, pH 7.0, and 0.3M NaCl; flow rate, 1.0 ml/min; detection, at UV 280 nm).

8. Febrile reaction in rabbits

The filtered and concentrated ascites was separated into 12 fractions by HPLC, which were then lyophilized. After dissolution in 31 ml of normal saline, 15 ml was intravenously injected into two rabbits, each weighing 3 kg. At the same time, 1 ml each was used for a Limulus test.

RESULTS

1. Reinfusion of ascites

Reinfusion of ascites was performed 213 times in total in the 63 cases, an average of 3.4 per case. The average volume of ascites per collection amounted to 2,476 ml, the average protein content was 27.5 g, the average volume of reinfusion was 343 ml.

2. Effect of the filter used at the time of reinfusion on the prevention of febrile reaction

The changes in body temperature following reinfusion of ascites were studied. The peak body temperature was recorded for each post infusion day, and the control temperature was the average of temperatures recorded during the 5-day period prior to the therapy.

The individual cases in the non-filter, SQ40S and SQ08 groups are compared in Table 1 (left). The temperature rose significantly less in the SQ40S than in the non-filter group, but there was no significant difference between the SQ08 and non-filter groups. The temperature increase after reinfusion in the non-filter, SQ40S and SQ08 groups is summarized in Table 1 (right). The temperature rose significantly less in SQ40S and SQ08 groups than in the non-filter group. However, there was no difference between the SQ40S and SQ08 groups. The frequency of the febrile reaction on reinfusion of ascites was lower with the SQ40S than with the SQ08.
Reinfusion of Ascites

Table 1. Difference in the Peak Temperature of Non-filter Group, SQ40S Group and SQ08 Group

<table>
<thead>
<tr>
<th></th>
<th>Each case</th>
<th>Each reinfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.517 ± 0.597°C</td>
<td>0.487 ± 0.711°C</td>
</tr>
<tr>
<td></td>
<td>(n = 30)</td>
<td>(n = 113)</td>
</tr>
<tr>
<td>Non filter</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>SQ 40S</td>
<td>0.128 ± 0.409°C</td>
<td>0.069 ± 0.557°C</td>
</tr>
<tr>
<td></td>
<td>(n = 15)</td>
<td>(n = 65)</td>
</tr>
<tr>
<td>SQ 08</td>
<td>0.241 ± 0.577°C</td>
<td>0.175 ± 0.658°C</td>
</tr>
<tr>
<td></td>
<td>(n = 18)</td>
<td>(n = 35)</td>
</tr>
<tr>
<td></td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

3. Analysis of SQ40S and SQ08 with an SEM equipped with an EDX

The data for the SQ40S are not included here as they have already been reported elsewhere (6).

Figure 1 shows the 1000-fold SEM pictures of an unused SQ08 (a) and an SQ08 (b) used for reinfusion. On EDX performed at the same time, Si, K, Fe, Cu and Ze were detected in trace amounts.

4. Immunological study of an SQ40S (Fig. 2)

By fluorescence microscopy, a deposit of fibrin was found on the SQ40S (Fig. 2a × 200).

However, in a control study (Fig. 2b), no fibrin was seen on the SQ40S (× 200).

5. Determination of endotoxin, serotonin, histamine

(a)

(b)

Fig. 1. 1000-fold SEM pictures of an unused SQ08 (a) and an SQ08 (b) used for reinfusion.

Fig. 2. By fluorescence microscopy, fibrin was observed deposited on the SQ40S (a) (× 200). However nothing was observed in the control study (b).
Table 2. Determination of Endotoxin, Serotonin, Histamine, and Prostaglandin E in the Filtered and Concentrated Ascites after Passage through SQ40S

<table>
<thead>
<tr>
<th></th>
<th>Normal range</th>
<th>Case No. 1</th>
<th>Case No. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endotoxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td></td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>filter (SQ 40S)</td>
<td>&lt;3 pg/ml</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>after</td>
<td></td>
<td>1.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Serotonin</td>
<td></td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>pre</td>
<td></td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>after</td>
<td></td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>filter (SQ 40S)</td>
<td>&lt;1.0 ng/ml</td>
<td>0.28</td>
<td>0.48</td>
</tr>
<tr>
<td>Histamine</td>
<td></td>
<td>0.31</td>
<td>0.64</td>
</tr>
<tr>
<td>pre</td>
<td>&lt;0.80 µg/dl</td>
<td>0.38</td>
<td>0.36</td>
</tr>
<tr>
<td>after</td>
<td></td>
<td>0.48</td>
<td>0.64</td>
</tr>
<tr>
<td>filter (SQ 40S)</td>
<td>&lt;1.0 ng/ml</td>
<td>0.28</td>
<td>0.48</td>
</tr>
<tr>
<td>Prostaglandin E</td>
<td></td>
<td>37 ~ 1,144 pg/ml</td>
<td>208</td>
</tr>
<tr>
<td>pre</td>
<td></td>
<td>137</td>
<td>189</td>
</tr>
<tr>
<td>after</td>
<td></td>
<td>126</td>
<td>50</td>
</tr>
<tr>
<td>filter (SQ 40S)</td>
<td></td>
<td>187</td>
<td></td>
</tr>
</tbody>
</table>

All values are within normal limits.

and prostaglandin E (Table 2)

All values in the filtered and concentrated ascites, after passage through an SQ40S, were all within normal range.

6. SDS polyacrylamide gel electrophoresis (Fig. 3)

SDS polyacrylamide gel electrophoresis failed to reveal clear differences among the following: 1) ascites before treatment, 2) filtered and concentrated ascites, 3) ascites before reinfusion, 4) ascites before passage through an SQ40S, 5) ascites after passage through an SQ40S.

The proteins noted had molecular weights of 11,300, 16,000, 22,500, 23,500, 56,000, 62,000, 72,000, 82,000, 108,000, 113,000, 126,000 and 150,000.

7. HPLC and fibrile reaction in rabbits

The filtered and concentrated ascites was analyzed by HPLC (TSK gel G3000SWXL) and separated into 12 fractions.
Table 3. When the fractions were intravenously injected into two rabbits, both rabbits showed a febrile reaction to fraction 4. They were also both found to be Limulus test positive.

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>rabbit (fever up)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td></td>
</tr>
<tr>
<td>Limulus test</td>
<td>(-)</td>
<td>(±)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(++)</td>
<td>(++)</td>
<td>(±)</td>
<td>(±)</td>
<td>(+)</td>
<td>(±)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

analyzed using HPLC (TSK gel G3000SWXL), and separated into 12 fractions, as shown in Fig. 4. When the fractions were intravenously injected into rabbits, both rabbits showed a febrile reaction to fraction number 4 (Table 3). Both were also found to be Limulus test positive (Table 3). Similar HPLC analysis of human albumin on a G3000SWXL column revealed a peak at the same position as fraction number 4.

**DISCUSSION**

Reinfusion of ascites has been studied primarily as a therapeutic method for refractory ascites in liver cirrhosis (9–11). However, there have been a few reports on the reinfusion of ascites for refractory ascites due to malignant tumors (1–8).

We previously reported the utility of reinfusing ascites in cases of ascites due to malignant tumors (4–8). Reinfusion of ascites has the following advantages: 1) the technique is simple and convenient; 2) there is no possibility of protein loss or hyponatremia; 3) there is little influence on the circulatory system; 4) it can be a substitute for a blood preparation when the overuse of the blood preparation is an issue; 5) there is little risk of developing hepatitis in comparison with the use of frozen plasma; 6) the effect of survival prolongation can be expected combined with the use of biological response modifiers.

On the other hand, reinfusion of ascites has been reported to cause such adverse reactions as fever, tachycardia, elevated blood pressure, pulmonary edema and disturbed consciousness (3). Fever is the most frequent reaction and it is considered that the usefulness of reinfusion of ascites would be further increased if this adverse reaction could be overcome.

We considered the use of a microfilter which is employed in blood transfusion for reinfusion therapy. The incidence of complications arising from transfusion of blood products is thought to be 0.5–1.0% (12), the most frequent being a non-hemolytic febrile reaction (13). Microaggregates were noted as one of the etiologies of the above-mentioned complication (14, 15). Microaggregates are comprised of degenerated platelets, granulocytes, protein and fibrin fibers, and their formation starts within 24 h of blood storage. The filtering of blood through a 40 µ microfilter was reported to be effective for preventing this complication (16). There are two types of microfilters, namely, screen filters and depth filters. The former block the passage of microaggregates which are larger than their pore size physically, and the latter adsorb microaggregates through the surface active effect of dacron, nylon or polyurethane (15). We found that microaggregates participate in the febrile reaction occurring on reinfusion treatment, as they do in the febrile reaction on blood transfusion.

Thus, the filters SQ40S and SQ08 were examined regarding the prevention of the febrile reaction occurring on reinfusion therapy. As previously mentioned, for reinfusion therapy we typically use a PVA filter to remove all cellular elements, including cancer cells and bacteria. This technique, employing SQ40S and SQ08, reduced the incidence of the febrile reaction drastically compared with the previous method, which did not involve cell removal. But the formation of microaggregates may not be preventable, because processed ascites are generally reinfused after 24 h or more. In the present study, a depth filter with a pore size smaller than that of the screen filter was used at the time of reinfusion of ascites, for comparison with the SQ40S. However as with the SQ40S, it was impossible to completely
Katoh et al

prevent the fever observed at the time of reinfusion of ascites. The results with the depth filter were inferior compared to those with the SQ40S. Although the reason for the difference is not known, the failure to prevent fever with a depth filter with a pore size of 0.8 \( \mu \) would suggest that the combined use of the filter has limitations. As the frequency of the febrile reaction was lower with the screen filter, the substance related to fever which was removed by the screen filter was immunologically identified to be fibrin. Fibrin, which has been reported to be one of the microaggregates noted at the time of blood transfusion, was considered to be a causative substance for the fever at the time of reinfusion of ascites.

However, fibrin alone cannot explain the fever at the time of reinfusion of ascites; it is suggested that there was a pyrogen in the filtered and concentrated ascites which passed through the screen filter. Thus, the level of endotoxin and other pyrogens present the filtered and concentrated ascites after passage through the screen filter was determined, but all were found to be within the normal range.

Little has been revealed about the mechanism underlying the febrile reaction on reinfusion of ascites and its possible prevention. It has been reported that the febrile reaction can be prevented by decreasing the rate of drip reinfusion of ascites using the same system as we employ (3). However, fever is still present. Although the involvement of endotoxin has been reported, the present results failed to support this. A cytotoxic polypeptide which is present in cancerous ascites has also been reported to be involved (11), but this has been not clarified. The present results led us to conclude that there was a pyrogenic substance in the ascites filtered through the PVA filter or the microfilter, in addition to the pyrogenic substances removed by the microfilter. It is thought that the substance could originate from protein components or polypeptides present in cancerous ascites.

Then, the pyrogenic substances present in the filtered and concentrated ascites were analyzed by means of SDS polyacrylamide gel electrophoresis. It was expected that as there was little difference in the pattern of filtered and concentrated ascites before and after screen filtration, the substances related to fever are present in trace amounts. Thus, a HPLC study was conducted. A TSK gel G3000SW\(_{XL}\) column, which allows gel filtration chromatography at high speed (17, 18) was used. As hydrophilic silica gel is employed, the adsorptivity of the packing material is very low; the test sample is separated only by the difference in molecular size, therefore it is available for proteins and enzymes exhibiting physiological activity and is suitable for the separation of proteins with molecular weights of 70,000 to 300,000 or higher (17, 18). The filtered and concentrated ascites were separated on a G3000SW\(_{XL}\) column into 12 fractions, which were intravenously injected into rabbits. The febrile reaction was only observed with the fraction corresponding to the albumin fraction. It is not feasible that albumin itself causes fever and it is very difficult to understand the significance of a febrile reaction in rabbits injected with human albumin. Most of the HPLC fractions were positive using a Limulus test, which indicates that the HPLC fractionation was not performed in a completely clean system. However, on intravenous infusion of each fraction, only fraction No. 4 caused fever in rabbits, suggesting involvement of the fraction in the febrile reaction on intravenous reinfusion of ascites. We considered that a small amount of bacteria in the other fractions which showed a positive Limulus test did not cause a febrile reaction. Further study is required on this point. It seemed unreasonable to assume that the fever occurring on reinfusion of ascites is caused by a single substance, and thus further HPLC study is necessary. Such a study will serve to establish the safety of reinfusion of ascites.

In conclusion, in order to prevent the fever occurring on reinfusion of ascites, a screen filter and a depth filter were used in combination. The results were favorable with the screen filter. Fibrin was considered to be one of the substances removable by the screen filter. HPLC analysis of the filtered and concentrated ascitic fluids, after passage through the screen filter, revealed a fraction corresponding to albumin. Intravenous injection of this fraction into rabbits caused fever.

ACKNOWLEDGMENTS: We wish to thank Drs. J. Okuda, T. Katoh, and T. Kojima for their cooperation in the present study, and Dr. T. Konaka, Laboratory Tests Dept.
Reinfusion of Ascites

of Shionogi Pharmaceutical Co. for performing the HPLC.

REFERENCES


