On-Line Plasma Exchange for Sepsis in Neonates and Infants

TEIJI TAKAHASHI, YOSHIHiro ASANUMA, TETSUO KATO, TATSUO HEBIGUCHI, KENJI KOYAMA and KOUZOU KUDO*

The First Department of Surgery and *Department of Microbiology, Akita University School of Medicine, Akita 010

Takahashi, T., Asanuma, Y., Kato, T., Hebiguchi, T., Koyama, K. and Kudo, K. On-Line Plasma Exchange for Sepsis in Neonates and Infants. Tohoku J. Exp. Med., 1989, 159 (4), 249-256 — The present study was designed to assess the effectiveness of on-line plasma exchange (on-line P.E.) between septic infants and healthy adults using septic puppies. The plasma separation system consisted of a membrane plasma separator and a plasma treatment system, KM-9027 (Kuraray Co.) equipped with small 2 channel blood and plasma pumps. 5×10⁹ CFU/ml/kg of E. coli with endotoxin was injected intravenously into 22 puppies and they were divided into 4 groups, namely untreated group (n = 7), on-line P.E. group (n = 5), P.E. group (n = 5), sham group (n = 5). In the on-line P.E. group, about 80 ml/kg of plasma in septic puppy was replaced during 2 hr with fresh plasma simultaneously obtained from healthy adult dog. As the results, 4 of 5 survived in the on-line P.E. group and 1 of 5 survived in the P.E. group, while all other puppies died within 24 hr. In the P.E. group and on-line P.E. group, mean blood pressure and urinary output were significantly improved (p <0.05) by the treatment. The numbers of E. coli and endotoxin concentration in the blood were reduced significantly (p <0.05) only in the treatment with on-line P.E. opsonic activity recovered significantly (p <0.05) in P.E. group.

This system appears to be effective and applicable for sepsis in infants and neonates. ——— on-line plasma exchange; plasmapheresis; exchange transfusion; sepsis; endotoxin shock

Postoperative sepsis in Neonates and infants is associated with high mortality rates, and at present, exchange transfusion is considered the final alternative therapy. Over the past 6 years, we have performed 46 exchange transfusions on 19 neonates and infants with sepsis. The therapy was effective in 15 cases, 12 of which survived. However, exchange transfusion requires massive fresh blood and is associated with such complications as anti-blood cell antibody production, infection, and graft-versus-host disease. In an attempt to simplify the blood supply and to prevent anti-blood cell antibody production, we developed on-line plasma exchange (on-line P.E.) between septic child and healthy adult. The present study was designed to assess the effectiveness of on-line P.E. by perform-
ing plasma exchanges between adult dogs and septic puppies, using a small plasma separator suitable for use in neonates and infants.

**MATERIALS AND METHODS**

Preparation of a sepsis model (Hinshaw et al. 1968): *Escherichia coli* strain $5 \times 10^9$ CFU/ml/kg was intravenously infused over a 1-hr period to 22 mongrel puppies weighing 1.8-3.2 kg. They were divided into the following four groups:

- **Untreated group** ($n = 7$): The course of sepsis was observed without treatment.
- **On-line P.E. group** ($n = 5$): The on-line P.E. was performed for 2 hr between puppies and adult dogs weighing approximately 15 kg.
- **P.E. group** ($n = 5$): Plasma exchange was performed for 2 hr using fresh frozen plasma, which was separated by centrifugation from an adult dog one week previously and stored at $-40^\circ$C.
- **Sham group** ($n = 5$): Plasma separated from the puppies' own blood was returned to them over a 2-hr period.

**Methods of on-line P.E.**

In on-line P.E., blood cells separated from puppies were mixed with plasma separated from adult dogs, and this mixture was returned to the puppies. Plasma separated from puppies was discarded (Fig. 1). The plasma separation system consisted of a membrane plasma separator and a plasma treatment system, KM-9027 (Kurarey Co., Osaka) equipped with a small blood and pump. The membrane plasma separator, custom made by Kurarey Co., was made of a ployvinyl alchohol hollow fiber with an effective surface area of 0.9 m$^2$, mean pore size of 0.4 $\mu$m, and volume of 10 ml. The priming volume of the system was 30 ml. The operating conditions were set at a blood flow velocity of 10 ml/min and plasma filtration rate of 1.7 ml/min. The amount of plasma exchanged was equivalent to 50–110 ml/kg.

**Examination items**

The following items were measured before injection of *E. coli* at 3 and 6 hr after the start of *E. coli* injection: 1) Survival perides, 2) blood pressure, urinary output, and

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**Fig. 1.** On-line plasma exchange system between puppy and adult dog. Blood cells from puppies were mixed with plasma separated from adult dogs, and this mixture was returned to the puppies. Plasma separated from puppies was discarded. Arrows show blood and plasma flow.
arterial blood gas analysis, 3) peripheral hematology, 4) the numbers of *E. coli* in blood (mixed dilution culture method), 5) endotoxin concentrations in blood (measured using the Toxicolor test), and 6) opsonic activity (measured using the chemiluminescence test).

**Statistical analysis**

Results are presented as grouped mean and s.D.. Data were analyzed using unpaired Student *t*-test. Only values of less than *p* < 0.05 compared with values for sham and untreated groups were considered statistically significant.

**RESULTS**

1) All puppies in the untreated and sham groups died within 24 hr. In the P.E. group, four puppies died within 24 hr and the remaining 1 survived for one more week. In the on-line P.E. group, however, one puppy died within 24 hr, but the remaining 4 survived for more than one week (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of dogs which survived up to 12 hr, from 12 to 24 hr and longer than 1 week</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>-12 hr</td>
</tr>
<tr>
<td>Untreated (<em>n</em> = 7)</td>
<td>3</td>
</tr>
<tr>
<td>On-line P.E. (<em>n</em> = 5)</td>
<td>0</td>
</tr>
<tr>
<td>P.E. (<em>n</em> = 5)</td>
<td>0</td>
</tr>
<tr>
<td>Sham (<em>n</em> = 5)</td>
<td>4</td>
</tr>
</tbody>
</table>

**TABLE 1. Number of dogs which survived up to 12 hr, from 12 to 24 hr and longer than 1 week**

![Graph showing changes in mean blood pressure](image)

**Fig. 2. Changes in mean blood pressure.**

- ○—○, on-line P.E. group (*n* = 5); ●—●, P.E. group (*n* = 5); △—△, sham group (*n* = 5); ▲—▲, untreated group (*n* = 7).

* *p* < 0.05 compared with sham and untreated groups.

* *period of P.E. or on-line P.E.*
Fig. 3. Changes in urine volume.
- - - - , on-line P.E. group; - - - - , P.E. group; - - - - , sham group; - - - - , untreated group.
\* p < 0.05 compared with sham and untreated groups.
mean ± s.d.
\[ \text{period of P.E. or on-line P.E.} \]

Fig. 4. Changes in base excess.
○○○○, on-line P.E. group; ●●●●, P.E. group; △△△△, sham group; ▲▲▲▲, untreated group.
\* p < 0.05 compared with sham and untreated groups.
\[ \text{period of P.E. or on-line P.E.} \]
2) In the P.E. and on-line P.E. groups, mean blood pressure and urinary output were significantly improved ($p < 0.05$) by the treatment. Mean blood pressure in untreated and sham groups deteriorated up to 3 hr after *E. coli* injection, the mean blood pressure was kept constant at 40-50 mmHg for up to 6

![Fig. 5. Changes in viability of *E. coli* with time.](image)

- ○—○, on-line P.E. group; ●—●, P.E. group; △—△, sham group; ▲—▲, untreated group.
- *$p < 0.05$ compared with sham and untreated groups.
- ——, period of P.E. or on-line P.E.

![Fig. 6. Changes in plasma endotoxin concentration.](image)

- ○—○, on-line P.E. group; ●—●, P.E. group; △—△, sham group; ▲—▲, untreated group.
- *$p < 0.05$ compared with sham and untreated groups.
- ——, period of P.E. or on-line P.E.
hr. Mean blood pressure of the P.E. and on-line P.E. groups started to increase in the middle of the treatment and recovered to 70–80 mmHg at the end of treatment. The mean blood pressure continued increasing thereafter and reached about 100 mmHg (Fig. 2). Urinary output in untreated and sham groups deteriorated after E. coli injection, whereas urinary output in the treatment groups started increasing at the end of treatment (Fig. 3). Acid-base equilibrium improved significantly (p < 0.05) in only the on-line P.E. at 6 hr after E. coli injection (Fig. 4).

3) The numbers of E. coli and the Endotoxin concentration in the blood were reduced significantly (p < 0.05) only in the treatment with on-line P.E. (Figs. 5 and 6). Endotoxin was isolated in separated plasma with a sieving coefficient above 0.95, but E. coli was not detected.

4) Opsonic activity recovered significantly (p < 0.05) at the end of the treatment by on-line P.E. However, there were no significant differences between on-line P.E. group and P.E. group (Fig. 7). Opsonic activity of FFP from adult dogs was diminished to 73% that of fresh plasma.

**DISCUSSION**

In neonates and infants, the function of the immune system is not sufficient, so postoperative sepsis in neonates and infants is associated with high mortality rates. It is therefore important in pediatric surgery to treat sepsis and accompanied multiple organ failure. In these cases in neonates and infants, the efficacy of exchange blood transfusion is evident when applied in addition to the conventional treatment (Vain et al. 1980). We have treated 19 cases using

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**Fig. 7.** Changes in opsonic index obtained chemiluminescence test.

- ○—○, on-line P.E. group; •—•, P.E. group; △—△, sham group; ▲—▲, untreated group.

* p < 0.05 compared with sham and untreated groups.

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<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Oposinic index</th>
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<tbody>
<tr>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

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E. coli period of P.E. or on-line P.E.
exchange blood transfusion a total of 46 times, and 12 patients survived. However, exchange transfusion requires massive fresh blood and is associated with such complications as anti-blood cell antibody production, infection and graft-versus-host disease (Operz et al. 1973; Parkman et al. 1974). In an effort to increase the effectiveness of exchange transfusion and eliminate its shortcomings, we developed an on-line P.E. system for treating pediatric sepsis which is connected between adult and child. We reported previously that the on-line P.E. between a healthy adult dog and a jaundiced puppy is acceptable with biocompatibility, and seems to be satisfactory from the point of system performance (Asanuma et al. 1987). In the present experiment, the effectiveness of this system was assessed in a septic puppy model. All puppies in the untreated and sham groups died within 24 hr. In the P.E. group, four puppies died within 24 hr and the remaining 1 survived for one more week. In the on-line P.E. group, however, only one puppy died within 24 hr, but the remaining 4 survived for one more week. Blood pressure, urinary output, and acid-base equilibrium were significantly improved by the treatment with on-line P.E. Moreover, the number of E. coli and endotoxin concentration in blood was reduced and opsonic activity recovered significantly by treatment with on-line P.E. These results suggest that the elimination of toxic substances, primarily endotoxin, and the activation of humoral immunity due to the elevation of opsonic activity were two major mechanisms responsible for the effectiveness of on-line P.E. in treating the septic model. On-line P.E. was more effective for the septic model than P.E. Opsonic activity of FFP from adult dogs on the plasma exchange was diminished to 73% of that of fresh plasma, although there is no statistically significant difference in opsonic activity of puppies between the two groups after treatments. The difference between the two groups seems to be related to the opsonic activity. Our experiments proved the effectiveness of on-line plasma exchange between puppies and adult dogs in septic puppies and elucidated a part of its mechanism. This on-line plasma exchange between adult and child seems an effective new therapeutic method for sepsis in neonates and infants.

References