Formation of cytokines by retransfusion of shed whole blood

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SUMMARY

We have studied, in 10 patients undergoing hip replacement surgery, the release of cytokines (tumour necrosis factor-α (TNF-α), interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6) and interleukin-8 (IL-8)) in association with retransfusion of autologous shed blood. The patients were reinfused with whole blood collected after operation. The median volume returned to the patients was 300 ml whole blood (25–75% range = 300–425 ml). Before reinfusion, blood was filtered. Plasma concentrations of IL-6 increased 1 and 60 min after retransfusion (P < 0.05). The plasma concentrations of TNF-α, IL-1α, IL-1β, IL-4 and IL-8 did not change significantly after retransfusion of shed wound blood. However, there were increased concentrations of IL-1α, IL-1β, IL-6 and IL-8 in the collected blood (P < 0.001). The filtration procedure did not reduce significantly the concentrations of these factors. This study shows that whole blood collected from a surgical wound contains large concentrations of cytokines. Filtration of the shed wound blood did not reduce significantly these levels and retransfusion caused increased plasma concentrations of IL-6. (Br. J. Anaesth. 1994; 72: 422–425)

KEY WORDS


Perioperative and postoperative blood salvage is becoming a common technique for avoiding the risks associated with transfusion of homologous blood. Various types of equipment are available commercially for retransfusion of autologous blood in different types of surgery. Studies have shown that changes in shed blood will occur in association with the use of these techniques [1, 2]. The coagulation, fibrinolytic and complement systems are activated by retransfusion of autologous blood [3, 4]. A recent study demonstrated activation of leucocytes and release of PMN elastase in systems used for retransfusion of erythrocytes and that the washing procedure removed the secreted PMN elastase [5]. Complications that may occur during retransfusion of autologous blood include haemolysis, air embolism [6], fat embolism and formation of micro-aggregates [7]. The aetiology of these complications is not understood fully, but it is well known that when blood comes into contact with foreign materials the complement cascade is activated, as when dialysis membranes, heart–lung machines and contrast agents are used [8–10]. Activation of complement leads to activation of leucocytes and may lead to the release of cytokines [11, 12]. Cytokines are formed in patients undergoing dialysis and extracorporeal circulation [13, 14]. Cytokines are polypeptides and are produced mainly by macrophages and monocytes. The production of cytokines may be triggered by complement activation split products and by different neuromediators [11, 12, 15].

The aim of the present study was to determine if cytokines are released during blood collection and found in the blood collected and if the amount of cytokines contained in the blood is sufficient to influence plasma concentrations when reinfused into the patient.

PATIENTS AND METHODS

We studied 10 patients (nine females and one male) undergoing elective hip replacement surgery. Their ages ranged from 47 to 78 yr (mean 66 yr). All patients required blood transfusions and received autologous blood after operation. Indications for reinfusion of aspirated whole blood were the same as those for transfusion of bank blood. When the patients had a haemoglobin concentration less than 100 g litre⁻¹ or showed signs of hypovolaemia or circulatory instability caused by bleeding, the reinfusion was started. The patients did not require blood transfusion during the intraoperative period and they received 1 unit of shed whole blood after operation. Patients who needed more than 1 unit of transfused blood received additional units as homologous blood. Shed drainage blood was mixed with anti-coagulating dextrose solution (ACD) 40 ml (ACD solution contains citric acid 0.32 g, sodium citrate 0.88 g and glucose 0.98 g). A drainage suction system allowing reinfusion of aspirated wound blood was used (Solcotrans, Solco Basle Ltd, U.K.). An aspiration tube of length 10 cm and diameter 3 mm
was connected to the entry port of the blood reservoir. A maximum negative pressure of 100 mm Hg was applied. The tubes and reservoirs were made of polyvinylchloride. The blood was reinfused by gravity into the patient through a microaggregate filter (Pall SQ 49-S micron microporous filter). In contrast with the technique where harvested blood is centrifuged and washed before reinfusion as an erythrocyte concentrate (centrifuge-based cell salvage instruments), the blood is reinfused as whole blood when using the Solcotrans system. The reinfusion of wound drainage blood was commenced within 4 h from the start of blood collection and completed within 45 min from the start of reinfusion. No bank blood was given before reinfusion of collected wound blood.

Blood samples from the patients were obtained before operation, 1 min before the start of reinfusion and 1 and 60 min after completing the reinfusion. Blood samples were obtained also from the blood reservoir before and after filtration. These samples were obtained before 1 min after the start of reinfusion. All samples were obtained in tubes containing 0.054 ml of EDTA 0.34 mol litre⁻¹ for measurement of cytokines. To remove the cells, the tubes were centrifuged immediately and samples were frozen in separate tubes within 30 min and stored at -80 °C. All measurements were performed in duplicate.

Tumour necrosis factor-α (TNF-α), and the interleukins (IL) IL-1α, IL-1β, IL-4 and IL-6 were measured with commercially available ELISA systems (Biotrak cytokines human ELISA systems, Amersham). IL-8 was measured by a solid-phase, double-ligand ELISA [16].

Statistical analysis

Values are given as medians and 25–75 % range of the values. The Wilcoxon test for paired comparisons was used. Differences were considered significant when P was less than 0.05.

RESULTS

Median volume returned to the patients was 300 ml whole blood (25–75 % range = 300–425 ml).

Concentrations of TNF-α, IL-1α, IL-1β, IL-4, IL-6 and IL-8 in collected wound drainage blood are shown in table I. In collected blood there were increased concentrations of IL-1α, IL-1β, IL-6 and IL-8 compared with concentrations in systemic blood (P < 0.05, respectively). There were no increases in the concentrations of TNF-α and IL-4 in aspirated blood. There were no significant differences in the concentrations of IL-1α, IL-1β, IL-6 and IL-8 before and after filtration.

Plasma concentrations of TNF-α, IL-1α, IL-1β, IL-4, IL-6 and IL-8 before operation, 1 min before the start of the retransfusion and 1 and 60 min after completing retransfusion are shown in table II. There were increased concentrations of IL-6 at both 1 (P < 0.05) and 60 min (P < 0.05) after retransfusion compared with concentrations found before operation and 1 min before the start of the retransfusion procedure. There were no significant changes in plasma concentrations of TNF-α, IL-1α, IL-1β, IL-4 and IL-8 in blood 1 or 60 min after retransfusion.

DISCUSSION

Autotransfusion or autologous transfusion may be defined as the reinfusion of blood or blood products derived from the patient's own circulation [17]. The techniques for autotransfusion have been developed to avoid the risks and complications of homologous transfusions. An alleged advantage of autologous whole blood is that the plasma proteins are retransfused. On the other hand, it has been postulated that whole blood retransfusion is associated with infusion of toxic substances as the coagulation and fibrinolytic systems are activated in the surgical wound. This study demonstrates that reinfusion of autologous

### Table I. Median (25–75 % range) concentrations of TNF-α, IL-1α, IL-1β, IL-4, IL-6 and IL-8 in retransfused blood and in plasma (control)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Plasma (control)</th>
<th>Before filter</th>
<th>After filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>9 (0-12)</td>
<td>12 (9-13)</td>
<td>11 (6-12)</td>
</tr>
<tr>
<td>IL-1α (pg/ml)</td>
<td>0.7 (0-1.7)</td>
<td>1.2 (1-2)</td>
<td>1.9 (1.7-2.2)</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>0 (0)</td>
<td>7.7 (5.0-10.7)</td>
<td>5.0 (2.7-11.2)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>&lt; 4 (&lt; 4)</td>
<td>&lt; 4 (&lt; 4)</td>
<td>&lt; 4 (&lt; 4)</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>3 (3)</td>
<td>&gt; 300 (&gt; 300)</td>
<td>&gt; 300 (&gt; 300)</td>
</tr>
</tbody>
</table>

### Table II. Median (25–75 % range) plasma concentrations of TNF-α, IL-1α, IL-1β, IL-4, IL-6 and IL-8 in plasma

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Before op.</th>
<th>1 min before transfusion</th>
<th>1 min after transfusion</th>
<th>60 min after transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>9 (0-12)</td>
<td>9.5 (0-11)</td>
<td>10 (7-10)</td>
<td>9 (0-15)</td>
</tr>
<tr>
<td>IL-1α (pg/ml)</td>
<td>0.7 (0-1.7)</td>
<td>0 (0-0.5)</td>
<td>0 (0-0.7)</td>
<td>0.5 (0-0.6)</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>0 (0)</td>
<td>0 (0-0.5)</td>
<td>0 (0-0.85)</td>
<td>0 (0-1.1)</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>&lt; 4 (&lt; 4)</td>
<td>&lt; 4 (&lt; 4)</td>
<td>&lt; 4 (&lt; 4)</td>
<td>&lt; 4 (&lt; 4)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>3 (3)</td>
<td>56 (22-98)</td>
<td>170 (75-274)</td>
<td>130 (55-200)</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>0.2 (0.1-0.3)</td>
<td>0.3 (0.2-0.3)</td>
<td>0.4 (0.1-0.4)</td>
<td>0.3 (0.2-0.3)</td>
</tr>
</tbody>
</table>
whole blood is associated with infusion of large concentrations of cytokines (IL-1α, IL-1β, IL-6 and IL-8). Several studies have demonstrated that cytokines are released in septic shock, trauma and haemorrhage [18–20]. Cytokines are important mediators of septic shock [18]. For example, TNF has multiple effects similar to those of endotoxin infusion [21–23]. TNF-α antibodies may reduce lethality of endotoxin and bacteraemia [24]. Activation of the cascade systems occurs during the processing of the blood as it comes in contact with extravasated tissue in the wound and with foreign material in the processing equipment. During retransfusion of aspirated whole blood, the shed blood passes through a microporous filter. We have demonstrated that none of the cytokines investigated was removed by the filter and that large amounts of IL-1α, IL-1β, IL-6 and IL-8 were infused into the patients. Retransfusion of shed wound drainage blood caused increased plasma concentrations of IL-6 after the transfusion was completed. The plasma concentrations of TNF-α, IL-1α, IL-1β, IL-4 and IL-8 did neither change significantly when the maximum volume infused was only 425 ml.

The large concentrations of IL-6 in plasma were not surprising as tissue damage is the major determinant of circulating concentration of IL-6 [25]. The biological effects of IL-6 are mainly induction of T-cell activation and B-cell antibody production [26]. IL-6 seems to be the main glycoprotein responsible for inducing the synthesis of acute phase proteins [27]. Although IL-6 is less toxic than other cytokines, IL-6 concentrations in plasma were found to correlate well with outcome in sepsis [18]. Antibodies against IL-6 were also found to protect against lethality in endotoxic shock in mice [28]. In a recent case report, the development of upper airway oedema was reported in association with whole blood retransfusion [29]. Activation of complement and leucocytes with the release of cytokines has been shown to be an important mediator of adult respiratory distress syndrome and MODS after trauma, vascular surgery, thoracic surgery and sepsis. Trauma, vascular surgery and thoracic surgery are important indications for retransfusion of shed autologous blood. Retransfusion of blood with large concentrations of cytokines may therefore contribute further to the development of organ failure. Although a maximum of 425 ml shed was retransfused in the present study, a significant increase in IL-6 in plasma was observed 1 h after completing the retransfusion. The reinfused whole blood contained large concentrations of IL-1α, IL-1β, IL-6 and IL-8. Retransfusion of larger amounts of shed wound blood may therefore lead to changes in plasma concentrations of these cytokines and infusion of such blood may lead to serious complications. Studies have shown that 1–4 units may be transfused safely [4, 30]. If larger volumes need to be transfused, a centrifuge-based cell salvage device might be preferable, as studies indicate that inflammatory mediators are reduced effectively by washing [5]. The concentrations of IL-1, IL-6 and IL-8 found in retransfused blood in this study were similar to the concentrations reported in association with severe trauma and sepsis.

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REFERENCES


