Changes in inflammatory mediators during orthotopic liver transplantation

M. C. BELLAMY, H. F. GALLEY AND N. R. WEBSTER

Summary

Transplantation is associated with an inflammatory rejection response. Graft reperfusion causes typical haemodynamic and biochemical responses. In this study we have investigated the relationship between these haemodynamic responses and changes in circulating inflammatory mediators after graft reperfusion in 10 consecutive patients undergoing orthotopic liver transplantation. After reperfusion, systemic vascular resistance index decreased \((P=0.011)\) and cardiac index increased \((P=0.038)\). These characteristic haemodynamic changes of the reperfusion syndrome were accompanied by global increases in cytokine concentrations. Plasma concentrations of leukotrienes decreased, while thromboxane \(B_2\) and platelet activating factor remained increased throughout. Reperfusion-mediated changes in inflammatory mediators may account for the haemodynamic disturbance. \((Br. J. Anaesth. 1997; 79: 338–341)\).

Key words

Liver, transplantation. Polypeptides, cytokines. Immune response.

Liver transplantation is associated with a complex sequence of events, including immune activation, haemodynamic derangement and long-term immune rejection reactions. Reperfusion injury, thought to be a result of increased oxygen derived free radical release and characterized by loss of endothelial cell viability, occurs after cold ischaemic storage and subsequent reperfusion.\(^1\)-\(^4\) Deranged endothelial cell function may in turn result in leucocyte activation and release of inflammatory mediators which may then cause graft damage and rejection. While preservation injury of the graft may result from activation of Kupffer cells and consequent local injury,\(^5\) our study was concerned with the host systemic response to graft reperfusion.

Extra-hepatic effects of hepatic reperfusion result in haemodynamic deterioration and biochemical responses similar to those seen in sepsis. The causes of this response are poorly defined; animal models of ischaemia–reperfusion syndrome have demonstrated increased expression of tumour necrosis factor-\(\alpha\) (TNF\(\alpha\)).\(^6\) In these models, TNF\(\alpha\) and haemodynamic changes resemble those seen in sepsis, where release of TNF\(\alpha\) and a range of interleukins (IL) are associated with reduction in systemic vascular resistance and an increased cardiac index.\(^7\) Moreover, cytokines are involved in rejection reactions which are a complication of liver transplantation.\(^8\) Thus ischaemia–reperfusion syndrome is likely to have several important intra- and extra-hepatic effects on outcome. The mechanisms underlying reperfusion syndrome have not been elucidated. We have therefore examined changes in cytokines and arachidonic acid metabolites after reperfusion to investigate the relationship between inflammatory and haemodynamic responses.

Patients and methods

Approval for the study was obtained from the local Clinical Research (Ethics) Committee. We studied 10 consecutive adult patients undergoing elective orthotopic liver transplantation after obtaining written informed consent.

Peripheral venous and radial artery cannulae were inserted and anaesthesia was induced with alfentanil 0.1 mg kg\(^{-1}\) and midazolam 0.1 mg kg\(^{-1}\). Neuromuscular block was facilitated by a bolus dose of atracurium 0.5 mg kg\(^{-1}\) followed by an infusion of 0.4 mg kg\(^{-1}\) h\(^{-1}\). After tracheal intubation, the lungs were ventilated to normocapnia with oxygen-enriched air and 1 MAC of isoflurane. Anaesthesia was supplemented with an infusion of alfentanil 0.1 mg kg\(^{-1}\) h\(^{-1}\). A central venous cannula, pulmonary artery catheter and 18-F percutaneous cardiac bypass catheter were inserted according to our usual practice.\(^10\) Dopamine was infused throughout the procedure at a rate of 3 \(\mu\)g kg\(^{-1}\) min\(^{-1}\). All patients were established on veno-venous bypass at the beginning of the anhepatic phase, at a flow rate of 0.4×cardiac output to help maintain haemodynamic stability.\(^11\) Tranexamic acid 1 g was administered prophylactically, followed by an infusion of 300 mg h\(^{-1}\) until the end of surgery.\(^12\) After excision of the liver and before reimplantation, prednisolone 10 mg kg\(^{-1}\) was administered. Where necessary, adrenaline was given in incremental boluses of 50 \(\mu\)g to maintain haemodynamic stability at reperfusion.

Serum potassium concentration was maintained...
Inflammatory mediators during liver transplantation

Table 1 Changes in inflammatory mediators following graft reperfusion. Data are median (range) and were compared using Wilcoxon signed rank test

<table>
<thead>
<tr>
<th>Time III5 (pg ml(^{-1}))</th>
<th>Time III60 (pg ml(^{-1}))</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour necrosis factor-α</td>
<td>4.4 (4.4–12)</td>
<td>32 (14–154)</td>
</tr>
<tr>
<td>Tumour necrosis factor receptor 1</td>
<td>3033 (2437–5621)</td>
<td>8108 (5783–21940)</td>
</tr>
<tr>
<td>Interleukin-β</td>
<td>1.0 (1.0–1.3)</td>
<td>1.5 (1.0–1.50)</td>
</tr>
<tr>
<td>Interleukin-1 receptor antagonist</td>
<td>1324 (220–10010)</td>
<td>5467 (653–24660)</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>376 (82–26855)</td>
<td>2137 (272–24660)</td>
</tr>
<tr>
<td>Interleukin-8</td>
<td>121 (50–338)</td>
<td>743 (398–1575)</td>
</tr>
<tr>
<td>Platelet activating factor</td>
<td>978 (741–2577)</td>
<td>1096 (453–1853)</td>
</tr>
<tr>
<td>Thromboxane B(_2)</td>
<td>350 (120–620)</td>
<td>370 (120–900)</td>
</tr>
<tr>
<td>Leukotriene C(<em>{4}), D(</em>{4}), E(_{4})</td>
<td>800 (110–6800)</td>
<td>580 (40–1200)</td>
</tr>
</tbody>
</table>

in the range 4.0–5.0 mmol litre\(^{-1}\) and ionized calcium in the range 0.8–1.4 mmol litre\(^{-1}\). Blood and colloid (modified fluid gelatin) were infused at \(37^\circ C\) via a rapid infusion system to maintain the packed cell volume at 0.26–0.32 and central venous pressure at 6–12 mm Hg.

Cardiac output was measured by the triplicate thermodilution method, and cardiac index and systemic vascular resistance index (SVRI) were calculated 5 min before (III5), and 5, 10, 20, 30 and 60 min (III5, III10, III20, III30 and III60) after graft reperfusion. Blood samples (5 ml) were collected at III5 and III60 into plain tubes for assay of cytokines, and into EDTA tubes containing 30 l of indomethacin 0.04 mol litre\(^{-1}\) for measurement of leukotriene and thromboxane. These were centrifuged immediately and stored at \(-70^\circ C\) until assay. Samples were obtained at the same times for assay of platelet activating factor (PAF) and were treated as follows: 2 ml of blood were added immediately to 6 ml of ice-cold methanol and vortex mixed. After centrifugation at 3000 g for 20 min, the supernatant was frozen at \(-70^\circ C\) until assay. Enzyme-linked immunoassay was used to measure TNF\(_\alpha\), IL-1\(\beta\), IL-6 and IL-8. Thromboxane B\(_2\), total leukotrienes (LTC\(_4\), D\(_4\) and E\(_4\)), TNF receptor 1, IL-1 receptor antagonist (IL-1ra) and PAF were measured using radioimmunoassay. Samples from single patients were assayed in the same batch, and all assays were carried out within 1 month.

STATISTICAL METHODS

Data are expressed as median (range). Changes in cardiac index and SVRI with time were analysed using Friedman analysis of variance. Friedman is a non-parametric approach based on rank. We chose this approach because our numbers were small, and to protect against potential effects of individual “rogue” values. The difference between pre- and post-reperfusion biochemical data was examined using Wilcoxon signed rank test. All statistical tests were performed using SPSS for Windows (v 6.0). Changes were taken to be statistically significant when \(P<0.05\).

Results

One patient died during operation, and data from nine patients (five women, median age 49 (range 39–62) yr) are therefore presented. Their diagnoses were primary sclerosing cholangitis (three patients), chronic rejection after previous transplantation (two),

![Figure 1](https://example.com/figure1.png)

**Figure 1** Time course of haemodynamic changes (systemic vascular resistance index (SVRI) and cardiac index (CI)) during orthotopic liver transplantation in nine subjects. Results are shown as median (range) and were analysed using Friedman two-way analysis of variance.

![Figure 2](https://example.com/figure2.png)

**Figure 2** Time course of inflammatory mediator changes during orthotopic liver transplantation in nine subjects. Results are shown as median (range) and were analysed using Friedman two-way analysis of variance.
chronic active hepatitis, sero-negative viral hepatitis, alcoholic cirrhosis and haemochromatosis (one each).

There were significant changes in both cardiac index (P=0.008) and SVRI (P<0.0001) (Friedman) during transplant (fig. 1). Cardiac index increased from 3.3 (2.2–6.8) litre min$^{-1}$ m$^{-2}$ at III$\_5$ to 5.4 (2.3–8.4) litre min$^{-1}$ m$^{-2}$ at III$\_5$ (P=0.038, Wilcoxon). SVRI decreased on reperfusion of the liver graft from 1647 (930–2871) dyn s$^{-1}$ cm$^{-5}$ m$^{-2}$ to 883 (561–1727) dyn s$^{-1}$ cm$^{-5}$ m$^{-2}$ (P=0.011).

Concentrations of all cytokines and IL-1ra and TNF receptor 1 increased at III$\_5$ compared with III$\_5$ (table 1). Total leukotriene concentrations showed a decreasing trend with time (fig. 2). The increased leukotriene concentrations seen at III$\_5$ related to a single outlying value. Thromboxane B$_2$ and PAF concentrations, although increased, remained constant (table 1).

**Discussion**

We have confirmed a well-defined change in cardiovascular status after graft reperfusion during orthotopic liver transplantation, consisting of marked reduction in systemic vascular resistance and an increase in cardiac output. We have also demonstrated significant changes in circulating concentrations of mediators of inflammation, including cytokines and eicosanoids, after reperfusion.

The endothelium produces several substances which regulate inflammation, including cytokines and arachidonic acid metabolites. Orchestration of the inflammatory response depends on communication between cells by cytokines which regulate the severity and duration of the inflammatory response.

In this study we found increases in TNF$\_\alpha$, IL-1$\beta$, IL-6 and IL-8 1 h after reperfusion. This contrasts with models of endotoxin-induced sepsis, where increases in TNF concentration in the circulation usually preceded increases in IL-6 and IL-8 by several hours. A previous study also found early intraoperative increases in IL-6. A possible explanation in our patients could be cytokine activation as a result of veno-venous bypass. Cardiopulmonary bypass can trigger an early increase in IL-6 and neutrophil activity. Another possibility could be "priming" by high circulating concentrations of PAF seen in this study.

Cytokines produce a biological effect which is receptor mediated. Specific cytokine receptor expression is itself cytokine dependent. Additionally, the interaction between cytokine and receptor may be modulated by the effects of free (circulating) receptor molecules or by circulating antagonists to cellular receptors. The biological action of IL-1$\beta$ for example is regulated by its receptor antagonist (IL-1ra) and regulated independently by other cytokines as part of the inflammatory process. In addition to increased cytokine concentrations, predictable concomitant increases in IL-1ra and TNF receptor 1 (TNF-R1, a circulating TNF receptor possibly antagonizing the interaction between TNF and its cellular receptor) were found in our study after reperfusion.

Under conditions of ischaemia and reperfusion, oxygen-derived free radicals are produced by activation of xanthine oxidase. We have shown previously increased oxidant stress after graft reperfusion during liver transplantation, associated with haemodynamic changes.

In this study we have demonstrated elevation of thromboxane B$_2$ and total leukotrienes in varying proportions in different patients. Oxygen-derived free radicals mediate production of both thromboxanes and leukotrienes. These vasoactive compounds with oxygen derived free radicals act as chemoattractants for leucocytes. Moreover, eicosanoids have been implicated in the increases in intracellular calcium in the leucocyte which trigger cytokine expression.

Lipid mediators of inflammation include PAF and arachidonic acid metabolites. PAF primes macrophages to other inflammatory mediators and alters microvascular permeability. Although PAF did not increase as a result of reperfusion, concentrations were increased compared with previous findings in healthy subjects. Arachidonic acid metabolites, including leukotrienes and thromboxanes, have profound inflammatory and vascular actions and may regulate, and be regulated by, cytokines.

We have shown that in patients undergoing liver transplantation there was an inflammatory response associated with expression of several pro-inflammatory cytokines subsequent to reperfusion in humans. This was accompanied by activation of lipooxygenase and cyclo-oxygenase pathways suggesting ongoing inflammatory activation and endothelial damage. These changes occurred in association with dramatic characteristic haemodynamic changes of the reperfusion syndrome. These changes in cytokines, biochemistry and haemodynamic state resemble those seen in the sepsis syndrome, although unlike septic shock they are precisely predictable. Other workers have shown an association between increases in intraoperative TNF$\_\alpha$ and IL-6 concentrations with subsequent graft rejection and infection. An understanding of the biochemical and inflammatory processes surrounding graft reperfusion may therefore lead to strategies to improve outcome. We have not considered in this study the role of anti-inflammatory cytokines and further work is required.

**Acknowledgements**

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**References**


