Transpl Int (1997) 10: 401–404

BRIEF REPORT

Jörg Gerlach
Achim Jörres
Michael Schön
Roland Nohr
Axel Berger
Gabriele Spatkowski
Mark D. Smith
Peter Neuhaus

Systemic liberation of interleukin-8 in the perioperative phase of liver transplantation

Received: 12 September 1996
Received after revision: 27 February 1997
Accepted: 3 March 1997

J. Gerlach (✉) · M. Schön · R. Nohr
A. Berger · G. Spatkowski · P. Neuhaus
Department of Surgery,
Rudolf Virchow Clinic,
Humboldt University Berlin,
Augustenburger Platz 1,
D-13353 Berlin, Germany
Fax: +49 30 45 05 99 28

A. Jörres
Department of Nephrology,
Rudolf Virchow Clinic,
Humboldt University Berlin,
Augustenburger Platz 1,
D-13353 Berlin, Germany

M.D. Smith
University of Strathclyde,
Glasgow, UK

Abstract Serum levels of interleukin-8 (IL-8) were investigated in the perioperative phase of liver transplantation (LTx) in order to help determine whether this cytokine might serve as a parameter for preservation injury. In a study of 45 patients undergoing LTx, systemic IL-8 was estimated at the end of the anhepatic phase, at 30, 60, and 120 min after reperfusion of the graft, and 24 h and 7 days after LTx. A maximum mean concentration of 665 ± 135 pg/ml was seen 60 min after LTx. The minimum was found on the 1st postoperative day (POD 1): 328 ± 33 pg/ml. Significant changes were found between 60 min and PODs 1 and 7, as well as between 120 min and POD 1. Differences in cold ischemia time were not found to be significant. We conclude that monitoring of systemic IL-8 levels is not useful in the development of new liver preservation concepts.

Key words Liver transplantation, interleukin-8 · Liver preservation, interleukin-8 · Interleukin-8, liver transplantation

Introduction

Damage to the liver as a result of preservation and/or reperfusion is still associated with organ dysfunction in the early course following liver transplantation (LTx). Primary dysfunction (PDF) and primary nonfunction (PNF) are the most severe consequences [16, 17, 21]. These problems of the immediate postoperative period are currently being addressed by several research groups as they work on the further development of preservation concepts. In order to conduct comparative studies of preservation concepts, data about the outcome of preserved organs are essential. However, only a few sensitive serum markers have, as yet, been clinically established for the assessment of potential organ damage during the perioperative period.

Cytokines play an important role in the regulation of interactions between blood cells and such nonparenchymal liver cells as organ-associated leukocytes and endothelial cells [4]. These interactions include the recruitment of specific leukocyte subpopulations to hypoxic/injured tissue [3, 22, 24]. Interleukin-8 (IL-8) is part of this response and may contribute to leukocyte infiltration [19].

IL-8 is an inflammatory cytokine that is produced by various cell types, among them Kupffer [25] and endothelial cells [20], hepatocytes, neutrophils, and monocytes [23]. Important biological activities include potent neutrophil chemoattraction and activation. IL-8 may be part of the mediator cascade of graft destruction [2], in that allogeneic major histocompatibility complex antigens expressed on the allograft and/or on the passenger leukocytes induce IL-8 formation via CD4/T helper cell activation and IL-1 and IL-2-secretion, which is followed by IL-4 and IL-6 formation and ensuing tumor necrosis factor-α (TNF-α) production, which leads to,

among other things, IL-8 formation [2]. This may be only one aspect of cytokine interaction, which is part of the complicated balance of mediators.

Like other cytokines, IL-8 was found to induce its own formation [8]. Lateveer et al. [10] have calculated a half-life of 9.9 ± 2.2 min for circulating IL-8 using rhesus monkeys. An IL-8 mRNA half-life of 4.6 h was calculated using blood from normal human volunteers [28]. Interesting physicochemical properties of IL-8 include its extreme stability with resistance to proteolysis and denaturation, and prolonged biological activity in vivo [15]. These properties suggest that IL-8 may be a comparatively long-term acting mediator, rather than a short-term acting cytokine like, for example, TNF-α. Although other groups have reported on cytokine responses to LTx, no information about the perioperative course of IL-8 has yet been provided.

Forty-five patients undergoing orthotopic LTx were investigated during the preoperative, anhepatic, and postreperfusional phases. Systemic IL-8 concentrations were monitored to determine their potential value as a serum parameter for the evaluation of new preservation concepts.

Materials and methods

Liver allograft recipients

Forty-five adult patients (28 male, 17 female) consecutively underwent orthotopic LTx at our unit. The patients' age ranged from 20 to 62 years. Indications for LTx included chronic active hepatitis, alcoholic cirrhosis, fulminant hepatitis, cryptogenic cirrhosis, primary biliary cirrhosis, Budd-Chiari syndrome, hepatocellular carcinoma, autoimmune hepatitis, and PNF. The mean cold ischemia time, starting from the interruption of circulation during organ harvesting until the beginning of the anastomosis, was 633 ± 35 min (median 625 min, range 280–1023 min). The mean anastomosis time was 70.2 ± 3.2 min. Patients were randomized to treatment with cyclosporin A (CyA) or tacrolimus-based immunosuppression prior to transplantation.

Liver transplantation and concomitant treatment

The surgical procedure was performed as reported elsewhere [13], including preservation with University of Wisconsin solution and the use of venovenous bypass in all cases. Aprotinin administration, i.v. antibiotic treatment, selective bowel decontamination, and other forms of prophylaxis were performed as previously described [12].

Immunosuppressive protocol

Patients in the tacrolimus and CyA-based treatment groups followed an immunosuppressive regimen described elsewhere [14]. This included methylprednisolone (PRED), azathioprine, and antithymocyte globulin (ATG; Fresenius, Bad Homburg, Germany). PRED was administered by i.v. bolus injection at a dosage of 500 mg before and 6 h after reperfusion. PRED treatment was commenced on POD 1 at 20 mg/day and was reduced to 15 mg/day after 4 weeks.

Collection of samples

Heparinized arterial blood samples were obtained via a radial arterial catheter and were collected sterile. In a few cases, samples on POD 7 were taken from a central venous catheter. Plasma was collected following blood centrifugation at 4 °C and immediately frozen at –80 °C.

Time points of sampling were immediately preoperatively and at the end of the anhepatic phase in the recipient. Plasma was also sampled 30, 60, and 120 min post-LTx as well as 24 h and 7 days postoperatively.

IL-8 Assay

An enzyme immunoassay was performed as a sandwich-type ELISA in 96-well microtiter plates and coated with a polyclonal goat anti-human IL-8 antibody (British Bio-technology BD A26). IL-8 in standards (hr-IL-8, 30–100–300–1000–3000 pg/ml R&D systems #208-IL) and samples was detected by a series of incubations with (1) biotinylated (Pierce #21335) goat anti-human IL-8, (2) avidin-peroxidase (Calbiochem #189733), and (3) TMB substrate for color development. The signal was measured biochromatically at 450 and 630 nm (Dynatech MR5000). There was no cross-reactivity with recombinant human IL-1α, IL-1β, TNF-α, TNF-β, or IL-6. The limit of sensitivity was 50 pg/ml.

Statistics

Data analysis was performed using a notched box-and-whisker plot analysis [11]. The boxes in Fig. 1 show the range from 25 % to 75 % quartiles. The bars represent the 10th and the 90th percentiles. A notch is added to each box corresponding to the width of a confidence interval for the median. The results given in the text are expressed as mean ± SEM. Differences between values were calculated using the Mann-Whitney U-test. Significance was estimated in relation to the recipient values before LTx. Differences were considered significant when P was less than 0.05.

Results

During the postoperative course, 33 % of the patients in our study suffered from intra-abdominal infections, 20 % from bacterial pneumonias, and 3 % from CMV infection. Thirthy-two percent of the patients developed a mild rejection and 22 % showed a moderate to severe rejection. Two percent had initial nonfunction.

The systemic IL-8 concentrations measured at the end of the anhepatic phase, at 30, 60, and 120 min after reperfusion of the graft, and at 24 h and 7 days post-LTx were compared to preoperative and postoperative levels. Figure 1 shows the course of these IL-8 plasma levels. A wavelike course of concentrations with a peak in-between 60 and 120 min postreperfusion is seen. In the recipients, the mean was 451 ± 63 pg/ml. A maxi-

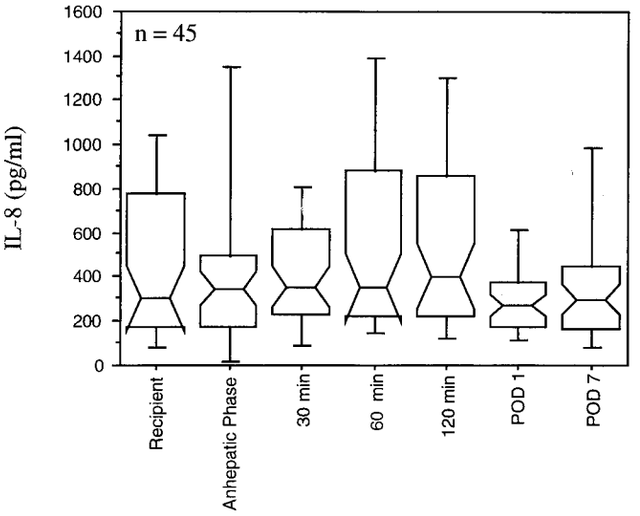

Fig. 1 Time course of systemic IL-8 levels in pg/ml in 45 patients during the perioperative phase of LTx. Significance was calculated for the recipients before LTx ($P < 0.05$). The values are given in notched-box plot-and-whisker diagrams. Time points of measurements were immediately before LTx (recipient), at the end of the anhepatic phase (anhepatic phase), 30 min, 60 min, and 120 min after reperfusion, as well as 24 h after reperfusion (POD 1) and on the 7th postoperative day (POD 7)

mum mean concentration of 665 ± 135 pg/ml was seen 60 min after LTx. The minimum – 328 ± 33 pg/ml – was seen on POD 1. Significant changes with respect to the recipient values before LTx were found between 60 min and PODs 1 and 7, as well as between 120 min and POD 1.

Patients were divided into two groups according to the cold ischemia times. In group 1, this was 240–600 min; in group 2, 601 –1023 min. No significant differences were observed.

The influence of the duration of anastomosis time on the cytokine response was also calculated. Again, the patients were divided into two groups. In group A, anastomosis time ranged from 30 to 70 min; in group B, from 71 to 107 min. IL-8 was not found to be sensitive.

Discussion

For clinical studies on liver preservation, noninvasive serum parameters that react quickly to consequences of preservation damage are of interest. IL-8 is a chemokine that is generated only after specific stimuli, such as oxidant stress [6] and bacterial lipopolysaccharide (LPS) [1]. This is of interest because oxidant stress has been shown to have little effect on the synthesis of the inflammatory cytokines IL-1β, TNF-α, and IL-6 [14]. Reactive oxygen intermediates induced by liver anoxia and reoxygenation are at least partly generated by intrasinusoidal enzyme systems [9, 18, 31]. LPS, one of the powerful stimuli for IL-8 synthesis, is interesting because of possible bacterial translocation in LTx with the subsequent risk of PNF [30]. A recently suggested aspect of the biological activity of IL-8 is haptotaxis [23]. It implies that leukocyte migration is directed by a gradient of insoluble, fixed chemokines.

Both IL-1 and TNF-α, at nanomolar concentrations, appear to be important early response cytokines that can stimulate the expression of IL-8 [23]. This study reveals that the signal responsible for the gene expression of IL-8 is related to the generation of reactive oxygen metabolites. IL-8 can also be produced in response to IL-6 and interferons.

In a study reported by Tilg et al. [26], IL-8 serum concentrations were elevated in liver allograft recipients during bacterial infection, cytomegalovirus (CMV) disease, and acute rejection, whereas patients with uneventful recovery showed IL-8 concentrations below the detection limit. Correlations between IL-8 and TNF-α were not seen in acute cellular rejection episodes or in other complications, such as bacterial infections or CMV disease.

Yagihashi et al. investigated systemic IL-8 concentrations in rats after experimental LTx [29]. Allogeneic LTx induced elevated IL-8 serum concentrations in the early postoperative period (PODs 0–4) without acute cellular rejection, while acute moderate cellular rejection resulted in a delayed increase in systemic IL-8 levels (PODs 13–19). A correlation between IL-8 peak levels and histological grades of rejection was not seen.

As suggested by Dallman [5], perioperative systemic cytokine levels may be interpreted mainly as a general response to inflammation that accompanies surgery, including inflammation due to ischemia and reperfusion, rather than as specific organ alterations like graft rejection. Moreover, cytokine formation and secretion depend to a great extent on the perioperative immunosuppressive treatment [7]. Surgical procedures alone influence cytokine levels [27].

The time points we chose focused on the early postreperfusional period. We found no significant changes, i.e., there were no statistical differences between patients with longer or shorter preservation times or between patients with different anastomosis times. This lack of significant changes in the IL-8 serum level in the immediate postreperfusional phase suggests that this mediator should not be used as a tool for the further development of liver preservation concepts.

Acknowledgement This work was supported by the Deutsche Forschungsgemeinschaft Ge 571/5–2.

References

1. Baggiolini M, Dewald B, Moser B (1994) Interleukin-8 and related chemotactic cytokines–CXC and CC Chemokines. In: Dixon FJ (ed) Advances in immunology. Academic Press, San Diego, p 105
2. Chandler C, Passaro E (1993) Transplant rejection – Mechanisms and treatment. Arch Surg 128: 279–283
3. Colletti LM, Remick DG, Burtch GD, Kunkel SL, Strieter RM, Campbell DA Jr (1990) Role of tumor necrosis factor-a in the pathophysiologic alterations after hepatic ischemia/reperfusion injury in the rat. J Clin Invest 85: 1936–1943
4. Cotran RS, Pober JS (1990) Cytokine-endothelial interactions in inflammation, immunity, and vascular injury. J Am Soc Nephrol 1: 225–235
5. Dallman MJ (1993) Cytokines as mediators of organ graft rejection and tolerance. Curr Opin Immunol 5: 788–793
6. DeForge LE, Preston AM, Takeuchi E, Kennedy J, Boxer LA, Remick DG (1993) Regulation of interleukin 8 gene expression by oxidant stress. J Biol Chem 268: 25568–25576
7. Emmel EA, Verweij CL, Durand DB, Higgins KM, Lacy E, Grabtree GR (1989) Cyclosporin A specifically inhibits function of nuclear proteins involved in T cell activation. Science 246: 1617–1620
8. Gesser B, Deleuran B, Lund M, Vestergard C, Lohse N, Deleuran M, Jensen SL, Pedersen SS, Thestruppedersen K, Larsen CG (1995) Interleukin-8 induces its own production in CD4(+) T-lymphocytes – a process regulated by interleukin 10. Biochem Biophys Res Commun 210: 660–669
9. Inauen W, Payne D, Kvietys PR, Granger DN (1990) Hypoxia/reoxygenation increases the permeability of endothelial cell monolayers: role of oxygen radicals. Free Radic Biol Med 9: 219–223
10. Lateveer L, Lindley IJD, Heemskerk DPM, Camps JAJ, Pauwels EKJ, Willemze R, Fibbe WE (1996) Rapid mobilization of hematopoietic progenitor cells in rhesus monkeys by a single intravenous injection of interleukin-8. Blood 87: 781–788
11. McGill R, Tukey JW, Larsen WA (1978) Variation of box plots. Am Statistician 32: 12–16
12. Neuhaus P, Bechstein WO, Blumhardt G, Wiens M, Lemmens P, Langrehr JM, Lohmann R, Steffen R, Schlag H, Slama K-J, Lobeck H (1993) Comparison of quadruple immunosuppression after liver transplantation with ATG or IL-2 receptor antibody. Transplantation 55: 1320–1327
13. Neuhaus P, Blumhardt G, Bechstein WO, Steffen R, Platz K-P, Keck H (1994) Technique and results of biliary reconstruction using side-to-side choledochocholedochostomy in 300 orthotopic liver transplants. Ann Surg 21: 426–434
14. Neuhaus P, Blumhardt G, Bechstein WO, Platz K-P, Jonas S, Müller AR, Langrehr JM, Lohmann R, Schattenfroh N, Knoop M, Keck H, Lemmens P, Raakow R, Lüsebrink R, Slama K-J, Lobeck H, Hopf U (1995) Comparison of FK506- and cyclosporin-based immunosuppression in primary orthotopic liver transplantation. Transplantation 59: 31–40
15. Peveri P, Walz A, Dewald B, Baggiolini M (1988) A novel neutrophil-activating factor produced by human mononuclear phagocytes. J Exp Med 167: 1547–1559
16. Ploeg RJ, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD, Hoffmann RM, Sasaki T, Sollinger HW, Belzer FO, Kalayoglu M (1993) Risk factors for primary dysfunction after liver transplantation – a multivariant analysis. Transplantation 55: 807–813
17. Pruim J, Woerden WF van, Knol E, Klompmaker J, Bruijn KM de, Persijn GG, Slooff MJ (1989) Donor data in liver grafts with primary nonfunction – a preliminary analysis by the European Registry. Transplant Proc 21: 2383–2384
18. Ratych RE, Chuknyiska RS, Bulkley GB (1987) The primary localization of free radical generation after anoxia/reoxygenation in isolated endothelial cells. Surgery 102: 122–131
19. Sekido N, Mukaida N, Harada A, Nakanishi I, Watanabe Y, Matsushima K (1993) Prevention of lung injury in rabbits by a monoclonal antibody against interleukin-8. Nature 365: 654–657
20. Sica A, Matsushima K, Van Damme J, Wang JM, Polentarutti N, Dejana E, Colotta F, Mantovani A (1990) IL-1 transcriptionally activates the chemotactic factor/IL-8 gene in endothelial cells. Immunology 69: 548–553
21. Southard JH, Belzer FO (1993) The University of Wisconsin organ preservation solution: components, comparisons, and modifications. Transplant Rev 7: 176–190
22. Steinhoff G, Behrend M, Schrader B, Duijvestin AM, Wonigeit K (1993) Expression patterns of leucocyte adhesion ligand molecules on human liver endothelia. Lack of ELAM-1 and CD62 inductibility on sinusoidal endothelia and distinct distribution of VCAM-12, ICAM-1, ICAM-2, and LFA-3. Am J Pathol 142: 481–488
23. Strieter RM, Koch AE, Antony VB, Fick RB, Standiford TJ, Kunkel SL (1994) The immunopathology of chemotactic cytokines: the role of interleukin-8 and monocyte chemoattractant protein-1. J Lab Clin Med 123: 183–197
24. Suzuki S, Toledo-Pereyra LH (1994) Interleukin-1 and tumor necrosis factor production as the initial stimulants of liver ischemia and reperfusion injury. J Surg Res 57: 253–258
25. Thornton AJ, Strieter RM, Lindley I, Baggiolini M, Kunkel SL (1990) Cytokine-induced gene expression of a neutrophil chemotactic factor/IL-8 in human hepatocytes. J Immunol 144: 2609–2613
26. Tilg H, Ceska M, Vogel W, Herold M, Margreiter R, Huber C (1992) Interleukin-8 serum concentrations after liver transplantation. Transplantation 53: 800–803
27. Tsukada K, Takenoshita S, Nagamachi Y (1994) Peritoneal interleukin-6, interleukin-8 and granulocyte elastase activity after elective abdominal surgery. APMIS 102: 837–840
28. Villarete LH, Remick DG (1996) Transcriptional and post-transcriptional regulation of interleukin-8. Am J Pathol 149: 1685–1693
29. Yagihashi A, Zou XM, Hirata K, Asanuma K, Tsuruma T, Matsuno T, Yamaguchi H, Yamashiro K, Koide S, Torimoto K, et al (1995) Evaluation of serum IL-8 concentrations after orthotopic liver transplantation in rats. Transplant Proc 27: 1632–1633
30. Yokoyama I, Todo S, Miyata T, Selby R, Tzakis AG, Starzl TE (1989) Endo toxemia and liver transplantation. Transplant Proc 21: 3833–3841
31. Zweier JL, Kuppusamy P, Lutty GA (1988) Measurement of endothelial cell free radical generation: evidence for a central mechanism of free radical injury in postischemic tissue. Proc Natl Acad Sci USA 85: 4045–4050