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Local liberation of cytokines during liver preservation

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Abstract In order to investigate locally produced mediators during the process of organ storage in liver transplantation, we collected the liver preservation solution effluent of 15 transplanted livers and compared it with serum samples taken preoperatively from donor and recipient, as well as 60 min after reperfusion. The mean ischemia time ± SEM was 10 h 10 min ± 53 min. Mean concentrations in University of Wisconsin preservation solution effluent were: interleukin-(IL-)1β 154 ± 77 pg/ml; IL-1 receptor antagonist (IL-1 ra) 1281 ± 309 pg/ml; IL-6 412 ± 90 pg/ml; and for tumor necrosis factor-(TNF-)α 74 ± 21 pg/ml. Cytokine levels in the donors were lower than those detected in the effluent. All measured cytokines showed higher concentrations in the effluent compared to those of the recipient prior to the operation. With respect to a comparison of donor and recipient values, no correlation is evident. Likewise, the ischemic time does not correlate with effluent values. Further development of liver preservation concepts requires information about the state of the graft before reperfusion. Data on cytokine liberation may serve as a helpful tool for the further development of preservation concepts because they enable an estimation of cell activation during preservation.

Key words Liver transplantation · Preservation injury · Preservation effluent · Cytokines

Introduction

Liver transplantation (LTx) has become accepted therapeutic practice in the recent years. Two major facts contributed to this development: the introduction of new immunosuppressive drugs (e.g. Cyclosporin A, 1979), and the improvement of preservation solutions (University of Wisconsin solution, 1987).

However, organ dysfunction following transplantation is still associated with liver injury during preservation and reperfusion [19]. Up to one fifth of the patients [27] suffer from poor initial liver function due to severe hepatocyte damage, with concomittant borderline synthetic and metabolic activity. This situation leads either to primary nonfunction (PNF) described in approximately 6% of LTx [27, 28] or to slow recovery [7]. Some parameters have been suggested for the perioperative prognosis of the function of liver grafts [36]. During organ reperfusion, the first milliliters of preservation solution effluent to be rinsed out of the organ represent the extracellular milieu of the graft before reperfusion. In order to assess graft quality prior to transplantation, some authors have determined enzyme activity in the effluent of liver grafts [17, 30]. Shimada et al. have concluded than an analysis of the effluent could serve as a predictor of hepatic graft viability [34]. Rauen et al. [30] and Lange et al. [17] have confirmed these findings. The employed assays emphasized the injury of hepatocytes, although endothelial cells play a major role in preservation- and reperfusion injury during liver transplantation.

Numerous studies have proven that non-parenchymal cells are significantly more vulnerable to cold storage and reperfusion than hepatocytes, and that they represent the primary target for liver graft damage upon reperfusion [3, 21, 22]. The aim of our study was to investigate whether data on cytokine liberation in liver preservation effluent could serve as a marker for preexisting cell damage of the graft.

During the perioperative phase of LTx, cytokines may play a pivotal role in the regulatory processes. The liver is one of the major target organs for interleukins (IL) [1, 11, 37, 41]. Among others, tumor necrosis factor (TNF)-α and interleukin-6 (IL-6) interact with blood cells, and non-parenchymal liver cells such as Kupffer cells and sinusoidal endothelial cells [40]. Several cell types of the liver express cytokine genes: sinusoidal endothelial cells [31], vascular endothelial cells [10], Ito cells [20], Pit cells [26], hepatocytes [1], and hepatic fibroblasts [9], while Kupffer cells [13], originating in the monocyte-phagocyte-system, represent the major source of cytokines in the body. Pharmacokinetic data suggest that, in the presence of an adequate stimulus, the induction of TNF-α and IL-6 occurs within 30–60 min [2, 16, 19, 29]. One conceivable theory for the development of instant postreperfusional alteration might be the result of the cytokine interactions. These mediators might be responsible for inducing both immunological impairment and exaggerated responses.

Several research groups have currently tackled the problem of poor postoperative liver function by working on the further development of preservation concepts, a problem that is seen as multifactorial in origin [18]. In search of appropriate parameters for such studies, apart from biopsies, we evaluated cytokines by measuring the concentration of IL-1β, IL-1 ra, IL-6, TNF-α in the preservation effluent. The results suggest that IL-1 ra, IL-6 and TNF-α may serve as a marker for preexisting non-parenchymal cell damage of the graft before reperfusion.

Materials and methods

Liver allograft recipients

At our unit, 15 adult patients consecutively underwent orthotopic LTx. The age of the patients ranged from 20–62 years, 9 were male. Indications for LTx included chronic active hepatitis, alcoholic cirrhosis, fulminant hepatitis, cryptogenic cirrhosis, primary biliary cirrhosis, Budd-Chiary syndrome, hepatocellular carcinoma, autoimmune hepatitis and PNF. Patients were randomized [24] to treatment with cyclosporin A (CsA) or FK506-based immunosuppression prior to transplantation.

Liver transplantation and concomitant treatment

The surgical procedure was performed as reported elsewhere, including preservation with University of Wisconsin solution and the use of venovenous bypass in all cases [25]. Aprotinin administration, i. v. antibiotic treatment, selective bowel decontamination, and further prophylaxis were performed as previously described in [23].

Immunosuppressive protocol

Patients in the FK506-based and the CsA-based treatment groups were treated according to an immunosuppressive regimen described elsewhere [24]. This included methylprednisolone (PRED), azathioprine and antithymocyte globulin (ATG, Fresenius, Bad Homburg, Germany). PRED was administered by intravenous bolus injection at a dose of 500 mg, before and 6 h after reperfusion. PRED treatment was daily, commencing on post operative day 1 with 20 mg and reduced to 15 mg/day after 4 weeks.

Collection of samples

Sterile, heparinized arterial blood samples were obtained through a radial artery catheter. In rare cases, samples from the organ donor 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 directly prior to explantation in the donor, directly prior to implantation in the recipient, and 60 min after reperfusion. Effluent samples were obtained through a sterile syringe at the moment of reperfusion. To remove blood cells, samples were centrifuged at 4 °C and immediately frozen at – 80 °C.

Assays

Enzyme immunoassays were performed in microtitre strips (Nunc, Roskilde, DK) as previously described for IL-6 and TNF-α [15]. The following antibodies were used: IL-1 ra assay: polyclonal anti-IL-1 ra antibody (R + D Systems, Bad Nauheim, FRG). IL-1β assay: monoclonal anti-IL-1β antibody (Biogenesis, Bournemouth, GB). IL-6 assay: mouse monoclonal anti-human IL-6 antibody (Serva, Heidelberg, FRG). TNF-α assay: mouse monoclonal anti-human TNF-α IgG antibody (Saxon Biochemicals, former Bissendorf Biochemicals, Hannover, FRG). After staining with 3,3',5,5'-tetramethyl benzidine (TMB) substrate buffer, absorbance was measured using a plate reader (Dynatech, Denkendorf, FRG). The limits of detection were 20 pg/ml for TNF-α, 50 pg/ml for IL-1β, 20 pg/ml for IL-6, and 50 pg/ml for IL-1 ra.

Statistics

Data analysis was performed by ANOVA. The bars in figures 1 and 2 show the mean ± SEM. Significance was determined to the effluent values. Differences were considered as significant when P was less than 0.05.

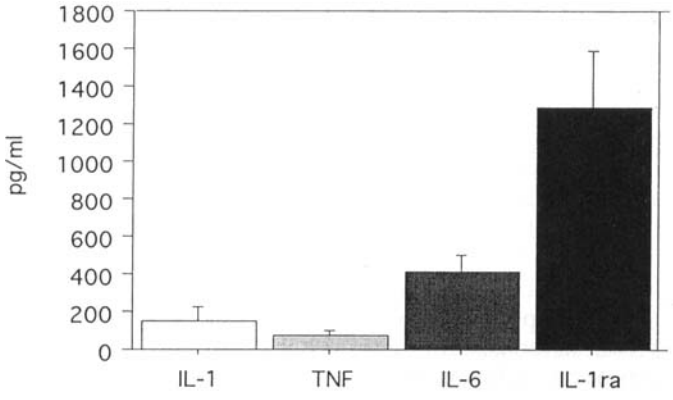


Fig. 1 Concentration of mediator levels in pg/ml in the preservation effluent

Results

In a study of 15 patients undergoing LTx, we measured selected systemic cytokines in the donors, in the preservation effluent, in the recipient before liver transplantation, and 60 min after reperfusion of the graft. We compared these values to preoperative and postoperative levels. The mean ischemia time ± SEM was 10 h 10 min ± 53 min. The results are summarized in figures 1 and 2. Levels of significance were calculated comparing the values of the preservation effluent. The results in the text are given as mean ± SEM, the results in the figures are given as mean ± SEM (see materials and methods).

Fig. 2 Time course of systemic cytokine levels in pg/ml from 15 patients during the perioperative phase of LTx. Significance was calculated by comparing values with those from the solution effluent ($^1 = P < 0{,}001$; $^2 = P < 0{,}01$; $^3 = P < 0{,}05$). The values are given in bar diagrams representing mean ± SEM (see Materials and methods). Time points of measurements were before explantation (*Donor*), immediately before LTx (*Recipient*), and 60 min after reperfusion

Figure 1 shows the concentration of mediators in the preservation effluent, the progression of systemic cytokine levels is shown in figure 2.

For IL-1β the mean concentration in the preservation effluent was 154 ± 77 pg/ml. 1281 ± 309 pg/ml was detected for IL-1 ra. The mean concentration of IL-6 was measured 412 ± 90 pg/ml. For TNF-α the mean concentration was 74 ± 21 pg/ml.

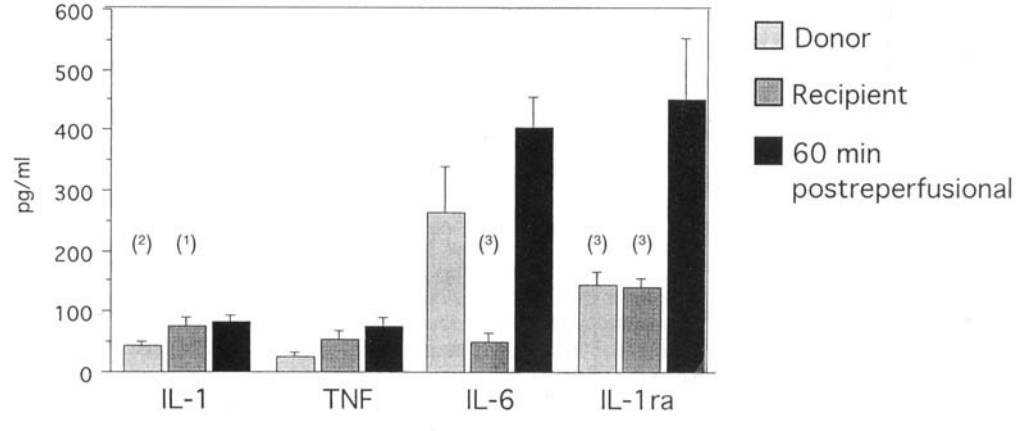
As indicated in figure 2, TNF-α levels did not differ significantly at all. Here the values for IL-1β in donor and recipient, and the IL-6 level in the recipient were significantly different from the levels in the preservation effluent. Additionally, the values for IL-1 ra in the donor and in the recipient were significantly different from the level in the preservation effluent.

We compared values of donors, recipients and effluents, and the corresponding ischemic time. No correlation between donor and recipient values became evident. Likewise, the ischemic time does not correlate with effluent values.

Discussion

Since the outcome of organ preservation is still a problem associated with poor initial liver function, many groups are working on modified liver preservation concepts. However, for clinical studies on liver preservation, serum parameters that react quickly to consequences of preservation damage are rare.

Originally, University of Wisconsin preservation solution was thought to work on a non-immunological basis [5]. However, immunological impact on the outcome of preservation is conceivable. The immunogenicity of the liver allograft is enhanced by preservation lesions [33]. These lesions attract inflammatory cells which could support acute rejection. Inflammatory cells locally release cytokines which enhance the expression of major histocompatibility complex (MHC) molecules [4, 14] and thus perpetuate the immunologic response. Ac-



cording to a further group [12], neither serum IL-1β nor TNF-α is raised systemically in hypoxic conditions, while production of both IL-1β and TNF-α is stimulated by following reoxygenation. Nevertheless, in the clinical situation, a rewarming injury has been inflicted additionally. Hypoxia induces enhanced binding of TNF-α to specific receptors on the endothelial cell surface in a time- and dose-dependent process. The authors think that enhanced TNF-α binding of endothelial cells during hypoxia is a secondary effect due to the perturbed integrity of the endothelial monolayer, followed by a biochemical conversion of the TNF receptor protein from the low to the high affinity state. As a consequence, the concentration of cytokines in body fluids is not the only determinating factor of the extent of biological activity.

Whether systemic cytokines can serve as markers for acute graft rejection is still in debate. Various efforts were made to show a relationship between changes of cytokine levels and postoperative rejections like rejection or infection [13]. Nevertheless, specific response patterns are not yet broadly accepted as valid predictors [6]. Dallman [8] claims that certain cytokines are formed as a result of the inflammatory process associated with surgery (e.g. explantation) whereas others, like IL-2 and IL-4, indicate a real activation of the immune system. Local cytokine formation and secretion during preservation may be an important feature of inflammatory processes during reperfusion. Investigation of the first milliliters effluent of the preservation solution gives information about local cytokine generation within the organ during storage. For testing new preservation solutions one needs to monitor graft function multimodally. Besides taking biopsies and measuring transaminases or detecting synthetic or metabolic function, the immunological status might be observed with the help of certain cytokines. However, since blood and effluent are different media that differ additionally in their volumes, the interpretation should be cautious.

As shown above, IL-1β and IL-1 ra appear to be sensitive parameters for the status of the graft before reperfusion. Rokita et al. [32] conclude that IL-1, an early mediator of inflammation, stimulates the IL-1 ra and IL-6 genes. Ulich et al. [38] have shown that IL-1 mRNA expression was followed by IL-1 ra mRNA expression, consistent with the hypothesis that IL-1 ra acts as an endogenous negative feedback mechanism to downregulate the proinflammatory effects of IL-1. Vidal-Vanaclocha et al. [39] blocked IL-1 receptor by use of IL-1 ra. Shito et al. [35] demonstrated that IL-1 secretion was very much higher after reperfusion in the rat.

TNF-α and IL-6, on the contrary, showed no significant alterations in the preservation effluent. Thus, investigation of liver preservation effluent could be an attractive and easily accessible method to obtain data about the status of the grafted organ before reperfusion.

For testing new preservation concepts one needs to monitor graft function multimodally. Our study indicates that the immunological status before reperfusion might be observed with the help of certain cytokines. Further studies have to be carried out to decide whether the determination of locally produced cytokines completes the methods of detecting transaminases and measuring the synthetic or metabolic function after reperfusion.

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