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Acute-phase response patterns in isolated hepatic perfusion with tumour necrosis factor α (TNF-α) and melphalan in patients with colorectal liver metastases

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Abstract

Background In this study, we have evaluated hepatotoxicity, secondary cytokine production and hepatic acute-phase response (APR) in patients who underwent isolated hepatic perfusion (IHP) with tumour necrosis factor (TNF) α and melphalan for irresectable colorectal liver metastases.

Design An extracorporeal veno-venous bypass was used to shunt blood from the lower body and intestines to the heart. Inflow catheters were placed in the hepatic artery and portal vein, and an outflow catheter in the inferior caval vein. The liver was perfused for 60 min with 0·4 mg of TNF-α plus 1 mg kg^{-1} melphalan (IHP_{TM} group, $n = 6$) or 1 mg kg^{-1} melphalan (IHP_M group, $n = 3$). The liver was washed with macrodex before restoring vascular continuity.

Results After the washout procedure, a TNF-α peak (169 ± 38 pg mL^{-1}) was demonstrated in the IHP_{TM} group only. Both groups demonstrated peak levels of interleukin 6 (IL-6) in the perfusate as well as systemically. These were significantly higher in the IHP_{TM} group. Acute-phase protein (APP) levels followed a similar pattern as has been demonstrated after major surgery, with no significant differences between both groups. The addition of TNF-α to the perfusate did not lead to a significant difference in APP levels and the time course between groups.

Conclusions IHP with TNF and melphalan is followed by a transient systemic peak of TNF directly after liver washout. Secondary IL-6 induction was seen in the present study after IHP with and without TNF, which was highest when TNF was added. This phenomenon cannot be extrapolated to APP induction, which appeared unaffected by the addition of TNF, presumably because the surgical procedure itself already causes maximal stimulation of APP production.

Keywords Acute-phase proteins, isolated hepatic perfusion, interleukin 6, tumour necrosis factor α.

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Introduction

The acute-phase response (APR) is a general systemic reaction induced by injury or various kinds of inflammatory states such as burns, infection or surgical procedures. As part of this reaction, the liver responds to several mediators by the increased synthesis of a series of glycoproteins called acute-phase proteins (APPs). The function of the APPs is pleiotropic: they may both mediate and inhibit inflammation, scavenge free oxygen radicals, act as transport proteins for products of the inflammatory process, or have an active role in tissue repair and remodelling.

Both tumour necrosis factor α (TNF-α) and interleukin 6 (IL-6) have been shown to induce the APP synthesis of the liver, of which the latter seems to be the major inducer

and mediator of this APP production [1–4]. TNF-α derives its name from the observation that it can cause haemorrhagic necrosis of tumours. However, TNF-α is also known to be an important mediator in shock. Systemic administration of small amounts of TNF-α leads to influenza-like symptoms; at higher dose levels septic shock syndrome develops, characterized by pulmonary oedema, adult respiratory distress syndrome and severe inflammatory response syndrome. For this reason, many phase I and II trials studying the systemic administration of TNF-α have failed to reproduce the experimental successes because the severe, systemic toxic side-effects of TNF-α limited the usable dose of TNF-α to a level at which no effective anti-tumour activity could be seen [5–7].

It has been shown recently that this concentration gap can be overcome by the application of cytostatic drugs in isolated perfusion systems. These allow safe increase in doses to $> 20\times$ the systemic maximum tolerated dose (MTD) [8]. This also holds true for the application of TNF-α in such systems. It has become firmly established that TNF-α can be applied safely and successfully in isolated limb perfusion (ILP) both in melanoma patients [9] and in patients with irresectable soft tissue sarcomas [10,11]. Furthermore, this remarkable anti-tumour effectivity of TNF-α in combination with melphalan, proved to be effective against a wide range of histologies [5].

The results of the ILP studies led to the development of isolated perfusion of other organs, such as isolated hepatic perfusion (IHP) [12,13]. With this technique, the liver circulation is completely separated from the systemic circulation, thus allowing high drug levels within the liver while keeping systemic exposure low. However, unlike limbs, the liver is a metabolically active organ that contains a large amount of tissue macrophages, the Kupffer cells. As Kupffer cells are known to release various cytokines in response to TNF-α, it could therefore be speculated that IHP with TNF-α might induce considerable (hepato)toxicity. Therefore, we were interested in the effects of IHP with TNF-α on hepatic function, secondary cytokine production and hepatic APR. To obtain further insight, we evaluated patients who underwent IHP with TNF-α and melphalan for colorectal metastases confined to the liver. The APR was evaluated regarding the levels and time dependency of TNF-α, IL-6, the APP C-reactive protein (CRP), α_1-acidglycoprotein, α_1-antitrypsin and transferrin.

Methods

IHP patient groups

From January to June 1995, nine patients underwent IHP for irresectable hepatic colorectal metastases. All gave informed consent before treatment in protocols approved by the hospitals' ethics committees. The study was carried out in accordance with the principles of the Declaration of Helsinki, as revised in Hong Kong in 1989. There were six men and three women with a mean age of 59·8 years (range 49–65 years). Inclusion criteria for IHP with TNF-α and melphalan included histological evidence of irresectable metastases of colorectal origin confined to the liver, Karnovsky performance status of $> 80\%$. Exclusion criteria included extrahepatic malignant disease, $> 50\%$ hepatic tissue replacement by tumour, liver cirrhosis, signs of significant hepatic dysfunction (abnormal levels of ASAT, ALAT or alkaline phosphatase $> 2\times$ norm) and ascites or portal hypertension.

ILP technique

The procedure of IHP has been described elsewhere [12]. Briefly, the vasculature of the liver was dissected free and isolated. After systemic heparinization ($200\,\mathrm{U\,kg^{-1}}$), an extracorporeal veno-venous bypass (VVB) circuit (aided by a passive centrifugal pump) was created to shunt mesenteric, renal and lower extremity blood around the liver to the heart. Next, inflow catheters were placed in the portal vein and hepatic artery, and an outflow catheter was placed in the infrahepatic inferior caval vein. These catheters were connected to a heart–lung machine, and the vascular isolation was completed by clamping the suprahepatic inferior caval vein and the suprarenal inferior caval vein. The liver was then perfused with a hyperthermic ($> 38\,^{\circ}\mathrm{C}$) perfusate consisting of a mixture of saline and erythrocytes. Once a stable perfusion was attained, leakage from the perfusion circuit into the systemic circulation was measured by the addition of $200\,\mu\mathrm{Ci}$ [^{131}I]-albumin into the perfusate and the continuous monitoring of radioactivity scintillation probes placed over the perfusate reservoir and the VVB. The leak rate was monitored for the duration of the perfusion and if the cumulative leak was greater than 15%, the perfusion would be halted and the perfusate flushed from the circuit. After the absence of leakage was confirmed, drugs (see treatment schedule) were administered as a bolus into the arterial line of the perfusion circuit. After a 60-min perfusion, the liver was washed thoroughly with a mixture of saline and macrodex, decannulated, and vascular continuity restored. Heparin was reversed with $1\,\mathrm{mg\,kg^{-1}}$ protamine sulphate (Novo-Nordisk, Rud, Norway) injection. Post-operatively, the patients were monitored at the intensive care unit for at least 48 h, primarily to evaluate evidence of systemic toxicity due to rhTNF-α.

Drugs

Recombinant human TNF-α (0·2 mg per ampoule) was a kind gift from Boehringer Ingelheim, Ingelheim am Rhein, Germany. The cytostatic drug melphalan (Alkeran) was obtained as a sterile powder (100 mg), which was dissolved aseptically using solvent and diluent by Burroughs Wellcome, London, UK.

Treatment schedule

In six patients ($\mathrm{IHP_{TM}}$ group), 0·4 mg of TNF-α was

administered as a bolus; melphalan ($1\,mg\,kg^{-1}$) was given directly after the TNF-α bolus. In three patients (IHP_M group) only melphalan ($1\,mg\,kg^{-1}$) was administered.

Sampling schedule

Blood samples were collected from a peripheral vein in siliconized 5-mL Vacutainer tubes (Becton Dickinson, Plymouth, UK) containing EDTA ($10\,nmol\,L^{-1}$) and soybean trypsin inhibitor ($100\,mg\,L^{-1}$) and benzamidine ($10\,nmol\,L^{-1}$) (Sigma Chemicals, Detroit, MI, USA). Samples were centrifuged immediately after collection, at 5000 rpm. for 5 min. Supernatants were stored at $-70\,°C$ until analysis. Perfusate was sampled at $t=0$ (i.e. upon drug administration), 10, 20, 30, 40, 50 and 60 min. Systemic plasma samples were collected the day before IHP, during IHP at $t=0$, 30 and 60 min and post perfusion (after release of the inferior caval vein clamp) at $t=1$, 5, 10, 20, 30, 60, 120 and 240 min, days 1, 3 and 7, and weekly thereafter, until 3 weeks post-operatively.

Assays

TNF-α levels were measured by a sandwich-type enzyme-linked immunosorbent assay (ELISA) using two monoclonal antibodies (Department of Immune Reagents, Central Laboratory of Blood Transfusion, Amsterdam, The Netherlands) raised against recombinant human TNF-α (courtesy of D. A. Creasey, Chiron, Emeryville, CA, USA). One MAb (MAb CLB-TNF-α-7) was used for coating at a concentration of $2\,\mu g\,mL^{-1}$. The other MAb (MAb CLB-TNF-α-5) was biotinylated and used in combination with streptavidin–poly-horseradish peroxidase conjugate (CLB, Department of Immune Reagent) to detect bound TNF-α. Stimulated human mononuclear cell supernatant was used as a standard for comparison with purified recombinant human TNF-α. Results were expressed as $pg\,mL^{-1}$ by reference to this standard [14]. The detection limit of this assay was $10\,pg\,mL^{-1}$.

IL-6 was measured by an ELISA modified from that described in detail before [15]. Briefly, purified monoclonal anti-human IL-6 antibody (MAb CLB-IL-6-16) was used as a capture antibody, and biotinylated sheep antibodies in combination with streptavidin–poly-horseradish peroxidase conjugate were used to detect bound IL-6. Results were expressed as $pg\,mL^{-1}$ by reference to a standard consisting of recombinant human IL-6. Normal healthy control subjects' values were $> 10\,pg\,mL^{-1}$, and the limit of detection was $5\,pg\,mL^{-1}$.

Acute-phase proteins

CRP, α_1-antitrypsin (α_1-AT), α_1-acidglycoprotein (α_1-AG) and transferrin (TRF) levels were measured by means of a nephelometric assay [16]. The antisera used were obtained from the Central Laboratory of the Blood Transfusion Service (CLB), Amsterdam, The Netherlands. Normal values (obtained from 100 blood donors) were CRP $<5\,mg\,L^{-1}$, α_1-AT $1{\cdot}4$–$3{\cdot}2\,g\,L^{-1}$, α_1-AG $0{\cdot}4$–$1{\cdot}3\,g\,L^{-1}$ and TRF $2{\cdot}3$–$4{\cdot}3\,g\,L^{-1}$.

Statistics

Results are expressed as means ± standard error of the mean (SEM). Comparison within groups were made by means of the Friedman non-parametric repeated measures test or by the Mann–Whitney test when appropriate. Correlations between maximum levels of parameters were calculated as Spearmann's rank correlations. A two-sided P-value $< 0{\cdot}05$ was regarded as significant.

Results

Operative procedure

The median duration of the operation was 8 h (range 6–10 h). In all patients, a stable perfusion was attained. In one patient, progressive systemic leakage resulted in discontinuation of the IHP after 43 min (cumulative leakage 20%). In all other patients, no leakage was demonstrated. Three patients in the IHP_{TM} group died in the immediative post-operative period as a result of surgical complications of the operation. As the purpose of this study was to describe APR in uncomplicated IHP, these patients were excluded.

Survival

In the six evaluable patients, the survival time ranged from 6 to 26 months. The median survival time was 10·3 months (mean 13·3 months).

Tumour response

The primary efficacy end points in the study were best tumour response observed [WHO response criteria: complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD)] and duration of the best response, calculated from the date the best response was observed until the date of progression. Tru-cut hepatic tissue samples and/or computerized tomography (CT) scan evaluations were used to describe the best tumour response. In the IHP_{TM} group five out of six patients demonstrated CR or PR, and one patient demonstrated SD. Patients treated with melphalan alone demonstrated PR or SD. The duration of best response ranged from 17·5 to 32·5 weeks (median 18 weeks).

Toxicity

Toxicity and adverse events were assessed and recorded

according to the WHO grading system (WHO Adverse Event Coding Thesaurus) [17].

General toxicity

In the IHP_{TM} group, all patients developed slight fever, hypotension, pulmonary toxicity and sinus tachycardia, which was reported as drug related. All patients demonstrated anaemia and thrombocytopenia (nadirs at day 3), returning to normal after 10 days post IHP ($P > 0{\cdot}05$ between groups).

Hepatotoxicity

All patients demonstrated significant initial elevations in liver enzyme levels, normalizing within the first 2 postoperative weeks ($P > 0{\cdot}05$ between groups). In contrast, bilirubin and alkaline phosphatase levels increased more slowly after IHP but remained elevated for a longer period. Overall, two (IHP_{TM} group) patients demonstrated grade III and three (IHP_{TM} group) patients grade IV according to the WHO classification (Fig. 1).

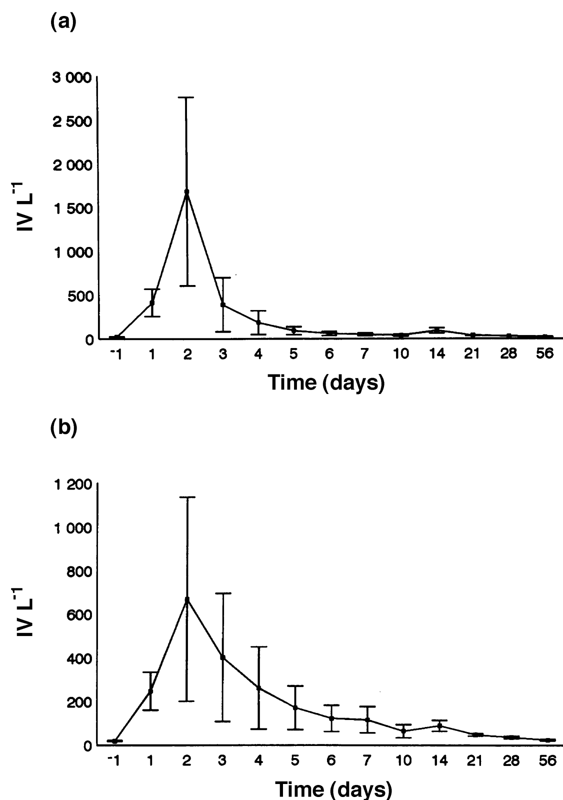

Figure 1 Time course of liver enzyme levels ASAT (a) and ALAT (b) as a function of time following IHP. Time is expressed in days on the x-axis, where -1 represents the day before the operation. Because liver enzyme levels were virtually equal in both groups, the IHP_M group levels are not shown for reasons of clarity.

Cytokine levels

TNF-α levels

In the perfusate, TNF-α levels in the IHP_{TM} group were $1{\cdot}8 \pm 0{\cdot}5\,\text{pg mL}^{-1}$ at $t = 0$ min and increased rapidly to $6{\cdot}7 \pm 0{\cdot}9 \times 10^4\,\text{pg mL}^{-1}$ at $t = 10$ min. Thereafter, TNF-α levels decreased to $3{\cdot}1 \pm 0{\cdot}3 \times 10^4\,\text{pg mL}^{-1}$ at the end of IHP ($t = 60$ min). In the IHP_M group TNF-α levels did not demonstrate any significant changes. (Fig. 2).

Baseline serum TNF-α levels were similar in both groups, although these levels were higher than normal ($4{\cdot}3 \pm 0{\cdot}5\,\text{pg mL}^{-1}$ and $4{\cdot}5 \pm 1\,\text{pg mL}^{-1}$ in the IHP_{TM} and IHP_M group respectively). During IHP, TNF-α levels did not change significantly, indicating that vascular isolation was effective. However, after washout, TNF-α levels increased rapidly to a peak value of $169 \pm 38\,\text{pg mL}^{-1}$ at $t = 1$ min in the IHP_{TM} group and normalized within the next 3 h. In the IHP_M group, TNF-α levels demonstrated a slight increase to $7{\cdot}2 \pm 5\,\text{pg mL}^{-1}$ within the first 30 min

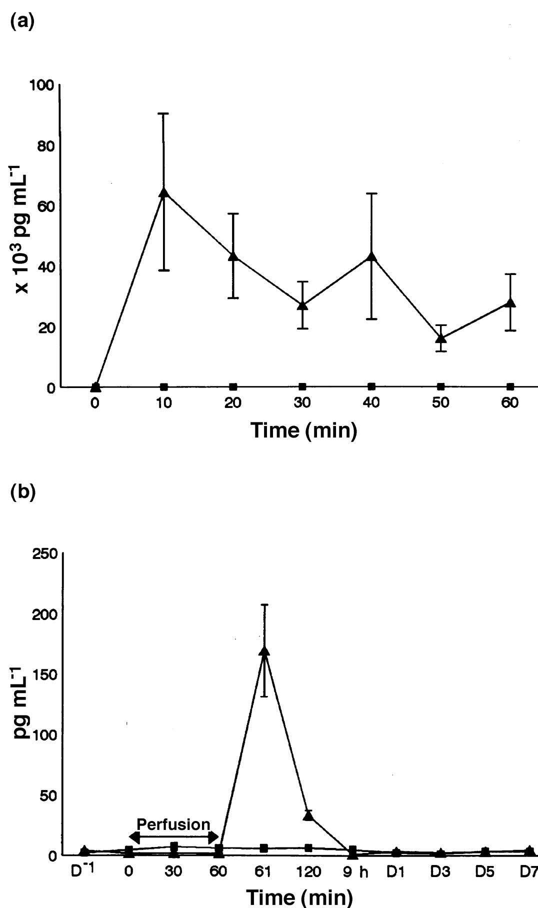

Figure 2 Time course of TNF-α levels before, during and after IHP. (a) Perfusate levels. (b) Systemic levels. D−1, the day before IHP; 0, 30, 60, 61, 120, 0, 30, 60, 61, 120 min after start of IHP (61 min represents the time just after vascular restoration, not shown in the following panels); 9 h, 9 h, after IHP; D1, D3, D5, D7, first, third, fifth and seventh days after IHP. ▲–▲, Patients receiving TNF and melphalan; ■–■, patients receiving melphalan alone.

after IHP. However, this elevation of TNF-α levels in the IHP_M group was not statistically significant (Fig. 2).

Interleukin-6 levels

In the perfusate, baseline IL-6 levels were elevated in both the IHP_{TM} and IHP_M groups ($91 \pm 45\, pg\, mL^{-1}$ and $94 \pm 58\, pg\, mL^{-1}$ respectively). During IHP, IL-6 levels increased over time to $2{\cdot}1 \pm 0{\cdot}7 \times 10^3\, pg\, mL^{-1}$ in the IHP_{TM} group ($P < 0{\cdot}01$) and to $227 \pm 128\, pg\, mL^{-1}$ ($P > 0{\cdot}05$) in the IHP_M group ($P < 0{\cdot}01$ between groups, Fig. 3).

Serum IL-6 levels at the start of the IHP procedure were elevated in both groups and rose slightly during IHP ($P < 0{\cdot}05$ between groups). However, after the washout procedure, IL-6 levels increased significantly, reaching the highest levels, 1 h after washout, in the IHP_{TM} group ($P < 0{\cdot}01$ between groups). Maximum IL-6 levels were $9{\cdot}8 \pm 0{\cdot}8 \times 10^3\, pg\, mL^{-1}$ and $2{\cdot}4 \pm 1 \times 10^3\, pg\, mL^{-1}$ in the IHP_{TM} and IHP_M group respectively. Thereafter, levels dropped and returned to preoperative levels within the first post-operative day (Fig. 3).

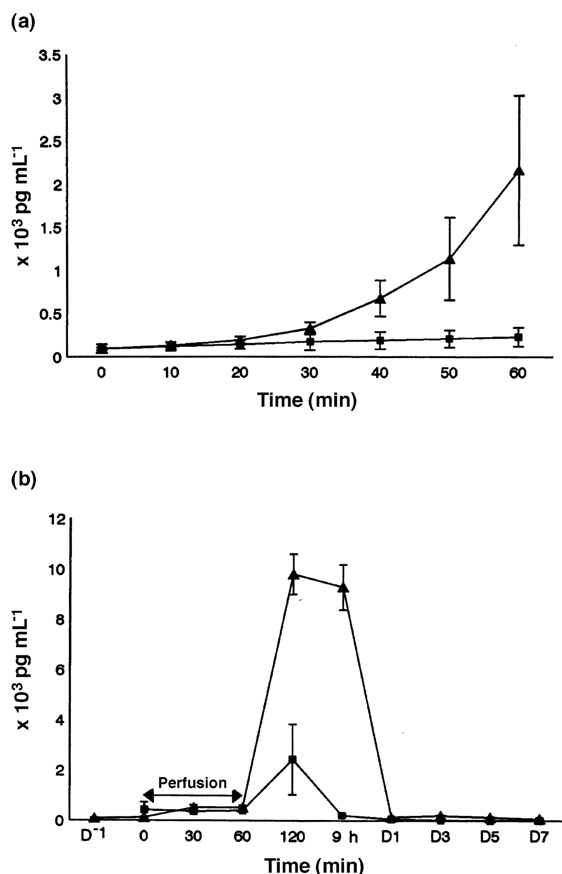

Figure 3 Time course of IL-6 levels before, during and after IHP. (a) Perfusate levels. (b) Systemic levels. D–1, the day before IHP; 0, 30, 60, 120, 0, 30, 60, 120 min after start of IHP; 9 h, 9 h, after IHP; D1, D3, D5, D7, first, third, fifth and seventh days after IHP. ▲–▲, Patients receiving TNF and melphalan; ■–■, patients receiving melphalan alone.

Acute-phase protein response

C-reactive protein levels started to rise 9 h after washout, and maximum levels ($143 \pm 3\, mg\, L^{-1}$ and $129 \pm 4\, mg\, L^{-1}$ in the IHP_{TM} IHP_M group respectively) were reached at the fifth post-operative day. Thereafter, levels dropped and normalized slowly within the next 2 weeks ($P > 0{\cdot}05$ between groups, Fig. 4).

From the start of the IHP procedure α_1-antitrypsin and α_1-acidglycoprotein levels first decreased in both groups. At 60 min after washout, levels increased gradually ($P < 0{\cdot}01$), and they were still elevated at day 10 ($P < 0{\cdot}05$ between groups, Fig. 4).

The 'negative' APP transferrin levels in both groups decreased until 30–60 min after washout, whereafter levels increased to normal values within the first post-operative days ($P < 0{\cdot}05$ between groups, Fig. 4).

Discussion

In this study, we describe the effects of IHP with TNF-α and melphalan, or melphalan alone, on secondary cytokine and APP production. After the washout procedure, IHP was followed by a TNF-α peak in the IHP_{TM} group only, normalizing within 2 h. TNF-α levels in the melphalan alone group remained virtually unchanged. In both groups, peak levels of IL-6 were observed 1 h after the washout, although levels were significantly higher in those patients with TNF-α added to the perfusate. IL-6 levels normalized at day 1 post-operatively. The APP production followed a pattern similar to that demonstrated after various kinds of surgery, including major hepatic surgery, with no differences between both groups.

Despite its promising *in vitro* anti-tumour effects, the *in vivo* administration of TNF-α has been demonstrated to be accompanied with dose-limiting systemic toxicity at dose levels at which no amti-tumour effect could be seen. Furthermore, these phase I and II studies demonstrated specific organ toxicity with increasing dosages of TNF-α [6,7]. We were particularly interested in the potential hepatotoxicity caused by IHP with TNF-α and melphalan. All our patients displayed moderate transient hepatotoxicity as demonstrated by transient elevated hepatic enzymes (Fig. 1). Since similar elevations were also observed in the IHP_M group, we believe that the addition of TNF-α did not lead to additional hepatotoxicity. These findings are in accordance with the results of our experimental IHP program in pigs [12]. Therefore, more likely, we think that the hepatotoxicity encountered in our patients is the result, at least in part, of the IHP procedure itself.

To avoid systemic exposure to chemotherapeutic agents or cytokines, the main goal of the isolation perfusion technique is the complete vascular isolation of the limb or

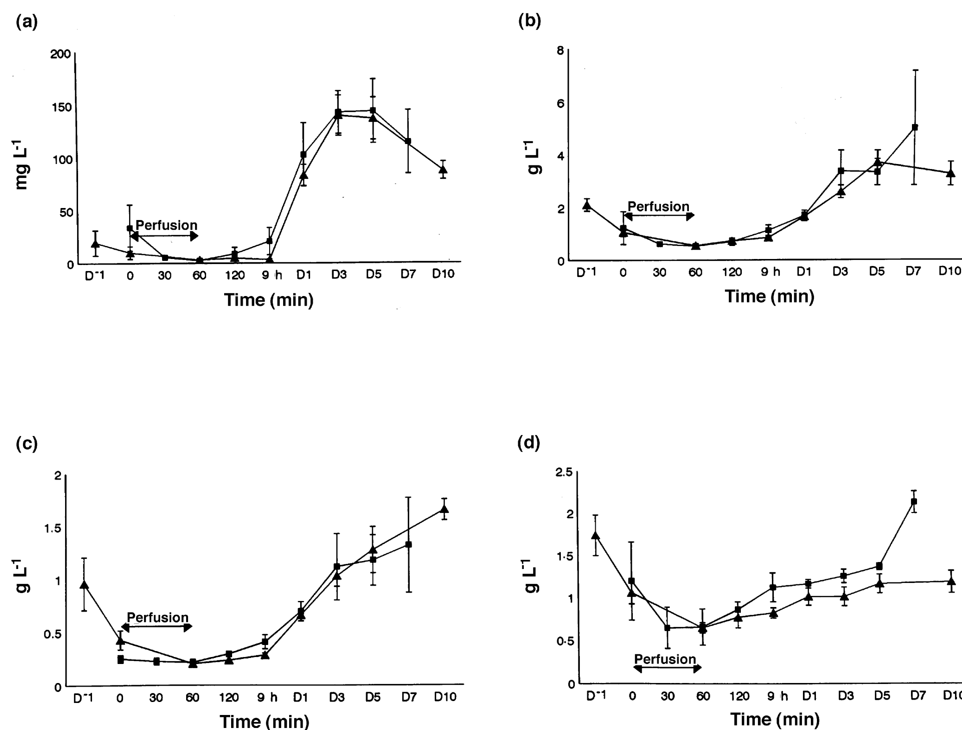

Figure 4 Time course of C-reactive protein (a), α_1-antitrypsin (b), α_1-acidglycoprotein (c) and transferrin (d) levels before, during and after IHP. Time is expressed on the *x*-axis as follows: D–1, the day before IHP; 0, 30, 60, 120, 0, 30, 60, 120 min after start of IHP; 9 h, 9 h, after IHP; D1, D3, D5, D7, first, third, fifth and seventh days after IHP. ▲–▲, Patients receiving TNF and melphalan; ■–■, patients receiving melphalan alone.

organ. In our study, vascular isolation of the liver was complete in all patients but one. In this patient, progressive systemic leakage (cumulative leakage 20%) led to premature termination of the IHP procedure after 43 min. However, despite this leakage, this patient did not demonstrate additional toxicity as demonstrated by clinical and biochemical parameters compared with the other patients studied. The same observation has been demonstrated by Pinsky and co-workers [18], who showed that the outcome of patients with septic shock did not correlate with peak levels but with the persistence of both TNF-α and IL-6 levels. In our eight patients without leakage, systemic TNF-α levels did not change significantly during IHP, indicating that vascular isolation was complete. After the washout procedure, however, systemic TNF-α levels in the IHP_{TM} group peaked rapidly and normalized within the next 2–3 h (Fig. 2). A possible explanation for this TNF-α peak in the IHP_{TM} group could be the release of remnant TNF-α in the liver after the washout procedure, a phenomenon also described in ILP with TNF-α and melphalan [16,19,20]. However, peak TNF-α levels in our study were much lower than those levels in ILP, indicating a more efficient washout procedure [19,21]. Furthermore, endogenous TNF-α production may have attributed to this peak, because surgery, extracorporeal circulation circuits and intravascular plastic catheters are known to induce TNF-α [22,23]. However, the last explanation may be of less importance because only mild elevation of systemic TNF-α levels could be demonstrated in the IHP_M group (Fig. 2).

TNF-α is also known as a proinflammatory cytokine able to stimulate the production of several interleukins such as IL-6. In this study, perfusate IL-6 levels increased significantly in all patients during IHP, with the highest levels in the IHP_{TM} group (Fig. 3). The same pattern has been demonstrated in the perfusate of ILP circuits in patients with melanoma stage III/IV or irresectable sarcoma of the limbs, although perfusate IL-6 levels in our patients (IHP_{TM} group) were much higher at a lower TNF-α dose [20]. Macrophages are known to produce various cytokines, e.g. IL-6, in response to TNF-α. As the liver contains the largest amount of fixed tissue macrophages, Kupffer cells, this induction of IL-6 production by the Kupffer cells could be an explanation not only for the difference in IL-6 levels between our study groups, but also between IHP and ILP. Furthermore, all patients demonstrated elevated systemic IL-6 levels at the start of the actual perfusion which further increased with perfusing time. Several studies have demonstrated increased IL-6 levels after various surgical procedures, including hepatic surgery [24–29]. Cruickshank *et al.* [29] described elevated IL-6 levels in patients undergoing elective surgery of varying severity. Levels of IL-6 were shown to increase with the extent of surgery. Several other studies confirmed this finding but peak IL-6 levels were usually less than 500 pg mL^{-1} [24–29]. In this context, it should be borne in mind that, at

the beginning of the perfusion, patients already had undergone a major surgical procedure, with subsequent increased IL-6 concentrations resembling levels described in other surgical procedures (Fig. 3, $t = 0$ min). The slow initial increase during IHP was followed by a further steep increase after IHP to a peak at 1 h after the washout procedure (Fig. 3). Although this peak occurred in both groups, it was significantly higher in those patients perfused with TNF-α and melphalan. Since both groups underwent the same surgical procedure with melphalan, the significant difference between groups can only be explained by the addition of TNF-α to the perfusate of the IHP_{TM} group. The same observation was reported by Thom *et al*. [20]. In their study serum IL-6 levels in patients with ILP with TNF-α, interferon-α and melphalan were significantly higher than ILP with melphalan alone, with a trend towards higher IL-6 levels with increased exposure to TNF-α. Taking into account the aforementioned Kupffer cell response to TNF-α, both the TNF-α peak after washout in the IHP_{TM} group and the hepatic IL-6 production could be an explanation for the difference in systemic peak IL-6 levels.

An important function of the liver is the production of so-called APPs in response to various stimuli. In all our patients a clear APR could be observed. Levels of all proteins first decreased, probably caused by haemodilution in combination with extravasation after the washout procedure. Thereafter the negative APPs normalized, whereas the positive APPs started to increase and remained elevated for at least 2 weeks with the exception of CRP, which normalized within this period (Fig. 4). Furthermore, there were no significant differences in APP levels between both groups. Several *in vitro* and *in vivo* studies demonstrated that IL-6 is secreted in the early stages of the APR and is the main mediator of the hepatic production of APPs such as α_1-AG, α_1-AT and CRP [2,4]. To emphasize the central role of IL-6, *in vivo* studies have revealed clear correlations between IL-6 and several APP levels [3,30–33]. Since in our study significant differences in systemic IL-6 levels were demonstrated, we speculated that this might lead to a different APP production profile between groups. In contrast, in our study, there were no significant differences in APP profiles nor peak levels between groups. Moreover, we could not demonstrate a significant correlation between peak IL-6 and CRP levels. Instead, the APP pattern, including peak levels, as described here is similar to patterns demonstrated after elective surgery of various extent, including ILP [16,31,32]. A possible explanation for the identical profile in both groups could be that the IHP, as a major surgical procedure (and identical in both groups), already caused a maximal induction of the APPs by the liver. Consequently, the addition of TNF-α to the perfusate, with subsequent short-lived systemic TNF-α peak and resulting IL-6 peak could not further influence the APR. Thus, the conditions under which TNF-α induced IL-6 production occurs may be essential to the extent to which the hepatic APR is induced [34].

In conclusion, IHP with TNF and melphalan is followed by a transient systemic peak of TNF directly after liver washout. Secondary IL-6 induction was seen in the present study after IHP with and without TNF, which was highest when TNF was added. This phenomenon cannot be extrapolated to APP induction, which appeared unaffected by the addition of TNF, presumably because the surgical procedure itself already causes maximal stimulation of APP production.

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