Systemic side effects of isolated limb perfusion with tumor necrosis factor alpha
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Chapter VII

Summary and concluding remarks

The main function of tumor necrosis factor alpha (TNF-α), a small polypeptide shared by all mammals, is probably protection against invading bacteria, parasites and viruses; killing of these microorganisms is facilitated in the presence of TNF-α. However, as its name suggests, TNF-α is also capable of killing tumor cells, in vitro as well as in vivo. This unique capacity has focused the attention on a possible role for this cytokine in the treatment of human cancer. It soon became apparent that its usefulness as such is seriously limited by its toxicity. In sensitive laboratory animals administration of even a very low dose of TNF-α will induce a sepsis like state, with hypotension, respiratory failure, hepatic necrosis and renal insufficiency. Higher doses may be fatal. Human clinical trials with TNF-α have confirmed its high toxicity, without showing any benefits in terms of tumor remissions.

Isolated limb perfusion was first described by Creech and Kremetz in 1968. It is based on the assumption that the circulation of a limb can be isolated completely from the rest of the circulation, while the tissues of the isolated limb are kept viable with a perfusate, that is oxygenated by a bubble oxygenator. With a system like this, it would be possible to administer high concentrations of antineoplastic drugs to tumors of a limb, while sparing the rest of the body the detrimental effects of chemotherapy. Moreover, the temperature of the perfused limb could be maintained between 39 and 40°C, which was expected to increase the antitumor effect of most antineoplastic agents. Many perfusions have been performed since, mainly with alkylating agents like melphalan, with variable results.

Lejeune and coworkers were the first to try recombinant TNF-α (r-TNF-α) in such an isolated perfusion system. They hoped to achieve sufficiently high concentrations of r-TNF-α in the tumor, with a systemic toxicity that would remain manageable. In their experiments, r-TNF-α was combined with melphalan, and with recombinant interferon gamma (r-IFN-γ), a cytokine that enhances the sensitivity of tumor cells to TNF-α by increasing the number of TNF-receptors on the cell surface. An impressive remission rate was achieved, especially in patients with locally irresectable sarcoma of a limb: 36% complete remissions and 51% partial remissions. In melanoma patients, the results also look promising, but formal testing of the efficacy of isolated perfusion with r-TNF-α for melanoma has not been completed. Systemic toxicity was manageable, but all patients required treatment in an intensive care unit for symptoms of sepsis.

The studies presented in this thesis exclusively deal with systemic toxicity; they do not report on remission- or survival rates. They were primarily undertaken to understand why patients treated regionally with TNF-α developed such serious systemic complications. The effects of TNF-perfusion on different organ systems are described in some detail. A better understanding of the toxicity of isolated limb perfusion with r-TNF-α might contribute to a better management of patients treated in this fashion. Because of the high remission rates that have been reported, their numbers can be expected to increase.

Apart from being a promising technique to treat some types of cancer, isolated limb perfusion with r-TNF-α presents an interesting model of sepsis and septic shock. In bacterial and fungal sepsis TNF-α is universally considered to be one of the prime mediators of shock and organ dysfunction. Cell wall products of these microorganisms, like lipopolysaccharide and peptidoglycan, stimulate macrophages
to produce excessive amounts of TNF-α. TNF-α directly interferes with cellular function but also sets of a cascade of inflammatory mediators that greatly enhance its deleterious effects. Clinical research of sepsis has traditionally been hampered by late diagnosis: once the syndrome is diagnosed the inflammatory cascade has fully developed and a plethora of damaging factors are at play. This makes it difficult to determine the role of any factor in particular. In isolated perfusion with r-TNF-α we have been able to show a considerable leak of r-TNF-α from the perfusion circuit to the general circulation. In fact, this type of treatment amounts to a 90 min. intravenous infusion of high doses of r-TNF-α, with a peak at the time when normal circulation is restored. Concentrations of systemic TNF-α are many times higher in these patients than those found in healthy volunteers after administration of what was considered to be acceptable doses of r-TNF-α. The pathology of the sepsis syndrome, induced by this cytokine, can thus be observed right from the start. Within its limitations, the model of isolated limb perfusion with r-TNF-α might prove useful in the study of sepsis.

In Chapter I the clinical experience with r-TNF-α in the treatment of cancer is described. The purpose of this chapter is to familiarize intensive care staff with the concept of isolated limb perfusion with r-TNF-α, a new modality in cancer treatment, that has attracted considerable attention. The systemic toxicity of this type of treatment, and of cancer therapy with r-TNF-α in general, is reviewed. This review is based on Phase I and Phase II trials published in the English language, along with supportive documentation and data on 64 patients treated with r-TNF-α in our own institution. Guidelines are offered for the successful management of this type of patient. Treatment with r-TNF-α results in a characteristic clinical syndrome, which resembles the sepsis syndrome. Hypotension and respiratory failure are the main features of this syndrome. Toxicity is largely independent of the route of administration. Very high serum concentrations of TNF-α, if short-lived, can be less toxic than sustained low serum concentrations. Treatment of patients who have undergone isolated limb perfusion with high dose r-TNF-α is feasible and effective in a modern ICU setting, even if high serum concentrations of TNF-α, due to leakage from the perfusion circuit, cannot be avoided. Most patients can be discharged from the ICU within 24 hours.

Chapter II describes the post-operative course of 25 consecutive patients, who underwent hyperthermic isolated limb perfusion with r-TNF-α and melphalan, following pretreatment with r-INF-γ, as treatment for recurrent melanoma, primary non-resectable soft tissue tumors, planocellular carcinoma or metastatic carcinoma. It is a retrospective, descriptive study, relating systemic TNF-α levels with indices of disease severity. All patients developed features of sepsis syndrome and required intensive care treatment. Most patients recovered quickly with a median ICU stay of 2 days (range 1-25). Maximum systemic TNF-α levels ranged from 2284 to 83000 ng/L (median 25409 ng/L) and returned to baseline values within 8 hours. Despite these high levels of TNF-α no patient died in the ICU, although the patient with the highest TNF-α level developed multiple organ failure and required continuous venousvenous hemofiltration for 16 days. Linear regression analysis showed a positive correlation between maximum TNF-α levels and systemic vascular resistance (p<0.01), cardiac index (p<0.02), lung injury score (p<0.02), prothrombin time (p<0.02) and activated partial thromboplastin time (p<0.05). It is concluded that hyperthermic isolated limb perfusion with r-TNF-α leads to high systemic levels of TNF-α, probably due to leakage of r-TNF-α from the perfusion circuit, mainly through collateral bloodflow. A sepsis like syndrome is seen in all patients. Despite high levels of systemic TNF-α, this sepsis syndrome is short-lived and recovery is rapid and complete in most patients.
Several investigators have reported that IFN-$\gamma$ can alter TNF-$\alpha$-induced effects in vitro. In Chapter III we have assessed in vivo effects of r-IFN-$\gamma$ on r-TNF-$\alpha$-induced activation of systemic blood coagulation in a non-randomized study in 20 consecutive cancer patients. Eight patients were treated with r-IFN-$\gamma$ prior to and during hyperthermic isolated limb perfusion with r-TNF-$\alpha$ and melphalan (IFN-$\gamma$ group). They were compared with twelve patients who did not additionally receive r-IFN-$\gamma$ (non-IFN-$\gamma$ group). Before start of perfusion, higher levels of TNF-$\alpha$, prothrombin fragment 1 and 2 (F$_{1+2}$) and thrombin-antithrombin complexes (TAT) were found in the IFN-$\gamma$ group. Fibrinogen and antithrombin III (ATIII) levels tended to be lower in this group. High TNF-$\alpha$ levels, due to leakage during perfusion, were associated with activation of coagulation in all patients, that became obvious after the end of perfusion, when heparin treatment had been antagonized. Activation, measured by increased F$_{1+2}$ and TAT levels, was significantly stronger in the IFN-$\gamma$ group. Monocytic tissue factor (TF) remained low, possibly due to shedding of TF positive vesicles and/or sequestration of TF positive activated monocytes against the vessel wall. In both groups F$_{1+2}$ and TAT levels declined 24 hours after the perfusion, whereas monocytic TF increased to levels that were higher in the IFN-$\gamma$ group. In conclusion, our data confirm a strong activation of coagulation induced by r-TNF-$\alpha$ in cancer patients. They suggest that r-IFN-$\gamma$ may lead to a slight activation of coagulation and augments TNF-$\alpha$ induced procoagulant activity. These effects may be due to r-IFN-$\gamma$ induced sustained monocytic TF activity.

The study described in Chapter IV was undertaken to determine the effects on systemic fibrinolysis of hyperthermic isolated limb perfusion with r-TNF-$\alpha$ and melphalan, with or without pretreatment with r-IFN-$\gamma$. Twenty patients were treated with r-TNF-$\alpha$ and melphalan; four patients, treated with melphalan only, served as controls. Of the twenty patients treated with both r-TNF-$\alpha$ and melphalan, eight received r-IFN-$\gamma$ for two days before the perfusion and as a bolus into the perfusion circuit. A significant leak of r-TNF-$\alpha$ from the perfusion circuit to the systemic circulation was observed in all r-TNF-$\alpha$ treated patients (mean maximum TNF-$\alpha$ 87227 ng/L versus 31 ng/L in controls, p<0.002). In these patients, but not in controls, there was an almost instantaneous rise in systemic tissue plasminogen activator (t-PA) activity (from 0.26 IU/ml to 5.28 IU/ml in 90 min), causing activation of fibrinolysis. After a delay of 90 minutes, plasminogen activator inhibitor-1 (PAI-1) antigen rose to high levels in the r-TNF-$\alpha$ treated group (mean maximum PAI-1 1652 ng/ml versus 211 ng/ml in controls, p<0.02), associated with a sharp decrease of tPA-activity and a slower decrease of plasminogen-antiplasminogen complexes (from 5.28 IU/ml to 0.02 IU/ml in 2 h, and from 1573 $\mu$g/L to 347 $\mu$g/L in 22 h respectively). No additional effect of r-IFN-$\gamma$ pretreatment on fibrinolysis could be demonstrated. These results suggest that in isolated limb perfusion with r-TNF-$\alpha$ and melphalan an initial activation of systemic fibrinolysis, induced by leakage of r-TNF-$\alpha$ from the perfusion circuit, is set off by a subsequent inhibition of the fibrinolytic system by PAI-1. This large increase in PAI-1 could place the patient at risk for deposition of microthrombi in the systemic circulation.

Chapter V describes renal function parameters in 11 cancer patients, who underwent 12 perfusions. Three patients, perfused with melphalan only, served as controls. All patients treated with r-TNF-$\alpha$ developed a sepsis syndrome and needed volume replacement and inotropes to remain normotensive; controls had an uneventful postoperative course. Creatinine clearance decreased transiently on the day of perfusion in both groups (mean preperfusion clearance 118 ml/min, mean postperfusion clearance 68 ml/min, p<0.02, n=15). Follow-up measurements of renal plasma flow and glomerular filtration rate in 9 r-TNF-$\alpha$ treated patients did not suggest permanent damage. One patient became hypotensive and developed transient multiple organ dysfunction with renal failure needing hemofiltration. In r-TNF-$\alpha$ treated patients, but not in controls, a transient increase in clearance of $\beta_2$
microglobulin (49 vs. 8171 ml/min, p<0.001) and urinary excretion of phosphate (12 vs. 48 mmol/24 hrs, p<0.05) was seen, compatible with proximal tubular dysfunction. These data suggest that hyperthermic isolated limb perfusion with melphalan decreases glomerular function, whether or not r-TNF-α is added to the perfusion circuit. Extension of the treatment regimen with r-TNF-α may result in additional proximal tubular dysfunction. If hypotension can be avoided this deterioration in renal function seems to be transient, with full recovery within weeks.

Finally, Chapter VI aims to analyze the mechanism of vasodilation and circulatory shock in patients who are treated with isolated limb perfusion with melphalan and r-TNF-α for locally advanced malignant tumors. The role of nitric oxide, if any, was determined by measuring plasma nitrite and nitrate levels. Eight consecutive patients developed sepsis syndrome due to leakage of r-TNF-α from the perfusion circuit to the systemic circulation. Despite the presence of very high systemic TNF-α levels during and immediately after perfusion and definite signs of hyperdynamic circulatory shock (increased heart rate, increased cardiac index, decreased systemic vascular resistance) nitrite and nitrate levels, measured in plasma at several time points, were not elevated. The hypothesis that, in humans, TNF-α induces vasodilation and shock through activation of inducible nitric-oxide synthase and subsequent formation of excessive quantities of nitric oxide is not substantiated by our results. Normal nitric oxide metabolite levels were found in the presence of high TNF-α levels and shock. Other mechanisms that do not involve the nitric oxide pathway are likely to play a role in the generation of hypotension and septic shock in this setting.

The experience with isolated limb perfusion with r-TNF-α, laid down in this thesis, has confirmed the feasibility of this type of treatment. The question whether r-TNF-α has a role in anticancer therapy has not been specifically addressed in this work. However, it can be assumed that the usefulness of r-TNF-α as an antineoplastic agent will be favorably influenced if its therapeutic index could be increased. Improvement of postoperative care is one way to achieve this. We speculate that with the current standard of anesthesia and postoperative intensive care, even higher dosages of r-TNF-α than have been studied thus far, can be tolerated by most patients. For treatment of malignancies not located on a limb, intravenous administration of r-TNF-α in dosages exceeding what has been considered the maximum tolerable dose (300 µg/m² over 30 min.) can be considered. Even more important will be the quest for safer TNFs; mutant TNFs that selectively bind to the 55 kd TNF-receptor (TNF-R1) share the anticancer potential of native TNF-α but have less pro-inflammatory activity.

Finally, basic research in sepsis will eventually supply us with drugs that can abrogate many of its deleterious effects. These drugs will be equally effective to counteract systemic toxicity in cancer treatment with r-TNF-α. The challenge here will be to reduce toxicity without reducing antitumor efficacy. Conversely, drugs that reduce toxicity in a model of isolated perfusion with r-TNF-α may be useful in the treatment of sepsis.