Melanoma
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Chapter II

Experience with isolated limb perfusion

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Introduction

Locally advanced melanoma or sarcoma of the limb was in the past surgically treated by an amputation of the affected limb. The majority of the patients died from their disease despite an amputation. This lead Klopp at the end of the forties to the idea to deliver a cytostatic agent, nitrogen mustard, direct to the tumor bearing limb through an intra-arterial injection, as a limb saving cancer treatment.\(^1\) Shortly afterwards Sullivan developed the technique of continuous intra-arterial infusion.\(^2\) The idea behind the intra-arterial chemotherapy delivery was the ‘first pass through effect’ of the cytostatic agent. Morton and Eilber developed a combined modality for locally advanced limb sarcoma with three days regime of continuously intra-arterial doxorubicin with preoperative high dose fractionation radiation schedules of different dose levels in the late seventies.\(^3\) Today we know however that intra-arterial chemotherapy has no advantage over systemic, intravenous, drug delivery.\(^4\)

The theory behind the delivery of isolated regional chemotherapy is that a high drug uptake by the tumor may be achieved with higher doses of chemotherapeutic agents without increasing the systemic toxicity. The technique of isolated limb perfusion, a regional cancer treatment that allows the direct infusion of high doses of chemotherapeutic agents in the arterial supply of a tumor-bearing area of a limb, came available with the development of an extra-corporeal circuit for cardiac surgery. In the mid fifties the surgical-oncologists Creech and Krementz of the Tulane University in New Orleans developed the technique of the isolated limb perfusion.\(^5\) In the sixties Cavaliere reported on selective heat sensitivity of tumor cells and added hyperthermia within the regional perfusion technique.\(^6\) The ultimate goal of hyperthermia within the perfusion setting was increasing the blood flow and permeability of the cell membrane, therefore the drug uptake within the tumor by a synergistic effect, leading to an ultimate tumoricidal effect.

The three major advantages of the delivery of chemotherapy within the isolated limb perfusion setting are: 1) the so called ‘first-pass’ effect resulting in an increased drug uptake, 2) hyperthermia facilitating drug uptake by increased blood flow and permeability of the cell membrane and 3) the use of cytostatic agents which cannot be used outside the isolated limb perfusion setting due to the high systemic toxicity. Key point in isolated limb perfusion is that the dose of
Chemotherapeutic agents used, can be 15-20 fold the maximum systemic tolerated dose, since vital organs are isolated from the perfusion circuit. Since the first isolated limb perfusion was performed in the late fifties, the complex surgical technique is only used in a few cancer centers in the United States, Europe and Australia for the limb salvage treatment of locally advanced melanoma or soft tissue sarcoma.

This chapter will review the technical aspects of the isolated limb perfusion procedure, the currently available cytostatic agents, the assessment of tumor response, the clinical results of adjuvant and therapeutic isolated limb perfusions for various malignancies of the limb, as well as the local and regional treatment toxicity and future directions.

Regional perfusion

Perfusion level

There are several levels for isolated limb perfusion in the upper and lower limb. Upper limb perfusions might be performed at the axillary level through the axillary artery and vein, or more distal upper limb perfusions through the brachial artery and vein. At the lower limb, perfusions might be performed at three perfusions levels, iliacal level through the external iliac artery and vein, femoral level through the femoral artery and vein, and at the distal thigh above the knee through the popliteal artery and vein. The level of perfusion is determined by the involved part of the limb and the kind of disease, e.g. skin malignancies versus sarcomas. For skin malignancies the most proximal site of cannulation is the best choice, since the whole limb is at risk. In sarcoma the perfusion level is defined by a distinction part of the limbs containing the tumor. In case of limb recurrence(s) repeated perfusions might be possible, preferably at another level, to reduce the risk of complications to the previous exposed and perfused vessels.

Perfusion technique

During the perfusion the limb is exclusively perfused. After dissection of the appropriate artery and vein and ligation of the collateral vessels, to control
collateral flow and prevent leakage, the patient is heparinized systemically (heparin 3.3 mg/kg body weight).

**Fig.1** *Five different perfusion levels for regional perfusion of the extremities.*

The limb is isolated from the systemic circulation by an esmarch bandage twisted around the root of the limb and fixed around a pin inserted into the head of the humerus (axillary perfusion) or iliac crest (iliac perfusion). An inflating tourniquet (300-400 mm Hg) is used for brachial or popliteal perfusions. The artery and vein are exposed, cannulated with 14 to 16 F catheters, connected to an extracorporeal circulation system and perfusion flow is initiated in the circuit. Thermister probes are placed in the subcutaneous tissue and muscle for continuous temperature monitoring. The perfused limb is wrapped in a thermal blanket to reduce heat loss. (Fig.2) The extracorporeal circulation perfusion system consists out of a roller pump, a membrane oxygenator, and a heat exchanger. The extracorporeal perfusion equipment improved during the last four decades with all
kinds of continuous data monitoring systems; recording of the temperature of the perfusate, the mean arterial and venous pressure in the perfusion canules, mean arterial pressure in the system, venous saturation and electronic balance of the perfusion volume. The perfusion group of the Groningen University Hospital further improved the perfusion technique by introducing the pressure-regulated perfusion, as well as safety by improvement in continuous leakage monitoring.\textsuperscript{8,9}

The perfusate priming used in Groningen is oxygenated by a membrane oxygenator DIDECO 902 (DIDECO, Mirandola, Italy) with a gas mixture of air and $\text{O}_2$, consisted of 250 ml Isodex in saline 0.9\% (NPBI International BV, Emmer Compascuum, The Netherlands), 250 ml white cell-reduced (filtered) packed red cells, 30 ml of 8.4\% $\text{NaHCO}_3$, 0.5 ml 5000 IU/ml heparin. Leakage into the systemic circulation is continuously monitored with radioactive tracers. A small calibration dose of radioactive Iodine-131 labeled human serum albumin, (RISA 0.5 MBq) and a dose of radioactive Technetium-99m labeled human serum albumin (RtcSA 10 MBq) are administered into the systemic circulation after surgical isolation of the limb is accomplished. The day before surgery the thyroid is saturated by the oral administration of iodine. A ten times higher dose of RISA (5MBq) is injected to the perfusion circuit. The 364-keV gamma rays emerging from the RISA and the 140-keV gamma rays from the RtcSA are measured with a NaI detector over the precordium. Leakage from the perfused limb to the systemic circulation results in an increase of the baseline level, continuously measuring the percentage of leakage from the perfusion solution into the systemic circulation. The risk of leakage is mainly related to the level of perfusion, and is in general less than 3\%.\textsuperscript{9}

The perfusions are flow regulated on the basis of arterial and venous pressure. To achieve adequate tissue perfusion during clinical regional perfusions, the extracorporeal circuit must be regulated at a delta pressure of 15 mm Hg between the pressure in the arterial and venous catheter.\textsuperscript{8} Cytostatic agents are added directly into the arterial line of the perfusion circuit as soon as a limb temperature of 38\degree C is reached. Perfusions are performed during 60 minutes under mild hyperthermia (39\degree-40\degree C). Adjustments of the flow rate and the pressure in the perfusion circuit by the perfusionist, as well as the blood pressure in the patient by the anesthesiologist, together with an optimal isolation of the limb by the surgeon, ensure a stable and therefore optimal perfusion.
Fig. 2 Schematic drawing of a regional perfusion circuit for an iliacal perfusion of the lower extremity; esmarch bandage around the hip with Steinman pin inserted in the iliac crest, arterial and venous perfusion catheters connected to the arterial and venous line, membrane oxygenator, heat exchanger, roller pump, leakage monitoring with scintillation detector placed over the heart, warm water mattress and thermo probes for skin and muscle temperature.

When there is an increase leakage to the systemic circulation (losing), the flow rate should be reduced and the systemic blood pressure increased, eventually the tourniquet tightened, while with a loss of the systemic circulation into the perfusion circuit (gaining) the tourniquet should be tightened, flow rate increased and outflow ‘occluded’. In case of too much leakage, losing or gaining, the perfusion should be terminated for technical reasons to prevent eventual loco-regional or systemic complications. After the perfusion the limb is extensively washed out with 4-6 L of saline and the limb filled with 250 ml white cell-reduced (filtered) packed red cells. The vessels are restored, first the vein and secondly the artery to ensure adequate outflow of the limb. Finally the heparin is antagonized with prothrombin and a fasciotomy is performed. Patients perfused with tumor
necrosis factor α (TNFα) are monitored during 24 hrs on the intensive care, while patients perfused with melphalan are observed on the ward. No prophylactic antibiotics are prescribed. Patients receive subcutaneous low-dose molecular heparine till a full mobilization is achieved.

Cytostatic agents

Cytostatic agents used in isolated limb perfusion must have appropriate pharmacokinetic profiles such as steep dose-response curves without requiring metabolic activation. The most optimal cytostatic agents must therefore have a high degree of extraction within the perfused limb and a high total body clearance in case of systemic leakage. The real advantage of cytostatic agents used in the perfusion setting should be expressed by comparing the area under the curve of the concentration versus time profile using the perfusion drug application versus the conventional intravenous drug delivery.  

The first drug used in isolated limb perfusion by Creech and Krementz was melphalan. A variety of other antineoplastic agents besides melphalan have been used during the last forty years in hyperthermic isolated limb perfusion such as dacarbazide (DTIC), actinomycin-D, thiotepa, mitomycine-C, doxorubicin and more recently cisplatin and carboplatin. The majority of the cytostatic agents were or ineffective, or the duration of response quite limited, or when a drug showed effectiveness the local toxicity hampered the further application. Doxorubicine showed effectiveness but disturbed the arterial vascularity of the limb, while cisplatin and carboplatin were too neurotoxic.

Mephalan (L-phenylalanine mustard, GlaxoSmithKline, London, England) is an alkylating agent of the bischloroethylamine type compromising of nitrogen mustard and phenylalanine. Phenylalanine is a metabolite of melanin and therefore melphalan specially targets melanocytes and melanoma cells. Its cytotoxicity appears to be related to the extent of its interstrand cross-linking with DNA. Like other bifunctional alkylating agents, it is effective against both resting and rapidly dividing tumor cells. Melphalan is widely used since the sixties and the standard, most effective drug in the isolated limb perfusion setting for melanoma.
The dose calculation of chemotherapeutic agents in the past performed was on body weight. The dosage melphalan was 1.0–1.5 mg / kg for the lower limb and 0.5–0.7 mg / kg for the upper limb. The maximally tolerated dose of melphalan by this method of calculation without regional toxicity is thought to be 1.75 mg / kg, while 2.0 mg / kg will lead to severe regional toxicity.\textsuperscript{15} Today the dosage is based on the perfused limb volume which seemed to be more appropriate.\textsuperscript{16} The volume of the perfused limb is determined before surgery by immersion. The current melphalan dosage used is 10 mg / L limb volume for the lower limb and 13 mg / L limb volume for the upper limb. The maximal tolerable dose is 16 mg / L limb volume. Doses greater than 150 mg per limb results in dose-limiting regional toxicity.\textsuperscript{17}

The systemic use of Tumor Necrosis Factor-alpha (TNF\textsubscript{α}; Boehringer-Ingelheim GmbH, Vienna, Austria) is a pleiotrophic cytokine was abandoned due to its vasoplegia effect resulting in a severe septic shock syndrome. To use the potential effect of TNF\textsubscript{α}, e.g. tumor vessel destruction while limiting the side effect, e.g. septic syndrome. In the late nineties Lejeune introduced TNF\textsubscript{α} in the perfusion setting together with melphalan in the treatment of locally advanced melanoma and sarcoma of the limb.\textsuperscript{18} The exact working mechanism of TNF\textsubscript{α} is still unknown, but progress is being made. TNF\textsubscript{α} attacks the neo-vascular endothelial cells in particular the tumor vasculature with rapid elimination of tumor blood flow during the treatment. Binding of TNF\textsubscript{α} to its endothelial receptors induces a cascade of mechanisms which suppress anticoagulant mechanisms and support thrombus formation in the tumor vessels, causing circulatory stasis and ischemia inside the tumor, followed by necrosis of the tumor. E-selectin, VCAM, ICAM-1, tissue factor and deactivation of an integrin (\(\alpha_v\beta_3\)) are all contributors in this cascade. Morphological changes of the endothelial cells with overlapping and elongation of the cell results in increased vessel permeability, facilitating the drug uptake of melphalan within the tumor cells.\textsuperscript{19}

Is it possible to hence tumor response to TNF\textsubscript{α}? Endothelial monocyte-activating polypeptide II (EMAP-II) is a novel tumor-derived cytokine that sensitizes tumor vasculature to the effects of systemic TNF\textsubscript{α}. Tumor necrosis factor receptor I (p55) is upregulated on endothelial cells by exposure to the tumor-derived cytokine EMAP-II. Furthermore EMAP-II induces apoptosis and has antiangiogenic effects. In an experimental rat-model Eggermont and co-workers showed indeed...
the improved antitumor response to isolated limb perfusion with TNFα after upregulation of EMAP-II in STS.\textsuperscript{20}

Since the introduction of TNFα in the perfusion setting various clinical and experimental studies have been performed to get more insight in the working mechanism of TNFα. Pre- and postperfusion angiography clearly showed that TNFα produces a selective destruction of the tumor-associated vessels, while the normal vasculature remained intact.\textsuperscript{21} The dosage of TNFα is 3 mg for the upper limb and popliteal perfusion, while 4 mg is used for the lower limb. None randomized studies showed however that 1 mg TNFα might be even effective as the usual used 3 or 4 mg dosage.\textsuperscript{22} Melphalan concentrations in the perfused limb decrease from time zero to 10-20\% of the initial dosage by 60 minutes. In the initial first 5-10 minutes there is a rapid decrease of melphalan due to the uptake by the tissue, while during the remaining perfusion time there is a continuously degradation of the drug as well as an adherence to the plastic tubes surface of the perfusion circuit. To receive an optimal effect of the melphalan a minimal perfusion time of 45 minutes is required. In contrast TNFα is almost immediately binded to the pathological endothelial cells, and a so-called ‘TNF priming time’ of 15-30 minutes prior to the intra-arterial delivery of melphalan seems appropriate. Based on experimental studies the clinically used perfusion time for melphalan alone is 45-60 minutes and for the combined TNF-melphalan perfusion 60-90 minutes.\textsuperscript{23}

\textit{Assessment of local tumor response}

Clinical assessment of tumor response is defined by the World Health Organization criteria.\textsuperscript{24} Complete response (CR) is defined as the disappearance of all measurable disease in the limb for longer than four weeks, partial response (PR) as regression of the tumor size by >50\% for longer than four weeks, and no change (NC) as regression of <50\% of the tumor or progression of <25\% for longer than four weeks. Assessment is possible by physical examination and/or radiodiagnostic imaging. An MRI is used to measure the response in size for sarcoma. Today there are techniques to measure non-invasive in-vivo responses available. The currently most widely and available technique is Positron Emission
Tomography (PET) which measures the tumor metabolism.\textsuperscript{25} New promising techniques such as bioluminescent imaging (BLI) are under development.\textsuperscript{26} The perfusion technique is a local regional cancer treatment with the ultimate goal to improve the limb salvage rate in locally advanced limb melanoma and sarcoma. There are no randomized studies performed as ultimate proof of the concept but the advantage of the technique in the limb salvage rate and local control of the disease is demonstrated in the various studies. The question if assessment of tumor response after isolated limb perfusion is a predictor of disease outcome is still unanswered. PET studies performed after TNF perfusions for locally advanced sarcomas provided insights in the effect of treatment, varying from CRs to NCs. Pre- and postperfusion Positron Emission Tomography (PET) showed as a result the rapid disappearance of tumor hypermetabolic areas linked to the hypervascularization.\textsuperscript{27,28} Extensive pathological examinations of resected soft tissue sarcomas showed however in the majority of the specimens ‘vital’ tumor cells at the surrounding of the necrotic specimen.\textsuperscript{27,28} Clinical, as well as in-vivo assessment of tumor response is a valuable predictor for the final effect of the regional treatment, which is a matter of course not translated to the overall outcome of the disease.

\section*{Isolated limb perfusion}

\textit{Melanoma}

In the beginning isolated limb perfusion was used as a therapeutic treatment for locally advanced melanoma, recurrences and/or intransit metastases. Later on adjuvant limb perfusions were introduced in the treatment of melanoma of the limb. Over the last three decades many papers on hyperthermic isolated limb perfusion for melanoma combining adjuvant perfusions with therapeutic perfusions, often with different treatment schedules have been published and no conclusion could be drawn.\textsuperscript{29} Finally a randomized trial showed (EORTC 18832/WHO-15/NAPG-1) no real benefit of hyperthermic isolated limb perfusion as an adjuvant treatment modality for patients with high-risk stage I melanoma (> 1.5 mm Breslow thickness).\textsuperscript{30} On the otherhand isolated regional perfusion with melphalan is a well accepted treatment for intransit metastases and/or recurrences.
in patients who are no longer candidates for local treatment, e.g. surgery, cryotherapy or laser treatment. Complete response rates of 40-60% with an overall response rate of 80% might be expected after therapeutic perfusions, while the addition of mild hyperthermia adds little to a normothermic perfusion. Although double perfusions might increase the response rate further, this will have however no effect on frequency, time to local recurrence as well as time to distant failures, or survival. Patients who did not achieve a complete response do worse than patients with a complete response. Roughly one third of the complete responders will recur, an additional one third will develop distant recurrences and the remaining one third will remain disease free. Perfusions performed in patients with recurrences after previous perfusions might render one third of these patients again disease free.

Other chemotherapeutic agents have been used in melanoma perfusions, showing lower subjective response rates and often higher toxicity. The most effective systemic agent in the treatment of melanoma is DTIC, but used in the perfusion setting this agent leads to complete response rate of 11% and a partial response rate of only 26%. Cisplatin is one of the most successful alternatives with of 50-60% response rates, but showed a too high frequency of peripheral neuropathy. After the initial successful experience in the early nineties of isolated limb perfusion for melanoma with TNFα, gamma interferon, and melphalan (TIM) with 90% CRs, Lejeune and co-workers initiated a prospective randomized phase II study of patients with advanced melanoma of the limb by comparing TIM-perfusion versus TM-perfusion. The study showed a 10% drop in CR when interferon was omitted, but the difference was not significant. A comparison with matched cases from a databank confirmed that melphalan only results in 52% complete responses. The study was early terminated due to the fact that TNFα and melphalan showed overall no real benefit, compared to melphalan alone with a median survival of 2.5 years. Patients with low tumor burden or small tumors showed equivalent results were documented (TNFα and melphalan versus melphalan), while TNFα and melphalan showed higher response rates in bulky melanoma. Therefore melphalan is the drug to be used in melanoma perfusion and combined with TNFα when bulky disease or recurrent disease after previous melphalan perfusion is the indication for perfusion. The key question if a TNF
perfusion has advantage over a perfusion with melphalan alone is still unanswered, and results from the American College of Surgeons melanoma perfusion trial (03-C-0137) are pending.

Sarcoma

Soft tissue sarcomas (STS) are relatively rare malignancies of different mesenchymal derivation, accounting for less than 1% of all cancer in adults. Roughly fifty percent of all sarcomas are located in the extremities, and fifty percent of them are over the age of 65 years. Although isolated limb perfusion was developed in the treatment of melanoma of the limb, the procedure was shortly after the introduction also applied in the treatment of soft tissue sarcoma of the limb. In the first experience Krementz showed an early response rate after melphalan of 83%, however complete regression of the tumor was hardly seen. The first breakthrough in the limb saving treatment of sarcomas was achieved in the eighties, when limb saving treatment, with or without adjuvant radiation treatment, showed the same disease free and overall survival as amputation. The isolated limb perfusion program at the Groningen University, as elsewhere, was stopped, when the results achieved with the complex perfusion technique were not better than after surgery and adjuvant high dose irradiation. Although the combined modality treatment of surgery and adjuvant radiation improved the limb salvage rate of limb sarcomas, an amputation of the limb was still unavoidable in 5-10% of these patients. Other perfusions agents in the treatment of limb sarcomas were therefore investigated. Rossi claimed efficacy of doxorubicin in the perfusion setting for limb sarcomas, while another study proved that doxorubicin alone was ineffective and combined with melphalan too toxic. Cisplatin showed also to be ineffective in the limb perfusion setting of sarcomas. Although adjuvant chemotherapy may reduce the local failure rate in some patients, (neo) adjuvant and adjuvant chemotherapy had no beneficial effect in improving the limb salvage rate, disease free and/or overall survival in extremity sarcoma. The second breakthrough in the treatment of locally advanced soft tissue sarcoma of the limb came with the introduction of TNFα and melphalan in the perfusion setting by Lienard and Lejeune in the early nineties. After TNFα perfusion, remarkable tumor shrinkage might be encountered within 6-12 weeks. Irresectable
sarcomas become resectable. Complete response rate of 18% and partial response rates of 64% by measuring the tumor size was achieved. Various reports have shown that a limb salvage rate of roughly 80% can be achieved in patients with primarily irresectable limb sarcoma. Since the resection margins in these tumors are minimal, and often viable tumor cells are encountered at the periphery of the tumor adjuvant radiation is applied to ensure local tumor control. Adjuvant irradiation is well tolerated after previous intensive combined modality treatment of perfusion and extensive surgical resection.

A large European study proved the ILP concept in the limb salvage procedures for irresectable STS with TNFα and melphalan. The objective response rate was 76%. A limb salvage rate of 71% was achieved with a minimal treatment related morbidity. An independent review committee considered that 80% of all enrolled patients in this study met indeed the criteria for irresectability and survival curves based on a match control study with cases of the Scandinavian Soft Tissue Sarcoma Databank showed that TNFα had no negative effect on survival. Further analysis showed that ILP-patients survived as long as matched controlled conventionally treated patients. The outcome of the ILP procedure in ‘elderly’ sarcoma patients was in general not different from the ‘younger’ sarcoma patients. Perfusions in elderly limb sarcoma patients can on the otherhand sometimes not be performed due to atherosclerotic changes in the main arteries and/or severe co-morbidity. For these patients an amputation of the limb is unavoidable. With the improved surgical perfusion techniques, the perioperative care and intensive care facilities, perfusion treatment related morbidity for limb sarcoma is minimal.

Lymph edema-associated angiosarcoma, the Stewart-Treves syndrome, may be diagnosed in patients who underwent a mastectomy for breast cancer or axillary dissection for melanoma. The Stewart-Treves syndrome is extremely rare and the pathogenesis is not completely understood. The TNFα perfusion is an excellent indication for the limb salvage treatment for patients who where previously candidates for ablative surgical procedures.

The results of the multicenter TNF study performed in the nineties lead to the approval of using Beromun® (Boehringer-Ingelheim GmbH, Vienna, Austria) and
melphalan. (GlaxoSmlthKline, London, England) for isolated limb perfusion treatment of locally advanced extremity sarcomas. Beromun® is not registered in the United States. Currently isolated limb perfusion with TNFα is worldwide available in more than 30 Centers. In 2002, 350 so called TNF-perfusions were performed. Why do not all the perfused sarcomas respond after a TNFα? Multidrug resistance is a major issue in chemotherapy treatment. Two groups investigated separately the expression of multidrug resistance in patients undergoing TNFα perfusion treatment. Hohenberger et al. investigated the expression of multidrug resistance genes major vault protein (MVP), MDR1, and MDR-associated protein 1 (MRP1) before, during and after isolated limb perfusion for sarcoma or melanoma. In 83% of the patients, MVP expression was induced during perfusion, while inductions of MDR1 and MRP1 were observed in only 13% and 27%. The temperature and the drugs were therefore unable to induce MDR1 and MPR1 in the majority of these tumors in the perfusion setting.51 Komdeur et al. investigated the expression of P-glycoprotein (P-gp), MDR1, and lung resistance-related protein (LRP) in relation to the clinical outcome of TNFα perfusion treatment for extremity sarcomas. The sarcomas were more often positive for P-gp, than for MRP1. The MDR status was not predictive for tumor response after TNF-ILP. Data of the study showed also that TNFα perfusion did not induce MDR positive sarcomas.52 This is an important finding, since systemic doxorubicin based polychemotherapy is currently investigated in soft tissue sarcoma patients after TNFα perfusion as an adjunct within the EORTC trial 62931. The combination of TNFα and melphalan is currently the standard drug combination in isolated limb perfusion for the limb salvage treatment of primarily irresectable soft tissue sarcoma of the extremities, but there are still 20-30% of the tumors that will not respond. Therefore agents synergistic with TNFα must be investigated such as doxorubicin, or actinomycin-D. The combination of TNFα and doxorubicin was recently clinically investigated showing no benefit when compared to TNFα and melphalan.54 Seynhave et al. showed in an experimental perfusion study in the rat-model that the combination of actinomycin-D and TNFα improved the tumor response in the soft tissue sarcoma bearing rats. The responses were unfortunately accompanied by severe, dose limiting, local toxicity; a synergistic anti-tumor response with idiosyncratic locoregional toxicity to the normal tissues.55 These
experiments don’t warrant the further clinical investigation of these two drugs in isolated limb perfusion for sarcomas.

**Miscellaneous tumors**

Isolated regional perfusions with TNFα have been successfully performed for in patients with locally advanced squamous cell carcinoma and Merkel carcinoma not amenable to local surgery with response rates of 87% (CR 60%, PR 27%). There is also a limited perfusion experience with cisplatin, as well as with TNFα for unresectable bony sarcomas. No conclusion can be drawn from these studies.

**Treatment toxicity**

The treatment toxicity can be categorized as side effects from systemic exposure to the cytostatic agent and as side effects due to the regional effects of the high-dose exposure and hyperthermia. The vast majority of perfusions can be performed with systemic drug exposure of less than 3%. The systemic exposure depends not only on the adequacy of the isolation of the limb during the perfusion, but is also related to the systemic exposure to the perfused agent during reperfusion. Although the limb is flushed after the perfusion, residual active agents still remain in the limb within the intravascular space or in the interstitial fluid, which results in a systemic peak of drug concentration following the re-establishment of normal vascular flow to the extremity. Systemic leakage of melphalan might cause mild nausea, bone marrow depletion, and fever. In case of leakage over 10% patients should have their white blood counts measured 7 to 14 days post-operatively to monitor bone marrow depression.

TNFα might induce secondary host mediators in contrast to the other drugs used in the perfusion setting. The most serious complication after TNFα perfusion is the systemic inflammatory response syndrome (SIRS) accompanied by fever, rise in cardiac output, fall in systemic vascular resistance and the need for fluid resuscitation and inotropes. If leakage exceeded the 2% limit during perfusion, there was less exposure of the tumor bearing limb to TNF and an increased exposure of the patient systemic circulation to TNFα, resulting in more systemic side effects. There is a direct correlation between maximum TNFα concentrations
and systemic vascular resistance and cardiac index.\textsuperscript{60,61} Severe toxicity might be encountered over a leakage of 5\%.\textsuperscript{18} In contrast Stam et al observed only a mild postoperative toxicity in the event of a significant leakage during perfusion.\textsuperscript{62} The more experience is achieved with the so called TNF\textsubscript{α} -perfusions, the better the transient, systemic side effects could be managed during the perfusion and postoperatively with appropriate resuscitative techniques. The leakage should not extend 10\% (as per licensee). Currently SIRS is only seldom seen, since all the institutions performing TNF\textsubscript{α} perfusions are experienced, achieve less leakage rates and use extensive washout procedure at the end of the perfusion procedure, to reduce the release of TNF\textsubscript{α} from the perfused limb to the main circulation after restoration of the limb circulation.

The normal tissues in the limb, skin, subcutaneous tissue, muscle, nerves, blood vessels, bone, and cartilage are all exposed to the same concentrations of cytostatic agents active against the tumor. Wieberdink developed a regional toxicity scoring system.\textsuperscript{15} The effects of the perfusate on normal tissues varied widely between individuals. Melphalan may cause skin toxicity, erythema and blistering, and serves as a document of the distribution of the perfusion.\textsuperscript{63} When a lymph node dissection is performed together with the perfusion, edema of the involved limb might be encountered. The most important toxicity is related to muscle and nerve damage, which might be avoided by a prophylactic fasciotomy, preventing the development of a compartment syndrome.\textsuperscript{64} All patients undergo some degree of skin reaction and lymph edema TNF\textsubscript{α} after the perfusion. This resolves in general within a month regarding the accompanied procedures, e.g. tumor resection and/or radiation. The Rotterdam perfusion group documented functional impairment of 20\% of the upper limb and 36\% of the lower limb after melphalan perfusion.\textsuperscript{64} In contrast Olieman and co workers showed no impairment of limb function after melphalan perfusion for melanoma compared to the unperfused limb.\textsuperscript{65} It might be expected that small proportion of patients (5-8\%) undergoing a limb perfusion for melanoma will have long-term limb symptoms, without severe impairment, secondary to their perfusion treatment.\textsuperscript{66} After perfusion for locally advanced sarcoma impairment of limb function is not related to the perfusion, but to the extend of the surgical resection of the soft tissue mass and eventually adjuvant irradiation. Another risk factor in isolated regional perfusion is not related to the cytostatic agents, but to the (elderly) patients' vascular status and the
extent of the soft tissue mass in case of perfusion for a locally advanced soft tissue sarcoma. Manipulation, cannulation and tight occlusion of sclerotic vessels might cause embolic events, arterial stricture after vessel repair, or arterial thrombosis, requiring reoperation or even amputation of the perfused limb. Beside the arterial complications, from the venous side deep venous thrombosis is sometimes encountered due to cannulation of the vein or the thrombogenic side effect induced by melphalan. Increase in limb temperature might slightly increase limb toxicity in melphalan perfusions. The addition of TNFα will significantly increase the limb toxicity due to increase of melphalan into the tissues and not to microembolization of tumor cells.57,68

Summary

Isolated limb perfusion has the potential to deliver high doses of chemotherapeutic agents to a tumor-bearing limb. There is no evidence-based indication for adjuvant isolated limb perfusions for high-risk limb melanoma, in contrast to therapeutic perfusions for extensive local recurrences or intransit metastases. The introduction of TNFα in combination of melphalan redefined the indication for therapeutic and palliative isolated limb perfusions in the limb saving treatment of locally melanoma and sarcoma of the limb, as well as multifocal skin cancers and drug-resistance bony sarcomas. The currently most widely used drugs in the perfusion setting are melphalan with or without TNFα. Various aspects of isolated limb perfusion treatment need to be further explored. The future developments in regional perfusion research should focus on new therapeutic perfusion agents and/or increasing the tumor sensitivity for TNFα without decreasing tumor response, as well as the further exploration of new anatomical areas in the body for regional cancer treatment, such the liver, lung, kidney and pelvis.
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