Different aspects of hyperthermic isolated limb perfusion
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Document Version
Publisher's PDF, also known as Version of record

Publication date: 2002

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Summary and conclusions
Samenvatting en conclusies
Chapter 1
Using technology to support extracorporeal circulation developed for cardiac surgery in the 1950s, the surgical oncologists Creech, Krementz, Ryan and Winblad of the Tulane University in New Orleans developed the technique of isolated limb perfusion (ILP). In this procedure the blood circulation of a tumor bearing limb is isolated from the circulation of the rest of the body by clamping the major artery and vein and tightening a tourniquet around the root of the limb. The artery and vein are subsequently connected to a heart-lung machine and a cytotoxic drug is administered through this isolated circuit. Key point in ILP is that the dose of chemotherapeutics used, can be 15-20 fold the maximum systemic tolerated dose since vital organs are isolated from the perfusion circuit. Cavaliere and co-workers investigated the addition of hyperthermia in the treatment of cancer and, as this appeared to augment the anti-tumor effects of melphalan, in doing so they laid the basis for hyperthermic isolated limp perfusion (HILP). The original patient population treated with HILP was a subgroup of melanoma patients with extensive local recurrence in the arm or leg. Later on also patients with soft tissue sarcomas of the extremities were treated. Throughout the years different chemotherapeutic agents were used in HILP, all with variable results.

William Coley, a surgeon who lived and worked in New York City during the second half of the 19th century, was the first to investigate the phenomenon of tumor necrosis, occurring in patients suffering from severe infections. By administering preparations of gram-positive and gram-negative bacteria or their products to patients with inoperable neoplastic diseases, Coley hoped to bring about an involution of the tumor. The side effects of Coley’s regimen were unacceptable, however, and his treatment ultimately fell into disrepute. The phenomenon that bacteria were capable of producing a tumor necrotisizing factor stimulated other investigators. In 1975 Old and co-workers identified a factor produced in mice pretreated with Bacillus Calmette-Guérin (BCG) and subsequently challenged with lipopolysaccharide (LPS). This factor was able to cause hemorrhagic necrosis of the meth A sarcoma, grown in the skin of a recipient animal. The factor was dubbed “tumor necrosis factor” (TNF). A lot of articles published both in scientific literature and in popular press claimed, that this molecule would prove to be a revolutionary tool in the battle against cancer. However, phase I and II clinical trials of systemic TNF were marked by a disappointing overall response rate of 1-2% and a dose-limiting toxicity of hypotension. This dose-limiting toxicity in patients kept the peak intravascular level achievable in humans 100-fold lower than the level needed to have an anti-tumor effect in a mouse model. Because it
seemed impossible to achieve effective systemic concentrations of TNF in patients, TNF was ideally suited for use in HILP where levels up to 10 to 20 times the systemically tolerated dose could be achieved. Ferdy Lejeune and Danielle Lienard, surgical oncologists working in Brussels at the time, were the first to observe the dramatic effect of tumor necrosis in humans using HILP with a combination of TNF, interferon (IFN) and melphalan.

The second part of the introduction describes the technique of positron emission tomography (PET). This is a non invasive, diagnostic imaging technique for measuring the metabolic activity of cells in the human body with the aid of short-lived positron emitting radiopharmaceuticals. Not only is it possible to visualize the metabolic processes of a tumor but it is also possible to quantify the metabolic processes. PET was used to evaluate tumor metabolism and with that tumor response before and after HILP with TNF, IFN and melphalan.

Chapter 2
Osteosarcoma is the most frequent occurring primary malignant bone tumor in human. During the past few decades, the use and further development of systemic neo adjuvant chemotherapy, e.g., including high-dose methotrexate (HD-MTX) and cisplatin, appears to have a definite influence on the disease free and overall survival for patients with osteosarcoma. However, the potential local tumor effect of this systemically administered chemotherapy is not always favorable. To increase the effect of cisplatin on locoregional osteosarcoma, the short term effect of HILP with cisplatin (30 mg/L extremity volume) was studied in 28 dogs with spontaneous osteogenic sarcoma using clinical, radiological, and histological parameters. Thirty days postoperatively mortality was 14.3 %. Total platinum levels at the start of perfusion were $28.2 \pm 14.3$ mg/L. A significant improvement ($p<0.001$) in the clinical score was observed in the overall group at 6 and 12 weeks after perfusion. The radiological parameter showed a stationary X-ray 2 weeks after perfusion and an improved X-ray 6 weeks after perfusion. Overall histological scores showed a moderate effect according to the Huvos classification. No additional therapeutic effect, according to the three parameters, could be demonstrated by increasing the perfusate temperature by 1°C. HILP with cisplatin is feasible in the local treatment of spontaneous osteosarcoma in dogs with acceptable locoregional toxicity. However, the histological results were modest, with none of the dogs showing a complete response 6 weeks after perfusion. Therefore the search for the ideal perfusion agent with substantial contribution to the limb sparing treatment in human osteosarcoma, continues.
Chapter 3
With the introduction of HILP with TNF, IFN and melphalan the question was raised as to whether the combination of TNF with cisplatin in HILP could improve the histological results after perfusion in dogs with spontaneous osteosarcoma. Before starting with perfusion in dogs with osteosarcoma the feasibility of TNF perfusion with and without cisplatin in healthy dogs was studied. During seven perfusions in six mongrel dogs (weight 32±2 kg) the technical aspects of HILP under mild hyperthermia (39-40°) were studied. In five experiments HILP was performed with TNF alone (0.5 mg/L extremity volume), and in two experiments TNF was combined with cisplatin (25 mg/L extremity volume). During the perfusions physiological parameters were monitored and TNF and total cisplatin concentrations were determined. Perfusion conditions (pH, PCO₂, PO₂, flow and pressure) remained within physiological ranges. Three dogs died within 24 hours despite a sublethal systemic concentration of TNF that leaked from the perfusion circuit. Three dogs were terminated; one dog after the second experiment in accordance with the Dutch ethical rules; one dog because it showed an invagination of the small bowel resulting in an ileus; one dog because of necrosis of the perfused limb. This feasibility study in healthy dogs demonstrated that HILP with TNF and cisplatin was associated with a high mortality rate and therefore does not allow us to treat dogs with spontaneous osteosarcoma with TNF and cisplatin HILP.

Chapter 4
In order to study the value of HILP with cisplatin in the management of locally advanced soft tissue sarcomas or metastatic bone sarcoma in humans, four patients were treated in this manner under mild hyperthermia. Toxicity in the perfused limbs was moderate, and the erythema and edema that occurred resolved spontaneously within 7-14 days as did the slight motor and sensory neuropathy over a longer period of time. Clinically, a reduction of pain was observed in all patients. Two weeks after perfusion, tumor biopsies were taken to evaluate the response. Two patients showed a pathological complete response, one patient showed >90% necrosis and one patient showed no response. The histological results of this study were modest and with the introduction of TNF in combination with melphalan as perfusion agents, no further research with cisplatin as a perfusion agent was done.

Chapter 5
The first patient treated at the University Hospital Groningen with the perfusion regimen of Lejeune, TNF with IFN and melphalan, had been treated with local resection and adjuvant external beam radiotherapy 3 years earlier. Radiotherapy
consisted of 40 Gy given 2 Gy per day in 4 weeks on the whole foot and a 20 Gy as a boost on the tumor bed. The first recurrence of the lesion was treated by HILP with cisplatin. After a second recurrence of the malignant fibrous histiocytoma and the patient refusing curative amputation, was treated with HILP with 4 mg TNF, 0.2 mg IFN and 45 mg melphalan. Already some hours after TNF perfusion, not only the tumor on the foot showed a bluish color, but also the area that had been irradiated three years ago. Nine days after TNF perfusion a lower leg amputation had to be performed because of severe necrosis of the foot. Histology showed complete necrosis of the tumor and marked thrombosis of the smaller vessels of the foot. TNF did not only have a damaging influence on the endothelial cells of the tumor, but also on the endothelial cells that developed after high dose irradiation therapy. An explanation of the observed dramatic effect was described and the case served to alert other surgeons in the field of TNF perfusions in treating patients with a history of irradiation therapy.

Chapter 6

In order to study the glucose metabolism of soft tissue sarcomas before and after HILP with TNF, IFN and melphalan, a FDG-PET study was performed in 20 patients before to, 2 and 8 weeks after HILP. After the final PET study, the tumor was resected and pathologically graded. Patients with a pathologically complete response (pCR) showed no viable tumor after treatment. Those with a pathologically partial response (pPR) showed various amounts of viable tumor in the resected tumor specimens. Seven patients showed a pCR (35%) and 12 patients showed a pPR (60%). In one patient, pathological examination was not performed (5%). The pre-perfusion glucose consumption in the pCR group was significantly higher than in the pPR group (p<0.05). Visual analysis of the PET images after perfusion showed a rim of increased FDG uptake around a core of absent FDG uptake in 12 patients. The rim signal contained a fibrous pseudocapsule with inflammatory tissue in the pCR group, but viable tumor tissue was seen in the pPR group. The glucose consumption in the pCR group at 2 and 8 weeks after perfusion had decreased significantly (p<0.05) compared with the glucose consumption in the pPR group. Based on the pretreatment glucose consumption in soft-tissue sarcomas one could predict the probability of a patient achieving a complete pathologically response after TNF HILP. FDG-PET indicated the pathologic tumor response to HILP, although the lack of specificity of FDG, in terms of differentiation between an inflammatory response and viable tumor tissue, hampered the discrimination between pCR and pPR.
Chapter 7

After we had studied the glucose metabolism of tumors before and after HILP and were confronted with the inability of FDG to discriminate between viable tumor tissue and inflammatory tissue we decided to study the protein metabolism of tumors. L-[1-11C]-tyrosine (TYR) was used as a tracer to study the protein metabolism before and after HILP. Seventeen patients (5 women, 12 men; age range 24-75 y; mean age 52 y) were studied. TYR-PET studies were performed before HILP, and 2 and 8 weeks afterwards. The protein synthesis rates (PSRs) in nanomoles per milliliter per minute were calculated. After the final PET study, the tumor was resected and pathologically examined. Patients with a pathologically complete response (pCR) showed no viable tumor after treatment. Those with a pathologically partial response (pPR) showed various amounts of viable tumor in the resected tumor specimens. Six patients showed a pCR (35%) and 11 patients showed a pPR (65%). All tumors were depicted as a hot spot on the PET study before HILP. The PSR in the pCR group at 2 and 8 weeks after perfusion had decreased significantly (p<0.05) compared to the PSR before HILP. A significant difference was found in PSR between the pCR and pPR group at 2 as well as at 8 weeks (p<0.05). Median PSR in nonviable tumor tissue was 0.62 and ranged from 0.22 to 0.91. With a threshold PSR of 0.91, sensitivity and specificity of TYR-PET were 82% and 100%, respectively. The predictive value of a PSR > 0.91 for having viable tumor tissue after HILP was 100%, whereas the predictive value of a PSR ≤ 0.91 for having nonviable tumor tissue after HILP was 75%. On pathological examination the 2 patients in the pPR group with a PSR < 0.91 showed microscopic islets of tumor cells surrounded by extensive necrosis. Inflammatory tissue after treatment did not interfere with viable tumor tissue on the images. Combining the results of the FDG and TYR-PET studies, we concluded that FDG-PET predicted the probability of a patient achieving a pathological complete response after perfusion and TYR-PET gave a good indication of the pathological outcome.

Chapter 8

With the introduction of TNF, the monitoring of leakage of the isolated circuit into the systemic circulation is mandatory since TNF levels in the perfusion circuit are approximately 10 times the maximum tolerated systemic levels. If significant leakage occurs during HILP the resultant TNF induced systemic inflammatory response syndrome (SIRS) could be fatal. The aim of this study was to analyze the value of continuous leakage monitoring with radioactive Iodine-131 labeled human serum albumin (RISA) in patients treated with HILP with TNF and melphalan. Forty-eight patients with melanoma (n = 14) or soft tissue sarcoma (n = 34) of an extremity
underwent 51 perfusions. Perfusion was performed at the iliac level in 22 cases, at the popliteal level in 16 cases, at the femoral level in 7 cases and in 6 cases at the axillary level. Leakage rates, perfusion circuit and systemic levels of TNF, interleukin-6, C-reactive protein (CRP) were determined, as were systemic hematological and metabolic profiles and tumor response. The mean isotopically measured leakage was 2.9 % (95% confidence interval 2.0 – 3.8%, range 0-15.5%). Systemic leakage was ≤2% in 28 perfusions (55%) and >2% in 23 perfusions (45%). The correlation between the maximal monitored leakage and the maximal systemic TNF levels was 0.7114 (p < 0.0001). The area under the curve (AUC) for TNF in the perfusion circuit, indicating the exposure of the perfused limb to TNF, was 18.7% lower in the >2% leakage group (p=0.0457). No significant differences in tumor response were found between groups. AUC for systemic TNF, indicating the exposure of the patient to TNF, was 18.1 times higher in the >2% leakage group (p<0.0001) resulting in a significant decrease in leukocyte and platelet count, hyperbilirubinemia, hypcholesterolemia and proteinemia. No beneficial effect of the systemically leaked TNF and melphalan was seen on the occurrence of distant metastasis during follow-up. There was a significant difference between perfusions performed at the iliac and femoral levels compared with leakage values at the popliteal level, p < 0.0001 and 0.0159 respectively. A good correlation between RISA leakage measurement and TNF exposure during and after HILP with TNF and melphalan was demonstrated. RISA leakage measurement serves as a good guide for the effectiveness of isolation during perfusion. If leakage exceeds the 2% limit during perfusion, less exposure of the tumor bearing limb to TNF, increased exposure of the patient systemic circulation to TNF, and more systemic side effects can be expected.

Conclusions

1. HILP with cisplatin in dogs with spontaneous osteosarcoma can be done safely with improvement of clinical and radiological parameters although histological results were modest.
2. Since we encountered an unacceptable mortality and morbidity rate in HILP with TNF and cisplatin in healthy dogs, an experiment in dogs with spontaneous osteosarcoma was not initiated.
3. HILP with cisplatin in patients with sarcomas of soft tissue and bone resulted in a reduction of pain after treatment. However, the histological outcome was moderate.
4. HILP with TNF, IFN and melphalan does not only have an effect on the vasculature of a tumor, but can also elicit an activation of the vasculature originated after irradiation therapy.
5. By studying the glucose metabolism of a tumor before HILP with TNF and melphalan with the aid of FDG-PET, an assumption can be made about the reaction of the tumor to TNF HILP.

6. In order to evaluate the result of HILP with TNF and melphalan one should perform a TYR-PET study to measure the protein metabolism of the tumor after HILP.

7. In order to determine leakage of TNF from the isolated circuit of the extremity to the systemical circulation of the patient, continuous recording with radioactive Iodine-131 labeled human serum albumin is an appropriate procedure.