Journal of Surgical Oncology 2014;109:332–337

Isolated Limb Perfusion with Melphalan for Melanoma

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Isolated limb perfusion with melphalan is a well-established and effective treatment for inoperable melanoma metastases of the extremities, with an overall response rate of 80% and a complete response rate of 54%. The surgical technique is complex and serious morbidity can occur, but with attention to detail major side effects can be kept to a minimum. This article reviews the technique, results and other aspects of this sophisticated form of treatment.

J. Surg. Oncol.2014;109:332–337 © 2014 Wiley Periodicals, Inc.

KEY WORDS: metastases; regional chemotherapy; limb perfusion; review

INTRODUCTION

Creech et al. [1] at Tulane University in New Orleans were the pioneers who developed isolated limb perfusion. In 1957, they described a 76-year old man with an extensive melanoma recurrence of his leg in whom a complete response was obtained. The man remained free of disease and died 16 years later from another cause. Subsequently, isolated limb perfusion became a well-established treatment option for inoperable melanoma metastases of the extremities. Perfusion exploits the ability of normal tissues in the extremities to tolerate higher drug concentrations than the vital organs. The rationale is that melanoma is sensitive to cytotoxic drugs, but the disease requires a higher dose than is customary in other types of cancer. In the isolated limb, drug concentrations of up to 20 times the level that would be tolerated in the rest of the body may be reached [2]. Therefore, perfusion with a high dose of cytotoxic medication may achieve regional tumor control without major toxicity to the normal tissues of the limb and without exposing the vital organs to high drug concentrations. This is achieved by isolating the limb from the body's circulation and establishing a separate oxygenated and heated extracorporeal blood circulation powered by a pump. Perfusion is a particularly useful technique for patients with in-transit metastases from melanoma. In-transit metastases are metastases that occur in the lymph vessels in the skin or subcutaneous tissue. These lesions are typical for melanoma and occur in some 6% of the patients. One or a few of these metastases can be excised but they have a tendency to recur, often in larger numbers. Perfusion provides the opportunity to treat the entire limb and remove not only the lesions that are evident but also the smaller ones that would become evident later. This article reviews the technique of isolated limb perfusion, its indications, results and toxicity. Finally, some thoughts on the future of this remarkable form of treatment are shared.

PATIENTS AND TECHNIQUE

Patients

Isolated limb perfusion can be considered in patients with an inoperable primary melanoma, a local recurrence, numerous in-transit metastases or frequently recurring in-transit metastases. The general condition of the patient should be assessed and the surgeon should be aware of concomitant diseases, allergies, and medication. A detailed examination of the skin, the subcutaneous tissues and the regional node field is performed, and the arterial blood supply to the limb is assessed.

The presence of regional dissemination should be confirmed by pathology examination. The stage of the disease should be determined and the combination of whole body FDG PET/CT and MRI of the brain is reasonable for the purpose. The finding of metastases elsewhere may change the treatment plan. Systemic therapy should then be contemplated, particularly now that new drugs are available that have been shown to improve survival.

Arteriography can be performed if the arterial blood supply appears questionable. Perfusion is not feasible if there is complete obstruction of the main artery of the limb. A tumor marker can sometimes be used to monitor the effect of the treatment and may draw attention to a recurrence at an early stage. Contraindications are listed in Table I. Advanced age or extensive arterial calcification are not contraindications per se [3].

Technique

Perfusion requires a concerted effort by the surgeon, the perfusionist, the nuclear medicine physician and the customary operating room team. L-phenylalanine mustard (melphalan) is the standard drug used. Phenylalanine has a key role in the synthesis of melanin. Incorporation of melphalan into them leads to destruction of melanoma cells. The dosage of the drug is adjusted to the requirements of the individual patient. The melphalan dose is usually based on the volume of the extremity, which can be determined using a water reservoir. Parameters such as gender and obesity may lead to adjustments in the dose. The standard dose is 10 mg/L perfused tissue for the lower limb and 13 mg/L for the upper limb [2]. The addition of tumor necrosis factor alpha has been reported to be beneficial in patients with bulky tumor nodules; perfusion with this drug combination is discussed in a separate article in

Conflicts of interest: none.

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Received 03 September 2013; Accepted 16 December 2013

DOI 10.1002/jso.23558

Published online 14 January 2014 in Wiley Online Library (wileyonlinelibrary.com).

TABLE I. Absolute and Relative Contraindications to Perfusion

Absolute contraindications
Obstruction of target artery or subsequent major artery
Diabetes with serious peripheral vascular disease
Child with open epiphyseal plates
Relative contraindications
Prior radiotherapy
Large superficial tumor with major tendon involvement
Brain metastases
Wound or ulcer with serious infection

this seminar edition. The use of other agents has been investigated, including cisplatin, vindesine, DTIC, fotemustine, interleukin-2, and lymphokine-activated killer cells, but only actinomycin (in combination with melphalan) has been widely used [4–12].

There is considerable variation in the perfusion technique between institutions. We describe here the procedure as it is carried out at The Netherlands Cancer Institute. General anesthesia is used. Epidural anesthesia is risky in a patient who is to be fully heparanized. It also induces vasodilatation and predisposes to leakage of blood from the systemic circulation to the perfusion circuit, and for these reasons is not recommended. Perfusion may be conducted in the lower limb at the level of the external iliac vessels, at the femoral level or the popliteal level and in the upper limb at the axillary or brachial level. The main artery and vein of the limb are dissected clear and collateral vessels are ligated to prevent leakage to and from the systemic circulation. Heparin is administered. The vessels are clamped proximally and distally, incised and the canulae are inserted. The canulae are connected to the perfusion circuit. Approximately 300 ml autologous blood is tapped from the vein and added to the priming volume of the extracorporeal circuit. Isolation is finalized by wrapping a rubber bandage or inflatable tourniquet around the root of the limb to compress the smaller vessels in the muscles and subcutaneous tissue (Fig. 1). The venous pressure is monitored through a distal vein. Thermistor probes are inserted into the subcutaneous tissue and a muscle compartment to monitor the temperature. The limb is typically cool at this stage and is wrapped in a heating blanket to attain the desired initial temperature of 37°C.

The perfusion circuit consists of a reservoir to collect the venous blood, an oxygenator, a heat exchanger and a roller pump. The melphalan is administered into this circuit. The highest possible flow rate is used without increasing the venous pressure more than 10 cm above its original value. Leakage of melphalan into the systemic circulation can cause substantial morbidity and is to be avoided. A small dose of a radiopharmaceutical like technetium 99 m-labeled serum human albumin is added to the perfusion circuit. Leakage of this tracer into the systemic circulation is monitored continuously by a gamma ray detector placed over the heart [14].

Tissue Temperatures

Physiological conditions are pursued as much as possible to minimize damage to the normal tissues. The tissue temperatures of the limb are kept between 37°C and 38°C during the procedure. This is called "controlled" normothermia. The temperature of the limb is typically below this range at the start of the perfusion. Warming the perfusate and wrapping the limb in a heating blanket help to reach the desired temperature and can prevent the limb from cooling during the procedure [15]. The drug is added to the perfusate when 37°C is reached. The benefit of the often-recommended "mild" hyperthermia with tissue temperatures between 39°C and 40°C is questionable because the results are not better [16]. Temperatures above 41.5°C have a specific cell-killing effect [17]. Melphalan perfusion with "true" hyperthermia implies temperatures between 41.5°C and 43°C and is not recommended because of unacceptable potentiation of melphalan, although reports of favorable responses have been published [18]. Interestingly, perfusion with true hyperthermia without cytotoxic drug is a reasonable option [19]. At our institution, a double perfusion schedule was tested with 2-hr true hyperthermic perfusion with tissue temperatures between 42°C and 43°C without melphalan, followed by normothermic perfusion with melphalan at regular dosage 1 week later [20]. The hypothesis was to kill cells in the hypoxic parts of the tumors with the hyperthermia and

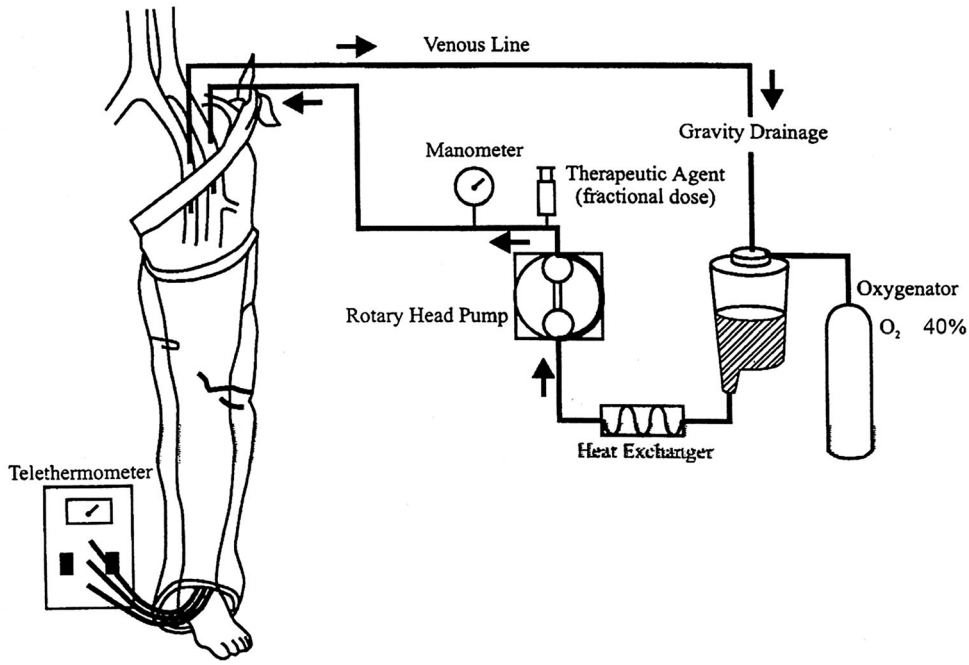

Fig. 1. Schematic drawing of isolated perfusion circuit (with kind permission of Springer Science + Business Media) [13].

the rest of the lesions with the melphalan in the subsequent week [21]. With this sequential schedule, both hyperthermia and melphalan were given at the maximum dose without encountering the substantial toxicity that simultaneous treatment would have caused. With this approach, a high complete response rate (63%) and a low limb recurrence rate (27%) were seen in seventeen patients with extensive, recurrent melanoma [22]. The morbidity was mild. This regimen could be considered as an alternative to perfusion with the combination of melphalan and tumor necrosis factor alpha in patients with extensive or bulky disease.

RESULTS

Locally Advanced Melanoma

Inoperable melanoma involvement of a limb is a generally accepted indication for isolated limb perfusion with melphalan. Although the majority of patients with extensive limb involvement will die from their disease, one should aim for cure if staging shows no metastases elsewhere. A review of the literature revealed an average complete response rate of 54% [6,23]. This is higher than the 45% at our institution [24]. This difference is probably due to a difference in tumor load. The number of lesions and their total surface area are important parameters that predict a response [25,26]. Necrosis of the metastases may become evident overnight but on average it takes three months for a complete response to develop [6]. It can take up to 9 months. Approximately 50% of patients with a complete response recur in the perfused limb after a median interval of 6 months following treatment. These recurrences can be managed by simple local treatment modalities such as excision or laser ablation in 70% of the patients. Ten-year survival in patients with a complete response is 49% [6]. Long-term survivors have a better quality of life than comparable control individuals [27].

About 25% of patients develop a partial response after isolated limb perfusion with melphalan [6]. Half of these can also be managed by simple forms of regional treatment. With this approach, our limb salvage rate in patients with truly unresectable disease is 96%. Amputation for intractable recurrence is required in 2.4% of the patients [28].

The presence of distant melanoma metastases does not preclude regional perfusion. Adequate palliation is often achieved in patients with symptomatic but unresectable local/regional limb involvement [29,30]. In particular, palliative perfusion should be contemplated in patients with distant cutaneous or subcutaneous metastases or distant lymph node metastases as they often survive for more than a year.

Double Perfusion

A double perfusion schedule was pioneered at our institution, based on the principles of fractionation and protraction [31]. This approach exploits the difference in tolerance to cytotoxic therapy between malignant cells and normal cells. The normothermic perfusions were performed at two levels of the lower limb. A high complete response rate of 77% was obtained in 43 patients with recurrent melanoma. The morbidity was acceptable. A shorter time interval between perfusions was associated with a better complete response rate. The limb recurrence rate was similar to that following single perfusions. Development of effective systemic consolidation therapy may decrease the recurrence rate.

Repeat Perfusion

A recurrence develops in 46–54% of patients after an initially successful perfusion [32]. Treatment of recurrence varies depending on the extent of disease, and may consist of excision, CO_2 laser ablation, radiotherapy with or without local hyperthermia, electrochemotherapy, or intralesional chemo- or immunoablation [33–36]. If the lesions are too large or too numerous, or recur too often, another perfusion with melphalan with or without tumor necrosis factor alpha may be considered to stave off amputation. Repeating a perfusion at the same level is technically challenging as the vessels are often embedded in rigid fibrosis. A different level is more attractive, for example, femoral instead of iliac. One may expect a favorable response from the second perfusion. We examined the results of repeat isolated limb perfusion with melphalan, using various schedules, both single and multiple, normothermic and hyperthermic perfusions [37]. A high complete response rate of 74% was obtained, with a limb recurrence-free interval of nine months. However, the associated regional toxicity was substantial.

Adjuvant Perfusion for High-Risk Primary Melanoma and Recurrent Melanoma

The favorable results in patients with extensive regional metastases generated the question whether perfusion could also be effective in adjuvant settings. A multicenter randomized clinical trial involving 852 patients examined perfusion as an adjunct after excision of high-risk primary melanomas, defined as a Breslow thickness of at least 1.5 mm [38]. The patients underwent wide local excision of their melanoma and were randomized to perfusion or observation. Initially, disease-free survival was significantly better for the patients in the perfusion group who did not undergo elective lymph node dissection ($P = 0.02$). Later, the survival curves came back together and in the end disease-free survival and overall survival were similar in the two groups. There was a beneficial impact of perfusion on the occurrence of in-transit metastases, which was reduced from 6.6% to 3.3% ($P = 0.05$). The incidence of lymph node metastases was reduced from 16.7% to 12.6%. Perfusion thus appeared to sterilize tumor cells in lymph vessels and nodes.

Another randomized study examined the value of adjuvant perfusion in 69 patients with resectable recurrent melanoma [39]. After radical excision of their local recurrence, satellites and/or in-transit metastases, patients were subjected to adjuvant perfusion or they were observed. Perfusion reduced the loco-regional recurrence rate from 67% to 45% ($P = 0.13$). The median disease-free interval was prolonged to 17 months after perfusion compared to 10 months after excision only ($P = 0.04$). The 44% 5-year overall survival in the perfusion group appeared slightly better than the 39% in the observation arm, but the difference was not statistically significant.

The conclusions of these two studies were that isolated limb perfusion is not an effective adjuvant treatment option. Still, one can also deduce that there is a cytotoxic effect on micrometastases, because perfusion postpones recurrence and reduces the number of recurrent lesions. We therefore hypothesized that adjuvant perfusion may have a place in patients with multiple and frequently recurring resectable in-transit metastases. The hypothesis was tested in 43 patients, in whom metastases had been excised at least three times [40]. The median limb recurrence-free interval in these patients had decreased significantly between time of the primary excision and the third or fourth limb recurrence. Perfusion was performed when the patients recurred once again. Afterwards, the median limb recurrence-free interval was 4.7 times longer than prior to the perfusion ($P < 0.001$). The mean number of subsequent lesions was 2.6 fold less compared to before perfusion ($P < 0.001$). Perfusion in this study thus lengthened the limb recurrence-free interval and decreased the number of recurrences significantly. These results justify the conclusion that perfusion is a valuable intervention in patients with repeatedly recurring in-transit metastases whose recurrence-free interval is steadily decreasing.

Perfusion in Elderly Patients

The mean life expectancy at age 75 is 9 years for males and 11 years for females [41,42]. The incidence of in-transit metastases increases with

advancing age [43]. Sometimes, we receive a call from a fellow surgeon who does not know what to do in a patient with non-resectable limb recurrence, and who says that "perfusion is not an option because of the the patient's advanced age." We have never regarded advanced age as a contraindication and have had a favorable experience with these patients. In a study of 202 patients combining data from two Dutch centers, the complete response rate in patients over 75 years of age was 56% compared to 58% in younger patients [3]. Approximately half of the patients with a complete response achieved long-term local regional disease control in either age category. Although the hospital stay was somewhat longer in the older patients, acute toxicity, postoperative complications and long-term morbidity were not related to age. The conclusion was that older patients can safely undergo perfusion and profit as much as younger people. Therefore, advanced age is not necessarily a contraindication for isolated limb perfusion.

MORBIDITY AND MORTALITY

Regional Morbidity

Perfusion usually results in slight postoperative erythema and edema of the limb. The edema and redness subside over a period of 2–3 weeks and give way to a tan discoloration that gradually disappears over the course of several months. Blistering is sometimes seen in the first few days. Serious postoperative complications are rare but they demand urgent intervention. Arterial thrombosis is known to occasionally happen in the first few hours. Thrombectomy should be performed and a venous patch is often needed to ensure sufficient flow. Acute muscle edema can lead to a compartment syndrome. The serum creatine phosphokinase level can be used to gauge muscle damage [44]. Timely fasciotomy of the involved muscle compartment prevents permanent damage. Some surgeons perform prophylactic fasciotomy routinely [45]. Postoperative muscle damage necessitates amputation in 0.9% of the patients [46].

There appears to be no relationship between limb toxicity and tumor response to the treatment [47]. Thus, there is no reason to push the drug dose to the limit of tolerable morbidity. The Wieberdink classification is used to quantify limb morbidity (Table II) [48].

The most important risk factors for severe acute regional toxicity are tissue temperatures above 40°C, a high melphalan peak concentration in the perfusate, female gender and obesity [18,49]. The higher melphalan uptake in muscle compared to fat is likely the reason why obese patients are more prone to morbidity. The dose of melphalan is based on the volume of the limb. As a result, muscle tissue in obese people is exposed to a relatively higher drug dose [50]. To avoid morbidity in these patients, one can lower the melphalan peak concentration without decreasing the absolute drug dose by using a larger priming volume and by prolonged or fractionated administration [51,52]. The dose of melphalan is often reduced by 10% in obese patients [49].

The degree of acute regional toxicity is related to long-term complications [53]. Long-term morbidity is seen in 44% of patients: recurrent infections 3%, neuropathy 4%, pain 8%, muscle atrophy or fibrosis 11%, limb malfunction 15%, or lymphedema 28% [53]. The lymphedema can often be attributed to concomitant lymph node dissection, however. Restriction of movement in the ankle joint is reported in 25% of patients [45,54,55]. Chronic pain is encountered in 5–8% of patients [53,55]. Long-term neuropathy is seen in 20% after axillary perfusion and in 2% after perfusion at the iliac level [56].

Systemic Morbidity and Mortality

Systemic toxicity can be avoided by adequate isolation of the limb. This can be assured by meticulous ligation of collateral vessels, avoiding a high flow rate, keeping the venous pressure in the limb low and stable, and by continuous monitoring of leakage. In 438 procedures, we measured a mean cumulative systemic leakage of $0.9 \pm 2.0\%$ (range 0–15.6%) after 60 min of perfusion [14]. A thorough wash-out of the limb at the end of the procedure limits the fraction of perfusate that reaches the systemic circulation to a few percent. With these precautions, systemic toxicity is mild or even absent [57]. Nausea and vomiting are the most frequently encountered side-effects. The perioperative mortality is less than 1% [46,57].

TABLE II. Wieberdink Classification of Postoperative Morbidity to Extremity[48]

1	No skin reaction
2	Erythema, edema
3	Blisters
4	Superficial necrosis, damage to deep tissues causing functional disturbance, threatening or manifest compartmental syndrome
5	Necrosis requiring amputation

The Future

Melphalan has been the standard drug ever since regional perfusion was introduced in 1958 [1]. Since then many new cytotoxic drugs have been developed and tested in the perfusion setting. Unfortunately, none could replace melphalan, but the utility of other drugs will continue to be explored.

Promising preliminary results of consolidation systemic biotherapy to extend the duration of complete remission in the limb have been published [12]. This combined regional and systemic approach deserves further study. A number of studies are addressing the combination of systemic targeted therapies with regional treatment strategies [58]. Targeted therapies like butathione sulfoximide and systemic ADH-1 (Exherin) as adjuncts to melphalan may disrupt various cell signaling pathways and may make the tumors more susceptible to a cytotoxic agent. This approach can possibly increase the response rate without causing additional toxicity [59]. Despite research efforts in the field, the standard regional perfusion procedure has changed little since the introduction of tumor necrosis factor alpha in 1992 [60].

Isolated limb *in*fusion is a more recent, minimally invasive procedure that was developed as an alternative to isolated limb *per*fusion. Infusion was pioneered at Melanoma Institute Australia (formerly known as Sydney Melanoma Unit) [61,62]. Recent reports from other centers suggest a lower fraction of complete responders compared to perfusion but no study to date has directly compared the two procedures [63–65]. The morbidity from infusion appears to be somewhat greater. Perfusion is the more challenging procedure of the two for a surgeon. It is a complex operation that requires a keen eye for detail because of the potential toxicity.

Will infusion replace perfusion? The relative ease of infusion is attractive to surgeons, but the seemingly higher response rate and lesser morbidity assure the place of perfusion in the therapeutic repertoire of melanomologists. Will perfusion be replaced by systemic therapy with new agents such as like vemurafenib and ipilimumab? The high 54% complete response rate of perfusion and its modest morbidity compare favorably with the 0.9% and 1.6% complete response rate with substantial morbidity of the new drugs [66,67]. We feel that at present perfusion (and infusion) remain the sensible first choice for patients with extensive disease limited to a limb. For patients who also have distant metastases, however, systemic therapy with the new drugs may be a more attractive option.

CONCLUSIONS

Isolated regional perfusion is an unusual form of therapy, specifically suitable for the biology of melanoma with its peculiar in-transit

dissemination. Perfusion is effective across the entire range of metastases from subclinical disease to bulky lesions. Isolated limb perfusion with melphalan results in a complete response in over half of the patients with extensive disease in an extremity. Such a response is durable in half of these individuals. Half of the patients with a complete response survive for ten years, most with an excellent quality of life. In patients who continue to develop in-transit metastases, perfusion can delay and diminish subsequent limb recurrence. Given the complexity of the technique and its potential toxicity, however, this form of treatment is best restricted to specialized melanoma treatment centers.

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