Hypoxic pelvic and limb perfusion with melphalan and mitomycin C for recurrent limb melanoma: a pilot study
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Hypoxic pelvic and limb perfusion by means of a balloon occlusion technique was evaluated in patients with recurrent melanoma of the lower limbs who were non-responders to isolated hyperthermic limb perfusion or who were not eligible for this procedure. A pilot study was performed in 17 patients, who underwent hypoxic pelvic and limb perfusion with 50 mg/m² of melphalan or 50 mg/m² of melphalan and 25 mg/m² of mitomycin C. Each procedure was followed by haemofiltration. A leakage monitoring study was performed in five of the 17 patients. The response rate and time to disease progression were the primary endpoints, with overall survival as the secondary endpoint. During the procedures there were no technical, haemodynamic or vascular complications, and no deaths occurred during surgery or in the postoperative period. Significant leakage (median 40%) was measured in the five patients studied. No severe systemic or regional toxicity was observed. After one course of treatment, the objective response rate was 47% (95% confidence interval 22.5–71.5%), the median time to disease progression was 10 months (range 2–40 months), and the 3 year overall survival was 20%. Hypoxic pelvic and limb perfusion seems to be a safe and effective treatment for patients with unresectable recurrent limb melanoma who are not eligible for isolated hyperthermic limb perfusion. Due to the non-homogeneity of the study, with some patients receiving a combination of melphalan and mitomycin C and others receiving only melphalan, it is not possible to make definite conclusions with regard to efficacy. Further studies are necessary to establish whether the response rates can be improved by using different drug regimens. Melanoma Res 13:51–58 © 2003 Lippincott Williams & Wilkins.

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Introduction
The preferred treatment of patients with in-transit metastases and/or recurrences of lower limb melanoma is isolated hyperthermic limb perfusion with melphalan-based chemotherapeutic regimens. This procedure gives complete tumour responses in 40–50% and objective responses in 70–90% of cases [1], while subsequent locoregional recurrence is seen in 20–40% of cases [2]. Sometimes, however, this procedure cannot be considered because of advanced age, co-morbid conditions or previous surgery. The treatment of patients not eligible for isolated hyperthermic ablative limb perfusion and/or patients refractory or with recurrences after this procedure is still controversial. Fortunately, these are unusual clinical situations.

With the objective of achieving the benefits of isolated hyperthermic ablative limb perfusion whilst avoiding the complexity, cost and patient morbidity, in 1994 Thompson et al. [3] proposed an alternative technique of hypoxic perfusion of the limbs, also using melphalan, for patients with recurrent melanoma. During hypoxic perfusion there are changes in the microenvironment, including tissue hypoxia and an endocellular decrease in pH. Some antitumour agents [4] are more cytotoxic in hypoxic conditions, in particular mitomycin C [4,5], but recent in vitro studies [6] and experiments in animals [7] have shown that the antitumour activity of melphalan is also increased in hypoxic conditions. In 1995, the technique proposed by Thompson et al. [3] was used by Fiorentini et al. [8] to administer mitomycin C in four patients with recurrent melanoma. In 1998, Thompson et al. [9] published data from 164 hypoxic perfusions performed for melanoma, recommending this technique because it was effective as conventional hyperthermic ablative perfusion, but was simpler and associated with less risk of complications and lower costs. This procedure is, however, not

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effective in patients with lesions in the inguinal or upper thigh regions because of the limits of the perfused compartment; the upper limit is located a few centimetres distal to the adductor hiatus.

This pilot study presents the preliminary results of a technique for hypoxic perfusion of the pelvis and lower limbs, as proposed by Aigner and Kaevel in 1994 [10], which is effective even in the presence of lesions located in the inguinal or upper thigh regions, in contrast to the method of Thompson et al. [3]. This technique was used in the treatment of melanoma patients with in-transit metastases and/or recurrences in the lower limbs when isolated hyperthermic antiblastic limb perfusion was not feasible because of advanced age, vascular disease, general frailty, or previous lymph node dissection of ipsilateral iliac and inguinal vessels. The treatment was also used in cases of non-response to or subsequent recurrence after hyperthermic perfusion. The drugs used were melphalan alone in accordance with the results of the trials on isolated hyperthermic perfusion [11], or melphalan and mitomycin C in view of their potential activity in hypoxic conditions. A leakage monitoring study was also performed.

Patients and methods

Patients

Written consent was obtained from 17 patients (six men and 11 women) after they were given complete information about the disease and the implications of the proposed treatment, in accordance with the ethical standards of the committees on human experimentation at our institutions (Table 1). The patients had a mean ± SD age of 56.5 ± 12 years (range 37–74 years), a mean body weight of 64.8 ± 13 kg, and a mean surface area of 1.71 ± 0.15 m².

All patients had multiple nodules of recurrent melanoma. In two patients the tumours were located in the upper part of the thigh. Although the more recent staging system for melanoma [12] would classify all the patients as stage III, for this study the patients were subdivided according to the less recent MD Anderson staging system [13] to make clear the regional node involvement (Table 1). The existence of an arterial blood supply was confirmed in all tumours by computed tomography (CT). All patients had lymphoedema in the involved limb.

Previous treatment of recurrence involved surgery in 15 patients (followed by systemic chemotherapy with dacarbazine and interferon-α2a in one patient) and interferon-α2a alone in two patients. Surgery included lymph node dissection of the ipsilateral iliac and inguinal vessels in nine patients. In two patients, recurrence occurred after surgery plus isolated hyperthermic antiblastic limb perfusion with melphalan.

All patients were free from metastases in other sites (based on CT evaluation), renal and liver failure, deep venous thrombosis, severe atherosclerosis or coagulopathy. They had a life expectancy of more than 3 months and were able to function with some independence (Karnofsky score of at least 60). During the month preceding the perfusion no chemotherapy was administered to the patients. Angiography of the aortoiliac tree and inferior vena cava was carried out before perfusion was performed.

This pilot study was undertaken after approval was obtained from the investigational review boards, bearing in mind that these patients had a disease with a predictable fatal outcome.

Anaesthesia

Before the perfusion was performed, patients received a short-term bowel preparation and hydration. After an overnight fast, each patient received a single subcutaneous dose of 12 500 IU of heparin calcium and a single intramuscular injection of 1.0 g of cefotaxime sodium. All patients were premedicated 45 min before surgery with an intramuscular injection of promethazine hydro-
chloride 50 mg and atropine sulphate 0.5 mg. Induction of anaesthesia was obtained with propofol at a dose of 1.5–2.5 mg after the administration of 0.1 mg of fentanyl citrate 5 min before surgery. Endotracheal intubation and controlled mechanical ventilation were initiated after the production of muscle relaxation with 1 mg/kg of succinylcholine chloride. Maintenance of anaesthesia was obtained with a mixture of oxygen and nitrous oxide at the rate of 1:3 to 2:3, isoflurane at concentrations varying from 0.5–1.5%, and pancuronium bromide at a dose of 0.05 mg/kg. During the procedure, either 0.7 mg/kg urapidil or intravenous nitroglycerin at a dose of 0.006 mg/kg per min was administered whenever arterial pressure levels exceeded 40% of baseline values. The bladder was emptied before the initiation of the perfusion.

**Hypoxic pelvic and limb perfusion technique**

The femoral artery and vein of the opposite side to the affected limb were exposed through a short longitudinal incision in the groin. After systemic heparinization (150 U/kg), a three-lumen balloon catheter (PfM, Cologne, Germany) was introduced into the inferior vena cava via the saphenous vein and a second one into the aorta via the femoral artery; these were positioned under fluoroscopic control below the renal vessels and above the aortic and venous bifurcation using a guidewire. Both balloons were filled with isotonic sodium chloride solution containing the radiopaque dye diatrizoate, and blocked. For isolation of the pelvis and limb, a large-cuff orthopaedic tourniquet, placed around the sound limb at the upper thigh just below the lower level of the femoral triangle, was inflated just before starting the perfusion.

The infusion channels of the arterial and venous stop flow catheters were connected to a hypoxic perfusion set on a roller pump. The set was primed with isotonic sodium chloride solution diluted in 250 ml of isotonic sodium chloride containing heparin (10 000 U). Once the flow was established (approximately 200 ml/min), the drug therapy was started. The drugs, diluted in 250 ml of isotonic sodium chloride solution also containing 16 mg of dexamethasone sodium phosphate, were administered into the aorta over 3 min. The extracorporeal circuit (Fig. 1) also included a haemofiltration system (Hemoflux 20; Gambro, Lund, Sweden) with a surface area of 2 m² and a heater-cooler unit (Polystan, Vaerlose, Denmark). The hypoxic perfusion circuit was maintained over 20 min (mean 23 ± 3 min). The temperature of the perfusate was approximately 38°C.

After perfusion, both the catheter balloons and the pneumatic cuff were deflated and the circulation restored. The haemofiltration section of the extracorporeal circuit was used for a further 80 ± 20 min. The catheters were then withdrawn and the vessels repaired.

**Chemotherapeutic regimens**

One course of perfusion was administered. A second treatment was performed in two patients with the purpose of overcoming melphalan resistance. Two regimens were used at random: (i) melphalan at a dose of 50 mg/m² (eight patients); (ii) melphalan at a dose of 50 mg/m² and mitomycin C at a dose of 25 mg/m² (nine patients). The doses and the duration of the locoregional drug exposure were determined based on the results of previous *in vitro* and *in vivo* studies [4,6,8,10,11].

**Leakage monitoring procedure**

In order to obtain the highest accuracy in leakage monitoring, a nuclear medicine technique using human serum albumin (HSA) labelled with technetium-99m (99mTc) was adopted. Leakage was detected using a precordial hand-held gamma probe. The amount of HSA injected into the extracorporeal circuit was normalized on the basis of 0.5 MBq/kg body weight. The correct individual dose was calculated by the nuclear medicine department from an accurate radionuclide dose-meter, taking into account the decay time of 99mTc (6 h). A simulation of the individual leakage monitoring was performed 72 h before the hypoxic perfusion using 10% of the HSA dose calculated for the specific patient with the purpose of measuring the maximum count rate (counts/min) for an hypothetical 10% leakage rate and to determine the optimal position in the heart area for the gamma probe. The count rate corresponding to a 1% leakage rate was then calculated. During the hypoxic perfusion, the HSA dose as calculated for the individual was injected into the extracorporeal circuit and the gamma probe was located in the predetermined position on the thoracic wall. Approximately 15–20 measurements were made during the 20 min hypoxic perfusion. The gamma probe was connected to a personal computer to visualize the leakage curve and record the data using appropriate software.

**Response and toxicity criteria**

Response was determined by CT when clinically indicated 30, 60 and 90 days after baseline, according to the following standardized criteria. A complete response was defined as complete disappearance of all directly or radiographically measurable disease, and a partial response was defined as a greater than 50% tumour regression (based on bidirectional measurements). Tumour regression between 25% and 50% was defined as a minimal response, non-response or stable disease was defined as 0–25% tumour regression, and tumour growth greater than 25% was classified as progressive disease.
Toxicity was graded according to World Health Organization (WHO) criteria. Limb toxicity was assessed on the scale proposed byWieberdink et al. [14] as follows: grade I, no visible effect; grade II, slight erythema and/or oedema; grade III, considerable erythema and/or oedema and blistering; grade IV, extensive epidermolysis and/or obvious damage to deep tissues with a threatened or actual compartment syndrome; grade V, severe tissue damage necessitating amputation.

**Statistical analysis**
The response rate and time to disease progression were the primary endpoints, with overall survival as the secondary endpoint. Survival analysis was assessed using the Kaplan–Meier method. All calculations were performed using SAS/STAT software (SAS Inc, Cary, North Carolina, USA).

**Results**
**Tolerability, toxic effects and leakage values**
During the procedures there were no technical (i.e. balloon rupture), haemodynamic or vascular complications, and no deaths occurred during surgery. In the postoperative period, no blood transfusions were required and there were no deaths. Postoperative complications included neutropenia in seven patients (grade 3 in four patients, grade 2 in three patients), which required granulocyte colony-stimulating factor for a
maximum of 5 days. Nausea and vomiting were limited by the routine use of a 5-hydroxytryptamine-3 receptor antagonist. Two patients developed a femoral vein thrombosis, Grade III limb toxicity occurred in one patient, and grade II toxicity in five patients. One patient developed a pulmonary thromboembolism after the second perfusion, which resolved with appropriate treatment. Total hospitalization time ranged from 6 to 21 days (median 9 days).

Leakage monitoring was performed in five of the 17 patients. A high variability in values was detected. The median value was 40% (range 10–85%), with a mean ± SD of 47 ± 30%.

Responses and survival
A partial response (more than 50% tumour regression) occurred in two patients (12%) with two and five melanoma nodules, respectively. Six patients (35.2%) obtained a minimal response, four of which had more than 20 melanoma nodules. Stable disease was observed in five patients. Disease progression occurred in three patients, all of whom had more than 20 melanoma nodules. The response rate was 47%, with a 95% confidence interval of 22.5–71.5%. No difference was found between the response rate of patients treated with melphalan alone and that of patients treated with melphalan and mitomycin C.

Limb oedema was decreased in seven patients, unmodified in four patients and increased in six patients (one case of grade III toxicity) but there was no convincing evidence of a developing compartment compression syndrome.

The median time to disease progression was 10 months (range 2–40 months). No difference was found between the median time to disease progression of patients treated with melphalan alone and that of patients treated with melphalan and mitomycin C. Median survival was 23.8 months (range 4–43 months). Based on cumulative probabilities of survival, 3 year overall survival was 20% (Fig. 2); using the log-rank test, the estimated proportion of surviving patients treated with melphalan was higher than that of patients treated with melphalan and mitomycin C, but the difference (P = 0.07) was not statistically significant.

Follow-up
Curative surgery became possible in two patients. Palliative cytoreductive surgery was performed in one patient who received further treatment with systemic chemotherapy (cisplatin, dacarbazine and vinblastine; CVD regimen). A second hypoxic pelvic and limb perfusion was performed in one patient after a minimal tumour response, producing stable disease. A second hypoxic pelvic and limb perfusion was also performed in another patient after progression of the disease, obtaining a temporary stable disease but disarticulation was necessary. In a patient with a bulky unresectable groin node metastasis, hypoxic perfusion was followed by shrinkage of the tumour mass; this permitted dissection of the node and isolated hyperthermic perfusion using melphalan and tumour necrosis factor-α, and after 30 days a complete tumour response was observed. Three patients received systemic chemotherapy (CVD regimen).

Among the 17 patients, three (17.7%) are alive without evidence of disease after 42, 11 and 10 months, respectively. Of the five patients alive with disease, one developed an abdominal recurrence, the treatment of which required a bowel resection, one refused any kind of chemotherapy, and three, as mentioned above, received systemic chemotherapy.

Discussion
The efficacy of isolated hyperthermic antiblastic limb perfusion in the treatment of patients with in-transit metastases and/or recurrences of lower limb melanoma is well documented. This procedure gives complete tumour responses in 40–50% of cases [1,11], and severe systemic toxicity is rare [15], while subsequent loco-regional recurrence is seen in 20–40% of cases [2]. In a recent multicentre randomized phase II study, an overall response rate of 95% and a median survival time of 29 months have been reported, and a regimen with tumour necrosis factor-α plus melphalan has been recommended as being superior to melphalan alone [16]. However, isolated hyperthermic antiblastic limb perfusion is inevitably associated with some vascular morbidity [17] and not insignificant regional tissue toxicity [18], especially when melphalan and tumour necrosis factor-α are used, and is followed by long-term functional morbidity [19] and neuropathy [20]. Moreover, isolated hyperthermic antiblastic limb perfusion is
a technically complex and costly procedure in which blood transfusion is routinely required. In addition, this procedure is not feasible in some patients because of advanced age, vascular diseases, poor general health, and previous lymph node dissection of ipsilateral iliac and inguinal vessels.

With the purpose of avoiding the complexity, cost and patient morbidity of isolated hyperthermic ablative limb perfusion, in 1994 Thompson et al. [3] proposed an alternative technique of hypoxic low flow perfusion of the limbs, termed isolated limb infusion, performed using percutaneously inserted catheters. In 1998, Thompson et al. [9] reported the results of 164 hypoxic limb infusions with melphalan for recurrent melanoma, confirming the low morbidity and concluding that the frequency and duration of responses after the hypoxic procedure were similar to the previously reported results achieved by conventional isolated hyperthermic ablative limb perfusion. These results have been recently confirmed by other authors [21]. Unfortunately, this alternative and simple technique is not applicable in the presence of lesions located in the inguinal and upper thigh regions.

Based on these considerations, this pilot study was planned to evaluate the technique of regional hypoxic normothermic low flow perfusion, as proposed by Aigner and Kaelvi in 1994 [10], which is applicable in the presence of inguinal and upper thigh lesions and in several patients in whom conventional isolated hyperthermic ablative limb perfusion would not have been considered because of particular patient conditions. As in isolated limb infusion, low flow perfusion should ensure more uniform drug distribution within the tissues in which vascular isolation has been achieved and should enhance the antitumour activity of melphalan as suggested by experiments in rats [7]. Because of possible toxicity to the normal tissues resulting from hypoxia and acidosis, a 20 min period of treatment was selected, taking into account that the uptake of melphalan is rapid and reaches a plateau after 10 min [22]. The choice of melphalan as the chemotherapeutic agent for this type of perfusion was based on the demonstration that the formation of DNA cross-links starts after 20 min of exposure [23], on data from both in vitro [6] and experimental animal studies [7] of drug activity in hypoxic conditions, and on the results of clinical trials [9,11]. The choice of mitomycin C was based on its enhanced activity in hypoxic conditions [4,5], on the demonstration that SK-Mel-28 human melanoma cells have high base levels of DT-diaphorase, a condition generally associated with greater sensitivity to mitomycin C [24], and on the results of a pilot clinical study [8].

The technique adopted in this study is based on the use of catheters introduced via femoral vessels, isolating the compartment to be perfused by endovascular balloon occlusion of the aorta and inferior vena cava. The procedure can be carried out percutaneously [25], but we preferred surgical exposure of the femoral vessels so that lymph node sampling in the contralateral groin could also be performed. The theoretical efficiency of the procedure has been recently demonstrated in a pharmacokinetic analysis evaluating nine treatments [26]. The mean value of the ratios between the melphalan area under the curve (AUC) of the perfused compartment (pelvis and limb) and the melphalan AUC of the systemic compartment was 8:1. However, high variability in the range of melphalan AUC ratio values (4.1:1 to 14.9:1) has been reported; this is attributable to the variability of conditions in different individuals (i.e. collateral venous leak from the perfused circulation to the systemic circulation), and explains why both the type of tumour response and the extent of toxic effects are not accurately predictable. The present study has demonstrated the existence of not insignificant leakage from the perfused compartment (approximately 40%). This is higher than the leakage reported for isolated hyperthermic ablative limb perfusion (approximately 10%) [27] or for the less conventional isolated limb infusion [9] when a same total dose of melphalan is infused. The theoretical consequences of this elevated leakage include more systemic toxicity and less tumour exposure to the infused drugs compared with both isolated hyperthermic ablative limb perfusion and isolated limb infusion. In the present series, severe systemic toxicity was not observed because of the protective action of haemofiltration performed at the end of the perfusion, the usefulness of which has been demonstrated in a previous study [26]. With regard to the theoretical suboptimal tumour exposure to chemotherapeutic agents, this seems to be confirmed by the lower response rates observed (approximately 50%) compared with those reported for both isolated hyperthermic ablative limb perfusion [1,2,11] and isolated limb infusion [9], if only stage IIIA and stage IIIB patients are considered. In particular, no advantage was found from adding mitomycin C to melphalan in terms of response rate, time to disease progression or survival. Our disappointing results may have been due in part to the lack of hyperthermia, which has been shown to have independent cytotoxic effects [28]. Our major problem was to maintain normothermia in the perfused compartment, and it is probable that several treatments were conducted under hypothermic conditions, which would lead to suboptimal perfusion of both normal tissues and tumour deposits.

The focus of interest of this article was the demonstration of a technique of hypoxic pelvic and limb perfusion that provides therapeutic options for palliation...
without relevant complications in a homogeneous group of patients with unresectable recurrent melanoma who are not eligible for isolated hyperthermic ablative limb perfusion or, at least in part, for the less conventional isolated limb infusion. Those patients in our series with recurrent melanoma in the leg could have undergone isolated hypoxic limb infusion, probably obtaining a tumour response. For all the other patients the only alternative would have been systemic chemotherapy. In a group of 53 patients with metastatic melanoma, Legha et al. [29] recently reported an overall objective response rate of 64% (21% complete responses) with two cycles of a biochemotherapy regimen (cisplatin 20 mg/m² daily for 4 days, dacarbazine 800 mg/m² on day 1, vinblastine 1.6 mg/m² daily for 4 days, interferon-α 5 MU/m² subcutaneously daily for 5 days and interleukin-2 9 MIU/m² by continuous infusion daily for 4 days). Legha et al. [29] claimed that the response rate was not influenced by the site of the metastases (soft tissue as in our series or other sites). In a more recent study on 40 patients, McDermott et al. [30] reported an overall objective response rate of 48% (20% complete responses) with a similar regimen, but modified by reducing the vinblastine dose (1.2 mg/m²) in order to limit treatment-related toxicities. Based on these data, the response rate seems to be not significantly lower in patients treated with our 1 day loco-regional chemotherapy (although no complete responses were observed) than in those receiving two courses of 5 days of systemic chemotherapy. Moreover, patients who received biochemotherapy experienced a wide range of side effects, several of which were cumulative, that reflected the combined toxicity of the CVD regimen and biotherapy. In our study, systemic toxic effects were limited by the use of haemofiltration and by the lower total doses of chemotherapeutic drugs used for the perfusion compared with those administered in the biochemotherapy regimens. The median time to disease progression in the present study was 10 months, but was reported as 5 months by Legha et al. [29] and as 8 months by McDermott et al. [30]. In addition, the median survival was 23.8 months in the three patients in our series who received systemic chemotherapy (CVD regimen) as further therapy, whereas Legha et al. [29] and McDermott et al. [30] reported median survival times of 11.8 and 11 months, respectively.

In conclusion, although some patients received a combination of melphalan and mitomycin C and others received only melphalan, this pilot study demonstrated that hypoxic pelvic and limb perfusion with melphalan can be an effective alternative for patients with recurrent limb melanoma when conventional hyperthermic perfusion is not feasible. For these patients, hypoxic pelvic and limb perfusion seems to be the best option when the lesions are localized in the groin and/or thigh, whilst higher response rates could be obtained by isolated limb infusion when the leg is the site of recurrent melanoma. On the other hand, because of not insignificant leakage, this procedure requires combined haemofiltration to reduce the systemic cytotoxic effects. In comparison with conventional hyperthermic limb perfusion, the hypoxic pelvic and limb procedure has a low morbidity, in particular reduced regional toxicity, is more repeatable, and greatly reduces the requirements for time, personnel and equipment in the operating theatre. Consequently, further studies are necessary to evaluate the use of other drugs that are active in hypoxic conditions and against melanoma cells, such as tirapazamine [31], as well as the subsequent use of biotherapy [29] with interferon-α and interleukin-2, with the aim of improving the response rates. Finally, further studies are necessary to evaluate modification of the technique by incorporating hyperthermia [32].

References


