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Results of a Double Perfusion Schedule with Melphalan in Patients with Melanoma of the Lower Limb

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From 1985 to 1990 43 patients with measurable locally inoperable or recurrent melanoma of the lower limb were treated according to a double perfusion schedule. The dose of melphalan given in the first perfusion was low (6 mg/l; 1 h; normothermic) in order to make it possible to carry out a second perfusion (9 mg/l; 1 h; normothermic) with a planned short interval of 3–4 weeks. The toxicity after the first perfusion was slight; after the second it was higher with a Wieberdink grade III reaction in 15 patients. A clinical complete remission (CR) was seen in 33 patients (77%) and a partial one in 6 patients. 16 of the 33 patients with a CR recurred in the perfused area after 5 months (range 1–29); the others remained limb recurrence-free (7–44+ months). The overall 3-year survival rate is 50%, 19 patients are alive with no evidence of disease. The double perfusion schedule shows a high CR rate, an acceptable toxicity and is technically feasible.
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INTRODUCTION

THE TECHNIQUE of regional isolation perfusion utilising an extracorporeal circuit was introduced in 1957 by Creech *et al.* [1]. This treatment modality has now become a standard procedure worldwide, with a well-established role in the management of locally inoperable and/or recurrent melanoma. The advantage of regional isolation perfusion is that a high dose of cytostatic drug can be delivered to the tumour, without producing the systemic side-effects. However, for technical reasons, the duration of each perfusion session is limited, and this modality cannot be repeated frequently. Consequently, in perfusion it is difficult to apply the theory of "fractionation and protraction", by which the difference in tolerance between malignant and normal cells is increased [2].

In an attempt to approach to some degree the principle of fractionation, we previously investigated the feasibility of a triple perfusion schedule in 9 patients with locally inoperable melanoma of the lower limb. This schedule consisted of three perfusion sessions, respectively on popliteal, femoral and iliac levels, each of 1-h duration with 7.5 mg melphalan/l perfused tissue. Based on schemes used in systemic chemotherapy, intervals of 3 weeks were chosen. The outcome of this study was that, due to toxicity, the intended intervals could be only realised in 4 patients. It was remarkable, however, that 3 of these patients showed a complete remission (CR) of the tumour mass.

Based on this experience, we decided to examine the feasibility and the efficacy of a double perfusion schedule. Preliminary results of this schedule focussing on feasibility have been published elsewhere [3].

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PATIENTS AND METHODS

All patients with measurable locally inoperable or recurrent melanoma of the lower limb were eligible for this study. Patients were staged according to the M.D. Anderson classification system (Table 1). None of the patients had undergone a perfusion treatment before. The lesions were left *in situ* in order to obtain objective response rates. Tumour response was defined according to the WHO criteria [4]. Tattoo marks were made beforehand to identify the site of the lesion in case a CR was obtained. About 12 weeks after the second perfusion a tissue sample was removed in order to obtain histological confirmation of response.

Our single perfusion technique has been described in detail [2] previously. Briefly, it aims to maintain normal physiological conditions in the limb during perfusion, allowing administration

Table 1. Staging of the patients according to the M.D. Anderson classification system

Stage	No. of patients
IA Primary intact	1
IB Primary excised	—
II Locally recurrent disease (in or < 3 cm from scar/skin graft)	6
IIIA Satellite/intransit metastasis (> 3 cm from scar/skin graft)	21
IIIB *Lymph node metastasis	5
IIIAB Satellite/intransit metastasis + lymph node metastasis	10
IV distant metastasis	—
Total	43

*Indication for perfusion: local recurrence (4 patients), inoperable primary (1 patient).

of a relatively high dose of melphalan with acceptable toxicity. In our dosimetric study the maximal dose of melphalan for lower limb perfusions was found to be 10 mg/l perfused tissue [5]. In the double perfusion schedule the technique is identical to the single procedure except for the dose of melphalan. During the first perfusion a relatively low dose of melphalan (6 mg/l perfused tissue) is administered in order to minimise toxicity, making it possible to carry out a second perfusion (9 mg/l perfused tissue) with a planned short interval of 3–4 weeks. Both perfusions are performed under controlled normothermic conditions (tissue temperatures during the whole hour of cytostatic circulation between 37 and 38°C) and last 1 h. Toxicity after perfusion was classified according to Wieberdink [5].

To investigate whether the number of lesions and the length of the interval in between the perfusions were influencing the response obtained, a statistical analysis using BMDP and SPSS software was performed concerning the relationship between these items using Cochran's linear trend test and the Mann–Whitney U-test.

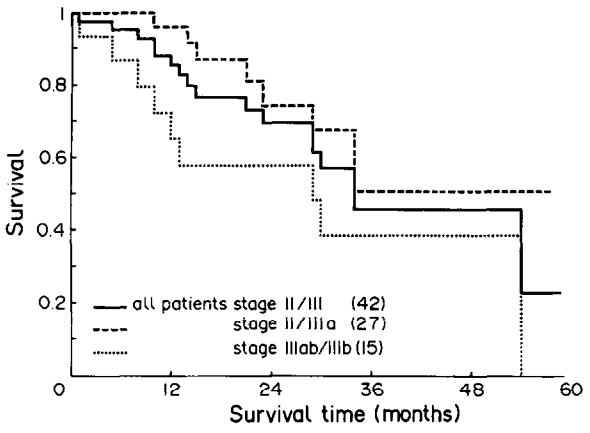

Fig. 1. Survival after the double perfusion schedule. The 3-year survival rate for 42 patients with stage II/III is 45%; for stage II + IIIa (27 patients) 50% and for stage IIIab + IIIb (15 patients) 38% ($P = 0.05$).

RESULTS

From 1985 to 1990 43 consecutive patients with locally inoperable or recurrent melanoma were treated according to this schedule. There were 35 women and 8 men with a median age of 64 years (range 39–76). The indications for perfusion were: neglected primary (2), local recurrence (4) and satellite and/or intransit metastases (37).

In the 41 patients with recurrent melanoma the primary had been removed 18.5 months (range 1–248) before the double perfusion schedule. In this interval, 25 of these patients experienced a median of 2 (range 1–7) previous limb recurrences. The median Breslow thickness of the primaries was 3.00 mm (range 0.70–20.20); the Clark level being III–V.

The extent of the disease, which was the indication of perfusion, is shown in Table 2. 16 patients had one lesion (median size of 10 × 10 mm); 19 patients had two to five lesions (median size 7 × 6 mm), 4 patients had six to 10 lesions (median size 5 × 5 mm) and 4 patients had extensive disease all over the extremity (median size 4 × 3 mm).

In 26 patients the first perfusion was at the iliac level and the second femoropopliteal. In 17 patients the reverse sequence was carried out.

Double perfusion was tolerated well. The toxicity after the first perfusion was slight: grade I (no toxicity) in 17 and grade II (slight oedema and/or erythema) in 26 patients; after the second perfusion it was higher: grade I in 2, grade II in 26 and grade III (considerable oedema and/or erythema with some blistering; slightly disturbed motility permissible) in 15 patients. The median interval between the perfusions was 28 days (range 14–48). The intended interval of 3–4 weeks could not be realised in 15 patients because of wound infection (4 patients) or logistics (11 patients). The long-term side-effects seen were: venous thrombosis within 3 months following the second perfusion in 2 patients, persisting slight oedema in 3 patients, a transient paralysis of the peroneal nerve in 3 patients, fibrosis/ankylosis not responding to therapy in 3 patients and persisting complaints of pain in 1 patient.

Table 2. Complete remission (CR) after the double perfusion schedule according to number and size of lesions

No. of lesions	Median size (mm) (range)	No. of patients	CR	
			Yes	No
1	10 × 10 (3 × 3–50 × 70)	16	14	2
2–5	7 × 6 (2 × 2–30 × 20)	19	15	4
6–10	5 × 5 (4 × 3–10 × 10)	4	3	1
Extensive	4 × 3 (1 × 1–25 × 30)	4	1	3
Total		43	33	10

Cochran's linear trend test $P = 0.02$.

The median duration of hospitalisation after the first perfusion was 10 days (range 8–30) and after the second 16 days (range 10–41), which is comparable with that of single perfusions. All except 4 patients were discharged from hospital in the interval between the perfusions.

The overall clinical response rate was 91%, with a CR in 33 of the 43 patients treated (77%) and a partial remission (PR) in 6 patients (14%). The remaining 4 patients showed no change (NC). 19 of the patients with CR started with more than one lesion (Table 2). Histological examination of the biopsy from the tattooed site, performed in 23 of the 33 patients, confirmed the CR in 19 patients but revealed vital melanoma cells still in 4 patients. 17 of the patients with a clinical CR had no recurrence in the perfused area after 7–44+ months follow-up. In the other 16 a relapse occurred after a median of 5 months (range 1–29); this included the 4 patients with a clinical CR but with vital tumour cells histologically. The median follow-up at this moment is 24+ months (range 2–58+). 17 patients have died, 14 months (range 2–54) after treatment and 19 patients are alive with NED. The overall 3-year survival rate after perfusion (Kaplan–Meier) is 50%. Excluding the patient with stage I disease, the 3-year survival rate is 45%, with a significantly better survival in patients with stage II + IIIA melanoma compared with patients with stage IIIAB and IIIB melanoma (50 and 38%, respectively; Mantel–Cox $P = 0.05$; Fig. 1).

Concerning the relationship between the number of lesions and the responses obtained, there was a decreasing number of CR with increasing number of lesions (Cochran's linear trend

Table 3. Complete remissions (CR) according to interval between perfusions

Interval (days)	CR		Total
	Yes	No	
14–21	16	3	19
22–28	8	2	10
29–35	6	1	7
36–48	3	4	7
Total	33	10	43

Cochran's linear trend test, $P = 0.07$.
Patients with CR had a mean interval of [mean (SEM)] 25 (1.2) days; patients without CR had a mean interval of 32 (3.3) days ($P = 0.05$).

Table 5. Response rate after regional isolated perfusion

Author	No. of patients			(%)	Tissue temperature* (°C)	Reference
	Total	CR	PR	CR+PR		
Rochlin (1965)	17	ns	ns	65	37–39	6
Rosin (1980)	80	21	29	62	39–40	7
Lejeune (1983)	23	15	6	91	39–41	8
Vaglini (1985)	32	18	8	81	40–41	9
Storm (1985)	26	21	0	81	40.5–42	10
Kroon (1987)	18	7	8	83	37–38	11
Skene (1990)	67	ns	ns	78	39–40	12

Only articles specifying lower and upper limit of tissue temperatures during perfusion are listed.
* = Lower and upper limit during perfusion; CR = complete remission; PR = partial remission; ns = not stated.

test, $P = 0.02$). This relationship could not be demonstrated after dividing the patients into two groups: one with a single lesion and the other with more lesions (χ^2, $P = 0.20$) (Table 2). A decreasing number of CR was also found, although not significantly, with an increasing length of interval between perfusions (Cochran's linear trend test, $P = 0.07$) (Table 3). For patients with more than one lesion, this trend was more pronounced ($P = 0.02$) (Table 4). Patients with CR had a significantly shorter time interval between perfusions than patients without CR [mean (SEM); 25 (1.2) and 32 (3.3) days, respectively]; Mann–Whitney U-test, $P = 0.05$ for all patients and 25 (1.5) days and 34 (3.7) days; Mann–Whitney U-test, $P = 0.02$ for patients with multiple lesions) (Tables 3, 4).

DISCUSSION

Our double perfusion schedule produced an overall clinical response rate of 91% (39/43 patients) and a CR percentage of 77% (33/43 patients), which is high compared with data from the literature (Table 5). Nearly all the listed data concern a single perfusion treatment. The summary shows that after perfusion with melphalan the average response rate obtained is about 80% with a CR percentage of about 40%. Most of the reports, however, relate to extensive disease, with a large tumour burden, as was not always the case in our patients. On the other hand, our data (Table 2) does not demonstrate a clear relationship between CR rate and extension of disease (one lesion vs. more than one lesion), as has been suggested in one study [13], in which a significantly lower response rate was reported in patients with more than one lesion. Our numbers are, however, too small to draw definite conclusions.

Table 4. Complete remissions (CR) according to interval between perfusions in patients with multiple lesions

Interval (days)	CR		Total
	Yes	No	
14–21	10	2	12
22–28	5	1	6
29–35	3	1	4
36–48	1	4	5
Total	19	8	27

Cochran's linear trend test $P = 0.02$.
Patients with CR had a mean interval of [mean (SEM)] 25 (1.5) days; patients without CR had a mean interval of 34 (3.7) days ($P = 0.02$).

In our schedule, perfusions were performed under normothermic conditions. As was reported earlier [2] and as can be seen from Table 4, the response rate of normothermic perfusions [11] seems to be similar to that of perfusions carried out at temperatures 1–2°C higher (mild hyperthermic perfusions) [6, 7, 12]. It seems therefore that the response rate of our double normothermic perfusion schedule is superior to that of single normothermic and single mild hyperthermic ones. From perfusions performed at high temperatures (tissue temperatures of about 41–42°C), high response rates are also reported, within one series, a striking number of CR [10]. In our hands, however, cytostatic perfusions at these high tissue temperatures are accompanied by an unacceptable degree of toxicity [14].

In four of the 23 tissue samples biopsied from the tattooed area, vital melanoma cells were still found. One problem with this type of response confirmation is that from microscopic examination it is difficult to assess the ability of melanoma cells to duplicate; this means that although melanoma cells appear microscopically vital, they could in fact be dead [15]. On the other hand this statement should be questioned since all 4 of these patients experienced limb recurrences later on.

In 16 of our 33 patients with a clinical CR (50%) melanoma recurred in the perfused area after 5 months (range 1–29). This recurrence rate is rather high compared with other reports [8, 12] citing a 30–35% limb recurrence rate in stage IIIA patients.

No other objective response data are known of a double perfusion schedule with a fixed short interval. In the adjuvant setting, however, there is a report from a study in Groningen [16] comparing two groups of patients with recurrent melanoma of the leg. In the latter study 56 patients underwent two perfusions and 37 patients underwent one perfusion. The cytostatic agents used were melphalan and actinomycin D and the temperature level was 39–40°C. The interval between perfusions was rather long, namely 6 weeks. The authors observed no difference in actuarial 5-year survival and limb recurrence rate, but because the groups were not matched, they may not have been entirely comparable. Also, the rather long interval between the perfusions may have had a negative impact on the outcome, as our data showed that a decreasing proportion of CR was

associated with an increasing length of interval. To our knowledge there is only one other report of a repeat perfusion schedule [17] whereby it is mentioned that a subgroup of patients with primary foot melanomas were perfused twice, also in the adjuvant setting, because of presumed bad prognosis. For this subgroup no specific data with regard to limb recurrence rate and survival were given, however.

Concerning toxicity, in our patients it was found that after the first perfusion (with a low dose of 6 mg melphalan/l perfused tissue), this was slight and only wound infections and logistics interfered with our intention to carry out the second perfusion within the planned short interval of 3–4 weeks. After the second perfusion grade III toxicity was seen in 15 of the 43 patients. This is more severe than encountered in our single perfusion routine, where in the period 1978–1990, grade III or higher toxicity was seen after only 38 of 354 perfusions performed. Also, the long-term morbidity after the double perfusion schedule seems to be somewhat more pronounced, although in our opinion still acceptable.

In summary, we conclude that the double perfusion schedule shows a high clinical CR rate, is well tolerated by the patients, has an acceptable degree of toxicity and is feasible technically. However, it should be taken into account that the improvement of the complete remission rate comes at a price: patients have to undergo two operations and have a longer duration of hospitalisation with its associated costs. Nevertheless, further extension of multiple perfusion schedules according to the "fractionation and protraction" principle seems warranted. Another way to approach this principle could be the combination of perfusion with repeat infusions as has been reported for osteogenic sarcoma of the extremities [18], or with tourniquet infusions [19]. Also, alterations in the duration of the cytostatic circulation or in dose scheduling are possibilities for further research, as is the option of the introduction of true hyperthermia in multiple perfusion methodology [14].

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