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Acknowledgements—This work was supported by the French Ligue Nationale contre le Cancer, Comité de L'Isère (1993). We wish to thank Pr. C. Agnius-Delord who provided us with the information on radioimmunoassays of PSA in the Centre Hospitalier Universitaire of Grenoble, and Dr G. Charriere who gave us the same information from the Institut Pasteur in Lyon. We are also greatly indebted to Pr Rambeaud of the Urology department, who gave us access to the information on ultrasonography and needle biopsies.

Pergamon

European Journal of Cancer Vol. 31A, No. 1, pp. 58–63, 1995
Elsevier Science Ltd

0959–8049/95 $9.50+0.00

0959-8049(94)00372-6

A Retrospective Comparative Study Evaluating the Results of Mild Hyperthermic Versus Controlled Normothermic Perfusion for Recurrent Melanoma of the Extremities

J.M. Klaase, B.B.R. Kroon, A.M.M. Eggermont, A.N. van Geel, H. Schraffordt Koops, J. Oldhoff, D. Liénard, F.J. Lejeune, R. Berkel, H.R. Franklin and A.A.M. Hart

The aim of this study was to investigate the role of mild hyperthermia (39–40°C) in isolated cytostatic perfusion for patients with recurrent melanoma of the extremities. A total of 218 patients treated with mild hyperthermic perfusion was compared to 166 patients perfused under controlled normothermic conditions (37–38°C). Only patients whose lesions had been excised before or at the moment of perfusion were eligible for this study. A variety of prognostic factors was controlled for in a Cox proportional hazards analysis. The application of mild hyperthermia did not influence limb recurrence-free interval nor survival (corrected *P* values 0.46 and 0.18, respectively). In this retrospective comparative study, no benefit for mild hyperthermia in regional isolated perfusion could be identified.

Key words: regional isolated perfusion, recurrent melanoma, mild hyperthermia, melphalan
Eur J Cancer, Vol. 31A, No. 1, pp. 58–63, 1995

INTRODUCTION

NINE YEARS after Creech and colleagues developed the technique of regional isolated perfusion for melanoma patients [1], Cavaliere and associates, in 1967, introduced the idea of combining hyperthermia with this cytostatic treatment modality [2]. At first, temperatures of above 42°C were used but, although this resulted in encouraging antitumour effects, it soon became clear that the application of this so-called true hyperthermia was

accompanied by an irresponsible degree of toxicity [3–5]. Since then, perfusions have been widely performed with lower tissue temperature levels of 39–40°C, so-called mild hyperthermia. However, the therapeutic gain from these slightly elevated temperatures remains questionable since it is generally known that the direct cell-killing effect of heat is only obtained at temperatures of 41.5°C upwards [6]. Perhaps secondary effects of the mild temperature elevation, such as drug potentiation and increased blood flow in the tumour, possibly resulting in increased drug uptake, play a role. One study reports objective response rates of normothermic perfusions (tissue temperatures between 37 and 38°C), which are similar to those of mild hyperthermic procedures [7]. Recently, patients with recurrent melanoma undergoing mild hyperthermic perfusion using melphalan showed a longer limb recurrence-free interval when compared to patients who had only received local excision [8]. Since no control group, treated with normothermic perfusion, was included in this study, the value of the mild hyperthermic component of this perfusion schedule could not be demonstrated [9]. In the Benelux countries, two perfusion centres (Amsterdam and Rotterdam) carry out perfusions routinely under normothermic conditions while two others (Groningen and Brussels) usually use mild hyperthermia. Here we present the results of a retrospective study comparing the patients treated in these four centres, whereby the focus is on the role of mild hyperthermia in perfusion for recurrent melanoma of the extremities.

PATIENTS AND METHODS

The perfusion centres of Amsterdam and Rotterdam make use of a computer-assisted database containing the comprehensive data of all 431 patients who underwent a total of 499 perfusions in the period 1978–1990. For this study, patients were eligible who had had recurrent melanoma excised before, or at the moment of, perfusion and who had received regional isolated perfusion under normothermic conditions (n = 166). This database was then extended to include the data of 326 patients with recurrent melanoma who had received mild hyperthermic perfusion during the period 1969–1990. Similarly, only the patients whose lesions had been excised before or at the moment of perfusion were selected for the present study (n = 284) (Table 1). All patients were classified according to the MD Anderson staging system (Table 2), which we adapted for stage II by defining local recurrence in contact with primary scar or skin graft as stage IIA and satellites within 3 cm of the primary site as stage IIB.

Regional isolated perfusion may be conducted in the lower limb at either the external iliacal- or the femoro-popliteal level, and in the upper limb at the axillary or brachial level. For the iliacal perfusions, the iliacal and obturator lymph nodes were removed, and for the axillary procedure, an axillary lymph node dissection was carried out.

The 1-h melphalan perfusion was performed at the normothermic tissue temperature level (37–38°C) as previously described [7]. This normothermia is termed controlled because special measures, such as warming the perfusate and applying a warm water mattress around the limb, are taken to prevent the limb from cooling down during the preparative surgery. Temperature control is monitored by four temperature probes inserted into the limb, both subcutaneously and intramuscularly at distal and proximal locations. The cytostatic drug perfusion period starts when a temperature of 37°C is reached at all four probes. Melphalan is given at doses of 10 and 13 mg/l perfused tissue for lower and upper extremity perfusions, respectively [10].

The technique of mild hyperthermic perfusion has also been described previously [11]. Temperature control is carried out in

Table 1. Number of patients and perfusion schedules

	Perfusion centre	
	Amsterdam/Rotterdam	Groningen/Brussels
Database content	n = 431	n = 326
Type of adjuvant* perfusion	Controlled normothermia (n = 166)	Mild hyperthermia (n = 284)
Cytostatics/dose	Melphalan	Melphalan†
	10 mg/l lower extremity	1.0–1.5 mg/kg lower extremity
	13 mg/l upper extremity	0.5–0.7 mg/kg upper extremity
		+ dactinomycin‡
		0.014 mg/kg lower extremity
		0.006 mg/kg upper extremity
		Melphalan dose as Am/Ro§
		Melphalan‖
		40 μg/ml perfusate lower extremity
		20 μg/ml perfusate upper extremity
Missing data	n = 0	n = 66
Total number evaluable	n = 166	n = 218

*Patients whose lesions had been excised before or at the moment of perfusion. †Groningen, 1969–1975. ‡Groningen, 1975–1990. §Groningen, 1982–1990. ‖Brussels. Am/Ro, Amsterdam/Rotterdam perfusion centres.

Table 2. MD Anderson classification

MD Anderson Stage	
IIA	Local recurrence
IIB	Satellite ≤3 cm from primary/scar/skin graft
IIIA	Satellite/in-transit >3 cm from primary/skin graft
IIIB	Regional node metastasis
IIIAB	Satellite/in-transit with regional node metastasis

Correspondence to B.B.R. Kroon.
J.M. Klaase, B.B.R. Kroon and R. Berkel are at the Department of Surgery, The Netherlands Cancer Institute, Amsterdam; A.M.M. Eggermont and A.N. van Geel are at the Department of Surgical Oncology, Dr. Daniel den Hoed Cancer Center, Rotterdam; H. Schraffordt Koops and J. Oldhoff are at the Department of Surgical Oncology, University Hospital Groningen, Groningen, The Netherlands; D. Liénard and F.J. Lejeune are at the Jules Bordet Institute, Brussels, Belgium; H.R. Franklin is at the Department of Biometrics and A.A.M. Hart is at the Department of Radiotherapy, The Netherlands Cancer Institute, Amsterdam, The Netherlands.
Revised and accepted 19 July 1994.

the same way as in the normothermic setting. However, during the course of cytostatic perfusion, the temperature is gradually elevated to 39–40°C and is kept constant for the rest of the 60 min of treatment. The cytostatics used in Groningen were melphalan and dactinomycin. From 1969 to 1975, melphalan was given as single agent at a dose of 1.0–1.5 mg/kg for lower limbs and 0.5–0.7 mg/kg for upper limbs. From 1975 to 1990, dactinomycin was added to the melphalan, at a dose of 0.014 mg/kg for lower limbs and 0.006 mg/kg for upper limbs. The maximum dose of dactinomycin was 1 mg. In 1982, the melphalan dosage was changed to 10 mg/l and 13 mg/l perfused tissue for lower and upper limbs, respectively. Patients with recurrent melanoma of the leg were generally perfused twice, first at the iliacal level and, after a planned interval of 6 weeks, at the femoro-popliteal level. Since no clear differences could be demonstrated between the results of the different dosage schedules nor between the single and double perfusion schedule in previous studies [12, 13], the mild hyperthermic perfusions performed in Groningen were considered together as one group. In Brussels, melphalan was used as single agent at a dose of 40 μg/ml perfusate for lower limbs and 20 μg/ml for upper limbs, which is comparable with respectively 10 mg/l and 13 mg/l perfused tissue.

The Wieberdink scoring system [10] was used in all hospitals to grade the toxicity reaction after the perfusion procedure. Grade II–III reactions were considered acceptable.

A multivariate Cox regression analysis was performed, using BMDP statistical software, to assess the relationship between prognosis after perfusion (expressed in limb recurrence-free interval and in overall survival) and the following variables: sex, age, stage of disease, Breslow thickness of the primary, Clark level of infiltration of the primary, site of the primary, time since the primary, number of previous recurrences, number of (excised) lesions forming the indication for perfusion and nodal status. The use of mild hyperthermia was included in the analysis as the eleventh variable. The term limb recurrence included all forms of metastasis occurring in the perfused limb. The stepwise regression analysis revealed, for each type of curve, which factors had a significant effect ($P \leq 0.05$). The nature of the effect (e.g. linear relationship) was investigated, and the optimal method of coding of the variable was assigned. Interactions between the variables were investigated. In order to see whether there was an advantage for mild hyperthermia within any of the subgroups, interactions between each of the variables and the factor mild hyperthermia were also tested in the model.

RESULTS

From the Amsterdam and Rotterdam database, a total of 166 patients who were treated with controlled normothermic perfusion complied with the selection criteria. There were 124 women and 42 men with a mean age of 50 years (median 52, range 17–79). The mean follow-up period of these patients was 50 months (range 6–151).

A total of 284 patients treated with mild hyperthermic perfusion in Groningen and Brussels also entered the study. For 66 of these patients, there were insufficient data on possible prognostic variables and so they were excluded from the analysis. The remaining patient group consisted of 154 women and 64 men with a mean age of 49 years (median 52, range 18–79). The mean follow-up period of these patients was 46 months (range 6–248).

To estimate whether the 66 patients excluded from the mild hyperthermic perfusion group could have produced a bias, rough limb recurrence-free and survival curves were computed. There was no significant difference between the curves of the excluded group and those of the study group (Figures 1 and 2).

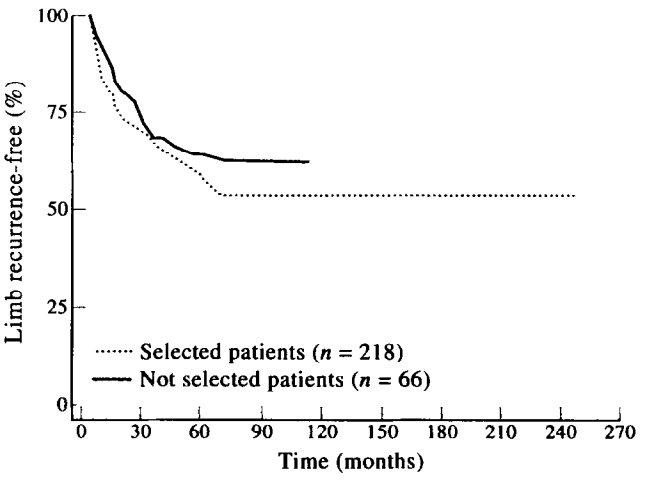

Figure 1. The limb recurrence-free interval of 66 patients excluded from the study, compared to the 218 evaluable patients treated with mild hyperthermic perfusion.

Table 3 shows the composition of the two groups of normothermic and mild hyperthermic perfusion with regard to the possible prognostic factors. There was a comparable distribution of the variables sex, age, number of previous recurrences, number of (excised) lesions forming the indication for perfusion and site of the primary melanoma. There were slight differences in the distribution of stage of disease, Clark level of infiltration and the Breslow thickness of the primary, time since primary and nodal status.

Limb recurrence-free interval

The regression analysis showed that five variables had a significant effect on limb recurrence. These were, in order of importance, stage of disease, number of previous recurrences, Breslow thickness, time since primary and nodal status (Table 4). There was a longer limb recurrence-free interval for patients

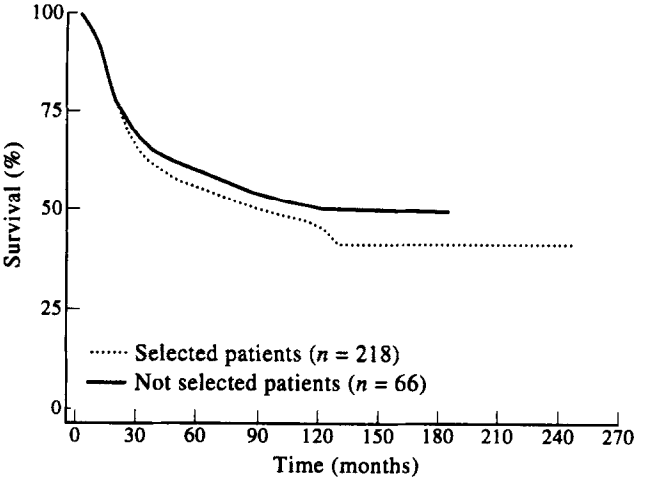

Figure 2. Survival curve of 66 patients excluded from the study, compared to the 218 evaluable patients treated with mild hyperthermic perfusion.

Table 3. Comparison of controlled normothermic and mild hyperthermic perfused patients according to possible prognostic factors

	Controlled normothermic (n = 166)	Mild hyperthermic (n = 218)
Sex		
Female	75%	71%
Male	25%	29%
Stage		
IIA	17%	7%
IIB	18%	13%
IIIA	28%	24%
IIIAB/B	37%	56%
Age (years)		
≤39	30%	23%
40–49	14%	19%
50–59	23%	23%
≥60	33%	35%
Mean	50 years	52 years
Median	52 years	53 years
Clark level		
II–III	17%	8%
IV	39%	44%
V	9%	6%
Unknown	35%	42%
Breslow thickness (mm)		
≤1.49	17%	11%
1.50–2.99	19%	14%
3.00–3.99	11%	6%
≥4.00	16%	23%
Mean	3.30 mm	4.10 mm
Median	2.62 mm	3.20 mm
Unknown	37%	46%
Number of previous recurrences		
0	80%	84%
≥1	20%	16%
Time since primary (months)		
<12	39%	55%
≥12	57%	44%
Mean	32.9 months	27.3 months
Median	12 months	9 months
Unknown	4%	1%
Number of (excised) lesions forming the indication for perfusion		
1	70%	74%
>1	30%	26%
Site of the primary		
Upper limb	16%	22%
Lower limb	60%	60%
Foot	20%	17%
Unknown	4%	1%
Nodal status		
Negative	63%	44%
One positive	12%	23%
> one positive	25%	33%

Table 4. Significant prognostic factors (in order of importance) in the proportional hazards regression analysis

	Significant prognostic factors	Corrected P value mild hyperthermia
Time to limb recurrence	Stage, number of previous recurrences, Breslow thickness, time since primary, nodal status	0.46
Survival	Stage, Breslow thickness, sex	0.18

with MD Anderson stage IIA, who had had no previous recurrences, with a Breslow thickness of less than 1.50 mm, and with a time since primary of ≥1 year. Figure 3 shows the time to limb recurrence corrected for these five variables. The application of mild hyperthermia was not shown to have any significant effect, neither as a single factor (P = 0.30) nor when corrected for the five variables (P = 0.46).

Survival

Variables influencing survival were, in order of importance, stage of disease, Breslow thickness and sex. Survival was longer for patients with MD Anderson stage II, with a Breslow thickness of less than 1.50 mm and for female patients (Table 4). Figure 4 shows the survival curve corrected for these three variables. The use of mild hyperthermia did not influence overall survival, neither as a single factor (P = 0.42) nor when corrected for the three variables (P = 0.18).

Summarising, mild hyperthermia was not a significant prognostic factor in either of the two types of analysis, nor was there any interaction of the other variables with that of perfusion.

Toxicity

In the mild hyperthermic perfusion group, the acute regional toxicity encountered was somewhat more pronounced (although

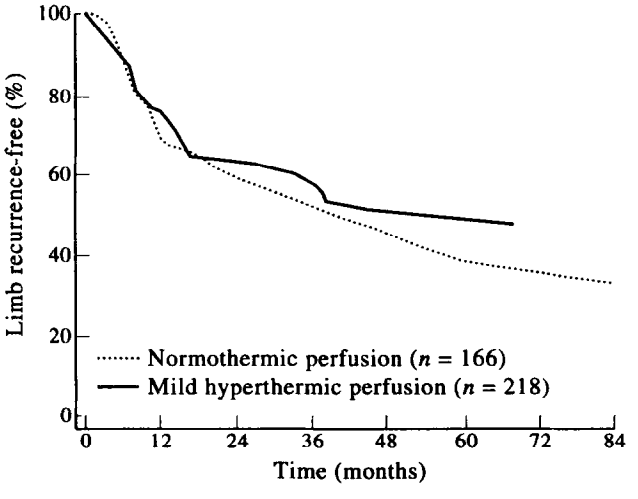

Figure 3. Limb recurrence-free interval of 218 patients treated with mild hyperthermic perfusion and 166 patients treated with normothermic perfusion, corrected for stage of disease, number of previous recurrences, Breslow thickness, time since primary and nodal status.

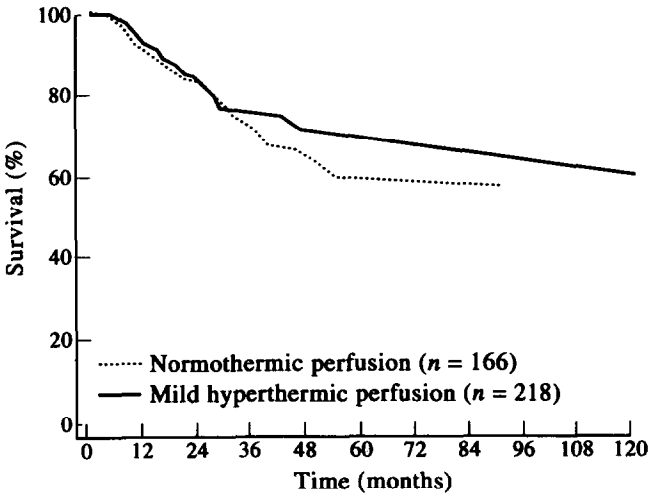

Figure 4. Survival curve of 218 patients treated with mild hyperthermic perfusion and 166 patients treated with normothermic perfusion, corrected for stage of disease, Breslow thickness and sex.

statistically non significant; $\chi^2 = 6.30$; $P = 0.28$) than that seen in the normothermic perfusion group. No toxicity (grade I) was seen in, respectively, 2 and 3% of the patients, a grade II reaction (slight erythema and/or oedema) was seen in 76 and 84%; a grade III reaction (considerable erythema and/or oedema with some blistering; slightly disturbed motility permissible) in 20 and 11%; a grade IV reaction (extensive epidermolysis and/or obvious damage to the deep tissues, causing definite functional disturbances; threatening or manifest compartmental syndromes) in both groups in 1% of the patients, and a grade V reaction (reaction which may necessitate amputation) also in both groups in 1% of the patients. There were no differences in the incidence of long-term complications such as fibrosis, ankylosis, persistent oedema and definite nerve injuries, altogether less than 5% in both groups.

DISCUSSION

The selection of possible prognostic factors for limb recurrence-free interval and survival after perfusion in this series was based on an analysis of 216 patients with recurrent melanoma, including 58 patients with tumour tissue left *in situ*, who were treated with controlled normothermic perfusion at the Amsterdam and Rotterdam perfusion centres [14]. In that study the factors seen to be linked with limb recurrence-free interval were, in order of importance, tumour tissue left *in situ*, number of previous recurrences and total tumour surface area. The factors influencing survival were stage of disease, sex, age, Breslow thickness, Clark level and number of lesions forming the indication for perfusion. The present study confirmed the prognostic significance of four of the above factors: number of previous recurrences for limb recurrence-free interval and stage of disease, Breslow thickness and sex for survival. However, the major issue of the present analysis was whether mild hyperthermia, as opposed to controlled normothermia, played a statistically significant prognostic role on the treatment outcomes. In this respect, the multivariate analysis, incorporating the use of mild hyperthermia as one of the tested variables, could not show any difference for limb recurrence-free interval nor survival. There were slight inbalances of distribution of some other tested variables between the two treatment groups—patients treated with mild hyperthermic perfusions more often had regional node involvement, deeper and thicker primary lesions and a shorter interval since the primary—but these variations were compensated for in the proportional hazards regression analysis.

Conclusive data on the potential enhancement of melphalan by rather slightly elevated temperatures is lacking [15]. Based on experimental studies, it seems that there is only a significant temperature enhancement ratio at temperatures of 41°C upwards [16]. In addition, a direct cell-killing effect cannot be expected from mild hyperthermia as this is generally only obtained substantially at temperatures above 41.5°C [6]. Below this level, it has been found that a 1°C decrease in temperature must be compensated for, in order to obtain the same effect, by an increase in treatment duration by a factor ranging from 2.7 to 33, with a mean value of 6 [17]. This time–temperature relationship means that at 39–40°C the heating period has to be at least six times longer than at 41.5°C in order to obtain the same results [15–17]. In addition, the suggested increase of drug uptake by a presumed better (sub)cutaneous tissue perfusion at elevated temperatures lacks scientific proof [3]. As has been put forward in earlier reports [7], the better results obtained by mild hyperthermic perfusions, when compared to perfusions without heating from the beginning of the perfusion era, may be explained by the fact that, unlike the present routine, no special measures were taken then to prevent the limb from cooling down during preparative surgery. In those days, tissue temperatures of the limb during perfusion would often probably have been as low as 32–33°C. In other words, mild hyperthermia was not in fact being compared to normothermia, but to hypothermia, a condition which most probably interferes with the effectiveness of chemotherapy.

True hyperthermia, with tissue temperatures ranging from 42 to 43°C remains attractive, as theoretically the hypoxic centre of the tumour is eradicated by the direct cell-killing effect of the heat while the well-vascularised periphery is attacked by the cytostatic drug. As was mentioned, however, this form of hyperthermia is seldom used for perfusion anymore, even though encouraging anti-tumour effects were achieved [3–5], because the resulting toxicity is unacceptably high. It is interesting in this respect to note that high response rates (superior to that of mild hyperthermic and controlled normothermic perfusions) have also been reported from perfusions using tissue temperatures at the borderline of true hyperthermia (40.5–42°C), and that these perfusions were well tolerated [18–21]. Recently, based on these good results, the WHO Melanoma Group has started a prospective randomised study to evaluate the role of this borderline true hyperthermia in an adjuvant perfusion setting for primary melanoma [22]. However, it is our experience (Amsterdam and Rotterdam centres) that the simultaneous administration of melphalan and borderline true hyperthermia has proved to be too toxic [23]. Therefore, in Amsterdam recently, a double perfusion schedule was developed using high-dose hyperthermia (tissue temperatures for 2 h between 42 and 43°C) and melphalan sequentially. By application of this procedure, both treatment modalities can be given at maximum dosage without an exaggeration of the toxicity. The first results of this schedule, in terms of response rate and toxicity, are promising [24].

In conclusion, in this retrospective comparative study, no clear benefit was seen for the application of mild hyperthermia (39–40°C) in regional isolated perfusion for recurrent melanoma compared to controlled normothermia (37–38°C). More research on methods for application of true hyperthermia in the direct

cell-killing temperature ranges of ≥41.5°C seems warranted, especially with respect to the reduction of toxic side-effects.

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Acknowledgements—We thank Prof. Ph. Rümke, internist, and the perfusion technicians of the four participating centres, who were closely involved in the perfusions and the research based on them. The help of the laboratories of nuclear medicine is kindly acknowledged. This study was financially supported by Boehringer Ingelheim B.V., Alkmaar, The Netherlands.