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Original Paper

A Comparison of Dosimetric Methods in Isolated Limb Perfusion with Melphalan for Malignant Melanoma of the Lower Extremity

D.S. Byrne,[1] A.J. McKay,[1] R. Blackie[2] and R.M. MacKie[3]

[1]Department of Vascular Surgery, Gartnavel General Hospital, 1053 Western Road, Glasgow G12 0YN; 2[2]Department of Medical Oncology, CRC Beatson Laboratories, University of Glasgow; and [3]Department of Dermatology, University of Glasgow, Glasgow, U.K.

The three dosimetric schedules currently used in isolated limb perfusion with melphalan for malignant melanoma of the lower limb were compared in a series of 51 patients. The doses prescribed by each of the three methods (based on total body weight (TBW), limb tissue volume (LTV) and total blood volume in the perfusion circuit (TBV)) were calculated for all patients and were then compared using Wilcoxon's signed-rank test. This revealed that the method based on TBV consistently prescribed much lower doses of drug than either of the other two methods. Pharmacokinetic profiles of melphalan obtained by HPLC analysis of blood samples during the procedure also showed that the method did not reliably predict the concentration of melphalan achieved in the perfused limb. The dosimetric schedule based on LTV prescribed slightly higher doses than that based on TBW. However, the technique is more difficult to practise due to the problems of measuring the limb volume by immersion. We conclude that the dosimetric schedule based on TBW is the most appropriate by virtue of its simplicity, the high doses of melphalan which it prescribes, and the well-controlled toxicity which it produces. Copyright © 1996 Elsevier Science Ltd

Key words: regional perfusion, melanoma, melphalan

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INTRODUCTION

THERE IS much debate on which melphalan (*L*-phenylalanine mustard; L-PAM) dosage schedule should be applied in isolated limb perfusion (ILP). Malignant melanoma has proved resistant to most forms of systemic treatment because unacceptable toxicity in normal tissues is encountered at drug doses which are too low to produce a clinical response. However, ILP offers the facility to exclude vital organs and tissues (e.g. liver, bone marrow) from the field of treatment, which allows higher, effective doses of cytotoxic agent to be administered. For this reason, it has generally been held that the optimal dose of melphalan in ILP is the highest dose tolerated by the normal tissues of the limb [1, 2]. Three dosage schedules are currently employed by various perfusion centres based on total body weight (TBW), the volume of the perfused limb (LTV) and the calculated total blood volume in the ILP circuit (TBV). In order to determine which dosage schedule most reliably prescribes the 'optimal' dose of melphalan for each patient, a comparative study of all three schedules was undertaken in a series of 51 iliac ILPs.

Correspondence to D. S. Byrne.
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PATIENTS AND METHODS

All the patients in this study underwent ILP at the level of the external iliac vessels. The operative technique has previously been described in detail [3]. The artery and vein were mobilised and isolated by ligating and dividing all branches and tributaries between the common iliac bifurcation and the inguinal ligament through an extraperitoneal exposure. The vessels were then cannulated and connected to an extracorporeal circuit comprising a heater/oxygenator and roller pump in series (Figure 1) and a tourniquet was applied to the root of the limb to complete the isolation. Isolated perfusion was then commenced, and after allowing

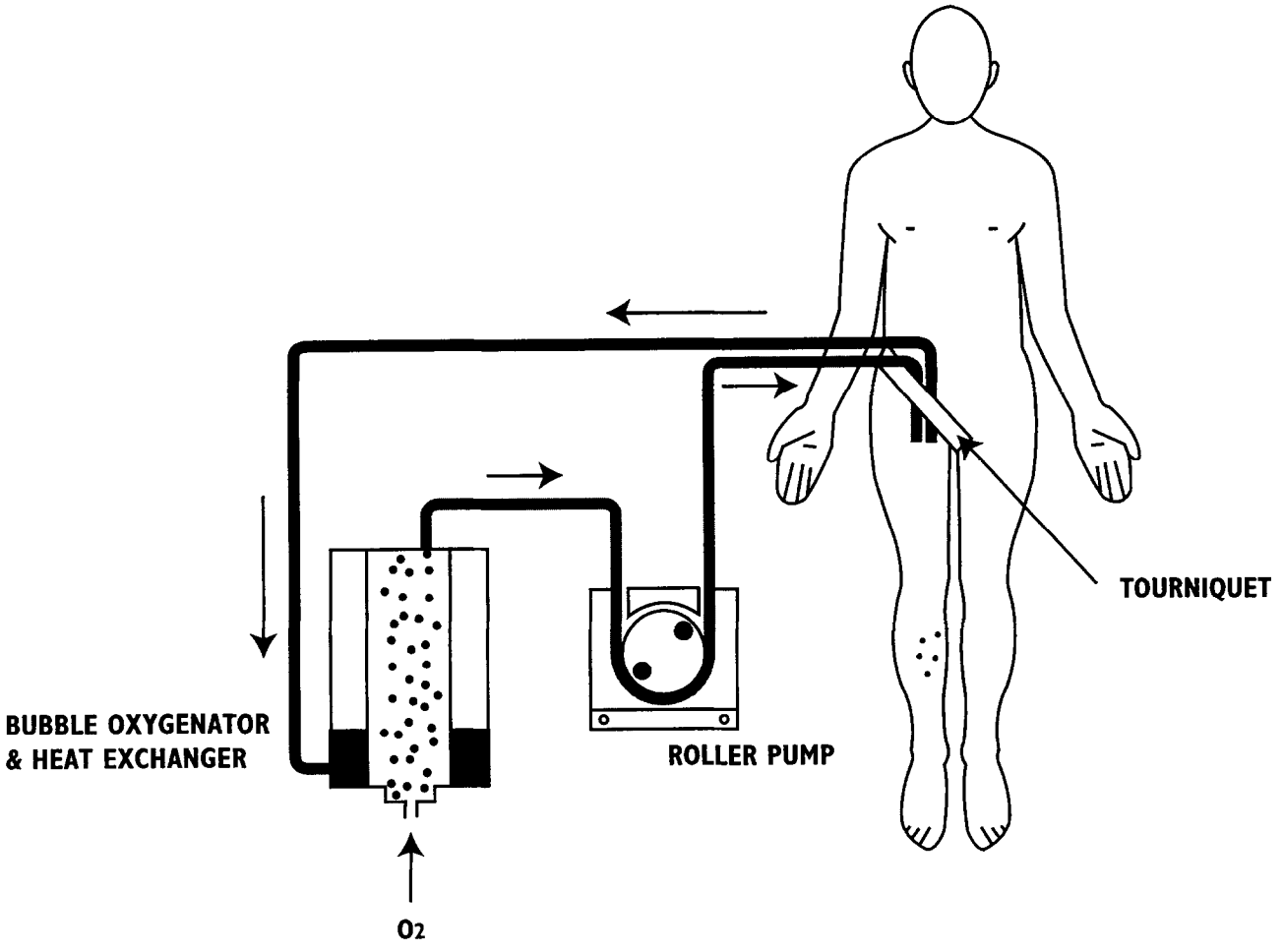

Figure 1. Components of the isolated limb perfusion circuit.

15 min for thorough mixing of the blood trapped in the limb at the time of isolation and the priming fluid in the extracorporeal circuit (1100 ml of Ringer's lactate and packed red cells, 2:1), melphalan was injected as a single bolus into the venous side of the oxygenator. This was then allowed to circulate for 1 h before rinsing the isolated circuit with 2 litres of Ringer's lactate and reversing the isolation procedure.

Melphalan dosage

The appropriate dose of melphalan for each patient was determined using each of the three dosage schedules as follows:

(1) *TBW.* All patients were weighed using the same scales and received 1.75 mg/kg of melphalan (maximum dose 150 mg).

(2) *LTV.* Limb volumes were measured by immersion according to the technique described by Wieberdink and associates [1]. The melphalan dose was then calculated at 10 mg/l of perfused tissue.

(3) *TBV.* The haematocritis of blood samples taken from the systemic circulation and the priming fluid in the pump reservoir immediately prior to isolation, and from the mixed blood in the ILP circuit after 15 min of perfusion were measured. Since the volume of the priming fluid in the extracorporeal circuit was known to be 1100 ml, it was then possible to estimate the total blood volume in the ILP circuit using the formula devised by Lejeune and Ghanem [4]. The dose of melphalan was then calculated at 40 mg/l.

Comparison of dosage methods

The three prescribed doses for each patient were compared using matched pairs tests. In order to test the reliability of the method for determining the total blood volume in the ILP circuit, a pharmacokinetic profile of melphalan in the perfusate was obtained for each patient. Serial perfusate samples were obtained at 5 min intervals throughout the ILP and were put into lithium heparin containers. The samples were placed on ice and were processed immediately after the end of ILP. Samples were centrifuged at 2500 rpm for 10 min. The plasma from each sample was then separated and stored at −20°C until melphalan assay could be performed. This was done using a sensitive and specific HPLC method which has been previously described [3, 5, 6]. The melphalan concentrations were then plotted on a logarithmic scale against time. The equilibrium concentration of melphalan in the perfusate was calculated by regression of the β-phase of the drug disappearance curve (i.e. the 20–60 min portion of the curve) and extrapolation of this line back to time zero. This value, the measured

equilibrium concentration of melphalan ($_{meas}[Mel]_{eq}$), was then compared with the equilibrium concentration which one would expect ($_{exp}[Mel]_{eq}$) if the calculation of TBV based on haematocrits were an accurate guide to the volume of distribution of the drug.

RESULTS

During a 2-year period, 51 patients undergoing iliac ILP were entered into this study. Owing to the very time-comsuming observations and sampling protocol, data collection was not complete for every patient. Nevertheless, 44 limb volumes were measured (86%), three haematocrits were obtained from 48 patients (94%), and 40 full pharmacokinetic profiles were derived (78%).

The dose of melphalan administered to each patient (based on TBW) and the doses prescribed by each of the other two methods are shown in Table 1. The mean calculated dose (S.D.) was 121.41 mg (23.68) by TBW, 126.16 mg (23.67) by LTV and 83.83 mg (24.91) by TBV. The three combinations of matched pairs were then analysed using Wilcoxon's signed-rank test (Table 2). This revealed that the dosage schedules based on TBW and LTV prescribed very significantly higher doses of melphalan than did the method based on TBV, even after allowing for multiple testing. Comparison of the TBW and LTV methods revealed a statistically significant but small difference in doses (LTV − TBW = 5.0 mg). In the clinical situation, an upper limit of 150 mg is applied to the administered dose of melphalan (see doses marked with an asterisk in Table 1). Analysis of these 'clinical' doses by the same method (Wilcoxon's signed-rank test) produced similar results, although the difference between the TBW and LTV doses was slightly less (LTV − TBW = 4.5 mg; see Table 2).

The pharmacokinetic profiles obtained from 40 ILPs were used to calculate the half-life ($t_{1/2}$) of the β-phase of the drug disappearance curve and the $_{meas}[Mel]_{eq}$ in each case. The mean $t_{1/2}$ of the curve was 34.12 min (S.D. = 10.37) and the mean $_{meas}[Mel]_{eq}$ was 47.99 μg/ml (S.D. = 26.79). Table 3 lists the $_{meas}[Mel]_{eq}$ and the $_{exp}[Mel]_{eq}$ for each case together with the estimated TBV based on the three haematocrit measurements and the volume of distribution of melphalan calculated from the administered dose and the $_{meas}[Mel]_{eq}$. There was very poor correlation between the $_{meas}[Mel]_{eq}$ and the $_{exp}[Mel]_{eq}$ ($r = 0.212$), and in most cases the $_{meas}[Mel]_{eq}$ was lower than the $_{exp}[Mel]_{eq}$, with a median difference of 15.89 μg/ml (95% CI (confidence interval) = 7.8–22.6; $P = 0.001$; Wilcoxon's signed-rank test). A similar comparison of the TBV with the calculated volume of distribution of melphalan confirmed a marked underestimation of the volume by the former method, with a median difference of 942.3 ml (95% CI = 574–1292; $P = 0.000$; mean value 921 ± 182 m/s) amounting to 31.5% of the mean volume of distribution. No correlation was found between this volume difference and either the LTV or the TBV ($r = -0.22$ and -0.27, respectively).

The toxic reactions encountered after ILP were graded according to the classification devised by Wieberdink and colleagues [1]. 4 patients had a grade I reaction, 25 a grade II and 22 a grade III.

Table 1. Doses of L-PAM calculated according to three dosimetric schedules for 51 patients undergoing ILP

Patient number	Schedule LTV	TBW	TBV
1	159*	140	99
2	137	163*	98
3	99	100	88
4	95	84	51
5	151*	166*	107
6	114	103	70
7	115	107	88
8	—	151*	81
9	—	137	101
10	93	85	53
11	116	105	88
12	116	112	88
13	144	133	71
14	134	119	147
15	155*	133	88
16		126	57
17	127	117	79
18	—	105	52
19	166*	145	82
20	97	100	50
21	111	98	69
22	170*	180*	101
23	139	144	–
24	97	105	69
25	157*	151*	73
26	111	119	121
27	151*	151*	95
28	129	119	132
29	169*	156*	99
30	138	119	70
31	112	109	66
32	121	128	73
33	143	135	66
34	105	91	95
35	154*	163*	167*
36	104	95	77
37	94	91	73
38	138	126	88
39	—	103	83
40	—	107	88
41	161*	137	70
42	90	96	66
43	111	107	50
44	99	89	70
45	134	117	132
46	119	103	59
47	104	112	94
48	146	158*	61
49	—	110	79
50	122	130	—
51	104	112	—

LTV, limb tissue volume; TBW, total body weight; TBV, total blood volume. Dose marked with an asterisk exceed the 150 mg upper liimit of administered melphalan applied in the clinical situation.

DISCUSSION

Of the three dosimetric methods assessed in this study of 51 patients, that based on TBV consistently prescribed a significantly lower dose than the other two methods. Calculation of the TBV led to inaccurate prediction of the

Table 2. Comparison of melphalan doses calculated by three dosimetric schedules

	TBW versus LTV ($n = 44$)	TBW versus TBV ($n = 48$)	LTV versus TBV ($n = 41$)
Prescribed dose	−5.0 (−9.0, −1.0) $P = 0.007$	+38.0 (29.5, 45.5) $P < 0.001$	+42.5 (33.5, 52.0) $P < 0.001$)
Clinical dose	−4.5 (−7.5, −1.0) $P = 0.001$	+36.5 (28.5, 43.5) $P < 0.001$	+40.0 (32.0, 49.5) $P < 0.001$

Data shown as median difference in mg (95% confidence interval).

equilibrium concentration of melphalan, contrary to Lejeune's experience [4, 5]. The difference in the volume calculations between this study and Lejeune's may, in part, be explained by his use of a greater priming volume [5], resulting in a relatively smaller contribution being made to the TBV by the blood contained in the limb at the time of isolation. This in turn would reduce the potential error in the volume calculations based on haematocrits. In fact, the estimated volume of distribution of the drug in this study was, on average, as much as 31.5% higher than the calculated TBV. The steep decrease in melphalan concentration which occurs during the α-phase of the drug disappearance

Table 3. Melphalan pharmacokinetics and estimated volume of distribution in 40 ILPs

Patient number	Administered dose	TBV	$[MEL]_{eq}$ exp	$[MEL]_{eq}$ meas	Volume of distribution
3	100	2200	45.45	35.41	2824
5	150	2671	56.16	38.16	3931
6	103	1760	58.52	28.77	3580
7	107	2200	48.64	28.21	3793
8	150	2017	74.37	41.13	3647
9	137	2514	54.49	49.91	2745
10	85	1320	64.39	26.79	3173
11	105	2200	47.73	30.43	3451
12	112	2200	50.91	41.27	2714
13	133	1787	74.43	70.60	1884
14	119	3667	32.45	40.06	2971
15	133	2200	60.45	35.41	2756
17	117	1980	59.09	62.81	1863
18	105	1300	80.77	56.85	1847
19	145	2043	70.97	85.51	1696
20	100	1238	80.78	109.19	916
21	98	1729	56.68	66.79	1467
22	150	2514	59.67	44.27	3388
23	144	—	—	45.38	3173
24	105	1728	60.76	31.05	3382
25	150	1833	81.83	55.66	2695
26	119	3025	39.34	30.94	3846
27	150	2383	62.95	75.44	1988
28	119	3300	36.06	119.40	997
29	150	2475	60.61	36.15	4149
30	119	1760	67.61	67.43	1765
31	109	1650	66.06	137.32	794
32	128	1833	69.83	54.38	2354
37	91	1833	49.65	27.03	3367
38	126	2200	57.27	47.87	2632
39	103	2063	49.93	24.96	4127
40	107	2200	48.64	22.90	4672
41	137	1760	77.84	52.62	2604
42	96	1650	58.18	19.89	4827
43	107	1238	86.43	36.74	2912
44	89	1760	50.57	24.24	3671
45	117	3300	35.45	32.74	3574
46	103	1467	70.21	25.47	4044
47	112	2338	47.90	24.77	4522
48	150	1513	99.14	35.77	4193

Units were as follows: dose (mg); TBV and volume of distribution (ml); $[Mel]_{eq}$ (μg/ml). TBV, total blood volume.

curve is believed to be due not only to equilibration of the drug between the intravascular and interstitial fluid compartments, but also to cellular uptake of the drug by the tissues of the limb, most of which occurs during this period. This is accompanied by loss of a proportion of the drug to the components of the extracorporeal circuit and to hydrolysis within the circulation. During the β-phase, intracellular transport of melphalan is believed to be matched by efflux of intact melphalan back into the interstitial fluid [3, 8]. Therefore, the volume upon which to base calculations of volume of distribution and desired equilibrium concentrations might more appropriately include TBV, the interstitial fluid of the limb, and a proportion of intracellular fluid, together with a correction factor to account for uptake by the components of the circuit. The estimated contribution of the patients' peripheral blood to the TBV (i.e. TBV-priming volume) in this study is slightly greater but of similar magnitude to that measured by Lejeune [4, 5] (mean ± S.E.M.: 952 ± 90 ml and 768 ± 72 ml, respectively). However, this study has identified an apparent further contribution of 921 ± 182 ml (mean ± S.E.M.) to the total volume of distribution of the drug which may represent these extra compartments into which the melphalan is distributed in addition to the potential errors in haematocrit-based volume calculations. No significant correlation was demonstrated between this extra volume and the LTV or the TBV. The TBV method of dose calculation may therefore be unreliable. Furthermore, the mean equilibrium concentration of melphalan produced by doses based on TBW in the present study was higher than Lejeune's recommended target of 40 μg/ml without producing any unacceptable toxic reactions. One can, therefore, conclude that even if the method achieved higher accuracy, this schedule would result in less than optimal dosage of melphalan.

Measurement of the volume of a limb by immersion is an attractive proposition since it allows the clinician to apply a numerical dosage formula where, in the past, he may have relied on a subjective impression of the patient's morphology when modifying the weight-based dose of melphalan to allow for features such as obesity, fair skin and hair colour [8–14]. However, the practice of immersing a whole leg is awkward and can at times be dangerous or impossible without the use of an expensive mechanical hoist (4 patients in this study could not tolerate the measurement due to limited hip movement or imbalance). Furthermore, there have been no studies to date to determine the accuracy and reproducibility of the limb volumetry protocol. Inherent in the method are several steps which can each contribute, to a greater or lesser degree, to potential error: lack of stability of the non-weightbearing limb if not using a hoist, perineal discomfort resulting in distortion of the patient's posture, visual estimation of two water levels, an arbitrary 10% correction factor to include the non-immersible part of the upper thigh and buttock in the calculation.

The main reason for seeking to introduce new dosimetric methods is the need to optimise and standardise treatment. The majority of perfusion centres in Europe currently employ the LTV method. This study shows that the doses prescribed on the basis of 1.75 mg/kg of TBW are only marginally lower than those based on LTV (10 mg/l). Previous reports have suggested that calculations based on body weight can result in wide variations of melphalan dose when expressed per tissue volume [1], and that this can produce variable, unpredictable and sometimes unacceptable toxicity to the normal tissues. However, in most studies, the doses are not strictly defined. They are, rather, selected from a range of dosage schedules according to build, complexion, hair colour and age [8–14]. In this study, the TBW-based dose, unadjusted for any of these factors and limited only to a maximum permissible dose of 150 mg, was equivalent to 9.63 ± 0.13 mg/l (mean ± S.E.M.) of perfused tissue, suggesting that a large part of the previously observed variability of dosage and toxicity may have resulted from the subjective assessment of the patients' morphological characteristics and from technical variations such as inconsistent temperature regulation in the ILP circuit. The toxic reactions in this study were largely acceptable with 47 falling into the 'desirable' grades II and III and the others all falling into the less toxic grade I (reactions graded according to Wieberdink's classification [1]).

The main aim of any dosimetric method is to allow the administration of the highest dose of cytotoxic agent consistent with acceptable morbidity. In order to standardise treatment, the method must ideally be easily reproducible. The results of this study suggest that, for ILP of the iliac region, a melphalan dosage schedule based on body weight is the most appropriate by virtue of its simplicity, the high doses which it prescribes, and the well-controlled toxicity which it produces. Further phase I studies of slightly higher doses than the currently advocated 1.75 mg/kg may allow the total dose to equal or exceed that prescribed by the LTV method. Limb volumetry by immersion is a somewhat awkward technique. It can be uncomfortable for the patient and may at times even be dangerous. The doses prescribed by the method based on TBV seem to be consistently lower than those prescribed by either of the other methods. The accuracy and reproducibility of this and of the LTV method have not been proven and may be suspect.

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