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Systemic toxicity after isolated limb perfusion with melphalan for melanoma

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Systemic exposure to melphalan is minimized during isolated limb perfusion (ILP) by isolating a limb from the rest of the body. Consequently, there should be no toxicity to vital organs. At present systemic toxicity after ILP has not been studied in detail. Therefore, the incidence, nature and risk factors of systemic toxicity was retrospectively studied in 368 patients who underwent a single ILP with melphalan between 1978–1990. Some form of systemic toxicity occurred in 98 patients (27%). Nausea and vomiting after the 1st post-ILP day was seen in 73 patients (20%), and in seven (2%) treatment was required. Bone marrow depression was encountered in seven patients (2%): WHO grade II in five, and grade III in two. Miscellaneous systemic side-effects, including fever and minimal scalp hair loss, occurred in 19 patients (5%). Leakage from the isolated circuit to the systemic circulation was measured with radioactive tracers. Mean cumulative leakage during ILP was 0.9%. Systemic toxicity was not increased in patients with leakage greater than 1% or 5%. Female sex was associated with an increased incidence of systemic toxicity ($P<0.05$). Age over 60 years ($P<0.05$) and more severe acute regional toxicity ($P<0.05$) were correlated with nausea and vomiting. The miscellaneous systemic side-effects were more frequently encountered in women than in men ($P<0.05$). In conclusion, systemic toxicity was rarely severe, with nausea and vomiting being the most frequently encountered side-effects. Age over 60 years, female sex and more severe acute regional toxic reactions were correlated with an increased incidence of systemic side-effects. Systemic leakage during ILP was not associated with toxicity, probably due to the low incidence of significant leakage.

Key words: **isolated limb perfusion; systemic toxicity; melphalan; melanoma.**

Introduction

Isolated limb perfusion (ILP) was introduced by Creech *et al.* in 1958.[1] Nowadays, it is an established procedure in the treatment of locally inoperable and recurrent melanoma.[2-8]

The main advantage of ILP is that a high dose of cytostatic drug (usually melphalan) can be given loco-regionally, thereby minimizing systemic exposure to the drug and subsequent toxicity to vital organs. The concentration of melphalan in the perfusion circuit is approximately 10 times higher than the concentration in blood after maximum-tolerated doses normally used for systemic administration.[9,10] During ILP, the melphalan peak concentration is about 150 times higher in the perfusate than in the systemic circulation.[11] Therefore, optimal isolation of the limb is of utmost importance to avoid leakage of the drug into the systemic circulation. Toxicity after systemic administration of malphalan includes bone marrow depression, gastrointestinal toxicity such as nausea and vomiting, diarrhoea and stomatitis, hair loss, maculopapular rashes and pruritis.[12]

Published data on systemic toxicity after ILP vary widely and, given the retrospective nature of most reports, all figures probably represent minimum estimates.[3] Mortality rates as high as 9% have been reported after ILP,[13] mainly from the beginning of the perfusion era.[2,14-16] Bone marrow depression is encountered in a wide range of 0–59% of perfused patients,[14,16-40] but data on other potential side-effects are scarce. Detailed information on aetiology and risk factors for systemic toxicity after ILP with melphalan is lacking. A better understanding of the influence of patient- and treatment-related factors on systemic toxicity could lead to improvements in ILP technology and to the prevention of side-effects.

Moreover, systemic toxicity has become of major concern with the recent introduction of tumour necrosis factor-alpha (TNFα) in ILP,[41] as TNFα has been associated with severe and sometimes life-threatening complications.[41-44]

In this study, we present our experiences with systemic toxicity after single ILP with melphalan with respect to incidence, nature and risk factors.

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Table 1. Distribution of patient- and ILP-related variables

	No. of patients (%) ($n = 368$)
Sex	
male	98 (27)
female	270 (73)
Age (year)	
<60	229 (62)
≥60	139 (38)
Level of isolation	
axillary	51 (14)
brachial	9 (2)
iliac	257 (70)
femero-popliteal	51 (14)
Tissue temperature	
37–38°C	306 (83)
39–40°C	44 (12)
41–42°C	18 (5)
Total dose of melphalan (mg)	
≤120	306 (83)
>120	62 (17)
Leakage to systemic circulation	
≤1%	298 (81)
>1%	39 (11)
unknown	31 (8)
Acute regional toxicity	
grade I–II	309 (84)
grade III–V	59 (16)

Patients and methods

A total of 499 ILPs in 431 patients with melanoma of the limbs were performed in our institutes in the period 1978–1990. ILPs performed after 1990 were not included in this study because of the frequent use of multiple ILP schedules and the introduction of TNFα. A computer-assisted database, containing all patient, tumour and treatment characteristics, allowed the selection of 368 patients who underwent a single ILP with melphalan. It comprised 270 women (73%) and 98 men (27%) with a mean age of 52 years (range: 17–83). One-hundred and fifty-eight patients (43%) underwent ILP for primary melanoma and 210 patients (57%) for recurrent melanoma. Patient and ILP characteristics are summarized in Table 1.

Our single ILP methodology has been described previously in detail.[8,45] Isolation of the limb was achieved by dissecting and cannulating the main supplying artery and vein, and ligating or clamping the main collaterals. For the axillary and iliac isolation levels, a Steinmann pin was inserted into the head of the humerus or anterior superior iliac spine, around which an occluding Esmarch rubber bandage was fixed and twisted around the root of the limb, proximal to the region of perfusion. This technique prevents collateral circulation in muscles, subcutaneous tissues and skin. In ILPs at the more distal brachial and femoro-popliteal isolation levels, an inflating tourniquet was used. Fifty-one ILPs (14%) were at the axillary, nine (2%) at the brachial, 257 (70%) at the iliac and 51 (14%) at the femoro-popliteal isolation level.

We essentially focused on the maintenance of normal physiology in the limb during ILP.[45] 'Controlled' normothermia (tissue temperatures between 37°C and 38°C) was routinely applied in 306 ILPs and 'mild' hyperthermia (39–40°C) was administered in 44, which were carried out in the framework of the prospective randomized WHO/EORTC trial for high-risk stage I melanoma.[46] Eighteen patients had been perfused using borderline 'true' hyperthermia (41–42°C).[47] Melphalan was routinely given in a dose of 10 mg/l tissues for lower and 13 mg/l for upper limb ILPs.[48,49] Mean melphalan dose given was 91.4 mg (range: 18.0–165.0 mg). Sixty-two patients (17%) received more than 120 mg melphalan in the perfusion circuit. The priming volume of the extracorporal circuit was approximately 750 ml and consisted of whole blood diluted with physiological electrolyte solutions to a haematocrit of approximately 30.

The perfusion procedure was flow-regulated. The optimal flow rate was considered to be the highest possible flow without increasing the venous pressure more than 10 cm above the starting point and with less than 5% leakage to the systemic circulation. A minimal flow rate of 40 ml/min/l perfused tissue is generally sufficient, and provides physiological blood gas values. The venous pressure in the isolated limb was monitored in a peripheral vein at a distal site. Any leakage to the systemic circulation was measured with a radioactive tracer. A small calibration dose of radioactively labelled human serum albumine (labelled with technetium-99m or iodine-131) was given to the systemic circulation and a higher dose of the same radiopharmaceutical to the isolated limb. Continuous monitoring was performed with a precordial scintillation probe. Systemic leakage was quantitatively expressed as a percentage, whereby a 100% leak was considered to give a homogeneous distribution of the tracer in the body.[50] The perfusion duration was 1 h in all ILPs. The mean cumulative systemic leakage after 60 min of ILP was $0.9 \pm 1.9\%$ (range: 0–15.6%). Systemic leakage was 1% or less in 298 patients (81%) and more than 1% in 39 patients (11%). Nine patients (3%) had more than 5% leakage during ILP, two of them (0.5%) having more than 10%. Leakage was not determined in 31 patients (8%), most of whom had ILP at a distal isolation level, where systemic leakage is seldom substantial.

After the ILP procedure, patients spent one night in the Intensive Care Unit. Prophylactic antiemetics were not administered. However, most patients required these drugs in the first 6–12 h post-ILP.

The acute regional toxicity after ILP was graded according to Wieberdink *et al.* (Table 2).[48] No, or only slight, skin toxicity after ILP (grade I–II) was found in 309 patients (84%) and 59 patients (16%) encountered more pronounced acute regional toxicity (grade III–V). The mean hospital stay after ILP was 23 days (range: 7–147 days).

As indicators of systemic toxicity we looked at the incidence of nausea and vomiting, bone marrow depression, and miscellaneous side-effects. Nausea/vomiting and bone marrow depression as expressed by white blood cell counts were graded according to the WHO-criteria (Table 3).[51] In 70 patients (19%), the number and/or timing of white blood cell counts was not sufficient to draw any conclusions concerning the presence or absence of bone marrow

Table 2. Classification of acute regional tissue reactions according to Wieberdink *et al.*[48]

Grade I	No subjective or objective evidence of reaction
Grade II	Slight erythema and/or oedema
Grade III	Considerable erythema and/or oedema with some blistering; slightly disturbed motility permissible
Grade IV	Extensive epidermolysis and/or obvious damage to the deep tissues, causing definite functional disturbances; threatening or manifest compartmental syndromes
Grade V	Reaction which may necessitate amputation

Table 3. Grading of systemic toxicity after perfusion according to WHO[52]

Nausea/vomiting	
grade I	nausea
grade II	transient vomiting
grade III	vomiting requiring therapy
grade IV	intractable vomiting
Bone marrow depression	
grade I	WBC 3.0–3.9 10^9/l
grade II	WBC 2.0–2.9 10^9/l
grade III	WBC 1.0–1.9 10^9/l
grade IV	WBC <1.0 10^9/l

WBC: white blood cell count.

depression. Leucocyte counts of less than 3.0×10^9/l were considered indicators of significant bone marrow depression (WHO grade II–III). Nausea and/or vomiting was considered as showing significant systemic toxicity when it occurred (or was still present) more than 24 h after ILP. The following patient- and treatment-related factors were tested using the chi-square method for their influence on systemic side-effects: sex, age (<60 vs ≥60 years), total dose of melphalan (≤120 vs >120 mg), systemic leakage (≤1 vs >1% and ≤5 vs >5%) and acute regional toxicity (grade I–II vs III–V).

Results

There was no post-operative mortality in the selected 368 patients. Some form of systemic toxicity during hospital stay occurred in 98 patients (27%), including nausea/vomiting after the first post-operative day in 73 patients (20% of the 368 patients), which required the administration of antiemetics (domperidon, metoclopramide, droperidol) in seven patients (2%) (Fig. 1). Bone marrow depression was encountered in 32 patients (9%): grade I in 25 (7%), grade II in five (1.5%) and grade III in two patients (0.5%) (Fig. 2). The white blood cell counts reached their nadir after a median period of 6 days in patients with grade I, and 14 days in patients with grade II and III bone marrow depression. Bone marrow depression was transient in all cases. Other systemic side-effects, such as fever and minor scalp hair loss, occurred in 19 patients (5%) and are listed in Table 4.

Table 5 shows the patient- and perfusion-related variables tested (χ^2-test) as possible risk factors for systemic toxicity. Sex appeared to be a significant factor associated with systemic toxicity ($P<0.05$), with women having a higher probability of developing systemic side-effects. Systemic toxicity also seemed to be somewhat higher in patients who received more than 120 mg melphalan in the perfusion circuit ($P=0.10$).

All variables were also tested for their influence on the different forms of systemic toxicity (Table 5). Age over 60 years ($P<0.05$) and more severe acute regional toxicity

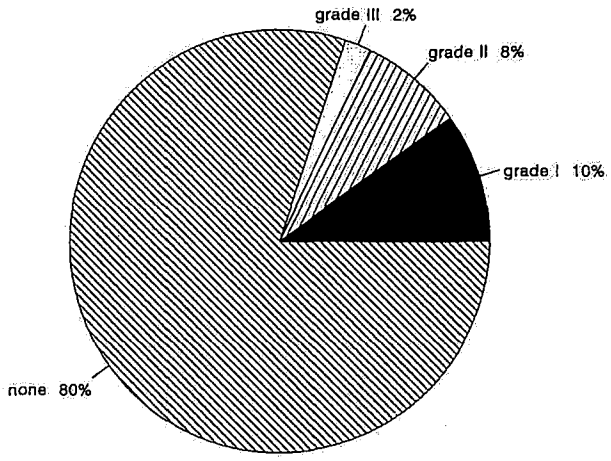

Fig. 1. Nausea and vomiting after ILP with melphalan.

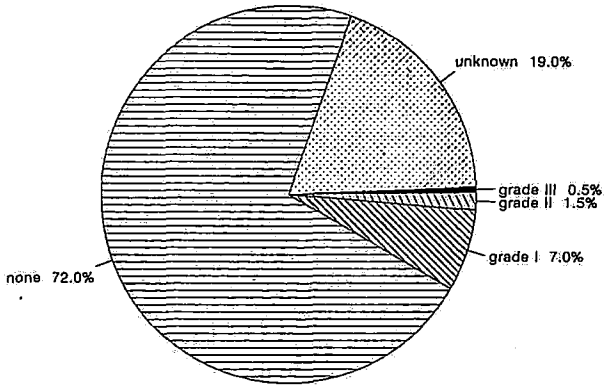

Fig. 2. Bone marrow depression after ILP with melphalan.

Table 4. Distribution of miscellaneous systemic side-effects ($N=19$)

Side-effect	*n*	(%)
Fever	10	(2.7%)
Minimal scalp hair loss	2	(0.5%)
Mild biochemical hepatotoxicity	2	(0.5%)
Reversible renal failure not necessitating dialysis	2	(0.5%)
Other (supraventricular tachycardia, myocardial infarction, gastric bleeding)	3	(0.8%)

($P<0.05$) were both correlated with post-operative nausea and vomiting. Bone marrow depression seemed to occur more frequently in patients who received more than 120 mg melphalan ($P<0.10$) and these patients tended also to have more severe limb toxicity ($P<0.10$). The miscellaneous systemic side-effects occurred significantly more frequently in women than in men ($P<0.05$).

No correlation was found between the occurrence of systemic toxicity and leakage greater than 1%. Also none of the separate forms of systemic toxicity was associated with leakage. Also leakage of 5% or more could not predict systemic side-effects. In the two patients with grade III bone marrow depression, systemic leakage was less than 1%. None of the seven patients with vomiting requiring therapy had a systemic leak greater than 1%. High systemic leakage (more than 10%) was found in only two patients (15.0 and 15.6%, respectively). Neither developed bone marrow depression but the latter appeared to have nausea and fever post-operatively.

Discussion

Mortality during the post-operative period was not seen in present series of 368 patients. In the literature, mortality rates after ILP with melphalan range from 0–9%[13] (Table 6),[14,16–40] with deaths mainly occurring in the early years of ILP experience.[2,14–16] Mortality usually results from pulmonary emboli, cardiac failure, renal failure, or bone marrow depression.[14,16–18,25,32,35–37]

The incidence of severe systemic toxicity after a single ILP with melphalan was minimal in the present series. Leucopenia (white blood cell counts $<1.0 \times 10^9$/l, WHO grade IV toxicity) was not encountered. Only two patients (0.5%) developed transient grade III bone marrow depression. Most patients had nausea and/or vomiting during the first 6–12 h following ILP. Intractable vomiting was not seen post-operatively, 2% of the patients required conventional antiemetic treatment after the first post-operative day with satisfactory results. Marked stomatitis, diarrhoea, maculopapular rashes and pruritis, commonly observed after the systemic administration of melphalan,[12] were not seen. Minimal scalp hair loss was seen in only two patients (0.5%), but without a striking aesthetic effect.

The absence of severe systemic toxicity can be explained by the low percentage of leakage to the systemic circulation during ILP in our series.[52] Leakage of cytostatic drug has been associated with the occurrence of systemic side-effects.[5,26,33,34,38] It is known from literature data that total leakage values during ILP up to 15% do not cause severe systemic toxicity.[53] When melphalan is given in a single dose at the start of ILP, as in our series, the concentration of melphalan in the perfusate declines rapidly during the early phase of drug circulation; there is a 40–50% decrease after 10 min of ILP and after 30 min almost all melphalan has accumulated in the tissues.[9,10,54] Therefore, it is not surprising that melphalan toxicity to the bone marrow was clearly related to greater leakages during the first 5–10 min of ILP in other studies.[26,38] Hence, melphalan should not be administered until haemodynamics have stabilized, and there should be no leakage of the perfusate into the systemic circulation.

On the other hand, systemic toxicity occurred to some extent in about one-quarter of perfused patients. In most cases this was gastro-intestinal toxicity with nausea, retching and transient vomiting which usually started in the immediate post-operative period lasting for more than 24 h. We feel that nausea and vomiting is more pronounced after ILP compared with other surgery of the same duration and extent. These symptoms can be particularly distressing after ILP at the iliac level (which requires a sizeable incision through the musculature of the iliac fossa) because they cause a great deal of additional pain.[55] Patients older than 60 years were significantly more frequently affected by this complication than younger patients in our series. However, these side-effects cannot be attributed completely to the melphalan alone as vomiting has also been reported in 10–50% of patients following routine surgery with general anaesthesia.[56] Besides, melphalan leakage during ILP was not associated with prolonged post-operative nausea and vomiting in the present study. Maybe the combination of melphalan, anaesthetic agents, major surgery and post-operative narcotic analgesics given to control pain, is a powerful emetogenic stimulus.[19,55] Pain itself and the release of waste products from the perfused limb may also be involved, as in our series more severe limb toxicity was associated with nausea and vomiting.

The recently developed serotonin antagonists provide excellent antiemetic control in patients treated with systemic chemotherapy or following surgery.[57–61] A single 8 mg dose of ondansetron (Zofren®) given intravenously at the time of anaesthetic induction has been reported to be effective in controlling nausea and vomiting in the great majority of patients after ILP.[55] As most of our patients required antiemetic therapy in the first hours post-operatively, it seems better to anticipate vomiting by the prophylactic administration of these drugs.

Due to differences in what investigators are willing to list as significant bone marrow depression following ILP, it is difficult to compare our results with those of others. In 9% of our patients white blood cell counts passed the lower limit of normal during hospital stay (4.0×10^9/l). However, for patients with grade I bone marrow depression the timing of the maximum fall suggested that it was related to fluid shifts rather than a drug effect.[62,63] The incidence of significant bone marrow depression (as expressed by white blood cell counts lower than 3.0×10^9/l) in our series (2%) is low compared with literature data, where bone marrow depression is reported in up to 59% of perfused patients (Table 6).[14,16–40]

As systemic leakage during ILP was not significantly associated with bone marrow depression, a phenomenon reported before,[5,19] other factors may have played a role in our series, such as the total dose of melphalan. This link between total drug dose and enhanced bone marrow depression has also been noticed by other investigators.[63,64] Despite very careful wash-out procedures, systemic peak concentrations of melphalan have been observed after reconnection of the perfused limb to the systemic circulation.[40,52] Systemic drug levels after ILP may derive from the intravascular components of the limb[11,40,52] or via

Table 5. Risk factors for systemic toxicity after ILP with melphalan

	Systemic toxicity	N/V*	BMD†	Miscellaneous
Sex (♂ vs ♀)	$P<0.05$	NS	NS	$P<0.05$
Age (years) (<60 vs ≥60)	NS	$P<0.05$	NS	NS
Total dose (mg) (≤120 vs >120)	$P=0.10$	NS	$P<0.10$	NS
Leakage (%) (≤ vs >1)	NS	NS	NS	NS
Regional toxicity (grade I–II vs III–V)	NS	$P<0.05$	$P<0.10$	NS

*Nausea and vomiting grade I–III according to WHO criteria.
†Bone marrow depression grade II–III according to WHO criteria.
NS = not significant.

release of the tissue-bound drug.[5] Therefore, the total dose of melphalan employed may be related to the amount entering the systemic circulation after ILP and so may be responsible for systemic toxicity.

In 3% of our patients fever (38–40°C) was noted in the immediate post-operative period without any evidence of infection. Fever has been reported to occur in up to 42% of patients following ILP with melphalan.[65] It could be a result of the surgical trauma itself, the inflammatory state of the perfused limb,[66] necrosis of muscle,[67] or induction of host-mediators.[68] Treatment is usually not required after exclusion of possible infectious aetiologies.

The reason why women had an increased risk on systemic side-effects is not understood and can only be speculated.

Our rather long mean hospital stay of approximately 3 weeks included a routine 3 day pre-operative work-up period

Table 6. Systemic toxicity after ILP with melphalan: review of the literature

Author	Period	*N**	BMD(%)	SHL(%)	Mortality(%)
Stehlin[17,18]	1958–1964	221	1.8	—	0.5
	1967–1974	185	—	—	1.1
Irvine[19]	1966†	29	31	—	0
Shingleton[20]	1961–1968	35	14	—	0
McBride[16]	1958–1969	678	1.2	—	0.7
Fontaine[21]	1974†	56	5	—	0
Golomb[22]	1959–1976	114	23	—	0
Hansson[23]	1966–1974	22	32	9	0
Jönsson[24]	1976–1981	80	0	—	0
Lejeune[25,26]	1983†	23	26	—	9
	1989†	206	1	—	1
Rege[27]	1962–1979	39	0	—	3
Minor[28]	1985†	28	7	—	0
Fletcher[29]	1965–1983	23	4.3	—	0
Krementz[14]	1957–1966	317	12.2	3.8	2.5
	1967–1974	278	4.0	0.7	0.4
	1975–1982	275	3.6	0	0
Vaglini[30]	1982–1985	95	5	—	0
Krige[31]	1976–1982	93	3	—	0
Di Filippo[32]	1969–1986	136	35	—	5.9
Santinami[33]	1981–1987	140	3.5	—	0
Kettelhack[34]	1982–1989	113	1.8	—	0
Pace[35]	1990†	111	18	3.6	0.9
Skene[36]	1979–1987	91	7	—	2
Hafström[37]	1981–1989	33	3	—	3
Neades[38]	1985–1989	26	31	8	0
Omlor[39]	1989–1991	36	—	6	0
Rauschecker[40]	1990†	44	59	—	—

*Number of ILPs (if not known: number of patients).
BMD: bone marrow depression.
SHL: scalp hair loss.
† Year of publication.

and reflects merely the duration of the acute regional toxic reactions in the perfused limb. During the study period, patients were only discharged when fully mobilized.

In contrast with the negligible incidence of severe systemic side-effects after modern ILP with melphalan, the complications associated with the recently introduced biological response modifier TNFα in ILP can be impressive. Application of high doses of TNFα, in contrast to melphalan, maintains high stable drug levels in the perfusate throughout the ILP,[41] and can lead to prolonged high systemic levels and induce a systemic inflammatory response syndrome comprising fever and chills, hypotension and ARDS.[41-44,69,70] This toxicity is directly correlated to leakage during ILP and can be almost completely prevented by adequate leakage control and extensive washout procedures at the end of the ILP.[41,71] It is also our experience that when leakage is abolished and thorough washout is performed, only a short-lived (less than 1 h) systemically measurable TNFα-peak is observed and systemic toxicity is restricted to the induction of fever and limited to the day of ILP.[72]

In conclusion, systemic toxicity occurred in one-quarter of our patients after ILP with melphalan but was rarely severe, probably as a result of the low systemic leakage percentage. Nausea and vomiting were the most frequently encountered side-effects. Age over 60 years, female sex and more severe limb toxicity proved to be factors associated with an increased incidence of systemic side-effects, drug leakage during ILP was not. These prognostic factors should be considered in attempts to decrease systemic toxicity after ILP.

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