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Kinetics of melphalan leakage during hyperthermic isolation perfusion in melanoma of the limb

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Summary. The kinetics of melphalan leakage into the peripheral blood were studied in 21 patients undergoing hyperthermic isolation perfusion of the upper or lower limb as an adjuvant treatment in high-risk melanoma; in 5 patients cisplatin was added. The melphalan concentrations in the peripheral blood rose predominantly during the first 20 min of perfusion and levelled out to an apparent steady state of about 0.28 μg/ml in upper extremity perfusions, and 0.34 (without cisplatin) and 0.37 μg/ml (with cisplatin) in lower extremity perfusions. Erythrocytes labelled with technetium Tc 99m, which were added concomitantly with melphalan to the perfusion medium, appeared in the systemic circulation of the patients at an almost constant rate of 0.32% (lower and upper limb perfusions without cisplatin and 0.37% (with cisplatin) of total tracer/min. This perfusate flow rate indicated by labelled erythrocytes completely explained the leakage of melphalan from the perfusion circuit into the peripheral blood. Peak concentrations of melphalan in the peripheral blood were observed immediately after reconstitution of normal hemodynamic conditions once isolation perfusion had been terminated. This fraction of melphalan might originate from tissue-binding sites, but also from vascular compartments; therefore, a thorough washing-out procedure might minimize this effect.

Introduction

Hyperthermic isolation perfusion is an established therapeutic modality for adjuvant treatment of high-risk malignant melanoma of the limb after wide excision of the tumor [9, 12, 14, 17]. The goals of regional perfusion are twofold: (1) to achieve very high local concentrations in the tumor-bearing area and (2) to minimize systemic drug concentrations at the same time. Thus, systemic side effects such as bone marrow depression, skin rash or other allergic symptoms, hemolytic anaemia, vasculitis and pulmonary fibrosis can be avoided. Local melphalan-induced toxicity such as muscle damage, edema formation, skin blistering, tissue necrosis or thrombophlebitis can usually be kept at a minimum by avoiding a temperature above 41° C in the subcutaneous tissue [9, 16, 17].

Unfortunately, it is very difficult to achieve a complete isolation of the tumor-bearing extremity during perfusion, even if meticulous surgical techniques are applied. As a consequence, a certain leakage of drug-containing medium into the systemic circulation cannot be avoided [11, 13]. At present, this loss of perfusate from the artificial circuit cannot be defined explicitly; furthermore, no data are yet available describing melphalan concentrations in the peripheral blood following termination of isolation perfusion.

The aim of the present study was to analyze whether melphalan concentrations in the peripheral blood can be assessed intraoperatively by a fast and simplified method using erythrocytes labelled with technetium Tc 99m as a marker for blood flow and average melphalan concentrations in the perfusion medium. Further investigations are required to evaluate the effect of the added cisplatin on the kinetic parameters of melphalan elimination and the importance of redistribution processes for the profile of systemic melphalan concentrations after termination of isolation perfusion.

Patients and methods

Patients. Nine men aged 21–74 years and 12 women aged 28–66 years were included in the study. Their body weight ranged between 50 and 93 kg. All patients suffered from histologically proven high-risk malignant melanoma of the limb (18 of the lower extremity and 3 of the upper extremity) with a vertical tumor diameter of ≥ 1.5 mm.

The hyperthermic isolation perfusion set consisted of a Ben V Bentley oxygenator, a roller pump and a heat exchanger. The perfusion medium was composed of 260 ml human erythrocyte concentrate, 500 ml human albumin solution (5%), 500 ml electrolyte solution (Eufu-

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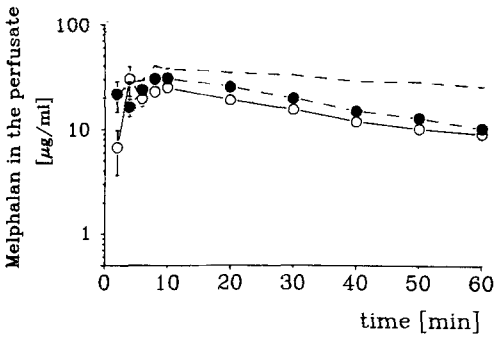

Fig. 1. Diminution of melphalan from the perfusion circuit of hyperthermic isolation perfusion of lower extremities. The perfusions were carried out at temperatures of 38° C in the subcutaneous tissue without (○——○, $n = 13$) or with (●- - - -●, $n = 5$) cisplatin. The results of a blank perfusion performed at 41° C in the perfusate were also introduced (– – –). Error bars were often smaller than symbol size

sol) and 50 ml $NaHCO_3$ solution (8.4%) at a final hematocrit of 15% ± 3% (X ± SE). The extremities were perfused via the external iliac or subclavian vessels at a mean pressure of 104 ± 7 mmHg and a mean flow rate of 418 ± 75 ml/min. To minimize leakage over skin collaterals a tourniquet was placed tightly around the hip or shoulder joint. After a subcutaneous temperature of 38° C had been obtained, melphalan (Alkeran; Wellcome GmbH, Burgwedel/Großburgwedel, FRG) and erythrocytes labelled with technetium Tc 99m (0.5 mCi) were injected into the oxygenator. The dose of melphalan was 10 mg/l limb volume for lower limb perfusions and 13 mg/l limb volume for upper limb perfusions. Five patients undergoing lower limb perfusion were treated additionally with cisplatin (20 mg/l). The volume of the limbs had been determined preoperatively by immersion. Hyperthermic perfusion was continued for 1 h; at the end of this period a subcutaneous temperature of 41° C was reached. Prior to disconnection of the limb from the perfusion circuit, the melphalan-containing perfusate was removed by a single-pass perfusion using 500 ml plasma expander (HAES-steril, 10%) mixed with 1,000 ml electrolyte solution (Eufusol) for both upper and lower extremity perfusions. Immediately before its reconnection to the systemic circulation, the limb was primed with 260 ml human erythrocyte concentrate in 250 ml human albumin (5%).

Methods. Erythrocytes of the patients were labelled with technetium Tc 99m in vitro according to Bauer et al. [3]. The [^{99m}Tc]-derived radioactivity in the blood and perfusate samples was counted in a well-type Beckman scintillation counter; the results were corrected for spontaneous radioactive decay. Serial samples were drawn from a peripheral vein and from the perfusion circuit as indicated in the figures. The samples were immediately placed on ice and plasma was stored at –20° C until further analysis.

Melphalan was quantified by high-pressure liquid chromatography (HPLC) using a Waters pump (6000 A) connected to a Hypersil-Phenyl analytical column (inside diameter, 250 × 4 mm; 5μm; Bischoff, FRG). The detection was performed using a Gynkotek fluorescence RF-530 detector set at 270/350 nm. Melphalan was eluted isocratically with 25% acetonitrile in 0.01 M NaH_2PO_4/H_3PO_4 buffer (pH 3; flow rate, 2 ml/min) at an ambient temperature. Melphalan (4-[bis(2-chloroethyl)amino]-L-phenylalanine), used as the reference compound for HPLC, was obtained from Sigma (Deisenhofen, FRG). Melphalan was extracted from plasma samples according to the procedure described by Woodhouse and Henderson [19]. The calibration curves showed linearity up to 100 μg/ml ($r = 0.9998$). The limit of detection was about 0.05 μg/ml (signal/noise ratio of 3 : 1) for sample volumes of 200 μl. For special purposes, i. e. low concentrations, it was necessary to increase the sample volume to 2 ml.

The elimination rate constant (k_{el}) and elimination half-life ($t_{1/2}$) were derived by least-squares regression analysis of the terminal phase of the concentration-time curve. Areas under the concentration time curve (AUCs) were determined by the trapezoidal method, including extrapolation to infinity. Clearance values were derived by dose/AUC or (for total body clearance) were estimated from the body weight and the mean clearance value of 5.2 ml/(min × kg) according to Benet and Sheiner [5]. The average concentration of melphalan in the perfusate, C_{av}, was calculated according to AUC/t (= 60).

The initial volumes of distribution were derived by Cl/k_{el} (melphalan) or by dose/C_o (^{99m}TC, C_o = extrapolated concentration at time zero). The systemic melphalan plasma concentrations were predicted according to C_{ss} = dose rate/Cl_{total}. The dose rate was estimated by the formulae:

A = ^{99m}Tc leakage/min × distribution volume of ^{99m}TC × C_{av} × (1-HK_{conn})

B = ^{99m}Tc leakage/min × exchangeable plasma volume × C_{av}

C = ^{99m}Tc leakage/min × melphalan dose.

The exchangeable plasma volume was estimated by the hematocrit method according to Lejeune and Ghanem [13] by the formula:

Volume of perfusate × (HK_{perf} – HK_{conn})/HK_{conn} – $HK_{pr\ op}$) × (1-HK_{conn}),

where Hk_{perf}, HK_{conn} and $HK_{pr\ op}$ represent the hematocrit in the perfusate (pre-and post-connection) and the preoperative hematocrit in the systemic blood, respectively. Statistical analysis was performed by a two-tailed *t*-test for independent samples.

Results

The kinetics of melphalan distribution and elimination were studied in 21 patients during hyperthermic isolation perfusion of lower and upper limbs. The cytostatic agent was removed from the perfusion circuit (Fig. 1, lower extremity) at apparent half-lives between 23 and 37 min

Table 1. Pharmacokinetic parameters of melphalan elimination in lower and upper extremities during hyperthermic isolation perfusion

	$T_{1/2}\beta$ (min)	Cl (ml/min)	V_D (ml/l)	V_{DTc} (ml/l)	C_{max}	C_{av}	C_{60}
Lower extremity:							
– Cisplatin ($n = 13$)	32.6 ± 2.1	82.3 ± 6.3	268 ± 41	254 ± 35	36.9 ± 7.2	23.8 ± 1.6	9.1 ± 0.7
+ Cisplatin ($n = 5$)	31.9 ± 2.6	73.4 ± 6.8	185 ± 16	164 ± 36	32.1 ± 1.8	27.4 ± 2.3	10.3 ± 0.9
Upper extremity:							
– Cisplatin ($n = 3$)	35.8 ± 8.3	29.3 ± 9.6	336 ± 57	314	23.2 ± 2.7	17.9 ± 2.3	7.5 ± 1.2

Values represent arithmetic means ± SE. $T_{1/2}\beta$, Elimination half-life; Cl, clearance derived by dose/AUC; V_D, volume of distribution of melphalan (ml/l limb); V_{DTc} = volume of distribution of erythrocytes labelled with technetium Tc 99m (ml/l limb; the volume of perfusate has been subtracted); C_{max}, C_{av}, C_{60}: maximal concentration, average concentration (AUC/60), concentration at 60 min

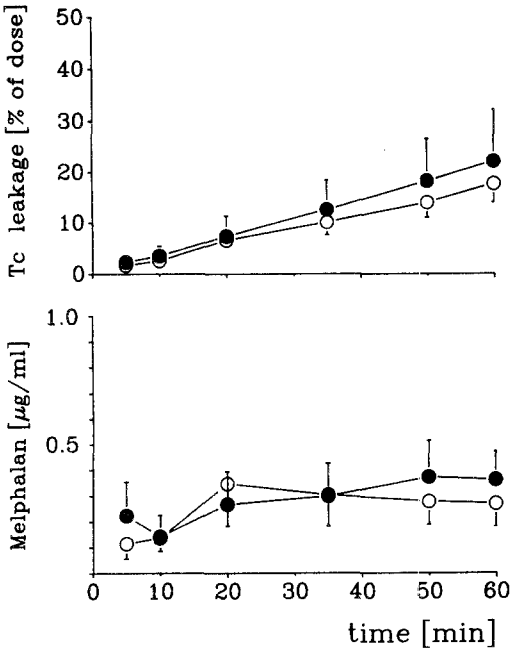

Fig. 2. Leakage of erythrocytes labelled with technetium Tc 99m into the systemic circulation (*upper panel*) and access of melphalan in the peripheral blood during hyperthermic isolation perfusion of lower or upper limbs (*lower panel*). The perfusions were carried out without (○——○, $n = 15$); or with (●——●, $n = 5$) cisplatin. The values for total leakage of ^{99m} Tc at 60 min ($P > 0.4$), with vs without cisplatin) and for melphalan concentrations (60 min; $P > 0.9$, with vs without cisplatin) were not influenced by the presence of cisplatin

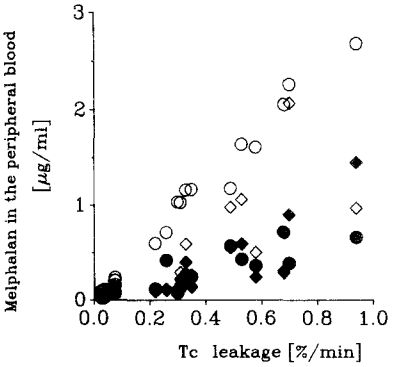

Fig. 3. Comparison of melphalan concentrations observed (●, median value) and predicted by methods A (◇), B (◆) and C (○), assuming infusion kinetics, as indicated by leakage of ^{99m} Tc cf. Fig. 2; melphalan steady-state concentrations = (drug leakage/min) × Cl^{-1}]. The drug leakage from the perfusion circuit was derived by:
A = melphalan C_{av} × %Tc/min × total V_D of erythrocytes tagged with technetium Tc 99m × (1-Hk_{conn})
B = melphalan C_{av} × %Tc/min × exchangeable blood volume × (1-Hk_{conn})
C = total dose of melphalan × %Tc/min,
where C_{av} = average concentration of melphalan derived by (AUC/60)1 %Tc/min = % of total ^{99m} Tc radioactivity/min appearing in the systemic circulation; V_D = volume of distribution (dose/C_o); (1-Hk_{conn}) = plasma fraction of the perfusate after connection; Hk_{conn} = hematocrit after connection

(Table 1), which exceeded the corresponding values reported for melphalan elimination from the total body [1, 5, 10]. The concentrations of melphalan decreased from the observed maximal value of 37 (23) μg/ml in the perfusate of lower (upper) limbs to 9 (7) μg/ml after 60 min. The clearance values for melphalan in the perfusion system were 82 ml/min for the lower extremity and 29 ml/min for the upper limb. None of these kinetic data was significantly influenced by the addition of cisplatin (Fig. 1, Table 1). It was unlikely that this diminution of melphalan concentrations was caused by spontaneous hydrolysis [6] because the drug levels decreased only slowly during the hyperthermic sham perfusion depicted in Fig. 1, which is in accordance with the results reported by Gera et al. [8].

In the perfusion system, initial distribution volumes were almost identical for melphalan and labelled erythrocytes. Values depicted in Table 1 (m/L limb) refer to the differences between the total volume, which was calculated by $c \times k_{el}^{-1}$ for melphalan and dose C_0 for Tc radioactivity, and the volume of perfusate used to prime the circuit. These data indicate (a) a low distribution of melphalan into the perfused tissue and (b) a relatively high distribution of labelled erythrocytes as compared with physiological conditions (total blood volume 75 ml/kg body weight, with skin plus resting skeletal muscle containing 14% of the total blood volume) [2, 15]. This effect may be explained by hyperthermia-induced vasodilatation.

As demonstrated in Fig. 2 (upper panel), an almost linear rise in Tc radioactivity amounting to 21% of the dose (range, 1%–56%) was observed in the peripheral blood. This loss of labelled erythrocytes, resembling an approximately constant flow, was paralleled by a leakage of melphalan, which was detectable thereafter in pheripheral venous blood (Fig. 2, lower panel). Again, when used as the second cytostatic drug in the combined therapy, cisplatin did not influence the dynamics of this leakage. Melphalan concentrations approached steady-state levels rather quickly, although the plateau phase would normally not be expected before 2 hrs, at which time infusion of melphalan at a constant rate is assumed.

The present study tried to predict melphalan concentration levels in the peripheral blood based on the data of labelled erythrocyte leakage and on melphalan kinetics in the perfusion circuit. The calculations were performed using formulae for infusion kinetics (for details see Patients and methods). The total body clearance was not determined individually, but was rather derived from the body weight and mean values published in the literature [5]. The rate of drug leakage was derived (methods A and B) from the plasma flow into the systemic circulation and the average melphalan concentration in the perfusate (C_{av}, Table 1). Alternatively (method C), it was derived from the fraction of radiolabel leakage (% of total tracer/min) and the total dose of melphalan.

The results shown in Fig. 3 (◇ = A, ◆ = B, ○ = C) were compared with the concentrations actually observed (Fig. 3, ●). Assuming identical redistribution of labelled erythrocytes and melphalan (method C), the calculated results did not correspond with the concentrations actually measured, which were many times lower than those predicted by this procedure. This indicated that melphalan was retained in compartments of the perfused tissue other than the labelled erythrocytes, although the initial volumes of distribution were quantitatively similar.

The best correspondence of predicted vs observed values was obtained by method B (Table 2). The plasma

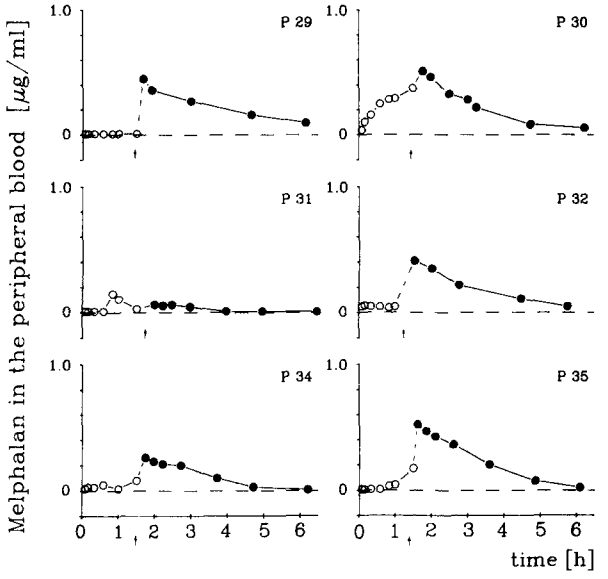

Fig. 4. Time course of melphalan plasma concentrations in the peripheral blood during hyperthermic isolation perfusion and after reconnection of the perfused lower limb. Profiles of 6 individual patients. The perfusions were carried out without cisplatin. From the rise of plasma concentrations and the average total volume of distribution (body weight × .62 l/kg), the following fractions of the melphalan dose gaining systemic access were calculated: P 29 = 18.02 mg (18%); P 30 = 5.3 mg (4.1%); P 31 (upper limb) = 1.5 mg (2.5% P 32 = 1.7 mg (1.4%); P 34 = 5.8 mg (6.4%); P 35 = 12.2 mg (8.2%)

flow was estimated from the leakage of labelled erythrocytes (% of tracer/min) multiplied by the exchangeable plasma volume calculated from the hematocrit values (according to Lejeune and Ghanem [13]; for further details see Patients and methods). Method A differred from method B simply in the assumption that the distribution volume of labelled erythrocytes is identical with the volume of perfusion medium after connection instead of using the value of exchangeable plasma volume. As depicted in Fig. 3 and Table 2, a good correspondence (method A) with observed concentrations was found only for those cases with a low Tc leakage. The accuracy of method A at a higher Tc leakage ranged between the results obtained from methods B and C. We interpreted this discrepancy, especially between methods A and B, as indicating that the distribution volume of labelled erythrocytes in the hyperthermic limb could not be measured accurately enough to enable its use as a parameter for this concept.

For removal of the major proportion of melphalan from the perfused limb before reconnection of the latter to the systemic circulation, the hyperthermic isolation perfusion was always terminated by rinsing the limb with 1,500 ml drug-free electrolyte solution. Afterwards, the blood volume was replaced by a solution (510 ml) consisting of erythrocytes and human albumin. In six patients, the melphalan concentrations in the peripheral blood were monitored for 6 h after reconnection. The results are listed as individual time courses in Fig. 4. In most patients the peak concentration of melphalan was observed 1.5 min after reconnection. During the observation period the concentration decreased, approaching the detection level of the assay after 6 h. Assuming a distribution of melphalan in the total body according to the kinetics of bolus injections, from the rise in plasma concentrations it was calculated that approximately 1.7–18 mg melphalan, equal to 1.4%–18% of the total dose, reached the systemic circulation after reconnection (individual results are given in the legend to Fig. 4).

In a separate study presently under investigation, the postoperative events were monitored in a larger group of patients. There was a correlation between the amount of labelled erythrocytes in the systemic circulation during perfusion and the extent and duration of postoperative leucopenia. Of 20 patients, 9 (42.9%) with a Tc leakage of <20% showed leucopenia between the 11th and 16th day after perfusion, with a nadir of 2,200 leucocytes occurring on the day 12. Of 24 patients, 17 (73.9%) with a Tc leakage of >20% became leucopenic after the 12th postoperative day, with a nadir of 1,800 leucocytes being seen on day 15.

Table 2. Leakage of erythrocytes tagged with technetium Tc 99m and of melphalan into the systemic circulation during hyperthermic isolation perfusion

	^{99m}Tc leakage		Plasma flow (ml/min)	Melphalan leakage[a] (%/60 min)	Ratio $C_{predicted}/C_{observed}$[b]		
	(%/60 min)	(%/min)			A	B	C
Lower extremity:							
– Cisplatin (n = 13)	19 ± 4	0.34 ± 0.06	9.7 ± 2.6	11.5 ± 3.2	1.7 ± 0.4	1.1 ± 0.2	4.7 ± 0.8
+ Cisplatin (n = 5)	22 ± 10	0.37 ± 0.17	7.6 ± 3.2	10.4 ± 4.2	1.4 ± 0.4	1.3 ± 0.5	3.2 ± 0.5
Upper extremity:							
– Cisplatin (n = 2)[c]	16.6 1.2	0.28 0.02	8.2 0.4	3.9 1.6	0.3 1.0	0.3 0.9	0.8 0.8

Values represent arithmetic means ± SE. Plasma flow was derived from ^{99m}Tc leakage (%/min) and ^{99m}Tc distribution volume corrected for the hematocrit value after connection.

[a] Total melphalan leakage within 60 min was calculated from plasma flow × 60 × C_{av} (C_{av} of Table 1)

[b] $C_{predicted}$ = melphalan plasma concentration in the peripheral blood predicted by:

A = plasma flow × C_{av} × Cl^{-1}

B = % Tc/min × exchangeable blood volume (cf. Patients and methods) × C_{av} × Cl^{-1}

C = % Tc/min × total dose of melphalan × Cl^{-1}

[c] Systemic Tc leakage was not estimated in one perfusion (cf. Table 1)

Discussion

Hyperthermic isolation perfusion of melanoma-bearing limbs has proven to be an effective adjuvant therapy in the treatment of high-risk melanoma patients [9, 12, 14, 17]. Its major goals are to enhance the sensitivity of tumor cells to chemotherapy by hyperthermia and to achieve high drug concentrations within the target tissue while simultaneously minimizing systemic side effects.

In the present study, we observed average concentrations of melphalan within the perfusion circuit that were about 100-fold of those in the systemic circulation. Thus, our intention of keeping the drug mainly within the perfusion circuit was realized. The appearance of melphalan in the peripheral blood, however, indicated that a certain amount of the drug was lost despite all efforts to isolate the limb completely during perfusion. Drug leakage into the systemic circulation has also been observed by other authors [11, 13]; our findings correspond with their observations that this cannot be avoided. The melphalan concentration in the peripheral blood during isolation perfusion exceeds the peak plasma concentrations of melphalan observed during systemic chemotherapy using conventional dosage regimens by 2–4 times [6, 7, 18, 20]. Manyfold higher levels of melphalan, however, are achieved by high-dose therapy [1, 10]. Therefore, it is conceivable that some postoperative events may at least in part be explained as melphalan-induced side effects.

The derived pharmacokinetic parameters of melphalan elimination from the perfusion circuit were in good agreement with data reported earlier [4]. In the present study, we intended to analyze the leakage of melphalan from the perfusion circuit into the patient's circulation in greater detail. The results clearly demonstrate that this process is mediated by an intra-individually almost constant blood flow, which obviously cannot be stopped by clamping major vessels with the additional use of a tourniquet. The systemic melphalan concentrations were greatly overestimated when the fraction of melphalan leakage from the perfusion circuit was predicted to be identical with the total radioactive fraction appearing in the peripheral blood (method C). This suggested that a major fraction of melphalan was retained in the perfused tissue and that redistribution from the tissue compartment into the perfusion circuit was slow. Furthermore, a non-blood-flow-mediated distribution between perfusion system and systemic blood stream, e. g. by lymphatic drainage, could be ruled out. The leakage of labelled erythrocytes as well as melphalan was not affected by the additional presence of cisplatinum in the perfusion medium. These findings correspond with results reported in the literature [21], whereby no effects of cisplatinum on the kinetic parameters of melphalan could be observed in patients suffering from ovarian cancer.

We obtained suitable results by predicting systemic melphalan concentrations using the average perfusate concentration and the calculated plasma flow rates. It was remarkable that the best prediction was obtained when the plasma flow rates were calculated from (a) the exchangeable plasma volume derived from hematocrit values according to Lejeune and Ghanem [13] and (b) the velocity of Tc leakage (method B). Both parameters could be measured without significant delay, in contrast to the application of HPLC for quantitative analysis of melphalan concentrations in the peripheral blood. The average melphalan concentrations in the perfusion circuit, which are also needed for quantitative prediction, did not vary greatly between comparable perfusions. These values may therefore be estimated in some perfusions and may then be used as a constant of that specific perfusion setup.

Theoretically, the determination of the total volume of perfusion medium after connection should also be possible by calculation of the initial distribution volume of labelled erythrocytes (method A). However, according to our experience, it is very difficult to extrapolate the data at time zero from the time course of labelled erythrocyte diminution in the hyperthermic limb. In interpreting our results, we came to the conclusion that the values were too high and that the predicted concentrations had therefore been overestimated, especially for perfusions during which a high Tc leakage was observed.

The melphalan concentrations in the peripheral blood approached steady-state levels rather quickly, although this is generally expected to occur after about 2 h, taking into consideration the constant infusion rate of melphalan. We explained this effect from the time course of melphalan concentrations in the perfusate, which initially exceeded the average concentration and dropped below average levels during the course of perfusion. Therefore, the relatively high drug load, especially during the initial phase of perfusion, quickened the increase in systemic melphalan concentrations. A comparison of predicted vs observed values, however, indicated that the average concentration is a suitable parameter for the estimation of drug leakage by this simplified procedure.

In a small number of isolation perfusions of lower limbs, we monitored the profile of melphalan concentrations for 6 hrs after reconnection. Our results show that the peak concentrations of melphalan appeared immediately after reconstitution of physiological conditions of hemodynamics. Therefore, the main portion of melphalan that appears in the peripheral blood gains access after termination of isolation perfusion. This amount of drug becomes of major importance in the consideration of melphalan-induced systemic side effects. The peak concentrations occurred hardly without any delay. At present, it is unclear whether this fraction of melphalan originated predominantly from tissue-binding sites or whether vascular compartments of the perfused limb are an additional source. The rise observed in melphalan concentrations was quantitatively different for individual patients, indicating that the washing-out procedure is the predominating factor for this effect. To minimize this late distribution of melphalan from the perfused limb, techniques for the washing-out phase should be improved.

In the present study we performed a detailed analysis of the kinetics of melphalan leakage into the patients' systemic circulation during hyperthermic isolation perfusion. Our results indicate that melphalan is lost from the perfusion circuit by blood flow only, e. g. via deep collateral vessels. A major proportion of melphalan is retained in the perfused tissue; thus, only that fraction of melphalan remaining within the perfusion circuit can gain systemic

access throughout the process of perfusion. The peak concentrations of melphalan in the peripheral blood, however, were observed immediately after reconnection of the perfused limb to the systemic circulation. This indicates that a considerable amount of the drug, which might be of major importance for melphalan-induced systemic side effects, reaches systemic access after normal conditions of hemodynamics have been reconstituted. To keep this unwanted escape of melphalan into the systemic circulation as low as possible we strongly recommend a very careful washing-out procedure.

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