Cancer Chemother Pharmacol (1999) 43: 35–42

ORIGINAL ARTICLE

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Pharmacokinetics of melphalan in isolated limb perfusion

Received: 23 February 1998 / Accepted: 2 June 1998

Abstract The pharmacokinetics of melphalan was studied by sampling of tissue and plasma in 72 rats that underwent isolated hyperthermic limb perfusion under different conditions. A miniaturized extracorporeal circulation system for small animals was used for perfusion of the rat hindlimb. Melphalan levels (L-phenylalanine mustard, L-PAM) were determined by high-performance liquid chromatography (HPLC). The temperature of the perfusate plasma and tissue, pH, administration method, and flow rate were modified and compared with regard to their influence on pharmacokinetic parameters. The highest tissue penetration of melphalan was observed under the following conditions: (a) pH range of the perfusate plasma between 7.3 and 7.7 (physiological environment), (b) temperature range of the perfusate from 40° to 41.5 °C (destruction of cellular carrier systems at higher temperatures and increased inactivation by hydrolysis of melphalan above 41.5 °C), (c) application of melphalan as a single dose into the reservoir of the extracorporeal circuit (optimal tissue penetration), and (d) reduced perfusate flow (prolonged contact time between perfusate and tissue).

Key words Melphalan · Pharmacokinetics · Hyperthermic limb perfusion · Melanoma

Introduction

Before isolated perfusion chemotherapy was first described in 1957 [10] and became an established therapeutic approach in subsequent years the only therapy for malignant melanoma of the limbs was wide excision of the tumor [21]. Mutilating surgical techniques involving amputation of the limb or compartment resection were often necessary to reduce the frequency of locoregional metastases. Melanoma tends to recur locally because of the microscopic involvement of local tissues, of regional lymphatics, and of in-transit metastases [25, 42]. Regionally recurrent disease significantly reduces the 5-year survival rate [23].

Isolation perfusion techniques combined with the additive cytotoxic effect of hyperthermia [6, 7] permitted the application of increased doses of melphalan to the limb, resulting in high local concentrations and at the same time avoiding systemic toxic effects such as suppression of hematopoiesis. Disadvantages of this method are the limited perfusion time, difficulties with increasing numbers of courses, and the amount of operative equipment involved [11, 22, 46]. The method is preferably used in the treatment of sarcoma and other primary tumors, of locoregional recurrences, and of in-transit metastases of malignant melanoma of the extremities. Prospective randomized and retrospective studies have demonstrated the advantages of isolation perfusion with regard to survival and disease-free rates [18, 24, 31, 39, 42].

Although this treatment has been established in cancer chemotherapy for several years [14, 28], studies on the pharmacokinetics of melphalan in isolation perfusion are rare [3–5, 30, 36]. The present study demonstrates the relationship between the pharmacokinetics of melphalan (L-PAM) and modified perfusion techniques for the evaluation of perfusion conditions that are optimal for the uptake of L-PAM into tissue. An important pharmacokinetic parameter for the cytostatic availability of melphalan is the AUC (*a*rea *u*nder the *c*urve, integral of the concentration-time curve over time), which should be high in tumor tissue and low in the systemic circulation.

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Materials and methods

Melphalan

L-Phenylalanine mustard (L-PAM) was donated by Wellcome (Burgwedel, Germany). The hydrolysis products monohydroxy- and dihydroxymelphalan were not analyzed. The internal standard solution (IS) was *N*-acetylprocainamide, which was supplied by Aldrich Chemicals (Milwaukee, USA). All substances were identified by mass spectrometry.

Workup

Blood and tissue specimens were stored at −80 °C until analysis. For determination the samples were melted and centrifuged. The samples were homogenized with a vortex-type mixer and sucked through a Sep-Pak-C18 cartridge (activated with 1 ml methanol) with a vacuum aspirator. The cartridges were washed with 5 ml deionized water and sucked dry, then the samples were eluated with 1.5 ml methanol into tubes. The elution fluid was evaporated and the samples were stored at −20 °C. For analysis the samples were dissolved in ice-cold mobile phase, homogenized for 30 s with a vortex, and centrifuged. Aliquots of 50 µl were injected into the high-performance liquid chromatography (HPLC) system [35].

HPLC system

Melphalan was assayed by HPLC. The system consisted of a reversed-phase C_{18} column (250 × 4.6 mm, Spherisorb S 5 ODS 1) and a precolumn (25 × 4.6 mm, Spherisorb S 10 P). Furthermore, an HPLC pump (Varian Liquid Chromatograph, Model 5000), a fluorescence detector (Shimadzu RF-530), and an integrator (Shimadzu C-R3A) belonged to the system. At an initial pressure of 180 bar the mobile phase (acetonitrile: phosphate buffer = 35:65, vol/vol) was eluated at 1.5 ml/min. The analysis time was about 8 min. The compounds were measured with the fluorescence detector at an excitation wavelength of 270 nm and an emisson wavelength of 350 nm. The phosphate buffer was made from 1.38 g NaH_2PO_4 (10 mM), 2.02 g sodium heptane sulfate (10 mM), and deionized water to 1000 ml. The buffer was titrated with 40% phosphate acid at a pH of 3.00 [35].

Calibration

The concentration of L-PAM was determined by the peaks of calibration curves, which were prepared before every test series. The calibration points were derived using a stock solution of L-PAM consisting of 1.5 or 10 µg melphalan dissolved in 1.5 ml plasma. The equation for the calibration curve was:

$$\text{Calibration factor} = \frac{\text{area IS} \times \text{amount L-PAM (1.5 or 10 µg)}}{\text{area L-PAM} \times \text{amount IS (tissue 2 µg, perfusate 5 µg)}} .$$

The concentration of the sample was calculated by:

$$\text{Concentration} = \text{calibration factor} \times \frac{\text{area L-PAM}}{\text{area IS}} .$$

Perfusion technique and experimental planning

About 100 rats underwent isolated hindlimb perfusion via a miniaturized extracorporeal circulation system for use in small animals. The system consists of a bubble oxygenator and heat exchanger and of commercially available roller pumps, polyethylene cannulas, and silicone tubes [34]. A total of 72 plasma and tissue samples of melphalan were selected for analysis with the HPLC system. The other 28 rats did not enter the evaluation because of technical problems encountered during the first perfusions and insufficient experimental data. The whole perfusion time was about 90 min. The temperature of the perfusate plasma and tissue, pH, flow rate, and administration method were modified depending on the specific test group of rats as demonstrated in Table 1. The assignment of the perfusion series to the different methods was performed by randomization. Blood samples of the circuit were taken from the venous system at 5, 15, 30, 45, 60, 75, and 90 min after the start of perfusion. Tissue specimens of constant weight (1.5 g) were taken at 5, 30, 45, 60, and 90 min respectively, from an identical site on the limb.

Melphalan was dosed per milliliter of perfused tissue volume. The priming volume of the system was 25 ml at every perfusion. The volume of the rat hindlimb was measured by water replacement [47]. Due to variation in extremity volumes the measured concentrations of L-PAM in blood and tissue of the perfusion circuit were standardized with a correctional factor for every perfused rat. The arithmetic mean value recorded for melphalan per milliliter of extremity volume was 363.7 µg/ml.

$$\text{Correctional factor} = \frac{363.7\ \text{µg/ml} \times \text{volume of extremity (ml)}}{\text{dose (µg)}}$$

Calculations

The pharmacological model for the calculations involved the isolated mathematical analysis of the distribution and eliminaton of L-PAM in the perfused tissue or in the blood of the perfusion

Table 1 Experimental planning and groups according to the modified perfusion method (n = Numbers of rats and perfusions)

Temperature of blood and tissue samples	Perfusion flow		pH		Application method	
37–38.5 °C	0.4 ml/min	$n = 9$	pH 6.0 ≤ 7.0	$n = 5$	Single bolus	$n = 6$
$n = 17$	0.5 ml/min	$n = 8$	pH 7.0 ≤ 7.3	$n = 8$	Continuous	$n = 6$
			pH 7.3 ≤ 7.7	$n = 4$	Reservoir	$n = 5$
38.5–40 °C	0.4 ml/min	$n = 8$	pH 6.0 ≤ 7.0	$n = 6$	Single bolus	$n = 6$
$n = 17$	0.5 ml/min	$n = 9$	pH 7.0 ≤ 7.3	$n = 5$	Continuous	$n = 6$
			pH 7.3 ≤ 7.7	$n = 6$	Reservoir	$n = 5$
40–41.5 °C	0.4 ml/min	$n = 9$	pH 6.0 ≤ 7.0	$n = 8$	Single bolus	$n = 6$
$n = 18$	0.5 ml/min	$n = 9$	pH 7.0 ≤ 7.3	$n = 3$	Continuous	$n = 6$
			pH 7.3 ≤ 7.7	$n = 7$	Reservoir	$n = 6$
41.5–42.5 °C	0.4 ml/min	$n = 10$	pH 6.0 ≤ 7.0	$n = 5$	Single bolus	$n = 7$
$n = 20$	0.5 ml/min	$n = 10$	pH 7.0 ≤ 7.3	$n = 8$	Continuous	$n = 7$
			pH 7.3 ≤ 7.7	$n = 7$	Reservoir	$n = 6$

circuit (one-compartment model). The AUC value for melphalan in perfusate and in tissue was determined by the trapezoidal method, including extrapolation to infinity [12]. The half-life ($t_{1/2}$) and the elimination rate constant (K_e) were determined by least-squares regression. Calculation was performed by:

$$t_{1/2} = \frac{\ln 2}{K_e} = \frac{0.693}{K_e} \quad .$$

The zero concentration of melphalan [C(O)] was calculated by extrapolation of the arithmetic means of the concentration-time curve. The average maximal concentration (C_{max}) and t_{max} of L-PAM were determined by comparison of the arithmetic means of the concentration-time curve of the test groups. Clearance values (CL) were derived by *dose*/*AUC*. The volume of distribution (V_z) of melphalan in the perfusate was calculated by clearance (CL)/elimination rate constant (K_e):

$$V_Z = \frac{\text{dose}}{K_e \times AUC_{5\ \text{min}-\infty}} = \frac{CL}{K_e} \quad .$$

The validity of the data was proved with Wilcoxon, Mann, and Whitney's test for randomized samplings [38].

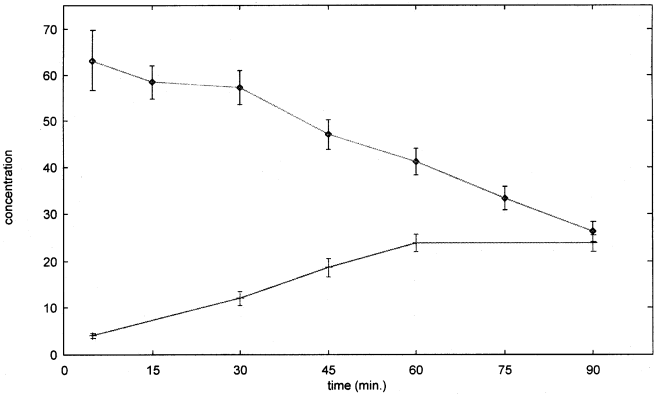

Fig. 1 Concentration-time curve generated for 72 perfusions. Presented is the concentration course of melphalan in the blood of the perfusion circuit (◆, μg/ml) and in the tissue (–, μg/g). Data represent mean values ± SEM

Results

Figure 1 shows the arithmetic mean values recorded for the concentration-time curves of all perfusions without regard to test arrangements into different groups. The mean dose of melphalan per milliliter of extremity volume at a perfusion time of 90 min was 365 μg.

Table 2 demonstrates how varied perfusion conditions changed the pharmacological characteristics $AUC_{5–90\ min}$, C(O), and C_{max} of the tissue during the perfusion time of 90 min. The alkalization of the perfusate to a pH range between 7.3 and 7.7 increased the uptake of melphalan into the bulk of the tissues. The extrapolated zero concentration C(O) of melphalan was significantly different in comparision with that recorded for groups with a lower pH range. In the perfusate, more pharmacokinetic parameters were analyzed (Tables 3, 4). In contrast to the increased tissue affinity of melphalan at pH ranges above 7.3, there was no statistically significant ($P < 0.05$) difference in the perfusate pharmacokinetics of the three pH ranges.

With a mean integrated area of 1832.0 μg min g^{-1} L-PAM under the concentration-time curve in the series with a tissue and perfusate temperature of 40–41.5 °C, the uptake of melphalan in the treated tissue was more than 60% higher than in the samples with perfusion temperatures above 41.5 °C. The extrapolated zero concentration C(O) was significantly different as compared with that recorded for the other groups (Table 2).

The disappearance of melphalan in the perfusate, mainly involving L-PAM's hydrolysis to monohydroxy- and dihydroxymelphalan at elevated temperatures [15, 30], is characterized by its half-life ($t_{1/2}$) and distribution volume (V_z) as demonstrated in Tables 3 and 4. Within the temperature range of 41.5–42.5 °C the half-life of L-PAM ($t_{1/2}$ = 36.3 min) was significantly shortened during a perfusion time of 90 min. In comparison, $t_{1/2}$ in the group with moderate hyperthermia (40–41.5 °C) was about 51.5 min. The distribution volume (V_z) of melphalan in this test series was about 48.8 ml. The highest C_{max} in the tissue was found within the temperature range of 40–41.5 °C after 67.5 min (Table 2). All these parameters demonstrate that appreciable drug uptake in

Table 2 Pharmacokinetic parameters of melphalan in tissue. Data represent mean values ± SEM

Modified perfusion conditions	$AUC_{5–90\ min}$ (μg min g^{-1})	C(O) (μg/g)	C_{max} (μg/g)	t_{max} (min)
pH 6.0 ≤ 7.0	1310.4 ± 177.2	3.8 ± 1.1	30.5 ± 4.6	70.6 ± 4.7
pH 7.0 ≤ 7.3	1353.3 ± 195.8	5.8 ± 1.8	27.1 ± 3.4	67.5 ± 4.4
pH 7.3 ≤ 7.7	1716.5 ± 187.5	*6.2 ± 1.2	34.9 ± 3.6	67.5 ± 3.5
37–38.5 °C	1364.5 ± 202.5	4.9 ± 1.8	27.0 ± 3.5	67.0 ± 4.8
38.5–40 °C	1659.9 ± 222.7	6.0 ± 1.9	34.6 ± 4.5	*60.8 ± 5.2
40–41.5 °C	*1832.0 ± 269.9	*6.3 ± 1.6	38.5 ± 6.0	67.5 ± 4.0
41.5–42.5 °C	1093.4 ± 136.2	4.2 ± 1.2	23.8 ± 3.1	77.2 ± 4.7
Single bolus	1376.4 ± 226.1	8.0 ± 1.9	24.6 ± 3.6	65.4 ± 4.8
Continuous	1439.5 ± 177.3	5.3 ± 0.9	28.5 ± 3.6	66.0 ± 4.0
Reservoir	1630.0 ± 144.5	*2.1 ± 0.3	*40.4 ± 3.9	75.0 ± 3.2
Flow = 0.4 ml/min	**1778.8 ± 167.8	***8.4 ± 1.4	*35.5 ± 2.9	66.2 ± 3.9
Flow = 0.5 ml/min	1172.8 ± 119.2	2.3 ± 0.2	26.1 ± 3.3	70.8 ± 2.8

*$P < 0.05$; **$P < 0.005$; ***$P < 0.001$

Table 3 Pharmacokinetic parameters of melphalan in the perfusate plasma. Data represent mean values ± SEM

Modified perfusion conditions	$AUC_{5–90\ min}$ (μg min ml^{-1})	$AUC_{5\ min–\infty}$ (μg min ml^{-1})	$t_{½}$ (min)
pH 6.0 ≤ 7.0	3650.2 ± 289.4	4966.5 ± 438.6	42.8 ± 3.24
pH 7.0 ≤ 7.3	4593.7 ± 397.5	6726.7 ± 661.9	45.1 ± 3.63
pH 7.3 ≤ 7.7	3779.9 ± 285.2	5760.2 ± 466.4	50.1 ± 5.21
37–38.5 °C	3951.9 ± 394.7	6328.1 ± 736.5	56.2 ± 5.4
38.5–40 °C	3814.0 ± 369.2	5216.1 ± 556.1	41.6 ± 2.8
40–41.5 °C	3912.1 ± 341.5	5986.3 ± 534.9	51.5 ± 5.6
41.5–42.5 °C	4306.8 ± 438.8	5744.1 ± 678.7	**36.3 ± 3.3
Single bolus	3779.9 ± 380.1	5094.6 ± 583.8	39.2 ± 2.8
Continuous	3543.8 ± 247.2	4920.6 ± 366.5	41.8 ± 4.3
Reservoir	*4794.6 ± 320.1	***7659.3 ± 493.7	**58.7 ± 4.1
Flow = 0.4 ml/min	4350.3 ± 283.6	*6506.8 ± 456.0	49.3 ± 3.6
Flow = 0.5 ml/min	3665.6 ± 253.8	5129.9 ± 407.6	42.8 ± 2.9

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 4 Pharmacokinetic parameters of melphalan in the perfusate plasma. Data represent mean values ± SEM

Modified perfusion conditions	V_z (ml)	CL (ml/min)	C_{max} (μg/ml)	T_{max} (min)
pH 6.0 ≤ 7.0	70.3 ± 8.6	1.2 ± 0.1	84.0 ± 6.8	18.5 ± 3.4
pH 7.0 ≤ 7.3	57.7 ± 5.6	0.9 ± 0.1	100.5 ± 11.9	25.8 ± 4.3
pH 7.3 ≤ 7.7	69.2 ± 7.2	1.0 ± 0.1	79.2 ± 8.5	30.0 ± 4.1
37–38.5 °C	75.1 ± 6.5	1.09 ± 0.2	77.4 ± 7.5	23.5 ± 3.6
38.5–40 °C	70.5 ± 10.3	1.17 ± 0.1	86.4 ± 10.5	30.0 ± 5.9
40–41.5 °C	71.3 ± 9.8	1.02 ± 0.1	89.1 ± 14.2	23.0 ± 3.8
41.5–42.5 °C	*48.8 ± 5.6	1.02 ± 0.1	96.6 ± 10.2	23.0 ± 4.8
Single bolus	70.5 ± 9.3	1.28 ± 0.1	*107.2 ± 12.5	*14.4 ± 3.7
Continuous	62.4 ± 6.4	1.11 ± 0.1	72.1 ± 5.8	32.1 ± 2.5
Reservoir	64.2 ± 5.1	*0.79 ± 0.1	83.9 ± 6.6	27.1 ± 4.8
Flow = 0.4 ml/min	60.1 ± 4.3	0.93 ± 0.1	92.3 ± 8.6	27.5 ± 3.3
Flow = 0.5 ml/min	71.4 ± 7.1	1.21 ± 0.1	83.5 ± 6.6	22.1 ± 3.1

*$P < 0.05$; **$P < 0.005$; ***$P < 0.001$

the treated tissue was found at a perfusion temperature of 40–41.5 °C and a perfusion time of more than 60 min.

In the next test series we compared the pharmacokinetic characteristics of L-PAM using different administration modes in the perfusate plasma (Tables 3, 4). In 25 perfusions the whole melphalan dose was given as a single bolus directly into the arterial line at the start of perfusion. In 25 cases, L-PAM was dosed as separate aliquots during the first 20 min of perfusion (continuous mode). In the last 22 samples the total melphalan dose was given as a bolus into the pump oxygenator reservoir of the extracorporeal circuit.

The mean maximal concentration (C_{max} = 107.2 μg/ml) in the perfusate was reached at approximately 14.4 min by administration of the whole melphalan dose as a single bolus into the perfusion circuit (Table 4). Despite this, the highest C_{max} of L-PAM in tissue was found at 75 min perfusion time with a significant reduction in the clearance (CL = 0.79 ml/min) of the perfusate after drug administration into the oxygenator reservoir. The mean maximal concentration in tissue was significantly reduced after bolus injection of melphalan at approximately 65 min of isolated perfusion as compared with the application of L-PAM into the reservoir (Table 2). Although we found significantly elevated $AUC_{5–90\ min}$ and $AUC_{5\ min–\infty}$ values for L-PAM in the perfusate plasma with a prolonged half-life ($t_{1/2}$) of 58.7 min in this group, there was no correspondingly high tissue level (Table 3, Fig. 2).

Due to the slow distribution and uptake of melphalan into the bulk tissues resulting from application of the drug via the reservoir, the mean extrapolated zero concentration [C(O) = 2.1 μg/g] was about 4 times lower than that obtained in the test series with bolus injection as shown in Table 2. Continuous injection of melphalan did not have any pharmacokinetic advantage in comparison with the other two methods (Fig. 2). These data show that there is no linear correlation between plasma and tissue levels of melphalan during isolated perfusion. The integrated areas under the tissue-concentration-time curve of melphalan show only slight differences for all application methods despite the significantly different $AUC_{L\text{-}PAM}$ obtained in the perfusates as demonstrated in Fig. 2. An appreciable uptake of melphalan after 60 min was achieved by administration of L-PAM via the oxygenator reservoir as shown by the corresponding C_{max} values, the extrapolated zero concentration of melphalan C(O), and the prolonged half-life ($t_{1/2}$).

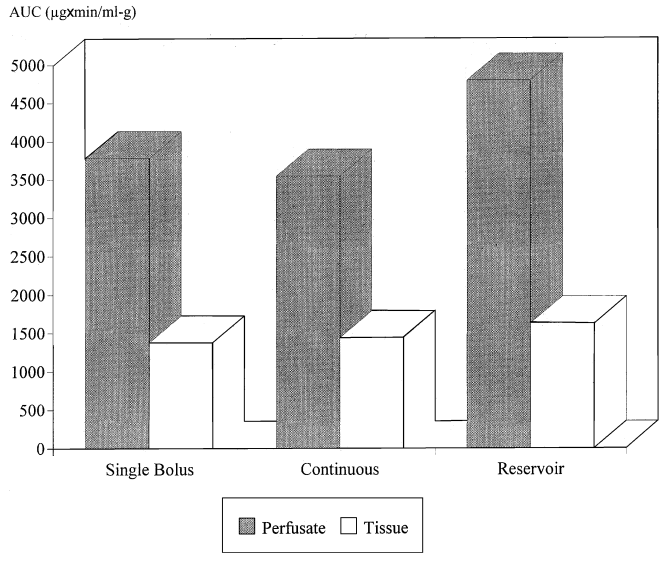

Fig. 2 $AUC_{L\text{-}PAM}$ determined in perfusate and tissue for the perfusion time of 90 min according to modified administration methods. Data represent arithmetic mean values (see Tables 2, 3)

The last series examined melphalan uptake with respect to different perfusion flow rates. In the first group, 36 animals underwent perfusion at a flow rate of 0.4 ml/min per 100 g weight. The other 36 perfusions were performed at a roller-pump flow rate of 0.5 ml/min per 100 g weight. The $AUC_{5\text{–}90\ min}$-extrapolated zero concentration C(O) and mean maximal concentration (C_{max}) of L-PAM in the tissue were significantly higher as compared with the data obtained with a flow rate of 0.5 ml/min as shown in Table 2. The $AUC_{5\ min\text{–}\infty}$ recorded for the perfusate in the lower-perfusion-flow group was about 1.3-fold higher than that obtained in the group with the higher flow rate (Table 3). Pharmacokinetic data noted for the perfusate, such as the distribution volume, clearance, C_{max}, and t_{max} of melphalan as presented in Table 4, were not significantly different. The results show that a lower perfusion flow rate increased the uptake of melphalan into the tissue at a perfusion time of 90 min.

Discussion

Hyperthermic isolation perfusion is an established treatment in adjuvant therapy of high-risk malignant melanomas of the upper and lower extremities after excision of the tumor or as the primary treatment of locoregional recurrent disease [7, 24, 27, 33]. The aim of locoregional therapy is to achieve a high drug concentration in the treated compartment and, simultaneously, to minimize systemic side effects such as bone marrow depression, hemolytic anemia, and gastrointestinal disturbances.

The present study evaluated the relationship between pharmacokinetic parameters of melphalan and modified experimental conditions during isolated perfusion in rats using a miniaturized extracorporeal circuit [34]. Melphalan concentrations were assayed using HPLC [35]. Pharmacokinetic data recorded for L-PAM in bulk tissues, such as the $AUC_{5\text{–}90\ min}$, C(O), C_{max}, and t_{max}, and in perfusate samples, such as the $AUC_{5\text{–}90\ min}$, $AUC_{5\ min\text{–}\infty}$, $t_{1/2}$, V_z, CL, C_{max}, and t_{max}, were analyzed to find out whether modification of application methods (varied pH ranges, temperature changes in perfusate/tissue, or different perfusion flows rates) might result in pharmacokinetic advantages.

The highest melphalan tissue penetration was found within the pH range of $7.3 \leq 7.7$, as the extrapolated zero concentration C(O) and $AUC_{5\text{–}90\ min}$ of the tissue indicated. At lower pH values the perfusion flow is disturbed by an increased rigidity of blood cells such as erythrocytes. As a result, drug uptake is decreased by occlusion of tissue capillaries [44]. Other authors have suggested that the blood flow at low pH ranges is inhibited by activation of clotting factors such as fibrinogen. Heparin could not fully antagonize this phenomenon [8, 9].

During chemotherapy the heat sensitivity of cancer cells is increased by reduced pH values in the tumor tissue [16, 32]. Elevated levels of lactic acid in a large tumor as a result of tissue hypoxia or nutritional deprivation, leading to severe tissue acidosis, enhances tumor regression [43]. Another study that analyzed the influence of pH by isolated normothermic limb perfusion of L-PAM with incubated human melanoma spheroids showed that higher pH values diminished the cytotoxic effect of melpahalan. A pH range of 7–8 did not have an influence on the regrowth delay of the melanoma spheroids and did not affect the hydrolysis of L-PAM [40]. In accordance, our study shows that at the initial phase of isolated perfusion, melphalan has an enhanced tissue affinity and sufficient cytotoxic effects at pH values ranging between 7.3 and 7.7.

The hightest drug uptake into the tissue, especially at the initial phase of isolated perfusion, was observed in our series with hyperthermia of 40–41.5 °C. This is shown by the area under the concentration-time curve ($AUC_{5\text{–}90\ min} = 1832.0\ \mu g\ min\ g^{-1}$ and the extrapolated zero concentration [$C(O) = 6.3\ \mu g/g$].

The significantly reduced distribution volume (V_z) and the short half-life ($t_{1/2}$) of melphalan above temperatures of 41.5 °C demonstrate the diminished tissue affinity and the enhanced hydrolysis of L-PAM at higher temperatures. Other studies showed that the rate of hydrolysis of L-PAM at 42 °C as compared with 37 °C was increased 1.5-fold in canine plasma and 1.9-fold in porcine plasma [37]. During isolation hyperthermic perfusion within the temperature range of 40–41 °C, hydrolysis of L-PAM yields 18.6% monohydroxymelphalan and only 1.9% dihydroxymelphalan [29, 30]. The therapeutic effect of the alkylating agent was not diminished, as only a small quantity of inactive

dihydroxymelphalan was found at this perfusion temperature. Other studies have shown that at a temperature of 42 °C the alkylating reaction of melphalan is enhanced by the formation of DNA interstrand cross-links in human malignant melanoma cells [48].

Melphalan, a derivative of the neutral amino acid L-phenylalanine, has been reported to be transported by active transport into tumor cells via two amino-acid carrier systems [2, 19, 45]. Other authors have demonstrated the facilitated transport of L-PAM by the above-mentioned carrier system through the blood-brain barrier [20]. To explain our findings, we conclude that at higher lethal temperatures above 42 °C this transmembranous amino-acid carrier system could be destroyed during hyperthermic perfusion, with no further melphalan being taken up into the treated tissues. In agreement with these reports and our findings, we conclude that the uptake and cytotoxity of melphalan is most efficient at a perfusion temperature of 40–41.5 °C.

In our study, melphalan was applied by three different modes. The highest tissue uptake was found when L-PAM was injected into the reservoir of the perfusion circuit, as the $AUC_{5–90\ min}$ and the mean maximal concentration (C_{max}) indicated. Via use of the reservoir, the whole dose of melphalan was given during the perfusion time of 90 min and the C_{max} could be detected after 75 min. Initially high concentrations in the treated extremity were obtained by direct application of L-PAM as a single bolus at the start of perfusion. Although we found high $AUC_{5–90\ min}$ and $AUC_{5\ min–\infty}$ values in the perfusate, there was no correspondingly high value in the tissue samples. Continuous administration of L-PAM in the first 20 min of the perfusion did not have any pharmacokinetic advantage.

No pharmacokinetic difference could be found in a study that analyzed the $AUC_{5–60\ min}$ of melphalan in the plasma of 15 patients treated with hyperthermic perfusion [41]. The total dose of L-PAM was given in several aliquots during the first 15 min of perfusion or as a single bolus into the arterial line at the beginning of the treatment. Our findings agree with these; although the highest mean maximal concentration (C_{max}) in the series using bolus injection of melphalan appeared after only approximately 14 min of perfusion time (t_{max}), the areas under the concentration-time curve ($AUC_{5–90\ min}$ and $AUC_{5\ min–\infty}$) of the perfusate did not differ significantly.

There is some controversy regarding the optimal administration method. Some authors prefer continuous application of melphalan in several aliquots during hyperthermic perfusion of 60 min to reduce severe intra- and postoperative complications such as limb edema, irreversible nerve damage, and venous thrombosis with amputation [26, 47]. A pharmacokinetic disadvantage for single bolus injection has been reported as a result of enhanced hydrolysis of L-PAM at the initial phase of perfusion and of diminished drug uptake during a total perfusion time of 60 min [27]. In our series we did not find differences in the distribution volume or elimination half-life of melphalan between bolus application and continuous injection into the perfusion circuit during a treatment period of 90 min.

Other authors have reported significantly reduced postoperative complications such as nerve lesions following the application of cytostatics as a single dose into the pump reservoir [1]. In agreement with these clinical findings and our pharmacokinetic data, application of L-PAM during isolated perfusion of 90 min should preferably be carried out as a single dose into the reservoir of the extracorporeal circuit.

In the last series we analyzed whether there would be a pharmacokinetic advantage with regard to different roller-pump-controlled perfusion flows in the extracorporeal circuit. At a reduced flow rate of 0.4 ml/min we found a sufficient uptake of melphalan into the limb tissues, as the $AUC_{5–90\ min}$, mean maximal concentration (C_{max}), and extrapolated zero concentration C(O) indicate. By reduction of the blood flow in the perfusion circuit, the contact time of the cytostatic agent with the tumor was prolonged, which increased the intracellular drug uptake [46]. Other authors reported that if the regionally treated organ had a high extraction rate of the cytostatic drug the regional pharmacokinetic advantage of treatment was enhanced by increased tumor regression [13].

In recent studies the blood flow in isolated perfusion therapy was not standardized. Values for perfusate flow varied from 80 to 300 ml/min for the upper extremity and from 120 to 600 ml/min for the lower extremity [11]. High perfusion flow rates increased limb edema or melphalan leakage during perfusion therapy [10, 11]. Some authors mentioned irreversible postoperative complications such as wound dehiscence and arterial necrosis resulting from low perfusion flow rates [6] and recommended a flow rate of 10 ml/min per 100 g of treated tissue [17]. Studies on pharmacokinetic aspects and improvement of drug extraction are rare. Considering our results, it would be advisable to use perfusate flow rates slightly lower than the physiological blood flow during isolated perfusion, controlled by the roller pump of the extracorporeal circuit. Further clinical studies are required to evaluate the pharmacokinetics of melphalan in melanoma cells as a result of modified perfusion methods or in combination with new chemotherapeutic agents to optimize the treatment method.

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