CLINICAL SCIENCE

Isolated limb perfusion with hyperthermia and chemotherapy: predictive factors for regional toxicity

João Pedreira Duprat Neto,[I] Fernanda Oliveira,[II] Eduardo Bertolli,[III] Andre Sapata Molina,[III] Kenji Nishinari,[IV] Luciana Facure,[V] Jose Humberto Fregnani[VI]

[I] Hospital do Câncer A.C. Camargo, Head of the Department of Skin Cancer of São Paulo, São Paulo/SP, Brazil. [II] Hospital do Câncer A.C. Camargo, Fundação Antônio Prudente, Medical Student-PIBIC (graduate research program-Conselho Nacional de Desenvolvimento Científico e Tecnológico). [III] Hospital do Câncer A.C. Camargo, Surgical Oncologist-Department of Skin Cancer, São Paulo/SP, Brazil. [IV] Hospital do Câncer A.C. Camargo, Head of Department of Vascular Surgery, São Paulo/SP, Brazil. [V] Hospital do Câncer A.C. Camargo, Research Nurse of Department of Skin Cancer, São Paulo/SP, Brazil. [VI] Barretos Cancer Hospital-Brazil, Coordinator of the Center for Researcher Support, Barretos/SP, Brazil.

OBJECTIVE: Isolated limb perfusion combined with melphalan is an accepted treatment for obtaining locoregional control in advanced melanoma of the extremities and other malignant neoplasias restricted to the limb. This study aims to examine the factors associated with toxicity caused by the regional method. We considered the technical aspects of severe complications associated with the procedure in an attempt to diminish the patient morbidity that occurs during the learning curve.

METHODS: We conducted a retrospective analysis of the records of patients who underwent perfusion at the AC Camargo Hospital in São Paulo, Brazil between January 2000 and January 2009. The Wieberdink scale was applied to classify local toxicity and its relation to clinical and laboratory variables.

RESULTS: Fifty-eight perfusions were performed in 55 patients. Most patients (86.2%) presented a toxicity level between I and III. Grade V toxicity was seen in five cases (8.6%), four of which occurred in the first 2 years. Creatine phosphokinase, an important predictive factor for toxicity, had an average value of 231.8 for toxicity grades I-III and 1286.2 for toxicity grades IV-V ($p = 0.001$). There was a relationship between the melphalan dose and toxicity, which was 77 mg (25 to 130 mg) for toxicity grades I-II and 93.5 mg (45 to 120 mg) for toxicity grades IV-V ($p = 0.0204$).

CONCLUSION: It is possible to prevent the toxicity associated with melphalan by adjusting the dose according to the patient's body weight (especially for women and obese patients) and the creatine phosphokinase values in the postoperative period.

KEYWORDS: Isolation Perfusion Cancer Chemotherapy; Hypoxia; melanoma; Regional chemotherapy; Melphalan.

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E-mail: jduprat@uol.com.br

Tel.: 55 11 3258-3636

INTRODUCTION

Isolated limb perfusion (ILP) is an alternative method that allows the regional administration of chemotherapy to patients with advanced melanoma and other malignant neoplasias restricted to the limb. ILP combined with melphalan is an accepted treatment modality for obtaining locoregional control in advanced melanoma of the extremities (1).

The use of regional perfusion combined with the administration of cytostatic drugs was suggested by Klopp et al. (2) in 1950 in an attempt to avoid the systemic toxic effects of increasing doses of nitrogen mustard. Briefly, a cannula was placed into the major artery supplying the tumor site, and the drug was then administered. In 1956, Luck reported that melphalan was an effective agent when combined with ILP to treat melanoma in mice (3). Three years later, Creech et al. (4) first treated a patient using the independent vascular blood circulation of the affected limb isolated from the systemic circulation with an extracorporeal machine using an oxygenator and high doses of melphalan. In 1967, the technical basis of ILP with hyperthermia was established, and Cavaliere et al. (5) described the selective susceptibility of cancer cells at high temperatures.

The mortality and regional toxicity rates associated with ILP surgery are considered low. Systemic leakage of the cytostatic drug(s) is negligible (6,7), although severe toxicity can occur (8-10).

Despite the common assumption of a relationship between side effects and drug response, the relationship

No potential conflict of interest was reported.

between toxicity and the response to ILP has never been shown (11). Considering that these patients have advanced disease, all efforts should be made to avoid negatively affecting their quality of life. Thus, severe treatment-related side effects must be avoided at all costs, as increased toxicity and its associated side effects are not associated with better treatment responses.

Many factors have been shown to affect toxicity, such as limb temperature above 40ºC, female gender, intense exchange of blood gases in the perfusion circuit, proximal iliac perfusion, and melphalan peak concentration (9,12-14).

It is possible to minimize acute regional toxicity by carefully considering these factors. Increased consideration of these factors should also decrease the incidence of long-term morbidity, especially ankle stiffness, limb malfunction, and muscle atrophy. A relationship between the severity of acute regional tissue reactions and later morbidity has been recognized (15).

Pasin et al. (16) described the treatment responses in 44 perfusions categorized as complete (43.2%), partial (36.4%), and unresponsive (20.4%). Although toxicity details were not described in the study, it was reportedly limited to slight erythema and edema. In one case, amputation was required because of the treatment.

In this study, we analyzed the risk factors associated with limb toxicity. Cases of amputation following ILP are seldom described in the literature. Here, we consider the technical aspects related to severe complications associated with ILP in an attempt to diminish the patient morbidity that has been observed during the ILP learning curve typically observed by health teams.

PATIENTS AND METHODS

A retrospective analysis was performed based on the medical records of 55 patients treated with the ILP technique at the A. C. Camargo Hospital in São Paulo, Brazil between January 2000 and January 2009. Cases for which no medical records were available were excluded. The casuistic included 46 patients with melanoma, four cases of skin squamous cell carcinomas, and five cases of soft tissue sarcomas. In the sarcoma group, two were classified as clear cell sarcomas, two as epithelioid tumors, and one as ganglioneuroblastoma. Almost all cases of melanoma were Stage IIIB or IIIC, and only one patient was in Stage IV.

Our perfusion technique has been described in detail elsewhere (17). Briefly, melphalan (Alkeran GlaxoSmith-Kline, Rio de Janeiro, Brazil) was used as a single agent in dosages of 10 and 13 mg/L of limb volume (previously measured) for the leg and arm, respectively. After verifying that no significant leakage had occurred and the intramuscular temperature was near 38.5℃, the drug was infused. The infusion lasted 15 minutes, and the target temperature of 40℃ was maintained for one hour, including the infusion period. After that, the limb was washed with at least 2 liters of Ringer solution, the cannulas were removed, and the vessels were repaired.

Data related to the patients' age, sex, type of disease, stage, affected limb, dose of melphalan received, limb temperature, blood gases, systemic and limb hemoglobin and hematocrit during the procedure, duration of post-operative hospitalization, degree of toxicity, creatine phosphokinase (CPK) level, day of the highest CPK level and response were tabulated in a spreadsheet and used as the nominal measurement level. The tumor's response to treatment and the procedure-related toxicity were analyzed according to the clinical and laboratory data.

Table 1 - Acute Regional Toxicity Grading System, according to Wieberdink et al. (18).

Grade	Reaction
I	No reaction
II	Slight erythema and/or edema
III	Considerable erythema and/or edema with some blistering; slightly disturbed motility permissible
IV	Extensive epidermolysis and/or obvious damage to the deep tissues, causing definite functional disturbances; threatened or manifest compartmental syndrome
V	Reaction that may necessitate amputation

Tissue toxic reactions after the ILP procedure and melphalan administration were classified according to Wieberdink's scale (Table 1) (18).

Tumor response was assessed according to the World Health Organization criteria: complete response (CR) corresponds to total regression of all injuries; partial response (PR) corresponds to at least a 50% reduction of the tumor volume; no response (NR) corresponds to a tumor volume reduction of less than 50% or disease progression (19).

The Mann-Whitney test was used to compare the mean values of several numerical variables according to the toxicity grade. Toxicity-rate comparisons based on categorical variables were conducted using Fisher's exact test and the chi-squared test. The significance level was set to 5% in all statistical tests.

All patients signed the informed consent form, and the local Ethics Committee approved the study.

RESULTS

Fifty-eight perfusions were performed with isolated limb hyperthermia and chemotherapy in 55 patients; three patients underwent the procedure twice. Population characteristics are shown in Table 2.

Toxicity of the method

Most patients progressed with a degree of toxicity ranging from I to III; however, toxicity grades IV and V were also present (Table 3). The learning curve associated with the procedure was included in the study period.

Table 2 - Characteristics of the population: Diagnosis and stage.

Characteristic	Number (%)
Diagnoses	
Melanoma	46 (84)
Sarcoma	5 (9)
Squamous cell carcinoma	4 (7)
Stage (melanoma)	
IIIC	30 (64)
IIIB	16 (34)
IV	1 (2)
Age (years)	
Limb	Median: 62; range: 23-84
Upper	8 (14.5)
Lower	47 (85.5)

Table 3 - Toxicity.

Toxicity	Number of patients (percentage)
I	1 (1.7)
II	45 (77.6)
III	4 (6.9)
IV	3 (5.2)
V	5 (8.6)
Total	58 (100)
Grade I-III	50 (86.2)
Grade IV-V	8 (13.8)

The relationship between gender and toxicity was analyzed. The patients were divided in two groups, a grade I-III group and a grade IV-V group. Although four of the five patients with grade V toxicity were female and 10 of the 12 patients with toxicity ≥ grade III were female, the difference between genders was not statically significant (Table 4).

Serum creatine phosphokinase (CPK) is an important parameter for assessing toxicity in these patients. The maximum median CPK values were 435, 324, 259, 206, and 285 (from the first postoperative day to the fifth day postprocedure). The relationship between toxicity and clinical and laboratory parameters is shown in Tables 5 and 6.

There was a clear relationship between toxicity and CPK values greater than 1,000. A total of 19.1% of patients with toxicity grades I-III had CPK values greater than 1,000, compared with 62.5% of patients with grades IV and V toxicity ($p = 0.02$).

There was no significant relationship between toxicity and other parameters, such as stage, systemic pO2, pCO2, pH, base excess, bicarbonate, hemoglobin, hematocrit, diagnosis (melanoma vs. nonmelanoma), and limb (upper vs. lower). There were 50 cases of lower limb tumors, of which 86% had grade I-III toxicity. This value was not significantly different compared to the toxicity observed in cases in which the upper limb was affected (87.5%).

There was a relationship between the melphalan dose and CPK values (highest and mean values, $p = 0.002$ and 0.003; Table 6) and systemic pH and CPK (highest and mean values, $p = 0.001$ and 0.001).

Patients with melanoma were hospitalized for an average of 9 days after surgery (range: 4 to 52 days). Nonmelanoma patients were hospitalized for an average of 7 days (range: 5 to 17 days).

DISCUSSION

Patients who are eligible for ILP had advanced-stage cancer. Although the objective of any medical treatment is to cure the patient, in many cases, the only benefit is maintaining a good quality of life. Thus, avoiding or limiting the side effects associated with ILP is imperative for preserving the patient's limb and minimizing patient suffering. In this study, we showed that it is possible to prevent the toxicity associated with high dosages of melphalan by adjusting the dose according to the patient's body weight (especially in women and obese patients), muscle temperature, and blood gas (pCO_2 and pH) levels and by considering the hematocrit and CPK values in the postoperative period.

Table 4 - Toxicity and gender; $p = 0.139$.

		Toxicity		Total
		Grade I-III n (%)	Grade IV-V n (%)	
Gender	Female	29 (58)	7 (87.5)	36 (62.1)
	Male	21 (42)	1 (12.5)	22 (37.9)
Total		50 (100)	8 (100)	58 (100)

Table 5 - Relationship between toxicity and clinical parameters (categorical variables).

		Toxicity		Total	*p*-value
		Grade I-III n (%)	Grade IV-V n (%)		
Limb	Lower	43 (86)	7 (14)	50 (100)	$p = 0.417$
	Upper	7 (87.5)	1 (12.5)	8 (100)	
Response	No response	8 (88.9)	1 (11.1)	9 (100)	$p = 0.363$
	Partial/complete	42 (95.5)	2 (4.5)	44 (100)	

Klaase analyzed 425 patients and described the following risk factors associated with toxicity (9):

a) Tissue temperature: In the group that achieved limb temperatures ≤37.9℃, 10% had a toxicity grade ≥ III. In the group that achieved limb temperatures ≥40℃, 36% had a toxicity grade ≥ grade III, with a steep increase in toxicity at temperatures above 41℃.

In most cases in the present study, the temperature was maintained at approximately 39 to 40℃. Furthermore, our group of patients was smaller than Klaase's group. In our study, limb temperatures were not significantly different between grades I-III and grades IV and V toxicity groups, most likely because of the smaller sample size. In the grades I-III toxicity group, the median temperature achieved was 39℃ (range: 36 to 39.5) and in the grades IV and V group, the median temperature achieved was 38℃ (range: 38 to 38.5℃); $p = 0.371$.

b) Gender: The muscle + skin/fat tissue ratio was different between genders. Because fat tissue has less vascularization than muscle, if the same dose is administered to a limb with a greater proportion of fat tissue, more of the drug will enter the muscle tissue, which results in higher toxicity. This may explain why the toxicity is different between genders. The ratio of patients with toxicity greater than grade II was 0.18 female:0.07 male. Recently, we corrected the dose according to ideal weight, which reduced side effects. In the present study, four of the five patients with grade V toxicity were female and 10 of the 12 patients with ≥ grade III toxicity were female.

The pH and hypoxia levels have been also been reported to affect the toxicity associated with ILP. By damaging endothelial cells, hypoxia increases the drug uptake and impairs the repairing of normal tissue. The association between hypoxia and acidic pH increases toxicity due to increased drug uptake and reduced melphalan hydrolysis (20).

Hematocrit values below 20% in the perfusate also increase the occurrence of toxicity. This phenomenon occurred in three out of six cases of grade IV toxicity (21). Animal studies have tested and confirmed that low hematocrit increases grades III and IV toxicity (22).

The side effects of melphalan are related to the amount of the drug that is administered. Pace et al. (23) compared three groups of patients: Group A was submitted to mild hyperthermia, melphalan (10 mg/l) and D-actinomycin;

Table 6 - Relationship between toxicity and clinical and laboratory parameters (numerical variables).

	Toxicity		*p*-value		
	Grade I-III	Grade IV-V			
Age (years)	Median: 62.5; range: 23 to 84	Median: 58.4; range: 25 to 76	$p = 0.385$		
Melphalan dose	Median: 77 mg; range: 25 to 130 mg	Median: 93.5 mg; range: 45 to 120 mg	$p = 0.204$		
Intramuscular temperature	Median: 39℃; range: 36 to 39.5℃	Median: 38℃; range: 38 to 38.5℃	$p = 0.315$		
CPK (highest value)	Median: 377; range: 65 to 16,558	Median: 1848; range: 594 to 46,863	$p = 0.001$	0.427 (cc)	0.002 (p)
CPK (mean value)	Median: 231.8; range: 63.5 to 5,835.5	Median: 1286.2; range: 481 to 35,867.3	$p = 0.001$	0.404 (cc)	0.003 (p)

cc: Correlation coefficient.

Group B was submitted to true hyperthermia (40 to 41.8℃); and Group C was submitted to true hyperthermia and an additional bolus of 5 mg/l of melphalan. Group C presented an increase in grade III toxicity. The authors observed 1.6% mortality (3 cases) in Groups B and C.

Thompson et al. (12,24) analyzed the use of a high-performance liquid chromatography (HPLC) system to measure the drug dosage in the perfusate during surgery and found that the peak dosage of melphalan influenced toxicity levels. Using an indocyanine green dilution method, it was possible to observe that perfusate volume is quite variable. Consequently, the peaks of dose, toxicity and effects are also variable (12,24). In the present study, patients with toxicity $\leq$ grade III had a mean melphalan dose of 77 mg vs. 93.5 mg in the group with toxicity grades IV and V; however, the difference was not statistically significant ($p = 0.204$), possibly because of our small sample. Muscle damage can occur as a result of ILP, and an important parameter for muscle damage is the peak value of creatinine phosphokinase (CPK). Lai et al. (25) were the first authors to analyze the CPK value at the day of its peak. They found that if CPK remained normal or peaked between the second and fourth days, the toxicity was mild. However, if peak values were greater than 1,000 after the fifth day, the toxicity was high. Vrouenraets et al. showed that if CPK remains normal or shows an early peak (before the 4th day), the chances of severe toxicity are lower. A ''late peak'' CPK pattern (i.e., a peak that occurs after the 5th day and is greater than 1,000 IU/L) indicates a higher probability of regional toxicity. Seven out of 15 limbs had severe toxicity with CPK values higher than 1,000 IU/L on the 2nd to 5th days after ILP. Other studies have confirmed this finding (25-27).

Vrouenraets et al. also tested CPK and other blood parameters (27). These authors analyzed lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT), and white blood cell counts (WBC). A strong correlation between toxicity and LDH peak was observed, but it occurred later than the CPK peak. The LDH peak occurred 4.7 days after ILP, which was 2.9 days after the CPK peak. The differences in ASAT levels did not reach statistical significance. WBCs increased between days 3 and 8 in grades IV and V toxicity compared with grades II and III. In the present study, there was a clear correlation between CPK levels (mean, highest level and number of cases with doses over 1,000 units) and toxicity ($p = 0.001$, 0.001, and 0.02, respectively).

Boesh et al. (21) showed that low hematocrit (below 20%) of the perfusate increases the occurrence of toxicity. This phenomenon occurred in three out of six Grade IV toxicity cases. We did not investigate this occurrence in our study.

Cause of Grade V toxicity

It is very important to explain the possible causes of grade V toxicity to minimize suffering in patients with advanced disease. Unfortunately, these causes have not been reported or investigated. In this study, we aimed to better understand the causes associated with high toxicity for each patient.

A 51-year-old man received two perfusions. Initially, he demonstrated a complete response and grade II toxicity, but he had a recurrence after 4 months. He received systemic chemotherapy with dacarbazine (DTIC), and his disease was stable for almost a year; after that, however, the disease in the affected limb worsened. A new perfusion was completed; after 1 week, he developed phlegmasia cerulea dolens and underwent amputation. It has been established that second perfusions increase toxicity from 21 to 58% (28).

Another patient was a 57-year-old obese female. For this patient, the calculated and administered dose of melphalan was 120 mg. At that time it was the usual calculated dose but recently the concept of correcting the dose by ideal weight was introduced. Another problem associated with this case was that the temperature probe should have been placed intramuscularly, but was likely placed in the subcutaneous tissue. While the registered temperature was 38℃, the actual temperature inside the muscle was probably higher.

Four cases of grade V toxicity occurred in our first two years of using ILP, indicating that we were at the middle of the learning curve. The fifth case occurred in 2005 (our fifth year of using ILP), and we have had no cases of grade V or IV toxicity in the subsequent six years.

In conclusion, the prevention of regional and systemic toxicity is essential for improving the quality of life of patients with advanced disease. It is possible to prevent the toxicity associated with high doses of melphalan by adjusting the dose according to the patient's body weight (especially in women and obese patients), muscle temperature, blood gas (pCO_2 and pH) levels, and by considering the hematocrit and CPK values in the postoperative period.

AUTHOR CONTRIBUTIONS

Duprat JP was responsible for study concepts and design; acquisition, analysis, and interpretation of data; quality control of data and algorithms; preparation, editing, and review of the manuscript. Molina AS contributed to study concepts, data acquisition and manuscript review. Fregnani JH contributed to study concepts and design, quality control of data and algorithms, analysis and interpretation of data, statistical analysis, and preparation and review of the manuscript. Bertolli E contributed to study design; acquisition, analysis, and interpretation of data; preparation and review of the manuscript. Oliveira F contributed to data acquisition, quality control of data and algorithms, and manuscript review. Nishinari K

contributed to data acquisition and manuscript review. Facure L contributed to data management.

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