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ORIGINAL ARTICLE

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Pharmacokinetic and phase II study of heated intraoperative intraperitoneal melphalan

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Abstract *Background*: Peritoneal surface malignancy resulting from local dissemination is a common manifestation of treatment failure of gastrointestinal cancers. Although the management of carcinomatosis has been improved with an aggressive surgical approach of extensive cytoreduction followed by heated intraoperative intraperitoneal chemotherapy, no patients are cured when there is residual disease after surgery. Melphalan (L-phenylalanine mustard) is a well-known antineoplastic alkylating agent which has markedly increased pharmacological activity with heat. The use of heated intraoperative intraperitoneal melphalan may provide a pharmacokinetic and clinical advantage in this group of gastrointestinal cancer patients who cannot be made cancer-free with cytoreductive surgery. *Methods*: Thirteen patients with residual disease following cytoreductive surgery for peritoneal carcinomatosis were included in this study. After surgical resection and prior to anastomotic reconstruction, patients received intraperitoneal melphalan (70 mg/m^2) in 3 l of 1.5% dextrose peritoneal dialysis solution at 41–42°C for 90 min. Concentrations of melphalan were assessed in the peritoneal fluid, blood, urine and tumor nodules using high-performance liquid chromatography. *Results*: During the 90 min of treatment 87.2±4.3% of the drug was absorbed from the perfusate/peritoneal fluid and 11.9±2.1% was excreted in the urine. The area-under-the-curve ratio of peritoneal fluid to plasma was 33.3±11.8 with an average peak plasma concentration of 0.82±0.24 μg/ml occurring at 28.5±13.1 min. Concentrations of melphalan in tumor nodules on the peritoneal surface were approximately ten times higher than in plasma with an average peak concentration of 7.2±4.2 μg/gm. The grade III/IV morbidity was 38%; there was no mortality. *Conclusion*: Approximately 90% of the drug was absorbed during the 90-minute procedure with a 30 times greater exposure of drug at the peritoneal surfaces than in the blood. Concentrations of the drug in peritoneal surface tumor nodules were approximately ten times greater than concentrations in the blood. These data demonstrate that heated intraoperative intraperitoneal melphalan could have a significant impact on the treatment of peritoneal surface malignancies.

Keywords Pharmacokinetics · Intraoperative chemotherapy · Intraperitoneal chemotherapy · Peritoneal surface malignancy · Carcinomatosis

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Introduction

The prognosis of locally disseminated peritoneal surface malignancies has been improved with an aggressive surgical approach with cytoreduction followed by heated intraoperative intraperitoneal chemotherapy. This combination of treatments has been extensively reported in the medical literature [1–4]. The morbidity is 28% with a 2% mortality [5, 6]. However, there are no patients cured when there is residual disease following surgery [1, 3, 4]. Clearly more adequate treatment of peritoneal surface malignancy is needed for this group of patients who cannot be made disease free by cytoreductive surgery.

Melphalan (L-phenylalanine mustard) is an effective and well-known antineoplastic alkylating agent that has been in use for over 50 years [7]. It has markedly increased pharmacological activity with heat, both in vitro and in vivo [8–10]. Clinically it remains the most effective single drug used in heated limb perfusion to treat in transit metastases from melanomas and advanced primary or recurrent extremity soft tissue sarcomas.[11, 12] Extensive phase I and phase II pharmacokinetic studies with normothermic intraperitoneal melphalan were

conducted by Howell et al. [13]. These studies showed that melphalan was tolerated up to a dose of 70 mg/m^2 with no local toxicity. The limiting toxicity was systemic myelosuppression and there were no deaths or major complications in this group of 19 patients.

The present study was designed to assess the pharmacokinetics and to evaluate the potential clinical advantages of heated intraoperative intraperitoneal melphalan as adjuvant therapy for patients with residual disease after cytoreductive surgery for peritoneal surface malignancies. Morbidity and mortality assessments were performed on all patients.

Methods

Thirteen patients with peritoneal carcinomatosis from colon cancer or appendix cancer were included in this study; in ten patients complete pharmacological studies were available. All patients signed an informed consent approved by the institutional research review board. All patients had cytoreductive surgery followed by 90 min of heated intraoperative intraperitoneal chemotherapy. The dose of melphalan was 70 mg/m^2 for all patients administered in 3 l of 1.5% dextrose peritoneal dialysis solution. The melphalan solution was maintained at a temperature of 41–42°C within the peritoneal cavity by a heater-circulator.

Administration of heated intraoperative intraperitoneal chemotherapy

After the surgical resection was complete and before surgical reconstruction, a Tenckhoff catheter and four closed suction drains were placed through the abdominal wall. One temperature probe was secured to the Tenckhoff catheter, and two other temperature probes were placed at the uppermost and lowest regions of the peritoneal cavity. The skin edges were elevated by monofilament sutures creating a reservoir within the peritoneal cavity to accommodate 3 l of the heated melphalan solution [14]. Recirculation of the heated melphalan solution was facilitated by roller pumps which forced the solution into the abdomen through the Tenckhoff catheter and pulled it out through the drains. A heater and heat exchanger were used to maintain the intraperitoneal fluid temperature at 41–42°C. During the 90 min of chemotherapy irrigation, the surgeon's double-gloved hand manually distributed the heated chemotherapy solution and vigorously manipulated all viscera in an attempt to maintain uniform exposure of all anatomic structures within the peritoneal cavity.

Collection of samples

Prior to and every 15 min during the 90 min of perfusion, samples of peritoneal fluid, blood and urine and small ($\leq$ 1 cm) tumor nodules, were obtained for high-performance liquid chromatography (HPLC) analysis of melphalan concentration. For each 15-min urine sample, the total volume of urine excreted was also recorded. At 90 min the perfusate was drained from the peritoneal cavity and the volume was recorded. An additional sample of blood and urine was obtained at 120 min. In a single patient samples of normal peritoneal surface tissues were also harvested to assess any difference in drug concentration between tumor and normal tissue. All samples were placed on ice immediately after collection and processed for storage immediately after the 120-min sample was obtained.

Processing and storage of samples

Peritoneal fluid and blood samples were centrifuged at 3,000 rpm for 10 min. The resulting plasma from blood samples and the supernatant from peritoneal fluid samples were transferred to capped polypropylene tubes for storage. Tumor and normal tissue samples were *'blotted'* with absorbent gauze pads to remove all surface fluid and placed in screw-capped polypropylene scintillation vials. All samples were stored at −20°C until HPLC analysis. For all patients HPLC analysis of samples was performed within 24 h of collection.

HPLC system

Melphalan concentrations were determined using a modification of the HPLC method as described by Norda et al. [15]. The HPLC system consisted of a Shimadzu LC7A instrument equipped with an SPD-6AV (UV-VIS) detector set at 270 nm and a C-R6a Chromatopac data processor. A *Dynamax* reversed-phase C_{18} column (150 × 4.6 mm^2) of Microsorb 100A° 5 μm particles was used coupled to a guard column of the same chemical consistency (Varian Associates, Walnut Creek, CA, USA). The mobile phase consisted of an isocratic mixture of 30% acetonitrile in 0.05 M NaH_2PO_4 with the pH adjusted to 3.5 with phosphoric acid. The flow rate was set at 1.2 ml/min and the volume of sample injections was 50 μl. All solvents used were HPLC grade (Fisher Scientific, Norcross, GA, USA).

Preparation of samples for HPLC analysis

All samples were prepared for HPLC analysis using the methanol extraction technique described by Wu et al. [16]. Briefly, peritoneal fluid and urine samples were diluted with methanol, as required, and filtered through 0.45 μm syringe filters prior to HPLC injection. Plasma samples were thoroughly mixed with a five times volume of methanol, using a vortex mixer. After centrifugation, 50 μl of the supernatant was injected directly into the HPLC system. For tissue samples, approximately 100 mg of each sample was accurately weighed and homogenised in 4 ml methanol. The homogenate was centrifuged and a 50-μl aliquot of the supernatant was injected for HPLC analysis.

Morbidity and mortality assessment

A morbidity and mortality database was maintained on each patient while in the hospital and for 6 months after surgery. The NCI toxicity grading was performed where grade I indicated that an adverse event occurred which did not require treatment. Grade II indicated that the adverse event required medical treatment. Grade III indicated that the adverse event required an invasive intervention, usually a radiologically guided procedure. A grade IV adverse event indicated a need for return to the operating room or surgical intensive care unit. A grade V adverse event resulted in a postoperative death.

Results

Table 1 and Fig. 1 present a summary of the pharmacokinetic data obtained from 90 min of heated intraoperative intraperitoneal melphalan treatment. The median total dose of intraperitoneal melphalan for all patients was 129.7±9.7 mg, which corresponded to a median body surface area of 1.86±0.14 m^2. The median total dose for males was 135.8±5.1 mg and the median dose for females was 125.7±10.2 mg. This difference was significant ($P = 0.037$). During the 90-min chemotherapy irrigation a median of 87.2±4.3% of total melphalan was absorbed. The median percentage of melphalan absorbed was 89.2±2.5% for males and 85.8±4.9% for females. This difference was not significant. However, the rate of drug absorption during the first 15 min was significantly higher for males than for females. Males absorbed 40.3±9.2% while females absorbed 27.8±6.5% ($P = 0.033$). The median amount of drug excreted in urine was 11.9(±2.1)% for all patients; males excreted 12.3±2.5% and females excreted 11.7±2.1%.

Concentrations of drug in the plasma reached a median peak level of 0.82±0.24 μg/ml in approximately 30 min. The median concentration of drug in plasma for males was higher than for females. The median peak plasma concentration was 0.96±0.29 μg/ml for males and 0.72±0.17 μg/ml for females. This difference was not significant. The median area-under-the-curve (AUC) ratio of peritoneal fluid to plasma was 33.3(±11.8). The AUC ratio of peritoneal fluid to plasma was greater in females (37.6±12.2) than in males (26.8±8.8). This difference was not significant. The median peak melphalan concentration in tumor nodules was 7.2(±4.2) μg/gm occurring at approximately 15 min. The median AUC ratio of tumor nodules to plasma was 8.1(±5). Figure 2 shows the difference in peritoneal fluid pharmacokinetics of males versus females. The initial rate of absorption of drug from peritoneal fluid is greater in male patients ($P = 0.033$).

In a single patient melphalan levels in normal tissues on the peritoneal surfaces were determined along with the tumor nodules, peritoneal fluid and plasma chemotherapy levels. A graphic summary of this data is presented in Fig. 3. Melphalan levels in normal tissues were approximately two times higher than in tumor nodules.

Morbidity/mortality data was available on 13 patients. There were no postoperative deaths. Four patients had no in-hospital adverse events. Five patients had a grade III or IV adverse event (leak from a rectal

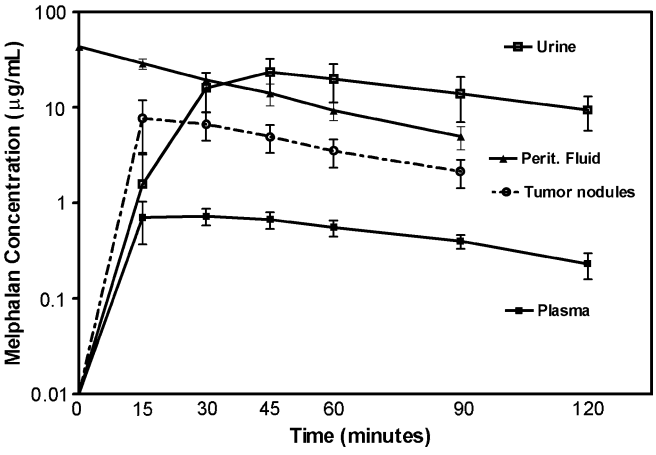

Fig. 1 Mean concentration versus time curves for peritoneal fluid, plasma, tumor nodules, and urine of ten patients during 90 min of heated intraoperative intraperitoneal melphalan perfusion with urine and plasma concentrations at 120 min. The melphalan concentration in tumor nodules is given in micrograms per gram

Table 1 Heated intraoperative intraperitoneal melphalan. A summary of the pharmacokinetic data in ten patients

Parameters	Average (10)	Males (4)	Females (6)	*P* value (males–females)
Age of patients (years)	47.5 (±15)	50.5 (±22.5)	45.5 (±9.3)	NS
Body surface area (m^2)	1.86 (±0.14)	1.93 (±0.05)	1.81 (±0.16)	0.054*
Total drug (mg) administered	129.7 (±9.7)	135.8 (±5.1)	125.7 (±10.2)	0.037
Total drug (mg) recovered at 90 min	16.5 (±5)	14.7 (±3.6)	17.7 (±5.8)	NS
Percentage of drug absorbed at 90 min	87.2 (±4.3)	89.2 (±2.5)	85.8 (±4.9)	NS
Percentage of drug absorbed within 15 min	32.9 (±9.7)	40.3 (±9.2)	27.8 (±6.5)	0.033
Percentage of drug excreted in urine at 90 min	11.9 (±2.1)	12.3 (±2.5)	11.7 (±2.1)	NS
Peak plasma level (μg/ml)	0.82 (±0.24)	0.96 (±0.29)	0.72 (±0.17)	NS
Time in min. to peak plasma level	30 (±12.2)	30 (±17.3)	30 (±9.5)	NS
AUC ratio (AUC-PF/AUC-PL)	33.3 (±11.8)	26.8 (±8.8)	37.6 (±12.2)	NS

The peak melphalan concentration in tumor nodules was 7.2±4.2 μg/gm at approximately 15 min. The AUC ratio of tumor nodules to plasma was 8.1±5

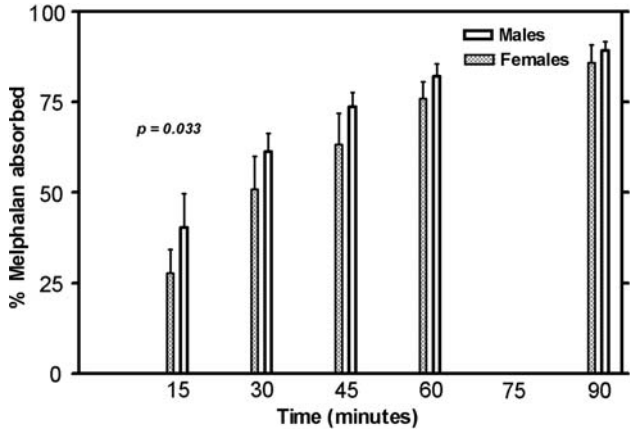

Fig. 2 The difference in peritoneal fluid pharmacokinetics in men versus women as expressed by the percent of melphalan absorbed during 90 min of heated intraoperative intraperitoneal melphalan perfusion. Differences were statistically significant at 15 min ($P = 0.033$)

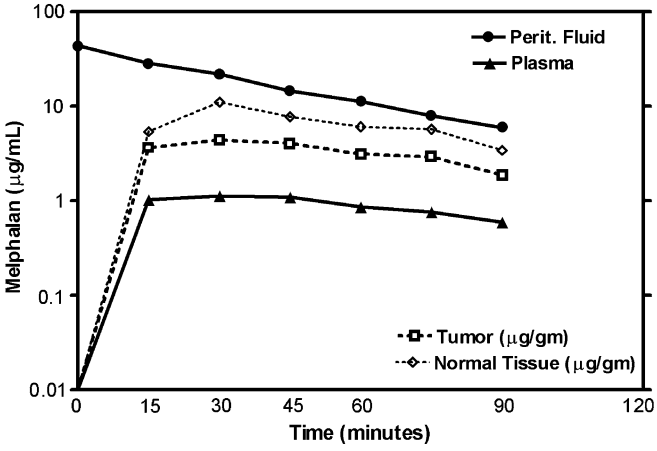

Fig. 3 Concentration versus time curves for peritoneal fluid, plasma, tumor nodules and normal peritoneal surface tissues in a single patient during 90 min of heated intraoperative intraperitoneal melphalan perfusion

stump, pancreatitis, myelosuppression, small bowel fistula and lower leg muscle compartment syndrome). Four patients had grade I/II complications.

Discussion

The pharmacokinetic advantage of intraperitoneal chemotherapy for the treatment of peritoneal surface malignancy has been well documented. Intraperitoneal administration results in sustained high local concentration of drug with corresponding low systemic levels. According to a distribution model proposed by Dedrick et al. [17] there is a predictable delayed distribution of drug into the systemic compartment following intraperitoneal administration. This phenomenon, which has been referred to as the peritoneal–plasma barrier, is dependent on diffusion of drug into tissues adjacent to the peritoneal space, the rate of removal of the drug by capillary blood flow within these tissues, and the rate of drug clearance from the blood [18]. With a mean AUC ratio of 33.3, melphalan is a promising drug by which to exploit the pharmacokinetic advantage of intraperitoneal administration.

In a study to assess the extent of thermal enhancement of chemotherapeutic agents, Urano et al. [8] demonstrated that melphalan had a maximal thermal enhancement ratio when heated to 41.5°C. Using an animal model for isolated limb perfusion with heated melphalan, Norda et al. observed that the highest tissue penetration of drug was obtained when (a) the *p*H of the perfusate was within the physiological range, (b) the perfusate temperature ranged between 40 and 41.5°C and (c) the perfusion time was more than 60 min [15]. From a theoretical perspective, not only a pharmacologic advantage from increased intraperitoneal concentration, but also a melphalan and heat synergy at the peritoneal surface suggest the possibility of remarkable cancer eradication with heated intraperitoneal melphalan.

The rate of absorption of melphalan during 90 min of hyperthermic intraoperative intraperitoneal administration was more rapid than the rate of absorption during 4 h normothermic intraperitoneal administration as reported by Howell et al. [13]. After 4 h of intraperitoneal dwell approximately 90% of the total drug was absorbed from 2 l of a normothermic melphalan solution. During only 90 min of heated intraoperative intraperitoneal administration approximately 90% of the total melphalan was absorbed. This increased rate of absorption may be attributed to a heat-activated increased diffusion of the drug into adjacent tissues [19]. Also the manual distribution of the chemotherapy solution by the surgeon's gloved hand may also support increased diffusion of drug into peritoneal surface tissues. The tissue penetration of melphalan was further evidenced by the high levels of drug in tumor nodules and tissues adjacent to the peritoneal surface. The exposure of drug to tumor nodules on peritoneal surfaces, as evident by the AUC, was approximately ten times greater than to plasma.

The median peak level of melphalan in plasma in our patients was 0.82±0.24 μg/ml. This was more than twice as high as the median peak plasma level of 0.306±0.289 μg/ml in four patients from the study of Howell et al. Both groups were treated with melphalan at 70 mg/m^2. Peak plasma concentrations occurred within the first 30 min during the hyperthermic intraperitoneal melphalan administration as compared to approximately 60 min with the normothermic study.

Howell et al. [13] showed that the dose of intraperitoneal melphalan could be increased to more than three times the maximum tolerated intravenous dose of approximately 20 mg/m^2 without acute local or systemic toxicity. Based on these findings, we used an intraperitoneal dose of 70 mg/m^2 of melphalan for patients in this study. The chemotherapy was administered in 3 l of 1.5%

dextrose peritoneal dialysis solution, the solution was maintained at a temperature of approximately 41°C during the 90 min of treatment, and continuous manual distribution of the chemotherapy solution maintained uniform distribution. This uniform methodology led to a grade III/IV morbidity of 38% with no mortality. A slight dose reduction to 60 mg/m^2 is suggested for future clinical use of hyperthermic intraoperative intraperitoneal melphalan to reduce the adverse events to the 20% range as is usually reported with this treatment modality [5, 6].

There were some differences between males and females in this study. There was a borderline significant difference in the body surface area of the two groups and a significant difference in the total drug administered. There was a significant increase in the rate of absorption of drug in males during the first 15 min of chemotherapy irrigation. This increased rate of absorption for male patients translated into higher plasma levels and a larger AUC peritoneal fluid to AUC plasma for females. Rubin et al. determined that there was a poor correlation of peritoneal surface area with calculated body surface, body weight and distance between the xiphisternal notch and symphysis pubis and suggested that it was unlikely that such a correlation would be found in the future [20]. However, the data from this study suggests that there might be a significant difference between the peritoneal surface area of males and females leading to a more rapid chemotherapy absorption in males.

This study supports the theory that heated intraperitoneal intraoperative melphalan perfusion can be a valuable asset in the treatment of patients with residual disease following cytoreductive surgery for peritoneal surface malignancies. A distinct pharmacological advantage was demonstrated with high levels of drug penetrating into tumor nodules on the peritoneal surface. The course of these patients will be carefully followed to determine the efficacy of this treatment protocol.

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