Pharmacokinetics of Melphalan in Clinical Isolation Perfusion of the Extremities

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ABSTRACT

The pharmacokinetics of melphalan in clinical hyperthermic isolation perfusion was studied in 16 patients with malignant melanoma. Analysis by computer-generated lines of best fit showed that the loss of melphalan from perfusate conforms best to a biexponential equation. The initial loss with a half-life (t1/2) of approximately 5 to 10 min is interpreted as rapid uptake of melphalan by the tissue of the perfused extremity. The terminal portion of the curve with a half-life of approximately 35 to 50 min is interpreted as due predominantly to the hydrolysis of melphalan, with a lesser component of loss due to absorption of melphalan to the filters and tubing of the perfusion apparatus. Determination of the area under the curve suggests that there is no appreciable uptake of melphalan by the tissue of the perfused extremity after 30 min.

INTRODUCTION

Isolation perfusion chemotherapy for advanced melanoma of the extremity has become a well-established procedure since it was first described by Creech et al. (5) in 1957. The bifunctional alkylating agent, melphalan, has been the most commonly used chemotherapeutic agent. Yet despite a large amount of pharmacokinetic information concerning p.o. and systemic administration of the drug, little is known of the pharmacokinetics in the perfusion system (5, 10). In their original paper, Creech et al. (5) used a bioassay system, and determined that active drug remained in the system for about 2 h at 37 °C. Klatt et al. (10) used a colorimetric assay for alkylating agents, and concluded that the initial loss of melphalan from the perfusion system was rapid for about 15 min, and then decreased at a steady rate. Since these early studies, mild hyperthermia of approximately 40 °C has been incorporated into the treatment regimens (1, 8, 17).

The response rate of melanoma to isolation perfusion with melphalan in advanced disease is 50 to 75%, with a complete response rate of 30% (12, 17). Such results are remarkable, considering the chemoresistant nature of melanoma and the ineffectiveness of melphalan administered systemically. These results occur because isolation perfusion can take advantage of the steep dose-response curve that characterizes many chemotherapeutic agents. The perfusate concentration of melphalan obtained in the perfusion setting has been estimated to be 10 times the level obtained after maximal-tolerated systemic administration.

The present pharmacokinetic study was undertaken to (a) evaluate the consistency and reproducibility of drug levels during clinical perfusion; and (b) provide base-line pharmacokinetic information which might permit modification of the presently accepted perfusion technique.

MATERIALS AND METHODS

Melphalan Analysis. Determination of melphalan levels in clinical perfusate and other solutions was performed by HPLC. One-ml samples of plasma or other fluid were vortexed with 2 ml of cold methanol, frozen at -70 °C in dry ice:acetone, and centrifuged at 0 °C at 2500 rpm for 10 min; the supernatant fluid was then injected into the HPLC apparatus (3). A 10-µm phenyl column (Waters µBondapak, Waters Associates, Milford, MA) was used with a flow rate of 2.5 ml/min with an isocratic system of 0.1% ammonium formate:acetonitrile (70:30). This is a slight modification of the system developed by Israel et al. (9) for the HPLC analysis of anthracyclines. The effluent was monitored at 254 nm, and the peaks were integrated with a Waters Model 730 data module. In addition to the measurement of melphalan in perfusate, analysis of melphalan in various solutions was undertaken to help standardize the assay, and facilitate interpretation of the perfusion data.

Samples of perfusate were withdrawn at intervals from the mixing chamber port of Shiley infant oxygenators (Shiley, Inc., Irvine, CA) during clinical perfusion. Perfusate samples were also taken during circulation of the blood:electrolyte solution through the perfusion apparatus itself to determine loss to the system.

Clinical Hyperthermic Isolation Perfusion. The technique of clinical perfusion therapy has been standardized in our institution, and is based on the well-described methods of several investigators (11, 12, 17, 18). Our technique of lower extremity perfusion is to first perform iliac and the tributaries of the external iliac vessels. After heparinization, the external iliac vessels are cannulated, and the cannulas are advanced to the proximal part of the leg (i.e., just distal to the inguinal ligament). A tourniquet is placed around the extremity and kept in position by 3 s.c.-placed Steinmann pins. Forequarter perfusion is performed by cannulating the proximal portions of the axillary artery and vein by a muscle-splitting incision. The tourniquet is placed about the entire forequarter and kept in position by 3 s.c.-placed Steinmann pins, one medial to the scapula, one just lateral to the sternum, and one at the posterior axillary line at the inferior aspect of the axillary fold. Once the tourniquet is in place, perfusion is then begun with a Travenol pump at a flow of 300 to 900 ml/min. A Shiley infant oxygenator is primed with 750 ml of Plasma-Lyte A (Travenol Laboratories, Deerfield, IL) and 300 ml of packed RBC. A 95% oxygen:5% CO2 gas mixture is used in the oxygenator. Blood gas determinations are made prior to injection of the drug, and adjustments are made if necessary. The temperature of the perfusate is raised to 43 °C initially. Heating blankets are also placed about the extremity. In addition, the temperature of the

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3The abbreviations used are: HPLC, high-performance liquid chromatography; AUC, area under the perfusate concentration versus time curve; t1/2, half-life.
operating room is raised to approximately 32 °C. We have measured the temperature of the perfusate at the arterial and venous sides and the mixing chamber, and the variation is in the 0.5–1.5 °C range, with the arterial side the highest. Radioactive iodinated serum albumin is injected into the pump circuit, and peripheral arterial blood samples are drawn and counted to determine leakage. The leakage for lower extremity perfusion is in the 0 to 3% range, and for forequarter perfusion, 20 to 40%.

Once the skin and muscle temperature is 39 to 40 °C, the perfusate temperature is lowered to approximately 40.5 °C, and melphalan (1.0 to 1.5 mg/kg body weight) is injected in bolus form into the mixing chamber of the oxygenator. The usual dose for lower extremity perfusion is 80 to 100 mg, and that for forequarter perfusion is 60 to 80 mg. Samples of perfusate are withdrawn from the mixing chamber and kept on ice, and the plasma is frozen at −20 °C until analysis. Perfusion with the drug is continued for 60 min, after which the extremity is rinsed with 2000 ml of heparinized Plasma-Lyte A. The tourniquet is then removed, the cannulas are withdrawn, and the vessels are repaired. The remainder of the indicated surgery is then completed.

Analysis of Results. Curve fitting for the purpose of pharmacokinetic analysis was done by means of the nonlinear estimation program BMDP3R (6). Data points obtained after 6 min were used for analysis, because mixing of drug and perfusate was complete after that period of time, and generated equations were more consistent if the early time periods were omitted. AUCs were determined by the log trapezoidal method (21).

RESULTS

Extraction of melphalan from plasma by using methanol was consistently 95%. The in vitro degradation rate constants (and $t_{1/2}$) for melphalan in electrolyte solution at pH 7.4 were 0.13 h$^{-1}$ at 20 °C ($t_{1/2} = 5.47$ hr); 0.61 h$^{-1}$ ($t_{1/2} = 1.13$ h) at 37 °C; 0.67 h$^{-1}$ ($t_{1/2} = 1.03$ h) at 39 °C; 0.74 h$^{-1}$ ($t_{1/2} = 0.93$ h) at 41 °C; and 0.84 h$^{-1}$ ($t_{1/2} = 0.83$ h) at 43 °C. Thus, although hydrolysis of the drug proceeds rapidly at elevated temperatures, within the temperature range of 39–41 °C used during clinical perfusion, there is little difference in the half-life of the drug ($t_{1/2} = 56$ to 62 min). For comparison, the $t_{1/2}$ of melphalan in distilled water at 40.5 °C is approximately 27 to 30 min. In distilled water, the loss of the parent compound is accompanied by the appearance and increase of the hydrolysis products of melphalan, monohydroxymelphalan and dihydroxymelphalan (7). Incubation of melphalan in Plasma-Lyte A, however, results in the appearance and increase of one other peak which is intermediate in polarity between melphalan and monohydroxymelphalan, and may represent an exchange (chloride ions for another anion in the Plasma-Lyte A).

It is well known that melphalan is stabilized in salt or protein solutions, as described by Chang et al. (2, 4). Chart 1 illustrates that the addition of protein to a melphalan electrolyte solution improves the stability of the drug beyond that seen with electrolyte solution alone (Curves A to D). The protein concentration in perfusate fluid is in the 2- to 4-g range when determined by the method of Lowry et al. (13). Curve E in Chart 1 has 2 components. The early and relatively rapid disappearance of melphalan is interpreted as being due to binding of the drug to the cellular components of blood. The latter portion of the curve represents the hydrolysis of the drug in plasma.

Table 1 summarizes the computer-generated values of the biexponential equations

$$C_p = Ae^{-k_1t} + Be^{-k_2t}$$

obtained in clinical isolation perfusion in the 16 patients.

Curves A and B of Chart 2 show that the half-life of melphalan in the perfusion system is approximately 50 min at 40.5 °C. This is less than the half-life of approximately 65 min that is obtained when melphalan is simply incubated in a water bath in the same solutions. The difference is interpreted as representing loss of

<table>
<thead>
<tr>
<th>Perfusion with melphalan</th>
<th>Mean ± SE</th>
<th>Range</th>
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<tbody>
<tr>
<td>Lower extremity (12 patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>38.3 ± 3.4</td>
<td>23.0–58.9</td>
</tr>
<tr>
<td>K</td>
<td>0.1986 ± 0.0229</td>
<td>0.0457–0.3063</td>
</tr>
<tr>
<td>B</td>
<td>17.7 ± 1.5</td>
<td>8.1–27.6</td>
</tr>
<tr>
<td>Ks</td>
<td>0.0174 ± 0.0010</td>
<td>0.0133–0.0243</td>
</tr>
<tr>
<td>Forequarter (4 patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>42.2 ± 7.1</td>
<td>23.8–55.3</td>
</tr>
<tr>
<td>K</td>
<td>0.2409 ± 0.0413</td>
<td>0.1364–0.3387</td>
</tr>
<tr>
<td>B</td>
<td>17.3 ± 2.6</td>
<td>11.0–22.7</td>
</tr>
<tr>
<td>Ks</td>
<td>0.0202 ± 0.0030</td>
<td>0.0125–0.0269</td>
</tr>
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MELPHALAN IN CLINICAL ISOLATION PERFUSION

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Chart 2. A, in vitro perfusion with 90 mg melphalan in Plasma-Lyte A. Flow, 800 ml/min; temperature, 40.5 °C; pH 7.4; terminal half-life, 52.2 min. B, in vitro perfusion with 100 mg melphalan in 1000 ml Plasma-Lyte A, 350 ml whole blood. Flow, 800 ml/min; temperature, 40.5 °C; pH 7.3; terminal half-life, 46 min by least-squares (Points 10 to 60) computer-generated line of best fit.

C, mean values of melphalan concentration in perfusate during lower extremity perfusion in 12 patients. Points, actual mean values. The curve is the computer-generated line of best fit plotted with the 95% confidence limits of the means.

Curve C of Chart 2 is computer generated, taking the means of the actual data points of the 12 patients who underwent lower extremity perfusion. The equation is similar to the equation in Chart 3.

It is also apparent from Chart 3 that during forequarter perfusion, the rate of loss of melphalan during the second portion of the curve is greater than that during lower extremity perfusion. This is most likely a dilution effect that occurs during upper extremity perfusion, the mixing of drug-free blood from the systemic circulation with the perfusate. During lower extremity perfusion, there is, by contrast, minimal leakage into or out of the pump oxygenator circuit (0 to 3%), due to the ease of obtaining complete isolation. Tourniquet placement for a forequarter perfusion usually does not result in complete isolation. Systemic leak during forequarter perfusion is in the 15 to 40% range. Leakage of perfusate from the pump oxygenator circuit to the peripheral blood pool will have no effect on drug levels in the closed circuit. However, blood from the periphery can leak into the pump oxygenator circuit via nonocluded or only partly occluded arteries, which will result in dilution. A constant level of perfusate in the oxygenator during forequarter perfusion implies a static volume. This can be misleading, because to attain a steady volume, the perfusionist must adjust flow to the point where the perfusion system pressure prevents inflow from the periphery, or at least balances outflow and inflow. Thus, drug levels may decline more rapidly during forequarter perfusion, and interfere with pharmacokinetic analysis.

It is evident by inspection of Chart 2 that the terminal half-life of the in vitro perfusions and that of the compositive curve are similar. We have elected to evaluate this further by calculating the percentage of AUC change with time, which reflects melphalan loss from the system, by using the equations from simple incubation of melphalan (Chart 1, Curve E), in vitro perfusion (2B), and clinical perfusion (Chart 2, Curve C). In Chart 4, the 60-min observation periods were divided into 2 parts, that is, 0 to 30 min and 30 to 60 min. The AUC from 0 to 30 min was taken
as 100%, and the percentage decrease with time plotted in Chart 4A. Differences in the profile of the plots were observed, particularly that of clinical perfusion. When similar calculations were done during the 30- to 60-min period, no differences were observed in the profile of the plots, suggesting that there is not an appreciable uptake of melphalan by the tissue during clinical perfusion.

Comparison of AUC obtained during perfusion to those obtained by conventional bolus i.v. injection requires some comment. Unlike the usual forms of drug administration, in the perfusion system, drug delivery ends abruptly. Total AUC$_{0-\infty}$ then is not accurate in terms of estimating exposure to drug. Even calculation of partial AUC$_{0-60 \text{ min}}$ might be expected to overestimate drug exposure, in that a portion of this area that is drug-containing perfusate, is discarded when perfusion is completed. An approximation of the lost or discarded drug could be obtained by calculating the rectangular area bounded by lines that have origin at the 60-min point concentration of melphalan and were projected back to the x and y axes. Subtraction of this rectangle from the AUC$_{0-60 \text{ min}}$ would seem to more accurately reflect drug exposure.

For example, the AUC$_{0-60 \text{ min}}$ for the equation in Chart 2C is 1240 min $\times$ $\mu$g/ml. With perfusion drug delivery is completed in 60 min, and the AUC$_{0-60 \text{ min}}$ for the equation in Chart 2C is 821 min $\times$ $\mu$g/ml. Because the drug remaining in perfusate is discarded, one might further subtract the rectangular area noted above for which the equation in Chart 2C would be 373 min $\times$ $\mu$g/ml. Therefore, the final AUC estimate would be 448 min $\times$ $\mu$g/ml. The AUC profiles of Chart 4 were done using this approach. If the AUC calculations were done using AUC$_{0-\infty}$ the results are similar, and the conclusion that there is little, if any, uptake of melphalan by tissue after 30 min is unchanged.

**DISCUSSION**

The present report demonstrates that the technique of isolation perfusion results in consistent levels of melphalan in the perfusate. A clinically relevant finding from the present studies is apparent from Charts 2 to 4. The similar rates of disappearance of melphalan during the latter portion of the clinical perfusion curve and that of the *in vitro* perfusion curves suggest that there is little, if any, further tissue uptake of melphalan from perfusate during the last 30 to 40 min of perfusion. These results were not unexpected, since *in vitro* studies with human melphalan cell lines have shown that the uptake of melphalan by the cultured cells reaches a plateau by 10 min (14). Cell culture studies have shown that melphalan uptake is an active process with a steady state cell:medium concentration of 5:1 in murine systems, and 3:1 in human melanoma (20). In addition, however, it has also been shown that the exodus of melphalan from L1210 murine cells occurs rapidly into a melphalan-free medium, and that only 50% of the drug is retained (20). Redwood and Colvin (15) found *in vitro* that 75% of the intracellular melphalan was free to leave the cell, and that, although most of the effect occurred in the first 20 min, it continued for approximately 2 h. Based on the foregoing, it would seem valuable to obtain data on tissue levels of melphalan and degree of DNA cross-linking with varying perfusion times. Recent work has also shown that the extent of DNA cross-linking in melphalan-sensitive and melphalan-resistant human melanoma cells in culture differed at melphalan concentrations up to 20 $\mu$g/ml, but reached the same maximum at 40 $\mu$g/ml (16).

Other work with murine L1210 leukemia cells has shown that the intracellular glutathione concentration is a major determinant of intracellular melphalan levels, and that resistant cells can be made sensitive by lowering intracellular glutathione levels (19). Possibly, perfusion with melphalan bypasses in part such mechanisms by the extremely high levels of drug attained.

This study demonstrates that clinical isolation perfusion with melphalan is a reproducible technique, and provides some basic pharmacokinetic information. Our data clearly suggested that perfusion with melphalan for longer than 30 to 40 min is unnecessary. Further studies to determine the tissue levels of melphalan and the amount of DNA cross-linking by melphalan with varied perfusion times are in progress.

**REFERENCES**


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