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Short Communication

Determination of melphalan in the perfusate from patients treated by regional perfusion chemotherapy

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Introduction

Melphalan is an important alkylating agent which has been widely used in the treatment of neoplastic diseases. In clinical practice, oral as well as intravenous administration of this drug is routine. However, in some cases administration via isolated regional perfusion offers a highly efficient form of chemotherapy with minimal systemic toxicity. This technique allows a ten-fold increase in melphalan concentration above usual levels and assures the easiest access of the cytostatic agent to the tumour and surrounding tissues. A combination of chemotherapy and hyperthermic perfusion yielded better results than with normothermic perfusions. Increasing the temperature of the tumour bearing region to 40–41°C potentiated the chemical action of the chemotherapeutic agents, and produced vasodilation allowing better perfusion of the tumour area. Research in hyperthermia has shown that it exerts a synergistic effect killing tumour cells when combined with either chemotherapy or irradiation. Hyperthermic perfusion chemotherapy has been used for the treatment of a variety of tumour types, but the most dramatic responses were seen in melanoma of the extremities [1].

Despite the effectiveness of regional perfusion therapy, little is known about drug levels in the course of the perfusion. Because of the rapid decomposition of melphalan [2–4] the measurement of its concentration changes in the perfusate are important for the optimization of this chemotherapy.

This paper is concerned with the determination of time–concentration profiles of melphalan in the course of perfusion, using HPLC. Procedures for the quantitation of melphalan have been recently reviewed [5]. Determinations in biological materials have

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been mainly performed by reversed-phase HPLC and a number of methods are available that differ in clean-up procedures, solvent composition and detection mode [2, 6–12]. Based on these reports a modified assay is described for the determination of the perfusate level of melphalan.

Experimental

Apparatus

Chromatography was performed using a Waters Assoc. liquid chromatography system, consisting of a Model M-45 pump, Model M441 UV detector and Data Module M730 for recording and quantitation of the chromatographic data. For digital data acquisition an Apple microcomputer was also used. The detector was operated at 245 nm with a sensitivity setting of 0.04–0.2 AUFS. Samples were injected using a U6K valve injector with a 200-μl sample loop. A 100 × 4.6 mm i.d. column packed with 5-μm Nova Pak C_{18} was obtained from Waters Assoc. The pH values were measured with a Corning pH-meter equipped with a combined glass electrode.

Chemicals

Melphalan and ethyl paraben were obtained from Sigma Chemical Co.; HPLC grade methanol, acetic acid and trichloroacetic acid (analytical grade) from Fisher Scientific Co. All chemicals were used as received, and double distilled water was used throughout.

Mobile phase

The mobile phase was water–methanol (1:1, v/v); its pH was adjusted to 3.2 using acetic acid. The mobile phase was passed through a 0.5-μm Millipore filter and degassed in an ultrasonic bath prior to use. All separations were carried out with a flow rate of 1.5 ml/min at ambient temperature.

Sampling and sample treatment

An entire upper or lower limb of a patient with malignant melanoma was subjected to isolated hyperthermic perfusion. The temperature of the isolated limb was maintained at 39–40°C. The mean dose of melphalan used was 80 mg (range 70–90 mg) depending on the extent of disease present and body weight. Perfusate samples were drawn from the injection site by an indwelling catheter attached to the arterial line of the extra-corporeal circulation. Both perfusate and systemic blood samples were taken from six patients during the 40 min period of perfusion. Each blood sample was immediately placed in ice, then centrifuged (3000 rpm; 10 min; 4°C) and the plasma collected. All plasma samples were stored frozen (−20°C) overnight until processed. A 0.5-ml aliquot of plasma was treated with 0.5 ml of cold (0°C) 5% trichloroacetic acid containing 2.5 μg/ml ethyl paraben as internal standard. The mixture was vortex mixed and centrifuged for 3 min at 15,000 rpm. The clear supernatant (80 μl) was then injected onto the column.

Standard curve

Aliquots of melphalan solution (1 mg/ml) were added to human blood obtained from a blood bank, to produce concentrations ranging from 2.0 to 115 μg/ml, and prepared for analysis by the same procedures as the samples. All injections were made at least in duplicate, with two unknowns being followed by two standard solutions. Quantitation

was performed by constructing a standard curve of the ratio of the peak areas of melphalan to the internal standard versus melphalan concentration.

In preliminary studies the conditions used during the sample work-up procedures were thoroughly investigated to minimise drug degradation. No significant change in the melphalan concentration was seen after storing or preparing samples at 20°C for 2 h which is similar to that previously observed [3, 4]. Since the total analysis time per batch was *ca* 1.5 h it can be assumed that the standard curve was not significantly affected by drug degradation.

Recovery

Spiked samples were prepared by adding to human serum aliquots of melphalan solution to obtain concentrations of 3.7, 58 and 88 μg/ml. The samples were then subjected to the sample clean-up and quantitation as described above.

Results and Discussion

Improvements in analytical methodology have been mainly directed at increasing the sensitivity of the assays. However, because perfusate levels of melphalan are in the μg/ml concentration range, sensitivity is only a minor problem here. Since the degree of sample preparation and purification required also depends on the concentration range of interest, the approach presented here is less complex and only uses protein precipitation with cold trichloroacetic acid. Despite this simplicity the procedure removed most of the interfering material from the plasma and has adequate sensitivity for this study. No degradation of melphalan was observed in the course of the sample preparation. It should be noted that the assay described is comparable with the early methods of melphalan quantitation [2, 6–7] with respect to the recovery and detection limits. The overall recovery was found to be 85%, and the limit of detection about 0.01 ppm.

The standard curve was linear in the concentration range studied (2–115 μg/ml in plasma). A least-squares analysis gives a correlation coefficient of 0.997, a slope of 0.484 with the relative standard deviation 2.88% and an intercept of 0.716 (n = 11).

Ethyl paraben was selected as internal standard due to its availability and convenience in handling and storage, and because it can be measured easily without impairing the separation. A representative chromatogram following the injection of a plasma sample with 13.1 μg/ml is shown in Fig. 1.

The samples of perfusate taken at fixed time intervals yielded chromatograms in which an additional peak appeared (t_R = 5.34), Fig. 2.

The dependence of the height of this peak on the sampling time as well as the comparison of retention time with previously published data [2, 6–7] indicate that the peak was formed by dihydroxymelphalan, although further work would be required to confirm this assumption.

Using this method the melphalan level in the perfusate from six patients treated by hyperthermal perfusion was determined, and the drug concentration measured at 5-min intervals. A representative log concentration–time profile is shown in Fig. 3. The systemic plasma concentration of melphalan during and after perfusion therapy was found to be less than 100 ng/ml.

The elimination half-life was derived from plots of log perfusate concentration against time. The average half-life was found to be 12.2 min, the rate constant 3.42 h^{-1}. There is little data describing the pharmacokinetics of melphalan in man. The half-life was

Figure 1
Chromatogram of a plasma sample containing 13.1 μg/ml melphalan. 1, ethyl paraben (internal standard); 2, melphalan.

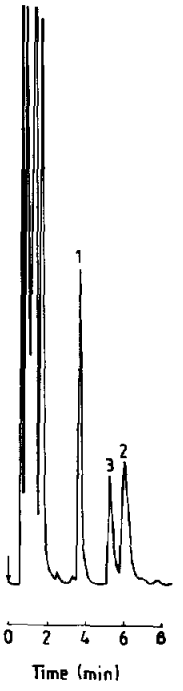

Figure 2
Chromatogram of a perfusate sample taken 15 min after onset of the perfusion. 1, ethyl paraben; 2, melphalan; 3, hydrolysis product of melphalan.

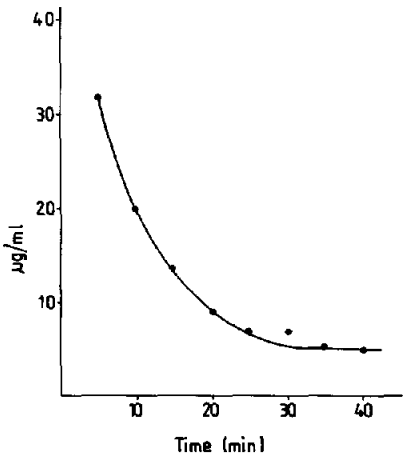

Figure 3
Semi-log plot resulting from perfusate levels of melphalan in a patient given 90 mg melphalan via regional hyperthermal perfusion; samples were drawn at 5-min intervals over 40 min.

reported to be 67 min after oral administration [13] and 21 min [14] after an intravenous one. Considering that the perfusions were performed at 40–41°C our estimates are in good agreement with those previously reported. Since melphalan undergoes a rapid spontaneous degradation even at room temperature [2–4], in pharmacokinetic studies both the hydrolysis and the enzymatic metabolism should be taken into consideration. Comparing the pharmacokinetic estimates with the *in vitro* rates of melphalan disappearance suggests that the major determinant of melphalan pharmacokinetics *in vivo* is its spontaneous hydrolysis.

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