Relative dispersion of intravascular transit times during isolated human limb perfusions for recurrent melanoma

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Aims We have characterized the relative dispersion of vascular and extravascular markers in the limbs of three patients undergoing isolated limb perfusions with the cytotoxic melphalan for recurrent malignant melanoma both before and after melphalan dosing.

Methods A bolus of injectate containing [⁵¹Cr] labelled red blood cells, [¹⁴C]-sucrose and [³H]-water was injected into an iliac or femoral artery and outflow samples collected at 1 s intervals by a fraction collector. The radioactivity due to each isotope was analysed by either gamma [⁵¹Cr] or beta [¹⁴C and ³H] counting. The moments of the outflow fraction-time profiles were estimated by a nonparametric (numerical integration) method and a parametric model (sum of two inverse Gaussian functions).

Results The availability, mean transit time and normalised variance (CV^2) obtained for labelled red blood cells, sucrose and water were similar before and after melphalan dosing and with the two methods of calculation but varied between the patients.

Conclusions The vascular space is not well-stirred but characterized by a CV^2 similar that reported previously for in situ rat hind limb and rat liver perfusions. A flow-limited blood-tissue exchange was observed for the permeating indicators. Administration of melphalan did not influence the distribution characteristics of the indicators.

Keywords: multiple indicator dilution, intravascular mixing, isolated limb perfusion, melphalan, pharmacokinetics

Introduction

Isolated limb perfusion (ILP) with cytotoxic agents has become an established method for the treatment of recurrent melanomas since its introduction in 1958 [1]. ILP with hyperthermia and melphalan administration is reported to be associated with a patient survival rate ranging from 79–92% [2–4]. However, the dosage regimens for cytotoxic agents in ILP remain largely empirical and ill-defined. The optimal dosage schedule should eventually be related to the physiology of the limb and the pharmacokinetics of the cytotoxic within that limb.

It is now well recognised that compartmental representations of organs are limited and that models of intravascular mixing which are based on the transit time dispersion of intravascular markers such as the inverse Gaussian distribution model may better define the disposition of solutes in isolated organs [5–10]. Such models may be extended to the whole body [11, 12]. The normalized variance (or relative dispersion) of vascular transit times is the main determinant of the transit time dispersion of drugs across organs, which

is also dependent on endothelial cell wall permeability, organ blood flow, plasma/tissue protein binding, vascular/tissue volumes and diffusion processes [10, 13–15]. A number of studies have now characterized the variance in vascular transit times for the liver [6], hind-limb [16] and other organs. However, there appears to be limited data available for human organs on which pharmacokinetic modelling can be based.

The present study investigated the variation in vascular transit times in the human ILP using the multiple indicator dilution technique [17], in which a bolus of perfusate containing [⁵¹Cr]-labelled red cells, [¹⁴C]-labelled sucrose and [³H]-water was administered into the femoral/iliac artery and the outflow fractions of each solute were measured at various sampling times. The multiple indicator dilution technique can be used to determine the volumes of intravascular, extracellular and intracellular space based on the estimated mean organ transit times (MTT) of the respective markers as well as their dispersion in the organ (CV^2) [5, 6, 16, 17]. Whereas the parameter MTT is solely dependent on steady state distribution, CV^2 acts as a measure of the dynamics of the distribution process within the system under study [11, 18]. The dispersion of the intravascular reference (⁵¹Cr labelled red cells) was assessed using a new

parametric model [10]. It is concluded that the dispersion obtained is comparable with that reported previously for animal models.

Methods

Isolated limb perfusions (ILP) with melphalan were conducted at 41.5°C with a 19% haematocrit as previously described [19] on three patients for the treatment of recurrent malignant melanoma. Each patient gave informed consent for the multiple indicator dilution (MID) studies to be carried out as part of their operation. The patient characteristics are shown in Table 1. The study was approved by the Human Ethics Committee of the Royal Brisbane Hospital, Herston, Queensland, Australia, in accordance with the Australian National Health and Medical Research Council's guidelines on human experimentation. Multiple indicator dilution studies were carried out in each patient before and after the administration of the cytotoxic agent, melphalan.

The multiple indicator dilution injection solutions were prepared after measuring the haematocrit ($19.2 \pm 0.5\%$) of the perfusate solution for each patient. The injection solution was made by adding 50 μl [^3H]-water (1 mCi ml^{-1} in water, Amersham, Sydney) to 500 μl [^{14}C]-sucrose (0.1 mCi ml^{-1} in ethanol water 9:1, Dupont NEN products, Boston, MA) and 950 μl sodium chloride 0.9% injection B.P. (Delta West, Perth, Australia). To this 1.5 ml of solution, 1 ml of [^{51}Cr]-labelled red blood cells (2.5 μCi) was added. Sufficient saline was then added to this sample to yield a haematocrit in the final injection solution equivalent to that in the perfusate. The injection volume was 2 ml. Bolus injections were given by syringe over as rapid a time (< 6 s) as possible to simulate an impulse input. A pump was used to divert outflow perfusate from the venous side of the ILP circuit and was operated at 1.5 ml s^{-1} . Effluent samples were then collected into sample tubes with a fraction collector at 1 s intervals for a total of 75 s. Bolus injections and fraction collection were also carried out in the absence of the limb to ascertain the impulse response contribution due to the catheter tubing used for marker administration and sampling.

Blood samples were analysed for ^{51}Cr content by gamma counting (Packard Instrument Co. USA). The [^3H]-water and [^{14}C]-sucrose content in plasma were assayed by scintillation counting (Minaxi β Tri-carb-4000, Packard Instrument Co. USA) following the addition of 50 μl supernatant to 5 ml scintillant (Emulsifier-safe[®], Parkard, Australia).

Data analysis

The concentrations of each indicator ([^{51}Cr]-labelled red blood cells, [^{14}C]-sucrose and [^3H]-water) in a given sample were expressed as an outflow fraction ml^{-1} (concentration in sample/dose of indicator). Two methods were used to analyse the data. In the first method, moments were calculated from the outflow fraction-time curve by standard techniques and corrected for catheter effects, the catheter effects including both that from the administration and sampling systems [16]. The area under the outflow fraction-time curve (AUC), the mean transit time (MTT), and the relative dispersion (CV^2) were calculated using a special numerical integration method with extrapolation to infinity [20, 21]. MTT and CV^2 were corrected for the catheter distortion [13, 16]. These parameters were also estimated using an empirical parametric model of the transit time density (i.e. the normalized outflow curve) of the vascular reference (mixture of two inverse Gaussian densities) which has been successfully applied in isolated rat hindlimb studies [10]. In order to account for the catheter effects, the unit impulse response of the catheter system was first fitted in the time domain using an inverse Gaussian function as an empirical model. The parameters MTT_{Cath} and $\text{CV}^2_{\text{Cath}}$ estimated for different perfusion rates were then held fixed and the outflow profile of the limb was fitted to a function given in the Laplace domain by the product of the catheter function and the mixture of inverse Gaussians (limb transit time density) using a numerical inverse Laplace transformation algorithm in the nonlinear regression program package SCIENTIST (MicroMath Scientific Software, Salt Lake City, USA) [10]. The availability F of each indicator was calculated from the product of AUC and the volumetric flow rate Q (recognising that AUC has been previously normalised to dose). The volume (V) accessible to the indicator was calculated from the product of MTT and Q , assuming that solutes have instantaneously diffused into red blood cells.

Results

Figure 1 shows a typical profiles for the MID in a system of the catheters alone (Figure 1a or the ILP Figure 1b). In the catheter system alone, the profiles for the indicators sucrose and water are similar with a peak time at approximately 4 s. In contrast, the profiles for the indicators in the catheters and limb show significant differences, the peak for the ^{51}Cr labelled red blood cells being higher than the other two indicators and a [^3H]-water outflow profile tailing substan-

Table 1 Patient characteristics for multiple indicator studies in ILP.

Patient	Gender	Age (years)	Weight (kg)	Site of arterial cannula	Arterial perfusion pressure (mm Hg)	Perfusion temperature ($^{\circ}\text{C}$)	Flow rate (ml min^{-1})
1	F	73	51	Femoral	110	37.0	650
2	F	45	74	Femoral	125	38.0	950
3	M	61	93	External iliac	118	41.7	1600

Figure 1 Representative outflow profiles for $[^{51}\text{Cr}]$ -labelled red blood cells (●), $[^{14}\text{C}]$ -sucrose (○) and $[^3\text{H}]$ -water (■) after bolus administration through catheters alone (a), a human limb isolated perfusion before administration of melphalan (b).

tially beyond that for $[^{14}\text{C}]$ -sucrose and $[^{51}\text{Cr}]$ -labelled red blood cells (RBC).

The parameters calculated for each indicator from the outflow profiles using the numerical integration method are shown in Table 2. The recoveries of RBC and sucrose are generally better than for water. The MTT of the three indicators were in the order of water > sucrose > RBC. A comparison of the moments obtained for each indicator before and after melphalan administration, revealed no significant differences (Table 2).

Table 3 shows a comparison of moments deduced by numerical integration method and non-linear regression of the parametric model [10]. The sum of two inverse Gaussian densities described the data in the tail region better than a single inverse Gaussian function and therefore represents a useful empirical representation of the data model. It is apparent that the parameter estimates obtained are comparable. In principle, the same agreement between the results of both methods was seen for sucrose and water, indicating that the transcapillary distribution of these substances is

flow-limited. This is also in accordance with the results obtained applying the complete convection-permeation-diffusion model to the sucrose and water data where parameter estimation failed due to high permeability and fast intratissue distribution in comparison with the noninstantaneous input process (flow rate, damping effects of the catheters).

Discussion

The knowledge of the impulse-response (IR) curve of a vascular reference is in general a prerequisite to the modelling of transcapillary distribution dynamics and elimination of drugs in any organ. It is shown in this study that the mixture of two inverse Gaussian density functions is a useful empirical model for the transit time distribution of the vascular reference in the human limb. This function was introduced as part of a new stochastic organ model which was applied to the isolated perfused rat hind limb [10]. The validation of this empirical model may serve as a basis for future applications of the complete convection-permeation-diffusion model [10] to the human hindlimb. In the present study the solute input rate was probably too low for this purpose leading to flow limited distribution. The results obtained with the parametric method (nonlinear regression) also serve as an independent validation of the numerical integration procedure.

The lower volumes observed for labelled red blood cells are at the lower flow rates (Table 2). Whilst one explanation may be the reported dependence of blood vessel volume and recruitment on flow rate [15], there are other reasons which may explain the results obtained. The extracellular and total water space in the human limb was estimated from the multiple indicator dilution technique to be 1.3 and 2.1 times that of the red blood cell space. The corresponding values reported for the perfused rat hind limb are 4.7 and 10.6 [16]. The lower ratios obtained for the human limb relative to that in the rat is suggestive of an incomplete distribution of solute in the tissues of the human limb, probably as a consequence of incomplete capillary recruitment with an effective increase in intercapillary distance in the human. Furthermore, the reduced recovery of $[^{14}\text{C}]$ -sucrose and $[^3\text{H}]$ -water relative to that of $[^{51}\text{Cr}]$ -labelled red blood cells may indicate that the observed tail part is not representative leading to an underestimation of MTT and consequently also the corresponding distribution volumes.

Dosages of cytotoxics in ILP remains somewhat empirical. There is a suggestion that the limb blood volume be used as a basis for dosing [22]. Kroon has stated that 'a dosimetric formula taking into account both the volume of the perfused region and the priming volume of the extracorporeal circuit is being waited' [23]. The approach described in this paper provides a method, although cumbersome, for the estimation of the perfused region and priming volumes. Such volumes can be used as a basis for estimation of doses when the distribution of the cytotoxics is flow limited. This work using a very short infusion time (<6 s), suggests that the CV^2 for the interstitial (sucrose) and cellular (water) markers is similar to the vascular reference, consistent with flow limited distribution for the markers. However, Wu *et al.* [24, 25] suggests that the distribution of melphalan into

Patient	Dosing time	Indicators	F	MTT (s)	V (ml)	CV ²
1	Before melphalan	RBC	0.86	28.9	313.1	0.46
		Sucrose	0.78	40.3	436.6	0.52
		Water	0.64	60.8	658.6	0.82
	After melphalan	RBC	0.85	27.1	293.6	0.43
		Sucrose	0.81	41.4	448.5	0.57
		Water	0.71	62.4	676.0	0.76
2	Before melphalan	RBC	0.81	40.1	634.9	0.35
		Sucrose	0.74	49.3	780.6	0.43
		Water	0.69	82.8	1311.0	0.85
	After melphalan	RBC	0.83	39.8	630.2	0.34
		Sucrose	0.78	49.1	777.4	0.52
		Water	0.70	89.4	1415.5	0.89
3	Before melphalan	RBC	0.83	38.0	1014.2	1.12
		Sucrose	0.64	51.1	1362.6	0.89
		Water	0.73	75.5	2013.3	1.25
	After melphalan	RBC	0.81	37.5	1000.0	1.06
		Sucrose	0.75	53.4	1424.0	0.98
		Water	0.68	73.4	1957.3	1.35

Table 2 Moment parameters obtained for each indicator in human ILP.**Table 3** Comparison of moment values for [⁵¹Cr]-labelled red blood cells before melphalan dosing estimated by numerical integration and nonlinear regression (model).

Patient	MTT (s)	MTT (s)	CV ²	CV ²	V (ml)	V (ml)
	PTTO	model	PTTO	model	PTTO	model
1	28.9	25.0	0.46	0.43	313.1	395.0
2	40.1	43.7	0.35	0.26	634.9	691.0
3	38.0	36.8	1.12	1.82	1014.2	981.0

various tissues is permeability limited. Whether the more complex vascular dispersion-permeation-diffusion model [10] needs to be applied to the definition of cytotoxic distribution in ILP is yet to be ascertained. The work of Wu *et al.* [24, 25] suggests that an overall dosage regimen needs to account for the overall duration of the ILP, limb tissue permeability and possible excessive cytotoxic concentrations in the vascular compartment.

The dispersion (CV²) of red blood cells observed is taken to reflect the branching of the vessels and interconnections, together with variations in the velocity and path lengths travelled by elements of blood. The values obtained for femoral artery cannulations are similar to those previously reported in the femoral artery cannulated rat hindlimbs which were estimated by either numerical integration [16] or by a parametric model [10]. The values are also similar in magnitude to those obtained for the liver [6]. The lack of significant difference in the estimated parameters for each of the indicators obtained before and after melphalan administration, suggests that administration of melphalan did not have a marked effect on the vasculature of the perfused limb during ILP. A slightly higher CV² is apparent in subject 3 where input was by the external iliac artery than by the femoral artery as for subjects 1 and 2. Whether the higher CV² for subject 3 reflects this injection site difference, the known peripheral vascular disease in this subject or the higher perfusion flow rate is not known.

Most studies on cytotoxic drug distribution during ILP have assumed a well-stirred vascular compartment. Whereas Benckhuisen *et al.* [26] examined melphalan pharmacokinetics in ILP using a two compartment model, Wu *et al.* [24, 25] assumed a well-stirred vascular compartment in conjunction with muscle, skin, fat and tumour compartments. The present analysis has shown that the vascular compartment is not well-stirred but characterized by a CV² of about 0.7. Whilst such a CV² is likely to be unimportant in designing dosage regimens for most cytotoxic drugs, vascular dispersion will influence the disposition of cytotoxics with a very high first pass extraction on perfusion through a limb with ILP. The principles defining such relationships are similar to that applying in hepatic elimination models for highly extracted drugs [5, 6].

The present data suggests that distribution kinetics reported for solutes in rats [16, 22, 27] cannot necessarily be translated directly into human studies. One substantial difference between the perfused rat limb model and human ILP studies is the presence of effective denervation in the rat preparation. In the ILP, an increase in perfusate flow or the use of vasodilators might increase capillary recruitment [16] and yield recovery, volume and CV² data more consistent with the previously reported rat data. However, an optimal approach to the treatment of malignant melanoma by cytotoxic administration during ILP is selective delivery of the cytotoxic agent to the tumour. Melanoma nodules are likely to show complete capillary recruitment at low perfusion flows as tumour blood flow is not normally under neural control. The preferential delivery of cytotoxics at low perfusion flows may therefore facilitate targeting to tumours as capillary recruitment in normal tissue is sub-optimal, as evidenced by the low recoveries and volumes of distribution for the extravascular markers used in this study. However, low perfusion flow may lead to hypoxia and subsequent tissue damage. The potential advantages of selective targeting over potential adverse physiological effects therefore need to be considered in choosing an appropriate perfusion flow rate.

A second implication of these findings is in the scaling of solute distribution kinetics in and between different species, including man. The applications of this concept to drug distribution have normally been based on the representation of organs as compartments in which it is assumed that the vascular and extravascular space is equivalent to a single well-stirred tank. More elaborate models based on the relative dispersion of vascular references in organs as a parameter of the intravascular mixing process [11, 12, 18], however, take the heterogeneity of the pharmacokinetic system at the organ level into account.

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