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Effect of L-leucine on oral melphalan kinetics in patients

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Summary. Melphalan uptake in the intestine has recently been shown to be an energy-dependent process which is affected by metabolic inhibitors. It is therefore theoretically possible that amino acids in food could reduce melphalan absorption by competing for uptake at the sites of absorption in the intestine. Since L-leucine has been shown to be the most potent inhibitor of melphalan transport into cells in vitro, this amino acid was chosen for the present study in patients. Oral melphalan (4.5 ± 0.5 mg/m^2) was given to ten fasting patients with and without a 2-g oral dose of L-leucine on separate randomized occasions at least 1 week apart. Melphalan plasma levels were measured by high-performance liquid chromatography (HPLC) for 5-h after dosing. L-Leucine plasma levels were measured by HPLC before and at 1 h after dosing. The area under the curve for melphalan was lower in seven of the patients after L-leucine. Plasma L-leucine levels 1 h after melphalan administration were 15.4 ± 3.7 μg/ml fasting and 35.4 ± 5.2 μg/ml after L-leucine. The results indicate that L-leucine can reduce plasma melphalan levels in some patients, probably through a reduction in absorption of the drug from the gastrointestinal tract. However, the effect, like that of food, is highly variable.

Introduction

Recently, we [5] and others [2] have shown that absorption of the antineoplastic agent melphalan (L-phenylalanine mustard) is reduced when the drug is taken orally with food. The median reduction in area under the plasma level-time curve (AUC) in our study of ten patients was 39%, but the reduction ranged from 0 to 100%. Bosanquet and Gilby [2] obtained a similar result in five patients, with a 54% reduction in AUC with food. A standardized breakfast was used in both studies, since melphalan is often taken as a single daily dose with breakfast.

The mechanism by which food reduces oral melphalan absorption is unknown, but the high chemical reactivity of the drug suggests that direct reaction with bowel contents could provide one explanation. An interesting alternative which we had proposed previously [5] was that melphalan transport across the gut wall could be inhibited by structurally related amino acids originating from ingested food. Melphalan is a mustard analogue of L-phenylalanine, but L-leucine and, to a lesser extent, L-glutamine have been shown to be potent inhibitors of melphalan transport into cells [7].

Recently, Adair and McElnay [1] demonstrated that melphalan uptake by the gut wall was an active, energy-dependent process which was affected by metabolic inhibitors. They also concluded that amino acids in food might compete with melphalan at the sites of absorption, thus reducing the efficiency of drug absorption. We have tested this proposal in a randomized study in patients by administering a dose of L-leucine with oral melphalan.

Materials and methods

Patients. Ten patients receiving oral melphalan (3.6–5.3 mg/m^2) for the treatment of various neoplastic disorders were studied. Patient characteristics are shown in Table 1. The drug was given in monthly courses consisting of single doses each day for 7 days. All patients had received several courses of melphalan prior to the study. Concurrent medication was the same on both study days for each patient. Patients gave informed consent before proceeding with the study. The usual morning dose of melphalan was given to each patient on two separate occasions at least 1 week but less than 1 month apart. Patients fasted for 12 h prior to the study. On one occasion the melphalan tablet was taken simultaneously with 4 capsules each containing 500 mg L-leucine. This L-leucine dose was approximately equivalent to the L-leucine content of the standardized breakfast given in our previous study [5], assuming complete hydrolysis of the protein content. The order of each study was randomized between patients. Blood samples were taken from a heparin lock immediately before administration and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 5 h after administration for melphalan assay. Blood samples were placed on ice immediately after collection and centrifuged within 1 h at 4° C. Plasma was stored at below −20° C until assay. The pre-dose and 1-h post-dose samples were also used for L-leucine determinations.

Assay methods. Plasma melphalan levels were assayed by high-performance liquid chromatography (HPLC) with fluorescence detection [5]. The limit of detection of this method was 2 ng/ml. Plasma L-leucine levels were also determined by HPLC [4].

Pharmacokinetic analyses. The terminal rate constant (K) and half-life ($t_{1/2}$) of melphalan were estimated by nonlin-

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ear regression analysis. The total AUC for melphalan was determined from the sum of the AUCs from 0 to 5 h after administration, estimated using the linear trapezoidal method, and that from 5 h to infinity, estimated from the 5-h melphalan concentration divided by K. The peak melphalan plasma level (C_{max}) was recorded.

Results

Mean melphalan plasma levels are shown in Fig. 1 and pharmacokinetic parameters, in Table 1. Seven of the ten patients had a substantial reduction in melphalan AUC when the drug was given with L-leucine. However, the effect was highly variable, with essentially no change in melphalan AUc in two patients and an apparent increase with L-leucine in patient 6. Peak melphalan plasma levels reflected changes in melphalan AUC. The mean (± SD) melphalan AUC was 277 ± 92 ng/ml.h fasting and 229 ± 98 ng/ml.h with L-leucine. Similarly, melphalan C_{max} was 112 ± 49 ng/ml fasting and 87.5 ± 43 ng/ml with L-leucine. The melphalan half-life was 80.6 ± 19 min fasting and 106 ± 87 min with L-leucine. None of these differences was statistically significant, and larger numbers of patients would be required to establish significance. The variability in melphalan half-life seemed to be higher with L-leucine. L-Leucine levels were similar before administration fasting (14.9 ± 3.0 μg/ml), before administration with L-leucine (13.4 ± 2.3 μg/ml) and 1 h after administration fasting (15.11 ± 3.7 μg/ml). The levels were approximately three-fold higher 1 h after L-leucine dosing (35.4 ± 5.2 μg/ml).

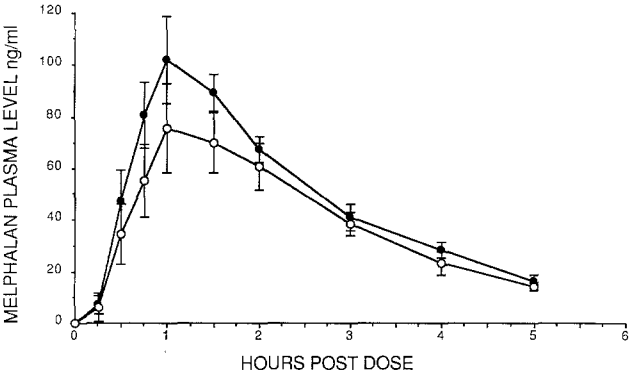

Fig. 1. Mean (± SE) melphalan plasma levels in all patients, both fasting (●) and when the drug was given with 2 g oral L-leucine (○) approximately 1 week later. Melphalan dosage was approximately 4.5 mg/m²

Discussion

Administration of oral L-leucine with oral melphalan can reduce melphalan plasma levels in some patients. However, as we have previously shown with food, the effect is highly variable and some patients may not demonstrate any appreciable change. All patients showed a substantial rise in plasma L-leucine levels 1 h after dosing, suggesting that the amino acid was absorbed at approximately the same time as melphalan. Melphalan peak levels were attained approximately 1 h after dosing in this and in previ-

Table 1. Patient characteristics and pharmacokinetic parameters

Patient	Sex/age (years)	Body surface area (m²)	Disease	Dose (mg/m²)	L-Leucine level (μg/ml)				AUC ng/ml · h		C_{max} (ng/ml)		$t_{1/2}$ (min)	
					Fasting		+ Leucine							
					0 h	1 h	0 h	1 h	Fasting	+ Leucine	Fasting	+ Leucine	Fasting	+ Leucine
1	F/64	1.9	Lymphoma	4.3	18.7	17.3	14.3	31.7	278	196	105	55.7	66.3	83.4
2	F/65	1.7	CGL	4.7	13.8	15.1	13.0	33.1	270	174	158	88.7	51.0	51.0
3	M/65	2.0	Multiple myeloma	4.0	14.5	11.8	14.1	27.0	250	264	88.0	72.5	83.7	107
4	F/55	1.7	Multiple myeloma	3.6	12.4	22.9	–	41.3	351	228	102	67.1	81.4	121
5	F/70	1.7	Multiple myeloma	5.0	9.8	10.5	9.4	30.1	495	411	223	169	79.7	76.7
6	F/75	1.7	Multiple myeloma	4.8	12.8	12.3	11.4	36.0	280	341	76.6	125	102	104
7	M/62	2.0	Multiple myeloma	5.0	20.0	19.5	14.8	39.6	243	221	131	105	65.8	55.7
8	F/64	2.0	Multiple myeloma	4.0	15.3	14.4	15.3	33.0	215	71.9	89.4	35.1	82.8	53.4
9	M/67	1.9	Waldenstrom's lymphoma	5.3	16.0	14.4	17.2	42.6	155	127	46.8	33.8	117	344
10	M/70	1.8	Waldenstrom's lymphoma	4.4	16.1	15.6	11.5	39.3	233	258	98.1	124	79.8	68.4
Mean	66	1.8		4.5	14.9	15.4	13.4	35.4	277	229	112	87.4	80.6	106
SD	5	0.1		0.5	3.0	3.7	2.4	5.2	92	98	49	43	19	87

ous studies [5]. It is probable that the reduction in melphalan levels in seven of the patients was due to a reduction in melphalan absorption. Studies by Adair and McElnay [1] have shown that melphalan is taken up by an active, energy-dependent process in the rat intestine. This seems to be consistent with known mechanisms of amino acid uptake in the human gastrointestinal tract [6]. Of the amino acids, L-leucine is the most potent inhibitor of melphalan transport into cells, either inhibiting uptake or enhancing transport out of the cell [3, 7]. For this reason, L-leucine was chosen for the present studies.

Although it is highly probable, it cannot be definitely concluded that a reduction in melphalan plasma levels seen with L-leucine is due entirely to a reduction in melphalan absorption. Other mechanisms are also possible. Melphalan clearance may have been enhanced by L-leucine, although an increase in the volume of distribution would need to have accompanied this change in order to explain the fact that the drug half-life was essentially unaltered. Since L-leucine might be expected to reduce both renal clearance and the volume of distribution of melphalan, if, as could be predicted, these are also active transport processes, then the observed fall in AUC is unlikely to be primarily a result of these mechanisms. Parallel i. v. studies conducted with and without L-leucine would be required to resolve this issue.

The present study has shown that L-leucine may play a significant role in the reduction of melphalan bioavailability seen with food. This effect could operate in conjunction with a reduction in tumour cell uptake of melphalan occurring due to a transient rise in extracellular L-leucine levels soon after food intake. The combined effect may be sufficient to completely exlude melphalan from the site of action. For these reasons, melphalan should not be given with food. The potential effects of total parenteral nutrition on melphalan uptake into tumour cells should also be taken into consideration.

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