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Pharmacokinetics of peptichemio in myeloma patients: release of *m*-L-sarcolysin in vivo and in vitro*

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Summary. Peptichemio (PTC) is a mixture of six synthetic oligopeptides, each of which contains the alkylating residue *m*-[di(2-chloroethyl)amino]-L-phenylalanine (L-mSL). The fate of PTC was investigated in eight patients with multiple myeloma after intravenous infusion of the drug. The quantitative analysis of the plasma samples was performed by liquid chromatography with fluorometric detection. L-mSL was rapidly released from the peptides and reached its maximal plasma concentration at the end of the infusion. Its median elimination half-life was 1.73 (range, 0.72–2.41) h. It was possible to follow the concentration of only one of the peptides, L-mSL-L-Arg(NO_2)–L-Nval·OEt, during and shortly after the infusion of PTC. The stability of L-mSL and the peptides was studied in buffer solution (pH 7.3), plasma, and blood. The stability of some of the peptides was drastically decreased in blood, the degradation half-lives being only about 1 min. We conclude that L-mSL plays an important role in the mechanism of action of PTC.

Introduction

Synthesis of oligopeptides containing a nitrogen mustard group has been one of numerous attempts to obtain antitumor agents of higher selectivity. Melphalan [22, 23], *N,N*-bis(2-chloroethyl)-*p*-phenylenediamine [3], or *m*-[di(2-chloroethyl)amino]-L-phenylalanine (*m*-L-sarcolysin) [4, 5] have constituted the alkylating portion of the peptides.

Peptichemio (PTC) is a mixture of six synthetic oligopeptides, each of which contains *m*-L-sarcolysin (Table 1). PTC has shown activity against several hematologic and solid tumors [1]. Promising results have been obtained using PTC in neuroblastomas resistant to first-line treatment or at relapse [18]. It has also been used in combination chemotherapy for advanced chronic lymphocytic leukemia [9]. An intriguing observation is that PTC apparently does not show cross-resistance to other alkylating agents such as cyclophosphamide [18, 19] in solid tumors and melphalan [2, 12, 15, 16, 24] in multiple myelomas.

To our knowledge pharmacokinetic data concerning PTC have not previously been published. It has recently been observed that PTC rapidly disappears in the presence of red blood cells, this effect being attributed to physicochemical interactions with the cell membranes [17]. Furthermore, after its intravenous administration to rabbits and rats, a tripeptide (PTT 119) with a similar structure was rapidly converted to *m*-L-sarcolysin, and no intact peptide could be detected [20].

The aim of the present study was (1) to study the pharmacokinetics both of the peptides of PTC and of *m*-L-sarcolysin after the administration of PTC to patients and (2) to compare the stability of the individual peptides of PTC with that of *m*-L-sarcolysin both in buffer systems and in human plasma and whole blood.

Patients and methods

Patients. The clinical characteristics of the myeloma patients who took part in the present study are given in Table 2. Patients 1–5 and 8 received PTC as third-line therapy. The first-line therapy was composed of intermittent oral melphalan courses. Second-line therapy consisted of combination chemotherapy comprising vincristine, doxorubicin, cyclophosphamide, and prednisone courses. Patients 6 and 7 received PTC as second-line therapy after melphalan and prednisone. PTC was administered as a single agent. The drug (0.6 mg/kg body weight) was mixed with 100 or 250 ml 5% glucose solution and was given via a central venous catheter [14] over 30 min. An infusion pump was used for patients 1–5.

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Table 1. Peptide composition of peptichemio

Peptide	Composition
P1	L-Ser–L-pFPhe–L-mSL·OEt
P2	L-Pro–L-mSL–L-pFPhe OEt
P3	L-pFPhe–L-mSL–L-Asn·OEt
P4	L-mSL–L-Arg(NO_2)–L-Nval·OEt
P5	L-pFPhe-Gly–L-mSL–L-Nval·OEt
P6	L-mSL–L-Arg–L-Lys–L-mSL–L-His·OMe

Table 2. Characteristics of patients with multiple myeloma undergoing treatment with peptichemio

Patient	Peptichemio dose (mg)	Age (years)	Sex (M/F)	Weight (kg)	Serum creatinine (μmol/l)
1	40	50	M	71	87
2	40	45	M	58	58
3	28	70	M	47	83
4	36	82	M	61	118
5	33	81	M	50	106
6	40	56	M	60	88
7	50	54	M	67	72
8	40	71	F	45	141

Table 3. Pharmacokinetic parameter for *m*-L-sarcolysin

Patient	t_0 [a] (min)	C_{max} (ng ml^{-1})	$t_{1/2}$ (h)	AUC (μg h ml^{-1})	AUC $dose^{-1}$ [b] (mg kg^{-1})
1	3	400	1.58	0.66	2.95
2	5	447	1.72	0.68	2.47
3	6	309	2.14	0.73	3.06
4	3	424	1.74	1.08	4.24
5	4	513	1.94	0.99	3.76
6	ND[c]	506	1.54	0.82	3.08
7	ND[c]	805	0.72	0.78	2.60
8	ND[c]	455	2.41	1.55	4.37

[a] Values were obtained by extrapolation of initial plasma concentrations (0–15 min) to zero concentration

[b] Calculations were based on the relation that 40 mg PTC corresponds to 16 mg *m*-L-sarcolysin

[c] Not determined since PTC was given by drip infusion

Drugs. PTC was purchased from Istituto Sieroterapico Milanese S. Belfanti (Milan, Italy). The individual pure peptides and *m*-L-sarcolysin were obtained as a gift from the same company. The structures of the peptides are given in Table 1.

Blood sampling. Blood (5–7 ml) was collected in glass tubes (Vacutainer) containing 250 IU heparin (freeze-dried) and were immediately placed on ice. After centrifugation at 4° C, the plasma fraction was removed and stored at –20° C until analysis. Blood samples were obtained immediately prior to treatment and at 5, 10, 15, 25, 35, and 45 min and 1, 1.25, 1.5, 2, 3, 4, and 6 h after the start of the infusion.

In vitro studies. Blood collected in Vacutainer tubes, fresh plasma, or buffer solutions were mixed with either the peptides or *m*-L-sarcolysin to a final drug concentration of 20 μg/ml. The samples were incubated at 37° C, and aliquots were taken at appropriate times for analysis by liquid chromatography as described below. The analysis of blood was carried out after separation of the plasma fraction by centrifugation (4° C).

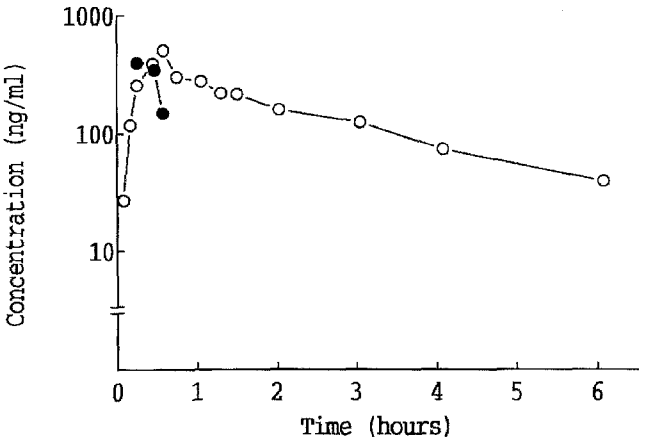

Fig. 1. Plasma concentrations of *m*-L-sarcolysin (○) and peptide P4 (●) in patient 5

Analytical method. The PTC peptides and *m*-L-sarcolysin were isolated from plasma using Sep-Pak (C_{18}) cartridges (Waters Associates, Milford, Mass. USA) containing 360 mg packing material. The cartridges were activated with 2 ml methanol and washed with 5 ml distilled water. Plasma (0.5–1 ml) was slowly (≈1.5 min) sucked through the cartridge, which was subsequently washed with water (5 ml) and dried using compressed air. The compounds were eluted with 1 ml ethanol : phosphoric acid (1 M) (99 : 1, v/v). After the addition of 0.5 ml water, the sample was filtered (0.45 μm) and 0.2 ml was taken for analysis by liquid chromatography (pump, Beckman 110B; injector, Rheodyne 9125). The separations were carried out using a Nucleosil 4 μ C_{18} column (Waters Associates; length, 10 cm; inside diameter, 0.5 cm). The mobile phase (flow rate, 0.4 ml/min) was acetonitrile : ethanol : phosphoric acid (1 M) : water (50 : 5 : 1 : 44, by vol.). The detection was performed using a Shimadzu RF-535 fluorescence detector (260/360 nm).

The recovery of *m*-L-sarcolysin (25 ng/ml) was 87% ± 7%. The recovery of individual peptides P1–P5 was >50%, and that of peptide P6 was about 20%. The capacity factors were: *m*-L-sarcolysin, 3.3; P1, 16.8; P2, 28.0; P3, 7.0; P4, 9.6; P5, 25.8; and P6, >100.

Pharmacokinetic analysis. The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule, and the residual area was extrapolated to infinity. The percentage of extrapolated AUC was in the range of 5.1%–23.6% (mean, 13.4%).

Results

After the administration of PTC, *m*-L-sarcolysin was rapidly released (Fig. 1). Extrapolation of the initial plasma concentrations during the constant infusion to zero concentration revealed a lag time in the appearance of *m*-L-sarcolysin in the range of 3–6 min (Table 3). The highest concentration of *m*-L-sarcolysin was obtained at the end of the infusion, and there was no tendency toward a sustained release of the compound since the concentration of *m*-L-sarcolysin rapidly declined after the end of the infusion. The median terminal half-life was 1.73 (range, 0.72–2.41) h. The AUC values showed a 2-fold variation between the patients (Table 3).

The concentrations of the intact peptides were evaluated in four of the patients. The limit of detection of the analytical technique was about 20 ng/ml for individual peptides P1–P5 but was considerably higher for peptide P6. Due to

Table 4. Degradation half-life for *m*-L-sarcolysin and the peptides of peptichemio at 37° C

Compound	Degradation half-life (min)		
	Phosphate buffer pH 7.33	Plasma[a]	Blood[a]
mSL	58	382	267
P1	54	146	1.6
P2	57	407	1.1
P3	43	41	7.2
P4	54	46	21.2
P5	69	98	1.3
P6	20	48	21.0

The concentration of *m*-L-sarcolysin (mSL) and the individual peptides was 20 μg/ml

[a] Blood and plasma were obtained from the same donor in all experiments

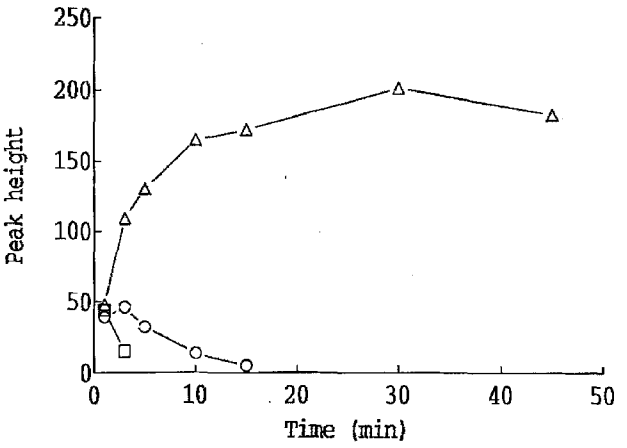

Fig. 2. Time course for peptide P2 (□), an intermediate degradation product (○), and *m*-L-sarcolysin (△) in blood at 37° C

this analytical sensitivity, it was possible to determine the concentration of only peptide P4 during and shortly after the infusion of PTC (Fig. 1). The maximal concentration of the peptide ranged from 100 to 500 ng/ml. The identity of the peptide was based on a comparison of the chromatographic retention and the absorbance spectrum (190–360 nm) recorded by a photodiode-array detector with a reference of peptide P4.

The stability both of *m*-L-sarcolysin and of the peptides of PTC is given in Table 4. In buffer solution the degradation half-life was about 1 h for *m*-L-sarcolysin and peptides P1–P5 at 37° C. Peptide P6 containing two *m*-L-sarcolysin residues had a half-life of about 0.5 h. The stability of the compounds in plasma either was increased (*m*-L-sarcolysin, P1, P2, and P6) or remained virtually unaffected (P3, P4, and P5). The stability of peptides P1, P2, and P5 was drastically decreased in whole blood, the half-lives being only about 1 min. The time course for degradation of peptide P2 and formation of the degradation products is given in Fig. 2. A degradation intermediate was rapidly formed but was below the limit of detection after about 30 min. It has not been established whether the compound might represent deesterified peptide P2 or a dipeptide from which the prolyl or the *p*-F-phenylalanyl residue has been split off. About 70% of the *m*-L-sarcolysin content of peptide P2 was accounted for as free *m*-L-sarcolysin after 30 min, which shows that the major portion of the intermediate is rapidly converted to *m*-L-sarcolysin in the blood.

Discussion

The present study shows that intravenous administration of PTC results in a rapid liberation of *m*-L-sarcolysin. Peptidase activity obviously plays an important role in the degradation process, since the half-life of some of the peptides in blood was on the order of minutes and the degradation resulted in free *m*-L-sarcolysin. The rapid degradation of the peptides in blood must be due to enzymatic activity connected with the blood-cell fraction as the stability in plasma was considerably higher. This observation is in accordance with early findings that erythrocytes and leukocytes contain a high level of peptidase activity toward di-, tri-, and tetrapeptides [21]. The increased stability both of some of the peptides and of *m*-L-sarcolysin in plasma as compared with buffer solution at pH 7.3 is most probably due to the lower rate of hydrolysis of the nitrogen mustard group bound to plasma albumin, an effect that has previously been observed for chlorambucil [7] and melphalan [6].

Due to the insensitivity of the analytical technique, it was possible to follow the concentration of only one of the peptides in plasma after the administration of PTC. Peptide P4 reached concentrations of 100–500 ng/ml, but the levels declined rapidly after the end of the infusion and it was not possible to make meaningful calculations of the elimination half-life.

Most probably, *m*-L-sarcolysin plays an important role with respect to the effects observed in patients treated with PTC. This conclusion is based on the following observations:

1. The dose-corrected AUC for *m*-L-sarcolysin is in the range of 2.47–4.37 μg h ml^{-1} (Table 3), which is not significantly different from the value we previously observed [8] following intravenous administration of melphalan (1.43–8.16 μg h ml^{-1}).

2. *m*-L-Sarcolysin exerts higher cytotoxic activity than does melphalan towards both lymphoblasts [10] and a human melanoma cell line [11].

3. *m*-L-Sarcolysin shows activity in Hodgkin's disease and multiple myeloma [13].

It should be of interest to compare the pharmacokinetics of *m*-L-sarcolysin given as the free drug with that obtained after the administration of PTC and to evaluate the fraction of PTC being converted to *m*-L-sarcolysin in vivo.

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