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Mitoxantrone and 5-Fluorouracil Modulated by the Pure (6S) Stereoisomer of Leucovorin as Second-line Chemotherapy for Advanced Breast Cancer

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GIVEN THE long natural history of advanced breast cancer, second-line chemotherapy is frequently necessary in patients. The combination of mitoxantrone, leucovorin and 5-fluorouracil has been tested in several studies with good results [1–6]. We report the results obtained with this regimen, modulated by leucovorin in the form of (6S)-5-CHO-FH_4, its active levorotative stereoisomer, in a series of patients resistant to the classic anthracycline-based combination FEC (5-fluorouracil, epidoxorubicin, cyclophosphamide).

To be eligible for the trial, patients were required to fulfil the following criteria: histologically or cytologically documented advanced breast cancer, with measurable or evaluable lesions, previously treated with FEC [5-fluorouracil 600 mg/m^2, epidoxorubicin 60 mg/m^2, cyclophosphamide 600 mg/m^2, all intravenous (i.v.) on day 1 every 3 weeks]; recovery from previous chemotherapy, surgery and/or radiotherapy, with WBC count $\geq$3500/mm^3 and platelet count $\geq$100000/mm^3; performance status (PS) 0–2 (ECOG scale); no brain metastases.

Chemotherapy was administered on an out-patient basis every 3 weeks for a maximum of six cycles. Mitoxantrone (12 mg/m^2) was administered on day 1, 5-fluorouracil (350 mg/m^2) and (6S)-5-CHO-FH_4 (150 mg/m^2) were given as i.v. bolus on days 1, 2 and 3. The cycle was delayed until marrow recovery if WBC count was <3500/mm^3 or platelet count was <100000/mm^3. Responses and toxicity were categorised according to WHO criteria [7].

24 patients were entered into the trial. Median age of patients was 53 years (range 41–75): 10 patients were premenopausal and 14 postmenopausal. The dominant sites of metastases were the bones in 14 (58.3%), viscera in 8 (33.3%) and soft tissues in 2 (8.3%). Previous treatments included FEC in all patients, as required by admission criteria; 13 patients (54.2%) had also received adjuvant CMF (cyclophosphamide, methotrexate and 5-fluorouracil), 17 (70.8%) had been treated with hormone therapy as adjuvant therapy (15 patients) or for the treatment of metastases (2 patients).

A total of 85 cycles of chemotherapy were administered.

Table 1. Toxicity

	No. of patients with WHO grade:			
	I (%)	II (%)	III (%)	IV (%)
Alopecia	6 (25.0)	7 (29.2)	1 (4.2)	0
Mucositis	3 (12.5)	4 (16.7)	0	0
Diarrhoea	3 (12.5)	4 (16.7)	2 (8.3)	0
Nausea and vomiting	7 (29.2)	2 (8.3)	0	0
Myelosuppression				
Neutropenia	5 (20.8)	6 (25.0)	3 (12.5)	0
Thrombocytopenia	1 (4.2)	1 (4.2)	0	0

Thirty-two cycles (37.6%) were delayed because of leucopenia, and seven (8.2%) were administered with a 25% dose reduction in 3 patients with persistent fever and leucopenia.

No complete response was observed; 7 patients (29.2%) showed a partial response with a median duration of 12 months, 10 (41.7%) had stable disease (with minor responses being observed in 3 patients) and 7 (29.2%) progressed. Table 1 shows toxicity results.

Published papers investigating the application of a combination of mitoxantrone, 5-fluorouracil and leucovorin in pretreated patients with advanced breast cancer have been recently reviewed [8]. Response rates ranged from 29 to 65%, with acceptable toxicity: obviously, higher response rates may be expected in chemotherapy-naive patients [5], but when used as second-line treatment, the regimen has also proved consistently active. In our own study, the role of this combination in pretreated patients has been further investigated by selecting a highly homogeneous group of patients, all of whom had previously received the same anthracycline-containing regimen FEC. Moreover, we employed the newly available, pure (6S)-stereoisomer of leucovorin, instead of the racemic (6R,S) natural form. Only the levorotatory (6S)-5-CHO-FH_4 is transformed into active folate cofactors and a higher activity may be expected theoretically, even if optimal doses and schedules of the 5-fluorouracil/folinate combination are not yet fully defined [9]. This combination appeared safe, easily administrable in an outpatient setting, and moderately active. However, many other regimens show similar activity in metastatic breast cancer: randomised comparative studies, analysing the results in terms of response rate, survival and quality of life, would be of interest.

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A Rapidly Progressing Leiomyosarcoma Expressing Drug-resistance Markers Failed to Respond to Cyclosporin A-associated Chemotherapy

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PROGNOSIS OF metastatic sarcoma remains poor and overall survival rates at 2 years range from 20 to 40%. Tumour cells resist cytotoxic drugs by means of multiple mechanisms, and this resistance is thought to be a major cause of therapeutic failure. Multidrug resistance (MDR) due to an increased level of P-glycoprotein (Pgp) has been found in approximately 30% of human sarcomas [1]. Cyclosporin A (CsA) is able to block Pgp-related resistance *in vitro* and *in vivo* [2]. However, other mechanisms of resistance are also involved in sarcomas. We report on a woman presenting with a rapidly progressing metastatic leiomyosarcoma that expressed several resistance markers, detected by reverse transcriptase polymerase chain reaction (RT-PCR), and our attempt to reverse drug resistance by adding CsA to the chemotherapeutic regimen [3].

A 42-year-old Algerian woman was admitted to the Institut Curie Hospital in October, 1992, with the diagnosis of unknown primary leiomyosarcoma with breast and pulmonary metastases. Upon admission, she was jaundiced due to liver metastases. Insertion of a T tube resulted in a 3-week delay in the onset of chemotherapy during which bladder, skin and contralateral breast metastases appeared. A skin metastasis was biopsied before each cycle of chemotherapy and subjected to semi-quantitative RT-PCR, using β_2-microglobulin as the internal standard to determine the levels of Pgp, multidrug-related protein (MRP), topoisomerase IIα (Topo IIα) and gluthatione-S-transferase (GSTπ). Because of MDR1 mRNA overexpression at the time of diagnosis, we decided to give three cycles of vincristine, deticene, ifosfamide and doxorubicin associated with CsA (5 mg/kg loading dose followed by 15 mg/kg/day continuously infused for 48 h during doxorubicin infusion). A pharmacokinetic study of CsA levels (0, 15 and 20 h) was performed for each cycle and showed that an effective reversing level (mean > 2 μg/ml) could be obtained in the serum, mean 3.8 μg/ml (range, 2.4–5.1 μg/ml), while the CsA content in the skin metastasis (cutaneous punctures at 0, 15 and 20 h) was low, mean 30.5 ng/ml (range, 25–36 ng/ml). Despite three cycles of this treatment, her disease continued to progress.

Table 1. Drug-resistance markers detected by RT-PCR

mRNA	At diagnosis	Before second cycle	Before third cycle
MDR1	0.19*	0.32	0.43
MRP	0.022	0.018	0.022
Topo IIα	0.88	0.95	0.98
GST π	1.3	1.1	1.1

*Values represent the ratios of yield of the amplified target gene/yield of the amplified internal standard, β_2-microglobulin.

The addition of CsA to this chemotherapeutic regimen did not reverse the MDR phenotype (Table 1). Indeed, the MDR1 mRNA transcript continued to increase after two cycles (the ratio mdr1/β_2 was similar to that obtained for a 5-times doxorubicin resistant breast cell line, MCF7 taken as a reference). Despite a high serum CsA level, the tissue CsA level remained low, which could by itself explain the lack of effect on MDR. Before considering a 'reverser' as being ineffective, it must first be determined, as we did, that it enters the target cells. Furthermore, this inefficacy could also be due to the high level of GSTπ. However, the resistance of this rapidly progressing tumour cannot be attributed to the MRP gene, which encodes a 190-kDa membrane non-Pgp, that did not pass the threshold of positivity nor to the relative stability of the topo IIα-mRNA transcript [4]. The intrinsic and acquired multifactorial resistance of metastatic sarcoma may explain the inability to halt the progression of this patient's disease.

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