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Timed Sequential Chemotherapy Following Drug-induced Kinetic Recruitment in Refractory Ovarian Cancer*

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Abstract—*Kinetic recruitment of cancer cells can seldom be monitored in human solid tumors. Repeated tumor sampling in ascitic ovarian cancer has been exploited to study tumor cell kinetic recruitment following treatment with the alkylating agent iphosphamide (IFX). The treatment schedule of the study was designed to administer the antimetabolic agents MTX-5FU at the time of the recruitment peak. Kinetic studies by the labelling index (LI) assay could be performed during and after the IFX treatment in four out of eight patients because of sampling difficulties. Experimental results showed that the IFX effectiveness reduced the proliferating cells, followed by cell kinetic recruitment from the resting pool. The antimetabolic treatment in concomitance with the proliferative peak (day 12) has been reduced with respect to the original schedule due to the heavy leukopenia occurring to the patients. It is likely that the reduced drug dosage might have contributed to the poor clinical response. However, the recruited cells exhibited an increased* in vitro *chemosensitivity to adriamycin in comparison to tumor cells studied before the IFX treatment.*

INTRODUCTION

THE SENSITIVITY of tumor cells to chemotherapeutic compounds can only be estimated on a statistical basis since there are no tumor markers or kinetic features that can be exploited for chemosensitivity prediction. However, in individual tumors non-dividing quiescent cells are usually less sensitive to cytotoxic agents than corresponding proliferating cells [1–7]. Therefore various attempts have been made to recruit resting tumor cells into the proliferating pool in order to increase cell susceptibility to antineoplastic agents [5–9]. Cell recruitment has been obtained following treatment with X rays, hormones and alkylating agents, and cycle-specific drugs given in concomitance with the recruitment peak have shown increased antitumor activity [10–20]. Most studies have been carried out in patients with hematological malignancies since tumor specimens are readily obtainable [10–12, 14, 15, 18–20]. On the other hand, serial biopsies can seldom be obtained from solid tumors; thus cytokinetic scheduling of treatment must be based on indirect findings from either animal tumors or human blood tumors.

In the present study kinetic recruitment has been studied in ascitic ovarian cancer patients since repeated tumor sampling is possible. Moreover, since resistance to or relapse after *cis*-platinum first-line therapy makes response to conventional second-line treatment unlikely [21], we planned a chemotherapeutic schedule based on cell kinetic recruitment for patients failing *cis*-platinum. An *in vitro* antimetabolic assay to predict drug chemosensitivity provided the experimental evidence of increased tumor susceptibility to antineoplastic compounds subsequent to kinetic recruitment [22].

MATERIALS AND METHODS

Patients and treatments

Eight patients with advanced ovarian cancer, refractory to or relapsing after prior poly-

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chemotherapy that included *cis*-platinum, entered this study of cell kinetic recruitment with alkylating agents. Six out of eight patients had sufficient ascitic fluid to allow monitoring of tumor kinetics during treatment. Tumor cells were obtained whenever possible from ascitic fluid collected through a draining catheter. In six patients a minimum of two samples were obtained. Patient characteristics are listed in Table 1. Patients underwent chemotherapy as follows: iphosphamide (IFX) 1.5 g/m^2 i.v. days 1–5, methotrexate (MTX) 40 mg/m^2 and 5-fluorouracil (5FU) 600 mg/m^2 day 12 q 28 days. After a few cycles it was evident that in our heavily pretreated series marrow recovery did not occur on day 12; the IFX dosage was therefore reduced to 1.5 g/m^2 i.v. days 1–3. IFX urotoxicity was counteracted by sodium-2-mercaptoethane sulfonate (MESNA) 600 mg p.o. every 8 hr days 1–3 [23].

Table 1. *Patient characteristics*

Patient No.	Age	PS*	Disease status	Previous therapy
1	40	3	P	D, M, F, A
2	43	3	P	D, M, F, A, MP
3	56	2	R	D, M, F, A, AK, C
4	41	3	P	D, A, C
5	57	3	P	D, A, C
6	61	3	P	D, M, F, AK, C, A
7	49	2	P	D, M, F, A, C, MP
8	48	2	P	D, M, F

*P = progression; R = relapse; D = *cis*-platinum, m = methotrexate; F = 5-fluorouracil; A = adriamycin; MP = medroxyprogesterone; AK = melphalan; C = cytoxan; PS = ECOG performance status.

Labeling index (LI)

Tumor cells obtained from ascitic fluid were washed twice in cold phosphate-buffered saline (PBS), checked for viability by the trypan blue dye exclusion assay and resuspended in RPMI 1640 medium (Flow Lab, U.K.) supplemented with 10% fetal calf serum. Cells (2 × 10^6/ml) were incubated with 10 μCi/ml [^{3}H]-thymidine ([^{3}H]-dThd, sp. act. 5Ci/mM, Amersham International, U.K.) for 60 min at 37°C in a Dubnoff shaker. Radiolabeling was stopped by adding cold PBS, the cells were washed twice in PBS and cytocentrifuged onto microscopic slides. The autoradiographic procedure was performed according to the stripping technique [24]. For evaluation of LI at least 1000 cells were scored for each sample by two independent observers.

In vitro *chemosensitivity*

In vitro chemosensitivity was assessed in one tumor by a biochemical antimetabolic assay (BAA) [22]. Briefly, 2 × 10^6 cancer cells were resuspended in CMRL 1066 medium with 20% FCS and antibiotics and stirred for 2 hr at 37°C with 1, 3 or 9 μg/ml adriamycin (ADR). The dosage of ADR was established according to the achievable plasma concentration *in vivo*. Incubation was continued for 1 hr in the presence of 4 μCi/ml of [^{3}H]-dThd (sp. act. 25 Ci/mM), cultures were centrifuged and three fractions were extracted with 100% trichloracetic acid (TCA) and one with ethanol. The pellets, stored at −20°C overnight, were resuspended in NaOH 0.3 N and incubated at 40°C for 4 hr. Test tubes were added with perchloric acid (PCA 2 N) and albumin (50 μg/ml) and incubated at 4°C for 30 min. Pellets were resuspended in PCA 0.5 N and hydrolyzed at 90°C for 20 min. Two hundred microliters of supernatant containing labeled DNA were dissolved in 2 ml of Instagel (Packard) and counted in an automatic scintillation counter. The ADR inhibition of the [^{3}H]-dThd incorporation was evaluated as follows:

$$\% \text{ inhibition} = 100 - \frac{\text{cpm samples}}{\text{cpm controls}} \times 100.$$

RESULTS

Kinetic features of eight ascitic ovarian cancer patients were studied after a three-day treatment with IFX. Patient characteristics including prior treatment and response to therapy are reported in Table 1. Experimental findings are reported in Table 2. The LI could be measured before and after IFX treatment in six out of the eight cases.

The protocol required three LI measurements, before, during and after chemotherapy, but tumor cells were available at the established times in only four patients. During the course of alkylating chemotherapy the percentage of S-phase tumor cells in the four evaluable patient dropped with respect to samples obtained before IFX therapy. Moreover, at 10–14 days after IFX administration a peak of proliferative activity was seen in four samples. On the other hand, no increase in proliferative activity after IFX was seen in the two cases with the highest basal LI. Experimental results, as reported in Table 2, show that four out of six samples, although obtained from heavily pretreated patients, could be recruited into the proliferating pool following treatment with an alkylating agent. Table 3 reports the number of kinetically designed chemotherapeutic cycles administered, and the effects of treatment on peripheral WBC counts and on the clinical evolution of disease. Cell-cycle-specific drugs were administered when a S-phase tumor cell 'peak' could be predicted since at

Table 2. Thymidine labeling index (LI) during therapy

Patient No.	LI% (C.I.)* Day 0	Day 3	Day 6–9	Day 11–14	Day 18–21
1	5.1(3.8–6.7)	0.4(0.1–0.9)	5.7(4.3–7.4)	15.5(13.3–17.9)	1.2(0.7–2.1)
2	1.2(0.7–2.1)	0.3(0.2–0.9)	4.8(3.6–6.4)	9.1(7.4–11.1)	2.6(1.7–3.8)
3	0.6(0.2–1.3)	0.2(0.1–0.8)	—	3.9(2.9–5.3)	—
4	4.6(3.4–6.1)	—	—	7.4(5.9–9.2)	—
5	6.3(4.9–8.0)	2.2(1.4–3.3)	—	5.8(4.5–7.4)	1.7(1.0–2.7)
6	18.7(16.4–21.2)	—	—	17.0(14.8–19.5)	—
7	3.8(2.7–5.2)	—	—	—	—
8	4.1(3.0–5.6)	—	—	—	—

*C.I. = confidence interval (J.L. Fleiss).

this time cells are often highly responsive to the effects of cytotoxic agents. IFX-induced recruitment was followed by cycle-specific polychemotherapy with MTX–5FU in concomitance with the LI peak. Unfortunately leukopenia was severe and only 9/19 treatment courses with MTX and 5FU could be administered on day 12.

Table 3 also shows that overall therapeutic response was poor, but in the three patients who received at least two complete cycles one SD and one PR were observed.

Figure 1 shows that, in the same patient (patient No. 2), the *in vitro* chemosensitivity to ADR was maximal at the time of increased proliferative activity. The labeling indices in the figure are related to the second cycle of therapy not reported in Table 2. ADR was chosen because antimetabolites cannot be tested with the BAA and, moreover, ADR chemosensitivity might predict the sensitivity to unrelated drugs [25].

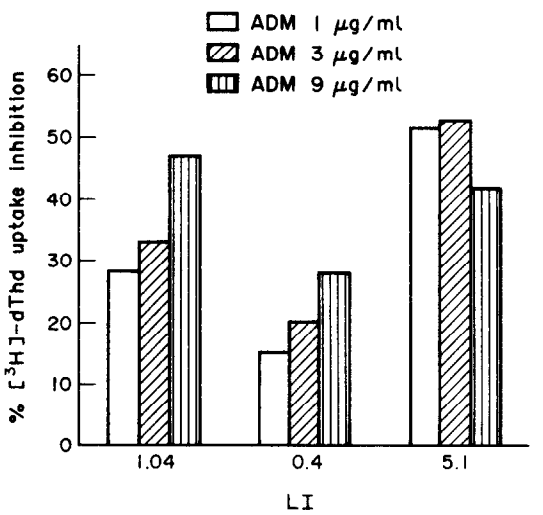

Fig. 1. LI and BAA in an advanced ovarian cancer patient.

DISCUSSION

Response rates observed in experimental tumors, human multiple myeloma and acute non-lymphocytic leukemia have been improved by scheduling drug administration on the basis of cytokinetic parameters [10–12, 14, 15]. A close relationship between proliferative activity of the tumor and its sensitivity to chemotherapy has been claimed [4–9]. In the attempt to improve the poor prognosis of either relapsing or progressive advanced ovarian cancer patients, a polychemotherapeutic schedule, including IFX days 1–3 and MTX and 5FU day 12, was given while tumor cell kinetics were monitored. The rationale for administration of MTX and 5FU can be summarized as follows: (1) both drugs are of proven effectiveness in ovarian cancer [26]; (2) in prior studies [17] peak cell recruitment subsequent to treatment with alkylating agents was observed about day 12.

Table 3. Chemotherapeutic cycles, mean WBC counts and responses

Patient No.	CT cycles Total	Completed	WBC (10^3) at day: 0	11–14	28	Clinical response
1	2	0	8.3	0.4	9.2	P*
2	2	0	4.8	0.6	6.3	P
3	3	0	6.2	1.4	4.3	SD
4	2	2	5.3	2.8	4.4	P
5	2	1	5.8	1.6	6.8	P
6	1	1	6.8	2.4	5.2	P
7	2	2	6.3	2.8	4.1	SD
8	5	3	4.5	3.7	4.2	PR → P

*P = progression, SD = stable disease, PR = partial response.

The kinetics of ovarian cancer cells subsequent to IFX treatment were in keeping with previous findings. Cancer cells from patients 5 and 6 were not susceptible to IFX recruitment. The high LI score prior to IFX treatment might explain recruitment failure. On the other hand, the cell recruitment peak might have occurred earlier than on day 12 and was missed in our observation. As Smets *et al.* [14] suggest, there could be an inverse correlation between the time needed for recruitment peak and the starting LI score. Moreover, it cannot be excluded that heavily pretreated advanced ovarian tumors, highly resistant to most antitumor agents, might no longer be responsive to IFX, and this drug cannot induce cell recruitment in rapidly progressive patients. It is important to note that leukopenia permitted administration of antimetabolites in only nine out of 19 treatment cycles scheduled. It is reasonable to expect that in advanced pretreated ovarian cancer patients, MTX-5FU-induced toxic effects would have been more severe than those experienced by less compromised hosts. However, although experimental evidence is lacking, proliferative IFX recruitment might also have occurred in the hemolymphopoietic system, thus accounting for hematologic toxicity.

A close relationship between proliferative activity and ADR chemosensitivity might be worthy of further investigation to obtain experimental data to support future chemotherapeutic planning. It must be underlined that the clinical predictivity of the antimetabolic assay has not yet been definitely established. Furthermore, the *in vitro* sensitivity to adriamicin obviously might not predict sensitivity to agents that belong to unrelated classes. In addition, this study was only concerned with one tumor type.

In conclusion, recruitment of pretreated ovarian cancer cells was obtained and timed sequential chemotherapy with cycle specific agents was given on the basis of kinetic, parameters. In the preliminary group of patients entered in this study the clinical response to MTX and 5FU following IFX recruitment was poor and associated with severe myelotoxicity. However, in one patient in which the *in vitro* chemosensitivity could be assayed, recruited cells were found to have increased sensitivity to chemotherapy.

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