ide and 4-MPR did not bind to cRABP [9]. Whether the metabolite at the 17 min peak in human tissue competes for cRABP or whether such binding has any functional significance is unknown.

The major portion of human breast tissue is fat. Since retinoids are lipophilic their concentration in mammary tissue may be primarily confined to the fat. However analysis of fenretinide and metabolites in epithelial and fat cells showed dramatic differences. Epithelial cells contained mainly parent drug whereas 4-MPR was principally found in the fat fraction. The results indicate that the retinoid may have been metabolised by the breast tissue. The parent compound and metabolites are then selectively distributed either to the epithelial or fat cells. Fat in turn may serve as a storage site for 4-MPR. The function of 4-MPR is not known. It would be of interest to find out whether 4-MPR can be metabolised back to fenretinide, as has been observed for the mouse mammary gland *in vitro* [9]. If so, 4-MPR could be delivered back to the epithelial cells when the retinoid is exhausted from the cells. In addition, the retinoid eluting at 17 min is principally located in the epithelial cells. The significance of the various metabolites is unknown; nonetheless, the differential distribution of fenretinide and metabolites within the mammary tissue may be important in understanding the action of this retinoid in breast cancer patients.

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Pilot Study of Teniposide in Combination Chemotherapy for Small Cell Lung Cancer

Giuseppe Giaccone, Giorgio Schmidt and Alessandro Calciati

Teniposide is one of the most active agents in small cell lung cancer (SCLC). Because of the experimental evidence of synergistic activity between teniposide and methotrexate and between vincristine and methotrexate, 34 SCLC patients were treated with a combination of teniposide, vincristine, methotrexate and cyclophosphamide. Chest radiotherapy was given to responding patients with limited disease and prophylactic cranial irradiation was given to complete responders only. Most patients had extensive disease and good performance status. The main side-effects were myelosuppression, mucositis and peripheral neuropathy, which were all common and often severe. A response rate of 78% with 22% complete responses was obtained in 32 evaluable patients. Median durations of responses and survival were 252 and 311 days, respectively. Patients with limited disease had a median survival of 556 days while extensive disease patients had a median survival of 240 days. 2 patients with limited disease have been in continuous complete remission for more than 2 years from start of treatment.
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INTRODUCTION

TENIPOSIDE is among the most active single agents in small cell lung cancer (SCLC) with response rates of 34–90% [1–4]. We have incorporated teniposide in a combination regimen including vincristine, methotrexate and cyclophosphamide in an attempt to exploit the synergy that has been described experimentally between methotrexate and teniposide [5] and between methotrexate and vincristine [6]. Teniposide is ten times more effective than etoposide in blocking methotrexate efflux from cells by enhancing formation of methotrexate polyglutamates. The best median survival of leukaemia L1210 bearing mice, was observed when teniposide followed 43 h after the administration of methotrexate [5]. A similar time-dependent synergistic antitumour effect was seen when vincris-

tine was given 16 h or longer after methotrexate [6]. Moreover, vincristine was more effective when given every 7 days than in a 3 week schedule [7].

PATIENTS AND METHODS

Untreated patients with histological or cytological diagnosis of SCLC, with adequate hepatic, haematological (white blood cells [WBC] 4000/μl or more, platelets 100 000/μl or more), renal and cardiac functions were entered. Eligibility criteria also included: age 70 or under, presence of measurable or evaluable lesions, ECOG performance status 3 or less, life expectancy of at least 2 months and informed consent.

Chemotherapy consisted of up to 6 cycles of a combination (VVMC) as follows: vincristine 1.4 mg/m^2 on days 1 and 8; teniposide 100 mg/m^2 (in 500 ml saline over 1 h) on days 1, 3 and 5; methotrexate 40 mg/m^2 on days 1 and 8; and cyclophosphamide 1 g/m^2 (in 250 ml 5% dextrose over 30 min) on day 1. Cycles were repeated every 3 weeks if WBC were 3000/μl or more and platelets were 100 000/μl or more. Folinic acid rescue (Lederfolin) 15 mg was given orally every 6 h four times, starting 24 h after methotrexate administration, because severe mucositis developed in the first few patients treated.

The dose of teniposide, cyclophosphamide and methotrexate was reduced by 25% if WBC and platelet nadirs were 500/μl and/or 20 000/μl or less, respectively. The methotrexate dose on day 8 was halved if WBC was 2000–2999/μl and/or platelets were 75 000–99 999/μl, and the drug was withheld if WBC fell below 2000/μl and/or platelets fell below 75 000/μl. The dose of vincristine was halved if peripheral neurotoxicity interfering with normal activities occurred and the drug was stopped if ileus or motor neurotoxicity ensued.

Response and toxicity criteria were those recommended by WHO. Patients with limited disease responding to VVMC received chest irradiation within 3 weeks of the last cycle, with 50 Gy in 25 fractions. Complete responders were given prophylactic brain irradiation at the end of treatment, with 30 Gy in 10 fractions. Duration of responses and survival was measured from the start of treatment.

RESULTS

From August 1986 to November 1987, 34 untreated patients entered the study. Patients had a good performance status (0–1 in 23 patients) and extensive disease (21 patients), bone and liver being the most common metastatic sites. 29 patients were male and 5 female and 15 had more than 5% weight loss. A total of 175 cycles of VVMC were given (median 6 per patient).

Side-effects of the treatment were mainly myelosuppression, mucositis and peripheral neuropathy (Table 1). 2 patients died shortly after starting treatment, 1 during an episode of sepsis and severe mucositis and the other from diabetic decompensation during severe myelosuppression. Neurotoxicity caused modification of the vincristine dose or suspension in about half of the patients. 41 cycles had to be delayed in 17 patients, because of myelotoxicity in 63% of cases. 67% of patients were given 90% or more of the planned dose of cyclophosphamide and teniposide; 43% were given 90% or more of the planned dose of methotrexate and vincristine.

Table 1. Toxicity (WHO grade)

	0	1	2	3	4
Emesis	0	6	14	13	1
Diarrhoea	18	5	9	1	1
Mucositis	14	8	9	3	0
Peripheral neurotoxicity	9	11	13	1	0
Constipation	27	3	4	0	0
Fever	30	2	2	0	0
Hair loss	3	0	2	29	0
Infection	19	8	4	1	2
Kidney*	32	2	0	0	0
Others†	29	2	1	1	1
WBC	3	1	10	15	5
Platelets	18	3	5	5	2
Haemoglobin	5	9	15	3	1

	Nadirs	
	Mean count/μl (range)	Day
WBC	2200 (400–5400)	13.9 (7–21)
Platelets	154 500 (800–340 000)	13.2 (7–21)

*2 patients with serum creatinine at 1.6 and 1.8 mg/dl.
† 1 panmucositis, 1 hyperglycaemia, 1 orchitis plus proctitis, 1 headache plus pruritis and 1 conjunctivitis.

Response rate was 78% in 32 evaluable patients, with 7 complete responses (22%), 18 partial responses, 5 stable disease and 2 progressions. The median duration of response was 252 days. Median survival time of all 34 patients was 311 days (44 weeks); limited disease patients survived a median of 556 days and extensive disease patients survived 240 days ($P<0.001$, log-rank test). 2 patients with limited disease are still alive and have been in complete remission for over 2 years (15% of those with limited disease).

DISCUSSION

On the basis of experimental synergy between some of the drugs included in our study, we anticipated severe haematological toxicity. However, severe non-haematological side-effects were also observed, including neurotoxicity which may have been due to interaction of vincristine with teniposide [8]. It is difficult to evaluate whether the synergistic effects described experimentally between methotrexate and teniposide and vincristine were achieved with our combination, which did not reproduce all the experimental conditions. However, response rate and duration of survival were at least similar to the best reported series [9].

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Modulation by Oestrogen and Progestins/Antiprogestins of Alpha Interferon Receptor Expression in Human Breast Cancer Cells

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Human breast cancer ZR-75-1 cells expressed 1516 (105) (mean [S.D.]) interferon (IFN) receptors (IFNR) per cell with K_d of 0.61 (0.15) nmol/l. Oestrogen independent ZR-PR-LT and tamoxifen resistant ZR-75-9a1 8 μmol/l cells expressed similar numbers of IFNR. ZR-75-9a1 cells, which had been maintained in the absence of tamoxifen or known oestrogenic activity for 46 weeks, expressed a significantly higher number of IFNR (3170 [315]). Exposure of ZR-75-1 cells to 10^{-9} mol/l 17β-oestradiol (E2) led to a consistent reduction in IFNR numbers whilst 10^{-6} mol/l tamoxifen slightly increased IFNR expression. Since IFN increases oestrogen receptors in this cell line, IFN and E2 appear to have opposite effects on expression of each others' receptor. 10^{-9} mol/l medroxy progesterone acetate and mifepristone significantly increased IFNR numbers whilst ORG 2058 decreased IFNR expression and ZK 98.299 had no effect. Progestin/antiprogestin induced IFNR increase in this cell line correlated with down-regulation of progesterone receptor (PR). Thus an IFN/ER/PR axis may exist in ZR-75-1 cells and variants.
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INTRODUCTION

ALTHOUGH INTERFERONS (IFNs) have significant antiproliferative effects on human breast cancer cells *in vitro* and *in vivo* [1, 2] results with IFNs in the treatment of patients with breast cancer have been disappointing [3, 4]. However, the combination of IFN and tamoxifen in the treatment of breast cancer may be of benefit since IFNs increase oestrogen receptor [ER] expression *in vivo* [5] and *in vitro* [6, 7] and increase the sensitivity of breast cancer cells *in vitro* to the growth inhibitory activity of anti-oestrogens [7, 8]. In a pilot study complete remission of lymph-node metastases was achieved in one patient with IFN-α_{2c} and tamoxifen [9].

IFN binds to high-affinity cell surface receptors [10]. We have investigated IFN receptor (IFNR) expression in ZR-75-1 human breast cancer cells and tamoxifen resistant and oestrogen independent variants and correlated IFNR content with ER and progesterone receptor (PR) expression. To establish whether heterospecific receptor modulation occurs we have also investigated the effect of oestradiol and progestins/antiprogestins on IFNR number.

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MATERIALS AND METHODS

Cells and culture conditions

ZR-75-1 human breast cancer cells were obtained from Flow Laboratories. Cells were routinely maintained in RPMI 1640 medium supplemented with 5% fetal calf serum (FCS), 100 IU/ml penicillin and 100 μg/ml streptomycin. Cells were grown in 5% CO_2 at 37°C. ZR-PR-LT cells were selected for their ability to grow in the absence of known oestrogenic activity [11] and were routinely maintained in RPMI 1640 lacking phenol red and supplemented with heat treated (53°C for 1 h) 5% FCS stripped by dextran-coated charcoal.

A tamoxifen resistant variant of ZR-75-1 cells, ZR-75-9a1 8 μmol/l, was routinely maintained in RPMI 1640 supplemented with 5% FCS and 8 μmol/l tamoxifen [12]. ZR-75-9a1 8 μmol/l cells which had been maintained for 46 weeks at the time of this