patients unlikely to respond to second-line therapy include progression of disease on primary therapy or relapse from remission within 3–6 months of conclusion of primary therapy. In our study, the interval between conclusion of previous cisplatin or carboplatin therapy and entry to this study for patients who were resistant to or failed to achieve complete response with primary therapy and ultimately experienced an objective response to iproplatin ranged from 2 months to 47 months (median 15).

This study has demonstrated that iproplatin can induce partial or complete responses in a limited proportion of cisplatin-resistant ovarian cancer patients and of carboplatin-resistant patients. Although response rates were low, it is noteworthy that these anticancer effects occurred in a subset of patients notoriously resistant to any treatment modality. In addition, response duration was appreciable (over 6 months) for several patients. The further clinical development of iproplatin is unlikely due to its modest antitumour activity in other gynaecological malignancies and solid tumours. However, its use as a second-line agent for platinum-complex resistant ovarian cancer might be rationalised in combination chemotherapy regimens with other active agents (e.g., etoposide, mitoxantrone, ifosfamide, and taxol). Iproplatin at this dosage and schedule cannot be recommended as an effective agent alone against platinum-resistant ovarian cancer.

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Distribution of Fenretinide in the Mammary Gland of Breast Cancer Patients

Rajendra G. Mehta, Richard C. Moon, Michael Hawthorne, Franca Formelli and Alberto Costa

Fenretinide has been used orally as a chemopreventive retinoid in a trial for women at risk of developing contralateral breast cancer. The levels of fenretinide and its metabolites were measured in the breast tissue obtained at surgery from women in the trial. Fenretinide was concentrated by breast tissue. Two major metabolites were detected in the tissue extract, one co-eluting with N-(4-methoxyphenyl)retinamide (4-MPR), and the other, more polar, eluting at 17 min under the conditions used. This metabolite remains unidentified. Division of the breast tissue into epithelial cells and fat fractions revealed that fenretinide and the metabolite at the 17 min peak were concentrated in the epithelial cells, whereas 4-MPR was principally localised in the fat compartment. Thus fat may serve as a storage compartment for the retinoid.
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INTRODUCTION

CONSIDERABLE ATTENTION has been directed towards the evaluation of retinoids as chemopreventive agents [1, 2]. Among the chemopreventive retinoids tested, fenretinide N-(4-hydroxyphenyl)retinamide, is the most efficacious in preventing chemically induced carcinogenesis in various target organs. Fenretinide is less toxic than retinyl esters, retinoic acid or any other synthetic retinoid at equimolar concentrations in rodents [3].

Fenretinide is being evaluated clinically for its effectiveness in preventing contralateral breast cancer in stage 1 patients who have received neither hormonal therapy nor chemotherapy. Patients receive 200 mg daily, a dose selected from the phase I

trial [4]. Fenretinide is well tolerated; however, plasma retinol is usually reduced as a result of the treatment [5]. This effect is reversible on interruption of drug administration, and the patients have a 3 day break from the treatment each month to increase plasma retinol levels. A similar reduction in plasma retinol was observed in rats [6].

Fenretinide is concentrated by various tissues in male and female rodents [7, 8], including in the mammary tissue of rats and mice [7]. Furthermore, the compound is metabolised to N-(4-methoxyphenyl)retinamide (4-MPR) as well as to other metabolites by mammary tissue in organ culture [9]. These *in vitro* findings reflect pharmacokinetic studies in rats and mice [7]. Moreover, both parent drug and 4-MPR have been found in plasma from patients treated with fenretinide [5].

In the present study, we investigated whether fenretinide is localised in the breast tissue of patients and the distribution of the drug and/or metabolites between the mammary epithelium and fat in this tissue.

PATIENTS AND METHODS

Fenretinide was supplied by McNeil Pharmaceuticals (Spring House, Pennsylvania) as 100 mg capsules. The patients were from the group of women participating in the phase 1 trial. The selection and exclusion criteria have been described [4].

Breast tissue or tumour samples were collected at the time of surgery for tumour removal or plastic reconstruction. The tissues were protected from light and immediately frozen in liquid nitrogen for storage at −70°C. Blood was collected a day before surgery. Blood samples were kept away from light and processed in the dark. Plasma samples were stored at −20°C and analysed within 3 weeks of collection as described [5].

Both normal breast tissue and breast cancer samples were studied. Breast tissues were divided into two portions. One half of the tissue was weighed, retinoids were extracted in methanol [10] and the extract was processed for the separation of metabolites. The second half was minced and incubated with 0.1% collagenase and 0.1% hyaluronidase for 2 h at 37°C in medium 199 containing antibiotics. Epithelial cells and fat fractions were separated by centrifugation and weighed and retinoids were extracted.

Retinoids were separated by reversed phase high-performance liquid chromatography (HPLC). Fenretinide and metabolites were separated by a linear gradient of methanol/water (70/30) to methanol (100%) in 30 min with a flow rate of 1.2 ml/min. Retinoids were detected and recorded at 350 nm [10].

Cellular retinoic acid binding proteins (cRABP) were measured in tissue from two patients who were not treated with fenretinide. cRABP was measured by sucrose density gradient centrifugation [11]. Briefly, mammary tissue cytosol was reacted with 50 nmol/l ^{3}H-retinoic acid alone or with 20 fold excess unlabelled all-trans-retinoic acid for 16 h in the dark at 4°C. After removal of unbound retinoid by dextran-coated charcoal, the sample was centrifuged at 360 000 *g* in a vertical tube rotor for 1.5 h on a preformed 5–20% linear sucrose gradient. cRABP is separated as a 2S component under these conditions.

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RESULTS

Seven uninvolved breast tissues and three mammary tumours were used for this study. In Table 1 the concentrations found in the plasma and tissues of each patient are reported with the length of treatment, the total dose received and the days of treatment interruption before surgery. In plasma, the concentrations of fenretinide decreased as the time since the last dose increased and small concentrations were still detectable after 83 days. The concentrations of 4-MPR were similar to those of the parent drug in those patients whose blood was collected 1 day after drug interruption whereas 4-MPR levels became higher (except in patient 3) as the time since the last dose increased. The concentrations of both fenretinide and 4-MPR in the tissues were higher than those in plasma, especially taking into account the fact that blood was collected 1 day before surgery. Despite a similar total dose and similar levels of fenretinide in the plasma of patients whose treatment was interrupted 1 day before surgery, mammary gland concentrations ranged from 410 to 2600 ng/mg.

The number of days of interruptions before collecting the tissues was correlated with tissue retinoid concentrations. In the tissues, 4-MPR concentrations were always higher than those of fenretinide, and became higher with the increase of time since the last dose, thus suggesting a slower release than that of parent drug. Little difference was found between fenretinide and 4-MPR concentrations in the tumour and in the mammary gland. In addition to elution of fenretinide at 23.6 min and 4-MPR at 28 min, a polar metabolite eluting at 17 min was also found (Fig. 1). The chemical nature of this polar retinoid is unknown.

To assess the distribution of fenretinide and metabolites between epithelial and fat cells, portions of mammary tissues were digested with collagenase and hyaluronidase to obtain epithelial and fat cell fraction. The percentage of parent drug, 4-MPR and the compound at the 17 min peak, considering their total areas as 100% and their distribution in epithelial cells and fat, was evaluated in five samples (Table 2). In the breast tissue as a whole, 4-MPR constituted 72% of the total retinoid, while fenretinide and the compound at the 17 min peak together represented 14% of total retinoid concentration.

These separate compartments of cells were extracted with methanol and subjected to HPLC. Figure 1 shows a retinoid metabolism profile for tissue, epithelial cells and fat from one of the patients. Fenretinide was concentrated in the epithelial cells, whereas 4-MPR was concentrated in the fat cell fraction. The fat contained 85% of the major metabolite, 4-MPR, whereas fenretinide and the metabolite at the 17 min peak constituted only 7% of total retinoids. On the other hand epithelial cells contained more parent drug and the metabolite at the 17 min peak than 4-MPR. This suggests a differential distribution of metabolites within the breast tissue.

In addition to samples obtained from eight fenretinide treated patients, mammary tumour and breast fat samples from two untreated patients were extracted with methanol and subjected to HPLC. No peaks were detected in the region where fenretinide, 4-MPR or the metabolite at the 17 min peak elute. Since the samples from the retinoid treated patients were used for the metabolism studies, cRABP were measured only in the control breast tissues from patients not treated with retinoid. Although binding was not evident in the fat, tumour samples did show 2S cRABP (data not shown).

DISCUSSION

Chemoprevention can be approached either by avoiding potential risk factors (high-fat diets), exposure to carcinogenic

Table 1. Plasma and tissue retinoid concentration in breast cancer patients

Patient	Treatment (mg per day)	Total dose (mg)	Days of interruption*	Plasma (ng/ml)			Mammary gland (ng/mg)			Mammary tumour (ng/mg)		
				FEN	4-MPR	Ratio	FEN	4-MPR	Ratio	FEN	4-MPR	Ratio
2	100/168 200/162	49 200	1	230	308	1.3	750	6250	8.3	—	—	—
7	200/195	39 000	1	388	374	1.0	410	2810	6.8	—	—	—
4	200/193	38 600	1	300	228	0.8	2600	13600	5.2	1200	8000	6.7
1	100/15 200/171	35 700	1	278	163	0.6	1150	2880	2.5	—	—	—
6	300/168 200/420	134 400	2	129	191	1.5	1970	3980	2.0	—	—	—
8	200/639	127 800	5	30	69	2.3	—	—	—	90	1730	19.2
3	200/23	4600	5	30	ND	—	76	1240	16.3	—	—	—
9	200/397	79 400	83	36	179	5.0	30	1040	34.7	130	3080	23.7

*Number of days treatment was interrupted before surgery. ND = not detectable. FEN = fenretinide.

substances (cigarette smoking) or by the consumption of potential chemopreventive agents in food. Yet another approach in high-risk groups (family history of cancer or patients currently disease-free) is pharmacological prevention [12]. To this end, retinoids have provided significant hope as chemopreventive agents. These compounds have shown efficacy against carcinogen-induced tumour development in a target organ specific manner. Among effective retinoids, fenretinide is efficacious against cancers of several organ sites and is better tolerated than most other retinoids [3]. Fenretinide has provided chemopreventive protection against mammary, urinary bladder, lung and skin carcinogenesis but has little effect against colon, liver and oesophageal cancers [13]. With these results in animal models, fenretinide was selected for clinical trial in Milan for prevention of contralateral disease in stage 1 breast cancer patients.

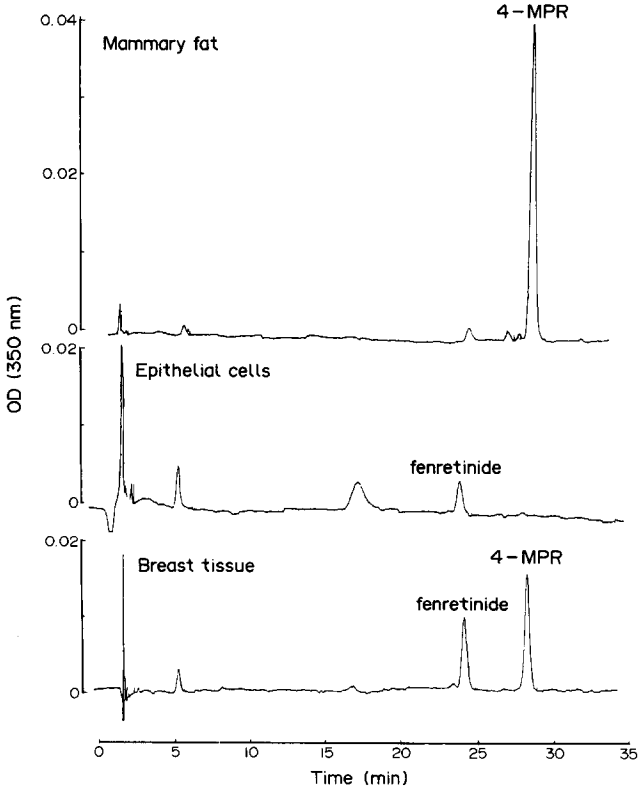

Fig. 1. HPLC profiles of fenretinide metabolism in human mammary tissue epithelial cells and fat. Breast tissue was obtained during surgery from patients treated with fenretinide 200 mg per day for 195 days.

Early results from this and other phase I trials indicated that fenretinide is absorbed from the gastrointestinal tract and is well tolerated. However, it was not known whether the compound accumulates in human breast tissue. The present results show that fenretinide is not only concentrated in the breast but is also metabolised there. As reported in the mammary tissues of rats and mice [7–9], fenretinide is metabolised to 4-MPR and an unidentified metabolite with a retention time of 13 min. In human breast tissue we observed 4-MPR as a major metabolite; however, instead of a 13 min peak, a metabolite eluting at 17 min was observed. No retinoid was eluted at 13 min. This may be due to species differences. Nonetheless, both the 13 and 17 min peaks are more polar than the parent compound. Earlier we observed that the metabolite at the 13 min peak in rodents competed for cRABP in the mammary gland, whereas fenretin-

Table 2. Distribution of fenretinide and metabolites in human breast tissues

Cell/tissue	Percentage retinoid (mean, S.D.)		
	FEN	4-MPR	17 min metabolite
Uninvolved breast tissue (n = 5)	14.2 (9)	72.5 (19)	13.6 (11)
Epithelial cells (n = 5)	29.0 (24)	28.0 (27)	42.4 (23)
Fat (n = 5)	7.2 (12)	85.0 (23)	7.5 (10)

Total area covered by fenretinide, 4-MPR and 17 min peak was considered as 100%.

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-