Eur J Cancer, Vol. 29A, No. 16, pp. 2269–2273, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00

Tamoxifen Enhances the Cytotoxic Effects of the Nitrosourea Fotemustine. Results on Human Melanoma Cell Lines

J.L. Fischel, V. Barbé, M. Berlion, P. Formento, J. Berrile, J.P. Bizzari and G. Milano

Fotemustine (Fote) is a new amino acid-linked chloroethyl nitrosourea which has been shown to be useful in disseminated malignant melanoma. The aim of the present study was to analyse the cytotoxic effects resulting from the combination of anti-oestrogens and Fote on human melanoma cell lines. The anti-oestrogens tested were tamoxifen (TMX, 5×10^{-7} mol/l and 5×10^{-6} mol/l) and 4OH TMX (5×10^{-8} mol/l and 5×10^{-7} mol/l). As a preliminary step, a series of nine human melanoma cell lines was screened in order to identify and quantify the presence of oestradiol receptors (ER) in these cell lines. This led to the selection of an ER-positive (+) cell line. The drugs alone or in combination were then tested against CAL 1 ER (+) and CAL 7 ER (−) melanoma cell lines. Different sequences of drug combinations were tested using clinically compatible drug concentrations. For CAL 1 cells, there was a growth inhibitory effect induced by the anti-oestrogens given alone. Overall, the presence of the anti-oestrogens resulted in higher cytotoxic effects than when cells were exposed to Fote alone. The lowest IC_{50} Fote values as compared to Fote alone were generated by the sequences in which the anti-oestrogens were administered before Fote. Significantly, these associations with anti-oestrogens enabled the IC_{50} values of Fote to be reduced by up to 80%. Globally, TMX and 4OH TMX had similar synergistic effects. TMX and 4OH TMX had a modest influence on Fote cytotoxic effects against CAL 7 ER-negative cells. These data may be useful for optimal planning of future clinical trials for malignant melanoma using anti-oestrogens and nitrosoureas.
Eur J Cancer, Vol. 29A, No. 16, pp. 2269–2273, 1993.

INTRODUCTION

THE NATIONAL Cancer Institute recently noted, in its annual statistical review, that in the years 1973–1989, melanoma incidence increased by nearly 85% [1]. Clearly, the incidence of melanoma is now rising faster than any other cancer. Unfortunately, the therapeutic success against advanced melanoma remains low, and thus new treatment modalities are eagerly awaited for improving response rate and survival in this malignant disease [2]. Dacarbazine and conventional nitrosoureas are the chemotherapeutic agents with the best established activity against advanced melanoma [3]. Based on the presence of oestradiol receptors (ER), which were reported in melanoma specimens [4], clinical trials with tamoxifen (TMX) have been undertaken and objective responses observed [5]. Apart from specific pharmacological effects through its binding to ER, TMX may interact with anticancer drugs at different cellular levels. First, TMX has been shown to be capable of restoring cell sensitivity to so-called multidrug-resistant (MDR)-related anticancer drugs, through interference with the cell membrane P-glycoprotein [6,7]. In addition, TMX has been shown to interact with non-MDR-related drugs, such as 5-fluorouracil (5FU) leading to final antagonistic effects [8,9], or cisplatin with, in contrast, a synergistic interaction [10]. A randomised trial was recently published concerning the comparison between dacarbazine and dacarbazine plus TMX in the treatment of metastatic melanoma [11]. The presence of TMX resulted in higher efficacy as evaluated by both the response rate and the median survival. Fotemustine (Fote) is a new amino acid-linked chloroethyl nitrosourea which has been shown to be useful in disseminated malignant melanoma [12]. The aim of the present study was to analyse the cytotoxic effects resulting from the combination of TMX and Fote on human melanoma cell lines. As a preliminary step, a series of nine human melanoma cell lines was screened in order to identify and quantify the presence of ER in cell lines. This led to the selection of an ER-positive (+) cell line. The drugs alone or in combination were then tested against ER+ and ER− melanoma cell lines. Different sequences of drug combinations were tested using clinically compatible drug concentrations.

Correspondence to G. Milano.
J.L. Fischel, V. Barbé, P. Formento and G. Milano are at the Centre Antoine-Lacassagne, 36 voie Romaine 06054 Nice cedex, France; and M. Berlion, J. Berrile and J.P. Bizzari are at the Servier Laboratories, 6 place des Pléïades, 92415 Courbevoie cedex, France.
Revised 8 June 1993; accepted 21 June 1993.

MATERIALS AND METHODS

Chemicals

The anti-oestrogen TMX and 4-hydroxy TMX (4OH TMX) were from ICI. 17β-oestradiol (E_2) was from Sigma (St Louis, Missouri, U.S.A.) and [2,4,6,7,-^{3}H]17β-E_2 (specific activity 315–407 $\times 10^{10}$ Bq/mmol) were from Amersham (Les Ulis, France). Dihydrotestosterone was from Sigma (StLouis, MO, U.S.A.). Fotemustine was obtained from the Institut de Recherches Internationales SERVIER (Courbevoie, France). All drugs were stored at −20°C and constituted the stock solutions. Dulbecco's modified Eagle medium (DMEM), L-glutamine, fetal bovine serum (FBS), RPMI 1640 were obtained from Gibco (Paisley, U.K.). Penicillin and streptomycin were supplied by Merieux (Lyons, France). Bovine serum albumin (BSA) was from Sigma. The MTT test was performed using 3-(4-5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide

(MTT) and dimethylsulfoxide (DMSO), both of which were obtained from Sigma.

Cell screening for the presence of ER

Nine different human melanoma cell lines were tested for their ER expression : CAL 1, CAL 7, CAL 23, CAL 24, CAL 52, CAL 86, CAL 103, CAL 109 and 77 V. All these cell lines had been obtained from our institute. The procedure for ER determination was derived from the Sutherland method [13]. At 90% confluence, cells grown in 24-well plates were rinsed three times with 500 μl RPMI 1640 containing 0.1% BSA at 37°C. After rinsing, 150 μl medium plus 50 μl ligand solution in the same vehicle were added. The final concentrations of [^{3}H]E_2 were 0.03, 0.04, 0.06, 0.1, 0.2, 0.4, 0.6, 1, 3, 8 and 15 nmol/l. To determine non-specific binding, an excess of unlabelled E_2 (1500 nmol/l) was added to the highest concentration of labelled ligand. Dihydrotestosterone (200 times more concentrated than labelled E_2) was added for saturating the binding to androgen receptors. Cells were incubated for 1.5 h at 37°C in 5% CO_2. Plates were placed on a tray with ice to stop the reaction, and the supernatant was removed for each well. Cells were washed three times with phosphate buffered saline (PBS) containing 5% BSA (4°C, 250 μl per well). The total amount of time required for rinsing was less than 20 min. After removal of the supernatant, cells were solubilised with 1 mol/l NaOH at 37°C (300 μl per well for 15 min). The radioactivity of each well was measured by β counting. Results were expressed in fmol per well. Scatchard analysis [14] was used to determine the number of receptor sites per cell and the dissociation constant (Kd). Each point of every Scatchard plot was carried out in quadruplicate or sextuplicate; the coefficient of variation was less than 10%. Cells were counted in three wells run in parallel, resuspended in 200 μl PBS at room temperature and counted with a haemocytometer. The validity of the Scatchard plot was verified by linear regression. We considered that there was significant binding for a given cell line when the difference between specific binding and non-specific binding was equal or higher than three times the standard deviation of the radioactivity counting. We considered a high affinity site for E_2 when the Kd value was below 1×10^{-9} mol/l.

Cell cultures

Two melanoma cell lines were cultured: CAL1 which was shown to be ER+ and CAL 7, ER−.

Cells were routinely cultured in a humidified incubator (Sanyo, Tokyo, Japan) at 37°C in an atmosphere comprising 8% CO_2 in air. Cells were grown in DMEM supplemented with 10% FBS, penicillin (50 000 U/l), streptomycin (86 μmol/l) and L-glutamine (2 mmol/l). Cells were grown in 96-well microtitration plates with flat bottoms. TMX concentrations tested were 5×10^{-6} and 5×10^{-7} mol/l, 4OH TMX concentrations tested were 5×10^{-7} and 5×10^{-8} mol/l. Fifteen fotemustine concentrations were tested between 2.5 and 2.5×10^3 μmol/l. The drug concentrations tested and the concentrations described in treated patients [15,16] were overlapping. Cells were exposed for 5 days to three different drug sequences (Sanyo, Tokyo, Japan): (i) Fote 2 h and then anti-oestrogens (TMX or 4OH TMX) for 120 h ; (ii) anti-oestrogens 60 h then Fote 2 h and again anti-oestrogens for 60 h and finally, (iii) anti-oestrogens for 120 h then Fote for 2 h. In addition, drugs were tested alone : Fote for 2 h, anti-oestrogens for 120 h.

Evaluation of cytotoxicity

The cytotoxic effects of the drugs tested alone or according to the above described combinations were assessed using the MTT semi-automated method [17]. For all the experimental conditions, the MTT test was conducted 5 days after the cells' exposure to Fote, whatever the sequence tested. Results were expressed as the relative percentage of absorbance as compared with that in untreated controls. Absorbance was set at 540 nm and was measured on a Titertek Twin reader. Each experimental point was determined in sextuplicate. For all experiments, the coefficients of variation ranged between 3 and 10%. IC_{50} was defined as the Fote concentration inhibiting 50% of the cell growth as compared with that in cells exposed to anti-oestrogens only. All experiments were conducted in duplicate within a 2–3-week interval.

RESULTS

Among the nine different human melanoma cell lines which were screened for their ER cellular content, only the CAL 1 cell line showed significant ER expression, having two families of E_2 binding sites (Fig. 1): one family with high affinity binding sites (Kd = 0.039 nmol/l, 2573 sites/cell) and the other one with low affinity binding sites (Kd = 12.5 nmol/l, 74 700 sites/cell).

Figure 2 illustrates the different growth inhibition patterns for CAL 1 ER+ cells resulting from the tested drug combinations. There was a growth inhibitory effect induced by the anti-oestrogens given alone (data not shown). Globally, the presence of the anti-oestrogens resulted in higher cytotoxic effects than when cells were exposed to Fote alone. The data for the CAL 7 ER− cells are shown in Fig. 3. In comparison with CAL 1 ER+ cells, the presence of anti-oestrogens did not markedly influence the cytotoxic effect of Fote on CAL 7 ER− cells. Unlike CAL 1, there was no growth inhibitory effect for CAL 7 induced by the anti-oestrogens used alone (data not shown). Table 1 summarises all the data resulting from duplicate experiments on CAL 1 and CAL 7 cell lines. Considering the CAL 1 ER+ melanoma cell lines, the lowest IC_{50} Fote values as compared to Fote alone were generated by the sequences with the anti-oestrogens administered before Fote ($P < 0.05$). It is worth noting that these associations with anti-oestrogens enabled the IC_{50} values of Fote to be reduced by up to 80%. For both anti-oestrogens tested, there was no significant difference in the Fote IC_{50} values when comparing the two doses which were tested. Globally, TMX and 4OH TMX had similar synergistic effects when combined to Fote, keeping in mind that 4OH TMX was used at one log concentration range below TMX. As already apparent from the

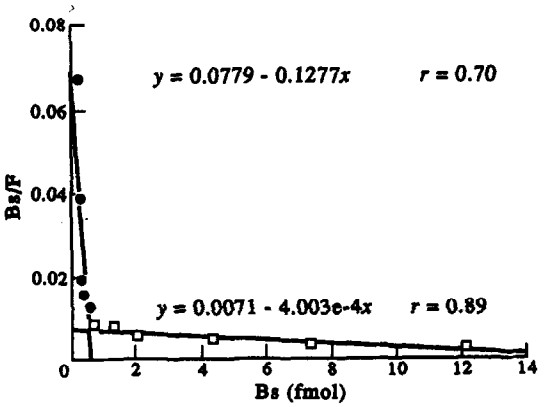

Fig. 1. Scatchard analysis of E_2 binding in CAL 1 melanoma cell line. Solid circles, high affinity binding sites; open squares, low affinity binding sites; r, linear coefficient of correlation. For methodological details see Material and Methods.

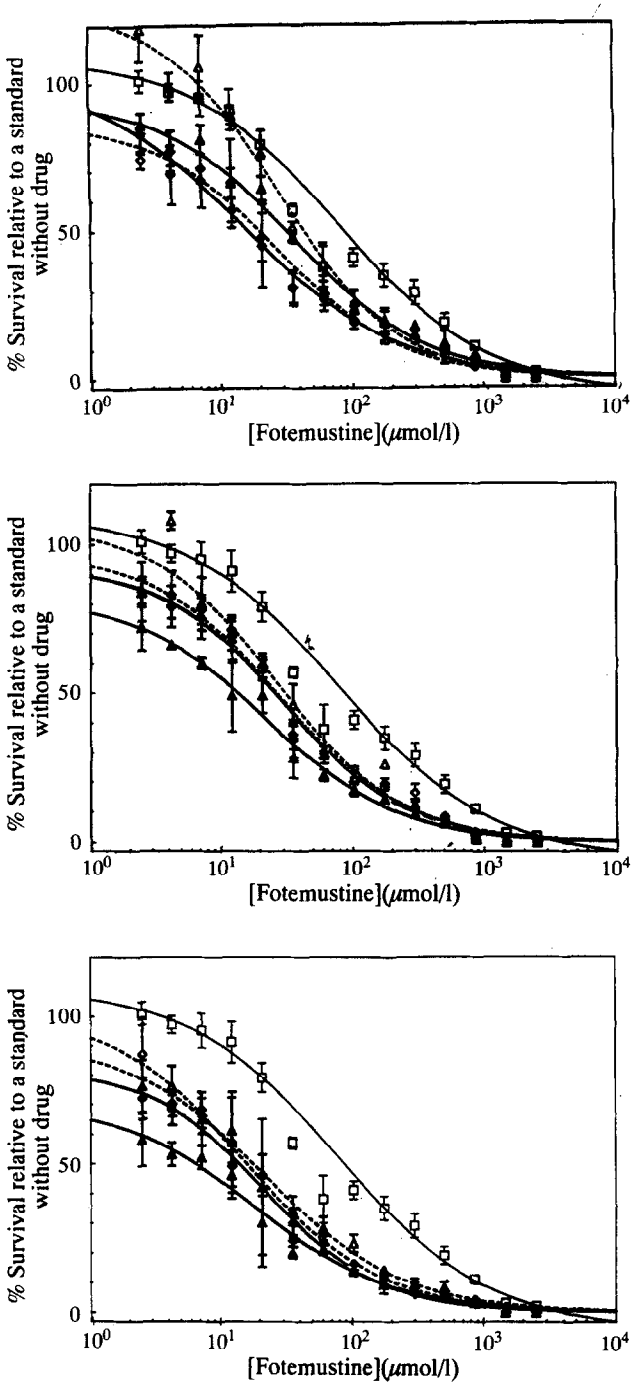

Fig. 2. Dose–response curves for Fote on CAL 1 ER+ cells. Upper panel shows drug sequence anti-oestrogens followed by Fote. The middle panel shows anti-oestrogens followed by Fote then anti-oestrogens. The bottom panel shows Fote followed by anti-oestrogens. Open squares, Fote alone; open triangles, Fote plus TMX 5×10^{-7} mol/l; closed triangles, Fote plus TMX 5×10^{-6} mol/l; open diamonds, Fote plus 4OH TMX 5×10^{-8} mol/l; closed diamonds, Fote plus 4OH TMX 5×10^{-7} mol/l.

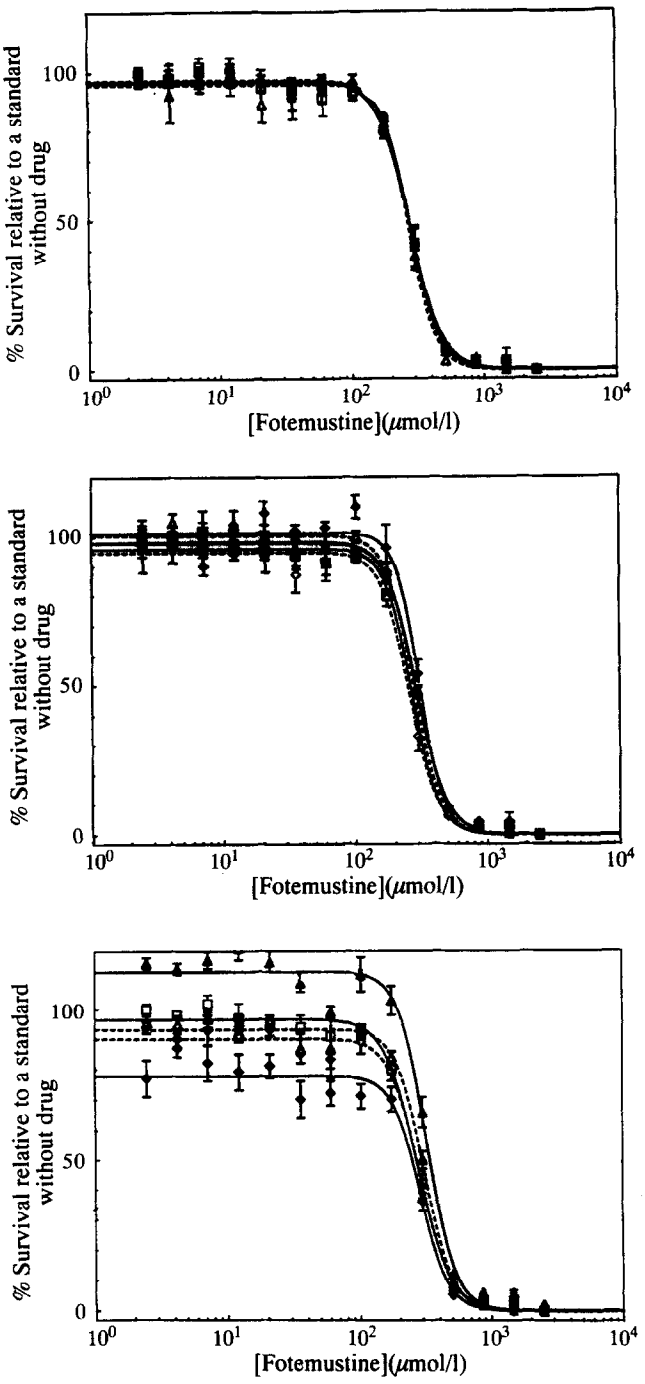

Fig. 3. Dose–response curves for Fote on CAL 7 ER− cells. Upper panel shows drug sequence anti-oestrogens followed by Fote. The middle panel shows anti-oestrogens followed by Fote then anti-oestrogens. The bottom panel shows Fote followed by anti-oestrogens. Open squares, Fote alone; open triangles, Fote plus TMX 5×10^{-7} mol/l; closed triangles, Fote plus TMX 5×10^{-6} mol/l; open diamonds, Fote plus 4OH TMX 5×10^{-8} mol/l; closed diamonds, Fote plus 4OH TMX 5×10^{-7} mol/l.

dose–effect curves shown in Fig. 3, the Fote IC_{50} values given in Table 1 confirm that both TMX and 4OH TMX had a modest influence on Fote cytotoxic effects against CAL 7 ER− cells.

DISCUSSION

This study shows that the combination of the anti-oestrogens TMX and 4OH TMX with the nitrosourea fotemustine leads to synergistic cytotoxic effects on human melanoma cell lines. The therapeutic benefit gained by combining TMX and chemotherapy in the treatment of advanced malignant melanoma has already been demonstrated. For instance, promising data were shown for a cisplatin–TMX combination [18], and more recently, when TMX was given in association with dacarbazine [11]. The synergistic anti-tumour effects of the combination

Table 1. Evolution of fotemustine IC_{50} values (μmol/l) according to the different associations with anti-oestrogens

		Drug combinations (first experiment)				Drug combinations (second experiment)			
			(I)	(II)	(III)		(I)	(II)	(III)
			T→F	T→F→T	F→T		T→F	T→F→T	F→T
		F	4OHT	4OHT 4OHT	4OHT	F	4OHT	4OHT 4OHT	4OHT
CAL 1 ER+		79.3				120			
T	5×10^{-7} mol/l		27.8 (66)	22.3 (72)	27.1 (66)		62.7 (48)	92.2 (23)	71.3 (41)
	5×10^{-6} mol/l		36.1 (55)	18.6 (77)	22.3 (72)		86.9 (28)	46.5 (61)	72.9 (39)
4OHT	5×10^{-8} mol/l		27.7 (65)	15.3 (81)	28.6 (64)		95.9 (20)	95.2 (21)	9.6 (25)
	5×10^{-7} mol/l		14.9 (81)	21.6 (73)	30.5 (62)		64.1 (47)	80.3 (33)	49.3 (59)
CAL 7 ER−		273.4				380			
T	5×10^{-7} mol/l		267.2 (2)	276.5 (0)	306.4 (0)		281.6 (25)	346.2 (9)	383.2 (0)
	5×10^{-6} mol/l		274.9 (0)	292.5 (0)	320.1 (0)		382.8 (0)	342.3 (10)	416.3 (0)
4OHT	5×10^{-8} mol/l		272.9 (1)	258.1 (5)	286.5 (0)		339.2 (10)	336.3 (11)	375.8 (1)
	5×10^{-7} mol/l		277.1 (0)	308.4 (0)	287.2 (0)		317.6 (16)	328.9 (13)	395.9 (0)

T, TMX; 4OHT, 4OH TMX; F, Fote; df, degrees of freedom; NS, non-significant. Numbers in parentheses are the percentage of IC_{50} reduction. In each case, IC_{50} values were determined as a function of the initial cell survival without Fote, which takes into account the growth inhibitory effect induced by the anti-oestrogens alone. For details concerning the experimental conditions see the Material and Methods section. Statistics: the statistical test used was the *t*-test for paired samples. Anti-oestrogen concentrations effects (all experimental conditions tested): TMX 5×10^{-7} vs 5×10^{-6} mol/l, $t = 1.26$, df = 11, NS. 4OH TMX 5×10^{-8} vs. 5×10^{-7} mol/l, $t = 0.61$, df = 11, NS. Drug combination effects (all experimental conditions tested): I vs. II, $t = 0.48$, df = 16, NS. I vs. III, $t = 2.48$, df = 16, $0.02 < P < 0.05$. II vs. III, $t = 2.38$, df = 16, $0.02 < P < 0.05$.

TMX–cisplatin on human malignant melanoma cells was recently confirmed and was shown to be independent of ER expression in these cells [19].

The present data confirm the relatively low frequency of ER expression in human melanoma [4], since among nine different tumours screened only one expressed a significant presence for ER. We disagree with the views of previous investigators who consider the ER expression in melanoma cell lines as a technical artefact [20]. The Kd value we found (0.039 nmol/l) in CAL 1 cells is proof of high affinity sites; it must be acknowledged that the absolute number of sites per cell (2573) is 10 times lower than that which we previously found in the classic human breast cancer MCF 7 cells [21]. Despite this relatively low level of ER expression in CAL 1 cells, it was possible to demonstrate a specific anti-proliferative effect of the anti-oestrogens TMX and 4OH TMX on these cells. Also, the presence of ER was the determining factor for a synergistic interaction between the anti-oestrogens and the nitrosourea tested. The ER-mediated effects of the anti-oestrogens is strengthened by the fact that 4OH TMX, used at one log concentration below TMX, exhibited similar synergistic effects to TMX; 4OH TMX is the most potent anti-oestrogen, and we have previously shown its affinity for ER, which is close to that of oestradiol, and 200 times stronger than TMX [16]. Considering the clinical application of the present observations, human melanoma expressing ER is the most likely to exhibit a therapeutic benefit when combining TMX and nitrosoureas. Although caution must be taken when extrapolating data from the bench to the clinic, significant therapeutic effects can be anticipated since the association with anti-oestrogens allowed the IC_{50} values of Fote to be reduced up to 80%. It is not easy to describe the pharmacological origin of the synergistic cytotoxicity observed in ER+ melanoma cells when combining the anti-oestrogens and nitrosourea. Nitrosoureas are preferentially active against cells in the G1 phase of the cell cycle [22]. One of the major cellular effects of TMX is to synchronise cells in the G1 stage of the cell cycle [23]. Thus, one possible explanation could lie in ER-mediated cell synchronisation by anti-oestrogens, allowing an optimal, subsequent cytotoxic effect of the nitrosourea on CAL 1 ER+ cells. This view concurs well with the fact that the most marked synergies were observed when anti-oestrogens were used before Fote. Other cellular targets, different from ER, may also explain the different behaviours of CAL 1 and CAL 7 towards the drug combinations tested. These targets may include the cellular membrane itself, whose structure may be altered at TMX concentrations in the micromolar range [24], and within the cell, the so-called type II oestrogen binding sites, whose interaction with TMX was shown to result in synergistic effects with cisplatin [10].

The presence of elevated concentrations of TMX has been shown in brain metastasis [25]. Fotemustine, due to its very good octanol–water partition coefficient, has a particularly high diffusion capacity in the cerebrospinal fluid [15]. Brain metastatic diffusion of human melanoma cells is a frequent complication of malignant melanoma which significantly compromises patient survival. This clinical situation could be particularly indicated to evaluate the drug combination presently described.

1. Scott R. Dermatologists warn nation of increased skin cancer risk. *J Natl Cancer Inst* 1992, **84**, 1696.
2. Legha SS. Current therapy for malignant melanoma. *Semin Oncol* 1989, **16** (suppl. 1), 34–44.
3. Patterson JAK, Geronemus RG. Cancers of the skin. In De Vita VT, Hellman S, Rosenberg SA eds. *Cancer Principles and Practice of Oncology*. Philadelphia, JB Lippincott Company, 1989, 1469–1542.
4. Fischer RI, Neifeld JP, Lippman ME. Oestrogen receptors in human malignant melanoma. *Lancet* 1976, **2**, 337–339.
5. Meyskens FL Jr, Vokes JB. Tamoxifen in metastatic malignant melanoma. *Cancer Treat Rep* 1980, **64**, 171–173.
6. Chatterjee M, Harris AL. Reversal of acquired resistance to adriamycin in CHO cells by tamoxifen and 4-hydroxy tamoxifen: role of drug interaction with alpha 1 acid glycoprotein. *Br J Cancer* 1990, **62**, 712–717.
7. Stuart NSA, Philip P, Harris AL, *et al.* High-dose tamoxifen as an enhancer of etoposide cytotoxicity. Clinical effects and *in vitro* assessment in p-glycoprotein expressing cell lines. *Br J Cancer* 1992, **66**, 833–839.
8. Hug V, Hortabagyi GN, Drewinko B, Finders M. Tamoxifen

citrate counteracts the antitumor effects of cytotoxic drugs *in vitro*. *J Clin Oncol* 1985, 3, 1672–1677.
9. Osborne KC, Kitten L, Arteaga CL. Antagonism of chemotherapy-induced cytotoxicity for human breast cancer cells by antiestrogens. *J Clin Oncol* 1989, 7, 710–717.
10. Scambia G, Ranelletti FO, Panici PB, *et al.* Synergistic anti proliferative activity of tamoxifen and cisplatin on primary ovarian tumours. *Eur J Cancer* 1992, 11, 1885–1889.
11. Cocconi G, Bella M, Calabresi F, *et al.* Treatment of metastatic malignant melanoma with dacarbazine plus tamoxifen. *N Engl J Med* 1992, 327, 516–523.
12. Calabresi F, Aapro M, Becquart D, *et al.* Multicenter phase II trial of the single agent fotemustine in patients with advanced malignant melanoma. *Ann Oncol* 1991, 2, 377–378.
13. Sutherland RL, Hall RE, Pang GYN, Musgrove EA, Clark CL. Effect of medroxyprogesterone acetate on proliferation and cell kinetics of human mammary carcinoma cells. *Cancer Res* 1988, 48, 5084–5091.
14. Scatchard G. The attractions of proteins for small molecules and ions. *Ann NY Acad Sci USA* 1949, 51, 660–672.
15. Lokiec F, Beerblock K, Deloffre P, Lucas C, Bizzari JP. Clinical pharmacokinetics study of fotemustine in different tumor types. *Bull Cancer* 1989, 76, 1063–1069.
16. Etienne MC, Milano G, Fischel JL, *et al.* Tamoxifen metabolism: pharmacokinetic and *in vitro* study. *Br J Cancer* 1989, 60, 30–35.
17. Carmichael J, De Graff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semi-automated colorimetric assay in assessment of chemosensitivity testing. *Cancer Res* 1987, 47, 936–942.
18. McClay EF, Mastrangelo MJ, Sprancho ID, Bellet RE, Berd D. The importance of tamoxifen to a cisplatin-containing regimen in the treatment of metastatic melanoma. *Cancer* 1989, 63, 1292–1295.
19. McClay EF, Albright KD, Jones JA, *et al.* Modulation of cisplatin resistance in human malignant melanoma cells. *Cancer Res* 1992, 52, 6790–6796.
20. Zava DT, Goldhirsh A. Estrogen receptor in malignant melanoma: fact or artefact? *Eur J Cancer Clin Oncol* 1983, 19, 1151–1159.
21. Etienne MC, Fischel JL, Milano G, *et al.* Steroid receptors in human osteoblast-like cells. *Eur J Cancer* 1990, 26, 807–810.
22. Meyn RE and Murray D. Cell cycle effects of alkylating agents. *Pharmac Ther* 1984, 24, 147–163.
23. Love RR. Tamoxifen therapy in primary breast cancer: biology, efficacy, and side effects. *J Clin Oncol* 1989, 7, 803–815.
24. Clarke R, Van der Berg HW, Murphy RF. Reduction of the membrane fluidity of human breast cancer cells by tamoxifen and 17 β-estradiol. *J Natl Cancer Inst* 1990, 82, 1702–1705.
25. Lien EA, Solheim E, Ueland PM. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. *Cancer Res* 1991, 51, 4837–4844.

Eur J Cancer, Vol. 29A, No. 16, pp. 2273–2279, 1993.
Printed in Great Britain
0959–8049/93 $6.00 + 0.00

Acute Effects of Growth Factors on T-47D Breast Cancer Cell Cycle Progression

Elizabeth A. Musgrove and Robert L. Sutherland

Growth factors play a major role in the control of human breast cancer cell proliferation but their acute effects on cell cycle progression have not been well studied in these cells. T-47D cells, growth-inhibited by serum deprivation, were induced to re-enter the cell cycle in a concentration- and time-dependent manner by addition of insulin, insulin-like growth factor (IGF)-I, epidermal growth factor (EGF), transforming growth factor α (TGFα) or basic fibroblast growth factor (bFGF). After a lag of ~10 h semi-synchronous entry into S phase was observed. The relative potencies of maximal concentrations of growth factors were in the order: insulin ≈ IGF-I ≈ bFGF > TGFα > EGF, identifying bFGF as among the most potent mitogens for these cells. Insulin or IGF-I alone resulted in growth rates comparable with those observed in fetal calf serum. These data demonstrate that single growth factors can induce a significant proportion of T-47D cells to traverse the cell cycle. The kinetics for entry into S phase were similar, indicating that the basis of differential sensitivity to the growth factors tested was the proportion of cells that responded and ultimately entered S phase.
Eur J Cancer, Vol. 29A, No. 16, pp. 2273–2279, 1993.

INTRODUCTION

BREAST CANCER cell lines respond not only to steroid hormones, but also to a wide range of peptide hormones and growth factors [1–3] and thus provide an experimental model in which the actions of a variety of mitogens can be studied. Members of the epidermal growth factor (EGF)/transforming growth factor α (TGFα) and insulin/insulin-like growth factor-I (IGF-I) family have been widely investigated using these cells, a reflection of the major role these growth factors are thought to play in the overall regulation of proliferation of breast cancer cells. Indeed, some of the earliest studies using breast cancer cells in tissue culture documented growth stimulation by insulin and EGF [4, 5]. However, there is evidence that other, less well-studied growth factors are important in aspects of normal breast and mammary gland development, as well as in the initiation or progression of tumours. For example, mammary epithelial cells respond to several fibroblast growth factor homologues [6–8]. Proliferation of MCF-7 and T-47D human breast cancer cells is also stimulated by both acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) [9, 10], although other studies have found no effect, even in the absence of other mitogens [11].

Despite the diverse growth factors to which responses have been recorded, relatively few studies have directly compared the effects of a range of growth factors on breast cancer cell proliferation. It is clear, however, that the degree of response differs between growth factors. For example, in one publication EGF, TGFα and bFGF promoted fewer cell population doublings of MCF-7 and T-47D cells than did insulin or IGF-I [9]. Similarly, another study using MCF-7 cells found insulin to be

Correspondence to E.A. Musgrove.
The authors are at the Cancer Biology Division, Garvan Institute for Medical Research, St Vincent's Hospital, Darlinghurst, NSW 2010, Australia.
Revised 23 July 1993; accepted 1 Sep. 1993.