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The Effect of Tamoxifen on the Growth of Human Malignant Melanoma *in Vitro**

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Abstract—*The effect of tamoxifen on the growth of malignant melanoma was investigated using human cell lines and single-cell suspensions prepared from patients' tumours cultured in soft agar. Tamoxifen stimulated both* [^{3}H]*-thymidine incorporation and cell numbers in all of the cell lines tested. Cytoplasmic oestrogen receptor (ER) was detected in one of the responding lines and progesterone receptor (PR) in another. Tumour colony formation in soft agar culture was satisfactorily established from tumour cell suspensions from* 13 *of* 21 *patients, only one of which had detectable cytoplasmic ER. Greater than* 50% *reduction in colony formation with* 5×10^{-7} M *tamoxifen occurred in two tumours, neither of which contained ER. These results indicate that tamoxifen has the potential to either retard or accelerate the growth of human malignant melanoma.*

INTRODUCTION

A CONSIDERABLE amount of evidence has accumulated in recent years to suggest that steroid hormones may influence the growth and survival course of malignant melanoma in humans. Several large epidemiological studies have individually confirmed the significantly more favourable prognosis in females [1–3]. Furthermore, one of these studies demonstrated that the survival advantage for females was not explained by differences between the sexes with respect to major prognostic variables, such as the thickness of the primary lesion [4].

The direct influence of steroid hormones was also demonstrated in the clinical reports of objective regression of disease in patients treated with progestational agents and oestrogens [5, 6] and in alteration of the *in vitro* growth of cultured human melanoma cells induced by oestrogens and progesterone [7].

The potential beneficial effects of the anti-oestrogen, tamoxifen, were subsequently explored by several groups with conflicting results. Nesbit *et al.* [8] reported a response in four of 26 patients and stabilization of disease in five others. Other workers have also observed disease regression in patients treated with tamoxifen [9]. These findings do, however, differ from those of Creagan and his co-workers [10], who observed no responses in patients treated with tamoxifen. The patient groups in the above series varied with respect to the degree of exposure to prior chemotherapy and to the distribution of visceral and soft tissue sites of involvement by disease. Steroid hormone receptor analyses were also lacking in these studies and thus the degree of activity and clinical role of specific oestrogen antagonists in malignant melanoma remain to be defined. The potential difficulties of predicting the response of human melanoma to oestrogen antagonists has been foreshadowed by the variable effect of oestrogenic hormones on human cell lines in tissue culture [7].

The aims of the present study were (i) to determine whether tamoxifen could consistently influence the growth of cultured human melanoma cells and (ii) to determine whether any observed variation in growth was correlated with the presence of oestrogen receptor activity in the cultured cells. The effects of tamoxifen on the growth of several human cell lines and on the growth of tumour cells prepared from specimens

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collected from patients undergoing surgery were studied. The latter specimens were cultured in soft agar and the plating efficiency determined. Steroid hormone receptor levels were performed on the cultured human cell lines and on the tumour specimens removed at surgery. The results show that tamoxifen may both stimulate and inhibit the growth of some human malignant melanomas and that the presence of oestrogenic hormone receptor activity does not predict the effect.

MATERIALS AND METHODS

Cells and tissue culture

The melanoma cell lines were obtained from Dr P. Hersey, Kanematsu Memorial Institute, Sydney Hospital, Sydney. The origin and characteristics of these lines and their freedom from mycoplasma have been reported [11].

The cell line Squire was derived from an inguinal lymph node metastasis in a male patient, and the Henderson line from axillary lymph node metastases removed from a male patient.

Cells were maintained as monolayer cultures in tissue culture flasks (Lux, Cat. No. 625375) in RPMI 1640 medium supplemented with glutamine and non-essential amino acids (Flow Laboratories, Sydney, Australia) in an atmosphere of 5% CO_2 in air at 37°C. The medium contained 10% foetal calf serum (FCS, Commonwealth Serum Laboratories, Melbourne, Australia), 100 units of penicillin and 100 μg streptomycin per ml. Cells were subcultured weekly after stripping from the flasks with 0.01% pronase in 0.02% EDTA in Dulbecco's phosphate-buffered saline (PBS).

Inclusion of steroids and drugs in medium

17β-Oestradiol (Sigma Chemical Co., St. Louis, MO, U.S.A.) or tamoxifen (I.C.I. 46474, gift from ICI Pharmaceuticals Division, U.K.) were added to the medium as ethanol solutions to give a final concentration of 0.1% ethanol. Controls contained 0.1% ethanol, which was shown to have no effect on growth of the cells. The medium for all monolayer growth and [^{3}H]-thymidine incorporation experiments was as described above. The endogenous steroids had been removed from FCS in the medium by two 45-min incubations at 50°C with dextran-coated charcoal [12]. The 'stripped' FCS was regularly assayed by Dr R. Seamark of the Department of Obstetrics and Gynaecology, University of Adelaide, and was found to contain no detectable oestrogen or progesterone.

Assessment of cell growth

Cells growing as monolayer cultures in flasks were harvested as above and resuspended in RPMI 1640 medium containing 5% 'stripped' FCS. After 6 hr to allow for recovery from the pronase treatment, the cells were again resuspended and counted in a haemocytometer. Cells were then diluted to a concentration of 1.25×10^4 cells/ml in medium containing 0.1% ethanol as control or an appropriate tamoxifen concentration. Four millilitres of each cell dilution (5×10^4 cells) were dispensed into six replicate wells of a 6 × 35-mm Petri dish plate (Limbro, Cat. No. 76-058-05) and incubated in an atmosphere of 5% CO_2 in air at 37°C.

Four hours prior to harvesting, the wells were pulsed with 20 μl of [6-^{3}H]-thymidine (10 μCi/20 μl, sp. act. = 20 Ci/mmol, Amersham, U.K.). At the end of the 4-hr pulse the medium was removed from the wells, the cells were harvested by pronase–EDTA treatment and resuspended in 1 ml of 0.2% FCS in PBS.

[^{3}H]-*Thymidine incorporation.* Two-hundred-microlitre aliquots of cells in FCS/PBS were harvested on glass fibre filters (Titertek, Cat. No. 78-105-05) using a Millipore 25-mm filter holder by both washing with distilled water and by using a 10-min precipitation in cold 10% TCA, subsequent washing with TCA and clearing with methanol. The filters were dried and placed in 10 ml of scintillation fluid and radioactivity determined in a Beckman LS 7500 Liquid Scintillation Counter.

Cell number determination. A further 200 μl of cells in FCS/PBS were diluted in 10 ml of Iston II (Coulter Electronics P.L., Sydney, Australia) and counted using a Coulter Counter, Model F.

Soft agar culture

Melanoma samples obtained at operation were collected in a sterile container containing 30 ml Hank's balanced saline solution and transported to the laboratory. Melanoma tissue was dissected free of any surrounding normal tissue and diced into 1–2-mm pieces. A single-cell suspension was prepared by enzymatic digestion using the method of Courtenay and Mills [13]. The number of viable, nucleated cells was determined in 0.1% trypan blue using a haemocytometer.

Cells were then set up in soft agar culture according to the method of Courtenay and Mills [13]. Briefly, cells were dispensed in quadruplicate in 0.3% agar in Ham's F 12 medium (Flow Laboratories, Sydney, Australia) containing 20% stripped foetal calf serum and 1/8 dilution of aged rat erythrocytes into tissue culture tubes at 3, 1 and 0.5×10^5 cells per tube. Tubes were gassed with 5% O_2, 5% CO_2 and 90% N_2 and incubated at 37°C. After 1 day one tube at each cell concentration was examined for clumping. Two millilitres of Ham's F 12 medium containing 20%

stripped foetal calf serum with and without 5×10^{-7} M tamoxifen was added to the remaining agar cultures. Medium was changed at weekly intervals thereafter and agar cultures were examined for colony growth after 21 days. Colonies containing greater than 50 cells were scored and plating efficiency (P.E.) was expressed at % of number of colonies/number of viable nucleated cells plated per tube. Colonies were harvested from the agar cultures and were examined both histologically and cytochemically for evidence of malignancy and melanin production (Masson–Fontana stain).

Hormone receptor studies

Cells for biochemical assay of receptors were removed from tissue culture flasks with a short incubation with pronase, washed with PBS containing 2% stripped FCS, pelleted and snap-frozen. The dextran-charcoal assay described by Tilley *et al.* [14] was used to quantitate cytoplasmic oestrogen and progesterone receptors in the cultured cells and in tumour specimens obtained at operation.

RESULTS

Effect of tamoxifen on melanoma cell lines

[^{3}H]-*Thymidine incorporation.* The incorporation of [^{3}H]-thymidine was determined for each cell line over a period of 7 days. The radioactivity was measured after a water lysis of cells and by measuring the incorporation into TCA-insoluble material. The results obtained with both methods in the case of MM200 are depicted in Fig. 1, which reveals an increased incorporation of [^{3}H]-thymidine in the cultures containing tamoxifen on all days apart from day 7, when incorporation is less than in the untreated control cultures. This was presumably due to the stimulated cultures approaching confluence by day 7, causing a decrease in the rate of growth. A marked increase was also observed consistently with both measurements of [^{3}H]-thymidine incorporation in the case

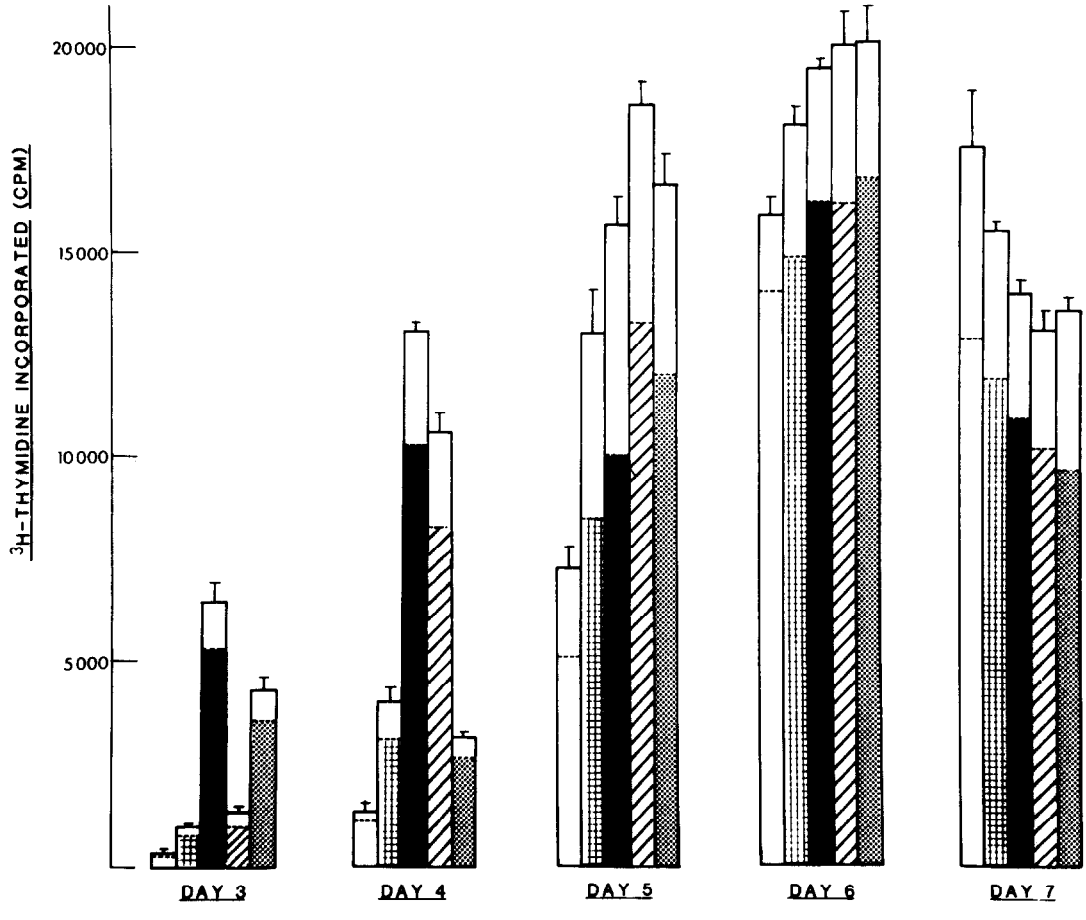

Fig. 1. The effect of tamoxifen on the incorporation of [^{3}H]*-thymidine in the MM200 melanoma cell line. Cells* (5×10^4 *per dish) in RPMI* 1640 + 5% *'stripped' FCS were inoculated on day* 0, *were pulsed with* [^{3}H]*-thymidine and harvested on the days indicated (see Materials and Methods). The solid lines represented the mean incorporation of* [^{3}H]*-thymidine into cells after water lysis and washing; the dotted lines represent mean incorporation into TCA-precipitated material. The treatments are shown as control* □, 10^{-9} M *tamoxifen* ▦, 10^{-8} M *tamoxifen* ■, 10^{-7} M *tamoxifen* ▨ *and* 10^{-6} M *tamoxifen* ▩. *The S.E.M. of six replicates is shown as* ⊤.

of the cell lines Squire and MM127. The results for TCA precipitated material of these experiments are shown in Figs 2 and 3 respectively.

Effect on cell numbers. The experiments were performed using each cell line simultaneously with those described above in the [^{3}H]-thymidine incorporation experiments. In the case of cell lines MM200, Squire and MM127 the inoculated cell number per well was 5×10^4 cells and in the case of Henderson it was 2×10^5 cells per well. The effect of tamoxifen at concentrations ranging from 10^{-10} M to 10^{-6} M on cell number measured at intervals over 7 days are shown in Figs 4–7.

In each case tamoxifen produced a stimulation of growth, which in some instances (eg. MM127, Henderson) amounted to a 100% increase in cell numbers compared with those in untreated control cultures.

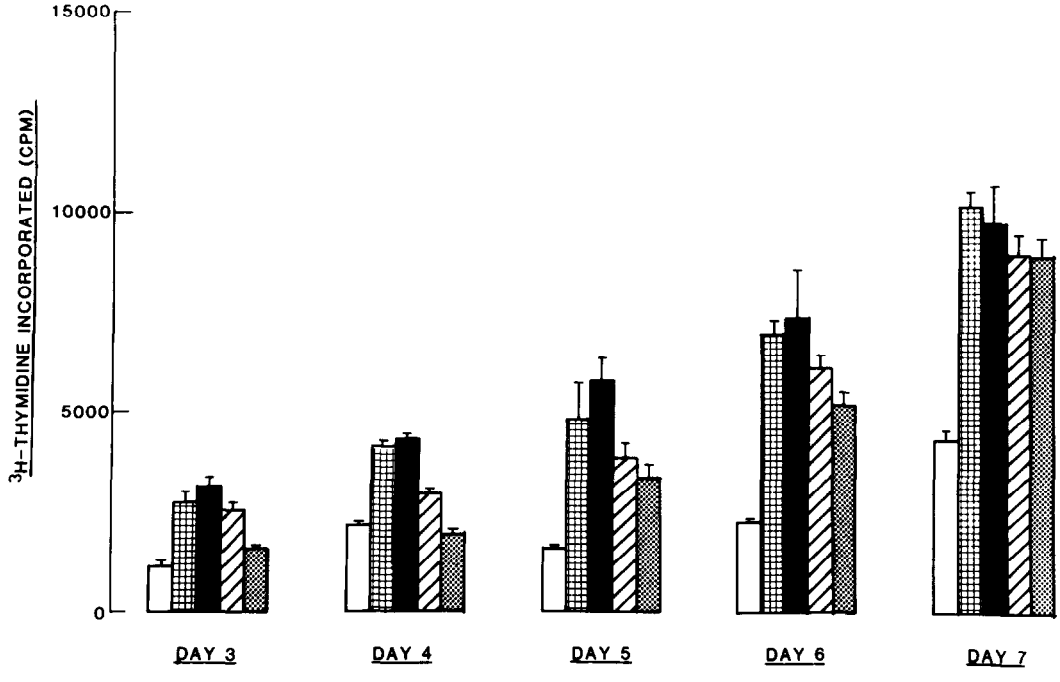

Fig. 2. The effect of tamoxifen on the incorporation of [^{3}H]*-thymidine into TCA-precipitated material in the Squire melanoma cell line. Tamoxifen concentrations are as for Fig. 1.*

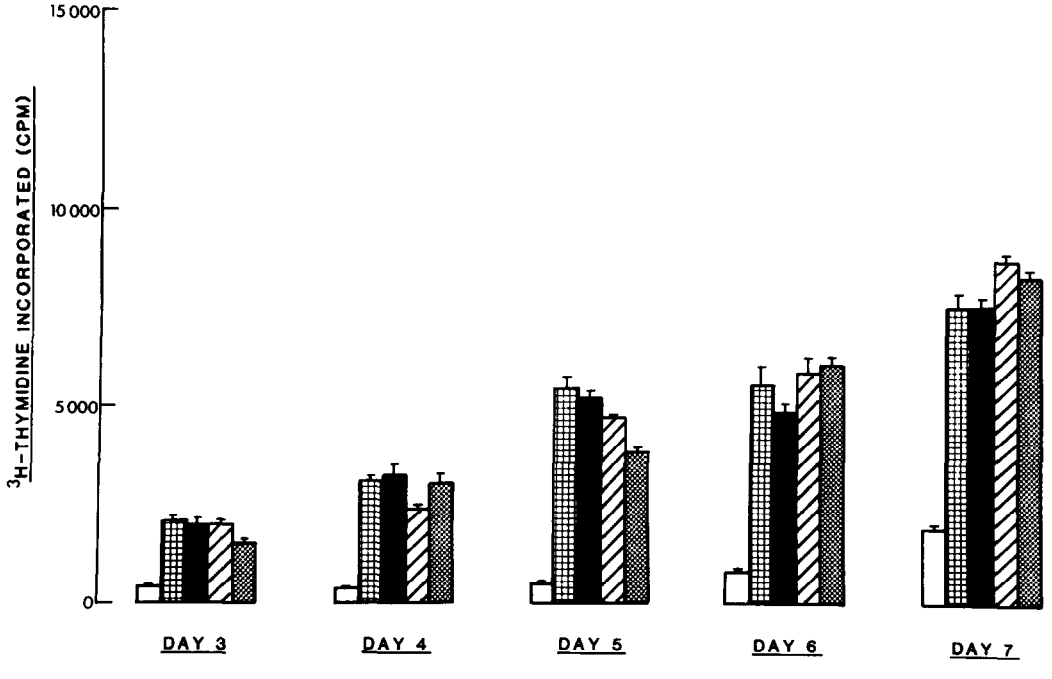

Fig. 3. Incorporation of [^{3}H]*-thymidine in the MM127 melanoma cell line, as for Fig. 2.*

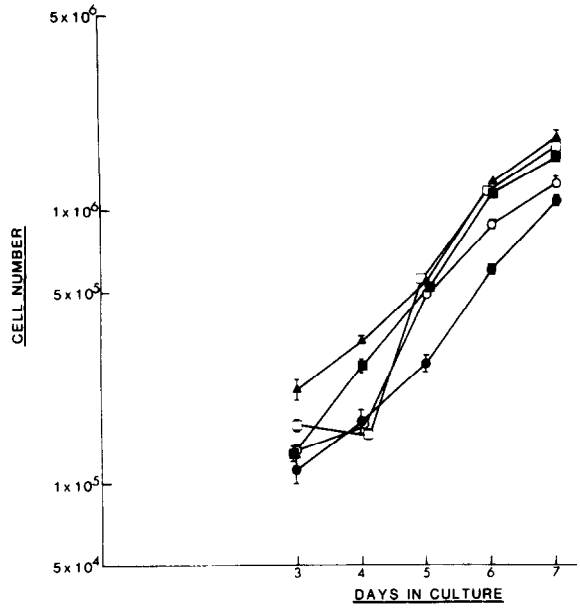

Fig. 4. The effect of tamoxifen on the time-course of the growth of MM200 melanoma cell line. Cells were plated on day 0 at a density of 5×10^4 *cells/dish in RPMI 1640 + 5% 'stripped' FCS, containing 0.1% ethanol as control* ●, 10^{-9} M *tamoxifen* ○, 10^{-8} M *tamoxifen* ▲, 10^{-7} M *tamoxifen* ■ *and* 10^{-6} M *tamoxifen* □. *Each point represents the mean of six replicate dishes and* ⊤ *is the S.E.M.*

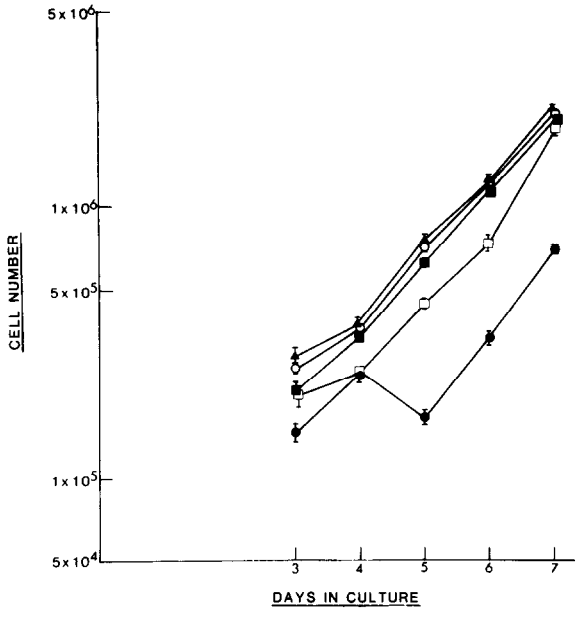

Fig. 5. The effect of tamoxifen on the time-course of the growth of the Squire melanoma cell line, as for Fig. 4.

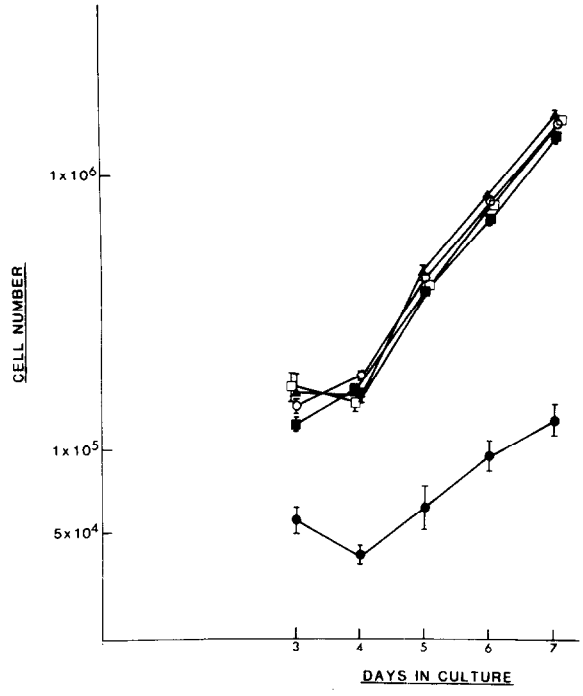

Fig. 6. The effect of tamoxifen on the time-course of the growth of MM127 melanoma cell line, as for Fig. 4.

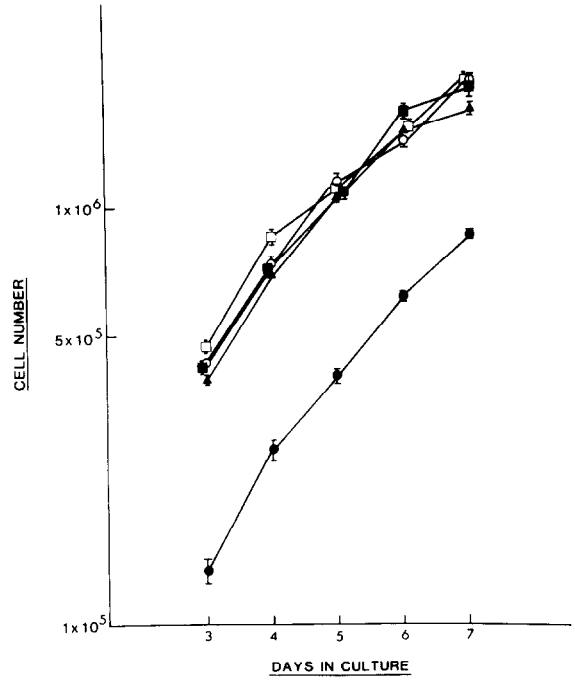

Fig. 7. The effect of tamoxifen on the time-course of the growth of Henderson melanoma cell line, as for Fig. 4 except that the cells were plated at a density of 2×10^5 *cells/dish.*

Effect of tamoxifen on growth in soft agar cultures of cells obtained from human tumour specimens

Tumour samples were collected at operation from 21 patients. In all cases the tissue was obtained from either lymph node or subcutaneous metastases and a tumour cell suspension was prepared and dispersed into soft agar culture using culture medium containing 5×10^{-7} M tamoxifen, as described in Materials and Methods. Control cultures were established in medium without tamoxifen. Tumour colony growth was effectively established in 13 instances, the plating efficiency ranging from 0.02 to 5.72%. The effect

of tamoxifen on the growth in soft agar has been expressed in terms of the P.E. of the tamoxifen-treated cells as a percentage of the P.E. in untreated control cultures from the same specimen. These results are shown in Table 1. There was a marked reduction in the P.E. in the cells obtained from 2 patients and a moderate reduction in one other. The growth of cells obtained from another patient was increased by tamoxifen, albeit not significantly.

Hormone receptor studies

These were performed on all cell lines used in these experiments and the results are shown in Table 2. No consistent correlation is evident between the presence of steroid receptor activity and alteration of either thymidine incorporation or cell numbers in these experiments.

In the case of the tumour specimens only 1 of 12 patients tested possessed cytoplasmic receptor for oestrogen (patient No. 12). Suppression of tumour colony formation by tamoxifen occurred in 3 cultures established from specimens, all of which were receptor negative.

Clinical effect of tamoxifen

Eight patients were given tamoxifen 20 mg b.d. until disease progression occurred. Two patients (Nos 7 and 12) had widespread disease and had received prior chemotherapy. The remaining six patients had either mainly subcutaneous or lymph node metastases or both and had received no prior systemic treatment. No patient achieved more than 50% regression of tumour. Patients Nos 1 and 3 experienced stabilization of subcutaneous nodules for periods of 11 and 16 weeks respectively.

Table 2. The concentration of oestrogen and progesterone receptors in the cultured human malignant melanoma cell lines determined by the dextran-charcoal assay

Cell line	Oestrogen receptor (fmol/mg protein)	Progesterone receptor (fmol/mg protein)
MM200	0	0
Squire	31	0
MM127	0	127
Henderson	0	0

DISCUSSION

The findings of the present study demonstrate that the anti-oestrogen tamoxifen has the potential to both stimulate and to inhibit the growth of human malignant melanoma. The occurrence of marked stimulation of cell numbers and DNA production in the cell lines which were cultured with tamoxifen suggest that some caution in its use in melanoma may be justified, although we did not observe clinically obvious acceleration of tumour growth in patients who received the agent.

The effects of tamoxifen using tumour colony formation in soft agar as the index of cell growth contained some encouragement. Three of 13 tumour specimens exhibited significantly reduced colony formation when grown in medium containing tamoxifen at a concentration similar to that achieved in the body fluids of patients treated with standard doses of the agent (10 mg b.d.) [15]. None of the three tumour specimens contained specific cytoplasmic binding for oestrogen or progesterone. The ability of tamoxifen to suppress the *in vitro* growth of tumours which do not contain detectable levels of

Table 1. Effect of tamoxifen (5×10^{-7} M) on the formation of soft agar colonies grown in oestrogen-free medium from human melanoma samples obtained at operation

Patient	ER (fmol/mg protein)	Plating efficiency of control cultures	% of untreated control (mean ± S.E.M.)	Probability
1	0	0.08	43.7 ± 3.5	<0.01
2	0	0.03	74.8 ± 6.7	<0.05
3	0	0.02	48.7 ± 10.1	<0.02
4	0	2.48	87.7 ± 8.5	NS
5	0	5.72	78.1 ± 7.3	NS
6	0	0.83	88.9 ± 13.5	NS
7	0	0.04	120.4 ± 13.3	NS
8	0	0.28	81.5 ± 5.9	NS
9	n.d.	1.14	86.9 ± 6.1	NS
10	0	0.99	70.7 ± 15.2	NS
11	0	0.96	104.8 ± 6.8	NS
12	8	0.02	136.0 ± 37.1	NS
13	0	0.02	87.4 ± 5.6	NS

n.d. = not done.

oestrogen receptors is consistent with the clinical reports of tumour regression in patients whose tumours lack cytoplasmic oestrogen receptors [16]. There are several possible explanations for these observations in addition to the potential problems associated with tissue sampling and the variation of receptor concentration in different areas of the same tumour.

The cytotoxic effects of tamoxifen against human breast cancer lines are apparent in the absence of oestrogen in the culture medium, which suggests that the anti-tumour effect may be mediated by mechanisms other than, or in addition to, oestrogen receptor blockade [17]. For example, there may be a specific receptor for tamoxifen which is distinct from that for oestrogen and which can mediate the anti-neoplastic effects of the agent. Such a molecule has been shown recently in human breast cancer cells [18] and the existence of similar molecules could explain the inhibition of growth of some human melanomas which lack oestrogen receptors.

It is also possible that these effects in the agar cultures reflect the action of tamoxifen on non-tumour cells in the prepared cell suspension (e.g. macrophages) which may then influence the *in vitro* growth of the tumour stem cells. It is significant in this regard, however, that two of the patients whose tumour growth was significantly reduced by tamoxifen *in vitro* displayed clinical arrest of rapidly progressive disease and minor regression of subcutaneous nodules of tumour.

The partial success of the hormonal therapy in a proportion of melanoma patients has been repeatedly exemplified by the description of the effects of hypophysectomy and castration [19], the effect of progestational agents [5], of oestrogen [20] and, more recently, of tamoxifen [8, 9, 21].

Our findings suggest that anti-oestrogens may benefit a small proportion of patients, but that the determination of oestrogen receptor binding in tumour specimens may not be helpful in the prediction of the response of melanoma cells to the agents. Significant cytoplasmic receptors for oestrogen were not detected in any of the patients' tumour specimens in this study. This differs from other studies and our earlier work [22], which suggested that hormone receptors were present in a significant proportion of human melanomas [6, 23]. It is, however, more consistent with the findings of Rümke *et al.* [24] and of Creagan *et al.* [25], who failed to detect significant levels of oestrogen receptors in any of their patients. Indeed, McCarty *et al.* [26] demonstrated that oestrogen binding in the tumours of their patients was associated with the presence of tyrosinase and the ability to produce melanin, and postulated that the oestrogen may bind to this enzyme in melanomas rather than to a specific cytoplasmic steroid receptor and so give false positive results for hormone binding.

These observations indicate that alternative mechanisms must be invoked to explain the occasional effect of tamoxifen on some human melanomas. Some of these possibilities have been discussed above.

It is possible that the agar culture system used in our experiments and which was described by Courtenay and Mills [13] is particularly suited to the growth of human melanoma and to the assay of its drug sensitivity, as opposed to its wider application to the range of human tumours. Indeed, the use of lower O_2 concentrations and of red blood cells in the tube cultures provided superior culture and assay conditions for human melanomas when the method was compared directly with the more widely used original technique described by Salmon *et al.* and discussed by Tveit *et al.* [27]. Furthermore, growth and drug sensitivity using the Courtenay system has been shown to correlate well with suppression of xenografts of melanoma *in vivo* [28]. The ease of preparation of tumour suspensions and cultures in the case of melanoma suggest that this particular soft agar method could eventually be used in the evaluation of sensitivity to hormonal agents and to anti-oestrogens as opposed to the determination of hormone receptor levels, for example. The latter do not appear to correlate well with observed clinical responses [16]. This may be particularly important as the empirical use of tamoxifen therapy has been associated with rapid acceleration of disease in two of eight patients reported by Meyskens and Voakes [9]. We observed increased colony formation when the cells from one patient's tumour were grown in medium containing charcoal-stripped FCS and tamoxifen. It is possible that these culture conditions permit a weak oestrogen agonist effect of the tamoxifen which would be masked by oestrogens present in medium containing normal FCS.

The frequency of melanoma and its resistance to chemotherapy justifies the continued exploration of the role of non-toxic therapies (such as tamoxifen) for subgroups of patients, despite the discrepancies in clinical response rates reported to date.

The study and interpretation of the effects of steroid hormones and their antagonists on cultured human melanoma cells would be simplified if we could maintain and culture the cells in serum-free conditions since the presence of serum can modify the balance between cytoplasmic and nuclear receptor levels [29] and affect cell growth due to the presence of non-steroidal

growth factors. Despite these current technical limitations regarding the present study, our results indicate that tamoxifen has the potential to either retard or to accelerate the growth of human malignant melanoma.

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REFERENCES

1. Davis N, McLeod R, Beardmore G, Little J, Quinn R, Holt J. The Henry Joseph Windsor lecture: melanoma is a word, not a sentence. *Aust NZ J Surg* 1976, **46**, 188–196.
2. Shaw HM, Milton GW, Farago G, McCarthy WH. Endocrine influences on survival from malignant melanoma. *Cancer* 1978, **42**, 669–677.
3. Rampen F. Malignant melanoma; sex differences in survival after evidence of distant metastases. *Br J Cancer* 1980, **42**, 52–57.
4. Shaw HM, McGovern VJ, Milton GW, Farago GA, McCarthy WH. Histologic features of tumours and the female superiority in survival from malignant melanoma. *Cancer* 1980, **45**, 1604–1608.
5. Johnson RO, Bisel H, Andrews N *et al.* Phase I clinical study of 6α-methylpregn-4-ene-3,11,20-trione (NSC-17256). *Cancer Chemother Rep* 1966, **50**, 671–673.
6. Fisher RI, Neifeld JP, Lippman ME. Oestrogen receptors in human malignant melanoma. *Lancet* 1976, **ii**, 337–338.
7. Chaudhuri PK, Walker MJ. Regulatory effect of steroid hormones on the growth of human malignant melanoma. *Proc Inst Med Chic* 1978, **32**, 45–47.
8. Nesbit RA, Woods RL, Tattersall MF *et al.* Tamoxifen in malignant melanoma. *N Engl J Med* 1979, **301**, 1241–1242.
9. Meyskens FL Jr, Voakes JB. Tamoxifen in metastatic malignant melanoma. *Cancer Treat Rep* 1980, **64**, 171–173.
10. Creagan ET, Ingle JN, Green SJ, Ahmann DL, Jiang M-S. Phase II study of tamoxifen in patients with disseminated malignant melanoma cell line. *Cancer Treat Rep* 1980, **64**, 199–201.
11. Pope JH, Morrison L, Moss DJ, Parsons PG, Regius M Sr. Human malignant melanoma cell lines. *Pathology* 1979, **11**, 191–195.
12. Lippman M, Bolan G, Huff K. The effects of estrogens and antiestrogens on hormone-responsive human breast cancer in long-term tissue culture. *Cancer Res* 1976, **36**, 4595–4601.
13. Courtenay VD, Mills J. An in-vitro colony assay for human tumours grown in immune-suppressed mice and treated *in vivo* with cytotoxic agents. *Br J Cancer* 1978, **37**, 261–268.
14. Tilley WD, Keightley DD, Marshall VR. Oestrogen and progesterone receptors in benign prostatic hyperplasia in humans. *J Steroid Biochem* 1980, **13**, 395–399.
15. Wilkinson R, Ribeiro G, Adam H, Patterson J. Clinical pharmacology of tamoxifen and *N*-desmethyl tamoxifen in patients with advanced breast cancer. *Cancer Chemother Pharmacol* 1980, **5**, 109–111.
16. Karakousis CP, Lopez RD, Bhakoo HS, Rosen F, Moore R, Carlson M. Estrogen and progesterone receptors and tamoxifen in malignant melanoma. *Cancer Treat Tep* 1980, **64**, 819–827.
17. Allegra JC, Lippman ME. The effects of 17β-estradiol and tamoxifen on the ZR-75-1 human breast cancer cell line in defined medium. *Eur J Cancer* 1980, **16**, 1007–1015.
18. Sutherland RL, Murphy LC. The binding of tamoxifen to human mammary carcinoma cytosol. *Eur J Cancer* 1980, **16**, 1141–1148.
19. Bodenham DC, Hale B. Malignant melanoma. In: Stoll BA, ed. *Endocrine Therapy in Malignant Disease.* London, W. B. Saunders, 1972, 377–383.
20. Neifeld JP, Lippman ME. Steroid hormone receptors and melanoma. *J Invest Dermatol* 1980, **74**, 379–381.
21. Masiel A, Buttrick P, Bitran J. Tamoxifen in the treatment of malignant melanoma. *Cancer Treat Rep* 1981, **65**, 531–532.
22. Thompson AJ, Cook MG, Gill PG. Immunofluorescent detection of hormone receptors in cutaneous melanocytic tumours. *Br J Cancer* 1981, **43**, 644–653.
23. Stedman KE, Moore GE, Morgan RT. Estrogen receptor proteins in diverse human tumours. *Arch Surg* 1980, **115**, 244–248.
24. Rümke P, Persijn PJ, Korsten CB. Oestrogen and androgen receptor in melanoma. *Br J Cancer* 1980, **41**, 652–656.
25. Creagan ET, Ingle JN, Woods JE, Pritchard DJ, Jiang N-S. Estrogen receptors in patients with malignant melanoma. *Cancer* 1980, **46**, 1785–1786.

26. McCarty KS Jr, Wortman J, Stowers S, Lubahn DB, McCarty KS Sr, Seigler HF. Sex steroid receptor analysis in human melanoma. *Cancer* 1980, **46**, 1463–1470.
27. Tveit KM, Endresen L, Rugstad HE, Fodstad O, Pihl A. Comparison of two soft-agar methods for assaying chemosensitivity of human tumours *in vitro*: malignant melanomas. *Br J Cancer* 1981, **44**, 539–544.
28. Bateman AE, Selby PJ, Steel GG, Towse GDW. *In vitro* chemosensitivity tests on xenografted human melanomas. *Br J Cancer* 1980, **41**, 189–198.
29. Zava DT, McGuire WL. Oestrogen receptor: unoccupied sites in nuclei of a breast tumour cell line. *J Biol Chem* 1977, **252**, 3703–3708.