7. van Hoff DD, Reichert CM, Cuneo R. Demyelination of peripheral nerves associated with cis-diammine-dichloroplatinum therapy. *Proc AACR* 1979, **20**, 91.
8. Houghton JA, Taylor DM. Maintenance of biological and biochemical characteristics of human colorectal tumors during serial passages in immunedeprived mice. *Br J Cancer* 1978, **37**, 213–223.
9. Steel GG, Courtenay VD, Peckham MJ. The response to chemotherapy of a variety of human tumor xenografts. *Br J Cancer* 1983, **47**, 1–13.
10. Shorthouse AJ, Peckham MJ, Smyth JF, Steel GG. The therapeutic response of bronchial carcinoma xenografts: a direct patient-xenograft comparison. *Br J Cancer* 1980; **41**, 142–145.
11. Fiebig HH, Schuchhardt C, Heuss H, Fiedler L, Löhr GW. Comparison of tumor in nude mice and in the patients. *Behring Inst Clin Res Comm* 1984, **74**, 343–352.
12. Harstrick A, Casper J, Guba R, Wilke H, Poliwoda H, Schmoll H-J. Comparison of the antitumor activity of cisplatin, carboplatin and iproplatin against established human testicular cancer cell lines *in vivo* and *in vitro*. *Cancer* 1989, **63**, 1079–1083.
13. Yagoda A, Mukherje B, Young C, *et al.* Bleomycin—an antitumor antibiotic; clinical experience in 274 patients. *Ann Intern Med* 1972, **77**, 861–870.
14. Rozencweig M, von Hoff D, Slavik M, Muggia FM. Cis-diamminedichloroplatinum—a new anticancer drug. *Ann Intern Med* 1977, **86**, 803–812.
15. Samuels MC, Howe CD. Vinblastine in the management of testicular cancer. *Cancer* 1970, **25**, 1009–1017.
16. Buckner CD. High dose cyclophosphamide for the treatment of metastatic testicular neoplasms. *Cancer Chemother Rep* 1974, **58**, 709–714.
17. Wheeler BM, Loehrer PJ, Williams SD, *et al.* Ifosfamide in refractory male germ cell tumors. *J Clin Oncol* 1986, **4**, 28–34.
18. Weiss HD, Walker MD, Wiernick PH. Neurotoxicity of commonly used antineoplastic agents. *N Engl J Med* 1974, **18**, 127–133.
19. Trump DL, Horvert L. Etoposide and very high dose cisplatin: salvage therapy for patients with advanced germ cell neoplasms. *Cancer Treat Rep* 1985, **69**, 259–261.
20. Williams SD, Einhorn LH. Etoposide salvage therapy for refractory germ cell tumors: an update. *Cancer Treat Rev* 1982, **9** (Suppl A), 67–71.

Eur J Cancer, Vol. 26, No. 8, pp. 901–905, 1990.
Printed in Great Britain

0277–5379/90 $3.00 + 0.00

Correlation of Drug Response in Patients and in the Clonogenic Assay with Solid Human Tumour Xenografts

Catherine C. Scholz, Dietmar P. Berger, Bernd R. Winterhalter, Hartmut Henß and Heinz-Herbert Fiebig

The potential of evaluating the preclinical response of solid tumours was studied in human tumour xenografts in the clonogenic assay. Tumour specimens surgically removed from cancer patients were implanted subcutaneously into thymusaplastic nude mice. Chemosensitivity of the mouse-grown tumours was tested with a modification of the double-layer soft-agar clonogenic assay . Tumour cells were tested against thirteen established cytostatic drugs at two dosages by continuous exposure. 62 retrospective *in vivo/in vitro* correlations were done. The clonogenic assay predicted correctly for clinical response in 16/27 (59%) and for resistance in 32/35 (91%). These correlation rates were similar to reported data for fresh solid human tumour specimens. The results support the clinical relevance of the nude mouse/clonogenic assay model.
Eur J Cancer, Vol. 26, No. 8, pp. 901–905, 1990.

INTRODUCTION

THE POSSIBILITY of individualizing chemotherapy is of special interest to oncologists since patients with identical histological tumour types frequently respond differently to the same chemotherapeutic regimen. Since 1978 research teams have attempted to assess the chemosensitivity of solid human tumours to various cytostatic agents preclinically with the clonogenic assay developed by Hamburger and Salmon [1–3]. Correct predictions for sensitivity are possible in about 69% and for resistance in 92% of diverse solid human tumours [4]. However, these data are difficult to interpret because of lack of standardisation of the experiments and inadequate quality control measures [5]. The key criterion, 'colony', has been defined by some as a minimum number of cells [6–8] but by others as a grouping of cells requiring a minimum diameter ranging from 40 [9] to 100 μm [10]. *In vitro* sensitivity has commonly been defined as inhibition of growth in the drug-treated plates to less than 30% of controls (T/C value under 30%). However, Link *et al.* [11] used a T/C value of 50%, Ajani *et al.* [12] used 35% and Mann *et al.* [8] used 25%. Relevant drug dosages present another significant problem with up to log 2 differences among various laboratories. Comparison of *in vivo/in vitro* correlation results demands standardisation of these key criteria.

We present *in vivo/in vitro* correlations with the clonogenic assay and with quality control measures and criteria for assay standardisation. Solid human tumour xenografts were the tumour source. The use of tumour material following passage

Correspondence to H.-H. Fiebig.
The authors are at the Department of Internal Medicine I, Division of Hematology/Oncology, University of Freiburg, Hugstetter Str. 55, D-7800 Freiburg, F.R.G.

in nude mice has the advantage of ample tumour material and assay reproducibility. Fiebig and Löhr [13] have shown that xenograft tumour material exhibited strikingly similar characteristics compared with the original tumour in terms of histology, chemosensitivity and tumour markers. In 80 *in vivo* chemosensitivity comparisons (nude mouse/patient) response was correctly predicted in 90% and resistance in 96% [14].

A combined approach of initial *in vitro* clonogenic assays and subsequent *in vivo* studies of selected human tumour xenografts in nude mice is being increasingly used in large-scale screens for new antineoplastic agents. A prerequisite to this approach is good correlation for chemosensitivity in the patient and in the clonogenic assay with human tumour xenografts.

MATERIALS AND METHODS

Tumours

Human tumour material from primary tumour or metastatic lesions, resected for diagnostic or therapeutic purposes, was placed into a sterile tube with RPMI 1640, HEPES buffer and glutamine. For the first serial passage, 16 tumour slices (5 × 5 × 0.5–1 mm diameter) were implanted subcutaneously into nude mice of NMRI genetic background. The animals were maintained as described by Fortmeyer and Bastert [15]. Approximately 50% of the tumours grew within 8–12 weeks to diameters of 1–2 cm, at which time they were removed under sterile conditions in a laminar air-flow system.

Single cell suspensions

Human tumours established in serial passage in thymuasaplastic nude mice were mechanically disaggregated and incubated with collagenase 0.05%, DNase 0.07% and hyaluronidase 0.1% at 37°C for 30 min. The cells were washed and passed through sieves of 200 and 50 μm mesh. Trypan-blue exclusion was used for a viable cell count in a haemocytometer.

Culture methods

A modification of the double-layer soft-agar assay [1] was used. The bottom layer consisted of 0.5% agar in 1 ml Iscove's modified Dulbecco's medium with L-glutamine and 10% fetal calf serum. 2–4 × 10^5 cells were plated in 35 mm culture dishes in 1 ml containing 30% fetal calf serum and 0.3% agar onto the bottom layer. Drugs were added in 1 ml medium containing 30% fetal calf serum. The cells were exposed to cytostatic drugs continuously during incubation at 37°C in 7% CO_2. Plates were examined under an inverted microscope for colony growth every other day. At the time of maximum colony formation (usually after 7–21 days in culture) a final colony count was done in an automatic image-analysis system ('OMNICON FASIII', Bausch & Lomb). 24 h before evaluation all cultures were incubated with 1 ml tetrazolium chloride dye (1 mg/ml), which stains vital cells and colonies with intact dehydrogenase activity.

Quality control

The following quality control measures were used [16]: (1) the median number of colonies in the control group dishes had to be 100 or more for a colony diameter of 60 μm, or 50 or more for an 80 μm diameter; (2) the cells were vitally stained with a tetrazolium chloride dye on day 0 and day 2 to determine the number of initial aggregates and their course during culture—the rate of aggregates had to be 20% of the control group count or less; (3) a positive reference compound (5-fluorouracil at a toxic dose of 100 μg/ml) had to effect a colony survival of less than 20% of the controls—the positive control drug was added to assess the maximum cytotoxic effect that could possibly be achieved; and (4) the coefficient of variation among the control plates had to be 50% or less. Only assays fulfilling these criteria were considered evaluable for *in vivo/in vitro* correlations.

Table 1. Cytostatic drugs and dosages tested

Drug	Relevant concentration (μg/ml)	High concentration (μg/ml)
Bleomycin	0.06	0.2
Cisplatin	0.1	0.3
Activated cyclophosphamide*	0.3	0.6
Dacarbazine	30.0	100.0
Doxorubicin	0.01	0.03
Etoposide	0.1	0.3
5-fluorouracil	0.2	0.6
Elmustine	6.0	12.0
Activated ifosfamide*	0.3	0.6
Mitomycin-C	0.005	0.015
Vinblastine	0.003	0.01
Vincristine	0.01	0.03
Vindesine	0.01	0.03

*Active metabolite was used (4-OH cyclophosphamide or 4-OH ifosfamide), supplied by ASTA Pharma, F.R.G.

Drugs

The thirteen cytostatic drugs listed in Table 1 were individually tested at two dosages in triplicate by continuous exposure. The 'relevant concentrations' predicting correctly for the clinical efficacy of the agents were determined for each drug separately by comparing the *in vitro/in vivo* response rates of sensitive tumour types both in patients and in nude mice. A 'high concentration' of the drug, usually triple this dose, was additionally given to test the reaction of the tumour cells at an unphysiologically high dosage. For compounds with steep dose-response effects (activated cyclophosphamide, activated ifosfamide and elmustine) the relevant concentration and twice this dose were administered.

Clinical correlations

A tumour was defined to be sensitive *in vitro* to a cytostatic agent if colony formation was reduced to less than 30% of the control value T/C ≤ 30%. The *in vivo* reaction of a patient's tumour to chemotherapy was evaluated by the attending oncologist without knowledge of the *in vitro* testing. A complete response was defined as the disappearance of all tumour manifestations for at least 1 month. A partial response required at least a 50% decrease of measurable tumour area, and a no change a less than 50% decrease or stabilization under therapy. Progression was defined as a more than 50% increase of measurable tumour area. For the correlation of *in vitro/in vivo* results, *in vivo* response was reduced to dichotomy. Only complete and partial responses were scored as *in vivo* sensitivity. No change and progression were considerd *in vivo* resistance.

Clinical correlations were possible if the patient received the chemotherapeutic agents that were also tested *in vitro*. Since most patients received combination chemotherapies for the treatment of their solid tumours, further clarification is necessary to explain how *in vivo/in vitro* correlations were done. Patients achieving clinical responses when treated with two or more drugs that were active *in vitro* were considered to have responded

only to the most active agent in the clonogenic assay. Thus, only one true positive correlation was recorded in such instances. Conversely, for patients showing clinical tumour resistance while treated with multiple agents, true negative correlations were established for all corresponding drugs being inactive *in vitro*. Patients who clinically progressed while receiving combination chemotherapy, but in whom one or more drugs were active *in vitro*, were considered to have true negative correlations to the inactive drugs but a false positive correlation to the drugs with *in vitro* activity. This method complies with that used by Bertelsen *et al.* [2] for the analysis of 258 *in vitro/in vivo* correlations.

For comparison, the results were analyzed with only one correlation for clinically resistant tumours. In this case, only the results for the least active compound were considered, further compounds tested showing lower T/C values were not evaluated.

RESULTS

44 patients' tumours could be assessed for *in vivo/in vitro* response to chemotherapy. There were 31 males and 13 females with a median age of 56 years (range 20–73). All patients presented with measurable disease. A broad spectrum of tumour histologies was studied, including non-small cell (7 cases) and small cell (8) lung cancer, melanoma (4), mesothelioma (2) as well as carcinomas of the colon and rectum (5), stomach (4), testes (4) and various histologies (10). However, the small numbers for each tumour type do not allow a comparison of correlation among various tumour histologies.

Table 2 shows the individual *in vitro* chemosensitivity test of a testicular teratoma (tumour designation TXF 881) tested after the fourth nude mouse passage. The 23-year-old patient received combination chemotherapy with cisplatin, bleomycin and vinblastine resulting in a complete disappearance of the abdominal lymph nodes under computed tomography follow-up. Bleomycin and cisplatin did not reduce colony formation in the clonogenic assay. The *in vitro* test suggested vinblastine as the effective cytostatic agent, with a reduction of colony formation at the relevant concentration to 26% of control.

Of 62 *in vivo/in vitro* correlations, 44 resulted from *in vivo* combination chemotherapies. The clonogenic assay predicted correctly for response in 59% and for resistance in 91% (Table 3). If only one *in vitro/in vivo* comparison was done for clinically sensitive and resistant tumours, a positive predictive value was 68% and the negative predictive value was 84%.

Table 2. Individual chemosensitivity testing of testicular teratoma TXF 881/4

Drug	Relevant concentration	High concentration
Doxorubicin	–	+
Bleomycin	–	–
Cisplatin	–	+
Activated cyclophosphamide	+	+
Decarbazine	+	+
5-fluorouracil	+	+
Elmustine	–	+
Activated ifosfamide	–	–
Mitomycin	+	+
Etoposide	+	+
Vinblastine	+	+
Vincristine	+	+
Vindesine	+	+
Total	8/13	11/13

– = inactive (T/C > 30%), + = active (T/C < 30%).

Table 3. In vitro/in vivo correlation of chemosensitivity

In vitro/in vivo correlation	Multiple comparisons per tumour	One comparison per tumour
R/R (true negative)	32	17
S/S (true positive)	16	16
S/R (false positive)	11	8
R/S (false negative)	3	3
Total no. of correlations	62	44
Positive predictive value*	59%	68%
Negative predictive value†	91%	84%

R/R = tumour resistance in clonogenic assay/tumour resistance in patient; S/S = tumour response in clonogenic assay/tumour response in patient; S/R = tumour response in clonogenic assay/tumour resistance in patient; and R/S = tumour resistance in clonogenic assay/tumour response in patient.
*(S/S) : (S/R + S/S).
†(R/R) : (R/S + R/R).

19/44 patients had *in vivo* sensitive tumours, yielding a positive accuracy of 71% and a negative accuracy of 86% [17]. In other words, the probability that a patient with an *in vitro* determined sensitivity will respond *in vivo* is 71%, and the probability that a patient without an *in vitro* sensitivity will not respond to clinical treatment with that drug is 86%.

Furthermore, a relation between the percentage decrease in colony number and the degree of *in vivo* response could be demonstrated (Table 4). Patients who went into complete remission showed the highest average inhibition of colony formation in the clonogenic assay (T/C 10%). The degree of inhibition of colony formation paralleled the clinical behaviour of the tumours *in vivo*. Tumours of patients showing progressive disease gave the lowest average T/C (54%). However, due to the small numbers in each group and broad spectrum of values, the differences in colony inhibition among adjacent groups were not significant (*t* test).

The correlation results were additionally analyzed for tumour origin. No significant difference could be seen in correlation results between tumour material taken from the primary tumour compared with that taken from a metastatic site ($P > 0.1$, Table 5).

Table 4. Comparison of clinical tumour response and inhibition of colony formation in clonogenic assay

In vivo tumour response	No. of tests	Inhibition of colony formation: Average T/C*	Inhibition of colony formation: T/C range
Complete remission	4	10%	0–34%
Partial remission	14	19%	1–84%
No change	4	31%	5–55%
Progression	35	54%	3–100%

*T/C = colony count in most effective treatment group/control group.

Table 5. In vitro/in vivo *correlation of chemosensitivity: influence of tumour site*

In vitro/in vivo correlation	Primary tumour	Metastatic lesion
R/R (true negative)	12	20
S/S (true positive)	7	8
S/R (false positive)	4	6
R/S (false negative)	1	2
Total no. of correlations	24	36
Positive predictive value	64%	57%
Negative predictive value	92%	91%

DISCUSSION

In a comparison of the chemosensitivity of solid human tumours *in vivo* in patients and *in vitro* in human tumour xenografts in the clonogenic assay, correct positive predictions for sensitivity were made in 59% of cases and for resistance in 91%. These results were similar to those reported for direct testings of *in vivo* tumours with fresh human tumour specimens. Bertelsen *et al.* [2] made 258 retrospective correlations and found correct positive predictions for sensitivity in 57% and for resistance in 92%. These data suggest that the combined model (nude mouse/clonogenic assay) can produce correlation results that are similar to the direct test. The combined model, however, will not find routine prospective application in predicting patient tumour response. Major drawbacks are the time required until chemosensitivity results are available (2–6 months) as well as the cost and expense necessary to maintain nude mice. Nonetheless, the advantages of sufficient tumour material and assay reproducibility, as well as the good *in vivo/in vitro* correlation support the use of the combined model in new drug screening.

Furthermore, the degree of clinical response *in vivo* paralleled the degree of colony inhibition *in vitro*. These data support the approach of using the most effective cytostatic drugs *in vitro* in the prospective treatment directed by the clonogenic assay of the cancer patient. However, the ultimate effectiveness of the clonogenic assay in this application requires randomized prospective *in vitro/in vivo* correlations.

Particularly disturbing in this respect were the three false-negative correlations made. Cytostatic drugs that were active *in vivo* would not have been recognized as active *in vitro*. Interestingly, two false-negative correlations were made for two ovarian carcinomas. Whether these results were attributable to synergistic effects of the combination chemotherapy *in vivo* not ascertained by individual *in vitro* drug testing [18] warrants further study.

The correlation results were also examined as regards tumour heterogeneity. The heterogeneity of solid tumours in their chemosensitivity has been a criticism of the clonogenic assay to predict *in vivo* chemosensitivity. Chemosensitivity differs between locations within an individual tumour [19], between primary tumour and metastatic lesions [19, 20] and between various metastatic lesions of the same primary tumour [18, 21]. The prospective use of the clonogenic assay in predicting tumour sensitivity *in vitro* before *in vivo* therapy becomes questionable, when primary tumour or metastatic lesion chemosensitivity is not representative of the entire tumour bulk. The chemosensitivity testing in our study used primary tumour or metastatic tumour material to ascertain sensitivity. No difference between *in vivo/in vitro* chemosensitivity correlations could be seen in differentiating primary tumour and metastatic lesion as tumour origin. This result may be explained by the small number of correlations made and is undergoing further study.

Retrospective *in vitro/in vivo* correlations of chemotherapy in patients and in the clonogenic assay have been widely reported. Our study was done to determine the value of human tumour xenografts for wide-range pretherapeutic cytostatic drug testing in a quality-controlled clonogenic assay. The correlation rate for the combined model (nude mouse/clonogenic assay) was similar to that for direct testing. In addition, patient's response could be predicted with the same accuracy, regardless of tumour origin from primary tumour or metastatic site.

1. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977, **197**, 461–463.
2. Bertelsen CA, Sondak VK, Mann BD, Korn EL, Kern DH. Chemosensitivity testing of human solid tumors. A review of 1582 assays with 258 clinical correlations. *Cancer* 1984, **53**, 1240–1245.
3. Salmon SE. Human tumor colony assay and chemosensitivity testing. *Cancer Treat Rep* 1984, **68**, 117–125.
4. Von Hoff DD. *In vitro* predictive testing: the sulfonamide era. *Int J Cell Cloning* 1987, 5, 179–190.
5. Shoemaker RH, Wolpert-DeFilippes MK, Venditti JM. Potentials and drawbacks of the human tumor stem cell assay. *Behring Inst Mitt* 1984, **74**, 262–272.
6. Alonso K. Human tumor stem cell assay. A prospective clinical trial. *Cancer* 1984, **54**, 2475–2479.
7. Hamburger AW, Salmon SE, Kim MB, *et al.* Direct cloning of human ovarian carcinoma cells in agar. *Cancer Res* 1978, **38**, 3438–3444.
8. Mann BD, Kern DH, Giuliano AE, *et al.* Clinical correlations with drug sensitivities in the clonogenic assay. A retrospective study. *Arch Surg* 1982, **117**, 33–36.
9. Von Hoff DD, Clark GM, Stogdill BJ, *et al.* Prospective clinical trial of a human tumor cloning system. *Cancer Res* 1983, **43**, 1926–1931.
10. Umbach GE, Poethen J, v. Matthiessen H, Bender HG, Koldovsky U. Experiences with the human tumor colony-forming assay in gynecologic malignancies. *J Cancer Res Clin Oncol* 1985, **110**, 234–237.
11. Link KH, Aigner KR, Kuehn W, Schwemmle K, Kern DH. Prospective correlative chemosensitivity testing in high-dose intraarterial chemotherapy for liver metastases. *Cancer Res* 1986, **46**, 4837–4840.
12. Ajani JA, Spitzer G, Baker F, *et al.* Human tumor cloning assay with prospective clinical correlations and association between *in vitro* sensitivity and the number of drugs tested. In: Salmon SE, Trent JM, eds. *Human Tumor Cloning*. Orlando, Grune & Stratton, 1984, 543–549.
13. Fiebig HH, Löhr GW. Wachstum menschlicher Malignome in der thymusaplastischen Nacktmaus. *Med Welt* 1984, **35**, 52–58, 81–86.
14. Fiebig HH. Comparison of tumor response in nude mice and in the patients. In: Winograd B, Pinedo H, eds. *Human Tumor Xenografts in Anticancer Drug Development*. Berlin, Springer, 1988, 25–30.
15. Fortmeyer HP, Bastert G. Breeding and maintenance of nu/nu mice and mu/rnu rats. In: Bastert G, Fortmeyer HP, Schmidt-Mattheisen H, eds. *Thymusaplastic Nude Mice and Rats in Clinical Oncology*. Stuttgart, Fischer, 1981, 25–28.
16. Berger DP, Henß H, Winterhalter BR, Fiebig HH. The clonogenic assay with human tumor xenografts: Evaluation, predictive value and application for drug screening. *Ann Oncol* (in press).
17. Makuch RW. Interpreting clonogenic assay results. *Lancet* 1982, **2**, 438.
18. Alberts DS, Salmon SE, Surwit EA, Chen HSG, Moon TE, Meyskens FL. Combination chemotherapy (CRx) *in vitro* with the human tumor stem cell assay (HTSCA). *Proc Am Assoc Cancer Res* 1981, **22**, 153.
19. Tanigawa N, Yoshifumi M, Takafumi H, *et al.* Comparison of drug sensitivity among tumor cells within a tumor, between primary tumor and metastases, and between different metastases in the

human tumor colony-forming assay. *Cancer Res* 1984, **44**, 2309–2312.
20. Von Hoff DD, Clark GM. Drug sensitivity of primary versus metastasis. In: Salmon SE, Trent JM, eds. *Human Tumor Cloning*. Orlando, Grune & Stratton, 1984, 183–195.
21. Bertelsen CA, Korn EI, Morton DL, Kern DH. Heterogeneity of human metastatic clones by *in vitro* chemosensitivity testing. *Arch Surg* 1983, **118**, 1406–1409.

Acknowledgement—This study was supported by the 'Bundesministerium für Forschung und Technologie'.

Eur J Cancer, Vol. 26, No. 8, pp. 905–907, 1990.
Printed in Great Britain

0277–5379/90 $3.00 + 0.00

Anti-proliferative Effects and Phenotypic Alterations Induced by 8-hydroxyquinoline in Melanoma Cell Lines

Jardena Nordenberg, Abraham Novogrodsky, Einat Beery, Miriam Patia, Lina Wasserman and Abraham Warshawsky

The effect of the transition metal chelator, 8-hydroxyquinoline (8-HQ), was examined on the growth and phenotype expression of B16 mouse melanoma cells. Micromolar concentrations of 8-HQ inhibited the growth of B16 cells as well as human melanoma cell lines. Removal of 8-HQ from the culture medium restored normal cell growth. Growth inhibition by 8-HQ was accompanied by phenotypic alterations that included changes in cell morphology, increased production of melanin and enhanced activities of the enzymes γ-glutamyl transpeptidase and NADPH cytochrome *c* reductase. These changes might be associated with a better differentiated phenotype. *Eur J Cancer*, Vol. 26, No. 8, pp. 905–907, 1990.

INTRODUCTION

THE TRANSITION metal cations, iron, copper and zinc, are involved in regulation of metabolic pathways related to cell proliferation [1, 2]. Several groups of chelating agents interfere with the growth of various cell types, including melanoma cells [2–4]. 8-hydroxyquinoline (8-HQ) and its halogenated derivatives are fungitoxic and amoebicidic [5, 6]. Yamato *et al.* have prepared tropolone derivatives, incorporating 8-quinolinol side groups. These compounds inhibit KB human epidermoid carcinoma cells *in vitro* and are highly potent against P-388 leukaemia in vivo [7–9]. We have evaluated the effects of 8-HQ on melanoma cell growth and phenotypic expression.

MATERIALS AND METHODS

Cell growth

B16 F10 melanoma cells were plated at 4×10^4 cells per ml 'RPMI 1640', supplemented with 10% fetal calf serum and antibiotics in tissue culture dishes (3 cm). In selected experiments 5×10^4 SKMEL-28 or RPMI 7951 human melanoma cells, MCF-7 breast cancer cells or human fibroblasts derived from ascitic fluid of an ovarian cancer patient were incubated in 0.5 ml growth medium in multiwell plates (0.9 cm). Cultured neonatal cardiomyocytes were used as non-proliferating normal control cells. Cells were incubated in the absence or presence of 8-HQ (Fluka Chemical Corporation) at 37°C in a humidified atmosphere of 5% CO_2 in air for different times. Cell growth was measured by counting cells in a Coulter counter after detachment with EDTA (1 mmol/l).

Phenotypic alterations

Cell morphology was evaluated by light microscopy after fixation of the cells with formalin and staining with haematoxylin-eosin. For assessment of melanin content and enzymatic activity of NADPH cytochrome *c* reductase and γ-glutamyl transpeptidase, 5×10^5 cells were plated in 10 ml culture medium in the presence or absence of 8-HQ for 72 h. Melanin and enzymes were extracted and measured spectrophotometrically [10–12].

Statistical significance of the results was evaluated with paired or unpaired *t* tests.

RESULTS

The number of untreated B16 F10 melanoma cells increased 23 fold over 92 h of incubation (Fig. 1). 8-HQ inhibited cell growth in a concentration dependent manner, leading to complete growth inhibition at 5 μmol/l. Cells remained attached and 95% viable at concentrations up to 5 μmol/l. Higher concentrations were cytotoxic towards the cells. The results in Fig. 2 show that 8-HQ also inhibited the growth of SKMEL-28 and RPMI 7951 human melanoma cells at micromolar concentrations. MCF-7 human breast cancer cells were also found to be highly sensitive to growth inhibition by 8-HQ—a 70% decrease in cell number was obtained at 5 μmol/l. The growth of human fibroblasts was inhibited by 40% at 5 μmol/l 8-

Correspondence to J. Nordenberg.
J. Nordenberg, A. Novogrodsky, E. Beery, M. Patia and L. Wasserman are at the Rogoff Research Institute, Beilinson Medical Center and Sackler School of Medicine, Petah Tikva 49100, and A. Warshawsky is at the Weizmann Institute of Science, Rehovot, Israel.