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A Novel Microculture Kinetic Assay (MiCK Assay) for Malignant Cell Growth and Chemosensitivity

V.D. Kravtsov

The THERMOmax™ microplate reader was adapted for monitoring the growth kinetics of human leukaemic OCI/AML-2 and mouse tumour J-774.1 cell lines in continuous culture. Fluid evaporation from wells, CO_2 escape and contamination were prevented by hermetic sealing of the microcultures in wells of a 96-well microplate, thus enabling the cells to grow exponentially for 72 h under the conditions of the incubated microplate reader. For both OCI/AML-2 cells, which grow in suspension, and adherent J-774.1 cells, a linear correlation was demonstrated between the number of unstained cells seeded in a given microplate well and the optical density (OD) of that well. Therefore, the OD/time curve of the culture could be deemed to be its growth curve. By the use of the linear fit equation, the actual number of the cells in the wells was computable at any time point of the assay. In the chemosensitivity test, an inhibitory effect of ARA-C on the growth of the cells could be estimated by viewing of the growth curves plotted on the screen. The maximum kinetic rates (*V*max) of the curves in the control and the ARA-C-treated wells were compared, yielding a growth inhibition index (GII). Comparison of results of the kinetic chemosensitivity assay with those of a [^{3}H]thymidine incorporation assay revealed that the novel assay is suitable for precise quantitation of the cell chemosensitivity, is more informative and has the added technical advantage of performance without recourse to radioactive or chemically hazardous substances.

Key words: malignant cells, growth kinetics, chemosensitivity
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INTRODUCTION

SEVERAL SPECTROPHOTOMETRIC non-clonogenic microculture assays have been developed to determine proliferation and chemosensitivity of malignant cells *in vitro*. These make use of tetrazolium-based colorimetric assay [1–6] or fluorimetric microculture cytotoxicity assay [7, 8], both of which are convenient for handling a large number of samples. However, the above methods obviously determine only the endpoint of the cell–chemotherapeutic drug interaction and do not enable continuous monitoring of the growth of drug-affected malignant cell populations *in vitro*.

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Incubated scanning microplate readers (e.g. THERMOmax™ Microplate Reader, Molecular Devices Corp., U.S.A. or iEMS Reader, Labsystems, Finland) have recently become commercially available. These machines are extensively utilised in microbiological laboratories for kinetic turbidimetric and chromogenic assays [9, 10]. To date, the use of incubated microplate readers for monitoring mammalian cell growth has not been possible because the incubated chamber of the reader does not preclude exposure of the cell microcultures to the ambient atmosphere, which results in evaporation of the warm fluid from the wells, escape of CO_2 from the media and possible contamination of the cell cultures with microorganisms.

We have developed a Microculture Kinetic Assay (MiCK assay) which overcomes the three problems of evaporation, sterility and pH maintenance in microcultures and allows at least a 3-day incubation of mammalian malignant cells in a microplate

reader at 37°C. This paper reports the MiCK assay application for continuous monitoring of the growth of the human leukaemic OCI/AML-2 and mouse tumour J-774.1 cell lines and for assessing their sensitivity to the chemotherapeutic drug, Cytosar (cytarabin, ARA-C). Comparisons are made between the results of chemosensitivity tests obtained with the MiCK assay and those of the conventional short-term thymidine incorporation assay (TIA).

MATERIALS AND METHODS

Cell lines

Cells of the human OCI/AML-2 cell line [11], courtesy of Dr M. Minden (Ontario Cancer Institute, Toronto, Canada), were cultured without added growth factors as a suspension culture. Mouse histiocytic J-774.1 cell line [12] was obtained from the American Type Culture Collection (ATCC) and maintained as a loosely adherent cell layer [13]. Both cell lines were cultured in 75-cm^2 flasks in RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum (FCS), 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a fully humidified atmosphere of 5% CO_2. Cell counts were performed with a haemocytometer and cell viability was determined by the Trypan blue exclusion technique.

MiCK assay for malignant cell growth

AML-2 and J-774 cells were harvested from the suspension of the stationary cultures, washed once and resuspended at the appropriate concentrations in RPMI-1640 medium without phenol red, supplemented with 10% heat inactivated FCS, glutamine and antibiotics (full medium). The cells were then vigorously vortexed and 250-μl aliquots were plated into a 96-well flat-bottomed microplate (Greiner, Germany). The plate was incubated at 37°C in a fully humidified atmosphere of 5% CO_2 for 1 h. 60 μl of sterilised light mineral oil (M-5904, Sigma) was then layered on top of the medium in each well. The plate was covered with a lid, placed in a THERMOmax microplate reader (Molecular Devices Corp.) with a chamber temperature of 37°C, incubated and the optical density (OD) was read at 600 nm over a 72-h period. To avoid clumping in the AML-2 cultures, the AUTOmix feature of the THERMOmax machine was used to shake the microplate before it was read. Reading of the adherent J-774 cells was performed without shaking. The microplate reader was calibrated to 0 absorbance using wells containing only medium without cells (blank wells). When this work was carried out, we had at our disposal only the SOFTmax software which allowed continuous kinetic reading of the culture over 24 h, i.e. every 24 h the programme was restarted and the readings were continued for the next 24 h. During each 24-h period 339 OD readings were taken automatically and plotted to diagram. Every 24 h the results of the last reading were copied from the data file of the software and used as 24-, 48- and 72-h OD endpoint readings when necessary. To quantitate the data on the growth kinetics of the cell populations, the maximum kinetic rate (Vmax) of the growth curves was determined with the same software package and expressed in mOD/min.

Drug. ARA-C (Cytosar, Upjohn, Puurs-Belgium) was dissolved in RPMI-1640 medium and used in three dilutions (5×10^{-8}, 1×10^{-7} and 1×10^{-6} M).

MiCK assay for malignant cell chemosensitivity

AML-2 and J-774 cells were suspended in the full RPMI-1640 medium (1×10^6 or 0.4×10^6 cells/ml, respectively), mixed with ARA-C solution to obtain three drug dilutions and 250 μl aliquots were plated out in wells of a 96-well plate in quadruple. Cells not treated with ARA-C were dispersed into control wells. The wells were sealed with 60-μl aliquots of the sterilised mineral oil, the plate placed into a THERMOmax microplate reader and the optical density read kinetically as above. The percentage of growth inhibition (growth inhibitory index, GII) was calculated in the wells using the following equation:

$$GII_{V\max} = (1 - V\max_{-\text{treated}}/V\max_{-\text{control}}) \times 100.$$

[^3H]thymidine incorporation assay (TIA)

The short-term assay by Dosik and colleagues [14] was used, with minor modifications. Briefly, 250-μl aliquots of the AML-2 and J-774 cells were plated in wells of a 96-well microplate. For the final 2 h of the first, second and third days of the assay, 10 μl of [^{3}H]thymidine (specific activity 41 Ci/mmol, Nuclear Research Center, Negev, Israel) were added to each well for a final concentration of 1 μCi/ml of cell suspension. The cells were collected on a filter paper with an automated cell harvester and [^{3}H]thymidine uptake was counted using a Liquid Scintillation Analyser (Packard, U.S.A.). The results were expressed as counts per minute (CPM) calculated per 10^3 cells. The percentage of the ARA-C-induced inhibition of the [^{3}H]thymidine uptake served as a measure for the antiproliferative effect of the drug [15] and was also expressed as GII:

$$GII_{CPM} = (1 - CPM_{-\text{treated}}/CPM_{-\text{control}}) \times 100.$$

Statistical analysis

The correlation between ARA-C induced growth inhibition determined by MiCK assay and TIA was described by linear regression analysis. Correlation coefficient was tested using a two-sided Student's t-test.

RESULTS

Relation between plated cell number and absorbance (OD)

A linear correlation between cell numbers and OD was demonstrated for both suspension AML-2 and adherent J-774 cells through the entire range of cell concentrations tested (Figure 1). In the J-774 cell population the linearity was equally good for suspension or adhered cells (Figure 1, b and c)

Utility of the incubated microplate reader for maintenance of mammalian cell growth

The optimal seeding concentration ensuring consistent exponential cell growth over 72 h was found to be 10×10^5 cells/ml for the AML-2 cells and 4×10^5 cells/ml for the J-774 cells. At these concentrations, AML-2 and J-774 cells were plated into wells of two 96-well microplates in twelve replicates and incubated either in a conventional CO_2 incubator or sealed as above and maintained in the THERMOmax machine at a chamber temperature of 37°C. During the 72-h incubation period, cell number, cell viability and pH in wells were determined daily.

As shown in Table 1, there were no significant differences in growth parameters between the two cell populations. No bacterial contamination was detected after inoculating media aliquots from the "reader-plate" wells into nutrient broth.

Real time kinetics of the AML-2 and J-774 cell growth

Figure 2 shows the SOFTmax display of the OD/time curves for the 72-h growth kinetics of the two cell lines. A detectable

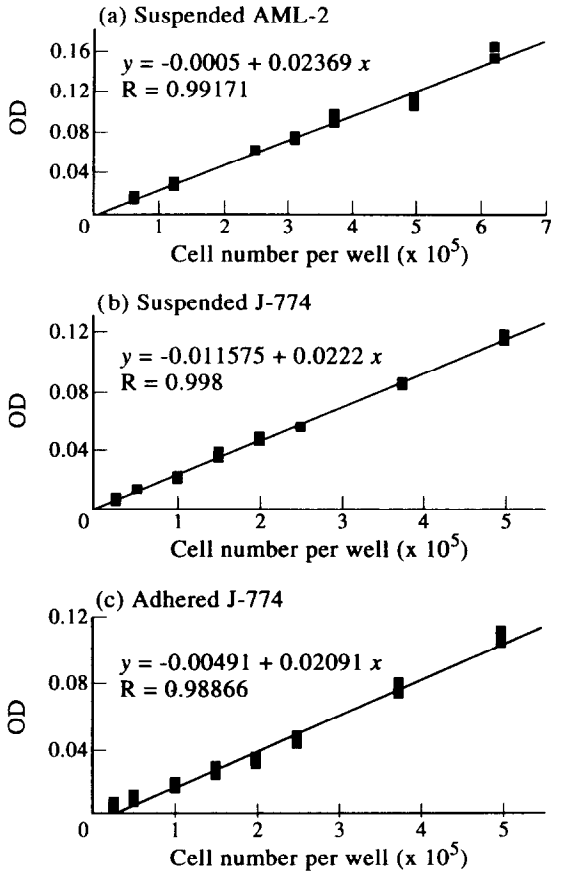

Figure 1. Relationship between plated cell number and absorbance (OD). Each point shows the OD measured in triplicates. The lines plotted are the best fit lines calculated by using linear regresssion analysis.

increase in the OD of the AML-2 cell population was noted at about 8 h after cell plating and continued for the next 64 h. The greatest growth rate of the AML-2 cells was during the first 24 h (Vmax = 0.034 mOD/min). Measuring AML-2 proliferation rate via TIA also pointed to maximal [^{3}H]thymidine uptake at the end of the 1st day (59.9 CPM/10^3 cells). A linear regression analysis was applied to compare Vmax and CPM, with a correlation coefficient (r = 0.951) showing statistical significance between the two assays ($P < 0.001$).

For the J-774 cells, the OD increase started at about 10 h after cell plating and continued for about 61 h before reaching a plateau. The greatest growth rate (Vmax = 0.027 mOD/min), as well as the maximal [^{3}H]thymidine uptake (100.1 CPM/10^3 cells), was noted on the second day of the assay. Regression analysis revealed good correlation between the Vmax and CPM (r = 0.900, $P < 0.001$).

Cell number/OD linear correlation offers an advantage of theoretical calculation of cell number in a well from the OD of the well at any time point of the assay. Table 2 compares cell counts determined using a haemocytometer or calculated from the linear fit equation as follows:

$$n = (\mathrm{OD} + A)/B.$$

where n is cell number per well, OD is absorbance of the well, A is the y-intercept and B is a slope empirically determined and equalling 0.0237 for suspension AML-2 cells and 0.0209 for adherent J-774 cells (see Figure 1, a and c). Excellent correlation between measured and calculated cell numbers was shown for both AML-2 (r = 0.997, $P < 0.001$) and J-774 cells (r = 0.991, $P < 0.001$).

Use of the MiCK assay and TIA for testing the sensitivity of the AML-2 and J-774 cells to ARA-C

In the MiCK assay, direct readout from the screen allowed preliminary estimation of the effect of ARA-C on cell growth kinetics. For better presentation, the OD data collected during the first, second and third days of the assay were extracted from the data file and combined through use of a spreadsheet programme (MicroCal Origin, U.S.A.) to yield continuous 72-h plots (Figure 3a and b).

In both cell populations, ARA-C induced a dose-dependent prolongation of the initial growth delay from 8–10 h in the control wells to 12–18 h in the drug-treated wells. In the AML-2 cell population, cell growth, once initiated, was observed at all concentrations of ARA-C used, and throughout the assay. However, in wells with 0.1 and 1.0 μM ARA-C, a brief growth curve plateau occurred by the end of the second day which persisted until the beginning of day 3, whereupon cell growth was resumed (Figure 3a).

Table 1. Growth parameters of the cell microcultures maintained under conventional conditions of a CO_2 incubator versus incubated microplate reader

		CO_2 incubator			Microplate reader		
Cell line	Time (h)	Cells/ml ×10^6	Cell viability* %	pH in wells	Cell/ml ×10^6	Cell viability* %	pH in wells
AML-2	0	1.0	98.1 ± 0.9	7.4	1.0	98.1 ± 0.9	7.4
	24	1.44 ± 0.1	96.1 ± 1.4	7.4	1.6 ± 0.2	97.0 ± 1.8	7.4
	48	2.48 ± 0.18	92.0 ± 1.6	7.4	2.62 ± 0.2	91.5 ± 1.3	7.3
	72	2.92 ± 0.2	90.5 ± 2.4	7.35	3.24 ± 0.2	90.0 ± 0.8	7.2
J-774	0	0.4	99.7 ± 0.2	7.40	0.4	99.7 ± 0.2	7.4
	24	0.84 ± 0.07	99.3 ± 0.5	7.40	0.92 ± 0.1	97.8 ± 1.5	7.35
	48	1.2 ± 0.1	97.8 ± 0.9	7.35	1.2 ± 0.1	97.5 ± 0.6	7.30
	72	1.38 ± 0.12	90.0 ± 1.3	7.35	1.4 ± 0.1	90.7 ± 2.1	7.30

* Mean ± S.D. of four replicates. At each time point the contents of 12 wells were combined to attain a volume of 3 mL; the pH of this mixture was measured via conventional pH-meter (Corning).

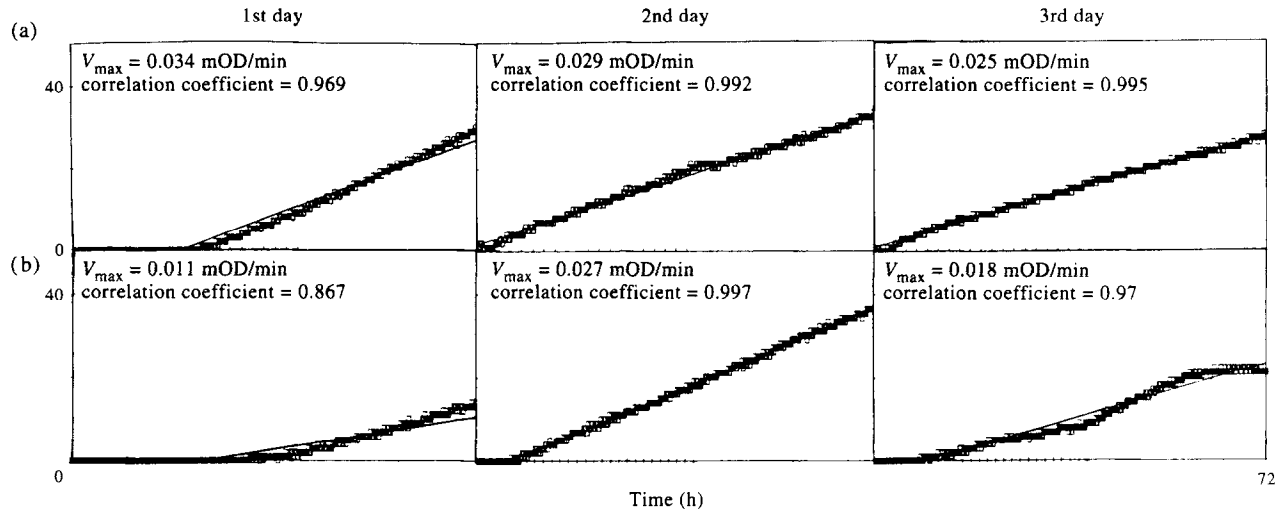

Figure 2. Montage of the SOFTmax displays showing the real time growth kinetics of the (a) AML-2 and (b) J-774 cells. A total of 1017 OD readings were taken during 72 h for each cell line. Each rectangle demonstrates the growth curve followed continuously for 24 h. The return to zero at the start of the second and third days was due to inevitable reloading of the programme after each 24-h run. In the upper left corner of the rectangle are given the *Vmax* and the correlation coefficient for the curve. OD limit used was 0.05 optical density units.

Table 3 provides quantitative data on the effect of ARA-C on AML-2 cell growth as measured by both the MiCK assay and TIA. By either GII_{Vmax} or GII_{CPM} calculations, the inhibitory effect of ARA-C was maximal in wells with 1.0 μM ARA-C during the first 24 h of the assay. The growth kinetics of the J-774 cells treated with 0.05 μM ARA-C was similar to that of control cells, but in wells with 0.1 and 1.0 μM ARA-C the growth rate was considerably lower and eventually stopped by day 3 (Figure 3b). Similar GII_{Vmax} and GII_{CPM} values were obtained (Table 4), indicating the maximal growth inhibition on day 3 in wells with 1.0 μM ARA-C.

DISCUSSION

We report herein on a novel microculture kinetic assay (MiCK assay) employing the THERMOmax microplate reader for continuous monitoring of malignant cell growth. The MiCK assay is based on the existing direct linearity between the number of unstained cells in a well and the OD of the well. Cell multiplication is followed by an increase in the OD that is scored by the reader automatically over a 3-day incubation period, and the OD data are stored on a disk. Where needed, the results on the OD measurements can be extracted from the data file and utilised for calculating the real number of cells per well through the linear fit equation.

Hermetic sealing of the CO_2-saturated cell microcultures with

Table 2. Cell numbers counted in wells by haemocytometer or calculated from the optical density of the wells

Cell lines	Time (h)	Optical density* of wells	Cell number† per well (×10⁵) Counted using a haemocytometer	Cell number† per well (×10⁵) Calculated from linear fit equation†
AML-2	0	0.062 ± 0.003	2.5	2.6 ± 0.14
	24	0.083 ± 0.003	3.67 ± 0.1	3.5 ± 0.14
	48	0.137 ± 0.003	6.1 ± 0.23	5.8 ± 0.14
	72	0.164 ± 0.005	7.2 ± 0.2	6.9 ± 0.3
J-774	0	0.019 ± 0.001	1.0	0.9 ± 0.01
	24	0.030 ± 0.001	1.5 ± 0.02	1.4 ± 0.01
	48	0.056 ± 0.005	2.9 ± 0.03	2.8 ± 0.01
	72	0.066 ± 0.004	3.3 ± 0.1	3.1 ± 0.04

* OD data extracted from the SOFTmax software data file. † OD = $A+B*X$, see Figure 1. Mean ± S.D. of two experiments, each performed in three replicates.

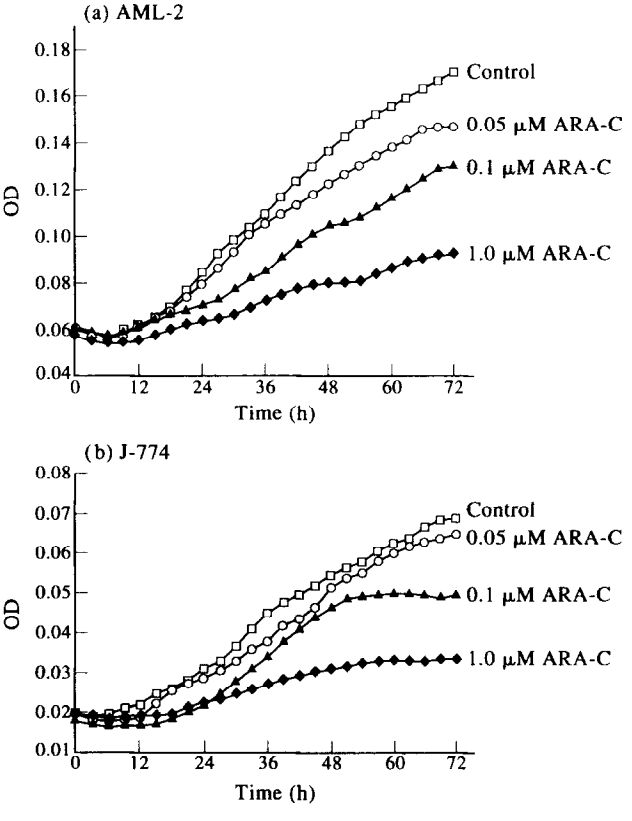

Figure 3. Effect of ARA-C on real time growth kinetics. Each point represents the mean of the four replicates. Coefficient of variation between equally treated wells never exceeded 8%. The OD limit used was 0.05 optical density units.

Table 3. Effect of ARA-C on Vmax and [^{3}H]thymidine uptake in the AML-2 cell population

Time (h)	ARA-C (μM)	*V*max* (mOD/min)	CPM per 10^3 cells†	Growth inhibition index (GII)	
				GII_{Vmax}	GII_{CMP}
0	Medium	–	64.0 ± 3.5	–	–
24	Medium	0.032 ± 0.004	59.9 ± 3.2	–	–
	0.05	0.025 ± 0.002	22.6 ± 1.2	21.8 ± 2.2	62.2 ± 4.2
	0.1	0.019 ± 0.006	21.3 ± 0.9	40.0 ± 4.6	64.4 ± 3.2
	1.0	0.012 ± 0.004	5.1 ± 0.1	62.5 ± 2.4	91.4 ± 3.2
48	Medium	0.029 ± 0.004	23.2 ± 1.3	–	–
	0.05	0.024 ± 0.001	17.1 ± 1.1	17.2 ± 1.9	26.3 ± 2.1
	0.1	0.023 ± 0.002	16.3 ± 1.4	20.6 ± 6.4	29.7 ± 2.3
	1.0	0.018 ± 0.001	7.2 ± 0.5	37.7 ± 6.4	69.0 ± 5.7
72	Medium	0.024 ± 0.001	13.5 ± 0.5	–	–
	0.05	0.020 ± 0.001	13.0 ± 0.2	16.6 ± 4.8	3.7 ± 0.2
	0.1	0.021 ± 0.001	12.2 ± 0.4	12.5 ± 3.6	9.6 ± 0.7
	1.0	0.010 ± 0.001	4.8 ± 0.1	58.3 ± 9.4	64.4 ± 3.1

* Mean ± S.D. of two separate experiments, each performed in four replicates. † Mean ± S.D. of two separate experiments, each performed in three replicates.

a sterile light mineral oil prior to their placement into the reader eliminates fluid evaporation, CO_2 escape and possible microbial contamination over 72 h. No significant differences in terms of growth parameters (number and viability of cells, and pH of the medium) were detected between paired cell microcultures maintained for 72 h in a conventional incubator and those sealed with sterile mineral oil and maintained for the same period in an incubated reader at 37°C.

While grown in the incubated reader, the two cell lines showed approximately three doublings of the growth curve during a 72-h assay, provided that the initial concentrations for the AML-2 and J-774 cells were 10×10^5 and 4×10^5 cells/ml, respectively. Replenishment of the medium in the wells restored cell growth for another 48 h (data not shown), suggesting that depletion of nutrients and probably also a lack of oxygen were the main cell growth limiting factors. It is of interest that growth kinetics of relatively slow proliferating OCI/AML-4 cells [16] seeded initially at 0.8×10^6 cells/ml could be followed for 7 days. Here, too, however, only three doublings of the growth curve were obtained before plateau (data not shown). In the present work, graphic display of both the AML-2 and J-774 growth curves evinced an 8-h and 10-h growth delay, respectively, which was probably the time required for the cells to enter the sensitivity window of the reader.

The MiCK assay is adaptable to the 96-well microplate format and can easily be applied to screening of a wide variety of agents for their effects on cell growth both rapidly and quantitatively. Results of the OD reading are fed into a computer and plotted immediately, so that the real time kinetics of the cell growth might be visualised while the data are collected. To quantitate cell growth kinetics, the maximum slope of the growth curve is measured automatically by the SOFTmax software and reported

Table 4. Effect of ARA-C on Vmax and [^{3}H]thymidine uptake in the J-774 cell population

Time (h)	ARA-C (μM)	*V*max* (mOD/min)	CPM per 10^3 cells†	Growth inhibition index (GII)	
				GII_{Vmax}	GII_{CMP}
0	Medium	–	75.0 ± 4.4	–	–
24	Medium	0.012 ± 0.001	60.5 ± 2.7	–	–
	0.05	0.009 ± 0.001	52.4 ± 5.6	25.0 ± 6.8	13.3 ± 1.2
	0.1	0.006 ± 0.002	47.2 ± 4.2	50.7 ± 6.5	21.9 ± 2.0
	1.0	0.004 ± 0.001	32.5 ± 3.6	66.6 ± 5.8	46.3 ± 4.1
48	Medium	0.026 ± 0.004	100.1 ± 8.2	–	–
	0.05	0.018 ± 0.001	88.0 ± 3.4	30.8 ± 5.4	12.1 ± 1.0
	0.1	0.015 ± 0.002	84.1 ± 3.1	42.3 ± 4.3	16.1 ± 1.2
	1.0	0.011 ± 0.001	9.8 ± 0.1	57.6 ± 1.1	90.2 ± 5.6
72	Medium	0.017 ± 0.002	64.6 ± 4.1	–	–
	0.05	0.015 ± 0.004	60.0 ± 2.1	11.8 ± 0.9	3.3 ± 0.2
	0.1	0.003 ± 0.004	8.5 ± 0.9	82.3 ± 0.9	86.2 ± 4.1
	1.0	0.001 ± 0.001	1.3 ± 0.4	94.1 ± 1.2	98.7 ± 0.7

* Mean ± S.D. of two separate experiments, each performed in four replicates. † Mean ± S.D. of two separate experiments, each performed in three replicates.

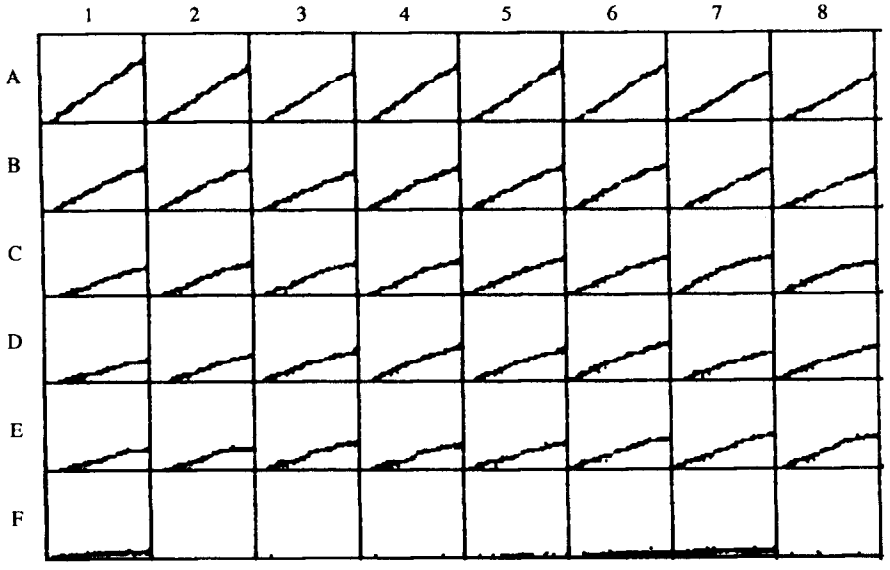

Figure 4. Direct readout of the SOFTmax display showing the effect of ARA-C on growth kinetics of the J-774 cells within the second day of the MiCK assay. Each rectangle represents the growth curve obtained in one well of the 96-well plate. Cells were seeded in eight replicates. A1–A8, control; B1–B8, 0.05 μM; C1–C8, 0.1 μM; D1–D8, 0.5 μM; E1–E8 1.0 μM ARA-C; F1–F8, medium without cells (blank).

as the maximum rate of the curve expressed in mOD/min (Vmax). Correlation between Vmax and [^{3}H]thymidine uptake (CPM) was observed for both AML-2 and J-774 cells, thus attesting to the applicability of the Vmax value in quantitating the growth kinetics of a cell population.

To evaluate the practical application of the MiCK assay, we used it for testing the sensitivity of the AML-2 and J-774 cells to the cycle-specific chemotherapeutic drug ARA-C (Cytosar). The dose dependent inhibitory effect of ARA-C on the growth rate of AML-2 and J-774 cells was evident from the behaviour of the respective growth curves (Figure 3 a, b) and could be quantitated by Vmax measurement and GII_{Vmax} calculation.

The MiCK assay apparently measures the antiproliferative effect of ARA-C on the entire cell population, without distinguishing between tumour clonogenic cells and their descendants. In this respect, the MiCK assay resembles the TIA, which estimates the DNA synthesising pool of tumour clonogenic cells as well as their progeny undergoing terminal division. The use of TIA for testing anticancer drugs *in vitro* dates back to the sixties [17, 18], and the merits and drawbacks of such assay have already been well documented [19, 20]. By now, TIA has undergone a number of technical modifications insofar as short-term chemosensitivity testing is concerned [14, 21–23]. Since the rate of [^{3}H]thymidine incorporation constitutes a measure for the proliferation of malignant cells [14, 23], the ARA-C-induced inhibition of [^{3}H]thymidine uptake can serve as a measure for the antiproliferative effect of the drug. We found it appropriate, therefore, to use the TIA as the standard test against which the MiCK assay for chemosensitivity could be compared. We have found that, by either method, growth inhibition by ARA-C was evinced by calculating the GII_{Vmax} or GII_{CPM}, and the similarity of the results attest to the fact that the MiCK assay is suitable for the quantitative determination of the effect of antiproliferative drugs on malignant cell growth. Moreover, the microculture kinetic assay has a definite informative advantage over the endpoint of TIA, does not require radioactive or chemically hazardous substances, and also bypasses the numerous preparative steps usually necessary to perform radioisotopic assay. Reproducibility of the results in the MiCK assay can easily be achieved by accurate pipetting of cell suspensions into wells of a 96-well plate (Figure 4). As can be seen from Figure 4, the growth curves for wells with identically treated cells display practically no difference. Successful use of the MiCK assay for monitoring of suspension and adherent cell cultures implies that the assay could be employed for chemosensitivity testing of a wide range of malignant cells capable of proliferating *in vitro*, whether from leukaemic patients or from patients with primary solid tumours or metastases.

In summary, the novel MiCK assay affords quantitative, informative and rapid assessment of the growth kinetics of malignant cells, and shows sufficient promise for future use in testing sensitivity of malignant cells to chemotherapeutic drugs. Further evaluation of the MiCK assay in comparison with clonogenic and colorimetric chemosensitivity tests is now under way.

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Feature Articles

The S-100-related Calcium-binding Protein, p9Ka, and Metastasis in Rodent and Human Mammary Cells

R. Barraclough and P.S. Rudland

INTRODUCTION

METASTASIS, the process whereby cancer cells spread from their site of origin to distant sites within the body, is responsible for the majority of deaths from solid cancers such as those of breast, lung, colon and prostate. In order to understand more fully the metastatic process, changes in gene expression in metastatic cells have been sought. Many such changes in metastatic cells result in an increase in expression of proteins which are associated with processes such as proteolysis and cell motility, and a reduction in proteins associated with processes such as cell adhesion [1–3]. However, it is an understanding of the co-ordination and regulation of such cellular processes that is likely to be important in understanding the primary lesions in a metastatic cell. Many intracellular processes are regulated in a highly specific manner by calcium ions acting through calcium-binding proteins. This article concerns a calcium-binding protein which can induce the metastatic phenotype in rat mammary tumour cells.

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UPREGULATION OF p9Ka AND ITS mRNA IN METASTATIC RODENT MAMMARY CELLS

p9Ka was first discovered as a polypeptide which is induced when certain cultured rat mammary epithelial cells change to an