Eur J Cancer Clin Oncol, Vol. 23, No. 3, pp. 273–276, 1987.
Printed in Great Britain

0277-5379/87$3.00+0.00

The ATP Assay is More Sensitive than the Succinate Dehydrogenase Inhibition Test for Predicting Cell Viability

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Abstract—*The succinate dehydrogenase inhibition (SDI) test and the adenosine triphosphate (ATP) assay, are both used for* in vitro *human tumor chemosensitivity testing.*

We exposed HeLa cells to various concentrations of mitomycin C for 1, 2 *or* 3 *days and found that the decrease in number of viable cells correlated with that of succinate dehydrogenase (EC* 1.3.99.1*) activity and that of intracellular ATP level of the viable cells. In the dead cells, the ATP level was extensively decreased, but the succinate dehydrogenase activity remained at a level of* 24% *of that of mitomycin C-untreated viable cells, even on day* 3*. Thus, the ATP level better reflected the cell viability. In clinical situations, the succinate dehydrogenase activity and the ATP level are assayed in whole cells following exposure to anticancer drugs, therefore the activity remaining in the dead cells must be taken into consideration for the chemosensitive prediction with the SDI test, but not with the ATP assay. This higher sensitivity of the ATP assay will enable a more accurate prediction of cell viability.*

INTRODUCTION

TISSUE culture systems are often used in studies related to chemosensitivity testing in cases of malignancy. Several biochemical methods, i.e. isotope incorporation method [1, 2], succinate dehydrogenase inhibition (SDI) test [3, 4] and adenosine triphosphate (ATP) assay [5] can be considered for research and clinical applications. The advantages of these methods are speed, technical simplicity and sensitivity. However, the isotope incorporation method needs an isotope laboratory set-up. The SDI test was introduced for clinical chemosensitivity testing, based on the correlation of the succinate dehydrogenase (EC 1.3.99.1) activity using tetrazolium salt as a hydrogen acceptor with the cell viability [6]. ATP is a basic source of intracellular energy and the intracellular ATP content correlates with the biomass of living cells [7]. Recently, Kangas *et al.* [7], Kuzmits *et al.* [8] and Garewal *et al.* [9] reported that the intracellular ATP level was a good index for evaluating the sensitivity to anticancer drugs.

Accepted 30 June 1986.
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We compared the biochemical chemosensitivity between the SDI test and ATP assay, using HeLa cells and following mitomycin C treatment.

MATERIALS AND METHODS

Cell

HeLa cells were routinely cultured in monolayers on plastic dishes using minimal essential medium (Nissui Seiyaku Co., Tokyo) with L-glutamine (292 mg/l), 10% fetal calf serum (Difco Laboratories, U.S.A.), penicillin (100 U/ml), streptomycin (100 μg/ml) and gentamycin (40 μg/ml). Two × 10^5 HeLa cells were plated in 60 mm plastic dishes in the absence of the drug and were incubated at 37° C in a humidified 5% CO_2 atmosphere for 2 days. The cells in each dish were then exposed to various concentrations: 0.3125, 0.625, 1.25, 2.5, 5.0 and 10.0 μg/ml of mitomycin C with medium changes and were then incubated for 1, 2 or 3 days. The cells were then divided into the floating ones and those attached to the dish. Almost all the floating cells were dead (≒ 100%) while over 95% of the attached cells were viable, determined using the novel dye exclusion method [10]. Both viable and dead cells were washed with phosphate-buffered saline (PBS, pH 7.2) and assayed for succinate dehydrogenase activity and intracellular ATP content.

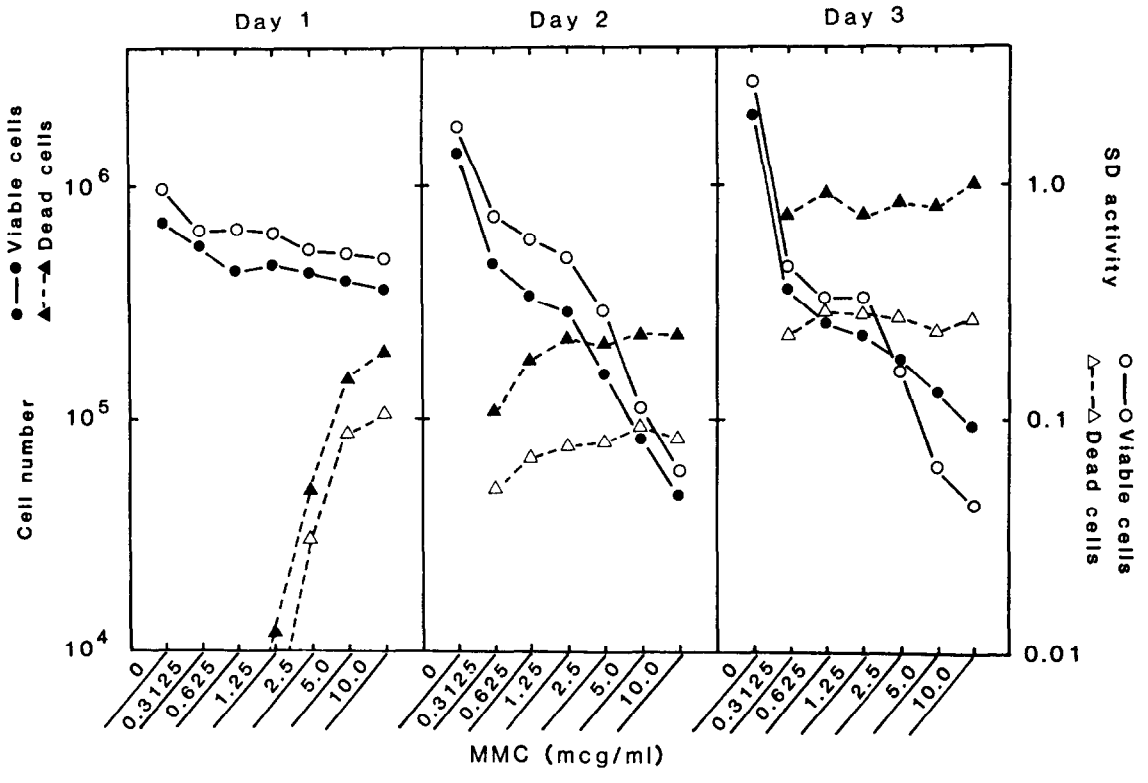

Fig. 1. Changes in cell number and succinate dehydrogenase activity after treating HeLa cells with mitomycin C. Two × 10^5 *HeLa cells were cultured in 60 mm plastic dishes for 2 days, then were exposed to various concentrations:* 0.3125, 0.625, 1.25, 2.5, 5.0 *and* 10.0 μg/ml *of mitomycin C for* 1, 2 *or* 3 *days. Floating cells (dead) and those attached to the dish (viable) were obtained. The cell number was determined by the novel dye exclusion method* [10] (●, *viable cells*; ▲, *dead cells*). *Succinate dehydrogenase (SD) activity was assayed using MTT as a hydrogen acceptor* (○, *viable cells*; △, *dead cells*).

Assay of succinate dehydrogenase activity

The reaction mixture, in a total volume of 1 ml, consisted of PBS, 10 mM sodium succinate and 0.04% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) [11], as the hydrogen acceptor. After incubation at 37° C for 1 hr, the cells in the reaction mixture were precipitated by centrifugation, the formed formazan from MTT was extracted with 3 ml of acetone containing 0.5% trichloroacetic acid and absorbance (optical density, OD) of formazan was measured at 565 nm, using a spectrophotometer. OD of 0.01 could be determined using a DU-50 spectrophotometer (Beckman Co., U.S.A.).

Assay of intracellular ATP

The intracellular ATP was measured using the luciferin–luciferase method [7, 8]. HeLa cells were suspended in 1 ml of 50 mM Tris–HCl (pH 7.75) and 4 mM EDTA, and a 100 μl fraction was transferred to a tube containing 300 μl ATP releasing reagent (Analytical Luminescence Laboratory, U.S.A.). After a vortexing for 15 sec the reaction was initiated by injecting 100 μl of firelight reagent with an automatic injection system. The ATP level was read in a luminophotometer (TD-4000, Labo-Science, Japan), using an internal standardizing technique. The ATP level of 1×10^{-12} moles could thus be determined with this system.

RESULTS

Change in succinate dehydrogenase activity after mitomycin C treatment

The succinate dehydrogenase activity of mitomycin C-untreated HeLa cells was 1.3 OD/ 1×10^6 cells, on each day (Fig. 1). The decrease of succinate dehydrogenase activity of the viable cells correlated with that of the viable cell number, after mitomycin C treatment. The correlation was $r = 0.9417$ on day 1, $r = 0.9933$ on day 2 and $r = 0.9994$ on day 3. The high dose of mitomycin C reduced the succinate dehydrogenase activity per viable cell number on day 3, that is 0.5 OD/ 1×10^6 cells at the concentration of 10 μg/ml. The dead cells retained succinate dehydrogenase activity at the level of 35% of that of mitomycin C-untreated cells on day 1, 27% on day 2 and 24% on day 3.

Change in intracellular ATP level after mitomycin C treatment

The intracellular ATP level of mitomycin C-untreated HeLa cells was 10.5 fmoles/cell, on each day (Fig. 2). The decrease in intracellular ATP level of the viable cells correlated with that of the

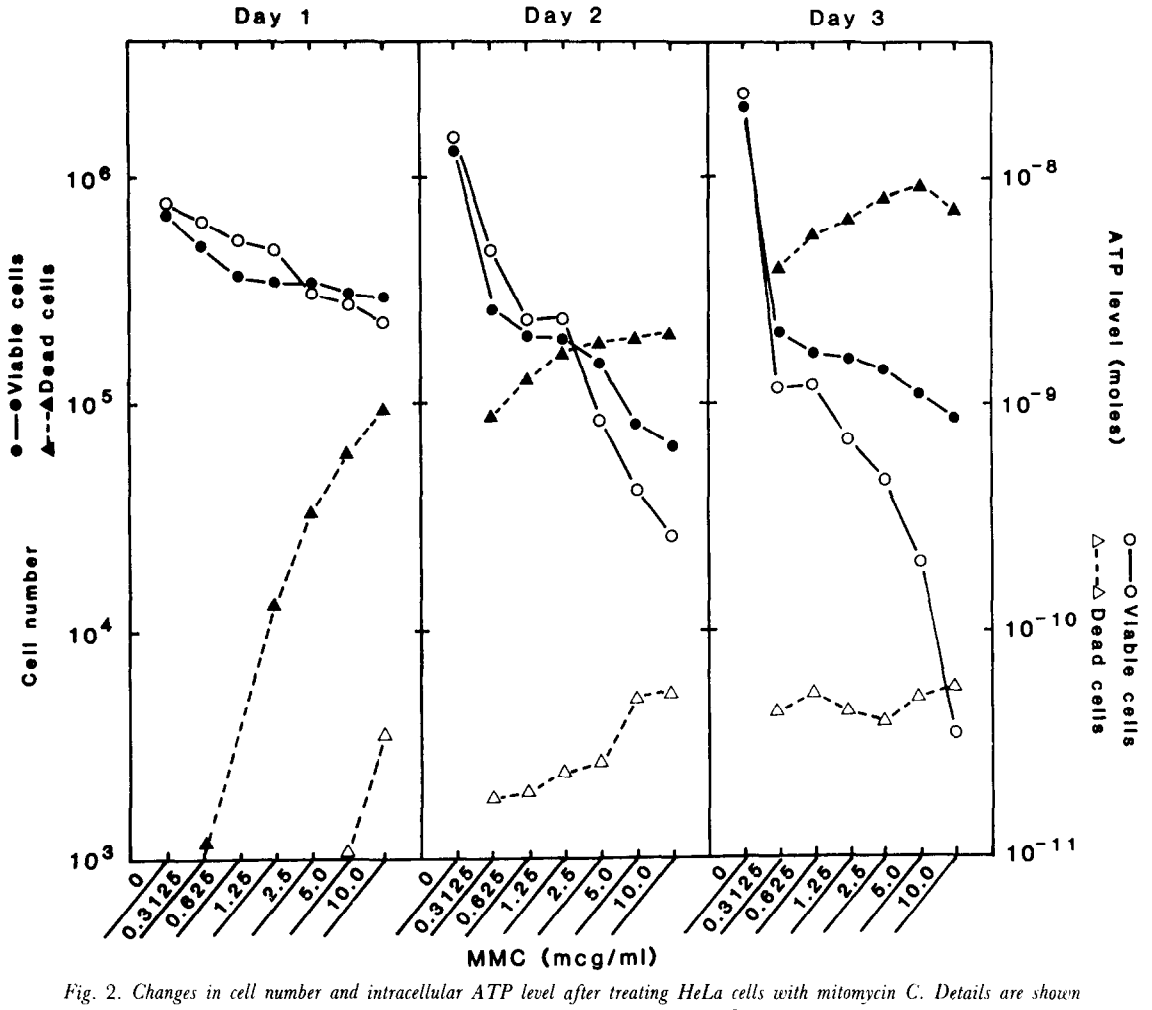

Fig. 2. Changes in cell number and intracellular ATP level after treating HeLa cells with mitomycin C. Details are shown in Fig. 1. The cell number was determined by the novel dye exclusion method [10] (●, *viable cells*; ▲, *dead cells*). *Intracellular ATP level was measured using the luciferin–luciferase method* [7, 8] (○, *viable cells*; △, *dead cells*).

viable cell number after mitomycin C treatment. The correlation was $r = 0.8666$ on day 1, $r = 0.9857$ on day 2 and $r = 0.9998$ on day 3. The ATP level of one cell was decreased in proportion to the concentration of mitomycin C, that is 0.41 fmoles/cell at 10 μg/ml on day 3. The ATP level of dead cells was significantly lower, i.e. two magnitudes lower than that of the viable cells.

DISCUSSION

In vitro test systems measuring changes in cell metabolism allow for prediction of anticancer drug effects [1–5, 12]. Succinate dehydrogenase is an ATP-producing key enzyme of the citric cycle and as the activity of this enzyme correlated well with the cell viability, it could be used to predict the clinical response [3, 4]. The SDI test is a simple, inexpensive and rapid technique for screening anticancer drugs. The number of viable cells correlated with the succinate dehydrogenase activity of the viable cells. However, the dead cells did retain 24% of the succinate dehydrogenase activity seen in the viable cells, even on day 3 after mitomycin C treatment. Much the same was noted in HeLa cells and Chinese hamster V79 cells following mitomycin C, adriamycin or 5-fluorouracil treatment (data not shown). As the succinate dehydrogenase activity is assayed clinically in whole cells in case of the SDI test, the activity remaining in the dead cells must be taken into consideration when assessing chemosensitivity.

On the other hand, the viable cell number correlated with the intracellular ATP level of the viable cells. When the cell died, the respiratory activity ceased and a rapid ATP depression followed. Kangas *et al.* [7] reported that the intracellular ATP level significantly correlated with cell number, viability, [^{3}H]-thymidine incorporation and clonogenic assay. The results can be obtained in a short time and the required number of cells is few with the ATP assay. One determination can be performed using 1000 cells in case of the ATP assay, while 100,000 cells are needed for the SDI test. Therefore, with the ATP assay, a repeat determination of ATP can be carried out at various times of incubation [8]. However, as the two assays

are time-consuming, and the SDI test is less expensive than the ATP assay, the SDI test may be advantageous for mass anticancer drug screening. As the intracellular ATP level of one cell was markedly decreased and the nuclei and cytoplasm were swollen and flattened (data not shown) in the case of 10 μg/ml of mitomycin C on day 3, the biological activity is probably decreased, even if cells are proved to be viable in the novel dye exclusion method.

Our results suggest that compared to succinate dehydrogenase, ATP is a more labile molecule and is rapidly destroyed upon cell death. Thus, the ATP assay has a higher sensitivity than the SDI test for the prediction of cell viability.

Acknowledgements—We thank Drs. Y. Nose and K. Akazawa for statistical analysis and M. Ohara (Kyushu University) for comments on the manuscript.

REFERENCES

1. Wilson AP, Neal FE. *In vitro* sensitivity of human ovarian tumors to chemotherapeutic agents. *Br J Cancer* 1981, **44**, 189–200.
2. Tanigawa N, Kern DH, Hikasa Y, Morton DL. Rapid assay for evaluating chemosensitivity of human tumors in soft agar culture. *Cancer Res* 1982, **42**, 2159–2164.
3. Kondo T, Imamura T, Ichihashi H. *In vitro* test sensitivity of tumor to carcinostatic agents. *Gann* 1966, **57**, 113–121.
4. Kondo T. Prediction of response of tumor and host to cancer chemotherapy. *Natl Cancer Inst Monogr* 1971, **34**, 251–256.
5. Grönroos M, Kangas L, Mäenpää J, Vanharanta R, Paul R. Steroid receptors and response of ovarian cancer to cytostatic drugs *in vitro*. *Br J Obstet Gynaecol* 1984, **91**, 479–482.
6. Dallne G. Viability and tetrazolium salt in ascites tumours. *Nature* 1960, **186**, 176.
7. Kangas L, Grönroos M, Nieminen A-L. Bioluminescence of cellular ATP: a new method for evaluating cytotoxic agents *in vitro*. *Med Biol* 1984, **62**, 338–343.
8. Kuzmits R, Aiginger P, Frass M, *et al.* Influence of cytostatics on ATP-levels of leukemic cells. *Adv Exp Med Biol* 1984, **165**, 383–388.
9. Garewal HS, Ahmann FR, Celniker A. The ATP assay: anti-cancer drug effects on malignant cell growth. *Sci Proc Am Assoc Cancer Res* 1985, **26**, 334.
10. Weisenthal LM, Marsden JA, Dill PL, Macaluso CK. A novel dye exclusion method for testing *in vitro* chemosensitivity of human tumors. *Cancer Res* 1983, **43**, 749–757.
11. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983, **65**, 55–63.
12. Weisenthal LM, Lippman ME. Clonogenic and nonclonogenic *in vitro* chemosensitivity assays. *Cancer Treat Rep* 1985, **69**, 615–632.