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In vitro Assays for Antitumour Activity: More Pitfalls to Come?

A.-R. Hanauske

CHOOSING A GOOD screening system to search for antitumour activity of chemicals is not a trivial problem. It is even more complicated if, in addition to screening for antitumour activity the objective is also to predict tumour response in the clinic. Until a few years ago, the search for new agents relied on tests that made use of mouse leukaemia cells and a limited number of mouse and human xenotransplants. These were mostly fast growing cells and led to the discovery of some active agents. However, these models might not adequately resemble the biology of slow growing human tumours and thus might produce false negative results. Indeed, the results were unsatisfactory for the development of clinically active drugs against common forms of human cancer, particularly lung, breast and colon cancer [1]. The screening of the vast number of available chemicals could only be expanded if there was a fast, simple, sensitive, reproducible and in expensive assay that could be automated. The MTT assay was most promising and was adapted for large scale screening [2–5]. With all the publicity associated with the assay it is important that we remind ourselves of its limitations, in particular that it does not provide a direct measure of cell growth.

In this issue of the *European Journal of Cancer*, a study by Pagliacci and coworkers addresses the limitations of the MTT

Correspondence to A. R. Hanauske at the Division of Hematology and Oncology, I. Department of Medicine, Klinikum rechts der Isar der TechnischenUniversität München, Ismaninger Str. 22, 8000 München, F.R.G.

assay [6]. The authors report that the isoflavone genistein, an agent known for its inhibitory effects on tyrosine kinase and topoisomerase II activity, effectively arrests cell growth in three malignant cell lines (MCF-7 breast cancer, Jurkat T-cell lymphoma, 929 transformed mouse fibroblasts) when direct cell counting is performed. However, if the effects of genistein are determined using the MTT assay, two cell lines (MCF and Jurkat) showed a stimulation of formazan production and the third failed to show any effect. This false negative result would have led to the conclusion that genistein is not affecting cell growth. Pagliacci *et al.* provide evidence that genistein induces a proliferation arrest at the G2/M transition and that the misleading results of the MTT assay may be due to an increased number and/or increased activity of mitochondria in arrested cells. Unfortunately, the authors have not expanded their studies to other agents that either inhibit G2/M transition or modulate the activity of tyrosine kinases or topoisomerase II. Of course, it would be important to know whether the observations can be generalised and to further elucidate the underlying mechanisms. Of particular interest would be to determine whether clinically established compounds would have been missed by the MTT assay.

The problems associated with antitumour drug screening can be considered at two levels. First, there are conceptual problems including the choice of clinically relevant drug concentrations, intratumour and intertumour heterogeneity if freshly explanted specimens are to be studied, predictivity for clinical activity of the cell lines used and whether this predictivity changes with increasing passage number. Other problems include interference of experimental conditions with the usual physiological microenvironment of tumour cells as they exist in the patient, selection pressure on tumour cells by the experimental system used, potential pharmacokinetic variations between patients and various disease states, and the clinical assessment of tumour response. The ultimate test for *in vitro* assays lies in the demonstration of accurate prediction of clinical activity. At the very least, the *in vitro* assay needs to correlate with *in vitro* cell death.

Second, there are specific assumptions to be made for each experimental system. Specifically, with the MTT assay it is assumed that the rate of formazan production is proportional to the total number of cells and that reductive reactions are similar in treated and untreated cells. Any agent that enhances or decreases the cell's reductive capacity with or without affecting cell number will have the potential for giving flawed results. Other investigators have earlier pointed to problems with the MTT assay and have attributed false negative results to differences in pH between the media [7]. In their study, interferon-α and interferon-γ failed to show any antiproliferative activity against human lung cancer cell lines. Direct cell counting, however, revealed a clear decrease in cell number. Interferon-treated cells showed increased cell size and increased mitochondrial activity. There was no change in cell cycle distribution.

Shall we lose confidence in *in vitro* screening in general or in the MTT assay? My view is that we should not. The MTT assay as well as other established assays like soft agar cloning of fresh or established tumour cells, thymidine incorporation etc. are still valuable tools for anticancer drug development. However, no assay gives the whole story, rather it is a piece in a mosaic. If we are aware of their limitations and devise intelligent strategies to compensate for them we will be able to get the best out of *in vitro* assays—for the benefit of our patients.

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