



A Fast Bioassay for the Evaluation of Anticancer Drugs

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- Are the cells sensitive or resistant to a particular anticancer drug
- How fast does a drug act on a cell
- Can different phases in the mode of action of the drug be distinguished

TAM can be used as an extremely fast bioassay that shows the depression of proliferation and metabolism immediately after the addition of a cytotoxic drug. In addition, dose-response curves can easily be constructed and the sensitivity to the drug can thus be evaluated.

INTRODUCTION

It is well-known that tumour cells grow fast with an enhanced rate of metabolism. When an anticancer drug is added to tumour cells the expected response is ceased growth and decreased metabolism finally

Thermal Activity Monitoring is a method that uses the heat flow as a measure of the rate of metabolism and growth. TAM enables the observation of cellular events directly upon addition of the drug. The action is monitored continuously and different phases in the mechanism of action can be distinguished. Moreover, dose-response curves can be constructed which makes it possible to judge if a cell line should be considered resistant to the drug.

EXPERIMENTAL IN SHORT

A stirred vessel with possibilities to make additions was loaded with a suspension of T-

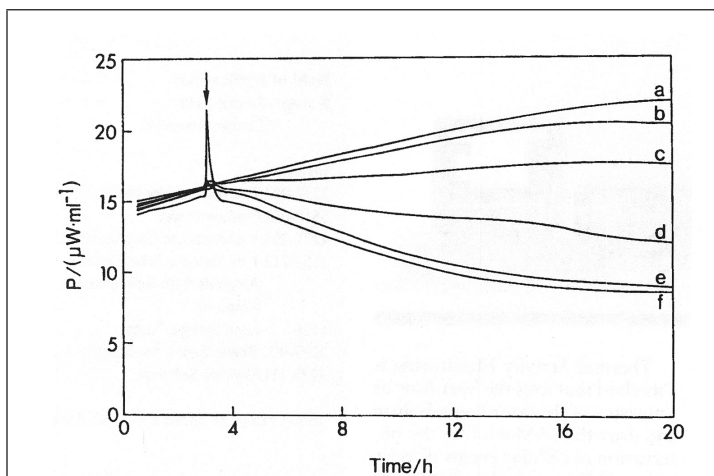


Figure 1. Heat flow, P , expressed in $\mu\text{W}/\text{ml}$ as a function of time recorded for 6 stirred cell suspensions. Methotrexate was injected at the time indicated by the arrow. The final concentrations of drug were here a) 0, b) 0.2, c) 0.5, d) 1.0, e) 2.0, and f) $4.0 \mu\text{mol}/\text{l}$. The cell concentration was 0.90×10^6 cells/ml.

lymphoma cells. The vessel was transferred to the measuring position of TAM and the metabolism and growth was recorded for about an hour. The desired amount of methotrexate was injected directly into the cell suspension and the change in metabolism was recorded continuously for another 20 hours. This application note presents results from the action of the anti cancer drug methotrexate on responding and resistant T-lymphoma cells.

RESULTS

The results of several experiments showing the heat flow produced by a cell suspension before and after addition of different amounts of drug can be seen in Fig.1. The change in heat flow caused by the drug was used to construct dose response curves. Such curves are shown in Fig. 2A for responding and in Fig. 2B for methotrexate resistant Tlymphoma cells, respectively. The 'resistant' cell line was calculated according to the TAM data to be 160 times less sensitive to methotrexate than the responding cell line.

The calculation method used for the construction of a dose response curve is described in detail in the literature references. In short the response, **R**, was derived by comparing the extreme values of the slopes before and after injection of drug.

CONCLUSION

With the bioassay described here the response of a drug can be seen within an hour after addition. The change in curvature recorded after drug addition takes into account all effects of the anticancer drug and can be used to construct a general dose-response curve.

These experiments were done with one drug and two different cell lines. The studies are still in progress, however, and researchers are now focusing on e.g. synergistic effects of two or several drugs used simultaneously.

REFERENCES

1. Bermudez.J., Bäckman, P. and Schön. A. (1992) Microcalorimetric Evaluation of the Effects of Methotrexate and 6-Thioguanine on Sensitive T-Lymphoma Cells

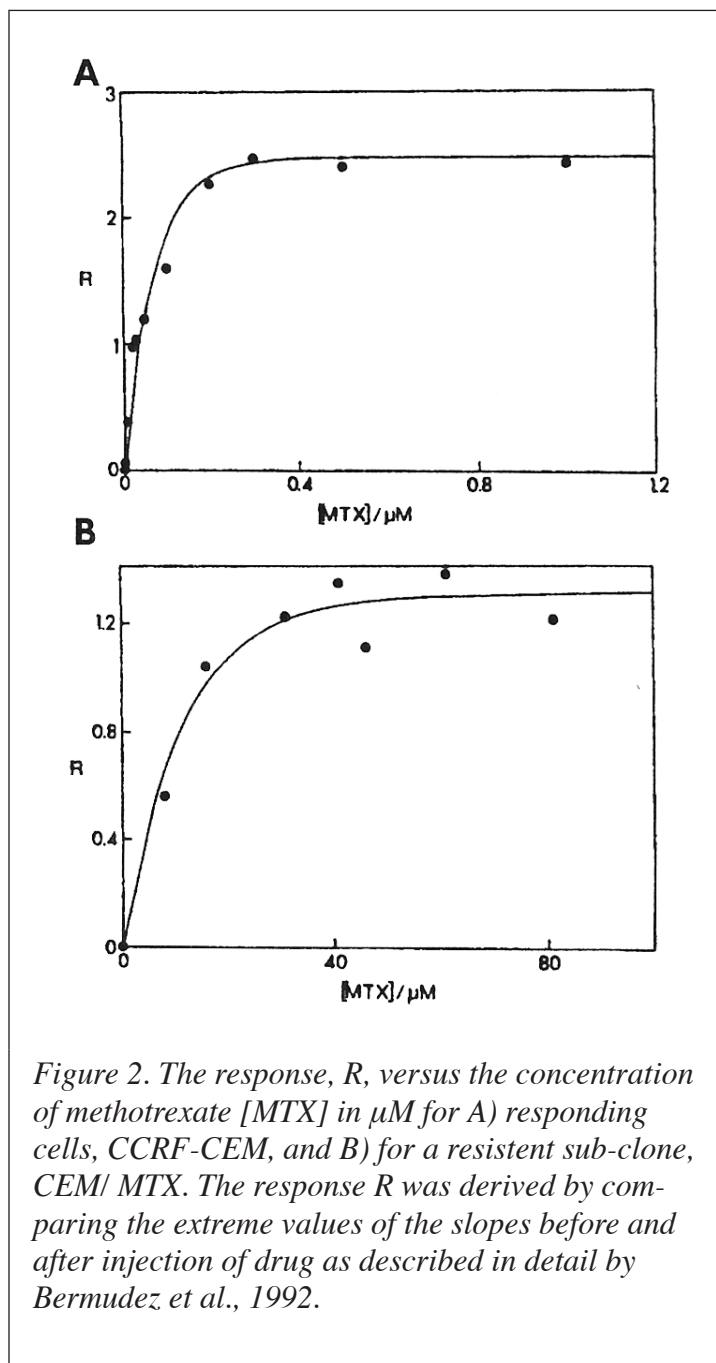


Figure 2. The response, R , versus the concentration of methotrexate $[MTX]$ in μM for A) responding cells, CCRF-CEM, and B) for a resistant sub-clone, CEM/MTX. The response R was derived by comparing the extreme values of the slopes before and after injection of drug as described in detail by Bermudez et al., 1992.

- and on a Methotrexate-Resistant Subline. *Cell Biophys.* 20. 111-123.
2. Roig, T. and Bermudez, J. (1995) Microcalorimetric Evaluation of the Effect of Combined Chemotherapeutic Drugs. *Biochim. Biophys. Acta* 1244, 283-290.