Diabetologia (2004) 47:2042–2043

Diabetologia

The use of tetrazolium salt-based methods for determination of islet cell viability in response to cytokines: a cautionary note

To the Editor: We have reason to believe that cell viability bioassays that utilise tetrazolium salts are unsuitable for assessment of islet cell viability following treatment with the cytokines IL-1 and IFN-γ. Tetrazolium salts, such as MTT and XTT, are reduced by living cells to highly coloured endproducts called formazans, and are frequently used as cell proliferation and cytotoxicity bioassays. Both MTT and XTT assays allow microplate analysis, requiring no cell transfers, and they produce a visible endpoint that can be quantified by an ELISA plate reader.

Our area of interest lies in the cytokine-induced death of beta cells. As beta cell metabolism [1] and viability in response to cytokines have previously been assessed with the MTT assay [2, 3, 4, 5], we used the MTT and XTT bioassays, expecting them to provide convenient and rapid read outs of beta cell survival in vitro. To our surprise, cytokine-induced beta cell death was not accompanied by a reduced MTT/XTT-signal. As shown in Figure 1, exposure of isolated rat islets and beta-TC6 cells to cytokines (50 U/ml IL-1β, 1000 U/ml IFN-γ and1000 U/ml TNF-α) for 24 h reduced the viability of the cells by 42% and 26% respectively, as assessed by propidium iodide staining followed by flow cytometry analysis. However, cells subjected to the same cytokine treatment did not significantly affect the MTT signal. Even more surprising is the fact that the XTT signal was significantly increased both when rat islets and beta-TC6 cells were treated with cytokines.

The failure of the formazan salts to reflect beta cell death in response to cytokines is probably the result of an inducible nitric oxide synthase (iNOS)-mediated reduction of MTT and XTT. It has been known since the fifties that enzymes with diaphorase activities are able to reduce tetrazolium salts [6], and in the nineties, it was reported that NOS possesses diaphorase activity [7]. Thus, it is possible that the cytokine-induced loss of beta-cell viability is masked by the iNOS tetrazolium-reducing activity. However, it is not clear why cytokines induce a significant increase in the reduction of XTT, but not MTT. MTT is reduced by NAD(P)H-dependent microsomal enzymes, whereas XTT is preferentially reduced at the cell membrane and requires, in addition to NAD(P)H, intermediate electron acceptors and possibly superoxide [8]. Thus, the different biochemical properties of XTT and MTT may be responsible for a more efficient iNOS-mediated reduction of XTT than MTT.

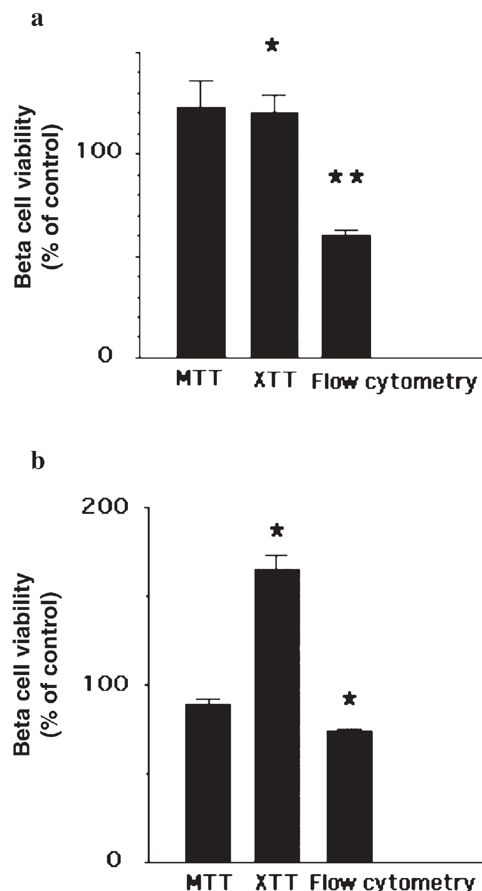

Fig. 1. Cytokine-induced beta cell death is not paralleled by a lowered MTT/XTT reduction. Rat islets (**a**) and beta-TC6 cells (**b**) were incubated for 24 h with or without 50 U/ml of IL-1β, 1000 U/ml of IFN-γ, plus 1000 U/ml of TNF-α. The number of remaining viable cells was determined by propidium iodide staining (10 µg/ml for 15 min) followed by flow cytometry analysis. The MTT and XTT bioassays were performed using the Cell Proliferation Kits I (MTT) and II (XTT) according to manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). The viability of the treated cells is expressed as a percentage of that of the control cells not exposed to cytokines. The data points show the means ± SEM for three independent experiments. * $p<0.05$ vs control cells; ** $p<0.01$ vs control cells using the Student's *t* test

An alternative explanation for the lack of correlation between MTT/XTT reduction and cell viability in cytokine-treated cells is that nitric oxide has a direct effect on the tetrazolium salts. To investigate this, we analysed tetrazolium salt reduction following the addition of the nitric oxide donor DETA/NONOate (2 mmol/l) for 24 h to the culture medium in the absence of cells. We observed a small increase (20% of the signal observed with control cells, data not shown) in MTT re-

DOI 10.1007/s00125-004-1566-5
Received: 9 September 2004 / Accepted: 21 September 2004
Published online: 1 December 2004
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duction, indicating that MTT interacts directly with nitric oxide or DETA/NONOate in the absence of cells. However, the amount of nitric oxide produced by cytokine-stimulated cells is considerably smaller than that generated by 2 mmol/l DETA/NONOate, which suggests that the contribution of the direct interaction between nitric oxide and MTT is negligible.

In summary, our findings call for caution when using tetrazolium salt-based methods, particularly the XTT method, to assess the viability of cells that express high levels of iNOS.

A. Barbu, N. Welsh
Department of Medical Cell Biology,
Biomedical Centre, Uppsala University,
Uppsala, Sweden

References

1. Janjic D, Wollheim CB (1992) Islet cell metabolism is reflected by the MTT (tetrazolium) colorimetric assay. Diabetologia 35:482–485
2. Janjic D, Asfari M (1992) Effects of cytokines on rat insulinoma INS-1 cells. J Endocrinol 132:67–76
3. Chen G, Hohmeier HE, Newgard CB (2001) Expression of the transcription factor STAT-1 alpha in insulinoma cells protects against cytotoxic effects of multiple cytokines. J Biol Chem 276:766–772
4. Lortz S, Tiedge M, Nachtwey T, Karlsen AE, Nerup J, Lenzen S (2000) Protection of insulin-producing RINm5F cells against cytokine-mediated toxicity through overexpression of antioxidant enzymes. Diabetes 49:1123–1130
5. Karlsen AE, Ronn SG, Lindberg K et al. (2001) Suppressor of cytokine signaling 3 (SOCS-3) protects beta-cells against interleukin-1beta- and interferon-gamma-mediated toxicity. Proc Natl Acad Sci USA 98:12191–12196
6. Farber E, Sternberg WH, Dunlap CE (1954) Tetrazolium stains for diphosphopyridine nucleotide (DPN) diaphorase and triphosphopyridine nucleotide (TPN) diaphorase in animal tissue. Proc Soc Exp Biol Med 86:534–537
7. Hope BT, Michael GJ, Knigge KM, Vincent SR (1991) Neuronal NADPH diaphorase is a nitric oxide synthase. Proc Natl Acad Sci USA 88:2811–2814
8. Berridge MV, Tan AS, McCoy KD, Wang R (1996) The biochemical and cellular basis of cell proliferation assays that use tetrazolium salts. Biochemica 4:14–19

A. Barbu (✉)
Department of Medical Cell Biology,
Biomedical Centre, Uppsala University,
P.O. Box 571, 75123 Uppsala, Sweden
E-mail: Andreea.Barbu@medcellbiol.uu.se
Tel.: +46-184714395, Fax: +46-184714059

Abbreviations: iNOS, inducible nitric oxide synthase