

Comparison between the TC10™ Automated Cell Counter and the Lactate Dehydrogenase (LDH) Assay to Assess Cellular Toxicity in Vitro

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Introduction

Determination of cell viability is an important issue when working with cellular models. Various assays, based on morphological (for example, electron microscopy) or biochemical (for example, caspase activation) characteristics of dying or dead cells, have been developed to assess cell death-related parameters. Among these assays, those measuring membrane integrity are the most commonly used even if they cannot discriminate between the different known forms of cell death. Loss of plasma membrane integrity is considered a point of no return while the occurrence of other biochemical events does not necessarily mean that cell death will ensue (Kroemer et al. 2009). The loss of membrane integrity can be measured by using exclusion dyes (that is, dyes, such as trypan blue, that cannot enter healthy cells but that are taken up by cells with permeabilized plasma membranes) or by measuring the activity of cytosolic enzymes (for example, LDH) released in the culture supernatants after rupture of the plasma membrane.

Here we present the results of a comparison study between the TC10 cell counter and the classic LDH assay to measure cell viability in suspension cells treated with two different toxic agents.

Materials and Methods

K562 cells were maintained in GIBCO RPMI medium (Life Technologies Corporation) supplemented with 10% fetal calf serum, streptomycin (100 µg/ml), and penicillin (100 IU/ml) at 37°C in a 95% air/5% CO₂ atmosphere with 100% humidity. For the experiments, K562 cells were incubated at a concentration of 1 x 10⁶ cells/ml.

Cell viability was estimated by using the TC10 automated cell counter (Bio-Rad Laboratories, Inc.), following manufacturer's instructions. An aliquot of the cell suspension (10 µl) was added to 10 µl of 0.4% trypan blue solution (Bio-Rad) in a test tube and mixed by gently pipetting up and down several times. Ten microliters were then transferred into the counting chamber of a TC10 counting slide and analyzed using the TC10 cell counter.

LDH activity was measured according to the procedure of Wroblewski and Ladue (1955), both in the culture medium and in the cell pellet obtained after centrifugation. Briefly, an aliquot of cell suspension (1 ml) was centrifuged at 1,000 x g for 1 min at 4°C. Five hundred microliters of cell-free supernatant were saved without disturbing the cell pellet. The remaining supernatant was discarded and the pellet was suspended in 1 ml of ice-cold phosphate buffer (pH 7.4). Both resuspended pellet and supernatant samples were treated by addition of Triton X-100 (2% final concentration) to lyse cells and were diluted threefold and twofold, respectively, with distilled water. Fifty microliters of these solutions were placed in 2.75 ml of phosphate buffer, in the presence of NADH (120 µM). Samples were heated at 37°C for 20 min and the LDH reaction was started by the addition of pyruvate (600 µM). NADH conversion to NAD⁺ was monitored spectrophotometrically at 340 nm. Cell viability was measured by calculating the ratio of released LDH activity to total activity. Results are expressed as 100% minus the ratio of released activity to the total activity.

Results and Discussion

K562 cells, an erythroleukemia cell line, were treated with 25 µM of doxorubicin, an anthracycline antibiotic commonly used to treat several types of cancers, and two concentrations of menadione (50 and 200 µM), a redox cycling agent that induces an oxidative stress. Twenty-four hours after treatment, cell viability was determined by measuring trypan blue exclusion using the TC10 cell counter and by measuring LDH activity. The two methods gave very similar viability results for the doxorubicin treatment experiment, showing a 40% decrease of cell viability. Similar cell viability results were also obtained with the two methods for the menadione treatment. However, cell viability reported by the TC10 cell counter was lower than that reported by the LDH assay (40% and 57%, for the LDH assay and the TC10 cell counter, respectively).

Based on these data, we propose that the TC10 cell counter can advantageously replace the LDH assay, both to measure the viability of cell suspensions and the cytotoxic effects of various compounds. Cell counting with the TC10 cell counter is faster than performing a long and time-consuming biochemical assay (a few minutes instead of several hours) and it avoids the classic pitfalls encountered with the LDH assay (for example, natural degradation of the enzyme, influence of the pH, inhibition by specific components of the culture medium).

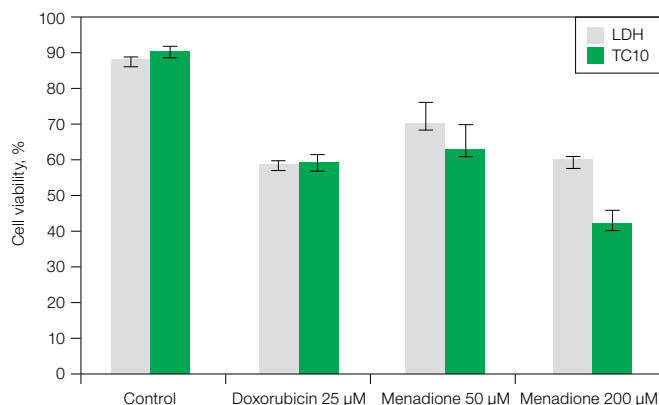


Fig. 1. Determination of cell viability after exposure to different toxic agents. K562 cells were incubated at a density of 1×10^6 cells/ml and exposed to the anthracycline antibiotic, doxorubicin, or the pro-oxidant, menadione, for 24 hr. Cell viability was determined as indicated in the Materials and Methods section.

Acknowledgements

The authors are grateful to Isabelle Blave and Véronique Allaëys for their excellent technical assistance.

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