Doxorubicin effects on TK1 protein levels in leukemia and breast cancer cell cultures measured using AroCell TK 210 ELISA: A tool for drug development.

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Introduction

➢Thymidine Kinase 1 (TK1) is a cytosolic enzyme involved in DNA precursor synthesis and its activity is cell cycle regulated.
➢Uncontrolled cell proliferation is a major characteristic of cancer progression which involves increased DNA synthesis and up-regulation of TK1 protein (1).
➢TK1 has been widely used to study cell proliferation and malignancies *in-vivo*, however, there have been few studies presented on its application to *in-vitro* cell culture methods, as are widely used in drug development.
➢Recent development of an ELISA by AroCell for determining TK1 protein levels in serum has extended the utility of TK1 as a biomarker for monitoring therapy and detecting recurrence in various malignancies (2).

➢Doxorubicin is an anti-cancer agent commonly used for the treatment of a variety of cancers. Oxidation of Doxorubicin results in production of reactive oxygen species which is a process that can lead to DNA damage and may result in cell death.

➢The aim of this study was to evaluate the effect of Doxorubicin on leukemia and breast cancer cell lines in-vitro using measurements of TK1 protein levels in cell extracts and culture media supernatants with the AroCell TK 210 ELISA kit.

Materials and Methods

Cell culture method

➢Human lymphoblastic leukemia cells (CCRF-CEM) were cultured in RPMI media with 10% fetal bovine serum and adenocarcinoma breast cancer cell lines (MDA MB-231) were cultured in DMEM media with 10% fetal bovine serum. in a humidified atmosphere containing 5% CO_2 at 37° C.

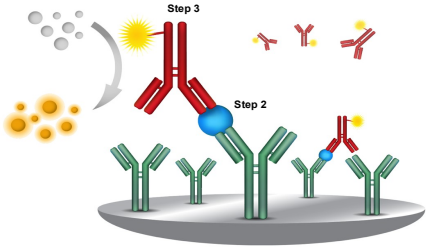
➢ Both cell lines were seeded into 96-well plates at concentrations ranging from 8000 cells to 800 cells per well. Cells were exposed to 0, 0.5, 1, 5 or 10 µM Doxorubicin for 24 hours.

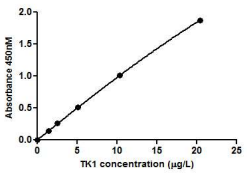
➢After 24 hours of exposure, culture media were centrifuged and the cell pellets lysed with a buffer containing 50mM Tris/HCl, pH 8.0, 150 mM NaCl, 0.5% NP-40 and 0.1% SDS.

➢The TK1 protein levels in cellular extracts and culture media were determined by using the AroCell TK 210 ELISA kit as described (www.e-labeling.eu/ARO1001-15-3) Figure 1).

AroCell TK 210 ELISA: A sensitive assay to measure the TK1 protein

- **Step-1:** Pre-incubation
- Cell extracts and culture supernatants are pre-incubated with AroCell sample dilution buffer, the buffer will expose the TK1 epitope that facilitate the binding of antibody to TK1 in samples.
- **Step-2:** The treated samples are added to a microtiter plate coated with anti-TK 210 monoclonal antibodies to which the TK1 complexes are bound.
- **Step-3:** The bound TK1 complexes are detected using a second biotinylated anti TK 210 monoclonal antibody followed by addition of a streptavidin-HRP conjugate.
- Following the addition of TMB substrate, the resulting optical density is proportional to the TK1 concentration in the samples and standards.
- The concentrations of TK1 in the samples are determined using a standard curve and a 4PL curve fit program.





Standard curve for TK 210 ELISA (absorbance of calibrators reduced from the blank)

Fig 1: Principle of TK 210 ELISA assay

The AroCell TK 210 ELISA kit is for research use only in the USA.
Not for use in diagnostic procedures.

15-3RE17001-02

CONCLUSIONS

✓ **These results demonstrate that AroCell TK 210 ELISA can be used for measuring TK1 protein as a biomarker in in-vitro studies, particularly with drugs targeting cell proliferation and DNA damage.**

Results

❖ To evaluate the linearity of the AroCell TK 210 ELISA kit, TK1 content from unexposed cells was plotted against the cell number. A linear relationship was found for both cell lines (Figures 2 A and 2B).

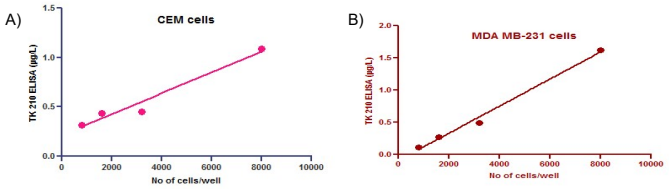


Fig 2: Relation between TK1 protein and cell number

❖ TK1 protein levels in CEM cell extracts increased about 2 fold with increasing concentration of Doxorubicin up to 1µM. Changes inTK1 protein were expressed as % change after normalization to the unexposed TK1 values as shown in Fig 3.

❖ However, TK1 protein levels in culture media only increased significantly at the 10 µM dose when a clear decrease in intra cellular TK1 levels was apparent (Figures 3 A and 3B).

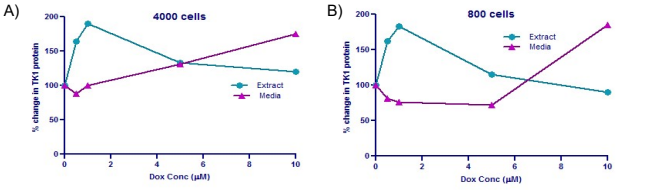


Fig 3: Percentage change in TK1 protein levels in CEM cells after doxorubicin treatment

❖ In MDA MB-231 cells, the intra cellular TK1 protein levels increased two fold, when the concentration of Doxorubicin was increased from 0 to 10 µM.

❖ Whereas, the TK1 levels in culture media only showed minor change at 2-10 µM Doxorubicin treatment (Figures 4A and 4B).

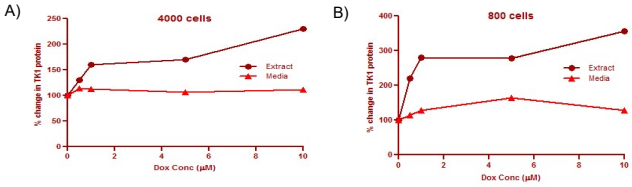


Fig 4: Percentage change in TK1 protein levels in MDA MB-231 cells after doxorubicin treatment

❖ These results show that changes in intra-cellular TK1 and in the media supernatant were detectable using only 800 cells/well (both CEM and MDA MB-231).

❖ The changes in the TK1 protein levels in response to Doxorubicin treatment indicate an induction of TK1 in cells probably due to DNA damage and a release of TK1 protein into the medium at higher doses due to cytotoxicity (3).

References

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