OMNI Life Science

Application Note: Cytostatic Drug Effects

Analysis of Cytostatic Drug Effects using the 24-channel microscope zenCELL owl

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Introduction

Assays designed to measure cytotoxicity in vitro are used to predict tissue-specific toxicity or to identify and classify leads for anti-cancer therapies. The cytotoxicity assay is widely used in the pharmaceutical industry and is a fundamental tool in the drug discovery process.

Conventional cytotoxicity assays make use of occurrences during cell death such as loss of membrane integrity (LDH release), activation of cell death-inducing enzymes (Caspases), loss of mitochondrial activity (MTT metabolism) or changes in intracellular ATP content.

One limiting factor of commonly used cytotoxicity assays is the determination of cell death by end-point parameters. The use of markers can have unpredictable consequences on the accuracy of those conventional assays. For example, the addition of organic or reducing agents during a determinable period of time can be toxic for cells. To avoid changes in cell cultures induced by the measuring method you need assays that can be conducted marker-free.

This application note describes the ability of the zenCELL owl 24-channel incubator microscope (Figure 1) to analyze effects of cytostatic compounds on cell growth and morphology in a non-invasive and continuous manner. Integrated image processing algorithms allow for a continually long-term observation and provide fast and concise results concerning the quality of the analyzed cell culture. A software especially developed for the zenCELL owl determines the current cell count and the cell coverage of the substrate's surface of the section enlarged by the microscope (1,2 mm x 0,9 mm) via a real-time data analysis. The output includes the number of cells attached to the substrate and the cells detached from it. Simultaneously the microscope documents the quality of each of these individual cell cultures using image recordings. Analysis are performed in all 24 Wells of a standard cell culture plate at the same time. The recording interval can be set from 10 min to several hours max.

The zenCELL owl is a 24-channel microscope designed for fast and automated cell culture microscopy. Combining stability and small size it is perfectly suitable for use in incubators. The modular design allows flexible configurations to ensure a secure analysis of biological samples. The small size leaves enough space in the incubator for other cell cultures or more zenCELL owl systems

Figure 1: zenCELL owl. 24-channel microscope with 24-well cell culture plate.

Cell culture and preparation

L929 mouse fibroblast cell line was seeded in 24-well plates at a density of 80000 cells/well and grown for 24 hours in the incubator to allow attachment to the substrate. Culture medium was DMEM supplemented with 5% FBS. Cytostatic drug treatment was performed with Chloroacetaldehyde (CAA) in concentrations of 25 µM and 50 µM and Doxorubicin (DOX) in concentrations of 2,5 µM and 5 µM. The further cell growth was observed by the 24 channel microscope zenCELL owl over a period of 48 hours. Incubation environment was maintained at a constant 37°C and a constant 5 % CO_2 in air in a humidified cell incubator.

Cytostatic drugs

Chloroacetaldehyde (CAA) and Doxorubicin (DOX), the cytostatic drugs used for this experiment, have been used to treat various types of cancer for over 30 years with CAA being the active metabolite of the cytostatic drug Ifosfamid. The use of cytostactic drugs results in cell death via a multitude of intracellular mechanisms including, most importantly, interaction with the DNA leading to broken DNA strands. Further effects consist of DNA-synthesis inhibition and the reduction of mitochondrial respiration and lipid oxidation shortly after the application of those drugs.

Results: Cytostatic drug effects

Chloroacetaldehyde (CAA)

Untreated cell cultures show proliferation activity (Figure 4) leading to a duplication of cell number (Figure 2) and formation of a confluent monolayer (Figure 3) over a time period of 24 hours.

Cell count (Figure 2) and microscope images (Figure 4) show an inhibition of cell proliferation as a result of the CAA treatment. Additionally, a dose-effect relationship can be observed. The higher drug concentration [50 µM] leads to visible morphologic changes within 3 hours. A couple of the cells show a round shape and have detached from the surface after 6 hours. As a reaction to drug treatment a loss of the original cell morphology and changes of intracellular granulation occur and over the further course of the measurement death of cells becomes visible (Figure 4). The cell number decreases to 50 % of the initial value within 6 hours (Figure 2).

The addition of a lower concentration of 25 µM CAA results in comparable effect occur with a time delay. Morphological changes can be observed after 6 hours and cell number decreases to 50 % of the initial value within 18 hours (Figure 2).

Analysis of cell coverage show an increase in the coverage of substrate surface from 75 % to a confluent monolayer (100 % coverage) for untreated cell cultures. In contrast, cell cultures treated with different CAA concentrations demonstrate decrease in cell coverage of around 15 % over the course of experiment (Figure 3). Summarized, the analysis of cell count and cell coverage yield comparable results.

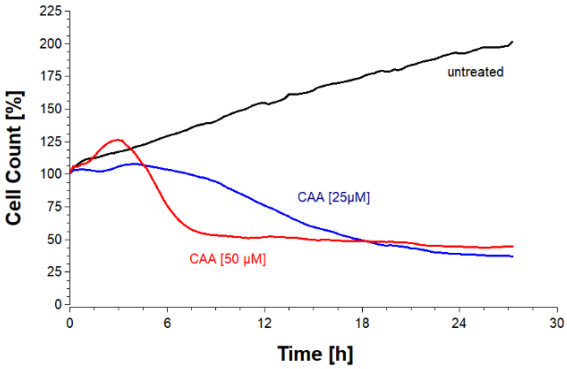

Figure 2: CAA effects on cell count. Dose-dependent decline of cell count. Standardized mean values for each condition are shown. n=8 for all groups

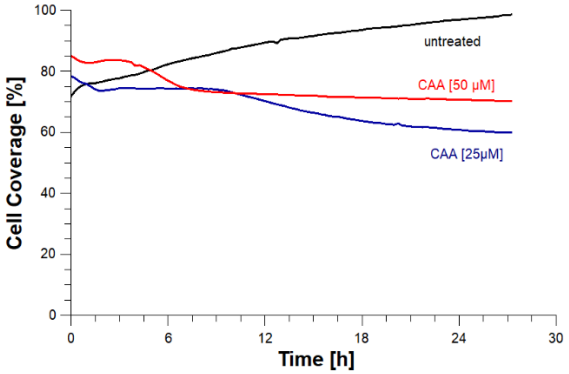

Figure 3: CAA effects on cell coverage. Standardized mean values for each condition are shown. n=8 for all groups

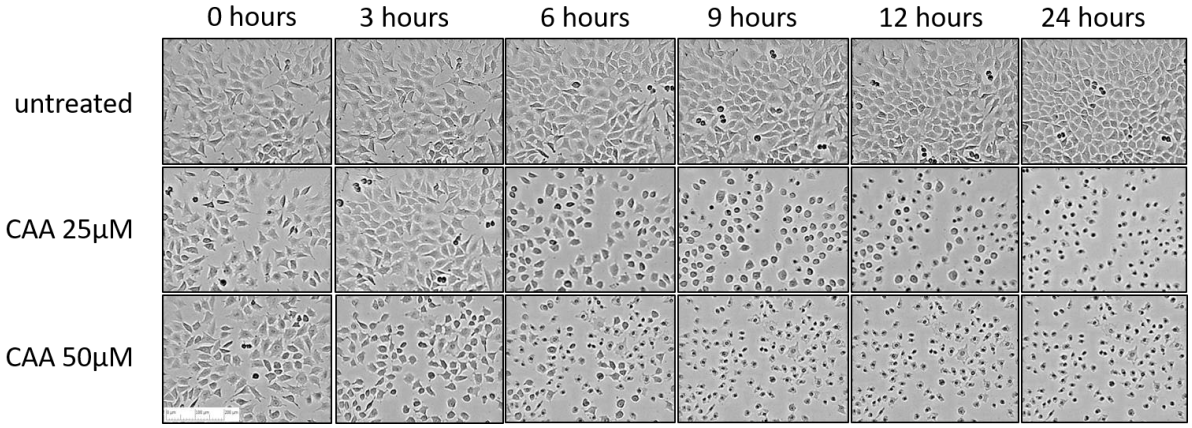

Figure 4: CAA effects on cell morphology. CAA treatment results in a dose-dependent growth inhibition, loss of cell morphology and cell death. Digital phase-contrast imaging of L929 cells. Scalebar: 200 µm

Doxorubicin (DOX)

Samples that have been treated with DOX also show the inhibiting effects on cell proliferation. Three hours after the start of the treatment, for both drug concentrations no dividing cells are visible compared to untreated cells which show proliferative activity (Figure 7). After approximately 24 hours morphological changes in both DOX concentrations is noticeable. Cells start to die off and cell debris detaches from the surface of the substrate. After 48 hours total both DOX concentrations don't show cells with original morphology anymore (Figure 7).

Cell count and degree of coverage during the course of the DOX treatment only show a low decrease. For a concentration of 2,5 µM DOX no serious cell count loss can be observed (Figure 5). The DOX concentration of 5 µM results in a cell count decrease of 25 % (Figure 5).

Coverage of the substrate surface remains the same for both concentrations during the course of the treatment (Figure 6). In comparison, the cell count of untreated samples doubles and the coverage of the substrate surface during the course of the treatment increases by almost 30 % (Figure 5 and 6).

A dose-effect relationship for treatments with DOX could not be observed in this experiment. Drug concentration seems to have a similar effect on L929 cells during the same time frame.

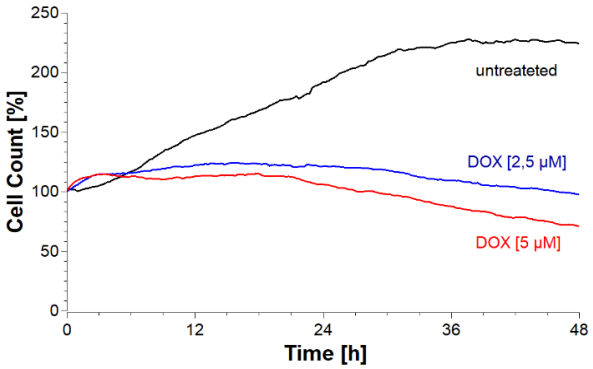

Figure 5: DOX effects on cell count. Changes in cell count resulting from DOX treatment. Standardized mean values for each condition are shown. n=8 for all groups

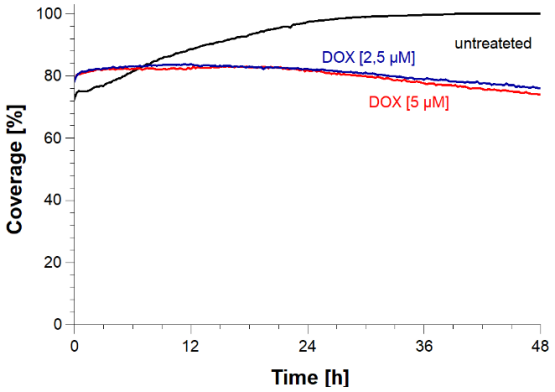

Figure 6: DOX effects on cell coverage. Standardized mean values for each condition are shown. n=8 for all groups

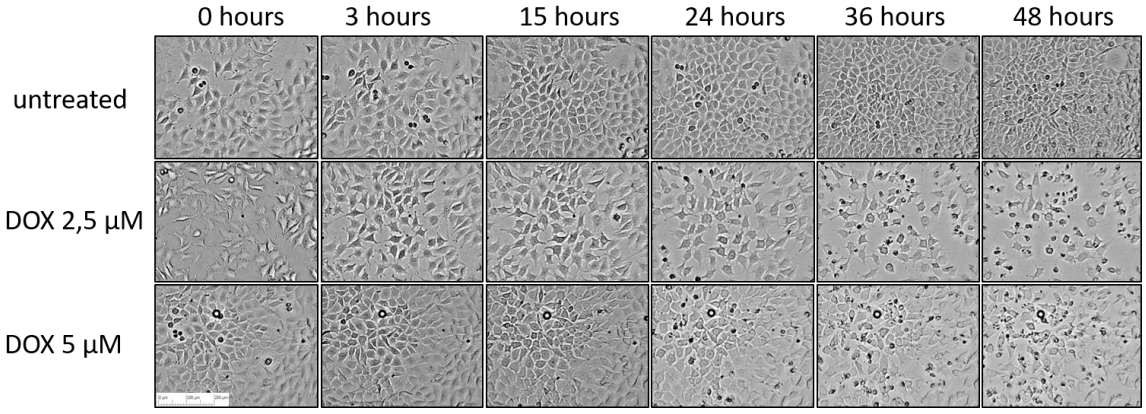

Figure 7: DOX effects on cell morphology. DOX treatment results in growth inhibition, loss of cell morphology and cell death. Digital phase-contrast imaging of L929 cells. Scalebar: 200 µm

Conclusion

Marker-free living cell analysis with the zenCELL owl is perfectly suited to observe dynamic processes inside a cell culture without potential side-effects of markers on cells. Measurements demonstrate the advantage in comparison to end-point methods since the development of cell count, degree of coverage and morphology can be displayed and analyzed separately for each well.

A dose-effect-related decrease of cell count and degree of coverage of the substrate surface can be observed. Cytostatic-induced morphological change including cell death demonstrate a dependence on the amount of dose. Simultaneous analysis of 24 cell cultures enables users not only to examine different test conditions at the same time and to compare them directly, it also rises reproducibility and provides the possibility for a statistical evaluation of research data.

zenCELL owl Live-Cell Imaging System

The zenCELL owl by InnoME is a compact 24-channel microscope system for automated cell culture microscopy. The zenCELL owl fits easily into your standard incubator and monitors your cell culture continually. The device for your automated, objective and reproducible long-term monitoring.

For more information about the zenCeLL owl please visit us at
www.zencellowl.com

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