

## Temporal analysis of the pharmacodynamics effects of cycloheximide

### INTRODUCTION

Pharmacodynamics implies the study of biochemical and physiological effects of drugs, the metabolism of drug action and the relationship between drug concentration and effect. These parameters provide important information about compounds and/or receptors to obtain pharmacologically relevant data. Using the **Bionas Discovery™ 2500 system** the action of cycloheximide on the human colon cancer cell line HT29 was investigated. Cycloheximide is an inhibitor of protein synthesis which interferes with RNA translocation. However, effects are rapidly reversed by removing the substance from the cell culture medium. Therefore, the system offers the advantage of an online and long-term monitoring of regenerative effects.

### MATERIALS & METHODS

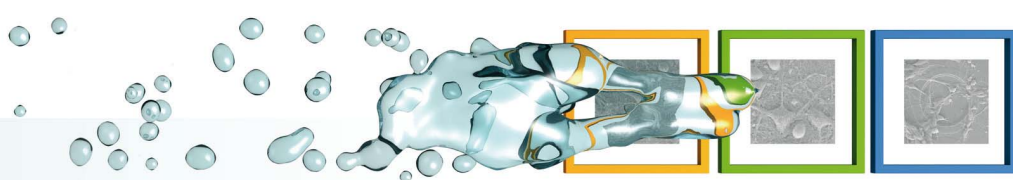
**Cell culture.** HT29 cells (Cell Lines Service, Germany) were cultured in MEM + 10% FCS + penicillin/ streptomycin at 37 °C and 5% CO<sub>2</sub>. For chip cultivation, the cell suspension was directly pipetted on the chip surface and cultured for one day prior to use. For measurement, the **Bionas® running medium** without bicarbonate buffer (RM) with 1 mM HEPES, 0.1% FCS and penicillin/ streptomycin was used. The pH of the running medium was adjusted to 7.4 and the osmolarity to 290 mOsm/kg H<sub>2</sub>O.

**Test Compound.** Cycloheximide (Sigma Aldrich, Germany) was used at varying concentrations ranging from 1 µg/ml to 20 µg/ml. As a control, the equivalent amount of DMSO (0.02%) was applied to the cells.

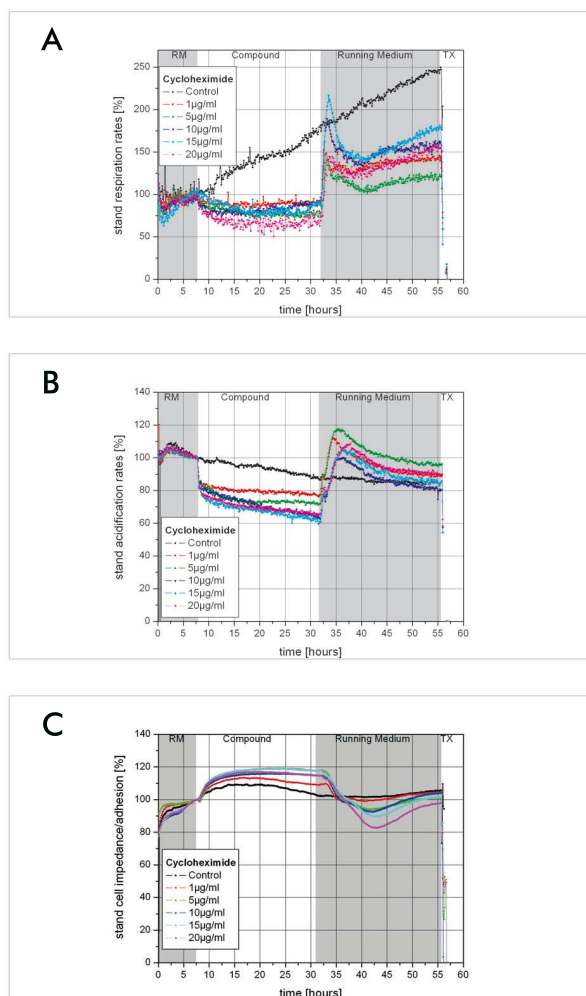
**Implementation of measurement.** As a first step the base lines for acidification, respiration and cell impedance/adhesion were determined. After a stabilization phase of about 3-4h cycloheximide was applied for 24h followed by a regeneration phase of 48h without compound. At the end of the experiment cells were killed by the addition of 0.2% Triton X-100 to get a basic signal without living cells on the sensor surface as a negative control.

**Analysis in the Bionas Discovery™ 2500 system.** With the beginning of compound application, an inhibition of protein synthesis became visible by a decrease of the acidification and respiration rates (Fig. 1A and B). Interestingly, cell impedance/adhesion (Fig. 1C) was increased with the onset of cycloheximide application whereas respiration and acidification of treated cells were reduced. While the acidification rate of untreated cells was just slightly reduced, the increase of respiration showed an interesting effect potentially caused by the addition of DMSO, which itself may have induced cellular reactions. After the removal of cycloheximide from the medium, a very strong increase of both measured metabolic parameters was visible. Application of increasing concentrations of cycloheximide induced increased reactions after the withdrawal of the compound. The increasing respiration rate reflected high energy consumption soon after the cells restarted to produce proteins. After a first peak, the respiration rate was at a higher level as in the prerunning phase. This effect was specific for the respiration rate, as the acidification rate returned to the basic level. Cell impedance/adhesion was increased up to 20% during cycloheximide application. 2-3h after withdrawal, cell impedance/adhesion values returned to the basic level, decreased within the next few hours and returned to starting levels.





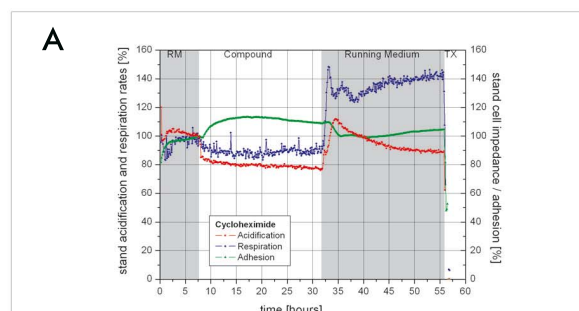
## EFFECT OF CYCLOHEXIMIDE ON HT-29 CELLS



**Fig. 1:** Standardized respiration (A) and acidification (B) rates and cell impedance/adhesion (C) of HT-29 cells incubated with varying concentrations of cycloheximide.

The results for the acidification and respiration rates as well as the cell impedance/adhesion of cycloheximide (1 µg/ml) are shown in Figure 2. The decrease of oxygen consumption and acidification at the start of compound treatment coincided with the increase of cell impedance/adhesion. Removal of cycloheximide resulted in a strong increase of measured parameters which did not coincide with each other as the acidification rate started to increase at the time point of decreasing oxygen consumption. The subsequent actions of cell metabolism let to the assumption that the mitochondrial activity was influenced first after removing cycloheximide from the medium. As a result of the higher energy consumption glycolysis was also enhanced with a brief delay.

## ACIDIFICATION, RESPIRATION AND ADHESION AT A GLANCE



**Fig. 2:** Standardized acidification, respiration and cell impedance/adhesion of HT-29 cells treated with cycloheximide (1 µg/ml).

## CONCLUSION

By the use of the **Bionas Discovery™ 2500 system** the temporal progression of cellular reactions caused by the application of cycloheximide and the adjacent recovery period without the compound was analyzed. Of major interest was the investigation of the kinetic parameters and the transient long-term effects up to 48h after removing cycloheximide. By inhibiting RNA translation and therefore protein synthesis the removal of cycloheximide resulted in a strong cellular reaction as indicated by an increase of the respiration and acidification. Therefore the **Bionas Discovery™ 2500 system** is a valuable tool to provide deeper insights into the dynamics of cell metabolism and of regenerative effects after compound treatment.

## KEYWORDS

Pharmacodynamics, cycloheximide, HT-29 cells, regenerative effects, protein synthesis

## REFERENCE

Thedinga E et al. On-line monitoring of cell metabolism for studying pharmacodynamic effects. *Toxicology and Applied Pharmacology*, 2007, 220: 33-44

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