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Evaluation of hepatotoxic effects of acetaminophen on HepG2 cells by parallel real-time monitoring in a multi-sensor analysis platform for automated cell-based assays

Authors: Heichler C, Nagy M, Wolf P

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Reviewer 2

Zuzana Červinková

Charles University, Faculty of Medicine in Hradec Kralove, Department of Physiology, Hradec Kralové. CZ

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*Only major points from review and responses included.

Reviewer 2

The manuscript deals with evaluation of hepatotoxic effect of acetaminophen on HepG2 cells using a multi sensor-based assay platform for a parallel and continuous long-term monitoring of living cells.

The goal of the article is expressed as follows: “to demonstrate capabilities of CYRIS analysis platform”. From this point of view, it will be important to compare results obtained by the authors with published data from other authors; in spite the authors statement, that there is no other similar system available. Some discussion about other systems like xCELLigence RTCA eSight or other automated microphysiometry facilitates for highly reproducible oxygen measurements is highly recommendable

Authors

Thank you for your comments, we gladly added a discussion of our data with published data from other authors studying the hepatotoxic effects of acetaminophen on HepG2 cells. These include the work of Piel et al. 2020, evaluating the mechanism of mitochondrial toxicity of APAP on primary human hepatocytes and HepG2 cells using the Oroboros O2k and Seahorse XF Flux Analyzer systems to measure cellular respiration. Furthermore, also a publication by Zhang et al. 2016, which demonstrated APAP-induced toxicity in HepG2 cells using classical cytotoxicity/cell viability assays such as MTT and LDH assay.

Added "Piel et al. 2020; Zhang et al. 2015; Rumack and Matthew 1975" and discussed similar effects of APAP toxicity (EC50, IC50) measured with different methods in chapter 3.1.

Reviewer 2

It would be also very useful to present data about reproducibility of the results. The new system was described in 2013, it will be important to mention other results obtained by the CYRIS platform in last 10 years.

Authors

We can understand that the reader has the impression that the analysis platform has already been providing comparable data for 10 years. After all, the original prototype of the platform is about this age. However, as a result of the resumption of the original development project with the Technical University of Munich and the founding of INCYTON GmbH, many things have changed in the last year. While the basic idea and fundamental measurement method remained the same, major components such as the sensory unit, microscope system, atmospheric chamber, and sensor plate have changed, along with a lot of smaller details.

We compared the data set of the current publication with a similar data set of a previous experiment where the procedure of measuring the controls of the two experiments was identical and performed on the same platform within 4 weeks. We calculated the Inter-Assay Coefficient of Variability (CV) for OCR and ECAR of the control groups. But such calculation results have so far only been collected internally and the results are not part of any publication (unpublished data).

Nevertheless, since Inter-Assay CVs (%) of less than 15 are generally acceptable, our calculation of a CV (%) of 6.1 for OCR and 11.8 for ECAR indicates a reliable reproducibility of the results. It should be noted that the initial phase (the first few hours) of the experiments, when the measured values first stabilize, hereby accounts for a particularly high proportion of the total CV. Therefore, we always have a pre-measurement phase, if possible, before we apply a substance.