

Toxicity analysis in expanded upcyte® liver cells on Nanion's CardioExcyte 96

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Summary

Drug induced hepatic toxicity is one of the main reasons for regulatory actions and market withdrawals in the last 50 years [1]. In Europe and the USA, the major cause of acute liver failure is indeed Drug Induced Liver Injury (DILI) [2]. Current existing *in vitro* models employed to predict DILI mostly focus on hepatocytes. Other cell types of the liver, such as liver sinusoidal endothelial cells (LSECs), are often overlooked. It is thus questionable whether, hepatotoxicity can be sufficiently predicted by analyzing hepatocytes only. LSECs are highly specialized endothelial cells forming the hepatic sinusoidal wall. Besides their high endocytic potential, LSECs have been demonstrated to markedly contribute to liver homeostasis, immunity, and may partially explain unexpected hepatotoxicity of selected drug candidates.

Several reports in the literature have highlighted a high sensitivity of LSECs towards hepatotoxic drugs [3]. It has been suggested that LSECs act as an early direct target for acetaminophen (paracetamol) induced toxicity, causing early swelling and loss of uptake activity and fenestrations before effects on hepatocytes are observed [4]. LSECs are further important cellular targets during sinusoidal obstruction syndrome (a distinctive and potentially fatal form of hepatic injury that occurs predominantly, if not only, after drug or toxin exposure).

The use of primary LSECs for comprehensive *in vitro* studies is compromised by poor cell yields, rapid dedifferentiation, contamination with other endothelial cells, and limited proliferation after isolation [5]. In this study upcyte® LSECs have been developed as a complementary tool to predict hepatotoxicity, uptake and drug interactions. In addition to conventional toxicity readouts such as ATP levels, upcyte® LSECs have been successfully tested using an impedance based system.

Results

The impedance signal recorded on the CardioExcyte 96 changes as a result of alterations in confluency, cell contact (morphological shape) and conductivity of adherent cells and thereby provides a measure of toxicity. Base impedance of expanded upcyte® LSECs (donor 462), upcyte® hepatocytes (donor 10-03) or co-cultured cells (donor 462 + 10-03) were cultured for three days at confluence, before test compounds were added (72 h post seeding). Classical liver damaging compounds were tested: acetaminophen, imipramine, tamoxifen and diclofenac. Medium containing these compounds was exchanged every 2 days (120 + 168 h post seeding). Normalized impedance values were recorded over 7 days (Figure 1A & B, data for tamoxifen and diclofenac are not shown).

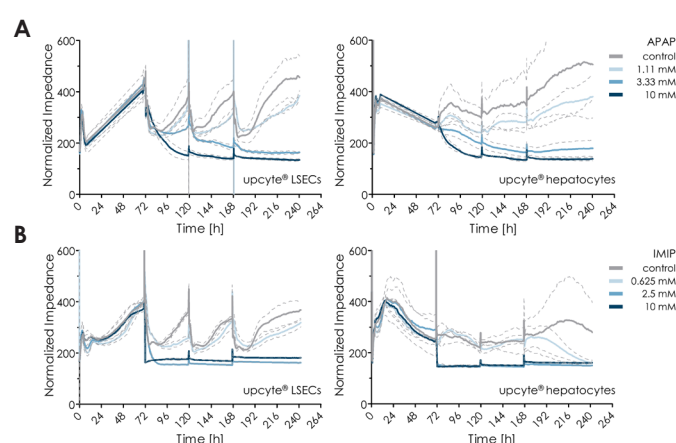


Figure 1: Real-time impedance monitoring of expanded liver cells treated with acetaminophen (APAP) or imipramine (IMIP). **A** Illustrated concentrations for APAP include 10mM, 3.3 mM, 1.11 mM, and 0.37nM and **B** for IMIP 10 mM, 2.5 mM, 0.63 mM and 0.16 mM. Values represent mean $\pm$ SD (dotted lines) from duplicate values.

Application Note

Acetaminophen and imipramine displayed a complete toxic effect on the liver cells (Figure 2A & B). Observing the IC_{50} values, acetaminophen was slightly more toxic towards LSECs compared to hepatocytes. Imipramine, on the other hand, demonstrated higher toxicity towards hepatocytes. Diclofenac showed no *in vitro* toxicity effect on both intracellular ATP levels and impedance, while tamoxifen induced only partial toxicity.

These initial results show that LSECs and hepatocytes respond differently to typical hepatic compounds and future *in vitro* applications should include more than one cell type.

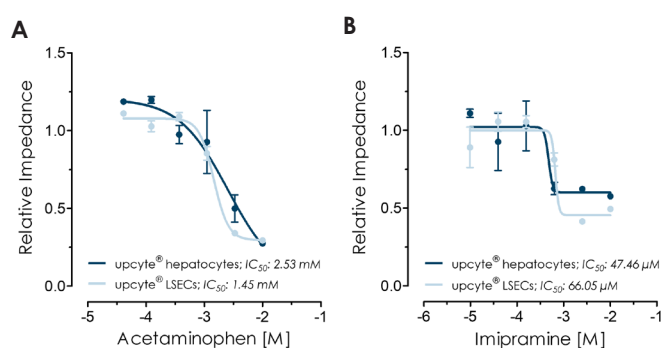


Figure 2: Impedance based dose response curves for expanded upcyte® LSECs and hepatocytes treated with hepatotoxic model compounds. A IC_{50} curve for acetaminophen (41.15 μ M - 10mM) and **B** imipramine (0.98 μ M - 1mM), with respective controls. Data represent mean $\pm$ SD summarized from one donor per cell type in technical duplicates.

References

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Methods

Cells

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	Cell type (upcyte®)	IC_{50} (ATP)	IC_{50} (impedance)
APAP	LSECs	1.71 mM	1.45 mM
	Hepatocytes	2.01 mM	2.53 mM
	Co-culture	2.82 mM	2.99 mM
IMIP	LSECs	83.21 μ M	66.05 μ M
	Hepatocytes	27.23 μ M	47.46 μ M
	Co-culture	44.27 μ M	44.5 μ M

Table 1: Comparison of IC_{50} values for expanded LSECs, hepatocytes and co-cultured cells analyzed by intracellular ATP levels vs. impedance.

Impedance based IC_{50} values represent mean values from a single experiment, while ATP-based IC_{50} values are summarized from three independent experiments, in technical duplicates.

APAP - acetaminophen; imipramine - IMIP.

In summary, upcyte® hepatocytes and LSECs are a reliable source of well characterized cells, facilitating their use in hepatotoxicity. Generation of large batches of these cells enables standardized, reproducible and cost efficient experimental settings. Classical hepatotoxic drugs had similar pharmacological IC_{50} values in impedance recordings compared to the intracellular ATP measurements, when applied to these cells (Table 1). This indicates that the impedance signal of the CardioExcyte 96 is a reliable, non-invasive and accurate measurement for testing hepatotoxicity, with an important advantage of time-resolved experimental design.

Impedance measurements

Impedance measurements were conducted according to Nanion's standard procedures for the CardioExcyte 96. upcyte® hepatocytes (Donor 10-03) and LSECs (Donor 462) and their co-culture were plated on NSP-96 plates and monitored for 3 days for the base impedance signal to stabilize. Acetaminophen, imipramine, tamoxifen or diclofenac were applied in the external media. About 2 hours before drug application the medium was completely removed from the wells and 200 μ l fresh medium was added. 6 concentrations were applied per substance, in technical duplicates. Cultures were monitored for 7 days, repeated dosing every 2-3 days. Cells were then analyzed for intracellular ATP levels or normalized impedance values.