The Bionas® system bridges the gap between *in vivo* and *in vitro*

The Bionas® 2500 system permits for the first time the long-term observation of cell lines, primary cells, tissue slices and biopsies in the presence of drug candidates under close-to-*in-vivo* conditions. The proprietary perfusion system allows the observation of regeneration effects.

We present a sensor system that simultaneously measures three metabolically relevant parameters, such as oxygen consumption, the extracellular acidification rate and adhesion of test cells. The Bionas system is easy to use and allows the noninvasive and label-free monitoring of compound effects on cellular metabolism. The readout is continuous for up to several days.

The Bionas system is a valuable tool for optimizing target validation studies as it places the targets in near-physiological context. The proprietary perfusion system allows the monitoring of both acute (short-term) and chronic (long-term) effects of drug candidates in various cell types, and thus facilitates the selection of the most promising compounds for development.

Prediction of hepatotoxicity

The Bionas system provides for the first time a close-to-*in-vivo* environment that allows scientists to better predict hepatotoxicity and thus make more informed decisions earlier in the drug development process.

Primary hepatocytes grow on sensor chips

The Bionas system, in combination with the HEPAC^2 cell cultivation method of primary hepatocytes (Primacyt GmbH), is a powerful tool for culturing primary hepatocytes and analyzing early signals of hepatotoxicity, a frequent side effect of pharmaceutical drugs.

To determine the most suitable cell type for predicting hepatotoxicity, we treated primary human and rat hepatocytes as well as human liver carcinoma cell line HepG2 cells with acetaminophen (AAP, paracetamol). AAP is metabolized to the toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) by the hepatic cytochrome P450 system.

We seeded the cells directly on precoated sensor chips and cultured them as described[1,2]. Monitoring of the oxygen consumption rates, acidification rates and cellular adhesion with the Bionas system revealed distinct differences between these cells (**Table 1**).

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Table 1 | Comparison of the effects of AAP on different cell types[2]

	Primary human hepatocytes	HepG2	Primary rat hepatocytes
O_2 consumption	Reduced	Increased (at low AAP) Reduced (at high AAP)	Reduced
Acidification	No effect	Increased (at low AAP) Slightly reduced (at high AAP)	Reduced
Cell adhesion	Slightly reduced (P1) No effect (P2)	Slightly reduced	Reduced
Regeneration	Yes (incomplete with high AAP only)	Not complete	No

Primary human hepatocytes are the best choice

The comparison of HepG2 cells and primary human and rat hepatocytes in response to AAP revealed the following:

- Primary human hepatocytes were more similar to HepG2 cells than to primary rat hepatocytes.
- Primary rat hepatocytes were more sensitive to AAP than the other cell types. Only rat hepatocytes showed strongly reduced cell adhesion and acidification rates in addition to a reduction of oxygen consumption.
- Only primary human hepatocytes showed complete regeneration with 1.0 mg/ml AAP, whereas rat hepatocytes were irreversibly damaged under these conditions.

The comparison of primary human and rat hepatocytes demonstrates that rat cells behave differently in response to AAP. Rat hepatocytes, and probably other rodent cells, are therefore not suitable for predicting hepatotoxicity in humans even though they are widely used.

Toxic effects are independent of the donor

We isolated primary human hepatocytes from two different donors (P1 and P2) and cultured them on precoated sensor chips before assaying the metabolic parameters in the presence of AAP. The results show that in both preparations, respiration decreased in a dose-dependent manner (**Fig. 1**). This inhibition took effect very quickly and reached maximum values within the first 10 min of AAP exposure. The effects were independent of the donor, indicating that the response may be an intrinsic feature of the primary cells.

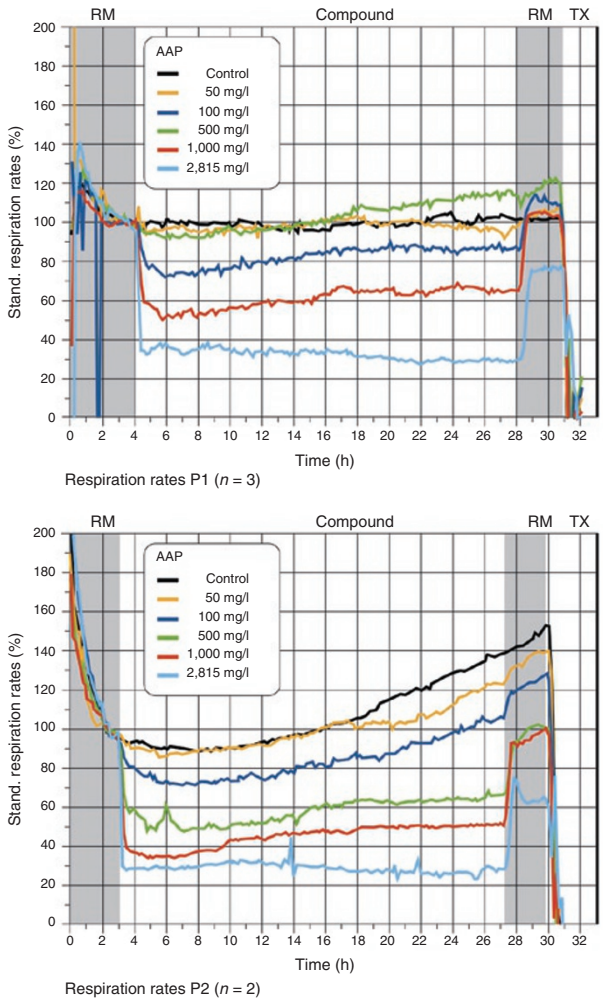

Figure 1 | Oxygen consumption rates of primary human hepatocyte preparations P1 and P2 treated with AAP. Cells were first exposed to running medium (RM) for 3–4 h, then to AAP for 24 h and again to RM for 3 h before they were killed with Triton-X 100 (TX).

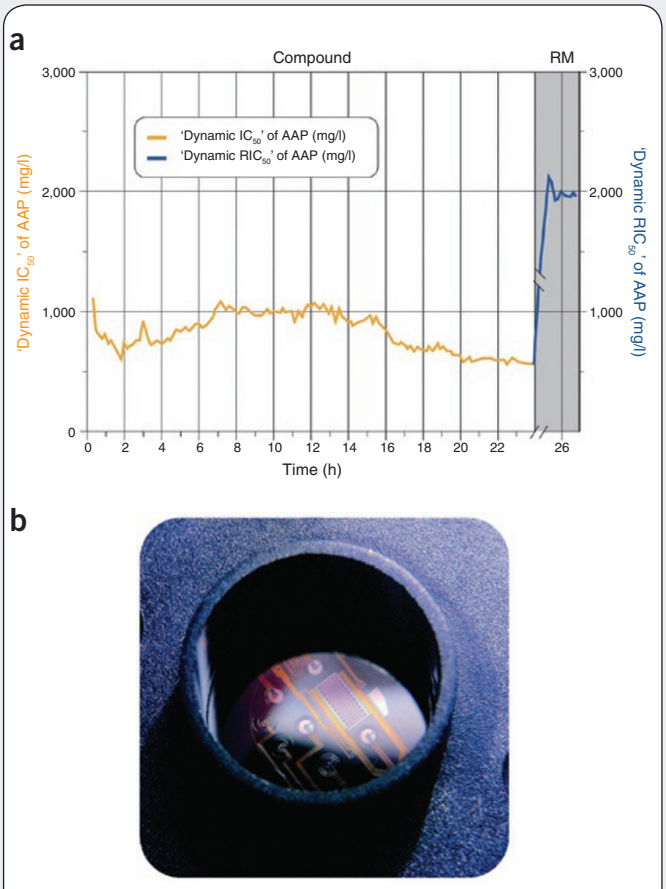

Figure 2 | IC_{50} values of AAP resulting from respiration rates of preparation P2 as measured with the Bionas sensor chip. (**a**) Variation of IC_{50} in the presence and absence of AAP ("RIC_{50}") with time. (**b**) The Bionas sensor chip.

After removing AAP from the medium, the hepatocytes regenerated very quickly. Neither acidification rates nor cell adhesion were significantly influenced by AAP in both preparations (data not shown).

Dynamic IC_{50} and RIC_{50}

Through the continuous respiration measurements (**Fig. 1**) taken by the Bionas system, it is possible to calculate the half-maximal inhibitory concentration (IC_{50}) at each time point. Plotting IC_{50} versus the exposure time to the substance generates dynamic IC_{50} curves that illustrate the rapid inhibitory effect of AAP on primary human hepatocytes (**Fig. 2**).

Notably, the dynamic IC_{50} values of AAP in primary human hepatocytes are around 1,000 mg/l and hence are much lower than those described in the literature (IC_{50}: 2,815 mg/l for a 4-h exposure, LS-L929 cells[3]) indicating greater than expected sensitivity. After removal of AAP, we observed an increase in the dynamic IC_{50} values reaching values around 2,000 mg/l. A residual inhibition of respiratory activity of 50% (residual IC_{50} or RIC_{50}) was observed after exposure to 2,000 mg/l AAP.

Conclusion

Continuous measurements using the Bionas analyzing system reveal distinct effects on metabolism and therefore allow deeper insight into cellular pathways than is possible with endpoint-based methods. Not only does this allow the analysis of dose response in cells and tissues, but in addition recovery, regeneration and adaptation effects are easily detectable.

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