# A micropatterned human hepatocyte co-culture model allows determination of toxicity at more relevant concentrations than hepatocyte sandwich cultures

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## Abstract

Use of human hepatocytes in sandwich cultures for determining toxicity potential is limited by the short functional lifespan of hepatocytes (~5 days). HepatoPac is a system of micropatterned human hepatocytes co-cultured with mouse embryonic 3T3-J2 fibroblasts enabling functional survival of hepatocytes for >30 days. This study was performed to determine if HepatoPac would allow for use of lower, more relevant concentrations in toxicity studies than used with sandwich cultures. Hepatocytes from 2 human donors were cultured in the HepatoPac system for up to 3 weeks or in sandwich cultures for 4 days and exposed to multiples of the human Cmax of pravastatin (PS), simvastatin (SS) or cerivastatin (CS). ATP levels, albumin secretion and urea synthesis were used to determine the viability and function of hepatocytes at various time points. Donor 1 results: In HepatoPac a slight decrease in ATP was seen with CS at 50x Cmax on day 3. Concentration-related decreases in ATP, albumin secretion and urea synthesis were seen with CS on days 7 and 15, beginning at 25x Cmax. No significant changes were observed with PS, and with SS only albumin secretion was affected at 50x Cmax on day 15. In sandwich cultures the only change observed was decreased albumin with PS and CS at 50x Cmax. Donor 2 results: In HepatoPac, concentration-related ATP depletion and decreased urea synthesis were seen with CS on days 8 and 15 from 10x Cmax, while albumin secretion was affected at ≥25x Cmax. The changes seen with Donor 2 were of a smaller magnitude than for Donor 1. Only urea was affected on day 15 with SS, and no changes were seen with PS. With sandwich cultures, very slight (<10%) decreases in ATP were seen at ≥50x Cmax for PS and at ≥10x Cmax for CS, with no changes in albumin or urea. Overall, the results show that extended functional life of hepatocytes may allow for determination of toxicity at more therapeutically-relevant concentrations than possible with sandwich cultures, and that there is donor variability.

## Materials & Methods

- HepatoPac from cryopreserved human hepatocytes (2 separate donors) were created and allowed to stabilize for 7 days
- Donor 1: Female, Caucasian, 57 years of Age, Smoker, Alcohol User, No Drug Use, Medications (Paxil, Clonipin, Synthroid, Trazadone), Cause of Death Was Anoxia
- Donor 2: Female, Caucasian, 61 years of Age, No alcohol, tobacco or drug use, Medications (Synthroid, Zocor, Boniva, Prilosec, Prednisone, Eyedrops), Medical History (Cardiac Arrest, C1-C2 fracture, Diverticulitis, GIRD, Hypothyroidism), Cause of Death Was Anoxia secondary to cardiovascular
- Sandwich cultures were created (rigid collagen substratum with Matrigel overlay) and allowed to stabilize for 2 days
- Cultures were dosed with Pravastatin, Simvastatin or Cerivastatin at Cmax, 10 Cmax, 25 Cmax and 50 Cmax (up to 200 Cmax for sandwich cultures) every 2 days with fresh compound dissolved in serum-supplemented culture medium (DMSO as vehicle) for HepatoPac and serum-free culture medium for sandwich cultures (per Industry Standard Protocol).
- Culture medium for assessment of albumin and urea levels, was collected 3, 8 and 15 days post-dosing for HepatoPac, while for 3 days post-dosing for sandwich cultures
- ATP levels were assessed at the same time points via CellTiter-Glo Assay from Promega
- An ANOVA one-way analysis of variance was utilized followed by Tukey's Post-Hoc Pairwise comparison test.
- Statistically significant differences (*)are defined by having P<0.05

## Introduction

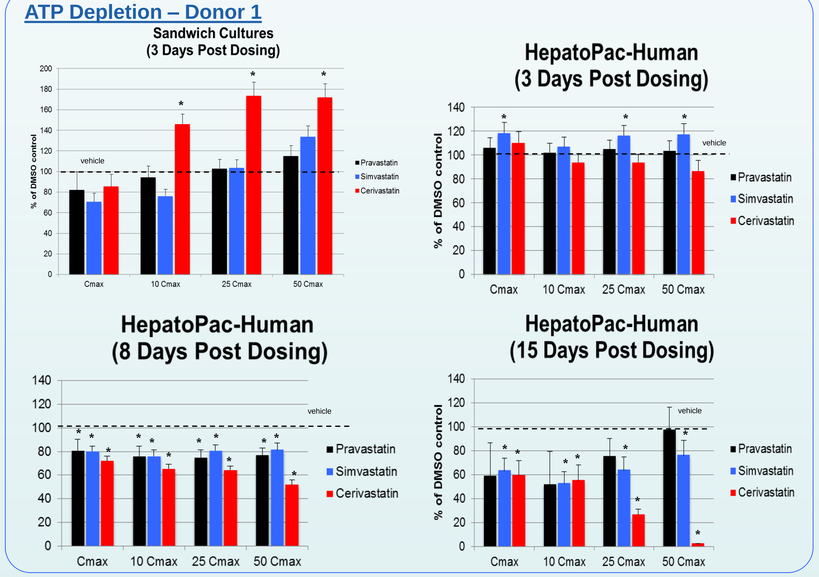
Drug-induced liver injury (DILI) is a major issue in pharmaceutical use and development. Animal models are not very sensitive for detecting human DILI potential, and human hepatocytes have a very limited lifespan for use in experiments. The short-duration exposures of hepatocytes to drugs necessitates the use of high concentrations that do not reflect actual drug concentrations in clinical use.

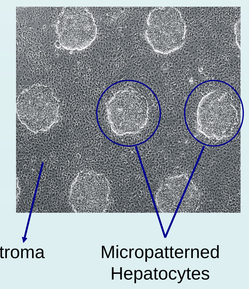
HepatoPac is a system of human hepatocytes and mouse embryonic 3T3-J2 fibroblasts co-cultured in a specifically engineered pattern to optimize the survival and function of the hepatocytes. Hepatocytes in the HepatoPac system can survive for >30 days with normal morphology and function, including maintenance of most CYP activities.

The hypothesis behind these experiments is that the ability to maintain human hepatocytes in culture for longer than the typical 3 days for sandwich culture methods would allow detection of toxicity potential at concentrations much lower than with sandwich cultures. This could allow detection of hepatotoxic compounds at more relevant concentrations, or that act through mechanisms that require longer times to manifest the toxicity.

## Results

### ATP Depletion – Donor 1

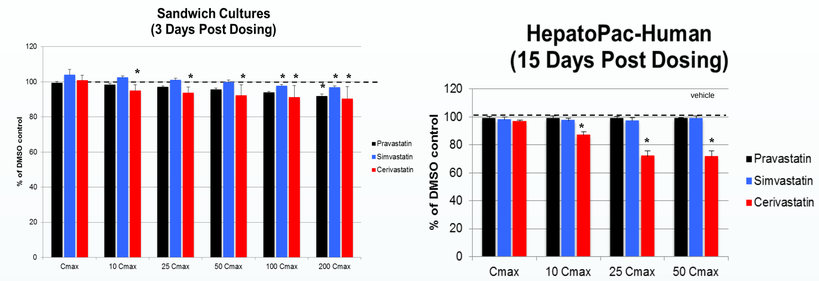


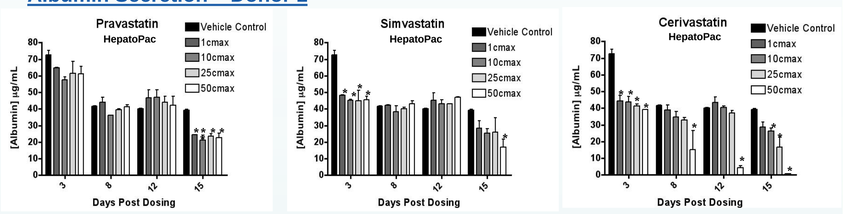

Typical HepatoPac culture system with islands of human hepatocytes surrounded by mouse fibroblasts in a precision engineered ratio

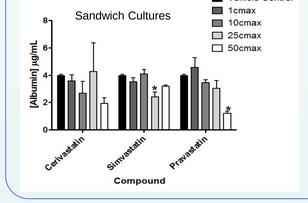
## Results, cont.

### ATP Depletion – Donor 2




### Albumin Secretion – Donor 1




Results for urea synthesis were similar to those for albumin secretion
- HepatoPac: No effect with pravastatin, no or small effect with simvastatin, significant decrease with cerivastatin on days 8 and 15
- Sandwich cultures: No effect in 3 days

Results for Donor 2 not shown:
- Similar results to Donor 1 for urea synthesis and albumin secretion, with changes of a smaller magnitude

## Conclusions

- In general, all statins compromised liver-specific functions to similar levels at 1-10 Cmax doses. However, at 25-50 Cmax doses, cerivastatin caused significant loss of liver-specific functions and hepatic viability, to a much greater extent than simvastatin and pravastatin. This loss of function and viability was not detected in 3 days with sandwich cultures.
- The results indicate an increased ability to detect toxicity with hepatocytes in the HepatoPac system, compared to sandwich cultures.
- Increasing the length of exposure allows detection of toxicity at lower concentrations.
- Albumin secretion and Urea synthesis (non-destructive assays) correlated well with the destructive ATP assay. This could potentially offer monitoring of the same wells over time for time-dependent effects.
- Variability in human donors is an important factor in detecting toxicity with hepatocytes. Multiple donors should be used to investigate human susceptibility.

## Reference

S.R. Khetani and S.N. Bhatia, Microscale culture of human liver cells for drug development. Nature Biotechnology 26(1), 120-126, 2008.