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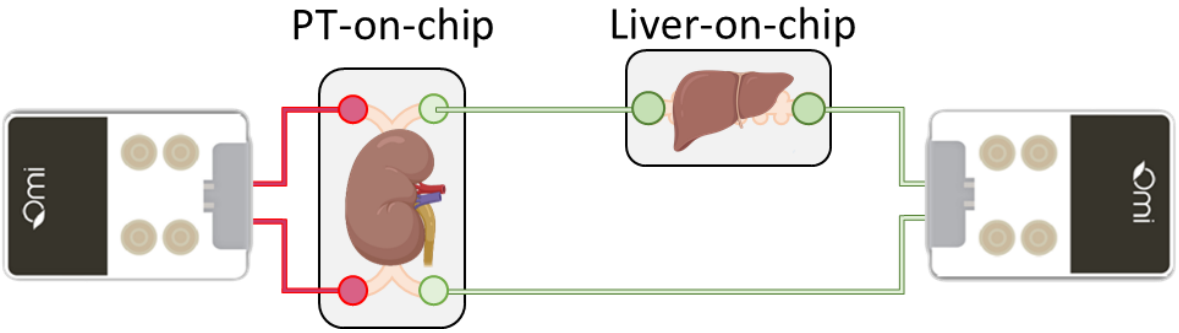
LIVER–KIDNEY ORGAN-ON-CHIP MODEL USING THE OMI™ DUAL PLATFORM TO INVESTIGATE DRUG DISPOSITION AND DRUG–DRUG INTERACTIONS



Tacrolimus, a commonly used immunosuppressant, is commonly prescribed for **liver transplant** patients but carries a high risk of nephrotoxicity due to its narrow therapeutic window and variable pharmacokinetics. Since both the liver and kidney play key roles in tacrolimus metabolism, distribution, and clearance, understanding cross-organ interactions is critical. A **dual liver-kidney organ-on-chip platform** provides a human-relevant model to study tacrolimus pharmacology, transporter dynamics, and potential drug-drug interactions to improve prediction of drug behavior and safety in clinical settings.

In this application note, the **Dual Omi Organ-on-a-chip Platform** maintained continuous recirculation of media and drug in the Liver-Kidney Model for 48 hours.

This application note is written in collaboration with University of Limoges Pharmacology and Transplantation UMR 1248, Isy Petit, Jean-Sebastien Bernard, Dr. Florent Di Meo and Dr. Nicolas Vedrenne.

PHARMACOLOGY & TRANSPLANTATION
PT
UMR 1248

1. UNDERSTANDING MODELING HEPATIC AND RENAL INTERACTIONS OF TACROLIMUS WITH ORGAN-ON-CHIP TECHNOLOGY

Loss of renal function is a major risk following liver transplantation. Although the exact reasons behind this association are yet unknown, nephrotoxicity brought on by immunosuppressive medications like tacrolimus is probably a contributing factor. A deeper comprehension of tacrolimus pharmacology is required. **Membrane transporters** are a major factor to consider, as they play a **central role in drug disposition** by regulating the key steps of the drug's absorption, distribution, metabolism, and excretion (ADME) properties. Also, changes in transporter activity can affect systemic medication exposure and, in turn, the effectiveness of treatment.

Tacrolimus is a widely used immunosuppressant characterized by a narrow therapeutic window **(tight dosing range)** and marked by important interindividual pharmacokinetic variability. Its elimination occurs mainly via hepatic metabolism followed by fecal excretion. It has also been shown that it can accumulate in the kidney, where transporter-mediated uptake and efflux might contribute to **long-term nephrotoxicity** (fig 1). Pharmacokinetic factors that influence tacrolimus exposure can be modulated by both endogenous metabolites and xenobiotics. Their activity can be altered by small compounds, leading to changes in the expression or function of drug-metabolizing enzymes and transporters, which in turn affect the local and systemic concentrations of tacrolimus. For example, metformin, an antidiabetic drug frequently co-administered with tacrolimus in the context of post-transplant diabetes, has been reported to alter these pharmacokinetic determinants. Despite this, the potential drug–drug interaction between tacrolimus and metformin remains poorly understood.

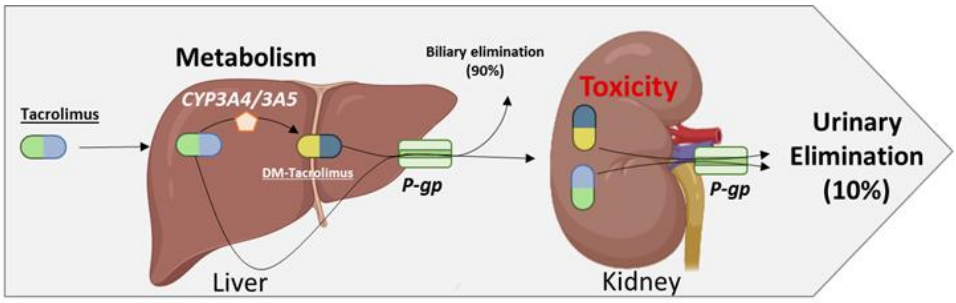


Figure 1. Tacrolimus pharmacology: liver and kidney involvement *Schematic representation of tacrolimus metabolism and elimination. Tacrolimus is metabolized by CYP3A4/5 in the liver, generating Demethyl-tacrolimus (DM-tacrolimus), with most of the elimination occurring via biliary excretion (~90%) and a minor fraction via urinary excretion (~10%). P-gp mediates efflux in liver and kidney compartments.*

Multi-organ on chip (OOC) models provide a unique opportunity to simultaneously investigate complex processes in a human-relevant context. By investigating hepatocytes and renal proximal tubule cells at the same time in a shared microfluidic circuit, the system allows for the study of hepatic metabolism, renal transporter dynamics, and cross-organ regulatory pathways.

2. MATERIALS & METHODS

2.1 Cell Culture and Preparation

RPTEC/TERT1 cells were maintained in DMEM:F12 supplemented with the RPTEC Growth Kit, antibiotics, and Geneticin, without FBS. Cells were cultured at 37°C, 5% CO_2 and used between passages 4–15. In the microfluidic chip, they were seeded at 130,000 cells/cm² on collagen I-coated Be-Doubleflow chips (Beonchip) and cultured on a rocker for 10 days with daily medium change before putting under flow.

HepaRG cells were expanded in hepatic growth medium for 2 weeks, then transferred to µ-Slide spheroid perfusion bioinert at 20,000 cells/well. Spheroids were maintained for 7 days in differentiation medium before use. The protocol is outlined in (fig 2.A)

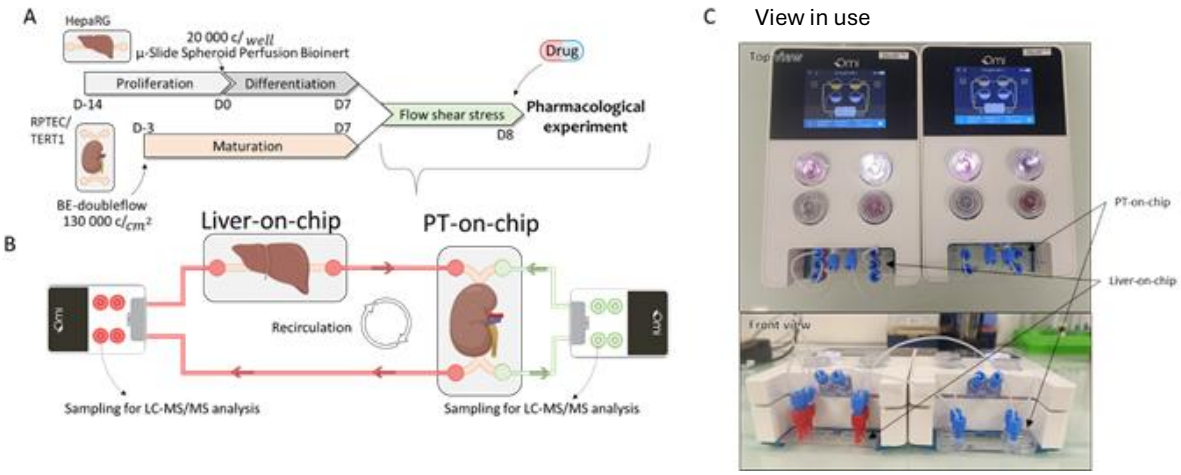


Figure 2. Overview of setup

(A) Diagram of the culture protocol. (B) Representation of the dual-organ model, the hepatic compartment in red and the urinary compartment in green. (C) View in use displaying the system, with the top view shown above and the front view below.

2.2 Omi Setup

An automated recirculation protocol was created using the Omi software and loaded onto the Omi device. The software guides each step through built-in, step-by-step instructions, including:

1. **Calibration:** Setting the reservoir level sensors to ensure accurate flow measurements.
2. **Sterilization:** Flushing the Omi fluidic path and experimental tubing with water followed by 70% ethanol.
   Note: Prior to use, all connectors and tubing were sterilized either by autoclaving or by storing in a sterilizing solution.
3. **Loading:** Pre-filling the fluidic path with experimental medium to remove air bubbles and ensure continuous flow.
4. **Chip Connection:** Both devices were connected to their respective microfluidic chips prior to recirculation (see Fig. 2).

5. **Dual-Mode Recirculation:** Each Omi device was linked to the appropriate channel for continuous perfusion:

Cells	Flow rate	Duration
RPTEC	10 µL/min	48h
HepaRG	20 µL/min	48h

2.3 Quantification of mRNA Transcripts

RNA was extracted, converted to cDNA, and analyzed by qPCR using gene-specific TaqMan assays. Expression was normalized to GAPDH and expressed as fold change relative to control.

2.4 Immunofluorescent Cell Staining

For 3D HepaRG spheroids, samples were fixed, permeabilized/blocked, incubated with primary antibodies for 24 h, then stained with secondary antibodies and DAPI. After washing, spheroids were mounted for confocal imaging.

2.5 Compound Quantitation by LC-MS/MS

Supernatants were mixed with acetonitrile containing an internal standard, centrifuged, diluted, and processed by solid-phase extraction. Metabolites were eluted, dried under nitrogen, reconstituted in methanol, and analyzed by LC-MS/MS.

2.6 Metabolomics Study

Extracts were analyzed by LC-MS/MS using a cell culture profiling method, with metabolite peak areas normalized to the internal standard.

3. RESULTS

3.1 Characterization of the models

Brightfield imaging under static and flow conditions showed no notable morphological differences in HepaRG cells. In contrast, RPTEC/TERT1 cultures displayed localized regions of maturation characterized by strong transporter expression (Fig. 3A), suggesting that proximal tubule cells develop spatially organized domains within the chip environment that may mirror functional zonation in vivo.

Immunostaining performed on HepaRG cells, cultured as spheroids, which are known to better express hepatic functions, confirmed the production of CYP3A4,P-gp, and OATP1B1 protein. OATP1B1 shows a clear membrane localization (Fig. 3B). Together, these findings indicate that the dual-organ system can sustain maturation of renal cells under flow while preserving hepatic cell structure and proper protein production, providing a physiologically relevant model to investigate drug metabolism and transporter-mediated processes.

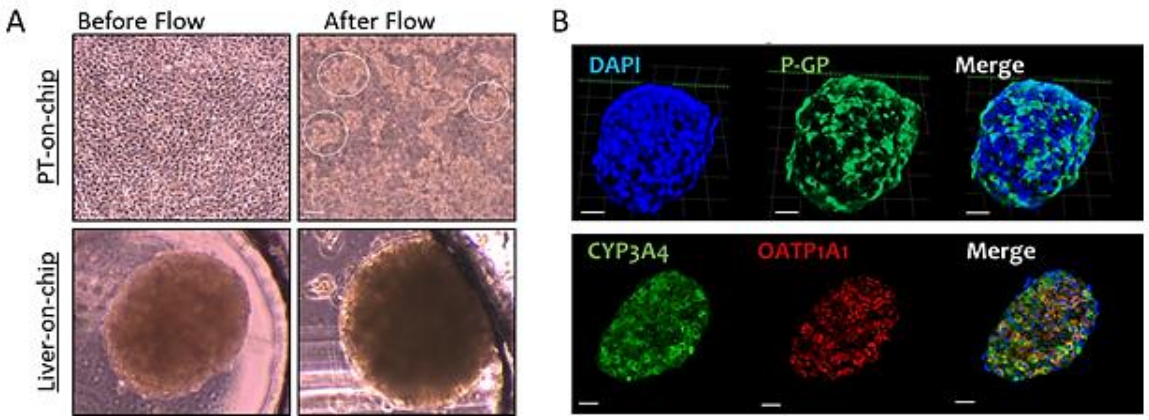


Figure 3. Brightfield and immunostaining

(A) Brightfield images of RPTEC/TERT1 (top) and HepaRG cells (bottom), before (left) and after (right) exposure to flow. White circles indicate regions of RPTEC/TERT1 maturation.

(B) HepaRG immunostaining after flow: top panels show nuclei (DAPI, blue), P-gp (green), and merged images; bottom panels show CYP3A4 (green), OATP1B1/3 (red), and merged images.

3.2 Metabolism and excretion of tacrolimus

The tacrolimus concentration decreased rapidly in the hepatic compartment before stabilizing after ~8 hours, a pattern that can be attributed to efficient uptake and metabolism by HepaRG cells, combined with potential renal excretion. However, given the physical-chemical properties of tacrolimus, one can also expect significant tacrolimus adsorption to system components. Appearance of tacrolimus concentrations in the urinary compartment reached a maximum peak at ~10 hours, but a lower concentration than in the liver-like compartment (10x fold). This supports the relevance of PT model for the limited renal clearance as compared with liver, as described by the *in vivo* predominance of biliary excretion.

The detection of DM-tacrolimus metabolites in the hepatic compartment highlights the relevance of the liver-biochip model to efficiently metabolize tacrolimus (fig 4.A). Interestingly, DM-tacrolimus metabolites were detected in the urinary-like compartment even though quantification was not possible. This highlights that the dual-organ system is able to recapitulate aspects of tacrolimus metabolism and elimination.

The co-treatment with metformin suggested a tendency toward reduced metabolism and excretion after 24 hours, although the variability observed across experiments precludes firm conclusions. This may reflect limited crosstalk in this overall system as well as insufficient exposure time to capture drug–drug interactions.

In the PT model, tacrolimus alone did not affect PXR expression, while co-treatment with metformin induced a slight upward trend. Expression of the transporters ABCB1/P-gp and ABCC4/MRP4 remained stable, whereas ABCC2/MRP2 was downregulated under tacrolimus, both with and without metformin. The lack of changes in the kidney injury marker KIM-1 indicates that neither tacrolimus nor tacrolimus plus metformin induced measurable nephrotoxic stress under these conditions, likely due to the relatively short treatment duration. Overall, these results show that co-treatment with metformin did not significantly alter transporter expression.

In the hepatic model, tacrolimus alone did not modify PXR expression, whereas co-treatment with metformin resulted in a modest increase. Since metformin is known to disrupt PXR activity, this rise may reflect a compensatory response to such disruption. However, the absence of consistent changes in CYP3A4 expression, combined with the transcriptomic increase in ABCB1, suggests that the effect is likely weak and potentially buffered by other regulatory mechanisms (Fig. 4B).

Taken together, these findings indicate that the dual HepaRG–RPTEC/TERT1 system successfully captures several mechanistic aspects of tacrolimus disposition, including hepatic metabolism and renal clearance. However, the modulatory effects of metformin appear limited and variable, underscoring the need for development and optimization of the method. This can be achieved by e.g., longer exposures, higher-resolution metabolic readouts, or integration with additional organ compartments (e.g., intestine) to better capture clinically relevant drug–drug interactions.

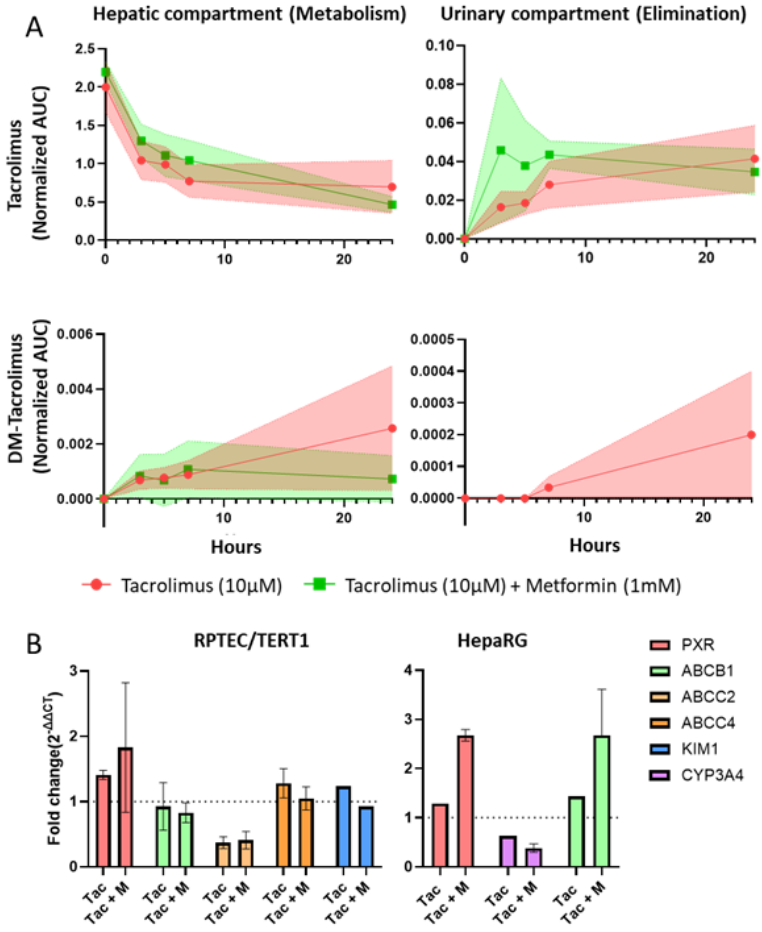


Figure 4 Tacrolimus effect onto the dual-organ models

(A) Area under the curve (AUC) for tacrolimus on the top panels and DM-tacrolimus on the bottom panels in the hepatic compartment on the left and the urinary compartment on the right (n= 3 for each condition*) during tacrolimus (10µM) treatment (red) and tacrolimus (10µM) + metformin (1mM) co-treatment (green) the color area represent the error bar range.*

(B) Relative mRNA expression levels of PxR, ABCB1, ABCC2, ABCC4, KIM1 in RPTEC/TERT1 and PxR, ACBB1 and CYP3A4 in HepaRG cells were measured in the dual-organ device after treatment with tacrolimus (10 µM, Tac) or in co-treatment with tacrolimus (10 µM) + metformin (1 mM, Tac + M). All data are expressed as fold changes relative to untreated controls and were normalized to GAPDH expression.

3.3 Metabolomics profile modulation under treatment

Under treatment, we observed pronounced changes in circulating metabolites in both hepatic and urinary compartments, highlighting a strong metabolic response. This was particularly striking in the urinary profile, where statistical analysis revealed clear differences between the three experimental groups (Fig 5.A).

Further analysis demonstrated distinct clusters of up- and downregulated metabolites, reinforcing the compartment-specific metabolic signatures (Fig 5.B). Liver samples showed differences under tacrolimus + metformin condition, whereas the renal compartment exhibited a different metabolomic profile with more differentially expressed metabolites, indicating that metabolic changes are more pronounced in the kidney. Interestingly, tacrolimus treatment alone induced more metabolite alterations than the tacrolimus +

metformin combination (Table 1), suggesting that metformin may limit tacrolimus-induced metabolic perturbations or increase variability in the response.

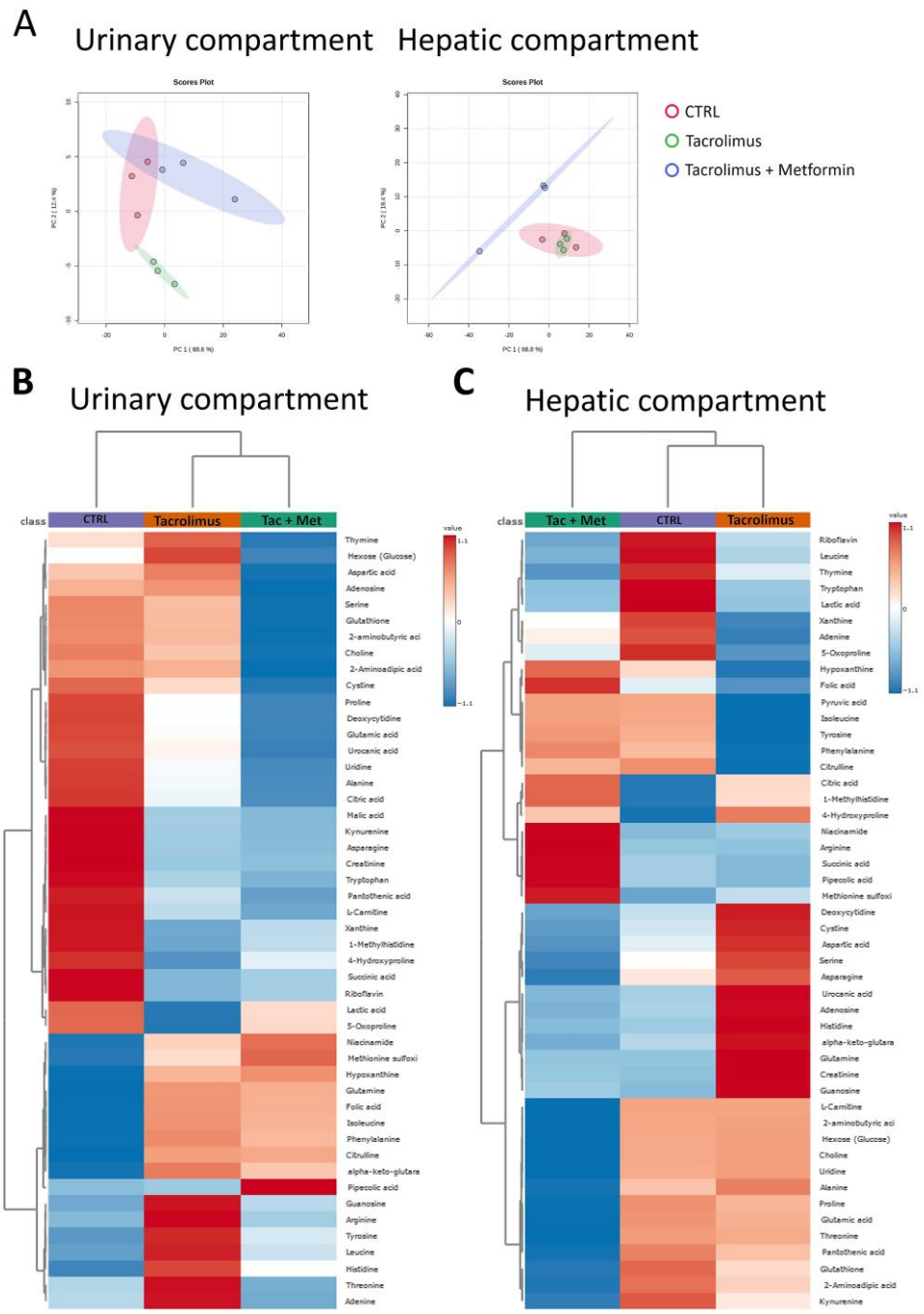


Figure 5 Metabolomics analysis under Tacrolimus and Tacrolimus + metformin treatment

(A) Principal component analysis (PCA) of metabolomics data from the urinary compartment (left) and hepatic compartment (right). Each point represents an independent replicate: red for untreated controls, green for Tacrolimus (10 µM) treated samples, and blue for Tacrolimus + Metformin (1 mM) co-treated samples.

(B) Heatmap of metabolites detected in the urinary compartment. Samples are colored by treatment group: blue for controls, orange for Tacrolimus, and green for Tacrolimus + Metformin. Data were square-root transformed, hierarchically clustered using the Ward method, and represent the mean of three independent replicates.

(C) Heatmap of metabolites detected in the hepatic compartment. Color coding and data processing are as described for (B).

Table 1 Differentially expressing metabolite (p. value <0.05)

Metabolites DE (P.value <0,05)		
Urinary compartment	**TAC**	**TAC + MET**
Alanine	-	ns
Asparagine	-	ns
Citric acid	-	ns
Creatinine	-	ns
Succinic acid	-	ns
Xanthine	-	ns
Niacinamide	+	ns
Threonine	+	ns
Guanosine	+	ns
Kynurenine	+	ns
2-aminobutyric acid	ns	-
Choline	ns	-
Hepatic compartment	**TAC**	**TAC + MET**
Adenine	-	ns
2-aminobutyric acid	ns	-
Choline	ns	-

CONCLUSION

The **dual liver-kidney organ-on-chip model** successfully captures key aspects of tacrolimus disposition, including hepatic metabolism, renal clearance. HepaRG and RPTEC/TERT1 cells maintained physiologically relevant functions, and metabolomics revealed compartment-specific responses, with tacrolimus alone inducing stronger metabolic alterations than co-treatment with metformin. **This platform provides a human-relevant system to study drug metabolism, drug and metabolite elimination and metabolic alteration.**

FUTURE DIRECTIONS

How do longer exposure times or repeated dosing affect tacrolimus metabolism and transporter regulation?

Can integrate additional organ cells. Compartments (e.g., intestine, immune system) improve prediction of systemic drug–drug interactions?

How can computational models or digital twins be leveraged to translate OOC readouts into human pharmacokinetic predictions?

What are the mechanisms underlying metformin's modulatory effects on tacrolimus-induced metabolic changes in different compartments?