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Leveraging a Human-Based Microphysiological System for In Vitro Pharmacokinetic Assessment of Oral Drug Formulations

A. Salminen[1], D. Dies[1], D. Austin[1], J.A. Willoughby, Sr[1], H. Walker[2], J. McKim[1]

[1]LifeNet Health Life Sciences, Virginia Beach, VA [2]Bayer Healthcare LLC, Whippany, NJ

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ABSTRACT

Background and Purpose: Pharmacokinetics plays a pivotal role in preclinical drug development, yet traditional in vivo studies are often limited by cost, complexity, and translational relevance. Microphysiological systems (MPS), which integrate reconstructed human organ models under dynamic flow conditions, offer a promising alternative for simulating absorption, distribution, metabolism, and excretion (ADME) in vitro. This study explores the utility of a three-organ MPS platform to evaluate the pharmacokinetics of an active pharmaceutical ingredient (API) delivered via a self-nano-emulsifying drug delivery system (SNEDDS), a formulation strategy designed to enhance oral bioavailability and protect APIs from degradation and first-pass metabolism.

Methods: A human-based MPS platform comprising EpiIntestinal™ (intestine), TruVivo™ (liver), and HK-2 (kidney) cultures connected through a series of semi-permeable membranes and a micro syringe pump (McKim et al., 2024) was employed to assess the pharmacokinetics of an API formulated in a SNEDDS. Preliminary cytotoxicity (MTT for intestine; ATP, Urea, LDH for liver; ATP, KIM-1, β-NAG for Kidney) and intestinal absorption studies were conducted using three SNEDDS variants (A, B, and C) at a final concentration of 0.4% v/v. Based on absorption performance and safety profiles, SNEDDS C was selected for full pharmacokinetic evaluation in the MPS. The API (200 µg/mL) was loaded in SNEDDS C in buffer and applied to the apical surface of the intestinal tissue, and media samples were collected from all organ compartments over 72 hours. API concentrations were quantified via liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results: Minimal to no cytotoxicity was observed in response to 24-hour exposure to the SNEDDS, whether delivered alone or loaded with the API (100, 10, and 1 µg/mL for the intestine mimicking oral exposure; 20, 2, and 0.2 ng/mL for liver and kidney mimicking predicted in vivo C_{max}). When compared to the API delivered in buffer, two of the three SNEDDS facilitated slightly increased intestinal absorption of the higher doses of the API (100 and 10 µg/mL) over the 24-hour exposure period, with permeability coefficient equal to 1.50 ± 0.03 $x10^{-6}$ cm/s, 1.68 ± 0.05 $x10^{-6}$ cm/s and 1.69 ± 0.04 $x10^{-6}$ cm/s for API alone, API in SNEDDS B, and API in SNEDDS C, respectively. In the MPS platform, the candidate SNEDDS, SNEDDS C, permitted sustained absorption of the API through the intestinal culture, which was subsequently distributed throughout the liver and kidney compartments.

Conclusions: This study demonstrates the value of MPS platforms for evaluating formulation-dependent pharmacokinetics in a human-relevant, interconnected organ system. While SNEDDS C modestly improved intestinal absorption, it did not significantly alter downstream pharmacokinetics, supporting its role as a protective oral delivery vehicle. Importantly, the findings underscore the MPS platform's potential as a translational tool for formulation screening, mechanistic ADME analysis, and physiologically based pharmacokinetic (PBPK) modeling, bridging the gap between in vitro and in vivo pharmacokinetics.

INTRODUCTION

A drug's pharmacokinetics are dictated not only by its physiochemical properties, but also by the formulation and method of delivery. Micelles are a promising drug delivery system with potential to promote drug retention in tissue, protect drugs from enzymatic degradation, enhance cellular uptake, and increase exposure to the target tissue of interest. Micelles can result in varying absorption and cytotoxicity; thus, it is critical to evaluate intestinal absorption and organ-specific cytotoxicity in the safety assessment of a drug when formulated in a micelle delivery system. In vivo pharmacokinetics studies are often not suitable for the high-volume screening necessary in early-stage formulation development. Further, traditional in vitro methods do not allow for generation of accurate kinetic absorption and bioavailability data sets along with organ toxicity via relevant routes of exposure. The LifeNet Health MPS system facilitates a more physiologically relevant approach, simulating the in vivo physiology by connecting biological barriers in vitro that mimic those known to be present in vivo. Here, an integrated, multi-organ platform containing human intestinal, human liver, and human kidney models interconnected via a simulated vasculature was utilized to evaluate absorption and cytotoxicity of an API, both alone and in a series of SNEDDS.

MATERIALS & METHODS

Cell Culture: EpiIntestinal™ tissues (MatTek) were equilibrated and maintained in SMI-100-MM culture media (37°C, 5% CO_2) in 12-well plates according to manufacturer's instructions until dosing.

TruVivo™ primary human hepatocyte cultures were established and maintained in culture (37°C, 5% CO_2) according to LifeNet Health instructions for use (IFU). Briefly, human feeder cells were thawed, resuspended in Feeder Cell Thawing Medium, and plated at a density of about 10,000 cells per well in plating media on a collagen-coated 96-well plate, or 200,000 cells per well in plating media on a collagen-coated 6-well culture cup for the MPS experiments. Following feeder cells attachment (~60 min), primary human hepatocytes were thawed and resuspended in Human Hepatocyte Thawing Medium, and the hepatocytes were plated atop the feeder cells at a density of 40,000 cells per well in TruVivo™ Plating Medium for the 96-well plates ($1.2x10^6$ cells per well for the 6-well culture cups for the MPS experiments). Hepatocyte attachment was monitored for 2-4 hours post-plating. Once hepatocyte attachment was observed, the media in each well was replaced with TruVivo™ Culture Medium (100 µL per well for 96-welll plates, 2 mL for 6-well cups). The culture was inspected daily, with media changes each day of culture until dosing (culture day 5).

HK-2 cells were thawed and plated on 96-well plates at a density of 20,000 cells per well and plated on 6-well cups at about $1.2x10^6$ cells per cup. The cells were maintained in culture (37°C, 5% CO_2) in Keratinocyte Serum Free Medium + 0.05 mg/mL bovine pituitary extract + 5 ng/mL human recombinant epidermal growth factor (Gibco) according to manufacturer's instructions until dosing (24 hours post-seeding).

Cell Viability: Viability of EpiIntestinal™ tissues was determined by MTT assay. Cell viability of TruVivo™ cultures was determined by assessing intracellular ATP (CellTiter-Glo® Cell Viability Assay; Promega) and extracellular lactate dehydrogenase (LDH) in the culture media (CytoTox-ONE Homogenous Membrane Integrity Assay; Promega). For HK-2 cells, intracellular ATP and extracellular LDH were assessed along with biomarkers for kidney injury; Kidney Injury Molecule 1 (KIM-1; KIM-1 ELISA kit; MyBioSource) and β-N-Acetylglucosaminidase (β-NAG; N-Acetylglucosaminidase Assay; Sigma).

Test Article Preparation: API was incubated with self-nano-emulsifying drug delivery systems (SNEDDS) solution in a glass vial, and vortexed for 2 minutes to prepare the API loaded SNEDDS. Test article solutions were revortexed for an additional 1 min prior to application to the test system. Unloaded SNEDDS were treated identically, without the API. In addition, API was prepared and dosed alone, in phosphate buffered saline (PBS).

MATERIALS & METHODS CONT'D

MPS System: Tissue models were connected by a simulated vasculature system (Phosphate Buffered Saline, pH 7.4, with 0.1% human serum albumin). The portion of the simulated vasculature system residing inside each organ compartment contains a semi-permeable membrane that allows for diffusion of the API into and out of the 'vessel'. The apical surface of the intestinal model was exposed to SNEDDS C + API at the maximum tolerable dose (200 µg API/mL) for 72 hours. Additional tissues were exposed to SNEDDS C alone (unloaded), in parallel for cytotoxicity assessment. Media samples were collected from the basolateral intestine, liver, kidney, and simulated vasculature compartments at 0-, 0.5-, 1-, 2-, 4-, 6-, 24-, 48-, and 72-hours post-dosing.

LC-MS/MS: Samples collected at each timepoint were analyzed using a Shimazdu Nexera XR LC-30AD UPLC system in-line with a SCIEX 4000 mass spectrometer via an electrospray ionization (ESI) interface. The API was separated on a Kinetex Biphenyl 30 x 2.1 mm, 2.6 µm column using 0.1% formic acid/H2O as mobile phase A and 0.1% formic acid/acetonitrile as mobile phase B. Data was collected using a SCIEX 4000 mass spectrometer via ESI and processed using Analyst 1.6.2 software.

RESULTS

Figure 1. Initial Cytotoxicity of SNEDDS Alone and API Loaded SNEDDS

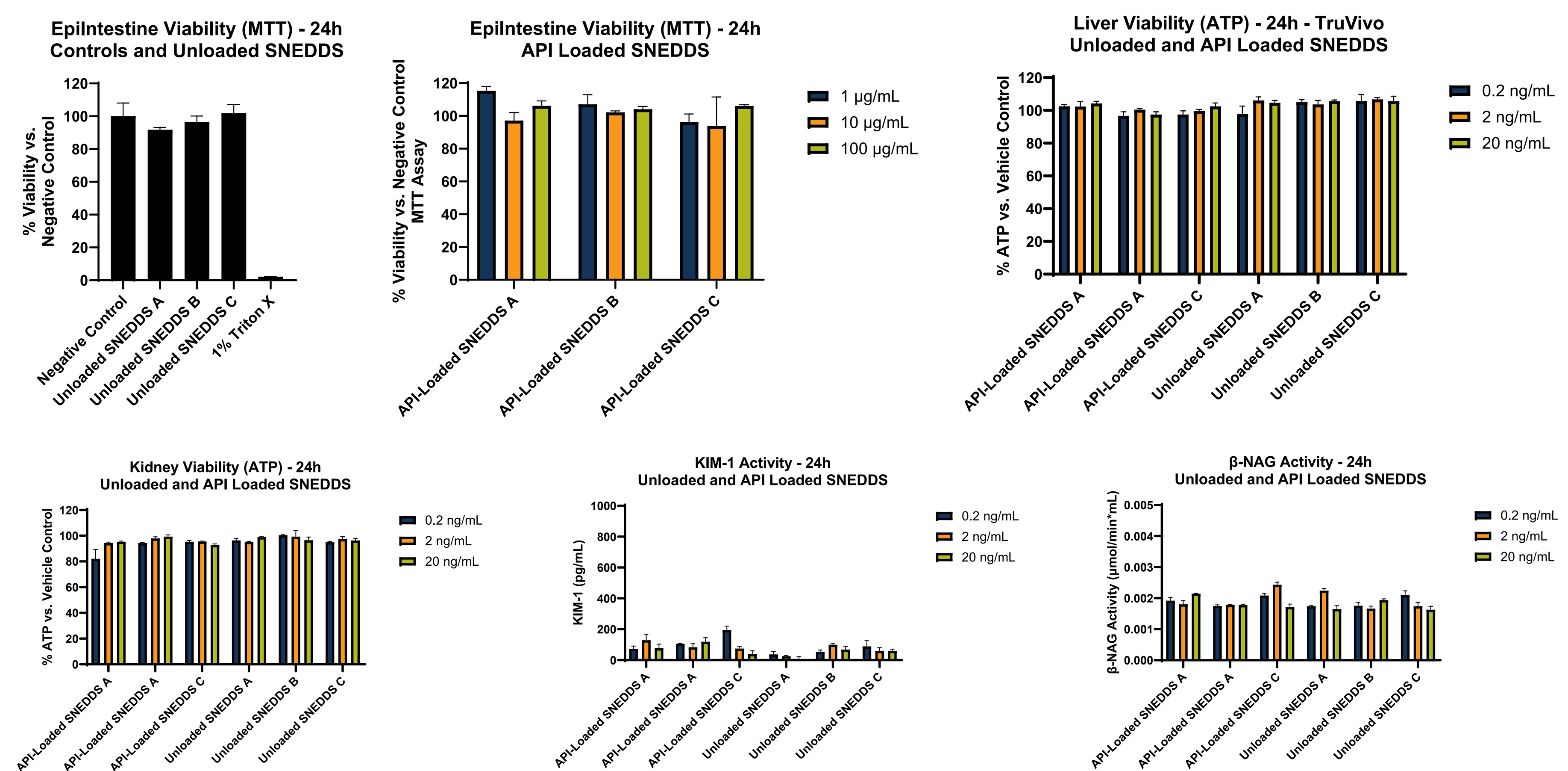

Fig. 1. EpiIntestinal™ tissues, TruVivo™ cultures, and HK-2 cultures were exposed for 24 hours at 37°C with 5% CO_2 to either SNEDDS alone or SNEDDS loaded with API. Viability was assessed by MTT in the EpiIntestinal™ tissues, ATP in the TruVivo™ cultures, and ATP, Kim-1, and β-NAG in the HK-2 cultures. Doses represented bracket oral dose levels of the API for intestine, and bracket C_{max} concentrations for the liver and kidney exposures. All exposures were conducted in triplicate (n=3).

Table 1. Apparent Permeability (Papp) of API Alone and with API Loaded SNEDDS Shows Slightly Increased Permeability with SNEDDS C

Timepoint (hr)	Concentration (µg/mL)	P_{app} ($x10^{-6}$ cm/s)			
		API Alone	API-Loaded SNEDDS A	API-Loaded SNEDDS B	API-Loaded SNEDDS C
0	1	N/A	N/A	N/A	N/A
	10	N/A	N/A	N/A	N/A
	100	N/A	N/A	N/A	N/A
0.5	1	N/A	N/A	N/A	N/A
	10	4.6 ± 0.8	N/A	N/A	N/A
	100	3.6 ± 0.0	3.2 ± 0.2	3.0 ± 0.0	3.5 ± 0.3
1.5	1	N/A	N/A	N/A	N/A
	10	4.6 ± 0.1	2.8 ± 0.1	3.0 ± 0.6	4.6 ± 0.8
	100	7.0 ± 0.3	6.6 ± 0.3	6.9 ± 0.4	7.4 (N=1)
24	1	0.7 ± 0.0	N/A	1.6 (N=1)	1.1 (N=1)
	10	1.3 ± 0.0	1.2 ± 0.01	1.3 ± 0.0	2.0 ± 0.1
	100	1.5 ± 0.0	1.5 ± 0.03	1.7 ± 0.1	1.7 ± 0.0

Table 1. Permeability coefficients of API in buffer (No SNEDDS) and API loaded into three different SNEDDS. EpiIntestinal™ tissues were exposed to 100 µL of each test article for 24 hours at 37°C with 5% CO_2. Samples were taken at 0, 0.5, 1.5, and 24 hours for LC-MS/MS analysis, and the Papp was calculated using standard calculations. Data are expressed as the mean P_{app} ± SEM of n=2 replicates, except where otherwise specified.

RESULTS CONT'D

Figure 2. Schematic of the LifeNet Health MPS System

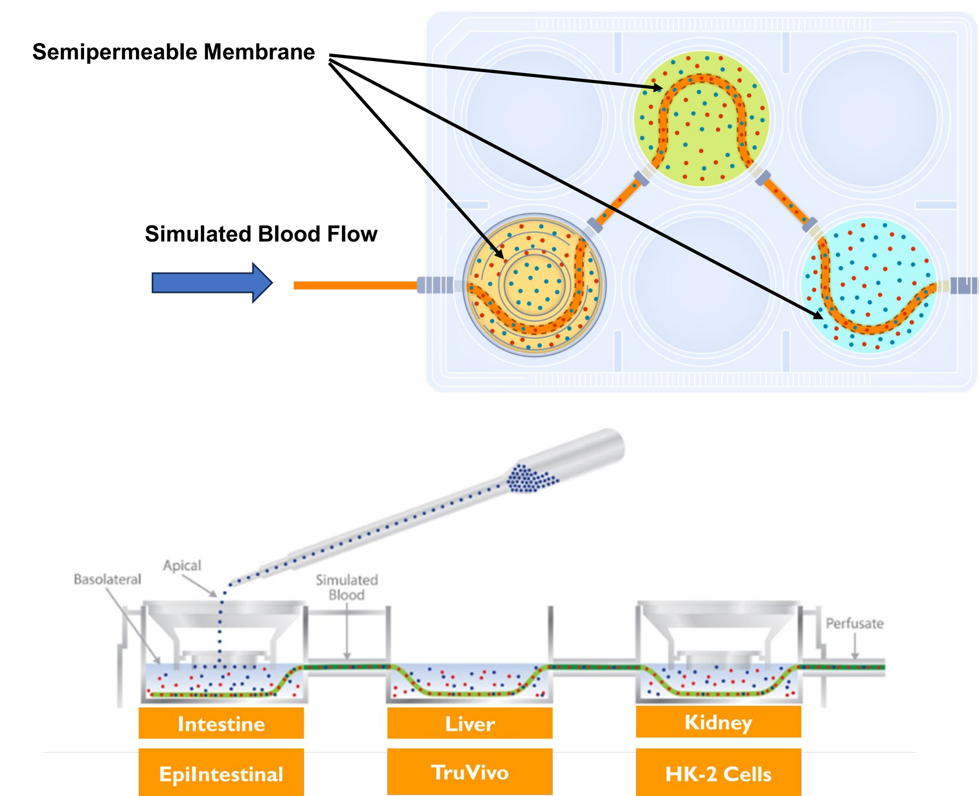

Fig. 2. Schematic of the Intestine – Liver - Kidney MPS System used in this study to assess API delivery, permeability, distribution, and toxicity.

Figure 3. Viability Assessment of Intestinal, Liver, and Kidney Models After 72-Hour Exposure to API Loaded SNEDDS in the MPS Platform

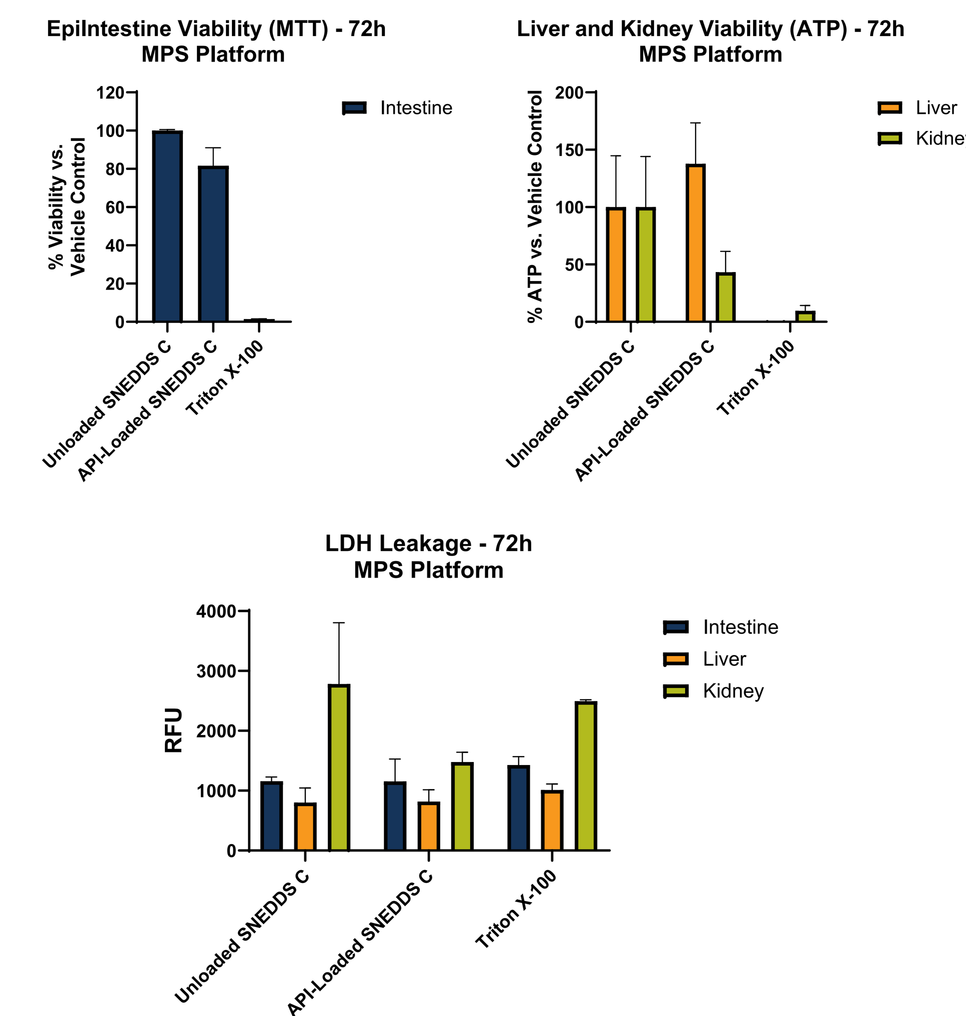

Fig. 3. SNEDDS and SNEDDS loaded with API were prepared, and 100 µL of the solutions were dosed to the apical side of the EpiIntestinal™ tissue. The system was exposed for 72 hours at 37°C with 5% CO_2. Viability of the intestine tissues was assessed using the MTT assay. LDH was assessed in the culture media. Viability of the liver and kidney was assessed using intracellular ATP. Data are expressed as % viability vs. vehicle control. LDH was assessed in the culture media. Bars represent the mean ± SEM of three replicates (n=3).

Figure 4. Pharmacokinetic Data of API in Each of the MPS Compartments Over 72 Hours of Exposure.

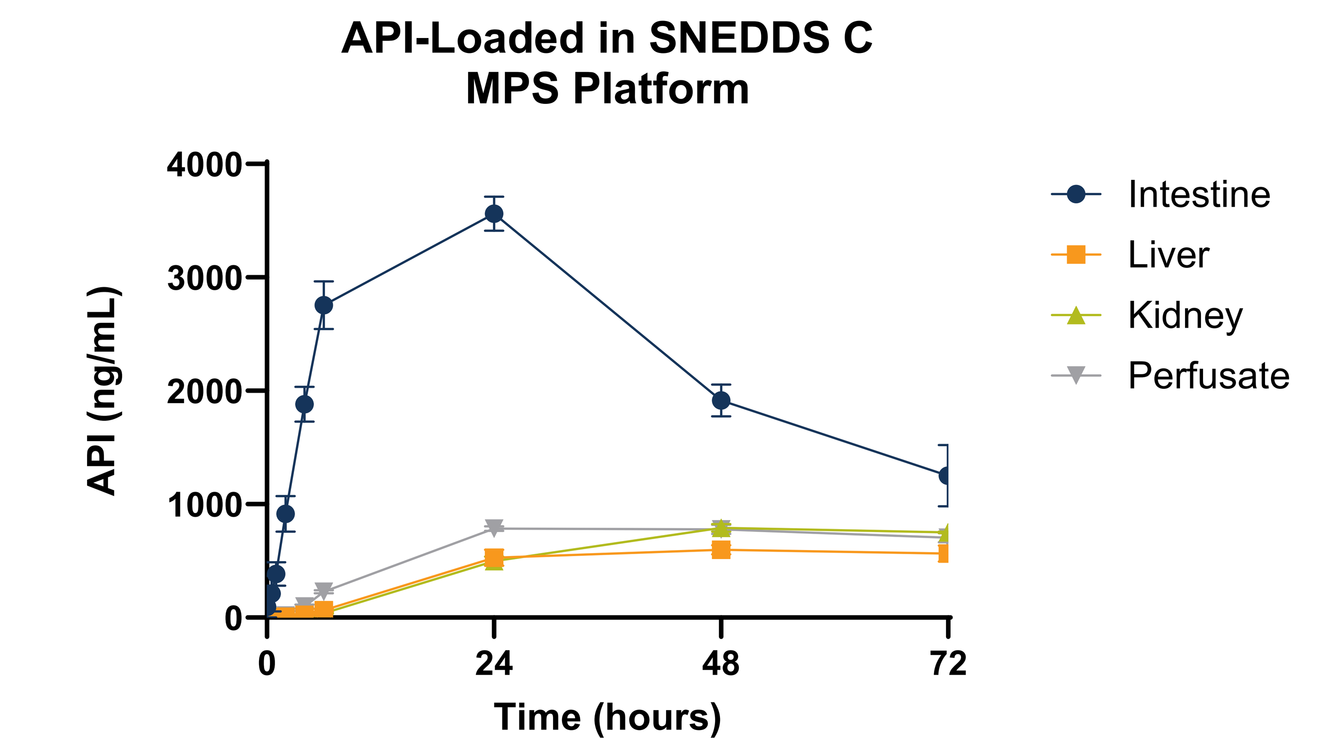

Fig. 4. The apical surface of the EpiIntestinal™ tissue was exposed to API-loaded SNEDDS C for 72 hours at 37°C with 5% CO_2 with samples being taken from each compartment at 0, 0.5, 1, 2, 4, 6, 24, 48, and 72 hours. Data are expressed as the concentration of API in ng/mL. Data points represent the mean ± SEM of N=3 replicates. Some error bars are too small to be seen.

CONCLUSIONS

- Comparable intestinal absorption profiles of API were observed when delivered in SNEDDS versus buffer. API loaded SNEDDS C showed lightly better absorption than with API alone.
- API had minimal cytotoxicity in all three models when initially assessed at 24 hours exposure.
- In the MPS system, intracellular ATP levels decreased and LDH leakage increased in the kidney model when exposed to API-loaded SNEDDS C for 72 hours, however, this was observed at concentrations higher than predicted C_{max}.
- LifeNet MPS system has been shown to accurately model pharmacokinetic and toxicological profiles of many compounds. Here we show the system is amenable to alternate delivery systems for APIs via oral absorption route.

REFERENCES / ACKNOWLEDGEMENTS

1. McKim, J. M., Austin, D. J., Sprando, R., Hermansky, S., Mattes, W., & Fitzpatrick, S. (2024). Developing Kinetic and Organ Toxicity Data with a Novel In Vitro Human-Based Multiple Organ MPS: Acrylamide as a Case Study. *Applied In Vitro Toxicology*, 10(2), 33-42. https://doi.org/10.1089/aivt.2024.0021.

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