Dynamic skin and liver co-culture to assess the effect of application routes on the metabolism of AHT

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Introduction

This project is based on the HUMIMIC Chip2 – a Multi-Organ-Chip from TissUse enabling the co-culture of skin (EpiDerm™) and liver models (consisting of HepaRG and stellate cells) – and aims to provide information about the influence of exposure scenarios on the bioavailability and metabolic fate of chemicals. The aromatic amine hair dye 4-amino-2-hydroxytoluene (AHT) was selected as a case study chemical to recapitulate a pre-clinical rat pharmacokinetics study.

Experimental Design Principles

The EpiDerm™ model by MatTek and liver spheroids consisting of HepaRG cells and human stellate cells were co-cultured for to 6 days in the Chip2. Half medium exchange was performed every day. AHT is an ingredient in hair dyes and was therefore applied one day after inoculation of the organ models in the chip once topically on top of the skin model (mimicking a real-life scenario) or systemically into the liver compartment, resulting in a concentration of 2.5 µM in the Chip2. The dynamic co-culture continued for 5 days. Samples of the medium were taken at various time points for analysis of LDH, glucose, lactate and albumin content, as well as for quantification of parent compound and metabolite.

Results

The morphology and metabolic activity of the organ models were well maintained for 6 days in the Chip2. Topical exposure resulted in a marked lower systemic concentration of the parent chemical compared to systemic application. The kinetics and quantitative profiles of several AHT metabolites were altered by topical application compared to systemic application, including N-acetyl-AHT and AHT-sulfate. Importantly, there was a higher peak concentration of N-acetyl-AHT after topical (1043 nM) compared to systemic (317 nM) application, indicating that a first-pass effect in the EpiDerm™ model had occurred. The AUC for this metabolite was also increased by 275% in case of topical exposure. In contrast to N-acetyl-AHT, the peak concentration and AUC of AHT-sulfate was lower after topical compared to systemic application.

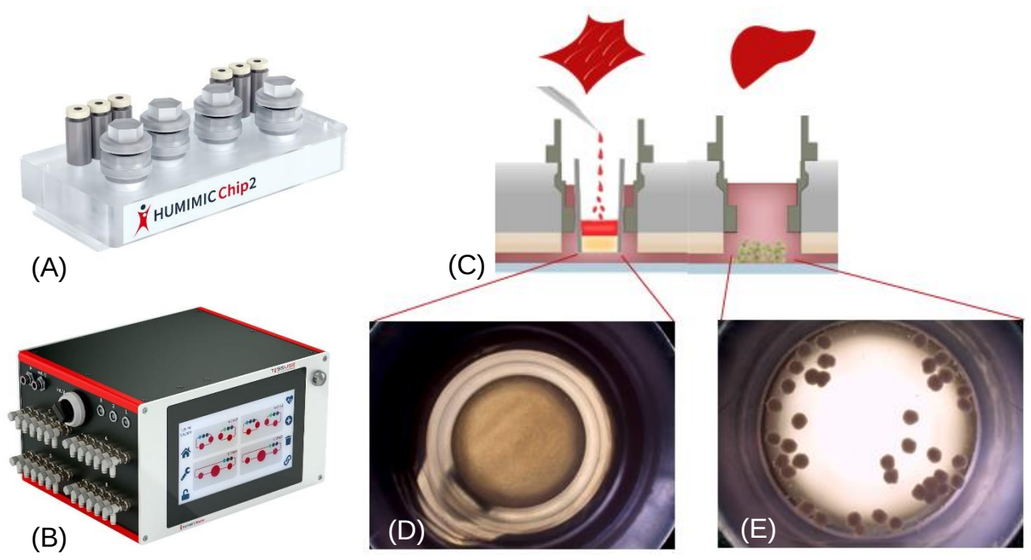

Fig. 1. (A) HUMIMIC Chip2 and (B) HUMIMIC Starter. All products are produced in-house. TissUse has been awarded the ISO EN 9001-2015 which is a globally recognized standard that certifies quality management systems focused on ongoing improvements, customer satisfaction and implementing a process approach in management. (C-E) **HUMIMIC Chip2 set-up.** (C) Schematic overview of the multi-organ-combination in the Chip2. (D) EpiDerm™ by MatTek in the skin compartment. (E) Liver spheroids (consisting of HepaRG cells and stellate cells) in the liver compartment.

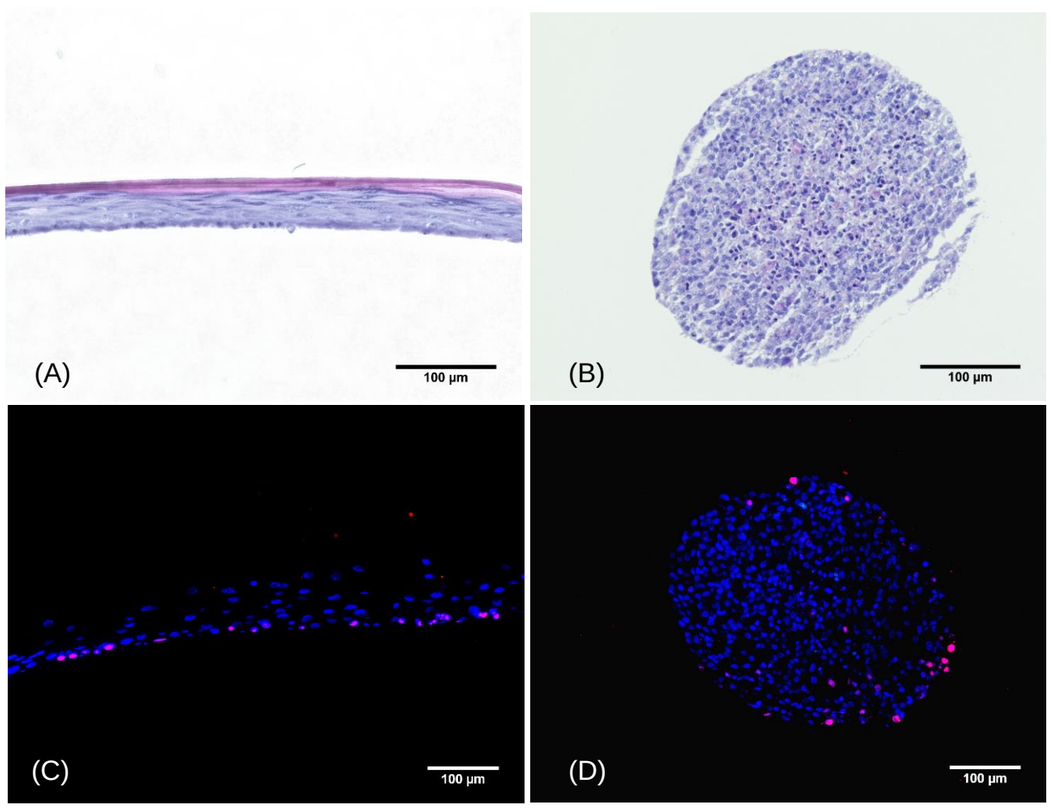

Fig. 2. Histology of the used organ organ models in the HUMIMIC Chip2. Hematoxylin stainings of (A) EpiDerm™ by MatTek and (B) liver spheroids. Dapi (blue) and Ki67 (red) staining of (C) EpiDerm™ by MatTek and (D) liver spheroids. Cryosections of 8 µM were used for all stainings.

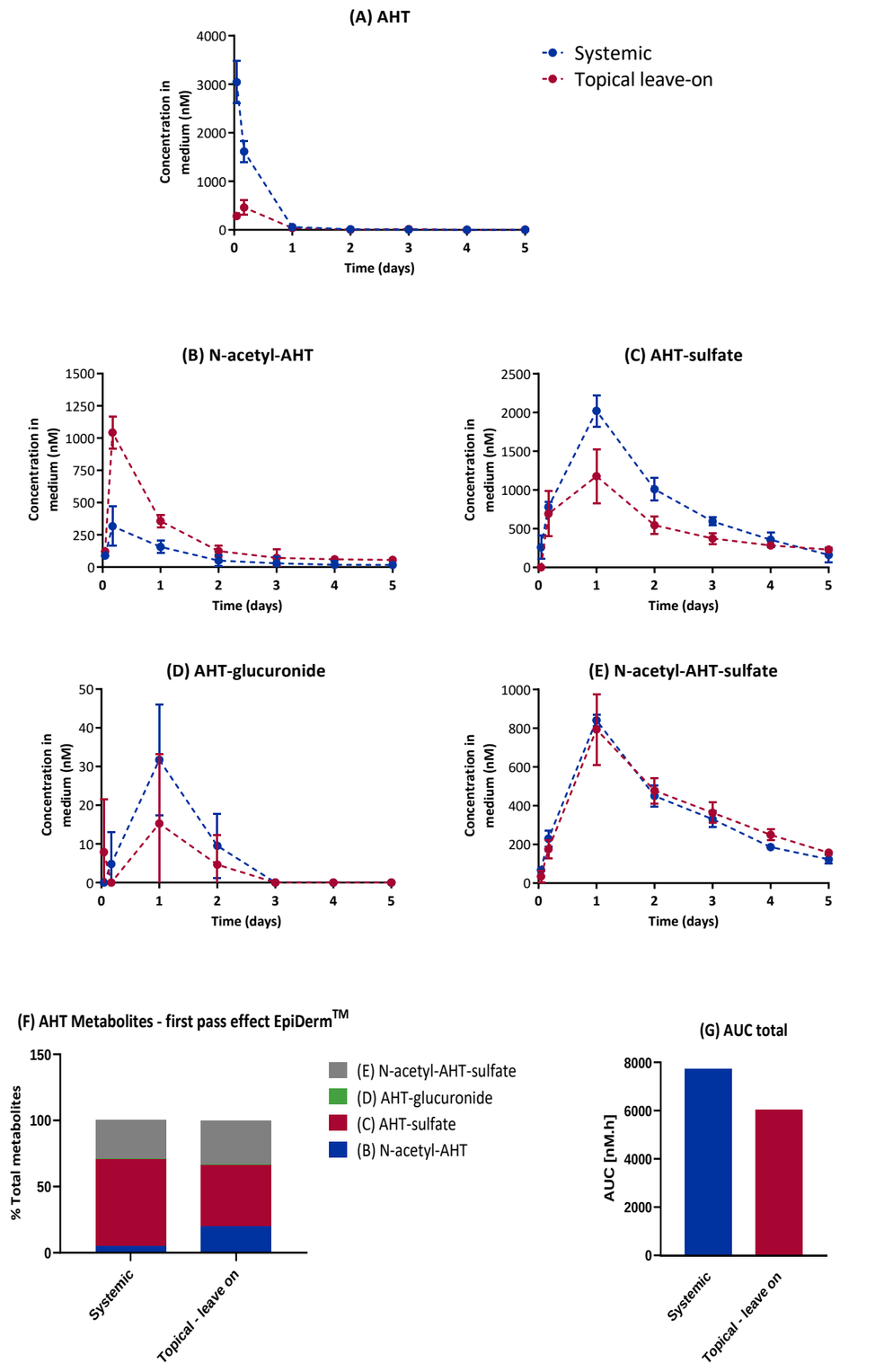

Fig. 3. Concentration of AHT (A) and its metabolites (B-4) in the Chip2 after systemic and topical application of 2.5 µM AHT. (F) Percentage of total amount of metabolites of AHT after systemic and topical application based on AUC. (G) Comparison of total AUC including AHT and metabolites of AHT. Values are from 3-6 circuits ± SD (SD only in A-E).

Summary and Outlook

We demonstrate that the Chip 2 is able to culture different organ models for several days and provide information about the influence of exposure scenarios on the bioavailability and metabolic fate of the cosmetics ingredient AHT. Findings of an *in vivo* rat study could be mimicked with the Chip2, which provides a dynamic culture closer to the *in vivo* situation. Here we show that this platform can provide additional and important information for risk assessment in the Consumer Products industry.

As part of Cosmetics Europe´s contemporary Long Range Science Strategy (LRSS), this project is aiming to, among other activities, establish the link between dermal and systemic exposure route. Beiersdorf and TissUse are the two independent collaborators in this project.

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