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Circulating bioactives resulting from TOTUM•854 absorption in humans protect primary human endothelial cells against lipotoxicity: lessons from an original *ex vivo* clinical trial

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Purpose

TOTUM•854 is a patented plant blend extract characterized for its polyphenol-rich content that supports hypertension prevention in preclinical studies. However, clinically validated approaches and further investigations on the mode of action at the cellular level especially in humans are required for optimized care management. In this study, we designed an *ex vivo* clinical innovative approach considering the circulating metabolites produced by the digestive tract following the ingestion of TOTUM•854 in humans.

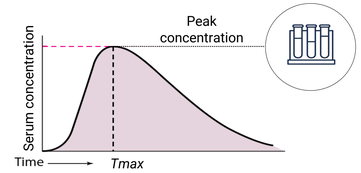
Methods

A pool of 10 healthy men volunteered for this study. To determine kinetic of TOTUM•854 bioactive molecules profile in the serum, volunteers fasted for 12h were given 3.71 g of TOTUM•854. Venous blood was collected from the median cubital vein before the ingestion and every 20 minutes for 240 minutes after the ingestion. Human circulating metabolites from polyphenols were quantified and characterized by UPLC-MS. Once the absorption peak was determined, volunteers were called back for the collection of a naïve serum and at the maximum absorption peak (Tmax) for TOTUM•854-metabolites enriched serum collection. Human serum enriched with metabolites deriving from TOTUM•854 absorption was further incubated with human endothelial cells (HUVEC), pretreated or not with palmitate (200 µM).

Clinical phase (Dose: 3.71g of TOTUM•854 in 8 capsules)

Step 1
Kinetics
10 volunteers, 4 hours

Step 2
Serum collection
10 volunteers



Serum enriched with polyphenolic compounds and derived metabolites

Ex vivo phase : Human Umbilical Vein Endothelial Cells

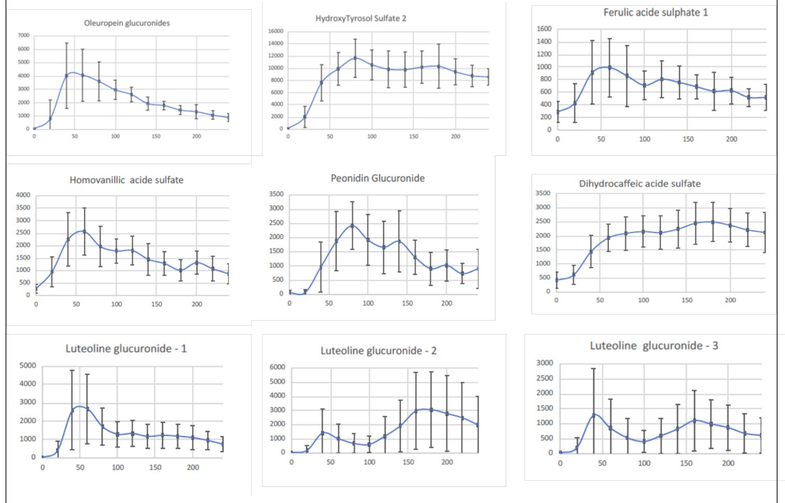
HUVECs were pre-incubated 24h in presence of either naive or TOTUM•854 sera before additional 24h incubation with the same condition but in presence or not of palmitate 200 µM to induce a lipotoxic stress. Following tests were performed:

- Cell viability assay to demonstrate no toxicity of metabolites-enriched sera;
- Evaluation of the Angiotensin Converting Enzyme 1 activity (ACE-1 activity);
- Evaluation of the oxidative stress environment (DCFDA and DHE staining, NO production, NOX2 expression);
- Measure of the inflammatory response (IL-1ß release);
- Evaluation of the potential cell recruitment (MCP-1 and VCAM-1 expression).

Conclusion

In the presence of the human metabolites from TOTUM•854, HUVECs were protected from an induced lipotoxic stress. HUVEC protection was characterized by a (1) decreased ACE-1 activity; (2) the limitation of an oxidative stress environment; (3) the inhibition of inflammatory response and (4) a decreased marker expression of endothelial reactivity. Using a pioneering clinical *ex vivo* approach, all together, these data give clues on the role of metabolites produced following TOTUM•854 intake in humans in endothelial cells protection.

Step 1: Results of kinetic profile of TOTUM•854 absorption in humans



The digestion and absorption profile of the extract was monitored through a kinetic of apparition of the metabolites following TOTUM•854 ingestion to determine the time frame of the absorption peak. We found 10 detectable circulating human metabolites including two oleuropein glucuronides isomers, three luteolin glucuronides isomers, one hydroxytyrosol sulfate isomer, one ferulic acid sulfate isomer, one homovanillic acid sulfate isomer, one dihydrocaffeic acid sulfate isomer, and one peonidin glucuronide isomer. Thus, these data strongly evidence an **efficient absorption and bioavailability of the product**. The Tmax (time to reach the maximum concentration observed in serum) ranged from 40min to 180min post absorption depending on the type of metabolites. According to the global absorption curve, enriched serum TOTUM•854 metabolites was collected at 60min post-ingestion for *ex vivo* investigation on HUVECs.

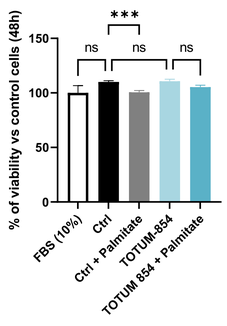
Disclosures

Doriane RIPOCHE, Yolanda F OTERO, Florian LE JOUBIOUX, Maxime BARGETTO, Véronique SAPONE, Murielle CAZAUBIEL and Pascal SIRVENT are employees of Valbiotis. Sébastien PELTIER is CEO of Valbiotis.
Thierry MAUGARD is a member of the scientific committee of Valbiotis.
Line BOUTIN-WITTRANT and Yohann WITTRANT are employees of Clinic'n'Cell SAS. Fabien WAUQUIER is CEO of Clinic'n'Cell SAS.

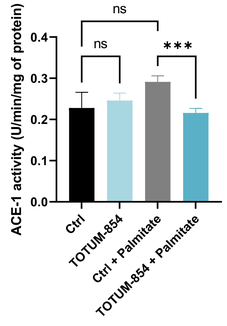
Step 2: Effects of TOTUM•854 metabolites *ex vivo* on human HUVECs under lipotoxic stress

In the presence of the human metabolites from TOTUM•854, HUVECs were protected from an induced lipotoxic stress. **No effect on cell toxicity** was detected in the presence of enriched sera. HUVEC protection was characterized by **(1) decreased ACE-1 activity** (-31% p<0.0001); **(2) the inhibition of oxidative stress** with decreased ROS (-25% observed by DHE fluorescent microscopy) as well as decreased Nox2 gene expression (p<0.01); **(3) the inhibition of inflammatory response**, with a decrease in IL-1β release (-41% compared to palmitate, p<0.001) and decreased MCP-1 et VCAM-1 gene expression (-86% p<0.001 and -70% p<0.001 respectively). All together, these data provide new biochemical mechanisms for a protective role of TOTUM•854 in human endothelial cells.

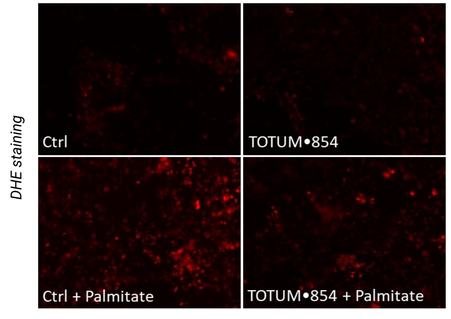
Cell viability at 48h



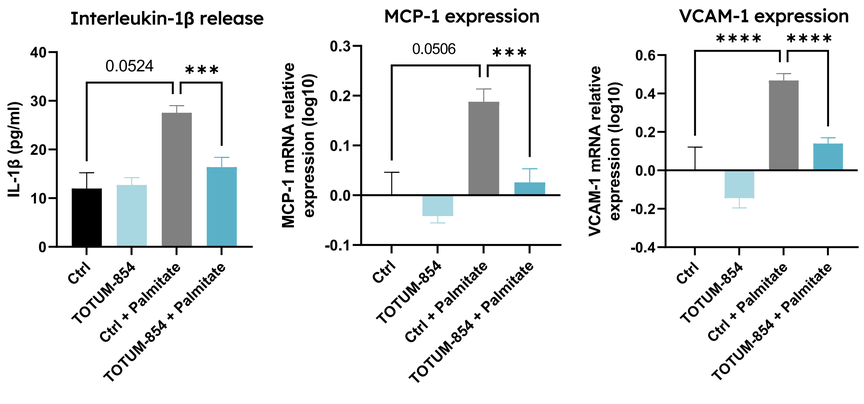
(1) Angiotensin Converting Enzyme 1 activity



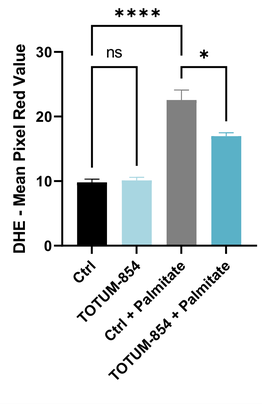
(2) Oxidative stress environment



(3) Inflammatory response



DHE staining



Nox2 expression