Metabolomics Evaluation of the Photochemical Impact of Violet-Blue Light (405 nm) on *Ex Vivo* Platelet Concentrates

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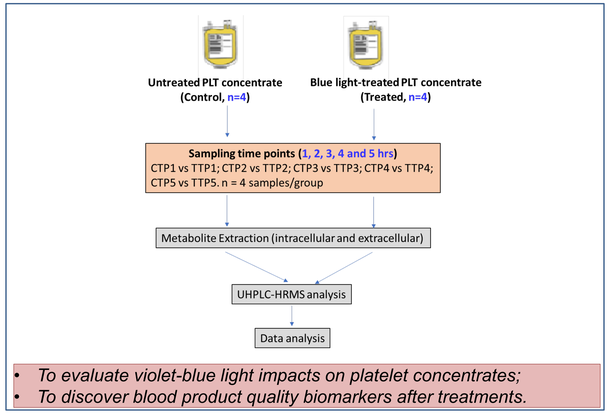
Abstract

Introduction: Ex vivo stored human platelet concentrates (PCs) are susceptible to bacterial contamination during the 5-7 day period before use at 22 ± 2°C in gas permeable bags. Current FDA-approved pathogen reduction technologies (PRT) use external photosensitizers in combination with UV light irradiation to reduce transfusion contamination risk. Since UV light is harmful to mammalian cells, we have been evaluating violet-blue light to determine whether it can serve as an alternate to PRT technology, without the need for additional exogenous photosensitizers. An LC/MS-based metabolomics analysis was conducted to evaluate the impact of violet-blue light on the platelets (PLTs).

Methods: Apheresis-collected human PCs from each donor were uniformly split into two transfer bags. One PC bag was used as control (no light treatment) and the other bag was used for the light treatment. PLTs in the bags were exposed to 405 nm light at an irradiance of approximately 54 J/cm^2/h. The protocol was approved by the FDA Research Involving Human Subjects Committee. LC/MS-based metabolomics analysis was conducted to identify the metabolic changes in both PLTs and plasma.

Preliminary Data: After 5h of treatment, no changes were observed in either platelet aggregation inhibitory factors or platelet activation factors. No changes were observed in lysoPCs or PCs, which indicated that the cell integrity was intact. After 5h of treatment, the distinctive changes were increases in hydroxy-fatty acids, OH-fatty acyl-carnitines, and aldehydes, indicative of induction of lipid peroxidation. Lower levels of glutathione, vitamin A and uric acid were observed due to counteracting the light-induced reactive oxygen species (ROS). Changes in a few endogenous photosensitizers suggested the anti-microbicidal potential of the light. In summary, the results indicate that platelet integrity, activation and aggregation potential-impeding biomolecules appear to be unaffected by the light treatment. However, a comprehensive functional analysis in the context of metabolome alterations is warranted to evaluate ex vivo PLT quality after the light (405nm) treatments.

Experimental Design



- *To evaluate violet-blue light impacts on platelet concentrates;*
- *To discover blood product quality biomarkers after treatments.*

Materials and Methods

Metabolite Extraction

Plasma (100 µL aliquot) was mixed with 300 µL methanol (−20 ºC) on ice for 20 min, then centrifuged at 13,000 rpm for 12 min at 4 ºC to precipitate proteins. The supernatant was transferred to autosampler vials for LC/MS analysis.

PLT pellet (~ 10^8 cells) was suspended in 200 µL ice-cold water. The cell suspension was transferred into a tube containing 600 µL methanol (−20 ºC), vortexed, kept on ice for 20 min, then centrifuged at 13,000 rpm for 12 min at 4 ºC to precipitate proteins. The supernatant was transferred to autosampler vials for LC/MS analysis.

Open Metabolic Profiling

The extracted metabolites were separated by a Thermo Vanquish Ultimate 3000 UPLC (Thermo Scientific, Milford, MA, USA) equipped with a Waters bridged ethyl hybrid (BEH) C8 column. The metabolomics data was collected with a Thermo Orbitrap Exploris 240 mass spectrometer (Thermo Scientific, Waltham, MA) operated in positive and negative ionization electrospray modes. Data were acquired in full-scan mode (*m/z* 70 to 1000) at a resolution of 120,000 for all samples.

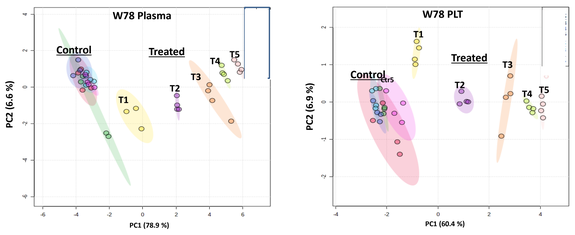


Figure 1. Principal component analysis (PCA) score plots of metabolome data from plasma and PLTs after the light-treated (T) and control (Ctr) samples from Donor W78.

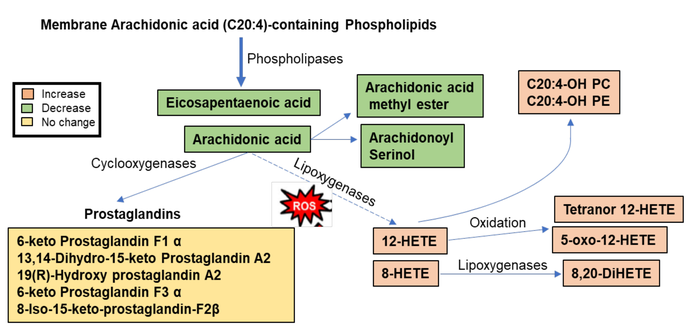


Figure 2. Identified plasma metabolites involved in the arachidonic acid (AA) metabolism after 5 h of light treatment.

Results

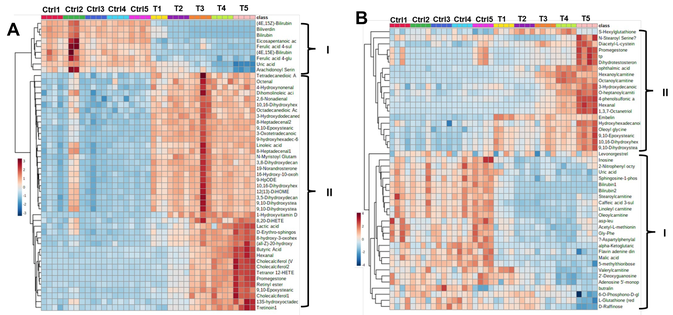


Figure 3. Heatmap of top-50 most changed plasma (**A)** and PLT metabolites (**B**) after 1, 2, 3, 4, and 5-hour light treatments, along with respective controls from donor W78. **Cluster I** consisted of antioxidant metabolites, endogenous photosensitizer metabolites and arachidonic acid related metabolites, which decreased after light treatments. **Cluster II** included light dose-dependent increases in aldehydes, vitamin D and oxidized vitamin A metabolites, lactate, and steroid metabolites.

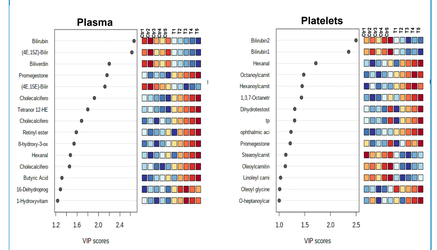


Figure 4. Top 15 variable importance in projection (VIP) plot of plasma and PLT metabolites after 1, 2, 3, 4, and 5-hour light treatments, along with respective controls from donor W78.

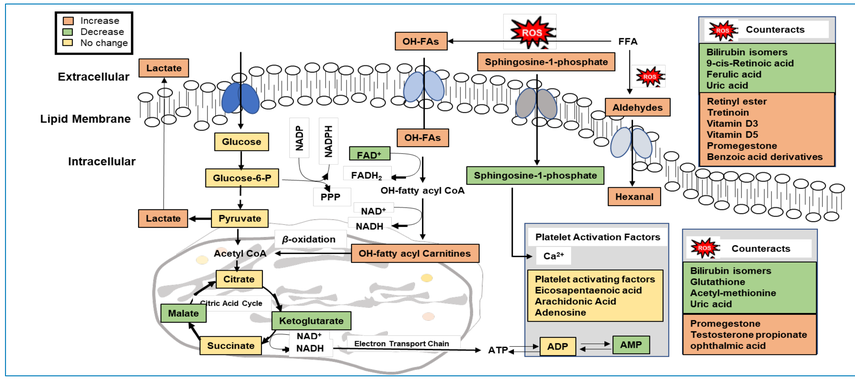


Figure 5. Summary illustration of the identified metabolites involved in the oxidative stress and energy pathways after a 5 h light treatment.

Conclusion

- After a 5 h-long light treatment, unaffected levels of prostaglandins, PAFs, and agonists indicated that the light (405 nm) illumination did not trigger, neither preventing nor promoting, the PLT aggregation process.
- After 5 h of light treatment, unaltered extra- and intra-cellular lysoPCs and phospholipids also indicated that 5 h light exposure did not affect PLT membrane integrity.
- Distinct increases in OH-FAs and aldehydes indicated that ROS were generated at high levels as early as 1h of light exposure.
- The observed decreases in antioxidant metabolites and increases in the corresponding oxidized metabolites were most likely caused by counteracting ROS, suggesting that ROS was likely generated by light illumination in PLT concentrates.
- Distinctly-changed endogenous photosensitizer metabolites observed as early as 1 h light exposure provides robust evidence as to why 405 nm light treatment alone is an effective microbicidal approach-and needs no additive external photosensitizers to reduce bacterial contamination risk as an alternate to current PRT technology.

This presentation reflects the views of the authors and does not necessarily reflect those of the U.S. Food and Drug Administration.