Microbicidal 405 nm Violet-blue Light Inactivates Leishmania Parasite in Ex Vivo Human Platelets Stored in Plasma*

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Abstract

Background: Violet-blue light of visible spectra at 405 nm is an effective microbicidal tool like ultraviolet (UV) light. Unlike UV light, 405 nm light is gentler on the host cells harboring microbes. To date, the violet-blue light has been shown to be effective against several blood-borne bacteria, HIV-L, and Trypanosoma cruzi parasite.

Purpose: To expand the scope of 405 nm light, microbicidal efficacy was evaluated on another blood-borne parasite, Leishmania donovani in experimentally contaminated platelet concentrates (PCs) stored in plasma.

Methodology: Apheresis-collected human PCs from six screened donors were used. The FDA Research Involving Human Subjects Committee approved the protocol. Platelets from three donors (n = 3) were each inoculated with L. donovani high titer (10E6/ml). Six bags with 40 ml of parasite-spiked platelets were prepared per donor. Three bags were used as controls (no light treatment, wrapped in tinfoil to prevent any light) and other three bags were treated with the light at irradiance of 54 J/cm2/h for 0h or 5h. Similarly, three additional donor PCs (n = 3) were inoculated with lower titer parasites (10E3/ml). All bags were placed in a closed LED system emitting narrowband 405 nm light, and maintained at 22°C, on shaker incubator at 60 rpm. Ten samples from each bag per time point were enumerated for parasites, using Neubauer hemocytometer chamber.

Results: Relative to controls, in the high titer parasite-spiked 5h light-treated samples, parasite counts were near zero, suggesting parasite inactivation. Similar results were observed with lower titer parasite-spiked 5h light-treated samples also. The observed parasite inactivation associated with the 5h light treatment demonstrates that the treatment was able to exert microbicidal effect on L. donovani in stored platelets. Based on previous reports, this is perhaps achieved through photocytotoxicity of purpurogallin and flavins present in the plasma which induces reactive oxygen species (ROS) that cause damage to pathogens.

Conclusions: This study revealed promising microbicidal role of the violet-blue light on L. donovani in platelets stored in ex vivo platelets stored in plasma and warrants further studies in vivo animal model to strengthen and validate these in vitro observations.

Figure 1. Overview of study design to evaluate efficacy of violet-blue light to inactivate Leishmania parasite in stored human platelets

Materials and Methods

- Study involving human subjects’ protocol was approved by FDA Research Involving Human Subjects Committee (IRB#s, Exemption Approval #11-0368).
- Human platelets collected through apheresis from six screened donors were used.
- Platelets were inoculated with L. donovani at high titer (10E6/ml) or low titer (10E3/ml).
- Six bags with 40 ml of parasite-spiked platelets were prepared per donor.
- Three bags were used as controls (no light treatment, wrapped in tinfoil to prevent any light) and other three bags were treated with the light at irradiance of 54 J/cm2/h for 0h or 5h.
- All bags were placed in an enclosed LED system emitting narrowband 405 nm light, and maintained at 22°C, on shaker incubator at 60 rpm.
- Ten samples from each bag per time point were enumerated for parasites, using Neubauer hemocytometer chamber.
- Three donor PCs (n = 3) were inoculated at each high and low titer parasite.

Results and Discussion

- The high titer-spiked 5h light-treated samples, parasite counts were near zero, with respect to controls, suggesting parasite inactivation.
- Similar results were observed with lower titer-spiked 5h light-treated samples.
- The observed parasite inactivation associated with 5h light treatment demonstrates that the treatment was able to exert microbicidal effect on L. donovani in ex vivo platelets.
- Based on previous reports, this is perhaps achieved through photocytotoxicity of purpurogallin and flavins present in the plasma which induces reactive oxygen species (ROS) that cause damage to pathogens.

Figure 2. Viable Leishmania donovani parasites (high titer spike) in platelets after 5 hrs of violet-blue light treatment

Figure 3. Viable Leishmania donovani parasites (low titer spike) in platelets after 5 hrs of violet-blue light treatment

Conclusions

- Microbicidal effect of the violet-blue light on L. donovani in stored platelets in plasma was demonstrated this study.
- Further optimization of duration of the treatment can be carried out to achieve equivalent reduction of L. donovani in ex vivo platelets.
- In vivo studies in a suitable animal model is warranted to strengthen and validate the in vitro observations.

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