C0680 COMPARISON OF PROTOCOLS IN METHYLENE BLUE-MEDIATED PHOTODYNAMIC INACTIVATION ON CUTANEOUS LEISHMANIASIS IN A MURINE MODEL USING REAL TIME BIOLUMINESCENCE

Fernanda Viana Cabral[1], Martha Simıes Ribeiro[1], Ismael Pretto Sauter[2], Mauro Javier Cortez Veliz[2]

[1]IPEN-USP Brazil
[2]ICB-USP Brazil

1 Background

Leishmaniasis is a chronic disease developed by parasites of the genus *Leishmania*. Cutaneous leishmaniasis (CL) has several different forms including destructive and ulcerated lesions. The available treatments are limited because of side effects, resistance and toxicity. Photodynamic therapy (PDT) has been explored as an alternative treatment once it is less expensive and no reports about resistance have been described. The aim of this work was to evaluate two protocols of methylene blue (MB)-mediated PDT on CL induced in mice using real time bioluminescence. In addition, we monitored lesion size progression and hyperalgesia.

2 Methods

Promastigotes of *L. (L) amazonensis* transgenic line expressing luciferase were used. Twelve BALB/c mice were infected in the left footpad with 1.10^6 promastigotes. After 4 weeks, mice were randomly assigned to experimental groups (n=4): Control (non-treated), G1 and G2 submitted to one and two PDT sessions, respectively. The second session was performed 24h after the first. PDT was performed using a red LED (λ= 660±22 nm), MB (100μM) and 150J/cm^2 fluence. Disease progression was evaluated once a week by measuring lesion size with a caliper and hyperalgesia with von Frey filaments. Parasite burden was evaluated through luciferase detection by bioimaging, every day, in the first 96h and then for the next 4 weeks. Prior to imaging, mice received 75mg/kg luciferin, intraperitoneally. Results were quantified with proper software, expressing the number of photons/s/cm^2. Statistically significant differences were considered when $p<0.05$.

3 Results

Statistically significant lesion size reduction was observed after 2 weeks post PDT compared to G1 and control groups. In 4 weeks, G2 demonstrated to be clinically more effective and no ulcer signs were detected. Hyperalgesia revealed a positive outcome after 2 weeks, with 50% nociceptive stimulus decrease. In 3 weeks there was a 75% reduction in G2, suggesting that 2 sessions were more efficient. Parasite burden was lower in the first 24h and 72h in both treated groups. Besides, G2 demonstrated statistically significant parasite burden reduction 96h after PDT compared to control.

4 Conclusions

The present study demonstrated that PDT reduced lesion size and hyperalgesia in BALB/c mice, suggesting that therapy probably influenced the inflammatory process. However, further studies are required to deep understanding about PDT mechanisms in cutaneous leishmaniasis treatment.