Liposomal zinc phthalocyanine as a potential agent for photodynamic therapy of leishmaniasis

Sir,

Developing a new therapeutic method for leishmaniasis, besides obtaining a prompt treatment response that prevents lesions' progression and scarring, is significant success in the treatment of the disease. Regarding the superficiality of the leishmaniasis lesions, there is anticipation that photodynamic therapy (PDT) could be beneficial in treating or at least preventing the lesions' progression.

Pharmacokinetic characteristics of zinc phthalocyanine (ZnPc) make this molecule a promising second-generation photosensitizer. ZnPc is not water-soluble, and aggregation of the molecules decreases its photosensitizing effect. In this paper, efficacy of PDT with liposomal ZnPc was assessed on Leishmania parasite.

ZnPc was purchased from Sigma-Aldrich (97% dye content). In order to formulate the photosensitizer in liposomal form, 300 mg of egg lecithin, 100 mg of cholesterol, 400 mg of glucose and a precise amount of zinc phthalocyanine were dissolved in 10 mL of pyridine. The solution was frozen using particular processes by dry ice and subsequently dried via freeze-dryer (Labco Co., USA) during two consecutive stages of −40°C and −25°C temperature designed for 24 hours.[1] Eventually, to prepare the final stable solution, 2 mL of distilled water was added. Based on the spectroscopic results from the liposomal suspension supernatant, the encapsulation rate of ZnPc was determined to be more than 85% and average of particle size was estimated to be 1.6 μm.

Leishmania major parasites (MRHO/IR/75/ER) were provided by Iranian Pasteur Institute, grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, pH 7.4, and antibiotic agents in a 25°C incubator.[2,3] Then the stationary-phase promastigotes underwent pre-planned *in vitro* treatments.[3] First, the parasite suspension was divided into four groups and incubated at the liposomal ZnPc concentrations (0,1,5,10 μM) for 3 hours at 25°C.[4] After parasite washing in phosphate buffer solution (PBS), it was suspended again in a complete culture medium and spread in the wells of two 24-well sterile plates. One of them underwent illumination, and the other was maintained in dark. An incoherent light source, such as LUMACARE, equipped with fiber-optic and filter of 670 ± 20 nm was utilized for illumination at intensity and fluence of 45 mW/cm^2 and 100 J/cm^2, respectively.

Twenty-four hours post-treatment, the parasite survival was determined by3-(4,5-Dimethylthiazol2-yl)-2.5-diphenyltetrazolium bromide (MTT) assay. The treated samples were incubated in a 96-well plate with the MTT reagent (10 mg/mL) for 40 minutes at 24°C, and optical density of the formazan crystals was measured at 545 nm using a 96-well microplate reader (AWARENESS, Model 3200). The experiments were repeated SPSS 16 using Kolmogorov-Smirnov normality test, ANOVA and Tukey test and analyzed three times.

As shown in Figure 1, among the groups which received complete PDT, minimum survival rate was obtained at 10 μM (12.4%), which was significant in comparison with the other concentrations ($P< 0.005$), while no significant reduction was confirmed between the 1-(Micromolar) μM group and control group. Without illumination, at 1 μM, reduction of parasite survival was significant in comparison with the control group, while it was not significant between 5 and 10 μM.

On the basis of an exponential trend line fitted on the PDT data ($R^2= 0.97$), ED_{50} and ED_{90} of liposomal ZnPc after 100 J/cm^2 illumination were obtained to be about 3.5 and 11.5 μM, respectively.

Regarding our data, liposomal ZnPc showed toxicity on the Leishmania parasite in darkness, but the

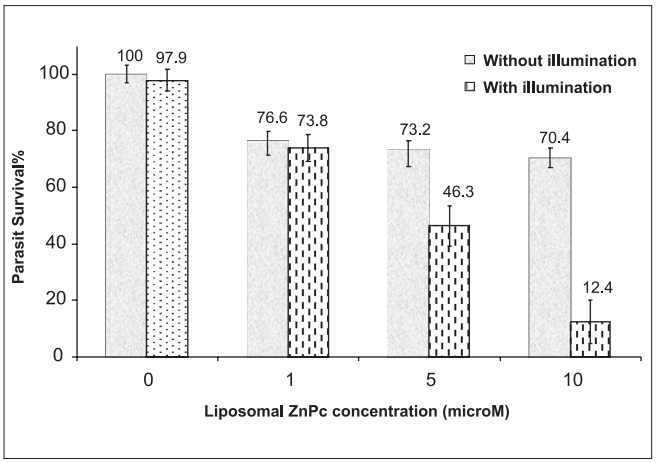

Figure 1: Leishmania parasites survival rate 24 hours after *in vitro* treatments at three concentrations of liposomal ZnPc; illumination dose: 100 J/cm². Each data represents the mean of the results of three separate experiments ± Standard Error (SE)

toxic effects did not increase with increasing dye concentration. This finding differs from the study by Scober *et al,* which reported 5% toxicity at a 15-μM of ZnPc concentration.[5] Also, they estimated ED_{50} and ED_{90} of ZnPc on two strains of Leishmania after 10 J/cm² laser irradiation. ED_{50} was 6.05 and 6.45 μM for *L. chagasi* and *L. panamensis*, respectively, and E_{90} was >15 μM for both strains. In our study, ED_{50} and ED_{90} of liposomal ZnPc after 100 J/cm² illumination were 3.5 and 11.5 μM, respectively. These differences could be due to the liposomal form of the dye, leading to an additional uptake by the parasite. In order to design a more efficient treatment scheme, we suggest this study be done with lower dye concentrations and a laser.

Dutta *et al,* demonstrated the cytolysis of *Leishmania amazonensis* with a combination of aluminum phthalocyanine chloride (AlPhCl) and light.[3] On the basis of their findings and in consideration of estimated ED_{50} of AlPhCl by Scober *et al,* (0.17 and 0.0033 after 10 J/cm² illumination),[5] it seems that ZnPc cannot compete with AlPhCl even as liposomal. Obviously, in order to make a confident judgment, another study in similar conditions using ZnPc and AlPhCl should be done.

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