Chlorin derivatives sterically-prevented from self-aggregation with high antitumor activity for photodynamic therapy

Irwin A. P. Linares[1], Kleber T de Oliveira[2] and Janice Rodrigues Perussi[1]

[1] University of São Paulo, Institute of Chemistry of Sao Carlos, Av. Trabalhador Saocarlense, 400 - CEP 13566-590, Sao Carlos, SP, Brazil

[2] Federal University of Sao Carlos, Department of Chemistry, Rod. Washington Luis, km 235, CEP 13565- 905, Sao Carlos, SP, Brazil

E-mail: janice@iqsc.usp.br

Photodynamic therapy (PDT) combine three per se harmless components: a light-sensitive molecule (photosensitizer), non-UV light corresponding to an absorption peak of the photosensitizer, and molecular oxygen generating reactive oxygen species (ROS) that lead to death of tumor cells and harmful pathogens [1]. In this study two new chlorins (8-a and 8-b) with L-type shape structure were synthesized from protoporphyrin IX dimethyl ester and 1-(2-hydroxyethyl)maleimide by the Diels-Alder reaction [2,3] in order decrease the tendency of chlorins aggregation in physiological medium, as well as to study the photodynamic action of these molecules on tumor (HEp-2 and HeLa) and non-tumor (Vero) cells when used as photosensitizers. These new chlorins were compared to a similar commercial chlorin, verteporfin (Visudyne®) used in the treatment of age-related macular degeneration by PDT. The chlorins were characterized by ^{1}H-NMR, ^{13}C-NMR, UV-Vis and high resolution mass spectroscopy (HRMS). Their photochemical properties such as quantum yield of singlet oxygen (ϕ_0) and fluorescence (ϕ_f) along with their photodegradation has been studied. The synthesized chlorins have a strong emission in 670 nm, very low photobleaching and no aggregation. The partition coefficient (log P) of the new molecules exhibited an important amphiphilic character wich is more adequated for PDT than verteporfin. The ϕ_0 is 0.75, similar to vertepofin, however the chlorins 8-a and 8-b were 57% and 77% more cytotoxic than verteporfin under irradiation, respectively (Fig. 1). Compounds 8-a, 8-b and verteporfin showed a lower cytotoxicity in normal cell lines (Vero) indicating a good selectivity for tumour cells. Fluorescence microscopy showed that the mechanism of cell death occurs by a highly apoptotic process. .

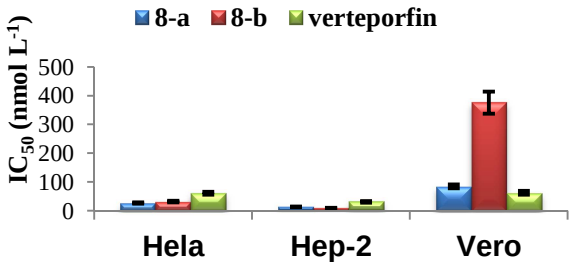

Figure 1: Medium inhibitory concentrations (IC50) of the chlorins 8-a, 8-b and verteporfin in HeLa, HEp-2 and Vero cells. The cells incubated for 2 h, following by red irradiation (660 nm) and light doses 6 J cm-2. The MTT assay was used to determine the cell viability after 48 h of irradiation. The results are expressed as means ± standard deviation (SD). *, α: statistically significant difference from Vero cells ($p<0.001$; $p<0.05$). □, β: statistically significant difference from HeLa cells ($p<0.01$; $p<0.001$).

The results suggest that these new chlorins derivatives exhibit strong potential as photosensitizer and may replace verteporfin with advantages.

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