Abstract

Photodynamic therapy is a therapeutic modality cabaple of inducing cell death by oxidative stress through activation of a sensitizer by light. Porphyrins proved to be photosensitizers with appropriate characteristics, and recent investigations showed that substitution with hydroxyl groups and the heavy atom effect may contribute to enhance the photodynamic activity.

This work compared the photodynamic activity of 5,10,15,20-*tetrakis*(2-bromo-3-hydroxyphenyl)porphyrin and 5,15-*bis*(2-bromo-3-hydroxyphenyl)porphyrin as sensitizers against human colorectal adenocarcinoma *in vitro* and *in vivo*. Fluorescence microscopy studies indicated that the subcellular localization of both sensitizers may be the mitochondria, and cellular uptake studies showed that TBr4HPP internalization is seventeen times higher than BBr2HPP. Despite this difference, the cell proliferation studies shown the higher photodynamic effect of BBr2HPP (IC50 at 24 hours: 180nm) in comparison to TBr4HPP (IC50 at 24 hours: 464nm), and that irradiation is essential for cytotoxicity. Both sensitizers induced death by necrosis in the WiDr colorectal adenocarcinoma cells, which may depend not only of the photodynamic treatment but also of the molecular profile of these cells.

In vivo studies showed that both sensitizers have effect in a low dose of 2mg/kg inducing a significant decrease in the growth of xenografts of WiDr cells implanted in *Balb/c nu/nu* mice. Results are consistent with *in vitro* studies pointing to the greater photodynamic effect of BBr2HPP. For this sensitizer, with an interval between injection and irradiation of 72 hours, we observed significant decrease in the growth of xenografts in 83.3% of cases, immediately in the second day of follow-up.

After the follow-up necessary to complete the *in vivo* studies, it was possible to excise the xenografts and proceed to histological and flow cytometry analysis. After the photodynamic treatment, it was still possible to find viable tumor cells, despite the

extensive areas of necrosis. This result indicates that, in the future studies, will be necessary to optimize the treatment protocol, in terms of concentration of the sensitizer and total energy and power of light to be applied.