# Photochemical internalization of bleomycin and temozolomide – *in vitro* studies on glioma cell line F98

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Here we evaluate the photosensitizer meso-tetraphenyl chlorin disulphonate ($TPCS_{2a}$) in survival studies of rat glioma cancer cells in combination with the novel photochemical internalization (PCI) technique. The tested anticancer drugs were Bleomycin (BLM) and Temozolomide (TMZ). Glioma cells were incubated with $TPCS_{2a}$ ($0.2\,\mu g\,ml^{-1}$, 18 h, 37 °C) before BLM or TMZ stimulation (4 h) prior to red light illumination (652 nm, $50\,mW\,cm^{-2}$). The cell survival after BLM (0.5 μM)-PCI (40 s light) quantified using the MTT assay, was reduced about 25 % after 24 h relative to controls, and 31 % after TMZ-PCI. The supplementing quantification by clonogenic assays, using BLM (0.1 μM), indicated a long–term cytotoxic effect: the surviving fraction of clonogenic cells was reduced to 5 % after light exposure (80 s) with PCI, compared to 70 % in the case of PDT. In parallel, structural and morphological changes within the cells upon light treatment were examined using fluorescence microscopy techniques. The present study demonstrates that PCI of BLM is an effective method for killing of F98 glioma cells, but smaller effects were observed using TMZ following the "light after" strategy. The results are the basis for further *in vivo* studies on our rat glioma cancer model using PDT and PCI.

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## Supplementary

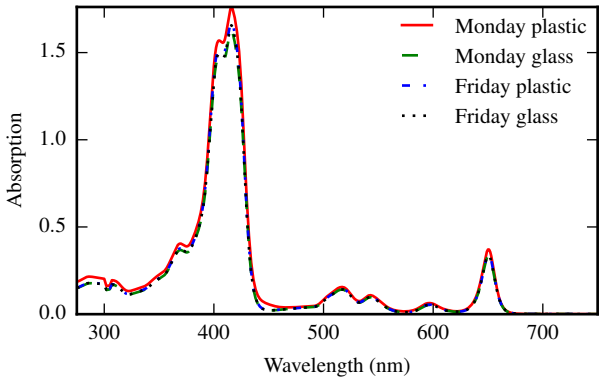


**Fig. 1** Absorption spectra of $TPCS_{2a}$ in methanol (150 µg ml$^{-1}$) recorded immediately after preparation and after 4 days. The solutions were contained in plastic or glass tubes.

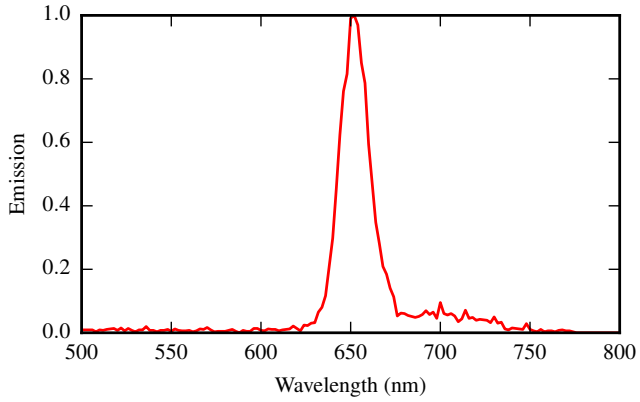


**Fig. 2** Emission spectrum of $TPCS_{2a}$ in methanol for excitation at 403 nm.

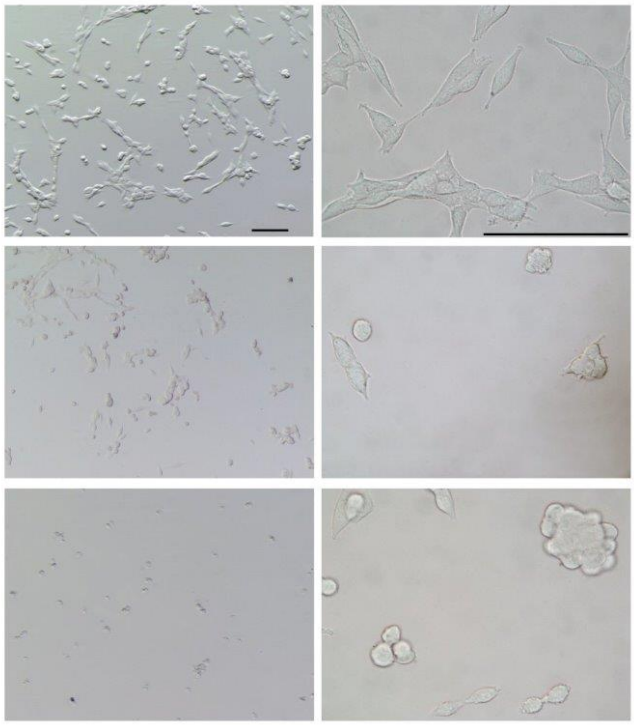

**Fig. 3** Light microscopy images of glioma F98 cells after $TPCS_{2a}$ incubation (0.2 µg ml$^{-1}$, 18 h) and different light exposure (Quanta System laser (652 nm, 50 mW cm$^{-2}$). Enlargement 40 times (left column) and 60 times (right column). Upper panels: Control cells (no treatment). Middle panels: cells at $LD_{50}$ dose (50 s of illumination). Lower panels: $LD_{100}$ dose (120 s of illumination). The viability was quantified using the MTT assay as described in Materials and Methods in the main manuscript. Scale bars in top panels are 0.1 mm.

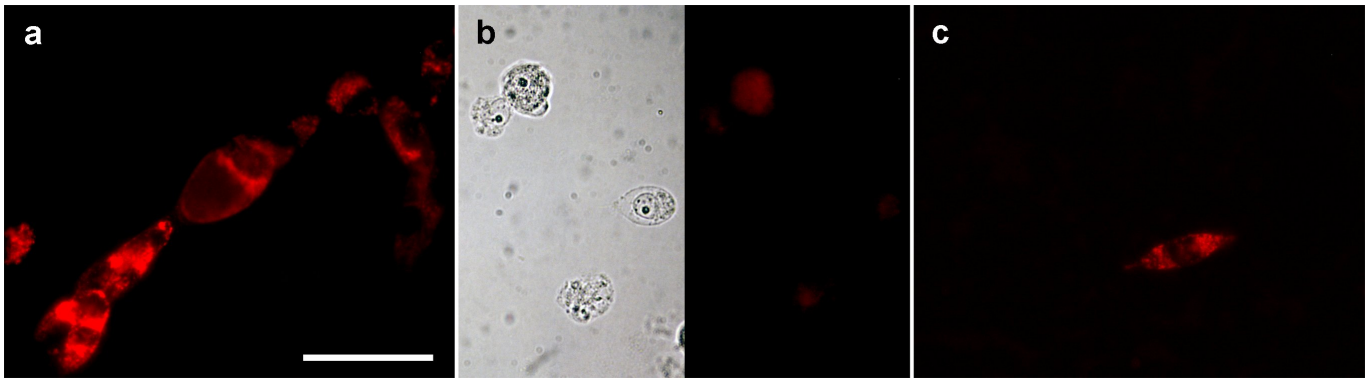


**Fig. 4** Subcellular localization of $TPCS_{2a}$ in F98 glioma cells after $TPCS_{2a}$ incubation (0.4 μg ml$^{-1}$, 18 h) in a) and after PDT (180 s, 652 nm, 50 mW cm$^{-2}$) in b) and after BLM-PCI (160 μM BLM, 4 h) in c). The cells were examined on an inverted microscope (Nikon Eclipse TE2000-S) 1.5 h after illumination as described in Materials and Methods, in the main manuscript. No auto fluorescence was detected neither in control, nor treated cells, as shown in Supplementary Fig. 5a). Scale bar is 50 μm

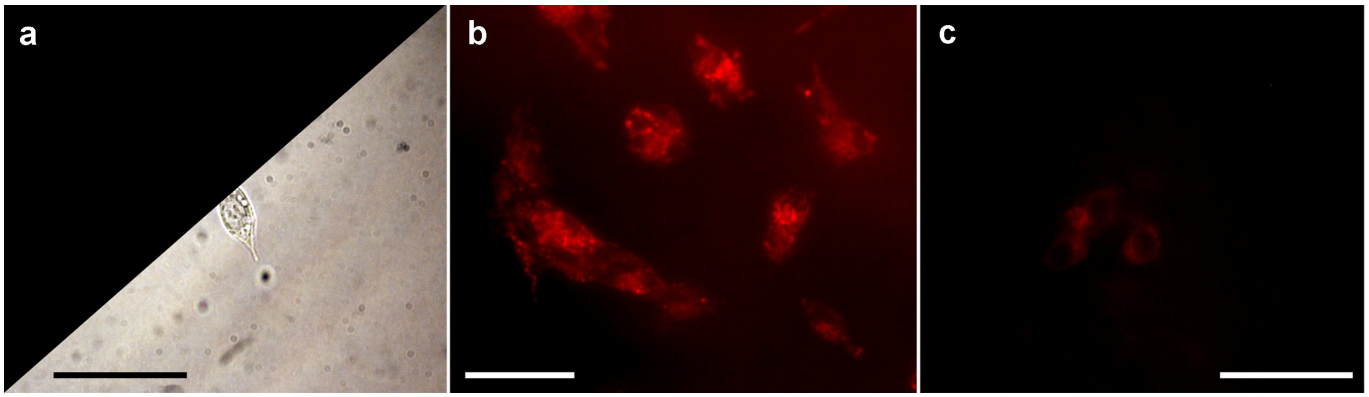


**Fig. 5** F98 glioma cells presented without any treatment shown by fluorescence and light microscopy detecting no auto fluorescence. $TPCS_{2a}$ treated cells (0.4 μg ml$^{-1}$, 18 h) prior to TMZ incubation (500 μM, 4 h) and without light were presented in b) and in combination with red light treatment (180 s, 652 nm, 50 mW cm$^{-2}$) in c). The cells were examined on an inverted microscope (Nikon Eclipse TE2000-S) 1.5 h after illumination as described in Materials and Methods, in the main manuscript. Scale bars are 50 μm.

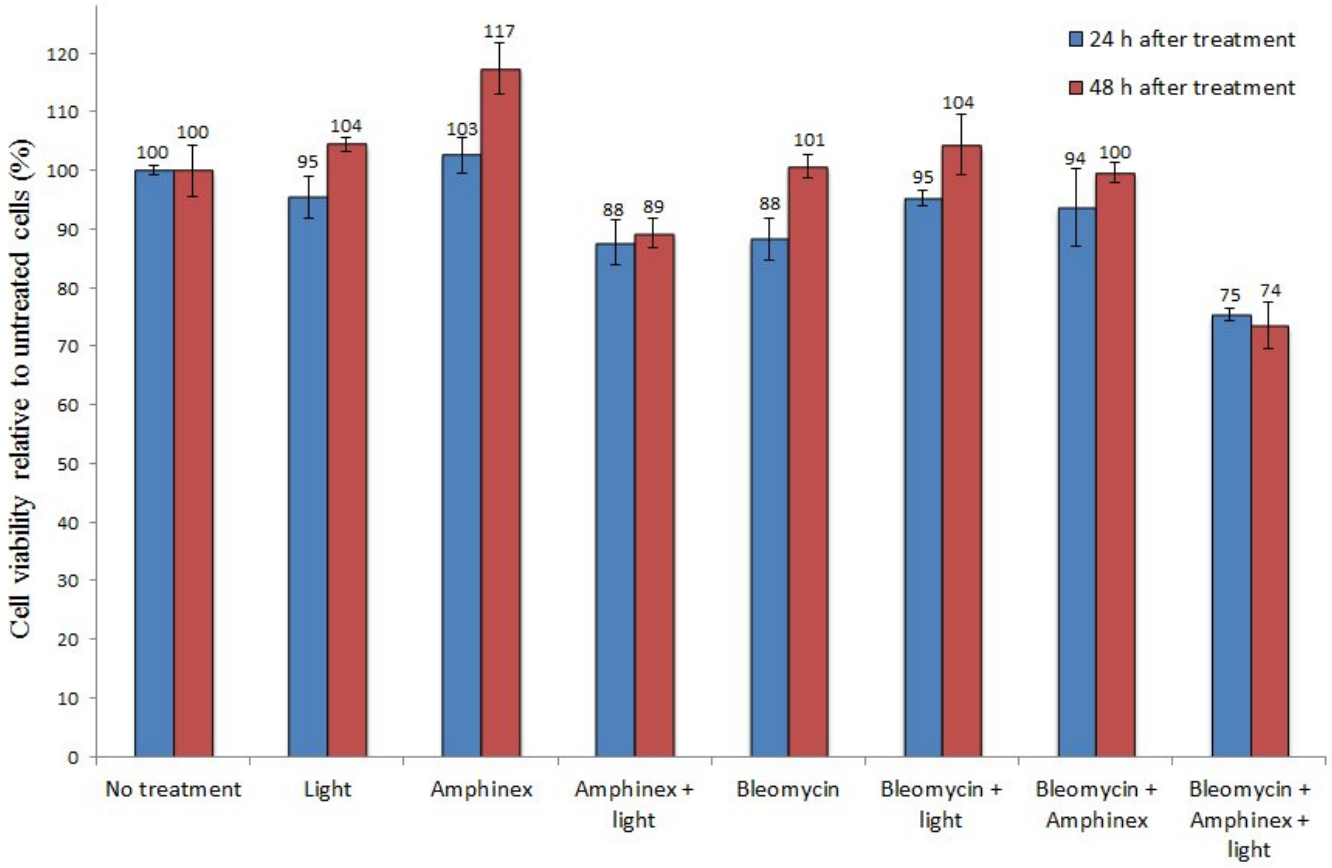


**Fig. 6** Viability of rat glioma cells (F98) exposed to different combinations of light from a Quanta System laser diode (40 s, 652 nm, 50 mW $cm^{-2}$), Amphinex ($TPCS_{2a}$) 0.2 μg $ml^{-1}$, 18 h and BLM (0.5 μM, ). Cell viability was measured by using MTT 24 h (blue bars) or 48 h (red bars) post treatment. Below each pair of bars, the relevant treatment combination is displayed. Error bars are based on two parallel runs.