

Rose Bengal - Phototoxicity versus Intrinsic Cytotoxicity

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Question:

Rose Bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein disodium salt; RB) is a fluorescent compound that has been in use in ophthalmology in the diagnostics of corneal damage. RB is a photosensitiser and its phototoxicity is well characterised. Recently, it has been tested as an intralesional agent (in the absence of light) for the treatment of cutaneous melanoma metastases and is currently undergoing further testing in a Phase II trial. However, the mechanism of action of RB on melanoma in the absence of light is not thoroughly understood.

Methods:

In addition to standard assays - such as 2D drug sensitivity assays, DNA content analysis, Annexin V staining, immunoblotting and confocal microscopy - we also made use of a number of more unique assays. We transduced melanoma cells with fluorescently labelled LC3 to visualise the accumulation of LC3-II in the membrane of autophagosomes. Further, we combined the fluorescent properties of RB with live/dead stains to perform three-colour fluorescence imaging of our 3D melanoma spheroids.

Results:

RB indeed had a dose-dependent cytotoxic effect on melanoma cells but not fibroblasts in the absence of light or upon exposure to red light (633 nm). In contrast, exposure to UV- or green light (561 nm) caused profound phototoxicity within minutes. In our 3D melanoma spheroid model, RB had a time- and dose-dependent effect on melanoma cell death of both proliferating and invading cells. In addition, RB exerted its toxicity through necrosis without perturbation of the cell cycle and the effects observed in the dark were independent of the phototoxic generation of ROS. Finally, we showed that RB induced autophagy in melanoma cells indicating a possible mechanism of action.

Conclusion:

In additions to its phototoxicity RB also exerts intrinsic cytotoxicity. In contrast, to the phototoxicity the intrinsic cytotoxicity has a wider therapeutic window. Here we showed that an interplay of cell necrosis and autophagy is one possible mechanism of action for RB