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Title: The current situation of 5-aminolevulinic acid mediated photodynamic therapy in bladder cancer

List of Authors: Dagmara Kobza-Mroczkowska[1,2], M. Ibrahim Khot[2]

Authors' affiliation:

1. School of Chemistry, University of Leeds, Leeds, UK
2. School of Medicine, St James's University Hospital, University of Leeds, Leeds, UK

Corresponding author:

M. Ibrahim Khot

Section of Translational Anaesthesia and Surgical Sciences,

Level 9, Wellcome Trust Brenner Building, St James's University Hospital,

Beckett St, Leeds, LS9 7TF, UK

E-mail: M.I.Khot@leeds.ac.uk

Dear Editor,

We have read with interest, the recent publication by Fahmy and Fahmy[1]. 5-aminolevulinic (5-ALA) is now recognised as an effective compound (through the intracellular production of fluorescent protoporphyrin IX) for mediating the intraoperative photodynamic diagnosis of bladder cancers[2]. In this study, the authors identify that 5-ALA mediated photodynamic diagnosis to aid transurethral surgical resection of the bladder tumour, is strongly established having track record as shown through multiple clinical studies. However, the definitive role of 5-ALA-mediated photodynamic therapy (PDT) in bladder cancers has largely remained unknown. The authors attempted to address this in their study by investigating the effects of 5-ALA PDT, in combination with the chemotherapeutic drug, Mitomycin C (MC). Following the surgical resection of superficial or non-muscle-invasive bladder cancers, MC is administered to patients as adjuvant treatment[3]. The T24 and RT4 cell lines were used to model bladder cancer *in vitro*. For both cell lines, the authors discuss that combined 5-ALA PDT and MC treatment demonstrated improved cytotoxicity. However, evaluating the results of the cell viability experiments, it could be argued that combined treatment did not prove to show any overall significantly improved cell killing, as compared to 5-ALA PDT or MC alone. Extrapolating these findings to a clinical setting, it would be difficult to justify the mediocre therapeutic synergy of combined treatment, taking into account the adverse toxicities of PDT and chemotherapy.

Oxidative stress through the formation of reactive oxygen species (ROS), is the primary mechanism through which PDT can elicit cell death[4]. In this study, the authors found 5-ALA PDT alone to produce the highest amount of ROS, compared to MC alone and combined 5-ALA PDT and MC treatment. This may give reason as

to why combined treatment, was not found to be more beneficial at cell killing than 5-ALA PDT alone. In addition, this study would have benefitted from exploring the intracellular uptake of 5-ALA and production of protoporphyrin IX. The activity and expression levels of known ROS scavengers and xenobiotic metabolising enzymes such as Glutathione-S-Transferases would also have been beneficial, to further understand reduced ROS formation in combined treatment[5,6].

It is also of interest for any subsequent studies to investigate specific intracellular changes, to elucidate the potential PDT-limiting effect of MC. One factor which will need to be studied, is the influence of multidrug resistance proteins such as ABCG2. ABCG2 is a well-documented transmembrane protein, which has been known to eliminate both protoporphyrin IX and MC from cells[7]. This will improve the understanding of the interplay between 5-ALA PDT and MC and how multidrug resistance proteins can modulate anti-cancer efficacy. We agree with the authors that 5-ALA PDT is potentially effective in bladder cancer patients[8]. However, the specific application of 5-ALA PDT will need to be further investigated pre-clinically and clinically validated as part of a multi-modal approach including surgery, radiotherapy and chemotherapy.

Finally, we would like to comment on some technical aspects of this study, which should be taken into consideration for any follow-up investigations. I) The authors use RT4 cells for cell viability experiments only, whereas T24 cells are used for all experiments. Ideally, the use of RT4 cells should have carried on throughout the publication, for comparison to results obtained from T24 cells. II) The authors state that *“the aim of this study is to investigate if 5-ALA has a phototherapeutic effect on bladder cancer cells”*[1], however the authors do not clearly state that PDT-inducing light treatment was applied throughout the publication, following the administration of

5-ALA. The authors will need to comment on the specifics of PDT experiments, which includes total light doses applied (in J/cm^2), conditions of light treatment and fluency rates.

Conflicts of interest

The authors declare no conflict of interest

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