

## News and Views

# In vivo action spectra, absorption and fluorescence excitation spectra of photosensitizers for photodynamic therapy

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Received 23 October 1994; accepted 31 October 1994

**Keywords:** Photodynamic therapy; Action spectrum; Absorption spectrum; Fluorescence excitation spectrum; Haematoporphyrin derivative; Photofrin; Phthalocyanine

Recently the importance of measuring in vivo action spectra for photodynamic therapy (PDT) with various photosensitizers was pointed out in this journal [1]. It was shown that the effect of PDT with zinc phthalocyanine tetrasulphonic acid ( $\text{ZnPcS}_4$ ) increases considerably when the wavelength of the activating light is increased from 680 to 692 nm. In this regard a further comparison with haematoporphyrin derivative/photofrin and other recently presented information about phthalocyanines may be useful. In particular we wish to point out in this note the possible relevance of the fluorescence excitation spectrum and the possibility to measure the in vivo absorption spectrum of a photosensitizer by reflection spectroscopy.

Preclinical and clinical studies on PDT with haematoporphyrin derivative (HpD) and photofrin have mostly been performed with light of 630 nm wavelength. The use of this wavelength is not supported by the in vivo action spectrum. The action spectrum for in vitro cell killing by HpD-PDT has a peak close to 630 nm [2]. However, the in vivo action spectrum for HpD-PDT (determined from damage to rat ears) has peaks close to 500 and 625 nm [3]. These wavelengths are the same as the wavelengths where the fluorescence excitation spectrum has a maximum [4]. Farrell et al. [5] have studied the wavelength dependence of the threshold for PDT-induced damage to rat liver and also found a maximum at 625 nm. Recently Potter et al. [6] (and personal communication) measured the absorption spectrum of photofrin in vivo in a mouse tumor by reflection spectroscopy and observed a maximum at 625 nm. Thus it appears that the in vivo PDT action spectrum, absorption spectrum and fluorescence excitation spectrum using HpD/photofrin all have a maximum at 625 nm, which differs somewhat from the in vitro PDT action spectrum. The difference between 625 and 630 nm is

not large, but it should be noted that in clinical protocols the wavelength for PDT is often prescribed at  $630 \pm 3$  nm. This means that 633 nm would in principle be acceptable. Since the in vivo HpD-PDT action spectrum has a relatively narrow peak [3], PDT at 633 nm has considerably less effect than at 625 nm.

Results similar to those of Griffiths et al. [1] for  $\text{ZnPcS}_4$  have recently been reported for aluminium phthalocyanine disulphonic acid ( $\text{AlPcS}_2$ ) by Cubeddu et al. [7]. The in vivo absorption spectrum of  $\text{AlPcS}_2$  peaked at 685 nm and a similar red shift was found for the PDT action spectrum. Incidentally, Cubeddu et al. [10] also measured the in vivo absorption spectrum of photofrin, which again showed a maximum at 625 nm.

The fluorescence excitation spectrum for  $\text{ZnPcS}_4$  in cells as reported by Griffiths et al. [1] has a broad maximum that does not correlate well with the PDT action spectrum. In view of the data on HpD/photofrin it should be worthwhile to measure both the in vivo fluorescence excitation spectrum and the in vivo absorption spectrum of  $\text{ZnPcS}_4$  to see which, if any, corresponds best with the PDT action spectrum. Another drug requiring such a study is zinc(II)-phthalocyanine in liposomes. This drug will soon be introduced for clinical PDT in a joint effort of Ciba-Geigy and Quadra Logic Technologies. Schieweck et al. [8] reported preclinical studies with this drug. Tumor necrosis required rather high light doses when PDT was performed with 671 nm light, the wavelength of maximum absorption in *N*-methyl-pyrrolidone. Van Leengoed et al. [9] excited liposomal zinc(II)-phthalocyanine with light of 675 nm wavelength and also needed rather high light doses ( $450 \text{ J cm}^{-2}$ ) to achieve tumor necrosis. If liposomal ZnPc shows the same red shift in the in vivo action spectrum as reported for other phthalocyanines

cyanines [1,7], the concomitant reduction in the required light dose for PDT, if performed at the optimum wavelength, has considerable clinical significance. The PDT action spectrum and/or the fluorescence excitation spectrum and the absorption spectrum of liposomal ZnPc should therefore be studied in vivo before this drug is tested clinically. Naturally this holds for any other new photosensitizer considered for clinical use.

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