

Pharmacokinetic study of FosPeg[®] in a mouse model using fluorescence imaging and absorption spectroscopy techniques

In photodynamic therapy (PDT), there exist many different photosensitizers [1], among which the second generation photosensitizers were developed to offer potential advantages over the first generation photosensitizers, including higher chemical purity, better tumor selectivity, a faster clearance, and a major absorption band at wavelengths greater than 650 nm offering better penetration of the treatment light. *Meta*-tetra hydroxyphenyl chlorin (m-THPC), also known as Foscan[®] containing the active ingredient temoporfin, is currently in clinical trials for diagnosing and treating cancers in USA, Europe and the UK. Furthermore it has a high extinction coefficient at 652 nm for treatment, which enables a lower drug concentration as well as a lower light dose comparing to the first generation photosensitizer like Photofrin [2].

In order to optimize the distribution properties of the photosensitizers, liposomes are designed as carrier and delivery systems. FosPeg is a formulation of mTHPC contained in pegylated liposomes. Liposomes with poly ethylene glycol (PEG) coating, a synthetic hydrophilic polymer, would improve their stability and prolong their half-lives in circulation [3].

Several techniques can be utilized to study the biodistribution and pharmacokinetic properties of photosensitizers. Optical techniques can be used *in situ* to follow the drug concentration as a function of time in each individual animal. The aim of my present study is to investigate the biodistribution of FosPeg (biolitec AG, Jena, Germany) in an experimental murine model by multispectral fluorescence imaging and absorption spectroscopy techniques. The ability to measure the Fospeg concentration with multispectral fluorescence imaging is evaluated with regression analysis.

The biodistribution and pharmacokinetics of FosPeg were investigated in 30 female NMRI nu/nu mice, inoculated subcutaneously into the left and right hind thigh with a suspension of HT29 human colorectal carcinoma cells. They were injected intravenously with m-THPC formulated in long-circulating poly ethylene glycol (PEG) modified liposomes (FosPeg). FosPeg was delivered at a dose of 0.15 mg/kg b.w. of mTHPC concentration through injection into the lateral tail vein of the mouse. At different drug-light intervals (30 min, 2h, 4h, 8h and 18h) after the photosensitizer injection the animals were sacrificed by cervical dislocation. The blood was removed rapidly by cardiac puncture and eight organs were excised (tumor, muscle, skin, liver, spleen, kidney, lung and heart) for *in situ* and *ex vivo* measurements.

Conventionally high performance liquid chromatography (HPLC) of excised tissues is used for analysis of pharmacokinetic behavior in animal experiments. The results are used as a gold standard to be correlated to the fluorescence measurements [4].

Fluorescence images were acquired using the setup depicted in Fig. 1. Excitation light at 405 nm is delivered through an optical fiber mounted above the target. The spot size is approximately 4 cm in diameter. The fluorescence was spectrally filtered using a Liquid Crystal Tunable Filter. Images of the fluorescence were acquired using the LCTF set to 450, 525, 630, 650 and 720 nm. An image was formed using a standard camera objective (f=50/1.8) and an EMCCD, set to run in CCD-mode.

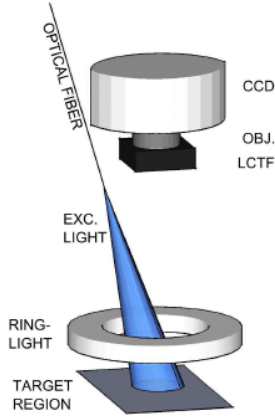


Fig. 1. Schematic picture showing the fluorescence imaging setup.

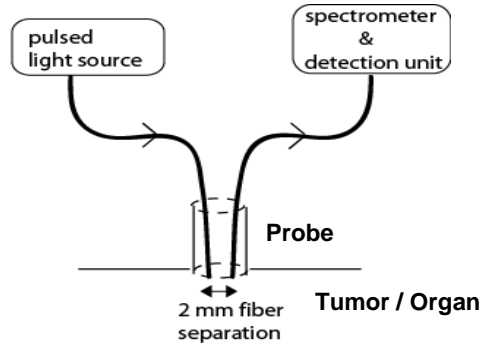


Fig. 2. The schematic diagram of the optical absorption setup.

All multispectral images at the wavelength of 450, 525, 630, 650 and 720 nm were prior to processing cropped to the size of the organ. Fluorescence at 652nm corresponds to the peak emission of m-THPC and 525 nm to the autofluorescence. Then the median value of each image across the organ was calculated and used in the subsequent statistical analysis. The optimum partial least squares (PLS) regression models based on the fluorescence imaging data are developed for the quantitative determination of FosPeg in different organ samples. In order to get high correlation coefficients and improve the prediction accuracy of the mTHPC concentrations, the blood absorption due to the presence of deoxy-(Hb) and oxyhemoglobin (HbO) are included in the PLS model. The concentrations of Hb and HbO are achieved by absorption spectroscopy. The optical absorption setup is schematically shown in Fig.2. The output from a pulsed xenon shortarc lamp was delivered by a 400- μm -diam optical fiber and, after interacting with the tissue, the transmitted light was collected by a 200- μm -diam fiber. The center-to-center distance between delivery and collection fibers measured 2.0 mm. An S2000 miniature spectrometer was used to disperse and detect the collected light. Wavelength-dependent fluctuations in source output and detector response were accounted for by taking a reference measurement from a spectrally flat diffuse reflector based on Spectralon material in connection to each measurement sequence. For source-detector separations in the range 1.5 to 2.6 mm, the path length of the collected photons has been shown to be relatively insensitive to variations in tissue scattering [5]. We assessed the changes in tissue absorption as following [4]:

$$\Delta A(\lambda) = -\ln\left(\frac{I_2(\lambda)}{I_1(\lambda)}\right) = -\ln\left(\frac{I_2(\Delta\mu_a + \mu_a^0(\lambda))}{I_1(\mu_a^0(\lambda))}\right) = \Delta\mu_a(\lambda) \cdot L_{\text{eff}}(\Delta\mu_a(\lambda) + \mu_a^0(\lambda)) \quad (1)$$

Where $I_2(\lambda)$ and $I_1(\lambda)$ are the transmission signal measured after and before the addition of an absorber, respectively. L_{eff} denotes the effective path length, which depends on the total absorption coefficient $\Delta\mu_a(\lambda) + \mu_a^0(\lambda)$ and the dependence can be determined by a nonsequential ray tracing software package (ASAP 8.0.3, Breault Research Organization, Tucson, Arizona) for a geometry matching the experimental setup.

Since we have known $\mu_a^0(\lambda)$ and the detected signal $I_2(\lambda)$, and $I_1(\lambda)$ is the signal when assuming that tissue absorption at 900 nm is dominated by water at a constant concentration of 60%, the change of the absorption coefficient can be solved by the function “lsqnonlin” in MATLAB.

$$\Delta\mu_a = \Delta c_{mTHPC}\epsilon_{mTHPC} + \Delta c_{Hb}\epsilon_{Hb} + \Delta c_{HbO}\epsilon_{HbO} + AM(\lambda) + \sum_{i=0}^2 \omega_i c_i \lambda^i \quad (2)$$

Where the chromophores included in the evaluation of the absorbance data were those of mTHPC, Hb and HbO. In addition, the mTHPC fluorescence spectrum was also included in the model. The last summation on the right-hand side of Eq.(2) was included to account for the background caused by the fact that the scattering parameters changed between the two measurements, and therefore the wavelength dependence of the collected light is changed [6].

After we got the concentrations of Hb and HbO in the samples, we included them in the PLS model by adding a term of $I(\text{Fluo})\exp(\Delta\mu_a L_{\text{eff}}(\mu_a))$ at each of the 5 wavelengths, in order to account for the imprint of fluorescent signals absorbed by blood. The effective pathlength L_{eff} can be achieved by Monte Carlo simulations. Reasonable PLS

components were selected by leave-one-out cross-validation. The PLS model can be improved by some preprocessing techniques like taking the ratios, or logarithms, or centering and scaling [7]. Our goal is to develop the models for different organs with a coefficient of determination in calibration (R^2) higher than 0.75.

The final result of my recent research will come up with a publication, hopefully soon.

References

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