Optimizing Light Dosimetry in Photodynamic Therapy of the Bronchi by Fluorescence Spectroscopy

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Abstract. Under identical conditions (drug and light dose, timing), the results of photodynamic therapy (PDT) of carcinomas of the bronchi with tetr(meta-hydroxyphenyl)chlorin (mTHPC) show large variations between patients. Before patients underwent PDT treatment, the mTHPC level was measured in the lesion, the normal surrounding tissue and the oral cavity, with an apparatus based on fluorescence spectroscopy. The fluctuations in degree of tissue reaction and tumour destruction between patients could be explained by individual variations in the mTHPC level in the mucosa of the bronchi. The patients who showed the highest mTHPC fluorescence signal also had the strongest response to PDT. In addition, a correlation between the mTHPC level in the oral cavity and bronchial mucosa was found. It is concluded that PDT can be improved by measuring the mTHPC level in the bronchi or the oral cavity before treatment by fluorescence spectroscopy, and then by adjusting the light dose to be applied to the observed mTHPC level.

INTRODUCTION

Photodynamic therapy (PDT) (1, 2) is a modality of cancer treatment based on the selective accumulation of a non-toxic photosensitizer (PS) in a tumour, as compared with the healthy surrounding tissue. In this study, the PS is mTHPC (3-5). Upon irradiation with light at a wavelength that is absorbed by mTHPC, this PS becomes phototoxic and can potentially destroy the tumour without major damage to the surrounding normal tissue. When applying a standard set of PDT parameters, this ideal situation has been attained only for a small fraction of the clinical treatments of early stage carcinomas of the bronchi with mTHPC. However, for the same set of experimental conditions (PS dose, fluence, fluence rate, wavelength, delay between injection and PDT, etc.) some patients were undertreated (ie no necrosis observed) and others overtreated (ie surrounding normal tissue was damaged to a significant extent). This variation in PDT efficacy from patient to patient probably has two major causes. First, the selectivity of the PS varies from one case to another. Second, the reaction of a tissue after irradiation is a function of several parameters, among which the light dose (which can be relatively well controlled), and the concentration of PS in the tissue to be treated are the most important. The latter parameter depends on the amount of PS injected, which was the same for all patients. However, the local PS concentration at the time of PDT is, in general, unknown due to inter-patient variation in pharmacokinetics. This implies that if one could measure the local PS concentration, or at least a parameter related to it, just before the PDT treatment, and change the light dose according to the observation, one might be able to obtain a much improved treatment for each patient.

Hence an efficient, simple, non-invasive and non-destructive method to estimate local drug concentration is needed. Conventional extraction (6) and radiolabelling methods (7) must be ruled out, being either time-consuming or difficult to use routinely in a clinical context. Furthermore, it should be realized that
whereas it would be valuable to have absolute mTHPC concentrations for this application, relative concentrations, when standardized between patients, are sufficient for the present purpose. Light-induced fluorescence (LIF) can meet these requirements (8) and, using standard endoscopic techniques, can be applied to the mucosae of most of the hollow organs of interest for PDT (9).

MATERIALS AND METHODS

Patients

Twenty-seven patients (two women and 25 men) participated in this study. Their mean age was 56 years (range 43–72 years). All patients had an early stage squamous cell carcinoma of the bronchi or the oesophagus. All these lesions were either staged as in situ (ie intra-epithelial and not invading the basal membrane) or micro-invasive (ie with an infiltration depth of less than 2 mm, which implies that there is no invasion of the muscle layers in the oral cavity, and no invasion of the cartilage in the bronchi). All patients were intravenously (i.v.) injected with 0.15 mg kg\(^{-1}\) of mTHPC. The LIF measurements used to establish the relative mTHPC concentration were made immediately before irradiation. PDT treatments and irradiation tests were effected 1 or 4 days after injection of the drug.

Enrolment was voluntary and written consent was obtained from all patients, as defined in the protocol approved by the Ethical Committee of the CHUV hospital in Lausanne. A complete explanation regarding the potential side-effects of mTHPC was given before enrolment, and each patient received detailed written and oral instructions concerning photoprotective precautions.

Photosensitizer preparation and administration

mTHPC was kindly supplied by Scotia Pharmaceuticals Ltd (Guildford, Surrey, England) as a powder and was stored in the dark at 4°C. Immediately before use, mTHPC was dissolved in 20% ethanol, 30% polyethylene glycol 400 and 50% H\(_2\)O. The solution was then administered i.v. through a bacterial filter under sterile conditions over a period of 10 min.

Instrumentation for LIF measurements

The overall configuration of the optical fibre-based spectrofluorometric apparatus has been described in detail previously (8). Briefly, the desired excitation wavelength is selected by passing the light of a short arc xenon lamp through a monochromator. This excitation light is transmitted through a beam splitter before injection via a microscope objective into a 0.6 mm core diameter quartz optical fibre. The distal end of the fibre is positioned on the surface of the tissue to be measured. The fluorescence spectrum induced in the tissue by light at the excitation wavelength is collected by the same optical fibre, reflected by the beam splitter, filtered to remove excitation light, and focused onto the entrance slit of a spectrograph which is coupled to an intensified diode array. The signal obtained is displayed on the optical multichannel analyser and can be further processed by a microcomputer.

Experimental protocol for LIF measurements

mTHPC LIF signals measured just before PDT are obtained in the following way: the distal end of the fibre is positioned on the surface of the tissue to be measured. The spectra obtained consist of both the autofluorescence spectrum of the tissue and the superimposed fluorescence from the injected PS. Knowledge of the autofluorescence spectrum before injection allows for its subtraction to obtain the ‘pure’ PS fluorescence spectrum before therapy. When the autofluorescence spectrum of the tissue cannot be measured before mTHPC injection, an average of the tissue autofluorescence spectra previously measured in different patients is used. In this case, a direct subtraction of the mean tissue autofluorescence spectrum is not possible because its intensity varies from patient to patient. The mean tissue autofluorescence is first scaled by a factor, which is the ratio between the LIF signal of the measurement composed of tissue autofluorescence and mTHPC fluorescence and the mean tissue autofluorescence at a given wavelength (10). The latter is chosen so that there is no mTHPC fluorescence or blood absorption peak in this part of the spectrum. The excitation and emission wavelengths are at 420 nm and 650 nm, respectively. The power at the distal end of the probe fibre is