Evaluation of fluorescence measurement techniques for tumour detection in vivo

Saarnak, A.E.

Publication date
1999

CHAPTER 10

Discussion and conclusions

The aim of this thesis was to evaluate fluorescence intensity detection of tumour-selective dyes in vivo and specifically the suitability of the Double Ratio (DR) method, developed in our group, for tumour diagnostics. This method was thought to be suitable not only for tumour diagnostics but for any application where a quantitative measurement of the concentration of a fluorescent dye in vivo would be useful. Since the DR value is independent of optical properties of the various tissues and is hardly influenced by variations in autofluorescence intensity (see chapter 9) it seemed suitable for quantitative measurements.

The research for this thesis was based on three questions (chapter 1). To answer the first question, *'Are the DR and NFR techniques suitable for tumour detection in vivo?'* , measurements in vivo were performed. In chapter 2 it was seen that the DR value in vivo was not influenced by colour of normal moles and normal skin of volunteers, despite the darker colour of the moles. Testing the DR technique on basal cell carcinoma (BCC) showed the same independence of colour but a clear difference in DR value between tumour and normal tissue due to the tumour-selectivity of the exogenous fluorophore mTHPC (chapter 4). In the two black coloured metastatic melanomas, a difference in DR value compared to normal skin could be seen as well. These results are encouraging for the possibility of early detection of dark lesions using the DR method.
For pharmacokinetics measurements described in chapter 4 the DR method was found to be less suitable since the mTHPC concentrations in vivo were most likely in the insensitive region of the DR where variations in concentration have no influence on the DR value. Here, a detection technique independent of optical properties and with an outcome proportional to fluorophore concentration was needed. Therefore, the Normalised Fluorescence Ratio (NFR) method was developed. With the NFR technique, pharmacokinetics behaviour could be studied and the measured tumour to normal tissue ratios agreed well with tumour to normal concentration ratios (chapters 7 and 8).
The results shown in chapters 4,7 and 8 show that the DR and NFR techniques are suitable for tumour detection in vivo.

Question 2 was *'Is the NFR value in vivo proportional to the fluorophore concentration?'*. This problem was investigated in vivo (chapter 8). Two different kinds of tissue were chosen, red liver and whitish tumour tissue. Averaging measurements of several rats, temporal behaviour of the NFR value agreed reasonably well with the fluorophore concentration pharmacokinetics behaviour determined by tissue extraction. In addition, tumour to normal tissue ratios were comparable between the two methods. However, in individual tissue samples comparison between the two methods showed a large spread in the fluorescence as well as the extracted fluorophore concentration. This complicates validation of the theory, which states that the NFR value has a linear relation to the fluorophore concentration. For these kinds of investigations it is of utmost importance that the gold standard of fluorophore concentration assessment is accurate. Our impression is that fluorescence measurements in vivo are more reproducible than concentration measurements by extraction. In conclusion, the linear relation that theoretically exists between the NFR value and fluorophore concentration could not be completely verified. However, it seemed to be valid when looking at the general behaviour of the NFR value and fluorophore concentrations averaged over a group of rats.
'Can the DR technique be used for absolute measurements of fluorophore concentration in vivo?' was the third question of this thesis. Obviously, if this would be possible, no extraction procedures would be necessary. During the work described in this thesis it became clear that for absolute

fluorophore concentration measurements using the DR technique the two factors α, dependent on the fluorescence yield of the fluorophore and autofluorophores and the concentration of autofluorophores, would have to be known. These factors depend on the fluorophore fluorescence yield and the autofluorescence intensity. Unfortunately, the α's may not be constant factors but may vary spatially and temporally. Determining the α in vivo proved to be complicated and consequently we were not able to use the DR technique for measurements of absolute fluorophore concentrations. However, the theoretical possibility still exists for these kinds of measurements if the α's can be determined reliably.

The choice between using the DR of the NFR technique should be based on the specific application. The DR technique can be used for any application where the malignant tissue has to be distinguished from normal tissue using a tumour selective fluorophore, while semi-quantitative estimation of the fluorophore concentration is not required. The advantage is that an increasing DR value in malignant tissue is only due to an increasing fluorophore concentration and not to variations in other tissue parameters, such as optical properties and autofluorescence. The DR value has been shown to be independent of absorption of dark skin lesions (chapters 2 and 4) and for the early detection of for instance malignant melanoma it might be an excellent diagnostic method. For semi-quantitative temporal fluorophore measurements, such as pharmacokinetics measurements, the NFR technique is better than the DR technique since the NFR value is proportional to fluorophore concentration. The NFR value is not only dependent on fluorophore concentration but on autofluorescence as well, which must be kept in mind when tumour to normal ratios are determined.

In conclusion and to summarise the answers to the questions which determined the research of this thesis (chapter 1):

1) The DR and the NFR techniques proved to be suitable for detection of malignant lesions (chapters 4 and 7). Clear differences between malignant lesions and normal tissue were seen using these methods, not depending on the colour difference between the two tissues.
2) The linear relation that theoretically exists between the NFR value and fluorophore concentration could not be completely verified. However, looking at the general behaviour in a group of rats, the temporal behaviour and the tumour to normal tissue ratios agreed well between the NFR value and the fluorophore concentration.
3) We were not able to accurately determine the factors α and therefore the DR technique could not be used to determine absolute fluorophore concentration measurements. However, the theoretical possibility still exists for these kinds of measurements if the α's are known.