In vivo absorption spectrum of disulphonated aluminium phthalocyanine in a murine tumour model

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Abstract

The absorption spectrum of aluminium phthalocyanine with an average disulphonation of 2.1 (hereafter called disulphonated aluminium phthalocyanine, AlS_2Pc) was measured in vivo in a murine tumour model by means of time-resolved reflectance. Mice bearing the L1210 leukaemia were administered 2.5 or 5 mg/kg body weight (b.w.) of AlS_2Pc intraperitoneally. Reflectance measurements were performed in the 650–695 nm range before and 1, 4 and 7 h after the drug administration. Fitting of the data with the diffusion theory allowed us to assess the absorption coefficient in both conditions (i.e. before and after). As a difference between the latter and the former data, the in vivo absorption spectrum of AlS_2Pc was evaluated. 1 h after the administration of 2.5 mg/kg b.w. AlS_2Pc, the absorption peak was centred at 685 nm, red-shifted about 15 nm with respect to the spectrum in aqueous solution. For the lower dose, the absorption line shapes 4 and 7 h after the administration remained very similar. The red shift of the absorption spectrum is consistent with the therapeutic efficacy of the photodynamic therapy which was measured at 672, 685 and 695 nm, and proved to be maximum at 685 nm for both the L1210 leukaemia and the MS-2 fibrosarcoma. With the higher drug dose, the absorption spectra taken from different animals showed significant differences. In particular, in some mice the line shape was similar to that measured with 2.5 mg/kg b.w., while in other subjects it showed a broadening or a second peak at shorter wavelengths. Measurements on some animals were performed also 18 and 24 h after the injection of 5 mg/kg b.w., leading to no time evolution or to a progressive line shape narrowing.

Keywords: Absorption spectrum; Photodynamic therapy; Photosensitizers; Phthalocyanine; Reflectance; Time-resolved reflectance

1. Introduction

The optical properties of tissues and exogenous substances administered for diagnostic or therapeutic purposes may be influenced by the functional state, metabolism, blood perfusion, and so on. Therefore in vitro measurements could lead to artifacts. This problem can be solved by means of time-resolved reflectance, which allows one to determine the optical properties (absorption and scattering coefficients) in vivo non-invasively [1].

In recent years, the photophysical and photochemical properties of disulphonated aluminium phthalocyanine (AlS_2Pc) have suggested its possible use as a second generation photosensitizer for the photodynamic therapy of tumours (PDT) [2–4]. Its absorption in solution in various environments is peaked at 672 nm or red shifted by no more than 4 nm [5].

Therefore 670–675 nm light was chosen for tumour irradiation and very satisfactory results were obtained [6,7].

However, the hypothesis of modifications of the absorption spectrum due to interactions of the sensitizer with the biological substrate cannot be completely ruled out only on the basis of measurements performed on solutions. Moreover, previous experiments [8] on the action spectrum for PDT in mice bearing the MS-2 fibrosarcoma or the B 16 melanoma resulted in a good therapeutic efficacy for wavelengths greater than 670 nm and up to 710 nm, in a range where the absorption measured in solution decreases rapidly.

As mentioned above, time-resolved reflectance allows one to check for eventual in vivo changes in the absorption properties. Therefore, using this technique, the absorption coefficient was measured in the 650–695 nm range in tumour-bearing mice before and after the administration of AlS_2Pc. The absorption coefficient of the incorporated drug was evaluated at each wavelength as the difference between the latter and the former values. The line shape of the spec-

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trum acquired in vivo peaks at 685 nm. This red shift with respect to the absorption maximum in solution is consistent with our previous data on the action spectrum [8]. That result was confirmed also in the present work, since the therapeutic efficacy in the treatment of both the L1210 leukaemia and the MS-2 fibrosarcoma at a lower dose proved to be significantly better at 685 nm than at 672 nm.

2. Materials and methods

2.1. Instrumentation

2.1.1. Time-resolved reflectance

The system is described in detail by Cubeddu et al. [9]. A cavity-dumped dye (DCM) laser (Coherent model CR-599, Palo Alto, CA) pumped by a mode-locked argon laser (Coherent model CR-18, Palo Alto, CA) was used as the light source. The pulse duration was less than 10 ps, with a repetition rate of 3.5 MHz.

1 mm plastic-glass fibre optics (Quartz & Silice PCS1000, Paris) were used for both illumination and collection. The laser light was coupled to the illumination fibre placed in contact with the animal skin by means of a fibre holder. The illumination power was less than or equal to 10 mW. The reflected light was collected by a second identical fibre, again placed in contact with the surface. The two fibres were set parallel to each other at a relative distance of 1 cm.

The distal end of the collection fibre was placed at the entrance slit of a scanning monochromator. The reflectance photons were then detected by means of a double microchannel plate photomultiplier (Hamamatsu model R1564U-01, Bridgewater, NJ) and the signal was suitably amplified by a fast pre-amplifier (EG&G model VT120A, Oak Ridge, TN) and a home-designed integrating amplifier. An electronic chain for time-correlated single-photon counting was used to measure the time-resolved reflectance. The experimental reflectance decay curves, collected by a multichannel analyser (Silena model Varro, Milan), were then transferred to a personal computer for data analysis.

The system response had a typical full width at half-maximum of 60 ps and its time drift was less than 1 ps min^{-1}. The spectral resolution was 2 nm.

A convolution procedure was used to evaluate the experimental data. The theoretical reflectance, as described in the diffusion model [10], was convoluted with the system response and fitted to the measured reflectance, once the uncorrelated background had been subtracted. The best fitting parameters were obtained by means of an iterative procedure to minimize the reduced χ^2. To judge the quality of the fitting, the weighted residuals and their autocorrelation function were always calculated.

2.1.2. Photodynamic therapy

The irradiation was performed with a continuous wave dye (DCM) laser (Coherent model CR-599, Palo Alto, CA) pumped by an argon laser (Coherent model CR-18, Palo Alto,

CA). The laser output was coupled to a 600 μm plastic-glass optical fibre (Quartz & Silice PCS600, Paris). The flat cut distal end of the fibre allowed approximately uniform irradiation of the tumour area. The power density was monitored at the fibre output.

2.2. Chemicals

Disulphonated aluminium phthalocyanine was kindly provided by Dr. A. McLennan (Paisley College of Technology, Paisley, UK) and diluted in saline at a concentration of 2.5 mg ml^{-1}. The mean degree of sulphonation of AlS$_2$Pc was 2.1, as ascertained by chromatography. The compound consists of a mixture of isomers with sulphonic groups in both adjacent and opposite positions.

2.3. Animals and tumour models

Hybrid (Balb/c×DBA/2 F$_1$) mice (Charles River, Calco, Italy) were used.

The chemically induced lymphoid leukaemia L1210 was maintained by weekly intraperitoneal injection. Tumour cells (10^6 cells per mouse) were injected intraperitoneally (i.p.) for the reflectance measurements and intradermally (i.d.) for the photodynamic therapy.

The MS-2 fibrosarcoma, originally induced by the Moloney murine sarcoma virus, was maintained by weekly intramuscular passage of tumour cell homogenate into the right hind leg. For treatment, tumour cells (10^6 cells per mouse) were injected i.d.

2.4. Experimental procedure

2.4.1. Time-resolved reflectance

Experiments were performed 6 days after the passage of tumour cells. One group of eight animals was used for each drug dose (2.5 and 5 mg/kg body weight (b.w.)). The mice were caged in a plastic box, with a hole centred at the tumour site (peritoneal region). The fibre holder was placed in contact with the abdomen skin and reflectance measurements were performed every 5 nm in the 650–695 nm range. Each acquisition, at a single wavelength, required 10 s to 2 min, that is the time to collect 10^5 counts. The total acquisition time for a whole spectrum was therefore 2–5 min. For each animal, after the first series of measurements, the fibre holder was moved and repositioned, and a second series was performed. Then mice were injected i.p. with 2.5 or 5 mg/kg b.w. of AlS$_2$Pc, and 1, 4 and 7 h later the whole measurement procedure was repeated.

Thus, at each drug dose, the absorption coefficient was evaluated twice as a function of wavelength both before and after drug administration, at the three selected times of observation. In order to assess the average absorption line shape of AlS$_2$Pc in vivo, the two spectra taken from each untreated mouse were averaged. The average was subtracted from each of the two spectra acquired from the same mouse after drug

administration. Then the resulting curves (total of 16 curves) relative to two repeated measurements on eight animals in the same experimental conditions (drug dose and uptake time) were normalized in area and averaged. The standard deviations were calculated at each wavelength for the normalized curves.

A further experiment was performed using the same procedure as described above on a third group of eight mice 1, 18 and 24 h after the intraperitoneal injection of 5 mg/kg b.w. of AlS₂Pc.

Each of the three experiments was performed at least twice.

2.4.2. Ex vivo absorption measurements

Six days after the i.p. passage of tumour cells, eight mice bearing the L1210 leukaemia were administered with 2.5 mg/kg b.w. of AlS₂Pc. 1 h later, the animals were sacrificed and the ascites was drawn from the peritoneum and diluted 1:10 in saline. The absorption spectrum was measured in the 640–720 nm range by a commercial spectrophotometer (Perkin Elmer model 554 UV–visible). The ascites of a control (untreated) animal was used as a reference, to minimize the strong contribution of scattered light to the measured signal.

The same experiment was repeated sacrificing eight animals 24 h (instead of 1 h) after the administration of AlS₂Pc. To check for eventual photobleach, after the acquisition of the absorption spectra, the ascites were irradiated with 100 mW cm⁻² for 10 min at 672 nm and then the absorption spectrum was collected again.

2.4.3. Photodynamic therapy

When the tumour mass was about 8 mm in diameter, mice were injected i.p. with 2.5 mg/kg b.w. of AlS₂Pc. Since the route of drug administration (intraperitoneal or intravenous) does not affect significantly the efficacy of PDT [11], the former modality was chosen for consistency with the reflectance measurements. 24 h later, each animal was caged in a plastic box, with a hole centred at the tumour site. The irradiation of the tumour area was performed with 50 or 100 mW cm⁻² (for the fibrosarcoma and the leukaemia respectively) of laser light for 5 min. For both tumour models, groups of eight mice were irradiated at 672, 685 or 695 nm respectively.

The therapeutic effect was evaluated through the median survival time (MST), in agreement with the evaluation parameters described by the in vivo anticancer drug screening programme of the National Cancer Institute [12]. The MST values obtained for treated and control (untreated tumour-bearing) animals were compared using the Mann–Whitney U test [13].

3. Results

Fig. 1 shows typical results of measurements performed on two different subjects before the administration of AlS₂Pc (A1 and B1). The absorption coefficient μ_a decreases gradually with increasing wavelength. The mean value decreases
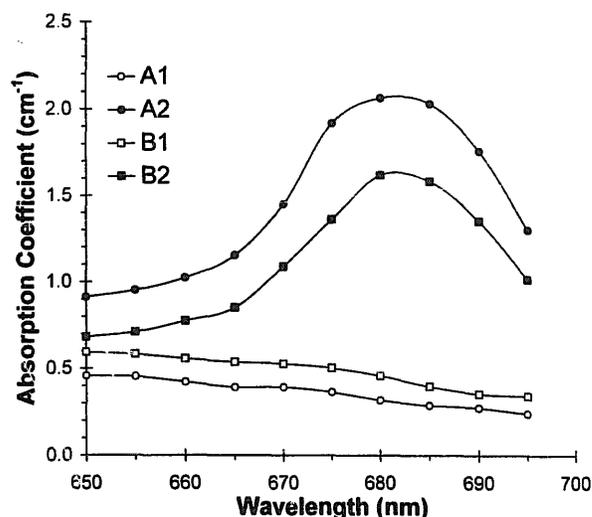

Fig. 1. In vivo absorption spectra of the abdominal region acquired from two different animals (A and B) before (A1 ○ and B1 □) and 1 h after (A2 ● and B2 ■) the administration of 2.5 mg/kg b.w. of AlS₂Pc.

from 0.5 cm⁻¹ at 650 nm to about 0.3 cm⁻¹ at 695 nm.

Following the administration of 2.5 mg/kg b.w. of AlS₂Pc, the absorption increases mainly around 675–690 nm. As an example, Fig. 1 displays also the spectra acquired from two different animals 1 h after the injection (A2 and B2). The spectra labelled A1 and A2 were collected from the same mouse and spectra B1 and B2 were from a second mouse. Significant differences (up to more than a factor of 2) were observed in the increase in absorbance after drug administration to different animals.

For both control and treated mice the variations due to repositioning of the fibre-holder in subsequent measurements on the same animal are usually smaller than the differences found when distinct mice are considered.

Fig. 2(a) shows the average line shape of the absorption spectrum of AlS₂Pc evaluated in vivo on eight animals, 1 h after the administration of the lower dose. The maximum absorption was measured at 685 nm, and a comparable value was obtained also at 680 nm. The standard deviation is less than 30% for $\lambda < 660$ nm and less than 15% for $\lambda \geq 660$ nm. The higher standard deviation for shorter wavelengths is related to the procedure used to evaluate the absorption coefficient. In fact, around 650–660 nm, the drug absorbance is a small number, calculated as a difference between similar values.

For comparison, the spectrum of AlS₂Pc (2.5 µg ml⁻¹) in aqueous solution, acquired with a spectrophotometer (Perkin-Elmer model 554 UV–visible), is also reported in Fig. 2(a). A red shift of about 15 nm characterizes the line shape measured in vivo, which is also slightly broader than that obtained in solution. Similar results were found 4 and 7 h after the administration, as shown in Fig. 2(b) for the latter case. The standard deviation is reduced slightly with time, being less than 10% for $\lambda \geq 660$ nm after 7 h.

With the injection of 5 mg/kg b.w., significantly different line shapes were measured from distinct mice already 1 h
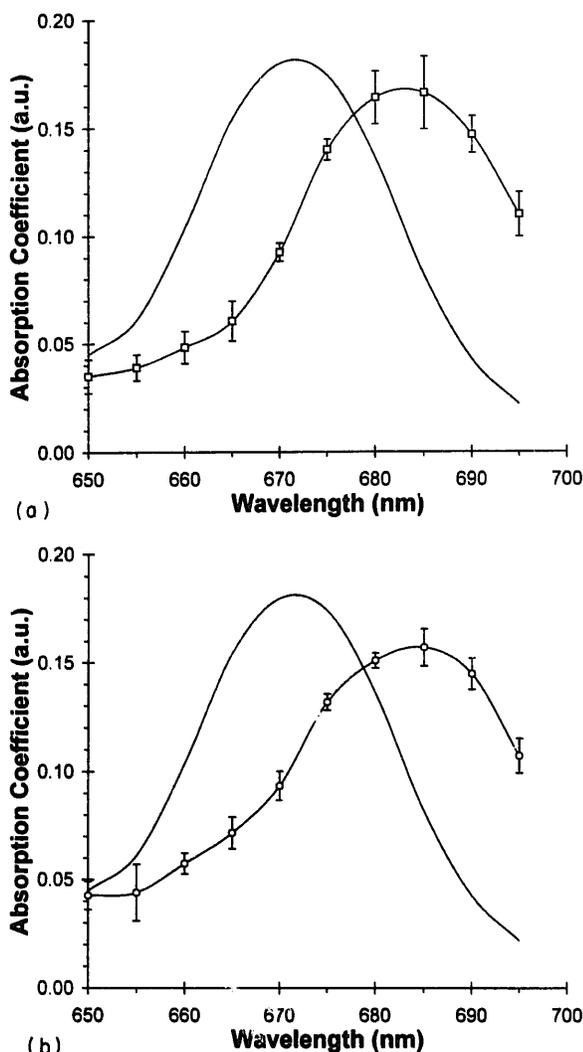
Fig. 2. Absorption line shapes of 2.5 mg/kg b.w. AlS₂Pc in vivo (a) after 1 h (□) and (b) after 7 h (○), and of 2.5 μg/ml AlS₂Pc in aqueous solution (——). All spectra are normalized in area.

Fig. 3. Absorption line shapes of 5 mg/kg b.w. AlS₂Pc acquired in vivo from different animals 1 h after the administration. All spectra are normalized in area.

after the administration. In some animals the increase in absorption due to administration of the higher dose extends to a wider wavelength range (665–690 nm instead of 675–690 nm, as observed with 2.5 mg/kg b.w.). In particular, in a few mice, the spectrum is very similar to that obtained after the injection of the lower dose, while in other subjects it is much broader or a second peak is observed around 670 nm. In most animals, very limited or no significant evolution with time is detected up to 24 h. However, in some cases the spectrum becomes progressively narrower with time or, when two peaks are initially present, the relative weight of the long-wavelength peak increases gradually. Three typical examples of line shapes measured from different animals 1 h after the administration of 5 mg/kg b.w. are reported in Fig. 3.

The increase in μ_a following drug administration ranges between 0.8 cm⁻¹ and 1.8 cm⁻¹ at 685 nm for both doses at any time. Therefore, under these experimental conditions, the absorption at 685 nm is due mainly to the sensitizer, with a minimal contribution from the tissue components. In some
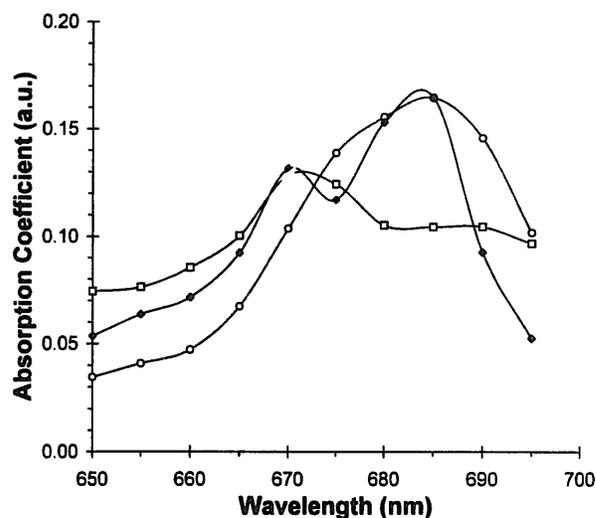
cases the higher dose leads to a comparable increase in absorption also at 670–675 nm.

Ex vivo absorption measurements were performed with a spectrophotometer from 640 nm to 720 nm on the ascitic tumour drawn from the peritoneum of animals administered with 2.5 mg/kg b.w. AlS₂Pc. 1 h after drug injection the absorption maximum in the range of observation is centred at 686–687 nm, as shown by the spectrum reported in Fig. 4. When the animals are killed 24 h after the administration of AlS₂Pc, a weaker signal is detected, but the spectral shape is still shifted to about 685 nm. To evaluate possible effects of photobleaching on the observed results, right after the absorption measurements the ascites were irradiated with a light dose of 100 mW cm⁻² for 10 min at 672 nm, that is twice the dose used for PDT experiments. After irradiation the absorption spectrum was acquired a second time. No differ-
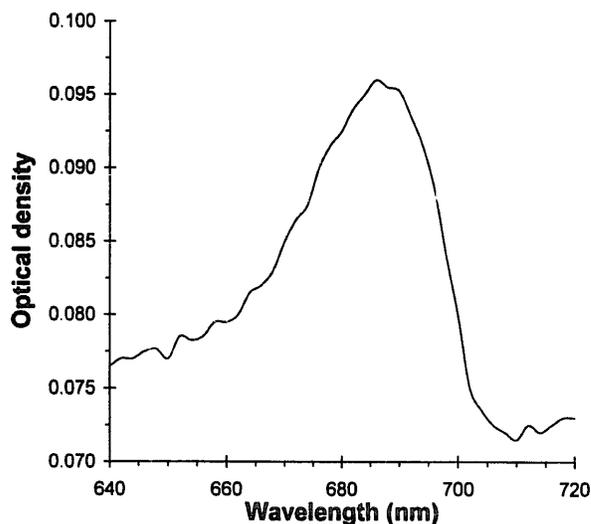

Fig. 4. Ex vivo absorption spectrum of an ascites drawn from the peritoneum 1 h after the i.p. administration of 2.5 mg/kg b.w. AlS₂Pc. The ascites was diluted 1:10 in saline.

Table 1
Therapeutic efficacy of PDT on the L1210 leukaemia after the administration of 2.5 mg/kg b.w. AlS$_2$Pc

Irradiation wavelength (nm)	Median survival time and range (days)	Dead animals/ Total
–[a]	8.5 (6–10)	8/8
672	14[b] (12–16)	8/8
685	17.5[c] (12–20)	8/8
695	16.5[c] (14–18)	8/8

[a] Control (untreated tumour-bearing) animals.
[b] $p < 0.001$ by Mann–Whitney U test vs. control group.
[c] $p < 0.001$ by Mann–Whitney U test vs. control group; $p < 0.05$ by Mann–Whitney U test vs. group treated at 672 nm.

Table 2
Therapeutic efficacy of PDT on the MS-2 fibrosarcoma after the administration of 2.5 mg/kg b.w. AlS$_2$Pc

Irradiation wavelength (nm)	Median survival time and range (days)	Dead animals/ Total
–[a]	43 (25–50)	8/8
672	60[b] (48–72)	6/8
685	88[c] (65–91)	5/8
695	70[d] (63–79)	6/8

[a] Control (untreated tumour-bearing) animals.
[b] $p < 0.01$ vs. control group.
[c] $p < 0.01$ vs. control group and group treated at 672 nm; $p < 0.05$ vs. group treated at 695 nm.
[d] $p < 0.01$ vs. control group; $p < 0.05$ vs. group treated at 672 nm.

ences were observed between the spectra collected before and after irradiation, concerning both the absorbance values and the spectral shape.

To check the consistency of the absorption spectrum and therapeutic response, the photodynamic therapy was performed on the same tumour model (L1210 injected i.d.) at different irradiation wavelengths 24 h after the administration of 2.5 mg/kg b.w. Table 1 reports the results obtained. As shown, the best therapeutic effect is achieved at 685 nm (MST 17.5 days). The efficacy is comparable at 695 nm (16.5 days), while it is significantly worse at 672 nm (14 days).

Photodynamic treatments at the same irradiation wavelengths were performed also on the MS-2 fibrosarcoma 24 h after the injection of 2.5 mg/kg b.w. AlS$_2$Pc. The results, reported in Table 2, are consistent with the absorption spectrum in vivo. After irradiation at 685 nm, the survival time is maximum (88 days). Even at the longest wavelength (695 nm), the MST (70 days) is significantly higher than at 672 nm (60 days).

4. Discussion

The action spectrum of AlS$_2$Pc for photodynamic therapy in two murine tumour models (MS-2 fibrosarcoma and B 16

melanoma) has been measured previously [8]. The protocol followed in that study included the administration of 5 mg/ kg b.w. AlS$_2$Pc. The therapeutic efficacy was found to increase from 640 to 672 nm and to remain almost constant in the 672–710 nm range. The behaviour observed indicates a red shift and a possible broadening of the action spectrum with respect to the absorption spectrum of AlS$_2$Pc in vitro. However, the hypothesis of a corresponding modification of the absorption spectrum in vivo is not obvious. In fact measurements by the authors and other research groups [5] showed that the spectrum in aqueous solution peaks at 672 nm, and only minor changes (a red shift of 4 nm at most) can be obtained in the presence of serum proteins or in other environments such as micelles. Moreover, no further insight seems to be provided by studies on cell cultures, since Moan et al. showed that neither the absorption spectrum nor the action spectrum are shifted significantly in two different cell lines [14].

Time-resolved reflectance measurements allow evaluation of the optical properties of tissue and of highly scattering media in general. Therefore this technique was used to evaluate the absorption line shape of AlS$_2$Pc in vivo, non-invasively, with no perturbations of the sensitizer properties.

The interpretation of time-resolved reflectance data is based on the diffusion theory [1,9,10]. Conditions of good applicability would require a semi-infinite uniform volume. The action spectrum was measured on solid tumours and their volume must be much smaller than 1 cm^3 to guarantee reasonable homogeneity. In fact, larger volumes are usually characterized by ulcerations and haemorrhagic or necrotic regions. Hence, for the reflectance measurements, an ascitic tumour model was chosen to attempt to deal with a wider volume. In tumour-bearing mice, owing to the ascitic fluid, the radius of the peritoneal cavity can increase by 3–5 mm so that, taking into account the optical properties, most of the injected photons are expected to remain within the tumour volume. Moreover, a higher homogeneity was expected, owing to the similarity between the ascitic tumour and a cell suspension. For better control of the drug dose at the tumour level, the sensitizer was injected intraperitoneally. Therefore the effective dose was higher than after intravenous administration, as performed in the previous work on the action spectrum for fibrosarcoma and melanoma [8]. On this basis, as a starting point the absorbance was evaluated with a reduced dose of AlS$_2$Pc (2.5 mg/kg b.w.). The absorption line shape measured in vivo, peaking at 685 nm, showed a significant red shift with respect to the spectrum in solution. In particular, the absorbance in vivo, at any longer wavelength up to at least 695 nm, is greater than at 672 nm. This spectral change seems to indicate a strong interaction between the sensitizer and the biological substrate. A modification of the chemical structure can perhaps be speculated, since various solvents and environments (e.g. micelles) could never lead to such a remarkable red shift of the AlS$_2$Pc absorption spectrum. Moreover the average degree of sulphonation is 2.1 and the sensitizer is not a pure disulphonated phthalocy-

anine. Therefore it is also possible that components with a distinct degree of sulphonation, characterized by somehow peculiar line shapes [4], give a different relative contribution to the global absorption spectrum in solution and in vivo.

The red shift observed is consistent with the results obtained by Farrell et al. on rat thigh muscle [15] and by Wilson et al. on cat thigh muscle [16]. In both cases only a brief description of the experiment is given, but the reported spectrum of aluminium phthalocyanine in vivo is shifted moderately with respect to the line shape in solution.

In the range of μ_a values of interest for the present study, the instrumentation allows us to assess the absorption coefficient with an absolute error of less than 20% [9]. The reproducibility of the measurement is better than 5%. These limits are definitely acceptable if compared with the standard deviations due to biological variability. Moreover, the absolute error increases progressively with the absorption coefficient. Therefore the relative error between measured values falling within a restricted range is significantly smaller than the absolute error [9]. This guarantees that the spectral line shape can be estimated with a precision significantly better than 20%.

With the tumour model chosen for the reflectance study, it was also possible to perform absorption measurements *ex vivo* with a spectrophotometer. The strong contribution of scattering was minimized by placing the diluted ascites of an untreated animal in the reference pathway of the spectrophotometer. Moreover in the wavelength range of interest the scattering coefficient is approximately constant, so it is not expected to alter the measured absorption line shape. The results are in good agreement with the data obtained in vivo and confirm the shift of the absorption maximum of AlS_2Pc after systemic administration. This allowed us to exclude the presence of any undesired effects or artifacts related to the application of time-resolved reflectance to the ascitic tumour model.

By means of reflectance, the absorption properties in vivo were also evaluated with a higher drug dose and a completely different situation was found. Clearly dissimilar line shapes were measured from distinct animals. Therefore an average spectral shape could not be obtained reliably. Moreover only in a few cases could an evolution with time be observed. When any change was present, it seemed to lead towards the line shape typical of the lower dose. If a second peak was observed, it was usually centred at about 670–675 nm, that is where the absorption is maximum for AlS_2Pc in aqueous solution. This might indicate that, owing to the high dose, and therefore to the high concentration of AlS_2Pc in the peritoneum, part of the sensitizer has no strong interaction with the biological environment. Moreover the behaviour observed might be at least in part due to an inhomogeneous drug distribution in the ascitic tumour, possibly related to the presence of phthalocyanine in two different binding conditions. This seems to be confirmed also by modifications of the absorption spectrum, observed in some cases, depending on the positioning of the fibre on the animal abdomen. Such

an uneven drug distribution would not satisfy the hypothesis of a homogeneous measurement volume, fundamental for the use of the diffusion theory in the interpretation of reflectance data, thus reducing the reliability of the absorbance values.

In cell-bound sulphonated phthalocyanines, Berg et al. observed a broad band underlying the typical line shape of monomers [4]. This unstructured absorption feature, which was attributed to aggregates, might also contribute to the spectral behaviour detected at the higher dose in our study.

When considering the absorbance data collected after the administration of 5 mg/kg b.w., it should be taken into account that the drug was injected i.p. and the measurements were performed on the peritoneum itself. Therefore, as mentioned above, especially in the first hours after injection, the drug concentration was significantly higher than at the level of the neoplastic region in a solid tumour model after intravenous administration of the same dose.

To avoid the possible saturation effect of the action spectrum observed previously in the case of the fibrosarcoma and melanoma [8], the therapeutic efficacy was evaluated again, but with the reduced drug dose of 2.5 mg/kg b.w., in the same fibrosarcoma and in the leukaemia used in the present study for the reflectance measurements. Three wavelengths were chosen: the absorption maximum in aqueous solution (672 nm), the absorption maximum in vivo (685 nm), and a longer wavelength (695 nm) where the absorbance is still high in vivo but much lower in solution.

For the fibrosarcoma, the MST reached its maximum value at 685 nm, and at 695 nm it was significantly higher than at 672 nm. Therefore the absorption spectrum of incorporated AlS_2Pc seems to be that obtained after the administration of 2.5 mg/kg b.w. of phthalocyanine. This confirms also that a saturation effect caused the flattening at long wavelengths in the action spectrum measured previously for the fibrosarcoma and the melanoma. In fact in that work drug and light doses were optimized to give the best possible therapeutic effect at 672 nm, where the absorption was supposed to be maximum. When the line shape measured in vivo is considered, it is evident that for any wavelength up to at least 695 nm, the absorption is higher than at 672 nm, and therefore the best efficacy, as obtained at 672 nm, is to be expected even in the whole range 672–695 nm.

For greater consistency with the absorption data, the photodynamic treatment at different irradiation wavelengths was performed also on the same tumour model as used for reflectance measurements. However, the L1210 cells were injected i.d., so that the irradiation could be performed and the curative effect of PDT evaluated as a prolongation of the survival time. Similarly to what was observed with the fibrosarcoma, the therapeutic efficacy proved to be significantly better at both 685 and 695 nm than at 672 nm, resembling the red shift of the absorption line shape obtained in vivo with the lower drug dose. The values at the two longer wavelengths are not significantly different from each other, since the possible increase in survival falls within a more limited range with the leukaemia than with the fibrosarcoma, and hence in the for-

mer case it is more difficult to achieve significant variations of the therapeutic effect with the irradiation wavelength.

For both absorption and action measurements the drug was injected i.p. However, the reflectance data were collected from an ascitic tumour in the peritoneum, while the therapy was performed on tumour cells of two different lines injected i.d. on the animal back. Therefore the consistency between in vivo absorption data and therapeutic effectiveness indicates that the sensitizer behaviour is similar, independent of the tumour model and location, at least for murine tumours.

Work is presently in progress to study the interactions leading to the red shift of the absorption spectrum in vivo. Time-resolved reflectance will be used to measure the absorbance of L1210 cells incubated in vitro with AlS_2Pc. This will help us to understand whether the spectral change is related to the intracellular or extracellular environment.

Acknowledgments

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