Comparison of Lasers for Photodynamic Therapy with a Phthalocyanine Photosensitizer

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Abstract. Three different lasers were compared under the same conditions for their effectiveness at producing photodynamic damage to normal colon following sensitization with aluminium sulphonated phthalocyanine (AlSPc). One laser was an argon ion pumped continuous wave (CW) dye laser and the other two were pulsed at 10 kHz (copper vapour laser pumped dye laser, and 5 Hz (flashlamp pumped dye laser). The CW and 10 kHz laser were equally effective at producing damage. The 5 Hz laser failed to produce a photodynamic effect, although occasionally caused a photomechanical effect when the laser fibre was placed touching the colonic mucosa. Quantitative analysis suggests that the high energy pulses of the flashlamp pumped dye laser saturate AlSPc, so very little of the available energy can be used to produce a photodynamic effect, in contrast to the two other lasers which do not produce saturation conditions.

INTRODUCTION

Photodynamic therapy (PDT) is attracting interest as a new technique with the potential for the selective destruction of malignant tumours. It is based on the systemic administration of certain photosensitising agents, that are retained in malignant tissue, and when activated by light produce a local cytotoxic reaction.

The most convenient light sources for PDT are lasers. There are two main reasons for this. First lasers can be focused into small flexible optical fibres at sufficient power levels for endoscopic therapy. Secondly lasers produce monochromatic light, so all the energy can be delivered at the absorption wavelength of the photosensitiser. There are two types of laser excitation for PDT. The most common is to use a constant irradiation intensity from continuous wave lasers. Alternatively, the laser light can be pulsed so that excitation occurs over a short time interval at high peak power. There is a wide range of possiblities for the pulse repetition rate and the duration and energy of each pulse. In this study the effectiveness of three laser systems, two pulsed lasers and one continuous wave (CW), is evaluated for PDT of the normal colon.

MATERIALS AND METHODS

Photosensitiser

The photosensitiser used in this study was aluminium sulphonated phthalocyanine (AlSPc). It was obtained from Ciba–Geigy and used as received after being dissolved in 0.9% saline for intravenous injection. The compound is a mixture with components containing different numbers of sulphonic acid groups, but with an average of 3 sulphonate groups per molecule. It is chemically and photochemically stable in aqueous solution at room temperature.

Light sources

Three different lasers were used in this study. All were tuned to emit laser light at the activation wavelength of AlSPc of 675 nm. The lasers were set to deliver 100 mW (pulsed lasers 100 mW average power).

1. ARGON ION PUMPED CONTINUOUS WAVE DYE LASER
(Aurora, Cooper Lasersonics)
The dye used was DCM (4-dicyanomethylene-2-methyl 6 (p-dimetylaminostyryl)-4 H pyran) dissolved in ethylene glycol and propylene car-

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bonate. The peak output of this dye was at 650 nm, but for use with AlSPc, the laser was tuned to 675 nm. The power output of the laser was coupled into a 200μ quartz fibre, and the laser was set to deliver 100 mW.

2. COPPER VAPOUR PUMPED DYE LASER (Oxford Lasers)
The dye used was oxazine 72 (0.7 mM) with rhodamine 6G (0.3 mM). The laser was pulsed with a repetition rate of 10 000 Hz with a pulse length of 40 nanoseconds. The laser was tuned to emit at 675 nm, with an average power of 100 mW. Each pulse was therefore 10 microjoules. The delivery system was again a 200 micron fibre.

3. FLASHLAMP PUMPED DYE LASER (Vuman Lasers)
The dye used was cresol violet and the laser emitted at 675 nm. It was also a pulsed laser with a repetition rate of 5 Hz and pulse length of 2 μs, giving a pulse energy of 20 mJ at an average output power of 100 mW. The same 200 μ optical fibre was used for light delivery.

The output of each laser was coupled into a 200 μ quartz optical fibre. The plastic cladding from the end of the fibre was cleared and the fibre cleaved to ensure a clean, circular beam prior to every treatment. The power output was measured in the same power meter that was able to record power from both pulsed and continuous wave lasers (Photon Control). Power measurements were taken before and after each treatment.

PHOTODYNAMIC THERAPY

Experiments were performed on normal male Wistar rats (180–250 g). They were injected with 0.5 mg/kg, 1 mg/kg or 5 mg/kg AlSPc by tail vein injection. Control experiments were also performed on unsensitised animals to assess the effect of laser light alone. The concentration of the solution was adjusted to give an injection volume of approximately 0.5–0.75 ml. The blue colour of AlSPc made extravasation easy to see. All procedures were performed under general anaesthesia with intramuscular Hypnorm (fentanyl and fluanisone).

Treatment was performed one hour after intravenous injection, which is the time of peak concentration in normal colon (1). At laparotomy a mobile portion of the colon was exteriorized onto the anterior abdominal wall. The laser was set to deliver 100 mW (pulsed lasers 100 mW average power). The laser fibre was inserted into the lumen of the bowel by puncturing the colonic wall, and was threaded along the lumen to a convenient point. It was held loosely in a clamp so that it just touched the bowel mucosa. The laser was switched on for the required exposure time, which was 500 s to deliver a light dose of 50 J. This energy was chosen because it was known that it would produce a large lesion with CW irradiation (1). After the laser was switched off the distance from the entry point to the treatment point was measured and the entry point marked with a silk suture. The abdomen was closed and the animals allowed to recover. Seventy-two hours after phototherapy they were killed by cervical dislocation. At post-mortem the area of treatment was identified and the whole colon removed and laid open and placed flat. The area of damage was normally a sharply demarcated oval area. The radius of damage was calculated by measuring the two radii at right angles to each other under an operating microscope (Wild M650) and taking the average. If no lesion was seen a careful search for any damage was made with the operating microscope.

In addition to this method, colon was treated by external irradiation (fibre not in contact with the tissue) to ensure that any effect or lack of effect was not caused by the method of irradiation. Pulses from the 5 Hz flashlamp pumped dye laser delivered with the fibre in contact with the target tissue are very similar to those used to fragment renal calculi. It was therefore felt necessary to check whether such photomechanical effects were also occurring in the colon. The fibre was held in a micromanipulator (Prior Instruments) 2.5 cm above the colon to irradiate an area 1 cm in diameter, the surrounding tissue being shielded. The laser was set to deliver 100 mW from the end of the fibre for 500 s. The power density on the colon was therefore 127 mW/cm^2, giving an energy density of 64 J/cm^2. The animals were treated 1 h after photosensitization with 5 mg/kg.

RESULTS

The results produced with 50 J of light delivered by internal irradiation at three different injected doses of photosensitizer are shown in Fig. 1. All animals were killed 72 h after phototherapy. No damage was produced by the laser pulsed at 5 Hz. There was no difference between the CW laser and the laser pulsed at 10 kHz.

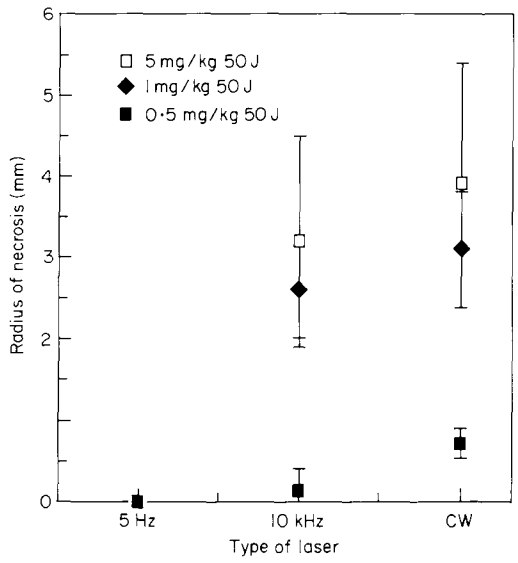

Fig. 1. Mean radius of photodynamic necrosis produced under the same conditions for three different lasers, 72 h after phototherapy. Irradiation was performed with the laser fibre placed just touching the colonic mucusa. Each point is the mean of five separate animals with standard deviations.

However, the laser pulse at 5 Hz produced an occasional photomechanical effect both in control and photosensitized animals, causing an intramural haematoma. It was clear that although these photomechanical effects occur only occasionally, they could represent a source of error. If blood vessels were disrupted photomechanically they may not be able to respond to PDT.

To exclude this, further experiments were performed using external irradiation. External irradiation produced similar results the 5 Hz laser again not causing any photodynamic or photomechanical effect. There was no difference between the damage produced by the laser pulsed at 10 kHz and the continuous wave laser (Fig. 2).

We have reported previously that light irradiation immediately after injection of 5 mg/kg AlSPc produced massive necrosis of tissue due to activation of the circulating sensitizer; irradiation of the mesenteric vessels caused ischaemic necrosis of the entire small bowel (1). We therefore used the 5 Hz laser to irradiate the colon and mesentery immediately after injection of 5 mg/kg to see if it was possible to activate circulating AlSPc. Again there was no photodynamic effect at all, the animals remaining totally unaffected.

DISCUSSION

The efficiency of pulsed and continuous wave light to produce a photodynamic effect on cells in tissue culture and a transplanted murine tumour with HpD has been compared by Cowled et al (2). They used a continuous wave argon ion pumped dye laser and a gold vapour laser with a pulse width of 50 ns and a frequency of 10–14 kHz. No difference between the two was seen.

Bellnier (3) compared a continuous wave argon ion pumped dye with a flashlamp pumped dye laser with a repetition rate of 4 Hz and a pulse width of 1.6 ms and peak pulse energies from 100–250 mJ for PDT of murine transitional cell carcinomas, using HpD as the photosensitiser. They encountered some experimental difficulties producing high tumour temperatures at high fluence rates with the pulsed laser.

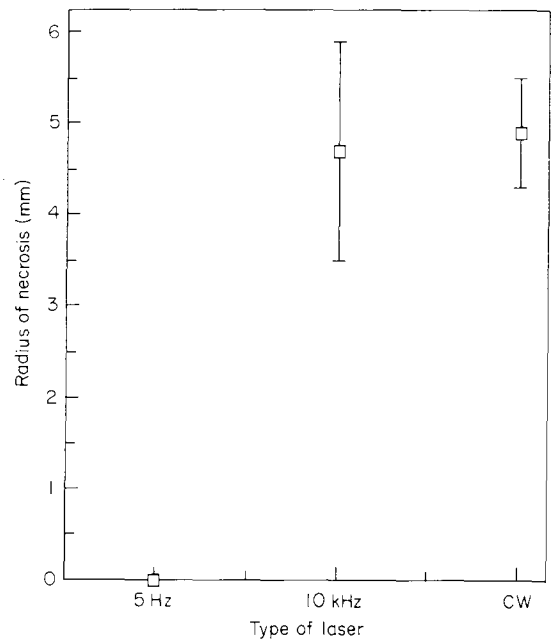

Fig. 2. Mean radius of necrosis produced under the same conditions by the three lasers, 72 h after phototherapy. Irradiation was by the external method to exclude the photomechanical effects occasionally caused by the 5 Hz laser. Each point is the mean of 5 separate animals with standard deviations.

However, they found that the pulsed laser light, when thermal effects were excluded, produced no photodynamic effect.

A novel approach, using a pulsed laser was reported by Andreoni et al (1982). They observed increased destruction of rat epithelial cells by pulsed light compared to continuous wave light. However, the mechanism of activation of HpD involved the two step activation of the sensitizer using a nitrogen laser providing pulses at 337.1 nm with a 10 ns duration and a peak power of up to 250 kW at a repetition rate of 30 Hz. They suggested that HpD radicals were formed and were the cytotoxic agents. They contrast this mechanism with photodynamic action where singlet oxygen is the cytotoxic species and they are careful in drawing this distinction.

There has been no work on different laser activation with AlSPc. It is important to try and identify the reason for the ineffectiveness of a PDT effect with the flashlamp pumped dye laser pulsed at 5 Hz with 20 mJ, 2 ms pulses. In the following discussion it is proposed that these observations could be explained by the onset of photosensitizer saturation during each laser pulse. Figure 3 shows a simplified diagram of the activation of AlSPc by light. Saturation occurs when the pulse energy is sufficiently high to pump the majority of the absorbing ground state to an excited state. At the onset of saturation during the laser pulse the ground state population is substantially depleted, with the result that much less of the remaining pulse energy can be absorbed by the photosensitizer, i.e. the pulse energy is not used efficiently because the photosensitizer is transiently photobleached.

Saturation pumping of phthalocyanines is a well-known process (5) from their use as saturable absorbers in Q switching of ruby lasers. This type of transient reversible photobleaching is distinct from photochemical decomposition of the photosensitiser.

The mechanism by which saturation occurs depends on the laser parameters and the photoproperties of the photosensitizer. For strong absorbers such as phthalocyanines, saturation is potentially more important since the rate of absorption depends on the extinction coefficient. However saturation is inhibited when the ground state can be efficiently repopulated on a similar timescale to the laser pulse duration. For a photosensitizer the rate of repopulation depends on the kinetics of the excited singlet and triplet states. Absorption by the ground state initially forms the singlet state which either decays via fluorescence back to the ground state or converts into a long-lived triplet state (Fig. 3). For AlSPc approximately 50% of the singlet molecules (5) decay via fluorescence on a relatively short timescale [fluorescence lifetime of AlSPc in aqueous solution is 5 ns, (6)] compared with the pulse durations used here. The other 50% populate the much longer-lived triplet state [triplet state lifetime of AlSPc in a degassed aqueous solution is 510 ms (6)]. The triplet state is believed to be the photoactive state relevant to PDT since it can generate singlet oxygen via quenching by molecular oxygen. Substances such as AlSPc must have significant triplet state yields to be useful as photosensitizers and it is possible that the long-lived triplet state can act as a 'sink' for the excited photosensitizer thus leading to the onset of saturation when sufficiently short and energetic laser pulses are used for excitation. To act as a 'sink' the triplet state must be sufficiently long-lived. The lifetime of the photosensitizer triplet state 'in vivo' depends on the bimolecular interactions with other species and in particular the rate of quenching by molecular oxygen. In order to estimate the triplet lifetime in vivo, the oxygen concentration and quenching rate constant must be known. Unfortunately 'in vivo' triplet state lifetimes of AlSPc have not been measured due to the inherent experimental difficulties. Evaluation of the oxygen concentra-

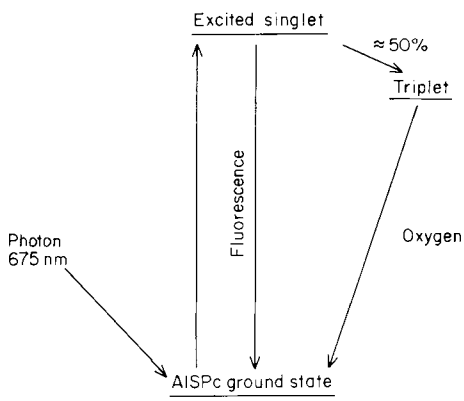

Fig. 3. Simple Jablonski diagram showing the possible excited states that can be populated when AlSPc absorbs red light.

tion 'in vivo' is also problematical since oxygen solubility can vary significantly according to the cellular site, since solubility tends to increase with the hydrophobicity. Therefore the microscopic distribution of AlSPc must be considered. AlSPc is a relatively polar species and dissolves readily in water to form monomeric solutions up to concentrations of 10^{-4}M. Consequently, it is reasonable to propose that AlSPc largely accumulates in hydrophilic cellular sites. Furthermore, the experiments were performed with light exposure 1 h after injection of the photosensitizer, so a significant amount of AlSPc would still be present in the circulating plasma. If it is assumed that the phototoxic agents such as singlet oxygen are largely generated from AlSPc excitation in hydrophilic or intermediate polarity environments then taking an oxygen solubility close to that for aqueous solutions would give an oxygen concentration of approximately 50 μM for a partial pressure of 50 mmHg. The rate constant for quenching of AlSPc triplet state is 2×10^9 M^{-1} s^{-1} (6) in aqueous solution; however in cells a lower value would apply owing to the lower rate of diffusion. For example the rate constant for oxygen quenching of triplet state haematoporphyrin in aqueous solution is reduced by a factor of ten when the haematoporphyrin is bound to human serum albumin (7). An estimate can now be made of the 'in vivo' triplet state lifetime of AlSPc:

$$\text{Triplet state lifetime/s} = [50 \times 10^{-6}\text{M} \times 2 \times 10^9\ \text{M}^{-1}\ \text{s}^{-1}]^{-1}$$

This gives a triplet state lifetime $> 10\,\mu$s, with the assumption that oxygen is the major quencher of the triplet state. Since this value is greater than the laser pulse durations used in these experiments, even if the oxygen concentration has been considerably underestimated, the possibility of saturation pumping of AlSPc 'in vivo' can be seriously examined. Our triplet lifetime estimate appears to be realistic in view of recent studies by Firey et al (8) on photoexcitation of zinc phthalocyanine in cultured myeloma cells. They observed that the triplet state lifetime of zinc phthalocyanine in the cells was about 10 μs using air-saturated culture medium which corresponds to an oxygen pressure of approximately 200 mmHg. Other secondary triplet deactivation processes such as electron transfer may occur, resulting in the formation of metastable phthalocyanine ions or radicals which may subsequently be reconverted to phthalocyanine ground state molecules.

The second main criterion for saturation to occur requires that the laser pulse fluence is sufficiently large to deplete the ground state population by conversion to the triplet state. Using external irradiation the incident fluences per pulse were 25 mJ/cm^2 (5 Hz flashlamp pumped dye laser) and 12 μJ/cm^2 (10 kHz copper vapour pumped dye laser). The number of photons at 675 nm are therefore 9×10^{16} photons/cm^2 and 4×10^{13} photons/cm^2 respectively. Considering absorption only by AlSPc the saturation energy fluence per pulse can be estimated from the saturation condition:

$$\text{Absorption cross section of AlSPc} \times n/2 = 1 \quad (9)$$

The absorption cross section of AlSPc is 6×10^{-16} cm/molecule in aqueous solution, n is the number of photons/cm^2 per pulse with a factor of 2 included to allow for fluorescence decay. From this condition the saturation photon fluence is derived as 3×10^{15} photons/cm^2 which corresponds to a pulse energy fluence of 1 mJ/cm^2. Inclusion of the competing tissue absorption and scattering would not greatly affect this figure, since the colonic wall is relatively transparent to 675 nm light. Thus, it is apparent that the pulse fluence from the 10 kHz copper vapour pumped dye laser is too low to result in saturation of the AlSPc whereas the pulse fluence from the 5 Hz flashlamp pumped dye laser is well above the saturation threshold. The conclusion is that much less AlSPc absorption and less photodynamic effect would occur with the 5 Hz system. The occurrence of fluorescence quenching would increase the saturation threshold but this effect is unlikely to alter our conclusion. Although the average power for each laser is similar, the fluence and pulse duration are the important parameters relevant to saturation. For the 10 kHz copper vapour pumped dye laser the time between pulses is 100 μs, which could be comparable to the triplet state lifetime in anoxic conditions, that is the triplet state population will approximate to a steady-state level, as with continuous-wave irradiation. Saturation with the copper vapour dye laser would only be possible at the energies used at the tip of the 200 μ fibre, where the energy density per pulse is the highest. This may explain the apparently lower response compared to the CW laser using 0.5 mg/kg as the injected dose.

A further point that must be considered is AlSPc concentration in the irradiated tissue. At a dose of 5 mg/kg, 1 h after injection the mean AlSPc level in normal colon is 4.7 μg/g of tissue

(1) which corresponds to a concentration of approximately 3×10^{15} molecules/cm^3.

It is clear that, for the 5 Hz flashlamp pumped dye laser pulse fluence of 9×10^{16} photons/cm^2, the number of photons delivered per pulse far exceeds the number of AlSPc molecules present in the directly irradiated zone. It has been assumed above that the threshold energy absorption to cause photosensitized tissue necrosis is comparable for pulsed and continuous wave irradiation. If for example the threshold for pulsed irradiation were substantially higher then the absence of a photodynamic effect with the high energy pulses from the 5 Hz flashlamp pumped dye laser could on that basis be explicable. However, the available evidence (10) with AlSPc indicates that the photodynamic threshold is reduced with increasing irradiation intensity, although these results were obtained in tissue culture with continuous wave irradiation.

We have concluded that the PDT effects with the continuous wave and 10 kHz copper vapour pumped dye laser are comparable. It is proposed that saturation of AlSPc photosensitizer absorption occurs using the high energy 5 Hz flashlamp pumped dye laser which reduces the fraction of the delivered light energy absorbed by AlSPc. It appears that a low repetition rate, high pulse energy source such as the flashlamp pumped dye laser is not an efficient irradiation source for PDT, especially with strong absorbing photosensitisers such as phthalocyanines. As described previously, HpD photodynamic therapy of murine tumours was ineffective when a flashlamp pumped dye laser was used (3), despite the fact that HpD is a relatively weak absorber in the red and in theory saturation should be less likely. However, it is known that the partial pressure of oxygen is relatively low in tumours compared to normal tissue, which would make irradiation of photosensitizers in tumours more susceptible to saturation using pulsed microsecond lasers.

Alternative explanations to saturation are conceivable, though unlikely. For example, it is possible that a cellular repair mechanism may become effective when using the low repetition rate flashlamp pumped dye laser. Such a mechanism would be difficult to establish but can not totally be excluded.

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