Non-invasive laser microsurgery selectively damages populations of labeled mouse neurons: dependence on incident laser dose and absorption

Roger D. Madison, Jeffrey D. Macklis and Matthew P. Frosch
Department of Neuroscience, Harvard Medical School, Children's Hospital, Boston, MA 02115 (U.S.A.)
(Accepted 15 September 1987)

Key words: Unfocused laser lesioning; Dye-targeting; In vitro; Neuronal death

Selective photothermolysis (SP) is a novel technique by which brief, unfocused laser pulses are selectively absorbed by, and cause selective thermal damage to, endogenously pigmented structures. This report describes the use of an exogenous non-fluorescent chromophore (Procion blue) to effect cellular damage by SP. Cultured dorsal root ganglia neurons were selectively labeled with Procion blue and subjected to varying doses of laser illumination. Progressive cellular damage was assessed by leakage of propidium iodide through damaged membranes. The neurons targeted via an exogenous chromophore sustained damage which was proportionate to laser dose and chromophore concentration. The results of these experiments demonstrated that the rate and extent of neuronal damage can be controlled by adjusting either the incident dose of laser energy or the amount of target chromophore within cells. Selective photothermolysis will provide an experimental tool for neurobiologists in particular and will find general use within the biomedical field.

INTRODUCTION

We recently described the use of unfocused, near-infrared laser illumination to kill populations of exogenously dye-targeted mouse neurons in vitro. Such long wavelength light penetrates many millimeters through rodent nervous system tissue and offers the future potential for non-invasive, selective lesions deep within the rodent central nervous system (CNS). In that initial report, we demonstrated selective delivery of an exogenous non-fluorescent chromophore to dorsal root ganglia neurons in culture. The dye-targeted neurons and the surrounding Schwann cells and fibroblasts were subjected to high doses of laser illumination (50 J/cm²), and the resultant cellular damage was assessed by leakage of propidium iodide through damaged membranes. The results from these first experiments clearly showed specific damage to the dye-targeted neurons; unlabeled non-neuronal cells in direct contact with targeted neurons remained intact. Control cells that received the high laser dose but were not labeled with the target chromophore sustained no more damage than control cells simply kept in the incubator. We now present additional studies which assessed the effects of varying laser dose and intracellular dye concentration in terms of neuronal damage in this model system in vitro. The experiments reported in this paper tested the hypothesis that the extent or rate of cellular damage could be controlled by adjusting either the dose of laser energy or the amount of target chromophore within cells. These experiments provide a necessary 'stepwise' foundation in vitro for our planned studies of selective targeting and non-invasive lesioning of neuronal subpopulations in vivo. Our goal is to produce exquisitely selective damage...
to subpopulations of neurons without damage to surrounding vascular, glia, or connective tissue, to study CNS development and plasticity.

Miller and Silverston\textsuperscript{10} were the first to show that cells could be damaged by filling them with fluorescent dyes and subsequently exposing individual cells to focused fluorescent light. Unfortunately, fluorescent light has several inherent limitations as a generalized lesioning tool. Light at these wavelengths penetrates only microns through soft tissue, and fluorescent light alone can cause damage to cells. Therefore, only cells directly in view can be exposed and damaged\textsuperscript{6}. Surrounding cells must not be exposed to focused fluorescent light in order to guard against non-specific damage.

Longer wavelengths of near-infrared light in the range of 650–850 nm are capable of penetrating several millimeters to centimeters through soft tissue in vivo\textsuperscript{12,13,15}. Such penetration is possible because the major endogenous chromophores, melanin and hemoglobin, display very little absorption at these long wavelengths. Extremely high energies (10\textsuperscript{6}–10\textsuperscript{7} W) of laser lights can penetrate several millimeters through unpigmented tissue, such as rodent CNS without absorption or damage\textsuperscript{2}.

Anderson and Parrish\textsuperscript{1–4} took advantage of similar optical properties of soft tissue at shorter wavelengths to develop a technique for use within the field of dermatology which they termed 'selective photothermolysis' (SP). They studied the optical properties of soft tissue and microvasculature in great detail and developed mathematical models of penetration, absorption, and radiation transfer as background for clinical uses of SP to lesion pathological structures containing endogenous melanin and hemoglobin\textsuperscript{4,13}. Within an absorbing structure, laser energy is converted into heat capable of disrupting proteins and membranes. Heating of particular structures is directly related to the optical absorption of those structures at a given wavelength.

If target structures have greater optical absorption than surrounding tissue, the thermal energy created is specifically confined to the target tissue. As target structures cool, they transfer heat to surrounding tissue mainly by thermal diffusion, which occurs over microseconds for cellular-sized structures. Given this 'thermal relaxation time' of cellular targets, sufficiently short pulse lengths can be chosen to raise target temperatures preferentially. Elegant studies by Anderson and Parrish\textsuperscript{1} showed that, by varying pulsewidth and wavelength, short pulses of high energy laser irradiation can selectively damage microvessels while leaving nearest-neighbor cells intact. They also showed selective damage to melanosomes while leaving the parent melanocytes intact, using shorter and higher energy pulses\textsuperscript{4}.

Our earlier report\textsuperscript{9} was the first demonstration of successful SP utilizing an exogenous chromophore. We chose Procion blue (PB) as our trial exogenous chromophore because it is non-toxic, non-fluorescent (allowing all energy to be transformed into heat), and highly absorptive at 650–700 nm. We chose longer wavelength, near-infrared laser energy to target cells containing PB while leaving tissue containing melanin and hemoglobin undamaged. All previous studies utilized endogenous chromophores as targets, e.g. melanin and hemoglobin. We attempted to expand the generalized experimental and therapeutic usefulness of selective photothermolysis by developing dye-targeting strategies using exogenous chromophores. We now demonstrate, again using PB as a trial exogenous chromophore for cellular targeting, that the rate and extent of neuronal damage can be controlled by adjusting either the dose of laser energy or the amount of target chromophore within cells. For the laser–dose experiments described below, Procion blue was again selectively delivered to neurons by conjugation with wheatgerm agglutinin (WGA). This plant lectin mediates the uptake of PB into a subset of neurons in vitro, conferring partial selectivity of targeting. For the dye–dose experiments, intracellular iontophoresis provided control of intracellular dye concentration.

MATERIALS AND METHODS

Conjugation of wheatgerm agglutinin and Procion blue

Wheatgerm agglutinin (WGA) was purchased from Vector Laboratories. Procion blue HB was purchased from Sigma Chemicals and 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide-metho-p-toluene sulfonate carbodiimide) was purchased from Aldrich Chemical Co. N-Acetyl-d-glucosamine (NADG) was purchased from Pfanzstiehl Chemical Co. Activated chromatography gel with NADG attached and pro-
tein determination reagents were purchased from Bio-Rad Laboratories. Procion blue (PB) was covalently linked to wheatgerm agglutinin (WGA-PB) with modifications of techniques used commercially to derivatize active protein species with the carbodiimide class of reagents (Vector and Bio-Rad Laboratories, personal communication).

We dissolved Procion blue in 10 mM sodium acetate buffer at pH 5.0 to form a 10% solution of the dye by weight. After filtering the dye solution through 0.22 μm filters, carbodiimide was added at a concentration of approximately 20 mg/ml. The reaction mixture was kept at 4 °C for 30 min. These reaction conditions were expected to yield near-quantitative coupling of PB to WGA.

Lyophilized WGA was dissolved at a concentration of 40 mg/ml in sterile 10 mM sodium acetate buffer at pH 5.0. To minimize the risk of steric alteration to WGA, and to saturate the active sugar-binding sites, 20 mM NADG was added to the WGA and allowed to stand at room temperature for 1 h. Activated PB solution was mixed with an equal volume of lectin solution and allowed to stand at room temperature for 4 h. The final solution (containing 5% PB, 10 mg/ml carbodiimide, 20 mg/ml WGA, and 100 mM NADG in 10 mM sodium acetate buffer at pH 5.0) was washed and dialyzed under high vacuum for 3–4 days against 100 mM sodium phosphate buffer at pH 7.4.

Electrophysiology

Microelectrodes were pulled on a Brown-Flaming microelectrode puller (Sutter Instrument Co., model P-77) using 1.0 mm o.d., 0.75 mm i.d. glass tubing with filament. Some electrodes were filled with a 2 M solution of potassium acetate to allow high quality intracellular recording (electrode resistance = 30–70 MΩ), and others were filled with a solution of 1.0% Procion blue in water. The Procion blue electrodes presented an input impedance that was too high to allow monitoring of more than resting membrane potentials. Electrodes were positioned using a 3-dimensional micromanipulator (Leitz). Intracellular recording, stimulation, and iontophoresis were performed with intracellular amplifier, stimulator, and isolation units (W/P Instruments models M707A, 301T, and 305, respectively). Activity was monitored on a high speed oscilloscope (Hewlett Packard) and recorded on a Brush chart recorder (Gould).

In vitro system

We removed dorsal root ganglia from C57BL/6J mice and cultured them as monolayers using standard techniques. Experimental cultures were exposed to WGA-PB in the medium for 1 h, washed, and kept at 37 °C for 40 h. Control cultures were treated the same way, without exposure to WGA-PB.

All experiments were performed at 37 °C in medium buffered for standard incubator conditions. We located fields with 5–20 neurons on an inverted microscope (Zeiss), noted their x–y coordinates, and videotaped their phase-contrast appearances with a silicon-intensified camera (Dege-MTI). We exposed areas approximately 0.1 cm² to 1 μs pulses of either 2.5 or 5.0 J of energy (25 J/cm² or 50 J/cm²) at 600 nm from a tunable dye laser (Candela) or 694 nm from a ruby laser (Lasermetrics, Fig. 1). Fields were centered within such areas of laser exposure. The locations of neurons, identified by their characteristic appearance under phase-contrast microscopy, were traced for each field on clear acetate sheets attached to the video monitor. Each neuron was numbered and followed during the time lapse experiments.

We assessed cellular injury by the leakage of propidium iodide through damaged membranes. Propidium iodide (PI) fluorescence was observed using a 488 nm excitation filter and a 590 nm high-pass filter. Propidium iodide was present in the dishes at a concentration of 0.05 mg/ml during laser exposure. Control experiments showed no cellular damage from propidium iodide in the dishes during laser exposure when compared to dishes exposed with normal medium alone. Fields of cells were exposed to laser energy either prior to or after the addition of PI. These control experiments showed no difference in cellular damage due to the presence of PI during laser exposure. We therefore carried out all experiments with PI in the dishes at the time of laser exposure.

After laser exposure, we relocated each field of cells using the x–y coordinates and videotaped the phase and fluorescence appearances at successive times after lasing. We minimized the chance of nonspecific fluorescence damage to cells by exposing fields to no more than 5 s at each observation time.
EXPERIMENTAL SYSTEM ARCHITECTURE

Fig. 1. General experimental system used to assess cellular damage secondary to selective photothermolysis (SP). At the center of the system is an inverted phase-contrast fluorescence microscope. Culture dishes were placed on the microscope stage and their x and y vernier coordinates recorded, so identical fields could be located repeatedly. Laser illumination from a tunable dye laser reached the stage via quartz fiber optics. Beam splitters were used to divert a portion of the laser beam to a power meter to record the amount of energy delivered to the microscope stage. A ruby laser, with nanosecond domain pulse durations, was directed to the stage via mirrors. Electro-optical shuttering and Q-switching controlled the pulse duration from the ruby laser. A silicon-intensified camera and video cassette recorder recorded phase and fluorescence video images at the rate of 30 frames/s. A micromanipulator (not shown) was used to guide a microelectrode during electrophysiological experiments. An intracellular amplifier recorded intracellular electrical potentials, and an intracellular stimulator was used to electrically ‘drive’ the cells and to inject dye. An oscilloscope and chart recorder were used to monitor and record electrical potentials during electrophysiological testing.

totaling less than 30 s during a 24–48 h experiment. All focal planes within a field were recorded at a rate of 30 frames/s. Cultures were returned to the incubator between observations.

In the experiments using iontophoresis to assess the dependence of cellular damage on intracellular dye concentration, we similarly identified and videotaped fields with 5–10 large neurons. Cells were treated in several ways, and individual fields contained cells from different groups. Each protocol was repeated between 5 and 15 times. Fluorescence exposure was minimized as described above.

Control cells were treated in 1 of 4 ways. Cells were evaluated for PI fluorescence and electrophysiological activity (intracellular resting potentials and stimulated action potentials) either: (1) prior to laser exposure, (2) after laser exposure, (3) after damage by either jagged microelectrodes or massive current injections (100–200 nA), or (4) after ‘sham’ iontophoresis with microelectrodes lacking PB followed by laser illumination.

The fluorescence and phase-contrast appearances of experimental cells were videotaped prior to any manipulation. Microelectrodes containing PB were introduced and current was injected (50 nA, 0.5 s, 1 Hz) until both phase and fluorescence images showed the cytoplasm to be darkly stained with PB. The electrodes were removed and cells were either: (1) immediately lased, (2) observed for 2 min before lasing, or (3) observed for 5 min without laser exposure. All cells were videotaped for later analysis.

Scoring system

Data analysis was performed in a double-blind fashion. Videotapes were set to unknown frames, index numbers recorded, and index counters reset. A second observer used the clear acetate sheets with outlines of neurons to score each neuron for PI fluorescence on a scale from 0 to 3. A score of 0 indicated an absence of fluorescence in all focal planes over the image of a cell. A score of 1 was given for faint cytoplasmic fluorescence, 2 for distinct cytoplasmic fluorescence, and 3 for distinct nuclear fluorescence. Scores were found to be highly reproducible by any single observer and between observers. Electrophysiologic correlation suggests that the scores indicate relative levels of cellular damage (Fig. 5).

RESULTS

Statistical analysis

We performed both non-parametric and parametric statistical analysis on the non-parametric scores for intracellular fluorescence. Non-parametric analysis of variance of ranked scores was performed, and differences between groups were compared using the Kruskal-Wallis test. We calculated averages and standard errors of the mean (S.E.M.) for each group at each time point. Both parametric and
Fig. 2. Means and standard errors of the mean (S.E.M.) are graphed for experimental and control groups at various times following laser exposure. The filled squares represent experimental cells labeled in vitro with WGA-PB as described in the text, and subsequently exposed to a 'high laser dose', 600 nm, 50 J/cm². The open circles represent experimental cells labeled with WGA-PB, and subsequently exposed to a 'medium laser dose', 600 nm, 25 J/cm². W represents control cells labeled with WGA-PB, that received no laser exposure. H represents control cells not labeled with WGA-PB, and subsequently exposed to a 'high laser dose', 600 nm, 50 J/cm². C represents control cells which neither were labeled by WGA-PB nor received any laser exposure. Data were analyzed statistically with analysis of variance using a general linear model. Differences between means were analyzed using the Student-Newman-Keuls test. Non-parametric analysis was carried out using analysis of variance of ranked scores. Differences between groups were compared using the Kruskal-Wallis test. Non-parametric analysis yielded the same results as parametric analysis. For simplification, only parametric statistics are reported. See text for details.

non-parametric analyses yielded identical results; we report only parametric statistics for simplicity (Fig. 2).

Neurons labeled specifically with WGA-PB and exposed to pulses of laser illumination sustained damage proportionate to laser dose. Fluorescence scores for cells in fields exposed to 50 J/cm² of laser energy increased earlier and more rapidly than scores for cells exposed to 25 J/cm². Nine hours following laser exposure, the average score for the 50 J/cm² experimental group (0.51 ± 0.07, mean ± S.E.M.) was significantly higher (P < 0.05) than for the 25 J/cm² experimental group or for any of the control groups. Twenty-eight hours after laser exposure, the average score for the 50 J/cm² group (1.24 ± 0.09) was significantly higher (P < 0.01) than that for the 25 J/cm² group (0.70 ± 0.06), and both experimental groups were significantly different from all of the control groups alone or in combination. Phase-contrast appearances of neurons deteriorated at a rate in correlation with the increase in intracellular PI fluorescence. This rate of deterioration was dependent on the dose of laser energy.

There was no statistical difference between scores for the 3 control groups: (1) unlabeled cells in fields exposed to 50 J/cm² of laser energy, (2) cells neither labeled nor exposed to laser energy, and (3) cells labeled with WGA-PB but not exposed to laser energy (Fig. 2). Exposure of unlabeled cells to energy densities of 50 J/cm² with 1 µs duration caused no damage above that found in unlabeled and unexposed control cells. Millions of watts of power were delivered to these unlabeled cells, but they did not absorb enough energy to cause either thermal or photochemical damage. Neither underlying Schwann cells and fibroblasts nor surface cells were damaged, as judged by PI fluorescence. As in our previous report, energies of 25–50 J/cm² penetrated at least two cell layers without significant non-specific absorption. Slight increases in average PI scores for control cells may be attributed to damage secondary to manipulation of culture dishes, multiple transfers from the incubator to the microscope stage, and unregulated stage temperature. The small, non-significant increment of additional damage to WGA-PB controls compared to the other control groups is presumably caused by the known mild toxicity of lectins toward neurons.

Iontophoretic results

All cells chosen for electrophysiologic assessment prior to any laser exposure were PI-negative and had healthy resting membrane potentials. Eighty percent displayed stimulated action potentials. Fifteen cells assessed following laser exposure were all PI-negative and had healthy resting membrane potentials; 10 produced action potentials when stimulated. Each of 10 cells damaged crudely by jagged microelectrodes or by high current injection lost both resting membrane potentials and action potentials immediately (see also Fig. 5). Each became PI-positive within 15–30 s. These results suggest that: (1) the cultures were healthy; (2) the laser energies used in these ex-
periments did not adversely affect electrophysiologic viability of unlabeled neurons; and (3) PI fluorescence was a reliable indicator of gross cellular damage.

Each of 13 cells exposed to laser illumination immediately after introduction of PB displayed rapid, dramatic damage under phase-contrast and became PI-positive within 15–30 s (Figs. 3, 4). They each became phase-dark, swollen, and poorly outlined, literally melting away with the loss of a visible membrane. As they melted, nuclear fluorescence due to PI entry and accumulation was so bright that it was visible during simultaneous phase contrast and fluorescence observation. One such cell was contiguous to an unlabeled cell which was entirely undamaged by the laser illumination, demonstrating the fine geographic selectivity possible with SP.

Two cells filled with PB were observed for 2 min, prior to any laser exposure, during which they remained PI-negative and intact under phase-contrast. They then received laser exposure and both reacted as the cells discussed above, 'melting' and becoming brightly PI-positive within 15–30 s. Two additional cells were injected with PB and followed for 5 min without laser exposure. By contrast, these cells remained PI-negative and intact under phase-contrast for the duration of observation.

DISCUSSION

The experiments on dose-dependence of SP in vitro reported in this paper provide further foundation for the feasibility and practicality of using exogenous chromophores to effect specific neuronal damage by selective photothermolysis (SP). Unfocused laser light generated heat specifically confined to structures which were targeted by an exogenously applied chromophore. This heat damaged neurons in a dose-related manner; damage can be controlled by varying either the amount of incident laser energy or the concentration of intracellular chromophore over wide ranges. This method is quite different from those using focused laser microbeams8 or fluorescent light9,10. The localization of damage can be as precise as with microbeam methods; however, SP can damage millions of targets simultaneously without precise aiming. The ability to control damage over such wide ranges of severity and time course allows future in vivo applications to conform to tissue abilities to remove cellular debris and toxic species.

The results of the experiments reported in this paper demonstrated several important aspects of using SP with exogenously applied chromophores. The single most important finding was that cellular damage was dependent on laser energy in a dose-related manner. This fact suggested that the extent and rate of damage was a function of the amount of energy absorbed by the target cells. Energy absorption can be controlled by adjusting either the amount of chromophore within the cell or the incident laser dose. Damage sustained by neurons specifically labeled with relatively small doses of WGA-PB was proportionate to laser dose (Fig. 2). Conversely, neurons heavily labeled by intracellular injections of PB displayed much more rapid and dramatic damage when exposed to the same laser dose (Figs. 3, 4).

Fig. 3. Sample video images of a DRG neuron that received an intracellular injection of Procion blue (PB). Propidium iodide (PI) was present in the medium at a concentration of 0.05 mg/ml. a: an overall view of the field is shown with a group of DRG neurons seen in the middle of the field, ×500. The lower right hand corner of the field is outlined in white and is shown at higher magnification in the succeeding panels. This phase micrograph was recorded immediately before laser exposure. The white arrow points to the DRG neuron which received an intracellular injection of PB. Note that this neuron had a darkened cytoplasm due to the PB injection, and that it was immediately adjacent to another DRG neuron which was not injected with PB. b: phase-fluorescence micrograph of the two neurons from (a) recorded immediately (4 s) after laser exposure (Ruby laser, Q-switched pulse 20–50 ns, 694 nm, 50 J/cm²). Note the distinct phase-dark and swollen appearance of the neuron injected with PB compared to the overall phase-bright appearance of the neighboring neuron. This micrograph was recorded with the field exposed to both phase and fluorescent light. and a dim nuclear PI fluorescence can already be seen in the neuron injected with PB. c: image with fluorescence alone of the same field as in (b). Note the "negative" fluorescence image of the neuron injected with PB, presumably from fluorescence quenching due to PB. Also note a brightly PI-positive glial cell immediately below the two neurons. d: phase-fluorescence micrograph of the same field 45 s after laser exposure. Note that the nuclear PI fluorescence for the injected cell was bright enough to be seen with the phase and fluorescent lights on. The neuron’s membrane literally 'melted' due to selective photothermolysis (SP) with an exogenously applied dye. The ‘next door neighbor’ neuron was still phase-bright and PI-negative. e: fluorescence image of the same field as (d). Note the brightly PI-positive fluorescence of the injected neuron and the PI-negative appearance of the neighboring neuron. The glial cell which was PI-positive in panel (c) is still clearly seen. Panels b–e, ×675.
Fig. 5. Intracellular recordings from a dorsal root ganglia neuron in monolayer culture (see text). This neuron underwent iontophoresis of Procion blue (PB) with propidium iodide in the dish (0.05 mg/ml). The cell was monitored with intermittent fluorescence illumination. a: hyperpolarization in response to current injection (5 nA, 0.5 s, 1 Hz) with visible anode break spikes. This record was made 100 s after entry into neuron. The cell was PI-negative. Large divisions equal 0.5 cm, chart speed equals 2 mm/s, and sensitivity equals 20 mV/cm. b: expanded (200 mm/s) time base showing action potentials evoked by 50 ms depolarizing current injections 200 s after entry (action potentials attenuated by response time of chart recorder). Current was injected for 12 min, the microelectrode was removed, and the cell was observed for 8 additional minutes. It remained PI-negative throughout. c: re-impalement of cell, by capacitatively 'ringing' the electrode, after total of 20 min from time of original entry. Arrow '1' indicates microelectrode touching neuron; arrow '2' indicates time of re-implantation. Action potentials were evoked by current injection indicating continued viability of the neuron. The cell was PI-negative. The neuron was then injected with massive current (200 nA, 0.5 s, 1 Hz). Both resting potential and evoked action potentials disappeared, and the cell became PI-positive within 30 s.

Another important finding was that very high laser energy delivered to unlabeled cells over time periods of 1 μs did not result in any damage.

The damage from SP under the experimental conditions reported in this paper was apparently non-reversible. We followed the incorporation of propidium iodide into more than 300 neurons; the relative ratings of all cells were seen to increase over time. No cell displayed a rating that decreased between successive observations.

Not all DRG neurons take up wheatgerm agglutinin; one expects neurons to become labeled with varying amounts of WGA-PB. We chose to follow every presumptive neuron in our fields which contained a continuum of unlabeled to densely labeled cells. We did not use a fluorescent tag as a second label to identify which neurons were labeled prior to laser exposure in order to avoid confounding our results with non-specific fluorescence damage. Scores for densely labeled neurons within experimental fields were statistically 'diluted' by scores for relatively unlabeled neurons. The result of this 'averaging' of lightly labeled cells along with densely labeled cells made our damage scores quite conservative. The real difference between control and experimental scores is probably much greater than our results suggest.

Long wavelengths of laser light in the near-infrared range of 650–850 nm penetrate several millimeters through mammalian tissue. In human and animal brain, both in vivo and post-mortem, 650–850 nm laser light has been reported to penetrate several centimeters. By contrast, most fluorescent dyes are excited by wavelengths of light lower than 500 nm which demonstrate little or no penetration through mammalian tissue. The use of these relatively short wavelengths of fluorescent light to damage tissue in vivo is necessarily limited to very superficial targets. In order to develop selective le-
sioning techniques for use in vivo with deeper structures, e.g. deep central nervous system nuclei, one must exploit the deep penetration of near-infrared light (650–850 nm) through soft tissue.

With an appropriate concentration of exogenous chromophore within or on a given class of cells embedded within a 3-dimensional matrix of other tissues, it is theoretically expected that the cells of interest can be killed in vivo, leaving all other cell types intact. With SP, there is no need to focus the laser energy, other than to insure that sufficient energy arrives at the targets. Selective damage with SP is dependent upon selective labeling of target cells with an appropriate chromophore, unlike the non-selective damage from microbeam techniques. Once selective labeling has been achieved in vivo, selective damage to target cells is practical and proven to be possible.

The results of the experiments reported in this paper show that the rate and extent of such selective damage can be finely controlled over wide ranges by adjusting either the amount of label in cells or the dose of incident laser energy. Such laser–dye dosimetry in vitro is essential for future experiments in vivo, since it is desirable to have in vivo damage from SP occur at a rate consistent with the tissue abilities to effectively dispose of cellular debris. Damage at a much faster rate could lead to undesirable non-specific side-effects secondary to necrosis and inflammation. It will be of interest to investigate biologic repair mechanisms following such highly selective damage.

Selective photothermolysis in vivo will provide an exquisitely sensitive tool for experimental neurobiologists. Potential therapeutic applications of SP targeted via exogenous chromophores include situations in which it is desirable to damage selectively pathological cells embedded within normal tissue. Our current experiments are focused on refining potential dye delivery strategies to be used for in vivo selective photothermolysis.

ACKNOWLEDGEMENTS

We are pleased to acknowledge the technical help of Mr. Jim Boll of the Wellman Laboratories. Drs. John Parrish, Rox Anderson, and Allen Osseroff of the Wellman Laboratories of Photomedicine, Massachusetts General Hospital provided many helpful discussions concerning this work. Supported by a grant from the Whitaker Foundation (R.D.M. and J.D.M.) and by a Medical Scientist Training Program Grant (M.P.F.).

REFERENCES