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Chloroaluminum Sulfonated Phthalocyanine Partitioning in Normal and Intimal Hyperplastic Artery in the Rat

Implications for Photodynamic Therapy

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Photodynamic therapy, the light activation of photosensitizers into cytotoxic mediators, has been a successful treatment for experimental intimal hyperplasia (IH). To understand the basis of the photosensitizer chloroaluminum sulfonated phthalocyanine (CASPc)-mediated photoinhibition of intimal hyperplasia in the rat common carotid artery model, we studied photosensitizer partitioning in hyperplastic as compared to normal arterial tissue. Serum clearance of CASPc is exponential with, a half-life of 300 minutes. Laser-induced fluorescence and spectrofluorimetric analyses of artery tissue demonstrated an approximately 60% lower uptake and retention of CASPc by normal arterial tissue as compared to arteries with IH; the differences become more pronounced at 24 h. Fluorescent microscopy of arterial tissue demonstrated increased uptake of the CASPc by the artery with IH. However, by 24 h it is primarily the IH tissue that has retained the CASPc, with clearance of the dye from the media of normal or hyperplastic arteries. These data demonstrate that IH, like neoplastic tissue, has an increased accumulation of CASPc compared to normal artery. The preferential partitioning into hyperplastic tissue has implications for therapeutic targeting of this cellular population with photodynamic therapy. (Am J Pathol 1993, 142:1898–1905)

Photodynamic therapy (PDT) is a technique that utilizes the light activation of an otherwise relatively inert photosensitizer drug (PS) to produce, in the presence of oxygen, free radical moieties that are reactive to a variety of cellular targets.[1,2] This photochemistry technique has been used primarily for its cytotoxic biological effects and, therefore, has had its greatest development in the treatment of cancer.[3]

In the studies relating to the efficacy of PDT, it has been known to exert its tumor-killing effect primarily through two mechanisms.[4] One has been by increased uptake and retention of the PS by the neovasculature endothelium, which, when treated, results in tumor vessel thrombosis. The other results from the abnormal neovascular endothelium of the tumor, thought to be more permeable to the PS than normal endothelium. This may be further augmented by increased uptake and retention of the PS by the metabolically active cells themselves.[5,6] It is the basis of this accumulation by the tumor mass as compared to normal tissue that provides a therapeutic window for the selective and successful treatment of tumors with PDT.

Since all of the known tissue partitioning and pharmacokinetics of PS pertain to neoplasms, and as new applications are formulated for PDT treatment, such as the treatment for intimal hyperplasia, it becomes important to further understand the behavior of some of these drugs in other tissue systems. Intimal hyperplasia (IH), a condition that occurs commonly in the

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arterial system following therapeutic interventions such as endarterectomy, angioplasty, and bypass grafting, results from migration and proliferation of vascular smooth muscle cells into the intimal layer of the artery.[7,8] This pathological process progresses in some individuals to a restenosis, commonly in the 6-month to 2-year time frame. To date, no clinically efficacious therapeutic option has been developed.

In the balloon-injured rat common carotid artery (CCA) model of IH, we demonstrated that PDT mediated by the PS chloroaluminum sulfonated phthalocyanine (CASPc) was an efficacious modality for either the prevention or the treatment of induced IH.[9] In the strategy of developing an optimal method for furthering this technique, it is important to better understand the differences in the uptake and retention of PS in normal arteries, as compared to arteries that have been manipulated to develop intimal hyperplasia. In this investigation, we studied the basis of CASPc-mediated photoinhibition of intimal hyperplasia. The partitioning of CASPc in the normal and injured CCA was determined at a tissue level using different techniques.

Materials and Methods

Surgical Induction of Intimal Hyperplasia

Intimal hyperplasia was induced in anesthetized 400 ± 50 g male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) by multiple passes of an inflated 2F Fogarty arterial embolectomy catheter through the left CCA, resulting in endothelial denudation and medial stretching. Animal care in this study complied with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals (NIH Publication 80-23, revised 1985). All animal procedures were approved by an independent institutional animal care committee.

Photosensitizer Administration

CASPc (CIBA-GEIGY, Basel, Switzerland) was diluted from a stock solution of 300 mg/ml in sterile water with phosphate-buffered solution to 5 mg/ml and stored at −70 C in the dark until used. The PS (5 mg/kg) was administered intravenously to the animals via surgical exposure of the femoral vein.

Laser-Induced Fluorescence

To analyze PS concentration in tissue versus time, a noninvasive laser-induced fluorescence (LIF) method was used (Figure 1).[10] The output of a nitrogen laser (VSL-337ND, Laser Science, Inc., Cambridge, MA) of 10 Hz, 3-ns pulses at 337 nm was coupled via a quartz lens into a single 600-μm-diameter core quartz fiber (Superguide G, Fiberguide Industries, Stirling, NJ) to emit 60 μJ/pulse. Fluorescence from the tissue was recovered using the same optical fiber and transmitted to a polychromator which spectrally disperses the wavelengths onto an intensified 1024-diode array controlled by an optical multichannel analyzer (OMA III, Princeton Applied Research, Princeton, NJ) for recording fluorescence intensities at wavelengths between 300 and 800 nm. Day-to-day variation in laser energy and fiber coupling efficiency was corrected by measuring the fluorescence from a cuvette of a stable dye (DCM, Exciton Inc, Dayton, OH) of

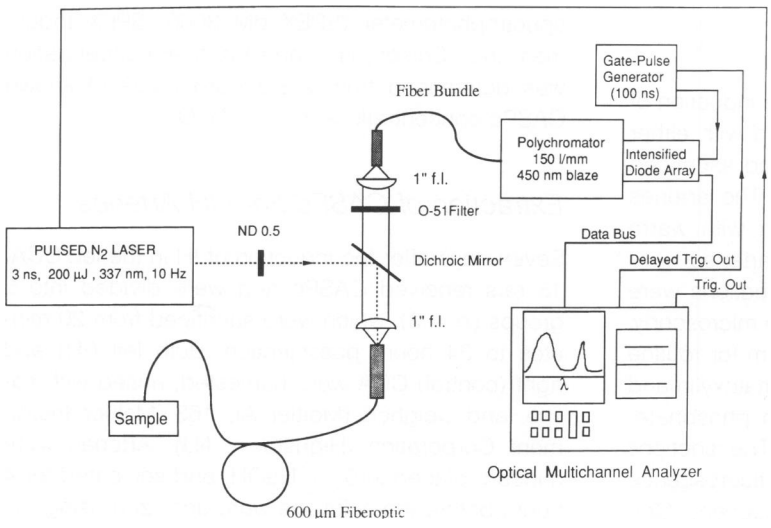

Figure 1. *Schematic of the laser-induced fluoresence apparatus. The pulsed nitrogen laser is triggered by the optical multichannel analyzer. The 337-nm light is passed through a neutral density filter and reflected on a dichroic mirror to a 600-μm fiberoptic, which interrogates the sample. A fluorescence signal is received through the same fiberoptic and after passing through a filter, the fluorescence signal is placed through a polychrometer and dispersed onto an intensified diode array gated to the laser pulse. The optical multichannel analyzer then digitizes the wavelength-dependent intensity signal for analysis.*

known concentration. Although the optical multichannel analyzer recorded a complete spectrum with each laser pulse, fluorescence of CASPc was measured at 682 nm. Each measurement is an average of 50 laser pulses.

The left CCA from 18 rats were balloon-injured as already described and divided into three groups. Group A received CASPc soon after arterial instrumentation, group B 2 days later, during medial smooth muscle cell proliferation; and group C 7 days later, in the presence of IH.

In groups A–C, LIF measurements started 5 minutes after CASPc injection and continued at increasing time intervals up to 6 hours. In one other group of animals injected shortly after artery manipulation, LIF measurements were performed at a single time point: day 1, day 3, and day 8 after CASPc injection.

To perform LIF measurements, both left and right CCA were exposed in the anesthetized animals through a midline neck incision. The surgical field was kept clean of blood and body fluids by saline irrigation aided with an operating microscope. The distal end of the optical fiber of the LIF apparatus was gently placed in contact with the arteries during the measurements. Six measurements in each artery were obtained per time point, moving the fiber to another portion of the vessel after each measurement. Alternating measurements were taken from the left and right CCA. Each data point was an average of at least three different animals.

Since no differences were noted between measurements of arteries with normal blood flow and arteries which were clamped and cleared of blood by saine irrigation, examination of arteries was performed without manipulation.

Fluorescence Microscopy

Seven days after balloon injury and the induction of IH as described, animals were injected with either saline (control) or CASPc (5 mg/kg) and sacrificed at 20 minutes, 3 hours, or 24 hours. The arteries were flushed with saline and injected with warm OCT at physiological pressures. The arteries were frozen and stored at −70 C. Frozen sections were cut at 10-μm thickness for fluorescence microscopy, and adjacent sections were cut at 6 μm for routine light microscopy after staining with hematoxylin and eosin. The arteries were covered with phosphate-buffered saline for index matching. The sections were examined with an epi-illumination fluorescence microscope (Axiophot, Zeiss, Oberkochen, Germany) equipped with a SIT video camera (DAGE-MTI SIT 66, DAGE-MTI, Inc., Michigan City, IN), image averager (Image-II, Nippon Avionics, Tokyo, Japan), video monitors, and a Macintosh II computer (Apple Computer, Cupertino, CA) with an A/D converter for digitization. The excitation filter was a 610–650-nm bandpass filter, and the emission filter was a long pass filter at 665 nm. The autogain circuit on the SIT camera was disabled so that the fluorescence intensity of different frames could be qualitatively compared.

Determination of CASPc Serum Concentration

To determine the serum pharmacokinetics of CASPc, blood (0.3 ml) was drawn from femoral vein punctures at time intervals between 5 minutes and 24 hours postinjection after IV CASPc administration. Blood samples were spun in an ultracentrifuge (20 minutes at 400 rpm; Eppendorf Centrifuge 5415, Eppendorf Geratebau GmbH, Hamburg, Germany), and the serum was stored at −70 C for subsequent analysis.

A spectrofluorimetric method was used for determining serum CASPc concentration. Serum was diluted 1:100 in 0.1 N NaOH. The excitation wavelength of the spectrophotometer (Spex 1680 0.22 m double spectrometer, SPEX Industries) was set at 610 nm, and the fluorescence was scanned from 640 to 720 nm, at 2-nm increments with a 0.5-second integration time. For each animal, the fluorescence signal of a serum sample drawn before CASPc injection was considered as background and was automatically subtracted from each reading. The peak area from 640 to 720 nm was calculated using the computer software connected to the spectrophotometer (SPEX dM 3000, SPEX Industries, Inc., Edison, NJ), and the final concentration was determined from a standard curve of known CASPc concentrations in 0.1 N NaOH.

Extraction of CASPc from IH Arteries

Seven days after the induction of IH in the left CCA, 15 rats received CASPc and were divided into 5 groups ($n = 3$), which were sacrificed from 20 minutes to 24 hours postinjection. Both left (IH) and right (control) CCA were harvested, rinsed with saline, and weighed (Mettler AE 163, Mettler Instrument Corporation, Highstown, NJ). Arteries were minced, placed in 0.1 N NaOH, and sonicated for 4 hours before the solutions were analyzed using the

described spectrofluorimetric technique and reported as ng/mg of tissue.

DNA Assay of Artery Segments

To improve the assessment of PS concentration in arteries with and without IH, DNA assays were performed on arterial segments to determine cellular content. Seven days after the surgical induction of IH corresponding to the time of CASPc artery extraction and LIF measurements, four rats were sacrificed, and the IH and control CCA were harvested and rinsed in saline. Artery lengths were measured, weighed, minced, and transferred into a homogenizer (Econo-grind homogenizer 613, Radnoti Glass Technology, Inc., Monrovia, CA) with 1 ml of digesting buffer (25 mmol/L EDTA, 10 mmol/L Tris-HCl, 100 mmol/L NaCl, 20 µl/ml Proteinase K), and incubated at 38 C. After 6 hours of incubation with periodic agitation of the samples, the tissue was almost indiscernible. Sonication was performed for 30 seconds before centrifuging the samples at 14×10^3 rpm for 5 minutes (Eppendorf Centrifuge 5415). An aliquot of the supernatant (350 µl) was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) and centrifuged at 14×10^3 rpm for 5 min.

The DNA was detected by a microfluorimetric determination using Hoechst 33258 compound.[11] A stock solution of Hoechst 33258 was prepared by dissolving 2 mg in 25 ml of distilled water, from which a 1% working solution was made in buffer (0.1 mol/L $NaPO_4$, 2 mol/L NaCl, 2×10^{-3} mol/L EDTA). A 10% solution of the aqueous tissue extract in the Hoechst working solution was used for fluorescence measurements. The excitation wavelength was set at 356 nm, and the fluorescence from the solution was scanned from 430 to 520 nm, at 2-nm increments with a 0.5-second integration time. A blank measurement, consisting of DNA-free digesting buffer plus Hoechst working solution was automatically subtracted from each measurement. The actual DNA concentration was determined from a standard curve.

Statistical Analysis

Statistical analysis between LIF measurements, CASPc, and DNA extractions of arterial tissue were compared between control and instrumented arteries using a Student's *t*-test. The pharmacokinetics of serum CASPc concentration was analyzed by minimizing the residual square error between data points and the curve generated from an exponential function, the validity of which is expressed by a correlation coefficient (r^2).

Results

Surgical Induction of IH

The efficacy of the arterial catheter instrumentation to promote IH growth was verified by histological analysis of perfusion-fixed CCA. Light microscopy of cross-sections at day 2 showed an absence of endothelium, increased numbers of smooth muscle cells in the media, and no IH, but at day 7, a 5–7-cell-thick IH was consistently present (Figure 2), which is known to progress to 15–20 cell layers by day 14.

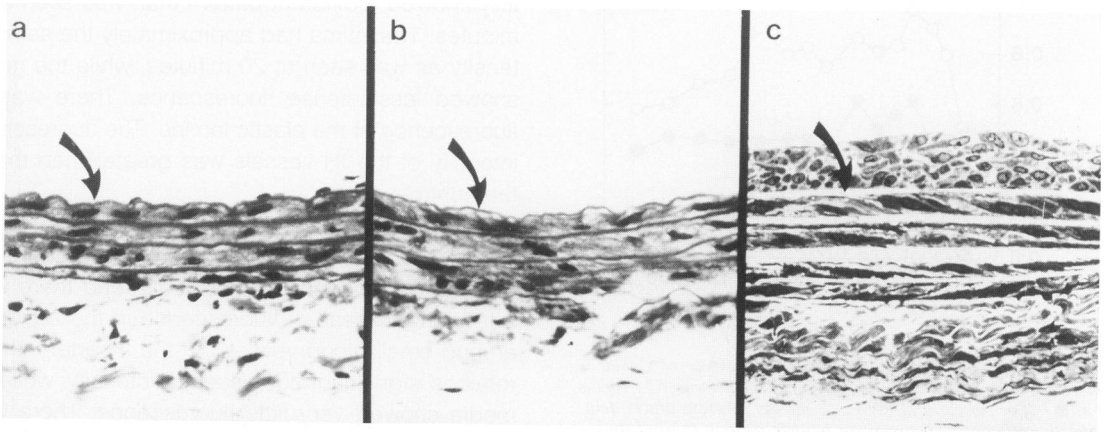

Figure 2. *Light photomicrographs depicting cross-sections of the control* (a) *rat common carotid artery, 2 days after balloon injury* (b) *and 7 days after balloon injury* (c). *Arrows point to the internal elastic lamina. In* a *there is endothelium which is absent at day 2* (b). *There is early proliferation and medial thickening at day 2* (b) *but no intimal hyperplasia, which becomes several layers thick by day 7* (c). *The internal elastic laminaes are aligned for comparison.* a,b, *hematoxylin and eosin;* c, *toluidine blue. Magnification, ×200.*

Laser-Induced Fluorescence Measurements

For each group, fluorescence intensities were converted to relative fluorescence by dividing each value by the highest value in each group, and comparison was performed between the instrumented and the control arteries. In group A (Figure 3a), in which CASPc was injected soon after CCA instrumentation, followed by LIF measurements over 6 hours, a significant difference ($P < 0.05$) in the fluorescence intensities between left and right CCA was only present up to 45 minutes postinjection. Additionally, the maximum peak of fluorescence intensities was reached earlier in instrumented CCA ($t = 5$ minutes) compared to control CCA ($t = 90$ minutes). The LIF measurements in animals followed at 1, 3, and 8 days later demonstrated low signals with no significant differences between the LIF of the instrumented and that of control CCA.

The LIF measurements from group B (Figure 3b), in which CASPc was injected 2 days after CCA instrumentation, were similar to the results from group A: significant differences ($P < 0.05$) in the fluorescence intensities between the instrumented (proliferating but not IH) and control CCA were present only in the first 60 minutes postinjection.

Animals from group C (Figure 3c), in which CASPc was injected 7 days after CCA instrumentation, LIF measurements demonstrated a significant difference ($P < 0.05$) in the fluorescence intensities between instrumented (IH) and control CCA, throughout the 6 hours of the LIF measurements. This was at a time in which there is a 5–7-cell-thick IH present in the manipulated arteries.

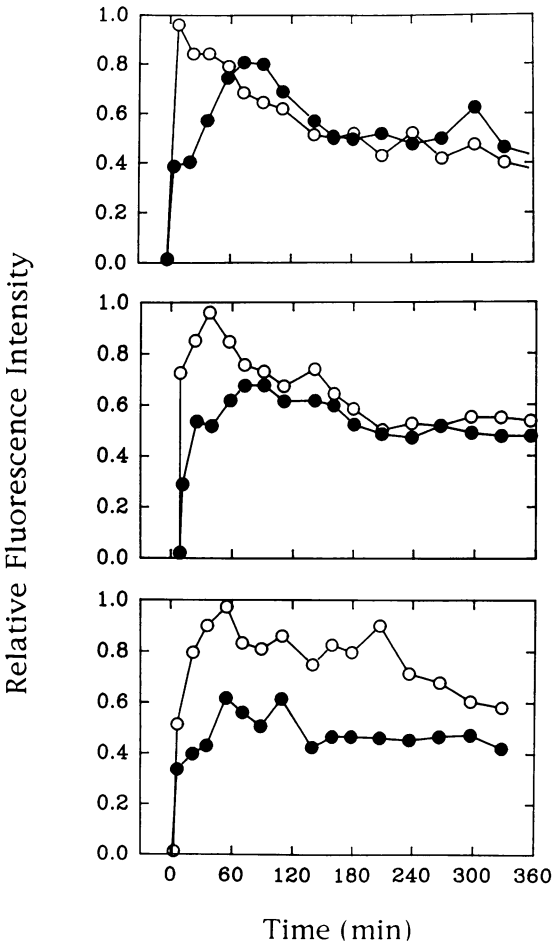

Figure 3. *Relative laser-induced fluorescence intensity of CASPc is plotted versus time in minutes for instrumented arteries (○) versus control (●) after injection of CASPc (5 mg/kg). The* top graph *demonstrates the differences between an acutely balloon-injured and a control artery; the* middle graph *demonstrates the differences between a 2-day-old balloon-injured and a control artery; the* bottom graph *demonstrates the differences between a 7-day-old balloon-injured artery with intimal hyperplastia and a control artery.*

Fluorescence Microscopy

There was no detectable fluorescence in animals not injected with CASPc. Figure 4 summarizes the findings: 20 minutes after injection of CASPc, fluorescence could be detected in the entire thickness of the artery wall. For both control and IH artery, the highest intensity was seen in the adventitia in the area of small blood vessels. There was an even distribution of fluorescence in the intima and media, with no fluorescence of the elastic lamina. The intensity of the fluorescence of the media was greater in the IH artery as compared to normal, whereas the fluorescence of the adventitia was approximately the same in both groups.

Three hours after injection of CASPc, the control artery showed less intense fluorescence but with a pattern similar to that seen at 20 minutes. The IH artery showed a different pattern than was seen at 20 minutes. The intima had approximately the same intensity as was seen at 20 minutes, while the media showed less intense fluorescence. There was no fluorescence of the elastic lamina. The fluorescence intensity of the IH vessels was greater than that of the control vessels.

At 24 hours after CASPc injection there was almost no fluorescence in the control artery, there was minimal fluorescence in the media, and there were a few bright areas of fluorescence in the adventitia around small blood vessels. In the IH artery, the IH retained significant fluorescence intensity, while the media showed very little fluorescence. There were some few areas of increased fluorescence in the adventitia, but this was diminished compared to the fluorescence at 3 hours.

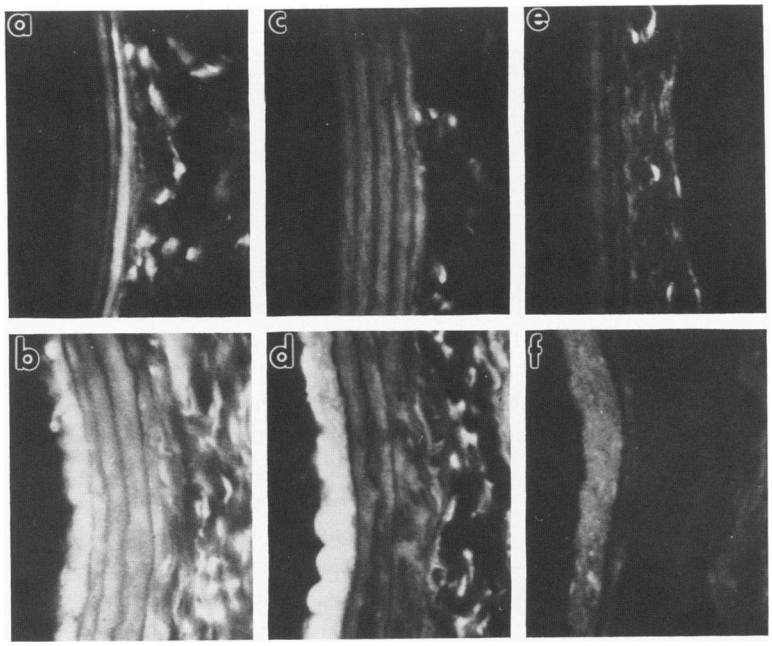

Figure 4. *Fluorescent micrographs of normal and intimal hyperplasia (IH), 7 days after balloon injury, rat carotid arteries at different time points after intravenous injection of 5 mg/kg CASPc. Excitation of unstained frozen artery cross-sections of 10 μm thickness was performed at 610–650 nm with a fluorescence capture over 655 nm. The lumen orientation is to the letter in each panel.* **a,c,e,** *normal arteries 20 minutes, 3 hours, and 24 hours after CASPc admininstration. Note scant medial fluorescence signal which elutes over time; however, there is a persistence of a few bright areas in the adventitia.* **b,d,f,** *injured arteries with IH 20 minutes, 3 hours, and 24 hours after CASPc administration. Note the early high-fluorescence signal in the IH tissue and media, the decrease in the signal in the media over time, and the persistence in the IH tissue. Magnification, ×400.*

Serum Concentration of CASPc

The pharmacokinetics of serum concentration of CASPc, measured starting at 5 minutes after intravenous injection, followed a single exponential decay (Figure 5) with a regression fit $r^2 = 0.993$. The serum half-life of CASPc was approximately 300 minutes.

Extraction of CASPc from Arteries

CASPc was extracted from IH and control arteries from 15 rats, at five different time points (Table 1). Peak tissue concentration of CASPc was present in each artery at 20 minutes, with wide variability and no significant differences between them. However, by 1 hour the variability decreased considerably,

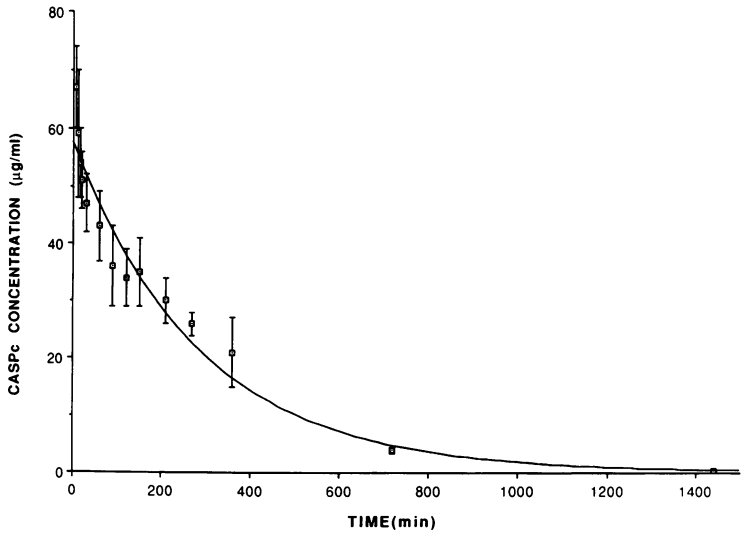

Figure 5. *Plot of chloroaluminum sulfonated phthalocyanine (CASPc) concentation (μg/ml) in rat sera versus time in minutes after intravenous injection of CASPc 5 mg/kg. The bars denote SDs. The exponential decay fit has an* r^2 *of 0.993.*

Table 1. *Chloroaluminum Sulfonated Phthalocyanine Extraction of Rat Carotid Arteries*

	20 minutes	1 hour	6 hours	12 hours	24 hours
Intimal hyperplasia (ng/mg tissue)	7.3 ± 2.4	5.1 ± 0.4*	4.9 ± 0.5*	4.9 ± 0.6†	3.6 ± 0.9†
Control (ng/mg tissue)	5.7 ± 1.6	3.2 ± 0.4	2.9 ± 0.2	1.3 ± 0.6	0.9 ± 0.3

* $P < 0.05$ imtimal hyperplasia versus control.
† $P < 0.01$ intimal hyperplasia versus control.

and the CASPc concentration in the IH CCA remained significantly higher than that of controls through 24 hours ($P < 0.01$). At 24 hours the retention of CASPc in the IH compared to the control CCA was 4 times higher than at 1 hour, and the rate of CASPc elution from the arteries was twice as high in the control arteries as in the IH.

Extraction of DNA from CCA

For the same length of artery, the DNA in the control CCA was approximately 65% of the IH CCA 7 days after ballooning. However, when analysis is made per unit weight of artery, there is no difference between the control and instrumented CCA: 2.95 ± 0.3 versus 3.03 ± 0.3 μg/mg of artery. This demonstrates that although there are more cells in the artery with induced IH, when taking into account the weight of the arterial segment, the cell numbers are equivalent.

Discussion

With the application of PDT for the therapeutic intervention of experimental IH, a better understanding of the uptake and retention of PS dyes is crucial for the development of a therapeutic strategy. In this study we have utilized a noninvasive method of laser-induced fluorescence along with tissue extraction and fluorescence microscopy to better understand the accumulation of the PS CASPc in normal versus IH arteries.

The serum concentrations of the dye followed first-order kinetic distribution, with an exponential decline over the first 24-hour period. Clearance from the blood is not very rapid, which substantiates previous data indicating that CASPc is rapidly bound to proteins, which would explain its endocytosis uptake and localization to the lysosomes.[12]

As noted by the LIF data, early tissue concentrations in arteries appear to be affected by the presence or absence of endothelium, which appears to slow the accumulation of CASPc and thus its fluorescence (Figure 3). However, because of sustained serum concentrations, this barrier appears to be of temporary importance for only the first 45–90 minutes postinjection in CCA without IH. After this period of time, there is equilibration between control and instrumented CCA.

At 7 days after balloon injury, in the presence of IH, LIF does demonstrate an elevated and persistent concentration of PS dye in the instrumented versus the control artery. In fact, there is only a 65% concentration of PS in the control artery versus the instrumented artery with IH. This LIF data compare remarkably well with the data obtained by the tissue extraction, which demonstrate a persistent elevated PS concentration in the IH artery versus the control over 24 hours. The control artery contains approximately 61% of the dye concentration compared to the artery with intimal hyperplasia.

Because of the greater number of cells present per unit length in the arteries with intimal hyperplasia compared to the control, the DNA analysis was undertaken to ensure that the differences in these measurements were not related purely to the number of cells in the arterial wall and were found to be similar to a previous report.[13] Although the weight per unit length of the artery was different in the intimal hyperplasia arteries compared to the control arteries, per mg of wet tissue the DNA concentration was equivalent at 7 days. Therefore, the approximately 60% difference in DNA content between the control artery and the IH artery is negated when the actual weight of the artery is taken into account. Thus, one would expect that besides the greater cellularity present in IH, there is still a much greater accumulation of CASPc by the proliferating cells in the neointima, as confirmed by the tissue extraction, LIF, and fluorescence microscopy data. These data agree with *in vitro* data, which demonstated that there was a higher accumulation of PS by highly proliferating cells.[14,15] Cultured human smooth muscle cells from stenosing lesions have also been found to be more sensitive to hematoporphyrin derivative-based PDT compared to smooth muscle cells from normal arteries.[16] These *in vitro* data support our study and indicate that PDT could be

exploited as a valuable therapeutic approach *in vivo* for a selective treatment of restenosis resulting from IH.

These data demonstrate increased uptake and retention of the PS CASPc in this experimental model of IH as compared to control arteries. Although the difference between normal arterial wall and arterial wall with intimal hyperplasia is 60% in the first 24 hours, there is special partitioning of the drug, primarily into the IH tissues as confirmed by fluorescence microscopy. Although further experiments are needed, this has important temporal significance for any therapeutic strategy that may be developed to improve PDT targeting of IH. With the primary late localization of the PS in the luminal location of IH tissue with little localization in the media, this bodes well for intraluminal light irradiation. Since the necessary components for cell cytotoxicity include light, oxygen, and PS concentrations, appropriate light dosimetry and spatial delivery could be determined at a late time point after CASPc administration to selectively remove the IH tissue and cause little to no cytotoxic effect on the media and adventitia of the irradiated artery. Other options such as receptor-based targeting of specific cellular populations within the IH can also be exploited to better identify their role in the biology of IH development and eventually in a clinical therapeutic strategy. Therefore, IH, a proliferating, nonneoplastic tissue, also demonstrates increased uptake and retention of PS that should provide it with a therapeutic window that would make it favorable for selective treatment of IH with PDT.

Acknowledgments

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References

1. Rosenthal I: Phthalocyanines as photodynamic sensitizers. Photochem Photobiol 1991, 53:859–870
2. Mosley ST, Goldstein JL, Brown MS, Falck JR, Anderson RGW: Targeted killing of cultured cells by receptor-dependent photosensitization. Proc Natl Acad Sci USA 1981, 78:5717–5712
3. Roberts WG, Klein MK, Loomis M, Welds S, Berns MW: Photodynamic therapy of spontaneous cancers in felines, canines, and snakes with chloro-aluminum sulfonated phthalocyanine. J Natl Cancer Inst 1991, 83:18–23
4. Roberts WG, Hasan T: Role of neovasculature and vascular permeability on the tumor retention of photodynamic agents. Cancer Res 1992, 52:924–930
5. Moan J, Steen HB, Feren K, Christiansen T: Uptake of hematoporphyrin derivative and sensitized photoinactivation of C3H cells with different oncogenic potential. Cancer Lett 1981, 14:291–296
6. West CML, West DC, Kumar S, Moore JVA: Comparison of the sensitivity to photodynamic treatment of endothelial and tumour cells in different proliferative states. Int J Radiat Biol 1990, 58:145–156
7. Ip JH, Fuster V, Badimon L, Badimon J, Taubman MB: Syndromes of accelerated atherosclerosis: Role of vascular injury and smooth muscle cell proliferation. J Am Coll Cardiol 1990, 15:1667–1687
8. Clowes AW, Reidy MA, Clowes MM: Mechanism of stenosis after arterial injury. Lab Invest 1983, 49:208–215
9. Ortu P, LaMuraglia GM, Roberts WG, Flotte TJ, Hasan T: Photodynamic therapy of arteries: A novel approach for the treatment of experimental intimal hyperplasia. Circulation 1992, 85:1189–1196
10. Schomacker KT, Frisoli JK, Deutsch TF. Medical applications of laser-induced fluorescence. Proc Society Photo-optical Instrumentation Engineers 1992, 1599: 296–308
11. Cesarone CF, Bolognesi C, Santi L: Improved microfluorimetric DNA determination in biological material using 33258 Hoechst. Anal Biochem 1979, 100:188–197
12. Roberts WG, Berns MW: *In vitro* photosensitization. I. Cellular uptake and subcellular localizations of mono-L-aspartyl chlorin e_6, chloroaluminum sulfonated phthalocyanine and photofrin II. Lasers Surg Med 1989, 9:90–101
13. Kocher O, Gabbiani F, Gabbiani G, Reidy MA, Cokay MS, Peters H, Hüttner I: Phenotypic features of smooth muscle cells during the evolution of experimental carotid artery intimal thickening. Biochem Morphol Stud Lab Invest 1991, 65:459–470
14. Figge FHJ, Weiland GS, Morganiello LOJ: Cancer detection and therapy: Affinity of neoplastic, embryonic, and traumatized tissues for porphyrins and metalloporphyrins. Proc Soc Exp Biol Med 1948, 68:640–641
15. Gomer CJ, Rucker N, Murphee AL: Differential cell photosensitivity following porphyrin photodynamic therapy. Cancer Res 1988, 48:4539–4542
16. Dartsch PC, Ischinger T, Betz E: Response of cultured smooth muscle cells from human nonatherosclerotic ateries and primary stenosing lesions after photoirradiation: Implications for photodynamic therapy of vascular stenoses. J Am Coll Cardiol 1990, 15:1545–1550