Effect of Photodynamic Therapy in Intimal Hyperplasia by Phthalocyanine Conjugated to the Scavenger-receptor Ligand, Maleylated Bovine Serum Albumin

Shigeki Ito, M.D., Tsuneyuki Nagae, M.D., Shin Ishimaru, M.D., Sara Chau*., Triet Dang*., Leslie Anne. Sabiniano*., Mizuho Zempo*., Mark C. Booth, B.S*., Lih-Huei L. Liaw, M.Sc*., Tatiana B. Krasieva, Ph.D*., Bruce J. Tromberg, Ph.D*., Johan E. van Lier, Ph.D#., Michael W. Berns, Ph.D*., Samuel E. Wilson, M.D*.

Department of Surgery, Tokyo Medical College, Tokyo, Japan,

*Beckman Laser Institute and Medical Clinic, Department of Surgery, University of California Irvine, CA.

*MRC Group in the Radiation Sciences, Faculty of Medicine, University of Sherbrooke, Sherbrook, Canada.

Correspondence to Shigeki Ito, M.D. (c/o Samuel E. Wilson, M.D.)

Department of Surgery (Building 53, Room 208)

101 The City Drive South Orange, California 92668

Phone: (714)-456-7246

Fax: (714)-456-8205

Key Word: Fluorescence, drug delivery, scavenger receptor, macrophage, photodynamic therapy, intimal hyperplasia.

SPIE Vol. 3033 • 0277-786X/97/$10.00

ABSTRACT

Purpose: Intimal hyperplasia (IH) is major cause of restenosis after vascular interventions for arterial occlusive disease. We reported that a fluorescenct probe, Texas Red, conjugated to the scavenger-receptor ligand, maleylated bovine serum albumin (mal-BSA) accumulated almost exclusively in the injured, hyperplastic sites[1]. The purpose of this study is to test the feasibility of enhanced drug delivery to the hyperplastic lesion by targeting the scavenger-receptors (mal-BSA-Phthalocyanine).

Methods: The abdominal aorta of Sprague-Dawley rat was injured by pulling an inflated balloon catheter. Photosensitizers (mal-BSA-Phthalocyanine, free Phthalocyanine) were injected 2 weeks after surgery. Four hours following photosensitizers injection, (1) abdominal aorta retrieved and frozen tissue sections were examined for arterial layer drug distribution using fluorescence microscopy. (2) photodynamic therapy (PDT) was performed on abdominal artery by using argon dye laser and 2 weeks after PDT, pathological analysis of the arteries were performed by measuring the ratio of IH thickness (IH thickness / Media thickness).

Results: Mal-BSA-Pc fluorescence from fully-developed neointimal tissue (1446.4 a.u.) is higher than abdominal media (1063.4 a.u.) and adventitia (680.0 a.u.). In contrast, Pc dose not selectively accumulate in the intimal hyperplastic lesion, eg; intimal hyperplastic lesion (597.5 a.u.), media (872.7 a.u.), adventitia (847.5 a.u.). Mal-BSA-Pc ratio of IH thickness is significantly lower (43.1 $\pm$ 2.2%) than Pc (59.9 $\pm$ 3.8%), Laser irradiation only (76.6 $\pm$ 4.1%) and control (105.6 $\pm$ 5.9 %) ($p<0.001$).

Conclusions: We conclude that molecules can selectively delivered to intimal hyperplasia via a receptor-mediated process.

INTRODUCTION

Intimal hyperplasia (IH) is a major complication after vascular interventions and bypass operation[2]. Monocytes adhere at the subendothelium several hours after arterial injury and smooth muscle cells (SMCs) in media are stimulated[3]. Activated SMCs change the phenotype from contractile to synthetic form[4,5]. Then SMCs migrate and proliferate in the intima to produce IH[4,5]. Macrophages and SMCs are a major contributing factor in the pathogenesis of IH[6].

Experimental and clinical studies have reported the possibility of inhibiting the development of IH using drugs such as anti-platelet, anti-coagulation, anti-hypertension and anti-inflammation. Although they have been not completely clinical effective in preventing or reducing the IH[7,8].

In the recent studies, photodynamic therapy (PDT) has been reported as a new effective treatment to inhibit IH[9,10]. PDT has the possibility to destroy any tissue which contains the proper combination of photosensitizer and laser light. Therefore PDT needs the photosensitizer which has the high accumulation in IH.

We have recently investigated that the feasibility of enhanced drug delivery to a intimal hyperplastic resion via targeting scavenger receptors located on macrophages and activated SMCs.These receptors have a high affinity for negatively charged molecules such as acetylated-LDL, oxidized-LDL, malondialdehyde-LDL, and maleylated albumin[11-14].

Maleylated bovine serum albumin (mal-BSA) has been reported to be utilized as a drug carrier for targeting scavenger-receptor-bearing neoplastic cells[15]. In the study we utilized the rat balloon injury model to evaluate the *in vivo* selectively of mal-BSA towards IH. Recently we have reported that a fluorescent probe, Texas Red (TR), conjugated to the mal-BSA selectively localized to the IH after 2 weeks of balloon injury and 4 hours after the introduction of fluorescence mal-BSA-Texas Red[1].

We selected a chloroaluminum-sulfonated phthalocyanine (Pc) as a photosensitizer, since it has been reported that Pc is the most effective photosensitizer at the intimal hyperplasic lesion[9]. Pc excitation spectrum has a peak at 675 nm, a wavelength at which light has high

tissue penetration[16]. In this study, we attempted to exploit this endocytic pathway by using Pc conjugated to mal-BSA and free Pc. Firstly we utilized the rat balloon injury model to evaluate the in *vivo* the selectivity of fluorophore uptake at intimal hyperplasia by using these drugs. Secondly these drugs-mediated PDT was investigated as a method for the treatment of IH in the rat abdominal artery model.

MATERIAL & METHODS

Model of Intimal Hyperplasia:

Male Sprague-Dawley rats (Charles River Lab., Wilmington, MA.) weighing 350-450 gm were anesthetized with an intramuscular injection 8 mg/kg xylasin (AnaSed)(Lloyd Lab, Shenandoah, Iowa) and 60 mg/kg ketamine (Ketaset)(Aveco Co. Fort Dodge, Iowa) and maintained with isoflurane (AErrance)(Anaquest, Madison, Wisconsin). The right iliac artery was exposed. A 2-F Fogarty catheter (Baxter Edwards Healthcare Corp., Irvine, CA) was introduced from right iliac artery and advanced superiority in the abdominal aorta. With the balloon inflated by injecting saline, the catheter was pulled through the abdominal aorta to produce endothelial denudation and stretch the media three times at a pressure of 300 mmHg. After injury, the catheter was removed and arteriotomy site was closed by continuous suture with 7-0 prolene (Ethicon Inc., Somerville, New Jersey). The rats were fasted 12 hours before the surgery. Only solid food was withheld. They had free access to water. After operation the rats were allowed to recover, after which they had free access to standard rat chow and water.

Animal care complied with the " Principles of Laboratory Animal Care " as formulated by the National Society for Medical Research and the " Guide for the Care and Use of Laboratory Animals " issued by the National Institutes of Health (U.S. Department of Health and Human Services, NIH Publication No. 80 - 23, revised 1985).

Maleylation of BSA-Phthalocyanine Conjugates:

Bovine serum albumin (BSA) was modified via a maleylation process in order to allow recognition by the scavenger receptor (binding affinity Ka ~ 10^{15} M^{-1} for mal-BSA). BSA-Phthalocyanine (BSA-Phthalocyanine, approximately 12 molecules of the photosensitizer Phthalocyanine per BSA, produced by Johan E. van Lier, Ph.D., MRC Group in the Radiation Sciences, Faculty of Medicine, University of Sherbrooke, Sherbrook, Canada) was allowed to react at concentrations of 5 - 10 mg/ml with 0.03 - 0.10 M excess amount of maleic anhydride (Sigma Chemicals, St. Louis, MO) at pH 8.5. The method of maleylation of BSA is described in detail by Butler and Hartley[17].

Fluorescence studies:

In this study, we compared the selectivity of fluorophore uptake in abdominal aorta (injured) for two kinds of the photosensitizers: (I) mal-BSA-Pc, (II) free Pc (produced by Johan E. van Lier, Ph.D., MRC Group in the Radiation Sciences, Faculty of Medicine, University of Sherbrooke, Sherbrook, CanadaPorphyrin Products, Inc. Logan, UT). Concentration of mal-BSA-Pc in phosphate-buffered solution (PBS) was determined by BCA assay (Pierce, Rockford, IL). Mal-BSA-Pc in PBS was injected intravenously via tail vein at 12 mg/kg concentration. In order to ensure the same dose of concentration of free Phthalocyanine was calculated below:

Free Pc conc = mal-BSA-Pc conc / MW BSA X Number of Pc / BSA X MW Pc

Final conc of Free Pc for each Rat =

Free Pc conc X (12 mg/kg X weight of Rat gm / mal-BSA-Pc conc)

conc : concentration (mg/ml)

MW BSA : 110,000 g/mol

Number of Pc / BSA : 12

MW Pc : 983 g/mol

These photosensitizers were injected at two weeks after balloon injury in dark room.

Three were injected PBS as control, three were injected mal-BSA-Pc, three were injected Free Pc. Four hours after drug administration in the darkroom, the animals were sacrificed upon retrieval of abdominal aortas. The arteries were embedded in Cryomolds filled with Tissue-Tek OCT compound 4583 (Miles Inc., Elkhart, IN) and stored at -70°C in the dark. Six micron-thick cross sections were cut from the frozen specimens in low diffuse light (Cryostat microtome, AO Reichert, Buffalo, N.Y.).

Imaging system:

The distribution and intensity of compounds (mal-BSA-Pc, Free Pc) in frozen sections were investigated using a fluorescence microscopy-digital imaging system. A slow-scan, thermoelectrically cooled CCD camera system (Princeton Instruments, model TE576/ST135, Trenton, NJ) coupled to Zeiss Axiovert 10 inverted fluorescence microscope (Carl Zeiss Inc., Germany) was used to examine arterial frozen sections. 10X objective (Zeiss Plan-neofluar, N.A.= 0.3 respectively) was used to visualize bright-field and fluorescence images. A 100W mercury are lamp coupled to a mechanical shutter (UniBlitz model T132, Vincent Associates, Rochester, NY) and filtered through a 660-680 nm, broad-bandpass filter provided excitation light. A dichroic mirror (Zeiss, FT 690) reflected the excitation light onto the samples and transmitted the fluorescence emission through a 700 nm broad-longpass filter onto the detector. Raw Fluorescence Images were normalized using the following algorithm in order to correct for non-uniform illumination and background noise:

Normalized Fluorescence Image=(Raw Fluorescence Image/Light Distribution Images)*

Mean Pixel Value of Light Distribution Image

Light Distribution Imags were acquired from blank slides with identical parameters (i.e. filters, exposure times) as the Raw Fluorescence Images. All images were corrected for signal contributed during the exposure time by subtracting dark noise obtained from the camera without source illumination. The mean pixel values of Light Distribution Images were calculated by averaging dark-noise-corrected Light Distribution Images over all pixels (

typically 2.2 x 10^5). Images acquisition, analysis and camera control were performed using a Macintosh computer with IPlab software (Signal Analytics Corp., Vienna, Virginia).

Normalized Fluorescence Images were analyzed by measuring the fluorescence intensity in the following structurally distinct tissue compartments: 1) the intimal hyperplasia, 2) the total media, 3) the adventitia. In each case the average fluorescence intensity per pixel over the entire tissue compartment was determined. Multiple fluorescence measurements (up to five sections) from individual animals were averaged and standard error (SE) calculated.

Photodynamic therapy in Intimal hyperplasia

These photosensitizers were injected at two weeks after balloon injury in dark room. Three were injected PBS as control, three were injected mal-BSA-Pc, three were injected free Pc. Four hours after drug administration in the darkroom, the abdominal aorta was surgically exposed in the anesthetized rat and optically isolated to avoid irradiation of surrounding tissue by gauzes. PDT was given to the abdominal aorta using an argon-pumped dye laser (model 920, Coherent, Palo Alto, Calif.) with wavelength 670 nm. The laser beam was delivered through a 400-μm quarts fiber optic (QSF 400 Quartz fiber) at a power of 100 mW/cm^2. Laser power was monitored from the output end of the optical fiber using a power meter (Coherent 210, Coherent) during procedure to deliver a uniform 2 cm diameter spot with an enagy density of 50 J/cm^2 at the surface of the abdominal aorta, 4 hours after drug injection . Four days after PDT, the rats were sacrificed by an overdose of pentobarbital. The aortas were then perfusion-fixed in situ with 1% glutaraldehyde and 4% formaldehyde in phosphate buffer solution, pH 7.4, at a hydrostatic pressure of 120 mmHg, for 5 minutes.

Histological analysis:

The adventitia of the irradiated segments of the abdominal aorta were stained with India ink for orientation of the direction of the laser beam and used pathological analysis. The abdominal aortic segments were rinsed in Ringer's lactate solution and cut three specimens under a dissecting microscope. The specimens were placed fresh fixative (3% glutaraldehyde in phosphate buffer) at 4°C for 24 hours. They were dehydrated and processed using a Fisher Histotech processor and embedded in paraffin. They were serially sectioned at 6 μm using an AO rotary microtome and stained with hematoxylin and eosin and examined under a light microscope. Photographs of light microscope images were taken (35mm Gold Super 200, Eastman Kodak Co. Rochester, NY). The pictures were scanned and displayed on the monitor screen by a scanner (Microtec International, Inc. Redondo Beach, CA) and Adobe Photoshop™2.0 (Adobe Systems, Inc. Mountain View, CA). Intimal and medial thicknesses were defined and calculated using a Macintosh computer with IPlab software (Signal Analytics Corp., Vienna, Virginia). The ratio of the thickness of intimal hyperplasia to media (IH thickness/Media thickness) was expressed as a percentage. Three sections were analyzed from each rat (9 sections per each group).

Statistical Evaluation:

Data are presented as the means ± standard error of the mean (SEM) with "n" equal to the number of animals studied. Comparisons were performed using one-way analysis of variance. Significant differences (*P* value less than 0.05) were detected by Fisher's least significant difference test.

RESULTS

Fluorescence studies:

Images of compounds (mal-BSA-Pc, Free Pc) in frozen sections were investigated using a fluorescence microscopy-digital imaging system. Intimal hyperplasia was fully formed two weeks after balloon injury (Figure 1a, Figure 1c). Fluorescence images were acquired under identical conditions and are shown normalized to the same scale. Intense fluorescence from the cross-sectional image indicates that mal-BSA-Pc appears to accumulate with great specificity in the intimal hyperplasia, compared to media or adventitia (Fig1b). The other side, free Pc fluorescence appears low accumulation in intimal hyperplasia, compared to media or adventitia (Fig1d).

The data of fluorescence intensity was summarized using digital image analysis. Mal-BSA-Pc fluorescence in intimal hyperplasia (1446.4 $\pm$ 441.7 a.u.) was higher than both media (1065.5 $\pm$ 85.5 a.u.) and adventitia (680.1 $\pm$ 52.7 a.u.), although there were no significant differences (Fig 2A). In contrast, Free Pc did not selectively accumulate in the intimal hyperplasic region, and fluorescence in media (872.7 $\pm$ 49.4 a.u.: $p<0.0001$) and adventitia (847.5 $\pm$ 44.7 a.u.: $p<0.003$) were significantly higher than that in intimal hyperplasia (597.5 $\pm$ 41.7 a.u.) (Fig 2B). We observed the better delivery of mal-BSA-Pc to intimal hyperplastic region than free Pc.

Photodynamic therapy :

Abdominal aortic sections which were injected mal-BSA-Pc had a loss of cells in the intimal hyperplastic region only. Smooth muscle cells (SMCs) and elastic lamina in the media remained and had no damages (Fig 3a). Abdominal aortic sections which were injected free Pc had a loss of cells in the intimal hyperplastic region and media. Also elastic lamina in the media had a damages (Fig 3b). Abdominal aortic sections which were given laser irradiation only had an increased number of cells in the intimal hyperplastic region and were not a loss of SMCs in the media (Fig 3c). Abdominal aortic sections from balloon-only as a control described the expanded intima by many cells (Fig 3d).

The ratio of the thickness of intimal hyperplasia to media was 43.1 $\pm$ 2.2 % (mal-BSA-Pc), 59.9 $\pm$ 3.8% (Free Pc), 76.6 $\pm$ 4.1% (Laser only) and 105.6 $^{+}$ 5.9% (control). Intimal hyperplasia was significantly inhibited by mal-BSA-Pc, compared to free Pc, laser only and control ($p<0.001$) (Fig 4).

DISCUSSION

Inimal hyperplasia often contributes to the long -term failure of many cardiovascular procedures used in clinical practice today. Such failures include re-stenosis of coronary arteries after balloon angioplasty or coronary artery bypass surgery; clotting of hemodialysis access fistulas; and re-occlusion of arteries after peripheral vascular procedures such as rotor atherectomy, balloon angioplasty, laser atherectomy, endovascular stenting, and surgical arterial bypass[2].

Macrophages and smooth muscle cells are a major contributing factor in the pathogenesis of intimal hyperplasia[6]. Monocytes, polymorphonuclear leukocytes and platelets adhere to the surface of the injured, de-endothelialized artery[18]. After monocytes, macrophages and platelets aggregate, they may release biologically active factors. Activation of smooth muscle cells from the contractile to the synthethic state occurs by monocyte / macrophage-derived growth factor (MDGF) and platelet derived growth factor (PDGF)[19,20]. The activity of synthetic smooth muscle cells migrate to the intima and proliferate, resulting in intimal hyperplasia. The scavenger-receptor mediated endocytic pathway has been localized to the macrophage in the intimal hyperplasia. We had reported that the scavenger receptor pathway of macrophages was exploited by conjugating a fluorophore agent to mal-BSA, a high affinity ligand in the scavenger receptor[1]. Photosensitizers were injected 2 weeks after surgery. Four hours following probe injection, abdominal aorta was retrieved. We had reported that after 2 weeks of surgery and 4 hours after the introduction of fluorescence mal-BSA-Texas Red (Texas Red: fluorescent probe), the drug accumulated almost exclusively at the injured, hyperplastic sites[1].

In this study, we selected phthalocyanine as photosensitizer and investigated the effect of it for intimal hyperplasia, since it has been reported that PDT was effective to prevent the intimal hyperplasia using phthalocyanine[9]. We attempted to exploit this endocytic pathway by using two different drug combination. One was mal-BSA-Pc. Another one is free Pc. The fluorescence of mal-BSA-Pc at the intimal hyperplasia was higher than at the media and the adventitia. Although it had no significant different. On the other hand, the fluorescence of free Pc was at the media and adventitia were significantly higher than at the intimal hyperplasia. After we confirmed the fluorescence sensitivity of mal-BSA-Pc at intimal hyperplasia, we investigated the effect of PDT at intmal hyperplasia. Several types of photosensitzer has been reported to prevent intimal hyperplasia[9,10]. Although there are no significant effect at IH. Four days after PDT, we found that PDT using mal-BSA-Pc reduced only cells at IH without damage of cells and elastic lamina at media. And IH was significantly inhibited by PDT using mal-BSA-Pc compared to PDT using free Pc, no drug and only balloon injury. Although PDT using free Pc reduce both cells of IH and media, further give a damage to elastic lamina at media. PDT of free Pc may cause fragility of the arterial wall and form the arterial anurythma.

Our result suggest that mal-BSA is taken up by recruited cells found in the developing intimal hyperplasia that are absent in noninjured arteries. Photosensitizer conjugated to mal-BSA ligand nay contribute to the application of photodynamic therapy for intimal hyperplasia without the risk of damage to surrounding tissue structures and cells compared to conventional arterial PDT. The drug delivery system *via* the scavenger receptor pathway may provide a basis for alternate treatment strategies.

REFERENCES

1. Nagae T, Tromberg BJ, Louie AY, et al: Targeted Drug Delivery for Intimal Hyperplasia, Proc. SPIE OE/LASE Los Angeles 1994; paper #2130-24

2. 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.

3. Haudenschild C, Studer A. Early interactions between blood cells and severely damaged rabbit aorta. Europ J Clin Invest 1971; 2: 1-7.

4. Hedine U, Thyberg J. Plasma fibronectin promotes modulation of arterial smooth muscle cells from contractile to synthetic phenotype. Differentiation 1987; 33: 239-245.

5. Hedine U, Bottger BA, Thyberg J, et al. A substrate of the cell-attachment sequence of fibronectin is sufficient to promote transition of arterial smooth muscle cells from a contractile to a synthetic phenotype. Dev Bio 1989; 133: 489-501.

6. Stadius ML, Rowan R, Fleischhauer JF, Kernoff R, Billingham M, Gown AM. Time course and cellular characteristics of the iliac artery response to acute balloon injury : An angiographic, morphometric, and immunocytochemical analysis in the cholesterol-fed New Zealand white rabbit. Arteriosclerosis and Thrombosis 1992; 12: 1267-1273.

7. Chervu A, Moore WS, Quinones-Baldrich WJ, Henderson T. Efficacy of corticosteroids in suppression of intimal hyperplasia. J Vasc Surg 1989; 10: 129-134.

8.Pan X-M, Nelken N, Colyvas N, Rapp JH. Inhibition of injury induced intimal hyperplasia by saralasin in rats. J Vasc Surg 1992; 15: 693-698.

9. Ortu P, LaMuraglia GM, Roberts G, Flotte TJ, Hasan T. Photodynamic therapy of arteries: A novel approach for treatment of experimental intimal hyperplasia. Circulation 1992; 85: 1189-1196.

10. Eton D, Colburn MD, Shim V, Panek W, Lee D, Moore WS, Ahn SS. Inhibition of intimal hyperplasia by photodynamic therapy using Photofrin. J Surg Res 1992; 53: 558-562.

11. Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: Implications for cholesterol deposition in atherosclerosis. Ann Review Biochem 1983; 52: 223-261.

12. Matsumoto A, Naito M, Itakura H, Ikemoto S, Asaoka H, Hayakawa I, Kanamori H, et al. Human macrophage scavenger receptors: Primary structures, expression, and localization in atherosclerotic lesions. Proc Natl Acad Sci, USA 1990; 87: 9133-9137.

13. Rohrer L. The Acetylated Low-Density Lipoprotein Receptor or Macrophage Scavenger Receptor. Atherosclerosis Reviews 1991; 23: 123-129.

14. Brown MS, Goldstein JL. Scavenging for receptors. Nature 1990; 343: 508-509.

15. Mukhopadhyay A, Mukhopadhyay B, Srivastava RK, Basu SK. Scavenger-receptor-mediated delivery of daunomycin elicits selective toxicity towards neoplastic cells of macrophage lineage. Biochem J 1992; 284: 237-241.

16. Parrish JA: New concept in therapeutic photomedicine: Photochemistry, optical targeting and the therapeutic window. J Inverst Dermatol 1981; 77: 45-50.

17. Butler PJG, Hartley BS. Maleylation of Amino Groups. Methods in Enz 1972; 25: 191-199.

18. Lucas JF, Makhoul RG, Cole CW, et al: Mononuclear cells adhere to sites of vascular balloon catheter injury. Curr. Surg. 1986; 43: 112-115.

19.Chervu A, Ahn SS, Moore WS: Current therapy of intimal hyperplasia. Endovascular Surgery (second edition): 571-589.

20. Ross R, Raines EW, Bowen-Pope DF: The biology of platelet derived growth factor. Cell 1986; 46: 155-169.

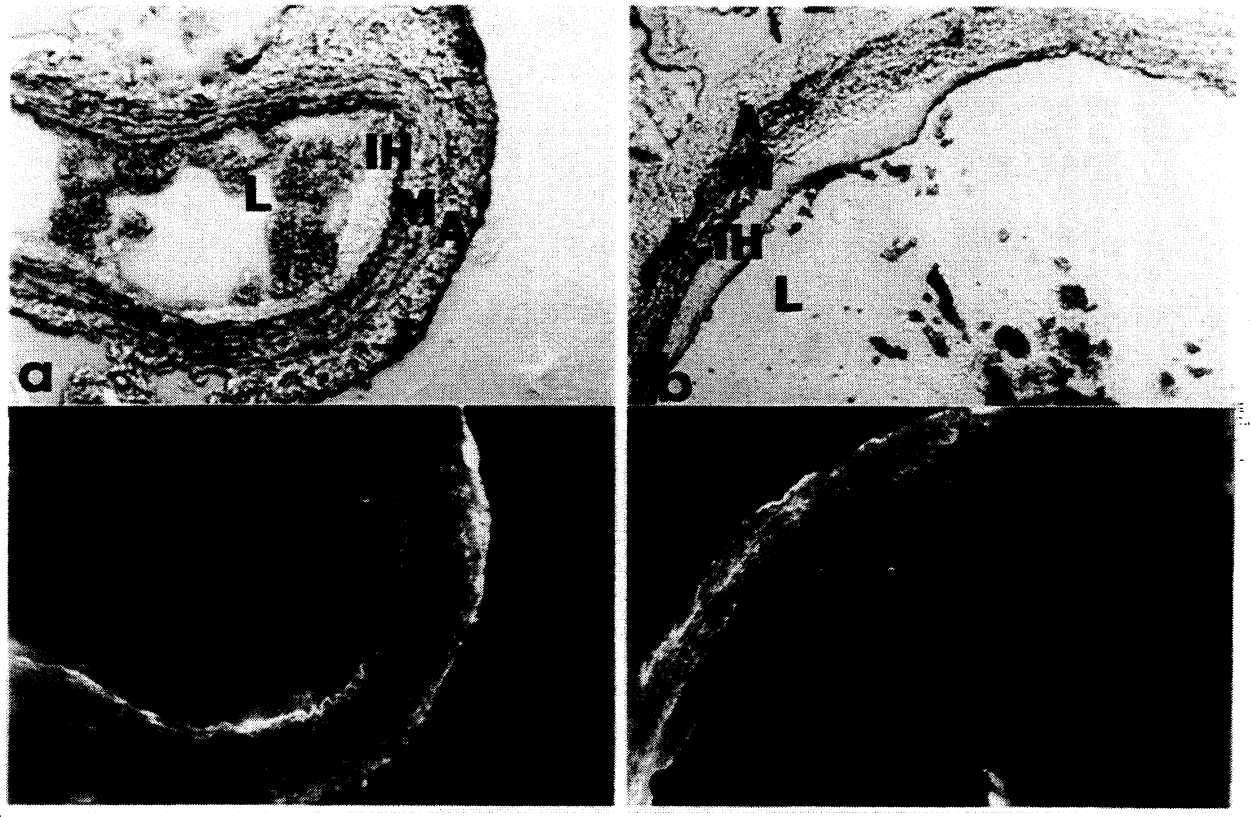
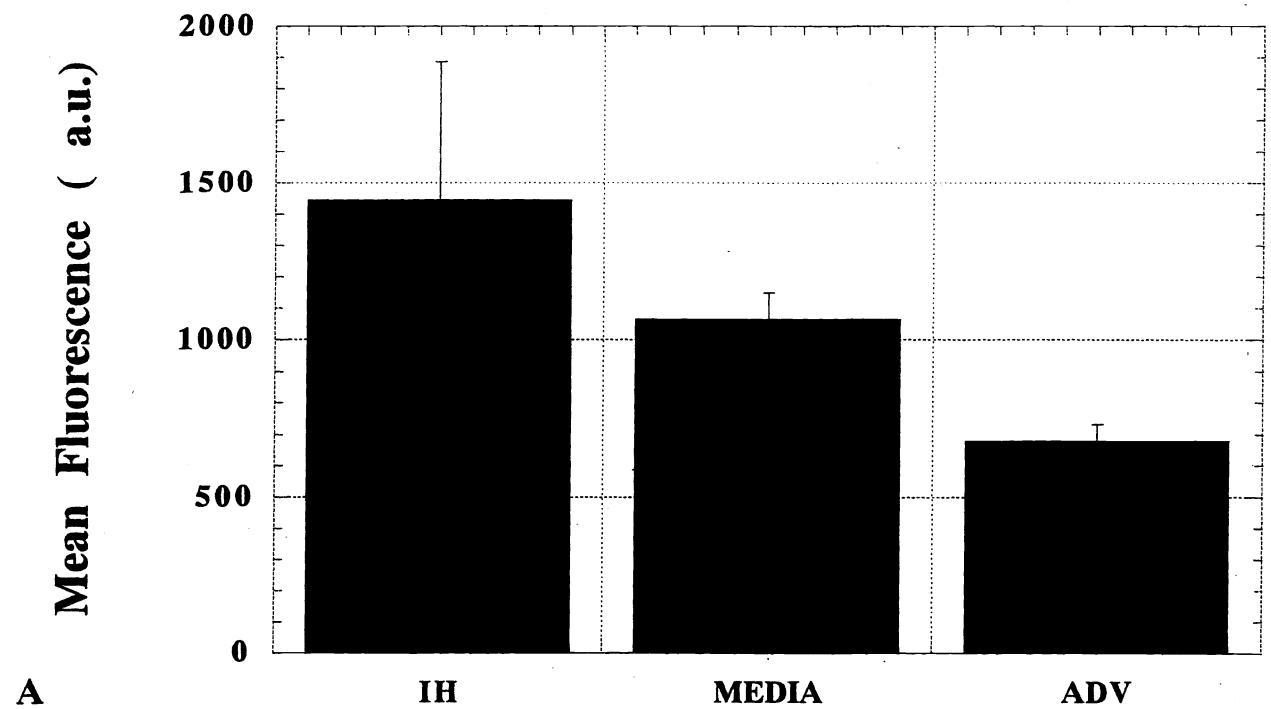
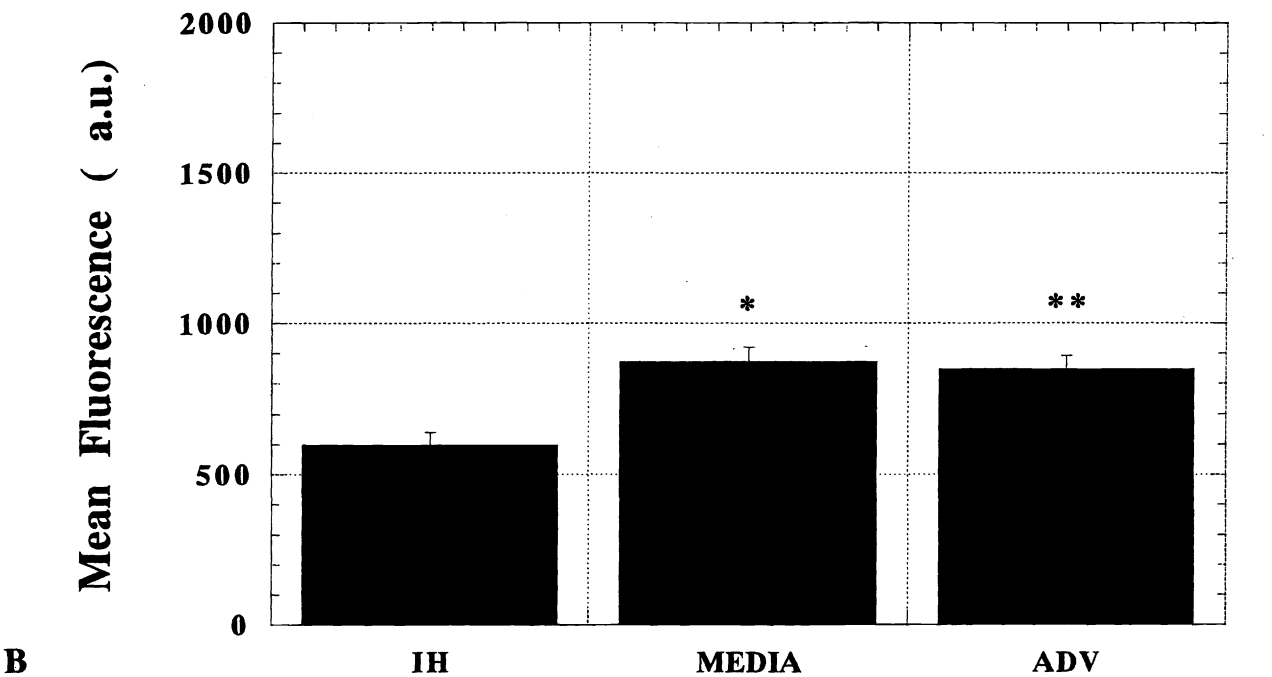


Figure 1. Cross-sectional images of injured (abdominal) arteries two weeks after balloon injury (10 x objective) showing: **a.** Bright field image of arterial section from animal receiving mal-BSA-Pc. **b.** Corresponding mal-BSA-Pc fluorescence image from panel a. **c.** Bright field image of arterial section from animal receiving free-Pc. **d.** Corresponding free-Pc fluorescence image from panel c. Adventitia(A), media (M), intimal hyperplasia (IH) and lumen(L) are labeled in panel a and c.



A



B

Figure 2. Distribution of fluorescence (arbitrary units, a.u.) in layers of injured abdominal arteries for animals receiving mal-BSA-Pc(A) and free-Pc (B).

* : $P<0.0001$ compared to IH. ** : $P<0.003$ compared to IH.

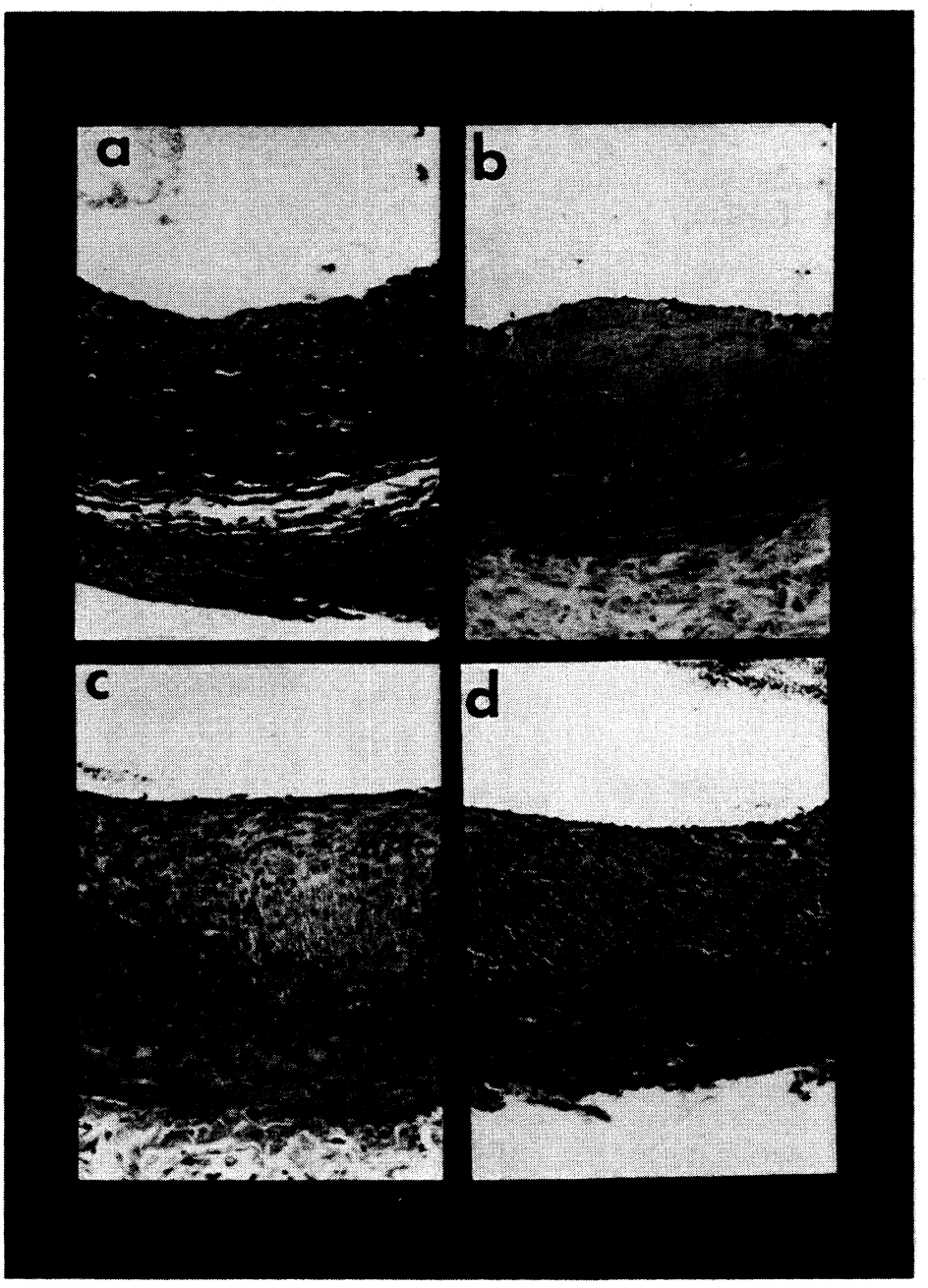


Figure 3. Light photomicrographs of histological cross sections of injured abdominal arteries of rats. **a:** Four days after PDT(mal-BSA-Pc). **b:** Four days after PDT(free-Pc). **c:** Four days after laser light only. **d:** Four days after no laser and no drugs as control. Arrows, internal elastic lamina. Magnification, X250.

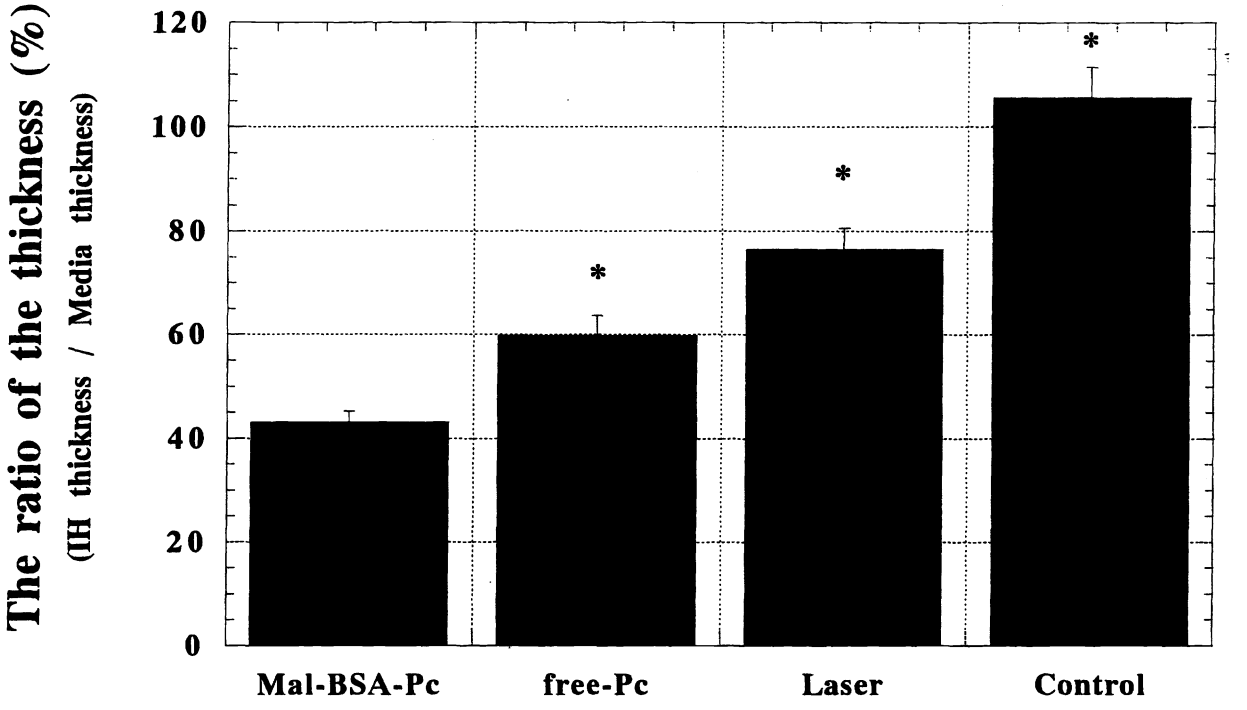


Figure 4. The ratio of the thickness (%) of intimal hyperplasia to media of abdominal injured arteries for animals receiving mal-BSA-Pc, free-Pc, laser light only and control (no laser and drugs).

* : $P<0.001$ compared to Mal-BSA-Pc.