Uptake of Hematoporphyrin Derivative by Atheromatous Plaques: Studies in Human in Vitro and Rabbit in Vivo

ARTUR M. SPOKOJNY, MD, JUAN R. SERUR, MD,* JOHN SKILLMAN, MD,† J. RICHARD SPEARS, MD, FACC
Boston, Massachusetts

Hematoporphyrin derivative, a photosensitive material used to identify and treat neoplastic tissue in humans, has been found to localize in atheromatous plaques in animals and has recently been found in postmortem human atherosclerotic plaques. It is not known whether human plaques take up hematoporphyrin derivative in vivo. In five patients undergoing surgical vascular procedures, specimens containing atheromatous plaques were removed and immediately incubated in autologous oxygenated blood at 37°C with hematoporphyrin derivative at a clinically relevant concentration for 2 hours. On exposure to ultraviolet light, porphyrin fluorescence was noted throughout each plaque, whereas adjacent plaque-free tissue showed no fluorescence.

To compare in vitro with in vivo hematoporphyrin derivative uptake by plaques, the fluorescence of three types of arterial lesions (induced by a high cholesterol diet, catheters or balloon injury) was studied in 16 New Zealand White rabbits. Each lesion fluoresced selectively with the same intensity whether hematoporphyrin derivative exposure was performed in vitro or in vivo. Fluorescence microscopy did not show a difference in the pattern of hematoporphyrin derivative fluorescence between in vitro and in vivo specimens.

The results suggest that human atheromatous plaques should take up hematoporphyrin derivative in vivo and are, therefore, potentially suitable for photochemical treatment as a new therapeutic approach to atherosclerosis.

From the *Charles A. Dana Research Institute and the Harvard-Thorn-dike Laboratory of Beth Israel Hospital, the Department of Medicine, Cardiovascular Division, the Radiology Research Center of the Department of Radiology, and the †Department of Surgery, Beth Israel Hospital, Harvard Medical School, Boston, Massachusetts. This work was supported in part by the National Heart, Lung, and Blood Institute Grant R01-HL3252-01. Part of this study was presented at the 9th European Congress for Cardiology in Duesseldorf, West Germany, July 1984.

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Address for reprints: J Richard Spears, MD, Beth Israel Hospital, Cardiovascular Division, 330 Brookline Avenue, Boston, Massachusetts 02215.

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Methods

Human Studies

From the carotid arteries of four patients undergoing endarterectomy, and from the popliteal artery of a patient undergoing a leg amputation, portions of the surgical specimens containing atheromatous and plaque-free arterial wall were immediately incubated in heparinized autologous blood at 37°C for 1 hour in chambers containing hematoporphyrin derivative (20 μg/ml). The chambers were gently mixed and were gassed with a 95% oxygen:5% carbon dioxide mixture. Immediately after incubation, each sample was rinsed with 0.15 M sodium chloride and examined grossly with a Wood’s lamp. Informed consent was obtained for the surgical procedures, including permission for pathologic studies of material removed from the body.

Rabbit Studies in Vivo

Twenty-four New Zealand White male rabbits, weighing about 4 kg each, were divided into three groups:

Group I: Diet-induced plaques. Six rabbits were fed a high cholesterol diet for a period of 6 months as previously described (1). One to 2 days before death was induced, hematoporphyrin derivative (5.0 mg/kg body weight) (Photofrin II) was injected intravenously. After rinsing the aortic luminal surface with phosphate-buffered saline solution, localization of porphyrin fluorescence was performed with a Wood’s lamp.

Group II: Catheter injury. In 10 rabbits, aortic lesions were induced mechanically by the presence of a chronically indwelling polyethylene catheter as described by Moore (5). The rabbits were anesthetized with diazepam and ketamine, and a cutdown over a peripheral artery was performed with lidocaine anesthesia. A polyethylene 90 tube was advanced to the level of the midthoracic aorta and, after balloon inflation with contrast medium to a constant pressure of 300 mm Hg, was withdrawn twice to the iliac bifurcation for endothelial denudation. Hematoporphyrin derivative (5 mg/kg) was given intravenously immediately after denudation. Two rabbits were sacrificed at 1 hour, and four rabbits were sacrificed at 48 hours after hematoporphyrin derivative administration, and the aorta was studied in the same manner as for Group I. Two additional rabbits were given 4 mg of 0.5% (wt/vol) Evans blue (Harvey Laboratories) intravenously along with hematoporphyrin derivative at the time of balloon injury and were killed 1 hour later.

Rabbit Studies in Vitro

The aortas of 12 rabbits were studied in vitro in the same fashion as the human specimens. Two aortas were obtained from normal control rabbits. In addition, catheter-induced lesions were produced, as described earlier, in four rabbits 2 weeks before sacrifice, and balloon de-endothelialization was performed in another four rabbits 1 hour before sacrifice for the in vitro study. Two rabbits that had had a high cholesterol diet for 5 months were used to provide a diet-induced atheromatous aorta for in vitro studies. Under pentobarbital anesthesia, the abdominal aorta of each rabbit was exposed by way of a longitudinal ventral incision, and 80 cc of blood was removed with an 18 gauge needle. The rabbits were killed with an overdose of intravenous pentobarbital, and multiple 1 cm normal and lesion-bearing aortic segments were incubated in 40 cc of heparinized autologous blood for 1 hour as described for the human specimens.

Microscopic studies. Fluorescence microscopy was performed on frozen sections of normal and lesion-bearing aortic segments from each rabbit model. Automatic exposure with a 35 mm Nikon single lens reflex camera facilitated fluorescent and white light photographic documentation of the findings as described previously (1). Additional sections of normal and lesion-bearing aortic segments from each rabbit model were fixed in 10% neutral buffered formalin, embedded in paraffin and treated with hematoxylin-eosin stain for light microscopic examination.

Results

Human Studies

In each human specimen, atheromatous plaque showed a fluorescence intensity intermediate between that of the balloon-injured segments and the experimental plaques in rabbits. Fluorescence was observed throughout the thickness of the plaque, and its intensity appeared to follow a concentration gradient from the luminal surface to the deepest layers of each plaque. Normal tissue adjacent to or below the plaque did not show any fluorescence. Calcium spicules within the plaque did not fluoresce and appeared white (Fig. 1).
Figure 1. Fresh human arterial specimen after incubation with hematoporphyrin derivative in vitro for 1 hour. On exposure to ultraviolet light, the atheromatous plaque fluoresces red in contrast to the plaque-free arterial wall.

**Rabbit Studies**

**In vivo.** The aortas of hypercholesterolemic rabbits injected with hematoporphyrin derivative appeared the same under normal and ultraviolet light, as described previously (1). Each rabbit aorta subjected to catheter-induced trauma showed a plaque that was continuous with the aortic intima and enclosed the distal end of the polyethylene tubing. On exposure to ultraviolet light, each plaque demonstrated an intense red fluorescence (Fig. 2) that appeared to be distributed throughout the thickness of each plaque, whereas the underlying aortic wall was free of fluorescence.

The gross pattern and intensity of porphyrin fluorescence was similar for rabbits killed 1 and 48 hours after balloon injury and injection of hematoporphyrin derivative. A homogeneous distribution of mild fluorescence was noted over the entire de-endothelialized luminal surface, whereas the proximal noninjured aorta showed no fluorescence. The distribution of the porphyrin fluorescence correlated closely with that of the Evans blue. The fluorescence intensity of the mechanically induced and diet-induced plaques was greater than that of the aortas subjected to balloon injury.

**In vitro.** Before incubation with hematoporphyrin derivative, no aortic segment demonstrated nonspecific fluorescence when examined with the Wood’s lamp. No fluorescence was noted in normal rabbit aortic segments incubated with hematoporphyrin derivative in autologous blood, whereas balloon-injured segments demonstrated mild fluorescence, and diet- and catheter-induced plaques showed intense fluorescence. Comparing the plaques of in vitro with in vivo specimens under ultraviolet light, no difference in uptake of hematoporphyrin derivative fluorescence could be seen (Fig. 2).

**Microscopic Studies**

**Light microscopy.** Light microscopic examination of the various types of aortic lesions demonstrated pathologic changes similar to that described by others (5,9) and ranged from the predominantly fatty streak-foam cell intimal thickening of the diet-induced plaque to the thrombo-fibro-fatty lesion of the catheter-injured aorta.

**Fluorescence microscopy.** The microscopic fluorescence pattern in the aortas of balloon-injured rabbits killed 1 hour after hematoporphyrin derivative injection was similar to that of the balloon-injured aortic segments incubated with hematoporphyrin derivative in vitro. A diffuse, nearly
continuous linear distribution of fluorescence in the subendothelium adjacent to the luminal surface and between elastic layers was evident in each case (Fig. 3).

Two days after hematoporphyrin derivative injection in the in vivo studies, the microscopic pattern of fluorescence was similar within all aortas, including catheter-induced and hypercholesterolemia-induced plaques and balloon-injured segments. This pattern differed markedly from that noted within injured aortas 1 hour after hematoporphyrin derivative injection and consisted of small clusters of granular fluorescence within the intima. The intensity of the granular fluorescence appeared greater than that of the homogeneous fluorescence noted 1 hour after hematoporphyrin derivative injection. The media of these tissues and all layers of non-injured aortas demonstrated no fluorescence.

**Discussion**

**Human studies.** Although the in vivo uptake of hematoporphyrin derivative by diet-induced arterial lesions in rabbits and in a patas monkey has been previously reported (1), it is unknown whether human atheromatous plaques...
also selectively concentrate hematoporphyrin derivative in vivo, because the histologic findings of human atheromatous plaque are variable and often differ markedly from those of commonly used experimental models (5). The uptake of porphyrins by postmortem human plaques was described by Kessel and Sykes (2). However, the design of their study may not permit extrapolation to the in vivo setting for several reasons. First, cadaver specimens might have undergone degenerative changes, thereby altering their affinity to hematoporphyrin derivative, which is known to be quite high in injured and necrotic tissue (10). Second, incubation of the specimens in Eagle’s minimal essential medium does not take into consideration the affinity of porphyrins for plasma proteins. The latter may play an important role in vivo in porphyrin bioavailability.

Rabbit studies. The aortas of all three models of aortic injury in the rabbit demonstrated the same intensity whether the specimens were exposed to hematoporphyrin derivative in vitro or in vivo, although the intensity differed among the various models. Fluorescence microscopy of both in vivo and in vitro incubated arteries revealed a similar pattern after 1 hour.

Mechanism of uptake. Because the exact mechanism and specific site of hematoporphyrin derivative uptake by tumors remains to be elucidated, it was beyond the scope of this study to define the precise mechanism and site of hematoporphyrin derivative uptake by atherosclerotic plaques. There are several theories that might explain the uptake of hematoporphyrin derivative by plaques. First, it has been suggested that porphyrins have an affinity for rapidly proliferating tissue, which would include malignant and atherosclerotic tissues (10). Second, it has been reported that non-neoplastic tissues, such as liver, kidney and spleen, which are characterized by capillaries with either a fenestrated or a discontinuous endothelium, have a higher affinity to hematoporphyrin derivative than do other normal tissues (11–13). This indicates that increased endothelial permeability, present in atherosclerosis, might account for hematoporphyrin derivative uptake. Third, an affinity of hematoporphyrin and other porphyrins for fibrinogen and platelets has been described (14), and the latter two may play an important role in atherogenesis. Finally, recent studies (15) suggest that mononuclear cell adhesion and migration play a central role in plaque progression, and mononuclear cell phagocytosis of porphyrins bound to lipoproteins may occur.

The findings of this study suggest that accumulation of hematoporphyrin derivative by human atheromatous plaques is likely to occur for three reasons. First, strong retention of hematoporphyrin derivative by experimental plaques, including the catheter-induced plaque which may bear a closer histologic resemblance to human plaques than to diet-induced lesions (5), occurs irrespective of plaque origin. Second, injury to the arterial wall is commonly accepted as a substrate for lesion progression (5,8,9,16), and the affinity of hematoporphyrin derivative for the aortic intima was enhanced experimentally by all forms of injury. Finally, because the in vitro studies in rabbits produced results that were both macroscopically and microscopically similar to those of the in vivo studies, it is likely that the in vivo uptake by human plaques of hematoporphyrin derivative will be similar to their uptake of hematoporphyrin derivative in vitro.

Potential use. Selective uptake of hematoporphyrin derivative by atheromatous plaques in humans may be useful clinically. In our previous experience with angioscopy (17), the boundary between plaque and the normal arterial wall could not be easily defined. However, on exposure to ultraviolet light, hematoporphyrin derivative fluorescence of atherosclerotic plaques could facilitate lesion identification. For therapeutic purposes, hematoporphyrin derivative could be used as a selective chromophore in laser angioplasty to enhance absorption of laser energy (for example, argon ion laser), thereby reducing potential adverse effects such as perforation (18).

Because the patchy distribution of granular fluorescence noted in the present study is similar to that found in biopsy specimens of tumors that have subsequently responded successfully to photodynamic therapy with hematoporphyrin derivative (T.J. Dougherty, personal communication), photochemical treatment of atherosclerotic disease might be possible. Because no significant adverse effect other than increased skin photosensitivity has been described for parenterally injected hematoporphyrin derivative, this drug may be beneficial for this purpose. However, in addition to the potential problems discussed previously (1), the exact mechanism of tumor destruction is unclear, so that the potential for successful treatment of porphyrin-laden plaques is difficult to estimate. Additional studies of porphyrin-mediated photodynamic tissue reactions in general are required to address these practical issues.

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