

Fluorescence of Experimental Atheromatous Plaques with Hematoporphyrin Derivative

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ABSTRACT Fluorescence of hematoporphyrin derivative (HPD) has been used clinically to localize malignant neoplasms because of its selective accumulation in these tissues. We tested the hypothesis that HPD may also be selectively concentrated within atheromatous plaques. 48 h after HPD injection in a variety of species, selective fluorescence of atheromatous plaques of the aorta was seen in each animal (rabbits and Patas monkey) exhibiting such lesions. No fluorescence could be demonstrated in aortic segments free of atheromatous involvement. Since the efficacy of photodynamic destruction of malignant tumors with HPD has been demonstrated in clinical studies, the observations of the present study may have therapeutic implications in atheromatosis.

INTRODUCTION

Naturally occurring porphyrins have been known for many years to accumulate in neoplastic tissue (1, 2). The fluorescence of parenterally injected hematoporphyrin and its derivatives (HPD)¹ within tissues exposed to UV light has been used to localize malignant tumors (3-7), and the cytotoxic effect of light-activated HPD has been used in cancer therapy (8-11). Although HPD may be photoactivated at a variety of wavelengths in the visible portion of the electromag-

netic spectrum, light at ~635 nm has recently been used because of its greater penetration at this wavelength (10). Upon HPD photoactivation, release of singlet oxygen with subsequent damage to the cell membrane may be the primary mechanism for the cytotoxicity (12).

Although several porphyrins have been shown, by fluorescence as well as by radiolabeling and chemical assay, to accumulate in nonneoplastic tissues (13-16), the affinity of other diseased tissues for HPD has received little attention. However, it has been suggested that rapidly proliferating tissues, in general, may preferentially concentrate HPD (17). In addition, evidence has recently been advanced that hydrophobic impurities in HPD, which is actually a complex mixture of porphyrins, may be responsible for the photosensitizing reaction in tumors (18).

Proliferation of smooth muscle cells, which are rich in lipids, (19-21), may contribute to the growth of atheromatous plaques (22-27). We therefore tested the hypothesis that HPD may be preferentially concentrated in atheromatous plaques, in comparison to the plaque-free arterial wall, by examining the postmortem aorta for fluorescence in experimental models of atherosclerosis 48 h after parenteral HPD injection.

METHODS

A variety of species was chosen for study, since the arterial tree in man could not be examined for HPD fluorescence. Four New Zealand White male rabbits were placed on a high cholesterol diet consisting of one egg yolk mixed in with Purina rabbit chow (Ralston Purina Co., St. Louis, MO) daily for 8 mo. Mean serum cholesterol levels on the diet ranged between 279 and 828 mg% for all rabbits, which weighed

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¹ Abbreviation used in this paper: HPD, hematoporphyrin derivative.

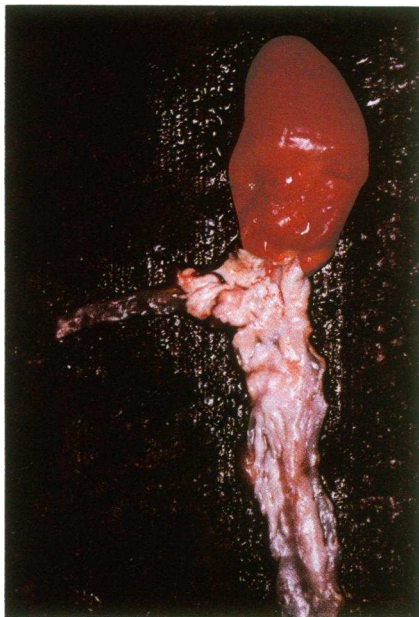


FIGURE 1 Luminal surface of rabbit aorta photographed under ordinary white light. Note the confluence of atheromatous plaques in the ascending aorta and arch with relative sparing of the innominate artery.

4 kg each at the time of the study. An 8 kg, 3.5-yr-old male *Erythrocebus Patas* monkey, which had been on a high lipid diet with a 0.5 mg% cholesterol content from birth to age two and subsequently had been on a regular diet, was obtained from the Primate Colony at Litton Biomedical, Inc., Kensington, MD. Additional species included a 10-kg female mongrel dog, a 2-kg chicken, a 1-kg male guinea pig, and a 0.5-kg white male Wistar rat (Charles River Breeding Laboratories, Inc., Wilmington, MA), all of which were retired breeders and had not been placed on special diets.

HPD was prepared by and obtained from Oncology Research and Development, Inc., Cheektowaga, NY. A method similar to that of Dougherty et al. (10) was used for the preparation, and 10 mg/kg i.v. was injected into each animal, except for the guinea pig, in which an intraperitoneal injection was performed. Animals were subsequently kept in a dark environment, to avoid a potential photosensitivity skin or mucous membrane reaction. One of the rabbits, which had been on a high cholesterol diet, served as a control and did not receive HPD. Another rabbit, which had been simply fed Purina rabbit chow, served as a low cholesterol control.

Each animal was killed 48 h after injection of HPD. The postmortem aorta was then dissected free of adjacent tissues and, after exposure of the luminal surface with a longitudinal incision and rinsing with saline, each specimen was examined and photographed under ordinary white light. A Wood's lamp was used to examine the luminal surface of each specimen for HPD fluorescence. A Corning 3-68 orange glass filter (Precision Glass, Enfield, CT) facilitated color photographic documentation of the fluorescence by filtering the activating blue light. Color photographs were obtained

with a 35-mm SLR Nikon EF camera (Nikon, Tokyo, Japan) with appropriate lenses and Kodak ASA 400 film (Eastman Kodak Co., Rochester, NY).

RESULTS

All four rabbits, which had been on a high cholesterol diet, on postmortem examination of the aorta demonstrated macroscopically evident atheromatous changes that varied from a few small scattered plaques in one rabbit with a serum cholesterol of 379 mg% to confluent plaques (Fig. 1) over most of the luminal surface in other rabbits, including one control rabbit, with higher cholesterol levels (828, 743, and 540 mg%, respectively). The other control rabbit, which had not been placed on a high cholesterol diet and had a serum cholesterol of 79 mg%, showed an aortic luminal surface grossly free of atheromatous changes.

The aorta of the *Erythrocebus Patas* monkey demonstrated several small fatty streaks that were raised <0.5 mm from the luminal surface. No atheromatous changes were evident in any of the aortas of the other species studied.

Upon exposure to UV light, an intense salmon pink fluorescence, which appeared orange when viewed through the Corning glass filter, was observed in all atheromatous plaques in the aortas of the high cholesterol-fed rabbits (Fig. 2), with the exception of the rabbit that did not receive HPD. Fluorescence was invariably seen to be evenly distributed throughout all three dimensions of each plaque (Fig. 3), while no plaque-free segment demonstrated any fluorescence. Although less intense than in the rabbits, fluorescence was also observed in the few fatty streaks in the *Patas* monkey, while the plaque-free arterial wall showed no fluorescence. In both species, the grossly normal arterial wall beneath each atheromatous plaque demonstrated no fluorescence.

The control rabbit with a low serum cholesterol, as well as all other species in which no atheromatous changes were apparent, demonstrated no fluorescence in any segment of the aorta. Likewise, except for fluorescence of the occasional lymph nodes identified in several rabbits, no other tissues examined within the abdominal and thoracic cavities of the animals that received HPD were found to fluoresce.

DISCUSSION

To our knowledge, HPD localization within atheromatous lesions has not previously been described. Experimental animals, such as the rat, which are commonly used for studies of malignant neoplasms, are likely to be resistant to atheromatosis and provide little opportunity to test the hypothesis that HPD may be selectively concentrated within atheromatous plaques.

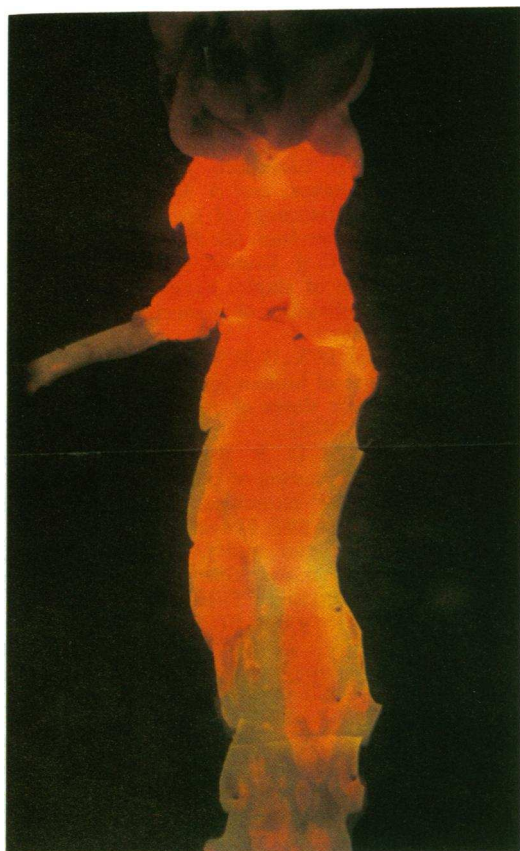


FIGURE 2 Same luminal surface as in Fig. 1, upon exposure to UV light and photographed through a Corning 3-68 orange filter. Note the selective fluorescence of all atheromatous plaques.

In addition, HPD fluorescence within tissue usually persists for no longer than 1 wk following parenteral injection, and postmortem examination of the arterial tree of patients who have received HPD for tumor detection and/or phototherapy has not been performed within this limited period of time.

Nonneoplastic tissues, including embryonic and granulating tissues, have been shown to accumulate HPD (17). The suggestion was made, therefore, that rapidly proliferating tissues in general may have an increased affinity for HPD. Evidence has grown that mitotic activity of smooth muscle cells plays an important role in the pathogenesis of fatty streaks and fibrous plaques experimentally (22-27). It may not be surprising, therefore, that in the present study HPD fluorescence was found within atheromatous lesions of the aorta in the rabbits and in the Patas monkey. Although lipophilic components of HPD, such as protoporphyrin, may be the photosensitizing agents required for cytotoxicity in tumors (18), the chemical characterization of HPD and the mechanism of its

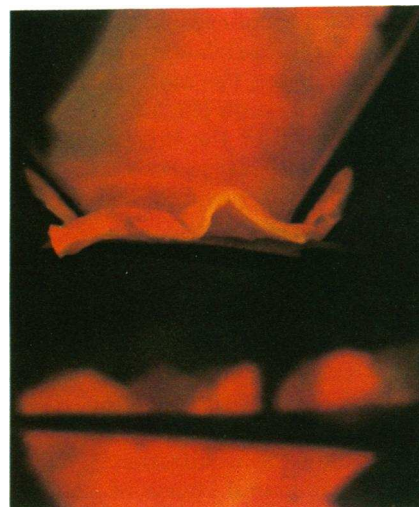


FIGURE 3 Transverse section of thoracic aorta of specimen shown in Figs. 1 and 2, upon exposure to UV light. The entire thickness of atheromatous plaques fluoresced homogeneously, while the underlying aortic wall, which was grossly free of atheromatous changes, demonstrated no fluorescence.

cellular uptake have not been well defined, so that an increased affinity for lipid-rich atheromatous lesions on this basis can only be surmised.

The time course of uptake and retention of HPD by atheromatous plaques in the present study is unknown, but the strong fluorescence observed 48 h after injection in the rabbits is consistent with the observation that radiolabeled HPD uptake by murine tissues is maximal within the first 2 d after injection (16).

Although lack of fluorescence may not indicate absence of HPD localization (15, 16), fluorescence has correlated with the potential for destruction of tissue (10). As found in this study, the absence of fluorescence of aortic segments free of atheromatous lesions may thus indicate that a favorable differential effect of HPD photoactivation on the plaque and adjacent plaque-free arterial wall may be possible.

The potential for HPD-mediated chemical destruction of plaques with light (photoatherolysis) to be clinically useful rests upon a number of untested hypotheses. Selective fluorescence of atheromatous plaques needs to be demonstrated in man. The recent development of an ultrathin fiberscope, which we have used for direct visualization of the arterial lumen (28), may make fluorescence angiосcopy possible in a manner similar to other recently developed endoscopic techniques (29-30), so that this hypothesis may be tested in vivo. If selective plaque fluorescence is found in man, plaque destruction following HPD photoactivation, such as might be accomplished with an intraluminal laser-transmitting optical fiber, may be lim-

ited to the cellular fibrous capsule that commonly surrounds an acellular lipid-rich material within a fibrous plaque, the latter being the most common lesion associated with clinical events (31, 32). Further problems might include control of the inflammatory reaction, usually noted before tissue necrosis, and the potential thrombogenicity of the plaque's acellular core upon exposure to the arterial lumen.

Irrespective of the uncertainty of the potential clinical utility of HPD localization in atheromatous plaques, the observations of this study are likely to provide impetus to further experimental investigations regarding the affinity of HPD for atheromatous lesions. In addition, intraluminal identification of HPD fluorescence with angioscopy, if feasible, might permit investigators to study the localization of atheromatous plaques and the temporal course of plaque progression/regression in vivo in a manner that has hitherto not been possible.

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