Uptake of hematoporphyrin derivative by valvular vegetations in experimental infective endocarditis

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ABSTRACT Drugs that localize in valvular vegetations may be useful in the diagnosis and treatment of infective endocarditis. We therefore tested the hypothesis that parenterally injected hematoporphyrin derivative (HPD), which is concentrated in tumors and atherosclerotic plaques, localizes in the vegetations of experimental infective endocarditis. In 14 rabbits, various bacteria were given intra-arterially immediately after injury to the aortic valve. In 12 additional rabbits, sterile vegetations on the aortic valve were produced by the trauma caused by an indwelling catheter that had been in place over a long period. HPD, 2.5 mg/kg, was injected intravenously 1 to 2 days before the animals were killed in six rabbits with sterile vegetations and in seven rabbits with infected vegetations. In all rabbits, multiple vegetations on the aortic valve leaflets were identified. On exposure to ultraviolet light, strong porphyrin fluorescence of all vegetations, whether sterile or infected, was observed only in rabbits given HPD. In two rabbits given HPD 10 weeks after catheter implantation across the aortic valve, however, only mild fluorescence could be detected in healing endocardial vegetations. In frozen sections of HPD-laden lesions, a patchy distribution of fluorescence was observed that was similar to the pattern of HPD localization in atheromatous plaques. Since vegetations in experimental infective endocarditis selectively concentrate HPD, porphyrins could be useful in the diagnosis and treatment of infective endocarditis.


THE MORTALITY and morbidity from infective endocarditis are still significant. Classic symptoms and objective signs of infective endocarditis are often lacking, and the initial clinical management of patients with this disease is often empirical, particularly when the results of blood cultures are either pending or negative. Therefore, it is important to investigate new techniques that may improve the diagnosis and treatment of infective endocarditis. Parenterally injected porphyrins have long been known to localize preferentially in certain pathologic lesions, including malignant neoplasms and mechanically injured tissues. The characteristic fluorescence of hematoporphyrin derivative (HPD) on exposure to ultraviolet light has been used to detect occult neoplasms. In addition, photodynamic therapy for tumors, consisting of light-activation of HPD with resultant local release of cytotoxic singlet oxygen, has recently been introduced clinically as a new therapeutic modality.

Recent studies have shown that atheromatous plaques in rabbits and humans selectively (as compared with the normal arterial wall) concentrate HPD, and that mechanical injury to the endothelial lining results in uptake of HPD by the arterial wall. Furthermore, the uptake of HPD by several species of bacteria has been demonstrated, and on exposure to light a bactericidal effect of about 99.5% could be achieved. Valvular vegetations, which characterize infective endocarditis, usually further traumatize valves that have previously suffered a variety of injuries. If valvular vegetations selectively concentrate HPD, in addition to the known uptake of HPD by bacteria, improvement in methods of diagnosis by the use of labeled HPD might be achieved and photodynamic therapy of vegetations might be possible. We therefore tested the hypothesis that sterile and infected vegeta-

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tions in a commonly used experimental preparation of infective endocarditis will, compared with the uninjured valve, selectively concentrate HPD after parenteral injection of this material.

Methods
Sterile vegetations. The experimental preparation chosen was similar to that described by Garrison and Freedman, which has been widely used in recent studies for determining the efficacy of various antibiotics in the treatment of endocarditis. Under ketamine and diazepam anesthesia, a longitudinal cutdown in the neck was performed in 14 New Zealand White male rabbits. Polyethylene 90 tubing was advanced under fluoroscopy via the carotid artery across the aortic valve over a guidewire, and the latter was removed. The catheter was filled with contrast medium to visualize and document the position of the tip, and the catheter was then fixed in position. Four to six days later in six rabbits, and 10 weeks later in two rabbits, HPD (Photofrin II, donated by Photofrin Medical, Cheektowaga, NY) was injected in doses of 2.5 mg/kg body weight iv. One day later, all 14 rabbits were killed and their hearts and aortic valves were examined.

Infected vegetations. In 14 other rabbits, a cutdown was performed as described above. Under fluoroscopic control, via the carotid artery, a 0.38 inch flexible guidewire was repeatedly advanced across the aortic valve to mechanically injure the valve. Immediately after injury, 1 ml of a Tryp-Soybrot (Scott, Inc., Fawkesville, RI) suspension containing bacteria in various concentrations was injected intra-arterially. Each type of bacteria was obtained from positive blood cultures of patients with known infective endocarditis. Eight animals were injected with Staphylococcus aureus in concentrations of 10^8/ml (n = 3), 10^9/ml (n = 3), or 10^10/ml (n = 2). Six animals were inoculated with Streptococcus viridans, 10^7/ml (n = 4) and 10^8/ml (n = 2), and in four animals either 10^7/ml (n = 2) or 10^8/ml (n = 2) of β-hemolytic Streptococcus was injected. Since the number of the days of survival correlated well with the concentration of bacteria injected, 2.5 mg/kg iv HPD was injected 1 day before expected death or sacrifice.

The heart and the aortic valves containing infected and sterile vegetations were exposed to ultraviolet light from a Wood’s lamp to detect HPD fluorescence. Aortic valves and vegetations from four rabbits with sterile vegetations and four rabbits with infected vegetations were stained with hematoxylin-eosin, formalin fixed, and embedded in paraffin. Tissue from three HPD-injected rabbits with sterile and two with infected (n = 2) vegetations was removed immediately after the animals were killed and fluorescence microscopic examination of frozen sections of the aortic valve and vegetations was performed.

Results
Sterile vegetations up to 4 mm in size appeared whitish and were usually identified on the aortic surface of the aortic valve. Fluorescence was detected upon exposure to ultraviolet light only in rabbits given HPD (figure 1). In the two rabbits in which the catheter had been in place for over 10 weeks, vegetations (1 mm) were detected, but only mild fluorescence was observed. Interestingly, weak fluorescence was also noted in traumatized valvular and myocardial tissues adjacent to the vegetations, although no gross abnormalities were apparent in those tissues under ordinary white light. Furthermore, myocardial injury due to infection (abscess) showed strong porphyrin fluorescence with clear demarcation from normal tissue.

Infected vegetations. In rabbits with infective endocarditis, the size of the vegetations varied with the species and concentration of the bacteria injected. Animals injected with 10^8/ml S. aureus died after 2 days with only minimal vegetations, while 10^9/ml S. aureus produced large vegetations (1 to 2 mm in size) that fluoresced only after prior injection of HPD. Rabbits injected with S. viridans either died or were killed 6 days after injection, and at necropsy were noted to have 2 to 3 cm long, 4 to 5 mm wide vegetations that frequently obstructed the aortic root. Similar findings were noted in rabbits infected with β-hemolytic Streptococcus; these animals were also killed or died 6 days after injection of bacteria.

Light microscopy. On hematoxylin-eosin stained sections, the vegetations were composed predominantly of fibrin, with occasional admixed platelets, leukocytes, and erythrocytes. In some areas, the endocardium immediately adjacent to the vegetations showed fibroblastic proliferation and neovascularity that focally extended into the base of the vegetations, consistent with early organization. Vegetations in rabbits with infective endocarditis appeared histologically similar to sterile vegetations, except for the presence of bacteria, as determined by Gram’s stain, and a large number of leukocytes. No difference in the histology of vegetations from HPD-injected and control rabbits was found. These findings are identical to those previously described for this preparation of infective endocarditis.

Fluorescence microscopy. The microscopic pattern of fluorescence consisted of small patches within the vegetation and the adjacent injured aortic valve. No difference in fluorescence between injected and sterile vegetations was observed.

Discussion
The effectiveness of antimicrobial therapy in valvular endocarditis has been studied by many investigators in a reproducible preparation of this disease in rabbits. Valvular injury is produced by a polyethylene catheter inserted into either ventricle via a jugular vein or carotid artery. The presence of the catheter in the right or left side of the heart provokes rapid deposition of fibrin-platelet vegetations on traumatized valves and endocardium. These sterile vegetations may then be infected by inoculation with microorganisms. Pathologically, the infected vegetations are remarkably similar to those found in man and consist of a
Upon exposure to ultraviolet light, only fresh vegetations in rabbits given HPD show intense fluorescence.

FIGURE 1. Upon exposure to ultraviolet light, only fresh vegetations in rabbits given HPD show intense fluorescence.

relatively amorphous mass of fibrin, platelets, platelet debris, occasional leukocytes, and bacteria. Using a similar rabbit preparation, we found the same histopathology previously described. Furthermore, we were able to produce infective endocarditis without the use of an indwelling catheter. Our findings confirm the hypothesis that HPD is selectively concentrated in sterile and infected vegetations, as compared with uninjured valve tissue, in an experimental preparation of infective endocarditis. To our knowledge, this observation has not been previously reported. The similarity of the pathology of valvular vegetations in the rabbit to the pathology of those in man makes it likely that human vegetations will also selectively concentrate HPD.

Since the exact mechanism and cellular site of HPD uptake by tumors and atheromatous plaques still remains to be elucidated, it is beyond the scope of this study to define the mechanism and site of HPD uptake by endocardial vegetations. It should be noted, however, that fluorescence microscopic examination of frozen sections of neoplastic and atherosclerotic tissues show patterns of HPD uptake similar to those in valvular vegetations, thereby suggesting analogous mechanisms. Recent evidence from our laboratory suggests that increased endothelial permeability of atheromatous plaques may be an important factor in the localization of HPD within this tissue, and endocardial vegetations that consist primarily of a fibrin-platelet mesh lack the endothelial barrier of normal valves. In addition, a variety of porphyrins, including hematoporphyrin, have been shown to have an affinity for fibrinogen and platelets, and fibrin matrixes are known to selectively concentrate HPD.

The diagnosis and treatment of infective endocarditis is often difficult, especially in subacute and chronic cases in which symptoms and signs are subtle or lacking. Gallium-64 scintillation scanning results in a high incidence of false-negative results because of poor spatial resolution and is, therefore, unreliable in the diagnosis of infective endocarditis. The usefulness of echocardiography is often limited by the small size of most vegetations and is unreliable in the diagnosis of prosthetic valve endocarditis. Hence, labeled porphyrins might be useful in the diagnosis of endocardial vegetations. However, the use of radiolabeled porphyrins might be limited by the inadequate spatial resolution inherent in radionuclear scintigraphy. Porphyrin labeling with paramagnetic metals such as Mn⁺⁺ could be helpful in the noninvasive diagnosis of endocardial vegetations, since nuclear magnetic resonance scanning with electrocardiographic gating may potentially provide sufficient spatial resolution to image vegetations 1 to 2 mm in size.

It should be noted, however, that in patients with atherosclerotic disease of the ascending aorta, identification of vegetations may be difficult, since atherosclerotic lesions probably would also take up HPD. To evaluate the specificity of HPD uptake in acute infective endocarditis vs that in areas of degeneration and fibrotic thickening of the valve, we examined the HPD uptake in rabbits in which an indwelling catheter was in place over a period of 10 weeks. The natural history of these noninfected vegetations is that they regress in size over period of time and endothelialize. Hence, despite previous injury to the valve and presence of a catheter, less fluorescence, when viewed with a Wood’s lamp, was observed compared with that in rabbits with fresh lesions. These findings suggest a quantitative difference in HPD uptake by fresh vs older lesions. Further studies are necessary to quantitate HPD uptake. If endothelial permeability or rapid proliferation are substantial for HPD uptake, it is not surprising that freshly grown lesions localize larger amounts of HPD.

Photodynamic therapy with HPD might be of potential use in the treatment of infective endocarditis for several reasons. It has been shown that this substance binds to cellular components of several aerobic and anaerobic microorganisms, including S. aureus, several strains of streptococci and Clostridium perfringens. Upon light activation, HPD was 99.9% bactericidal for gram-positive strains. Combinations of light-activated HPD and polymorphonuclear leukocytes had a 99.99% bactericidal effect on both intracellular and extracellular S. aureus. Hence, photodynamic sterilization of infected valves and myocardium might be possible with HPD by application of light, either with a fiberoptic system during cardiac catheterization or directly during open heart surgery.

In addition, since vegetations concentrate HPD, irrespective of the presence of bacteria, photodynamic treatment might reduce the integrity of the fibrin-platelet matrix barrier of the vegetation, which in turn would amplify the effect of antibiotic treatment, especially in infective endocarditis with gram-negative rods, which are not susceptible to HPD photodynamic therapy. However, potential HPD photoactivation within valvular vegetations might be limited by various factors. Hypoxia, if present within the vegetation, would reduce the photodynamic response, which requires generation of singlet oxygen. In addition, vascular reactions may play an important role in HPD-mediated photodynamic tissue reactions, and avascular portions of vegetations would not be amenable to therapy by this mechanism. Furthermore, even if a photodynamic response could be elicited, sloughing of necrotic debris might occur after treatment and would lead to peripheral embolization.

Although the potential utility of HPD in the clinical management of endocarditis is uncertain, our observation that vegetations in experimental infective endocarditis selectively (compared with normal adjacent tissue) concentrate HPD suggests that the use of HPD in the management of infective endocarditis merits further study.

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