

Bacterial calcification in infective endocarditis

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Summary: The present report is the first description to our knowledge of a clinical case of bacterial calcification in human infective endocarditic vegetations. Partial calcification of bacteria within vegetations may be a further mechanism of bacterial protection from host defences and antibiotics. Similar calcification has recently been reported in vegetations formed on porcine valvular prostheses implanted experimentally in sheep.

Introduction

The mortality of infective endocarditis remains about 30% in most series, despite the use of antibiotics.^{1–3} Factors such as the increasing age of affected patients, later diagnosis during the course of the disease, and the emergence of antibiotic resistant pathogenic organisms, are thought to explain this continuing high mortality.⁴ The vegetation is thought to harbour infection by protecting bacteria against circulating polymorphs, antibiotics and antibodies by surrounding bacteria in a fibrin-platelet clot.^{5,6}

Case report

The patient was a 72 year old woman with a 6-month history of malaise and lethargy. She denied previous rheumatic fever. At presentation she had a pyrexia of 38°C, sinus tachycardia of 100 beats/min, with a blood pressure of 130/60 mmHg. Initially she had a harsh harsh mid-systolic murmur, maximal at the cardiac apex, but no stigmata of bacterial endocarditis. Her blood count showed a normochromic, normocytic anaemia, with haemoglobin of 8.2 g/dl, white cell count of 17.7×10^9 /dl, and erythrocyte sedimentation of 57 mm/h. The electrocardiogram was normal and there was no evidence of cardiac failure. Large vegetations were observed by two dimensional echocardiography on the mitral and aortic valves.

A penicillin sensitive, slime producing *Streptococcus salivarius* was isolated from one set of six blood cultures. Intravenous penicillin with gentamicin was given for 12 days, followed by 8 days' treatment with oral amoxycillin which was stopped as a severe generalized maculopapular rash developed while on

this treatment. Oral erythromycin was given subsequently for a further 6 weeks. Adequate bactericidal antibiotic levels were demonstrated with each antibiotic or antibiotic combination used, with bactericidal levels at dilution of serum greater than 1:1024 for both pre- and post-dose levels.

After 5 weeks in hospital she was discharged at her own request because she appeared to have made a dramatic clinical improvement. Subsequently she was readmitted at outpatient clinic review with florid subungual petechiae and newly developed aortic regurgitation. Vancomycin and rifampicin, to which the originally isolated organism was also fully sensitive was substituted for treatment with erythromycin.

She died before valve replacement could be performed, following a subarachnoid haemorrhage, from a ruptured mycotic aneurysm. No further organisms were isolated despite repeated subsequent blood cultures.

The heart was removed at post-mortem and fixed in 10% formalin. Light microscopy of the valvular vegetations showed non-calcified Gram-positive cocci. For electron microscopy wax embedded valvular tissue was dewaxed, post-fixed in osmium tetroxide and embedded in Agar 100 resin (Agar Aids). Thin sections were stained in uranyl acetate and lead citrate, and examined in an AEI EM 801 electron microscope. The bacteria illustrated (Figures 1a and 1b) show evidence of septa, characteristic of division, and were typical of the bacteria present. The organisms were approximately 1µm in diameter and showed Gram-positive wall structure, comprising a single plasma membrane surrounded by a 65 nm thick, medium electron dense wall. Many organisms were totally surrounded by crystalline material (Figures 1a, 1b), in the form of electron dense, needle shaped crystals. A minority were only partially surrounded by crystals

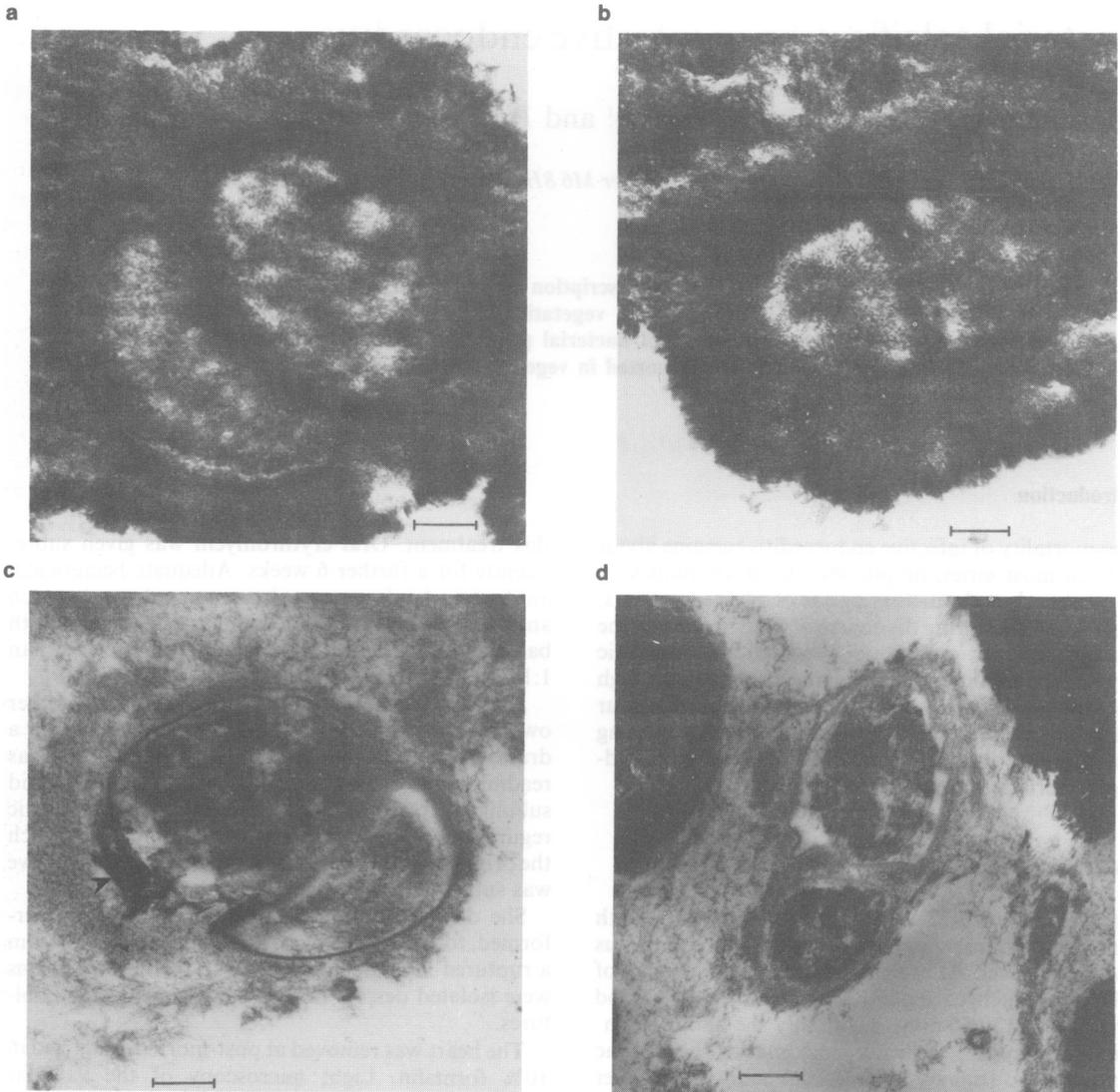


Figure 1 (a) Dividing coccoid bacterium with complete division septum. Note that the organism is completely surrounded by fine crystalline material containing calcium and phosphorous. Scale bar = 200 nm. (b) Completely calcified bacterium showing an incomplete division septum. Scale bar = 300 nm. (c) Partially calcified bacterium showing limited crystal deposition (arrowhead). The organism has Gram positive wall structure and is surrounded by filamentous material reminiscent of slime. Scale bar = 200 nm. (d) Uncalcified dividing organism showing evidence of protoplasmic lysis. Scale bar = 200 nm.

(Figure 1c). Some bacteria remained uncalcified (Figure 1d), but these bacteria showed evidence of protoplasmic lysis suggesting probable bacterial death. The presence of elemental calcium and phosphorous in the crystals was confirmed by X-ray microanalysis using an AEI Cora analytical electron microscope. Adjacent areas of valve tissue examined were not found to contain detectable levels of these two elements.

Discussion

Calcification of bacteria in infective endocarditis has not been previously reported in a clinical case. Most previous studies of endocarditis from the ultrastructural viewpoint have been confined to the examination of the external surfaces of heart valves and vegetations derived from clinically and experimentally obtained material, by scanning electron microscopy.⁷⁻¹⁰ Trans-

mission electron microscopy has not been used extensively in studies of infective endocarditis. In transmission electron microscopical studies, endocarditic vegetations appear to contain bacteria, surrounded by fibrin, collagen, platelets, polymorphs, bacterial and other cellular debris.¹¹⁻¹⁴

Bacterial calcification within endocarditic vegetations has only been previously noted in a single report. Thiene *et al.*¹⁵ investigated the effects of an agent capable of retarding degenerative calcification of porcine valvular prostheses implanted experimentally in sheep. They observed calcified bacteria found by chance in endocarditic vegetations during the course of their study, without commenting on their possible significance. It is possible that partial calcification of viable bacteria may promote persistence of infection within vegetations by acting as an impermeable barrier to antibiotics, neutrophils, and humoral defence mechanisms.

The calcification of bacteria in vegetations is probably a host response, although bacterial slime may also

have a role in the initiation of calcification. Bacterial calcification is also seen in urinary tract struvate stones which can harbour bacteria notoriously resistant to eradication by antibiotics.¹⁶⁻¹⁸ Bacterial slime, or alternatively the wall of the organism, may act as a nidus for precipitation of calcium and phosphorus. Furthermore, bacterial slime production has recently been demonstrated in clinical and experimental cases of bacterial endocarditis.^{14,19} The small amount of calcification present around the bacteria was not visible on light microscopy of routine haematoxylin and eosin stained sections taken from the vegetations, which might explain why such calcification has not been noted previously. It is probable that despite their apparently normal morphology, completely calcified bacteria are non-viable. The question of viability however remains unresolved as no post-mortem cultures were taken from the heart in this patient. Further studies are required to accurately determine the frequency and significance of bacterial calcification in infective endocarditis.

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