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EXTENDED ABSTRACT

Role of multispecies microbial biofilms in the occlusion of biliary stents

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Abstract

Endoscopic stenting is a standard palliative approach for the treatment of a variety of diseases involving biliary obstruction. However, the major limitation of this approach is represented by stent occlusion followed by life-threatening cholangitis, often requiring stent removal and replacement with a new one. Although it is generally believed that microbial colonization of the inner surface of the stent plays an important role in initiating the clogging process, so far available data are not enough for a full understanding of this phenomenon. In fact, it is known that when a biliary stent is inserted across the sphincter of Oddi, the loss of the antimicrobial barrier represented by the sphincter itself and the low pressure in the common bile duct allow reflux of duodenal content, thus promoting an ascending microbial colonization. The sessile mode of growth and the exopolysaccharide production, which leads to the subsequent establishment of a thick biofilm, provides microorganisms with an efficient protection from both antibacterial agents and phagocytic cells. The aim of this study was to analyze the tridimensional structure of the microbial biofilm grown in the lumen of 15 clogged biliary stents and to identify the microbial species involved in the clogging process. Scanning electron microscopy investigations revealed that sludge present in the stent lumen consist of a rich and assorted microbial flora, including aerobic and anaerobic species, mixed with a large amount of amorphous material containing dietary fibres, crystals of cholesterol and other precipitates of bacteria-driven bile salts.

Key words: *biliary stent, biofilm, microbial colonization, stent occlusion*

Introduction

Endoscopic stenting is a standard palliative approach for the treatment of a variety of diseases involving biliary obstruction (1). However, the major limitation of this approach is represented by stent occlusion followed by life-threatening cholangitis, often requiring stent removal and replacement with a new one (2). Although it is generally believed that microbial colonization of the inner surface of the stent plays an important role in initiating the clogging process, so far available data are not sufficient for a full understanding of this phenomenon (3,4).

In fact, it is known that when a biliary stent is inserted across the sphincter of Oddi, the loss of the antimicrobial barrier represented by the sphincter itself and the low pressure in the common bile duct allow reflux of duodenal content, thus promoting an ascending microbial colonization (5). The sessile mode of growth and the exopolysaccharide production, which leads to the subsequent establishment of a thick biofilm, provide microorganisms with an efficient protection from both antibacterial agents and phagocytic cells. The aim of this study was to analyze the structure of the microbial biofilm grown in the lumen of 15 clogged biliary stents and to identify the microbial species involved in the clogging process.

Materials and methods

Stents

Fifteen biliary stents were removed from patients who had undergone endoscopic stent insertion due to malignant or benign bile duct obstruction. Immediately after removal, segments of approximately

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1 cm were cut under sterile conditions from the distal, proximal and central portions of the stents, put into sterile tubes and immediately sent to the lab.

Microbiological analysis

For the isolation and identification of aerobic microorganisms, the segments obtained from the distal end of stents were bisected along their long axis, placed into PBS (pH 7.4) and sonicated in ice for 10 min at 2 μA (Soniprep 150, MSE). Then 0.1 and 0.01 ml of the suspension were plated on non-selective media and incubated for 24–48 h under aerobic conditions. Isolated microorganisms were counted and identified at the species level according to standard biochemical tests.

For the isolation and identification of anaerobic bacteria, all procedures were performed in an anaerobic cabinet. Each segment of the proximal portion of the stents was bisected along its major axis and the inner luminal surface of one section of the stent was scraped with a sterile wire loop to remove the sludge and adherent bacteria. Then, the suspension was serially diluted (1:10) in PBS and 100 μl of each dilution were spread on prereduced Columbia agar plates supplemented with 5% sheep blood, 0.1% vitamin K_1 and haemin and incubated anaerobically at 37°C for 72 h. The other half of the stent was transferred into prereduced BHI broth, vortex mixed and incubated anaerobically for 7 days. After appropriate dilutions, samples were streaked onto Columbia blood agar plates to determine the bacterial density (cfu). Individual colonies were selected on the basis of their morphology and plates were also incubated aerobically to exclude the aerobic growth. Anaerobes were identified with the RAPID ID 32A kit (Bio Mèrieux).

Scanning electron microscopy

Segments cut from the central part of stents and bisected as described above were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 0.1% ruthenium red (Sigma) at room temperature for 30 min. Following post-fixation in 1% OsO_4 for 30 min, samples were dehydrated through graded ethanols, critical point dried in hexamethyldisilazane (Polysciences Inc., Warrington, PA, USA) and gold coated by sputtering. Samples were examined with a Cambridge 360 scanning electron microscope.

Results and discussion

At the macroscopic level, all the examined stents were more or less occluded by a heterogeneous sludge.

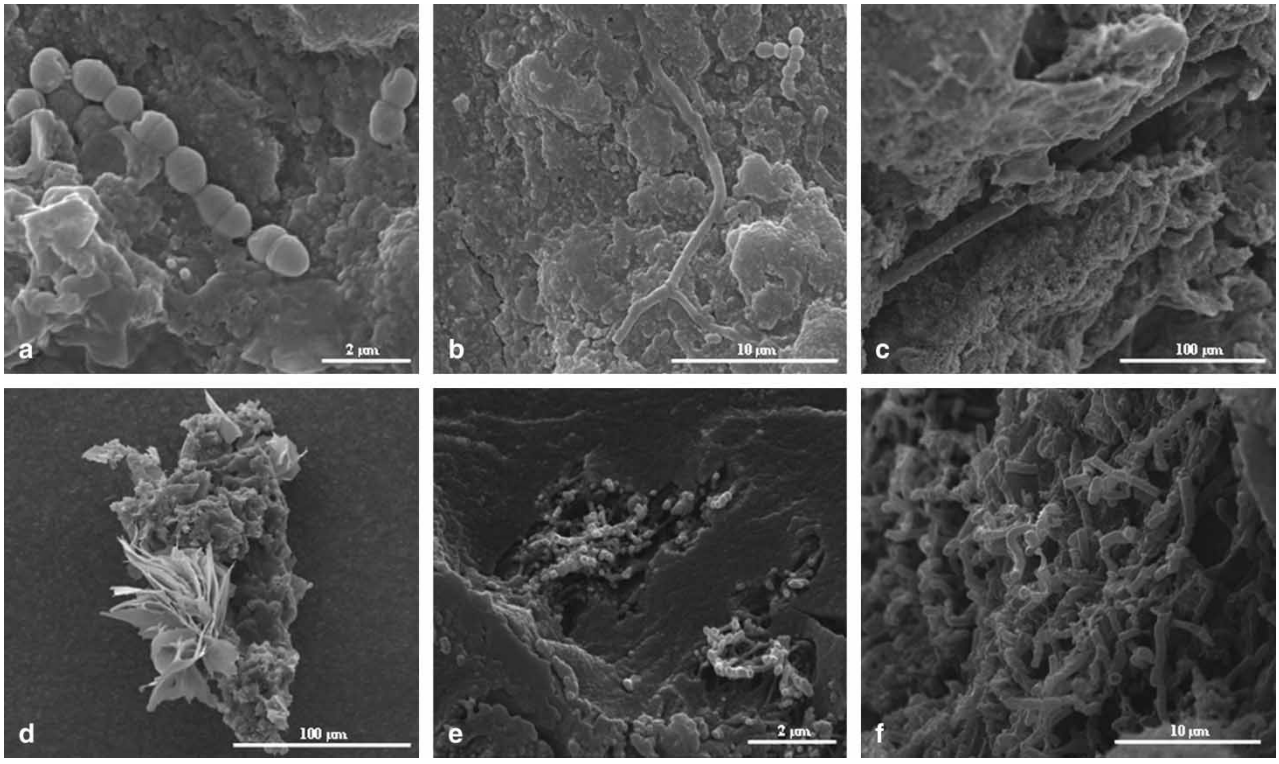

Figure 1. Scanning electron micrographs of biliary stent clogging material. Sludge present in the stent lumen consists of a rich and assorted microbial flora including enterococcal (a) and fungal (b) species mixed with a large amount of amorphous material containing dietary fibres (c) and crystals of bile salts (d). Observation of the surface of the clogging material in direct contact with the bile flow showed the presence of a dense amorphous material (e), probably mucus, in which several bacterial species were immersed to form a dense microbial biofilm (f).

Microbiological analysis revealed the presence of a mixed microbial colonization. Isolates belonging to both aerobic and anaerobic bacterial species, as well as fungi, were identified.

Among the aerobic bacteria, gram-positive *Enterococcus faecalis* was the most frequently isolated species followed by gram-negative *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp. and *Enterobacter* spp.

Bacteroides spp. and *Fusobacterium* spp. among gram-negatives and *Clostridium* spp. among gram-positives were the most frequently isolated anaerobes. As suggested previously, anaerobes may play an important role in the blockage of biliary stents (6).

Candida albicans and *Candida parapsilosis* were the only two fungal species isolated.

Scanning electron microscopy investigations, as previously reported (7–11), revealed that sludge present in the stent lumen consists of a rich and assorted microbial flora including enterococcal (Figure 1a) and fungal (Figure 1b) species mixed with a large amount of amorphous material containing dietary fibres (Figure 1c) and crystals of bile salts (Figure 1d). Observation of the surface of the clogging material in direct contact with the bile flow showed the presence of a dense amorphous material (Figure 1e), probably mucus, in which several bacterial species were immersed to form a dense microbial biofilm (Figure 1f).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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