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Clogging of biliary endoprostheses: a new perspective

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Abstract

Endoscopic palliation with biliary endoprostheses is now an established treatment for benign and malignant strictures of the biliary tree. These endoprostheses, however, tend to clog with time. We investigated this problem by undertaking in vitro studies on stents of different designs made of different polymer materials. The stent that performed best was then tested in an in vivo trial. There was a direct relation in vitro between the frictional coefficient of a polymer and the amount of encrusted material. Catheters perfused in bacterially contaminated bile, irrespective of material and design, accrued significantly more sludge than catheters perfused with sterilised bile. The presence of side holes significantly increased the amount of sludge in the stents, but eliminated any differences between the various materials. We therefore investigated the effect of omitting side holes in a clinical trial which consisted of two groups of 20 patients each. The group treated with conventional stents accrued significantly more sludge in the stents than the group treated with experimental stents without side holes ($p<0.05$). The absence of side holes did not cause incomplete drainage or increase morbidity. Side holes are detrimental to stent patency, which is adversely affected by other factors including bacteria and proteins.

Endoscopic insertion of biliary endoprostheses is now an established palliative treatment for both benign and malignant strictures of the biliary tree.[1–4] It is the treatment of choice in elderly and frail patients who are unable to tolerate major surgery.[5,6] A well known and unsolved problem is the tendency of endoprostheses to clog within a few months, after which endoscopic replacement becomes necessary.

The mechanism of stent blockage is not well understood. Wosiewitz *et al*[7] and Groen *et al*[8] showed that the major components in sludge derived from occluded stents were unconjugated bilirubin, protein, and food fibres. The material also contained large numbers of bacteria. Leung *et al*[9] and Speer *et al*[10] confirmed these results and proposed that bacteria had an important role in the initial phase of stent clogging. Recently, we investigated the effect of longterm treatment with antibiotics or aspirin on sludge formation in the first two months after stent insertion.[11] Both aspirin and doxycycline tended to decrease sludge formation, suggesting that indeed both bacteria and mucous glycoprotein play a part in sludge formation. The effects, however, were relatively slight, indicating that additional factors are probably important in the clogging process.

Patency is affected by stent diameter. Both Speer *et al*[12] and Siegel *et al*[13] showed that increasing the diameter of the endoprosthesis significantly increased its patency. The effect of the material used for stent construction on patency has not yet been rigorously tested. We investigated the effect of design and polymer used for stent construction on the rate of sludge formation in an in vitro perfusion system. Subsequently, the stent that performed best in vitro was tested in a clinical trial.

Methods

IN VITRO STUDIES

Hepatic bile from different patients was collected via T-tubes three to 10 days postoperatively, mixed into a common pool, and stored at −20°C. The mean viscosity of the bile was 1·1 centipoise.

An in vitro model was constructed to provide controlled recirculation of bile in a closed system connected to a reservoir. The flow rate was 0·5 ml/min, providing a total daily volume of 700 ml. All experiments were carried out at 37°C. Commercially obtained straight 10 French gauge catheters, internal diameter 2·4 mm, length 5 or 10 cm, were connected by polyvinyl chloride tubing. For each experiment the entire system of connecting tubing and catheters was changed.

Bile was contaminated artificially by adding a broth containing bacterial strains cultured from human bile samples. A total concentration of 10^5–10^6 colony forming units/ml was added. In some series of experiments, microbial growth was inhibited by supplementing bile with 0·05% (w/v) sodium azide.

The catheter materials studied were polyethylene (PBN Medicals, Denmark), polyurethane (Ethicon Inc, USA), Teflon (Chrompack Inc, USA), polyvinyl chloride (Technicon Inc, USA), and silicon rubber. The coefficients of friction of the materials were 0·75, 0·60, 0·15, 1·50, and 1·50 respectively. Four side holes with a diameter of 3 mm were made by a standardised procedure on each catheter.

After two weeks of continuous perfusion, the catheters were removed for biochemical analysis and scanning electron microscopy.

IN VIVO STUDY

The in vivo study was prospective and consisted of 40 consecutive patients with distal malignant bile duct obstruction, who were referred to our hospital unit between September 1988 and

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January 1989. They underwent palliative biliary drainage with either a conventional endoprosthesis (group A, n=20) or an experimental endoprosthesis (group B, n=20). The two groups were evenly matched for age, sex, serum bilirubin concentrations, and duration of obstructive jaundice. There were 21 women and 19 men (mean age 76 years, range 53–94 years), all with suspected carcinoma of the pancreas. Patients were excluded from the trial if they were considered for definitive surgery, had evidence of impending duodenal obstruction, or had undergone other treatment for the biliary obstruction. Informed consent was obtained.

The technique of endoscopic placement of a biliary endoprosthesis has been described in greater detail elsewhere.[14 15] After diagnostic endoscopic retrograde cholangiopancreatography and an endoscopic sphincterotomy, the endoprostheses were inserted over an atraumatic guidewire and coaxial catheter via a 4·2 mm channel video endoscope (Olympus TJFV10) by standard techniques. No prophylactic antibiotics were used after stent placement.

All the endoprostheses were 11 cm long straight 10 French gauge polyethylene stents (PBN Medicals, Denmark) with an internal diameter of 2·4 mm and slight tapering at the biliary tip. Each conventional endoprosthesis had four side openings, two near the ends of the stent and one each at the site of the anchoring sideflaps, made by a lateral incision of the stent wall. The side holes at both ends of the endoprosthesis had a diameter of 3 mm.

Experimental endoprostheses were manufactured from polyethylene tubing (PBN Medicals, Denmark) which were identical in shape and diameter to the conventional stents. They had no side holes and the side flaps were made by superficial cuts but the inner surface of the stent wall remained intact (Fig 1). After two months the endoprostheses were removed endoscopically. Simultaneously, a 3 ml bile sample was taken after selective cannulation of the common bile duct. These endoprostheses were immediately processed or stored at −20°C.

PROCESSING THE ENDOPROSTHESES

To determine the amount of encrusted material at the internal surface of each drain the deposits were removed by thorough rinsing for 12 hours with 8 ml distilled water, followed by lyophilisation in preweighted vials. The bile samples were cultured and bacteria were identified by standard microbiological techniques. The concentration of bile acid was measured using the 3 alpha-hydroxysteroid dehydrogenase assay.[16] Phospholipid was determined as described by Gurantz *et al.*[17] Cholesterol was measured enzymatically.[18] Free fatty acids were determined with acyl-coA synthetase using the NEFA-C test (WAKO Chemicals, Neuss, FRG). Bilirubin was determined by the method of Weber and Schalm.[19] Protein was estimated by the method of Bradford[20] after delipidation using the method of Wessel and Fluegge.[21] Mucin was measured in delipidated samples according to Crowther and Wetmore.[22]

Human and bacterial β glucuronidase activity of the bile was assayed as described by LaRusso and Fowler,[23] with slight modifications. Incubation of substrates occurred in two separate buffer solutions of pH 5·2 and 7·0. An enzyme activity at pH 7·0 of more than 20% of the human peak activity at pH 5·2 was indicative of contamination by bacterial enzyme.[24]

Scanning electron microscopy of the inner surface of endoprostheses made of different materials was performed as follows: longitudinal tube segments were mounted on cylindrical brass stubs, air dried, and sputtercoated with 50 nm gold palladium. Each sample was then examined using an ISI SS40 scanning electron microscope.

Statistical analysis was performed using Student's *t* test for unpaired observations and the Mann-Whitney U test for non-parametric analysis, and $p<0·05$ was considered significant. All results are given as the mean (SD).

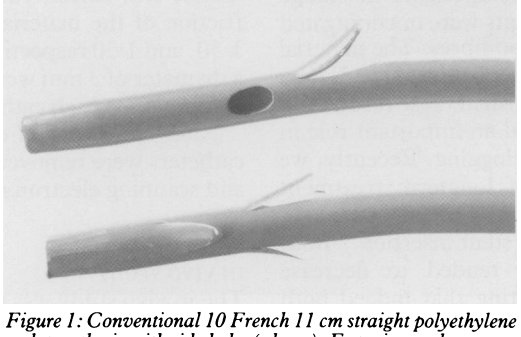

Figure 1: Conventional 10 French 11 cm straight polyethylene endoprosthesis with side holes (above). Experimental endoprosthesis without side holes and superficially cut side flaps (below).

Results

Teflon, polyurethane, polyethylene, polyvinyl chloride, and silicon rubber catheters were perfused with human bile for two weeks. At the end of this period the dry weight of the encrusted sludge was measured. Figure 2 shows that there

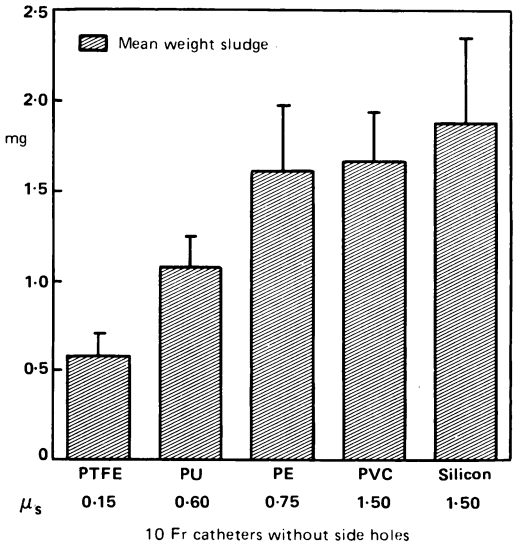

Figure 2: Results of different catheter materials tested in hepatic bile in vitro. The mean weight of sludge accrued in each material is shown, expressed as mean (SD) for 14 different experiments. μ_s is the coefficient of friction. Teflon (PTFE) accrued significantly less sludge than polyurethane (PU) ($p<0·05$) and all other materials tested. PE=polyethylene; PVC=polyvinylchloride.

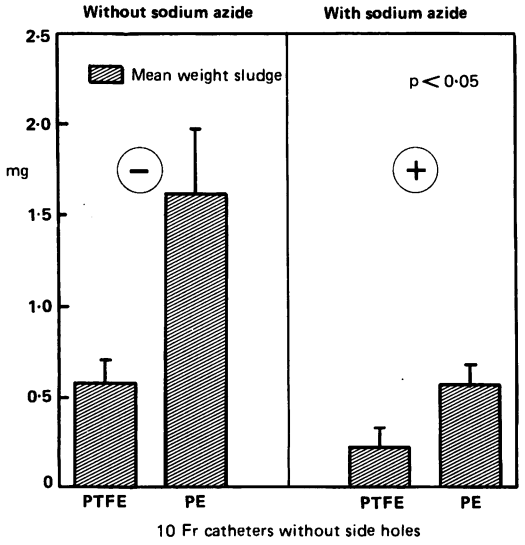

Figure 3: The amount of sludge accrued in hepatic bile without sterilisation is shown on the left. On the right microbial growth was inhibited with sodium azide. Data are expressed as mean (SD) for 14 different experiments. There was significantly less sludge in sterilised bile ($p<0·05$), irrespective of material and design. PTFE = Teflon; PE = polyethylene.

is a direct relation between the frictional coefficient of a polymer and the amount of encrusted material. Teflon was found to be significantly better than all other polymers ($p<0·05$). The effect of bacterial growth on the rate of stent occlusion was investigated by perfusing the stent with either sterilised bile or bile to which bacteria were added. All catheters perfused in the bacterially contaminated bile, irrespective of material and design, accrued more sludge ($p<0·05$) than catheters perfused with sterilised bile (Fig 3).

The effect of the presence of side holes on the rate of sludge formation was tested in stents made of polyethylene or Teflon. As shown in Figure 4A, stents with side holes performed poorly. The amount of sludge in the stents increased significantly in both the Teflon and polyethylene stents ($p<0·05$). The presence of side holes eliminated any differences between the two materials ($p=0·41$).

These results indicate that the detrimental effect of the side holes outweighs the effect of the stent material. We therefore tested the effect of side holes in an in vivo study using only polyethylene stents. Group A consisted of 20 patients who were treated with conventional endoprostheses and group B consisted of 20 patients treated with experimental stents without side holes. The stents were changed routinely after two months. Unfortunately, 10 patients in each group were not followed up for various reasons: four died, seven were terminally ill, three refused follow up, two developed duodenal obstruction, one had surgical biliary bypass, and three had their stents changed because there was no clinical response, though all the stents were patent. There was no difference in the distribution of these non-evaluable patients in the two groups.

At the end of two months all stents were functioning well endoscopically. Upon removal, they were all patent as judged by eye. All the stents were subjected to quantitative sludge analysis. Two stents from each group were scanned by electron microscopy of the inner

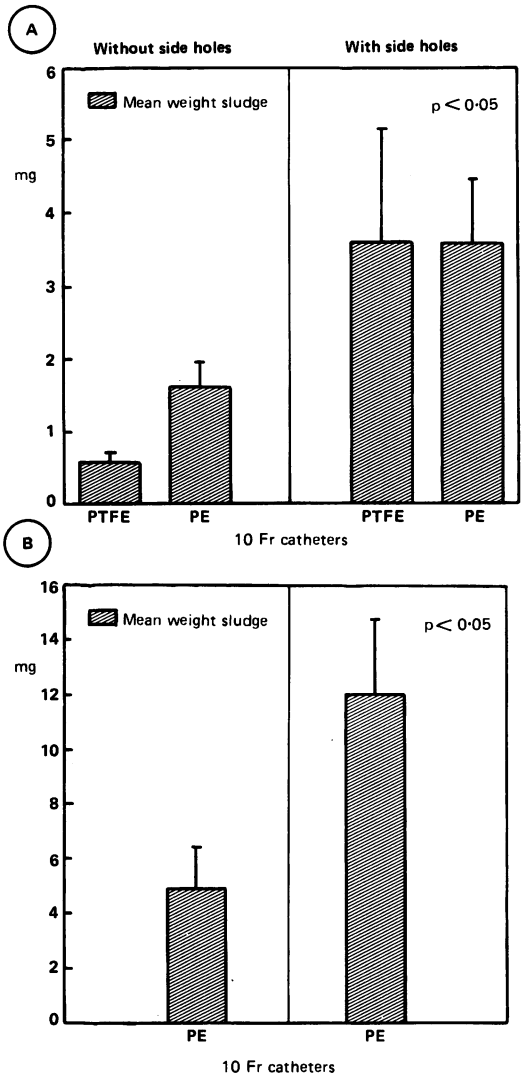

Figure 4: (A) Teflon (PTFE) and polyethylene (PE) catheters were tested in hepatic bile in vitro; (B) PE catheters were tested in vivo. Data are expressed as mean (SD); n=21 and n=10 respectively. In all instances significantly less sludge was formed in catheters without side holes ($p<0·05$). Note that in (A) PTFE accrued the same amount of sludge as PE when side holes were included ($p=0·41$).

wall, with special attention being paid to the areas around the side holes. Bile samples were successfully collected for bacterial culture and chemical analysis in 16 patients, nine from group A and seven from group B. The chemical composition of the bile tested and the spectrum of bacteria cultured from the two groups were not different. Mixed organisms were recovered from all bile samples.

The predominant bacteria were enteric flora with *Enterococcus* sp and *Escherichia coli* (Table I). Human β glucuronidase activity was detected in all bile samples collected. At pH 5·2, the

TABLE I *Distribution of bacteria isolated from bile*

Bacteria	*Bile samples cultured (n=16)**
Enterococcus	12
Escherichia coli	6†
Enterobacter sp	6
Klebsiella oxytoca	6
Streptococcus sp	5
Klebsiella pneumonia	4
Citrobacter freundii	2
Proteus sp	1

*All mixed cultures.
†Three of six *E coli* cultures produced β glucuronidas.

TABLE II *Composition of human bile collected at stent exchange (16 samples)*

Components	Concentration (mean (SD))
Bile acids (mM)	27·7 (14·1)
Phospholipids (mM)	6·9 (3·1)
Cholesterol (mM)	2·8 (1·1)
Protein (g/l)	2·1 (1·3)
Mucin (g/l)	0·12 (0·04)
β Glucuronidase activity (nmol/ml/min)	
pH 5·2	129·6 (97·3)
pH 7·0	17·2 (14·8)*
	41·3 (25·0)†

*No bacterial enzyme activity (n=13).
†Bacterial β glucuronidase activity (n=3).

optimal pH for human β glucuronidase in bile, the mean (SD) maximal velocity of the hepatic bile enzyme was 129·6 (97·3) nmol/ml/min. In 13 samples the enzyme at pH 7·0 was 17·2 (14·8) nmol/ml/min, which was 9–19% of its maximal activity at pH 5·2 indicating little or no bacterial enzyme activity.[24] Of the 16 bile samples, only three in which *E coli* was cultured showed higher bacterial enzyme activity with a mean of 41·3 (25·0) nmol/ml/min. In these samples, endogenous enzyme activity was also found to be relatively high (Table II).

The dry weight of sludge obtained from stents in group B was significantly less (p=0·02) than that obtained in group A (Fig 4B). Most of the stents without side holes contained too little material to determine the concentration of the individual components of the sludge. On scanning electron microscopy sludge which consisted of amorphous material and bacteria was detected along the entire inner surface of the endoprostheses. Sludge accumulation was greatest at the rims of the side holes (Fig 5).

Discussion

Clogging of biliary endoprostheses is caused by several factors. During the past few years it has become clear that two factors play an important part in the initial stage of this process: binding of biliary proteins and adherence of bacteria. In principle, both could be controlled with antibiotics or inhibitors of mucus production. Recently, we reported the results of an in vivo trial in which we assessed the effect of doxycycline and aspirin on the rate of encrustation.[11] Both treatments decreased the amount of material accrued in the stent but the effect was not impressive. In the present study we approached the problem from another angle. It is obvious that the type of biomaterial used for stent construction can play an important part in clogging. In addition, the rheological properties of the stent may be important. For instance, Rey *et al*[25] have shown that the presence of side holes decreases flow through the stent. Our in vitro studies indicate that in straight stents without side holes the frictional coefficient of a material determines the rate of encrustration. Teflon, which we used in this study, had the lowest coefficient of friction and was superior to materials such as polyurethane and polyethylene, which are generally used for stent construction. Unfortunately, Teflon is stiff and hard; it tends to cause more trauma to the bile duct or duodenal wall and presumably produces a higher duodenal perforation rate.[14] Therefore it may not be the most suitable material in practice.

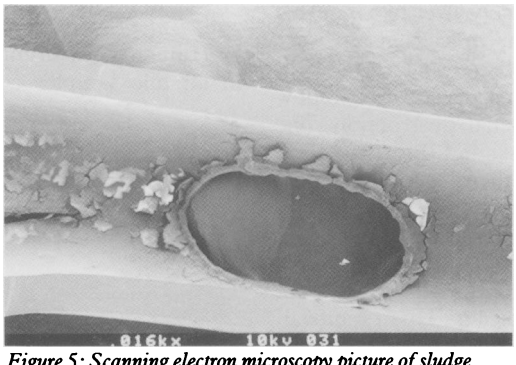

Figure 5: Scanning electron microscopy picture of sludge deposition in an endoprosthesis. Note sludge build up especially around the side hole rim (original magnification ×16).

The presence of side holes in the stent had a dramatic effect on the rate of encrustation. In fact, the superior effect of Teflon was overcome by the apparently more important factor of inner wall irregularity and possible micro-disturbed fluid stream generated by the presence of side holes. Bacteria were important in the accelerating effect of encrustation of the side holes. The addition of sodium azide to inhibit microbial growth greatly decreased sludge formation in both stents with and without side holes.

To investigate the relevance of these results in patients, a clinical trial was carried out in which the effect on encrustation of side holes in the stent was measured over a two month period. We chose not to await complete stent occlusion but rather to change the stents routinely after two months since occlusion is not always readily detectable clinically and jaundice may occur due to concomitant liver pathology. Furthermore, patients undergoing endoscopic stent treatment for malignancy are generally difficult to study, as the survival is short and they will often be treated palliatively at home and hence default. In our study the drop out rate at the end of two months was about 50%. A similar high drop out rate was observed in our previous study.[11] Even though the number of patients remaining in both groups was low, omitting the side holes in the stents had a noticeable effect. The amount of sludge decreased by more than 50% (p<0·05). There was no difference in the composition of bile in the two groups nor was there any difference in the activity of biliary β glucuronidase in the two groups. Although the mechanism of sludge encrustation had presumably not changed, having a stent with a smoother inner wall and no side holes greatly decreased the rate of the process.

Side holes are thought to be necessary in stents to facilitate biliary drainage, both in the hypothetical situation where the end orifice abuts against the ductal wall and when the terminal holes are occluded by cellular debris, blood clots, or mucus plugs. In the present study, however, we found no evidence for this. The absence of side holes in the experimental stents did not cause incomplete drainage or a higher complication rate.

Our data indicate that sludge formation in

stents is diminished by omitting side holes. We infer that their presence is detrimental to stent patency. The effects of different materials used in stent construction are nullified by the presence of side holes. If stents must include side holes to optimise drainage, perhaps for proximal biliary obstructions, their construction must be improved to ensure minimal inner wall irregularity.

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