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Heparinase production by anaerobic bacteria

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SUMMARY The production of heparinase by a wide range of anaerobic bacteria isolated from clinical specimens was investigated. None of the 29 strains of *Bacteroides fragilis* produced heparinase. Of 62 other *Bacteroides* tested, only two of four strains of *B ovatus*, two of three strains of *B thetaiotaomicron*, and two of four strains of *B uniformis* were heparinase producers. None of the 48 strains of fusobacteria or seven strains of *Veillonella* produced heparinase. The anaerobic cocci (19 peptococci and seven peptostreptococci) were also negative for heparinase production as were 46 *Clostridium* spp tested. It was concluded that heparinase production by anaerobic bacteria was unlikely to play a part in the regional thrombophlebitis that sometimes occurs in anaerobic infections.

Septic thrombophlebitis is a potentially serious complication of anaerobic infections, which may lead to the formation of metastatic abscesses. While the responsible agents are poorly defined, *Bacteroides* spp and anaerobic streptococci have been implicated.[1] In confirmed *Bacteroides fragilis* bacteraemia the vascular complications of emboli and thrombophlebitis occur in more than 50% of patients.[2]

Heparin is a naturally occurring acidic mucopolysaccharide that inhibits the clotting of blood in vivo and in vitro. It is found in many tissues, but its highest concentration is in the liver and lungs, and the granules of basophil leucocytes and mast cells.[3] The term heparinase has been used to describe several bacterial enzymes which are thought to act sequentially in the breakdown of heparin to monosaccharides.[4]

Heparinisation has been suggested as a safe and effective alternative to surgical intervention in the management of septic thrombophlebitis,[1] but septic thrombophlebitis associated with *Bacteroides* infection may be difficult to treat with standard heparin,[5] and this has been attributed to the production of heparinase by *B fragilis*.[6]

Recently, however, we examined 128 strains of *Bacteroides* isolated from clinical specimens for heparinase activity.[7] None of 59 strains of *B fragilis* degraded heparin and only strains of *B thetaiotaomicron* and *B ovatus* produced heparinase. These findings were in agreement with those of Steffen and Hentges[8] and Salyers *et al.*[9]

Although most anaerobic infections are polymicrobial, there have been few attempts to determine whether other anaerobic bacteria commonly associated with human infections produce heparinase. The aim of the present investigation was to examine a wider range of anaerobes to determine the extent of this property.

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Material and methods

BACTERIAL STRAINS

A total of 221 strains of anaerobic bacteria, representing nine genera and 49 species, were obtained from the culture collection of the Anaerobe Reference Unit of the Public Health Laboratory Service. The unit receives isolates of clinically important anaerobic bacteria for identification from medical and veterinary diagnostic laboratories throughout England and Wales. All isolates were identified using the methods and criteria of Phillips[10] and Holdeman *et al.*[11] They were stored in brain heart infusion broth containing 10% glycerol at −70°C. Working cultures were maintained in cooked meat medium at room temperature. Tables 1 and 2 list all strains used in the investigation, together with several reference strains which were included for comparative purposes. These were *B fragilis* (NCTC 9343, 9344, and 10581); *B melaninogenicus* (NCTC 9336); *B distasonis* (NCTC 11152); *B hypermegas* (NCTC 10572); *Fusobacterium necrophorum* (NCTC 10575); *Peptococcus indolicus* (ATCC 29427); *Peptrostreptococcus anaerobius* (NCTC 11460); and *Clostridium tetani* (NCTC 9570).

REAGENTS

Heparin was obtained from Evans Medical Ltd as sodium heparin at a concentration of 25000 U/ml.

Table 1 *Heparinase activity in Gram negative anaerobes*

Organism	*No tested*	*No positive*
Bacteroides fragilis	29	0
B vulgatus	9	0
B bivius	8	0
B ureolyticus	7	0
B melaninogenicus	6	0
B distasonis	5	0
B ovatus	4	2
B uniformis	4	2
B pneumosintes	4	0
B oralis	4	0
B thetaiotaomicron	3	2
B ruminicola	2	0
B capillosus	2	0
B disiens	1	0
B loeschii	1	0
B furcosus	1	0
B hypermegas	1	0
Fusobacterium necrophorum	23	0
F nucleatum	8	0
F gonidiaformans	4	0
F mortiferum	2	0
F necrogenes	1	0
F varium	1	0
F naviforme	1	0
Fusobacterium spp	8	0
Veillonella parvula	7	0

Table 2 *Heparinase activity in Gram positive anaerobes*

Organism	*No tested*	*No positive*
Peptococcus magnus	9	0
Pc indolicus	4	0
Pc asaccharolyticus	4	0
Pc prevotii	2	0
Peptostreptococcus anaerobius	5	0
Ps micros	2	0
Clostridium difficile	10	0
C perfringens	7	0
C sporogenes	7	0
C bifermentans	3	0
C septicum	3	0
C tertium	3	0
C sordellii	2	0
C tetani	2	0
C butyricum	2	0
C paraputrificum	2	0
C putrificum	1	0
C histolyticum	1	0
C fallax	1	0
C ramosum	1	0
C scatologenes	1	0
Propionibacterium acne	1	0
Actinomyces israelii	1	0
Bifidobacterium adolescentis	1	0

Toluidine blue was purchased from Sigma Chemicals and used as a 0·01% aqueous solution.

HEPARINASE ASSAY

The heparinase assay described by Riley and Mee[7] was modified so that the test could be carried out in a microtitre tray using an improved heparin broth. The development and assessment of the modified methodology form the basis of a report to be published elsewhere.[12] A strain of *B ovatus* (R1486) known to produce heparinase was used as a positive control throughout the study while a strain of *B fragilis* (R1308) was the negative control. Additional controls included wells of uninoculated media both with and without heparin.

Results

Table 1 shows the heparinase producing ability of Gram negative anaerobes. None of 29 strains of *B fragilis* produced heparinase. Of 62 isolates of other *Bacteroides* spp, only strains of *B ovatus*, *B thetaiotaomicron*, and *B uniformis* were positive. Two of four strains of *B ovatus*, two of three strains of *B thetaiotaomicron*, and two of four strains of *B uniformis* degraded heparin. None of 48 strains of fusobacteria or seven strains of *Veillonella* produced heparinase.

Table 2 shows the results of the heparinase assay for Gram positive anaerobes. The anaerobic cocci, including 19 *Peptococcus* spp and seven *Peptostreptococcus* spp, were negative for heparinase production, as were 46 *Clostridium* spp.

Discussion

The production of heparinase by an unspeciated *Bacteroides* was first described by Gesner and Jenkins.[13] Subsequently, it was concluded that heparinase production by *Bacteroides* in vivo may contribute to both the increased incidence of thrombophlebitis in patients with *Bacteroides* infections and the lack of success of standard heparin treatment.[2 5] Despite the paucity of evidence Finegold[6] attached some clinical importance to heparinase production by *Bacteroides* concluding "a heparinase produced by *B fragilis* may . . . lead to a requirement for an increased dosage of heparin sodium in treating septic thrombophlebitis with this organism." Recent studies, however, suggest that *B fragilis* does not produce heparinase.[7]

The present study confirms and extends our previous findings in relation to heparinase production by *Bacteroides* spp, other than *B fragilis*.[7] Thus in addition to *B ovatus* and *B thetaiotaomicron*, *B uniformis* was also found to degrade heparin. This is a not unexpected finding in view of the close taxonomic association between *B thetaiotaomicron* and *B uniformis*.[14] An additional five species of *Bacteroides*, including the important pathogens *B melaninogenicus* and *B ureolyticus*, were unable to degrade heparin.

As many anaerobic infections are polymicrobial it

seemed reasonable to examine anaerobes that occur in mixed infections other than *Bacteroides* for their ability to degrade heparin. Little is known about the production of hydrolytic enzymes by fusobacteria. In the present study none of 48 strains of fusobacteria degraded heparin. A single strain of *F mortiferum* was reported to show hyaluronidase and chondroitin sulphatase activity.[8] It thus seems that the production of this type of enzyme may have little to do with the virulence of fusobacteria. It was not surprising to find no evidence of heparinase activity among the anaerobic Gram positive cocci. Steffen and Hentges[8] found only one of 11 strains of anaerobic cocci with hyaluronidase activity, and none which produced heparinase. They suggested that because the anaerobic cocci were nutritionally fastidious, their lack of mucopolysaccharidase activity may have been a reflection of their more limited metabolic capabilities.

The clostridia are the most enzymatically active group of anaerobic bacteria, and several important virulence factors have been described.[15] Apart from well documented hyaluronidase activity by several different *Clostridium* spp, however, the production of other mucopolysaccharidases has received little attention. None of the 15 different species of clostridia tested in the present study was found to produce heparinase. These included known hyaluronidase producers such as *C perfringens* and *C septicum*, suggesting a narrow substrate specificity for the clostridial mucopolysaccharidases rather than the broad substrate specificity which has been reported for *Bacteroides*.[16]

In the light of these findings the role of anaerobic bacterial heparinase, particularly that produced by *Bacteroides* spp in the development of thrombophlebitis, needs reassessment. It seems unlikely that heparinase production by any anaerobic bacteria is responsible for this event. It may well be, as we have suggested,[7] that neuraminidase production by anaerobic bacteria is more important in this setting. Mixing normal human erythroytes with some bacterial neuraminidases results in exposure of Tk polyagglutination determinants on the erythrocyte membrane. Erythrocytes that carry these determinants are polyagglutinable as anti-Tk is present in many adult human sera. Neuraminidase activity is widespread among *Bacteroides*[17] and some clostridia.[18] It is possible that such a mechanism could lead to intravascular clotting and septic thrombophlebitis. Further investigations are required to determine the neuraminidase activity of other anaerobic bacteria and to establish an animal model to test these theories in vivo.

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