Journal of Pharmaceutical Research Vol. 10, No. 1, January 2011 : 30-33.

BINDING OF *STAPHYLOCOCCUS AUREUS* ONTO BOVINE INTESTINAL MUCIN

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Received on : 11.01.2011 Revised : 28.01.2011 Accepted : 31.01.2011

ABSTRACT
Mucins act as protection for the gastrointestinal tract against various invading organisms. They are also crucial in developing drugs against these organisms as well as for other therapeutic purposes. This study was carried out to investigate the binding of *Staphylococcus aureus* onto bovine intestinal mucin *in vitro*. The isolate of *Staphylococcus aureus* was added into graded concentrations of mucin ranging from 100 mg to 15000 mg. The mixture was agitated in test tubes for 5 min. The viable count of the bacteria in the mixture was taken at the end of the agitation period using the surface viable method and incubated along with control containing the same quantity of bacterial suspension at 37 ^{0}C for 24 h. After 24 h incubation, the count was taken so as to determine the cells adsorbed by the mucin. The result of the study indicates that bovine intestinal mucin had binding capacity and hence, a reduction in the total cell counts of *Staphylococcus aureus in vitro*. Thus mucins may enhance the effectiveness of antibiotics when used in combination as they may immobilize the organisms for ready destruction by the antibiotics.

Keywords: *Binding; Staphylococcus aureus; bovine mucin.*

INTRODUCTION

Mucins are the major macromolecular constituents of the mucous secretions that coat the oral cavity and the respiratory, gastrointestinal and urinogenital tracts of animals.[1] Secretory mucins are typically of very high molecular mass (over 1 mDa) and have hundreds of *O*-linked saccharine constituting between 50% and 80% of the molecule by weight[2]. The saccharides are based, at present, on seven core structures and can vary in length from disaccharides to oligosaccharides of approximated 20 monosacharides and exhibit astonishing diversity[3,4]. The biological relevance of this diversity is not fully understood, but one possibility is that they act as 'decoy' receptors for the prevention from binding of pathogens to epithelial cell[5,6]. Owing to the technical problems associated with deglycosylation of mucins, however, the biochemical characterization of the protein backbone of the large discrete mucins has been fraught with difficulties[7,8].

The integrity of the intestinal mucin may also help as a physical barrier to the entrance of bacteria into the underlying tissues. Thus organisms that produce enzymes capable of hydrolyzing mucins can easily establish infections. Thus, the microorganisms that produce sialidases are capable of hydrolyzing cervical mucin and such organisms have been implicated in the pathogenesis of sexually transmitted infections in the female genital tract[9]. Hence, the detection of these enzymes may be indicative of the presence of invading organisms and may be used as a diagnostic tool[10,11]. In this study, the binding of *Staphylococcus aureus* to purified intestinal mucin is presented.

MATERIALS AND METHODS

Materials

Acetone (BDH) and nutrient agar (Oxoid) were the main materials purchased. They were used without further purification. Chitosan was a Sigma-Aldrich product. Mucin was obtained from a batch processed in our laboratory.

Methods

Processing of the mucin

Cow intestine was bought from the abattoir after fresh slaughtering and was prepared in our laboratory. The mucoid layer was scraped from the intestine into chilled water. The material was washed to remove blood materials. The crude mucin so obtained was transferred into chilled acetone in a plastic container. The chilled acetone caused the precipitation of the mucin and the whitish wooly material was washed several times with fresh chilled acetone and collected on a Buchner funnel by means of suction from a vacuum pump. It was allowed to dry under vacuum desiccators containing calcium chloride for 3 weeks. The material so obtained was pulverized using an end runner mill and stored in airtight containers until used[3].

Standardization of the bacterial cells

Twenty-hour old culture of *Staphylococcus aureus* on nutrient agar slant was harvested with 2.5 ml of sterile distilled water to get an approximate concentration of $1x10^9$ CFU/ml. The standardization was done using the surface spread viable method by counting on the

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surface of an over-dried nutrient agar using the Miles and Misra method. This acted as the standard concentration. From this standard concentration, dilutions were made to obtain a suspension containing 10^3 CFU/ml and the count taken.

Preparation of bovine mucin suspension

Bovine mucin was prepared according to an earlier established method[12]. Various quantities of mucin, ranging from 100 mg to 1500 mg, were weighed using an analytical weighing balance. The weighed samples were suspended in 10 ml distilled water and were sterilized at 121^{0}C for 15 min using an autoclave. This was to eliminate foreign organisms.

Binding studies

Known volumes (20 ml) of the suspension of *Staphylococcus aureus* containing 10^3 cfu/ml (determined using the surface spread viable method) were aseptically transferred into the containers containing the mucin suspensions. The mixtures were then subjected to shaking in an Endecotts flask shaker for 5 min. At the end of the 5 min the bacterial count was repeated using the surface viable method. Mucin suspension without the addition of any bacterial cells was also treated similarly and the viable count repeated. Secondly, the suspension of bacteria was also treated like the other but without any mucin added and the viable count repeated.

Interaction of the Mucin with Chitosan

Various ratios of mucin were weighed and allowed to hydrate with various ratios of chitosan. This was allowed to equilibrate for 6 h before the interaction studies were carried out using a differential scanning calorimeter (DSC, ZetaSizer).

The surface viable count method

Sterile nutrient agar of 20 ml volume was aseptically poured into a sterile Petri dish and allowed to solidify. The dish was turned upside down and allowed to stand in an incubator for 30 min to obtain an over-dried nutrient agar medium. The back of the Petri dish containing the medium was marked into 8 parts using a marker. Sterile Miles and Misra pipettes were used to place drops of the treated materials on the marked sections and incubated at 37 ^{o}C for 24 h. At the end of the 24 h incubation period, the viable counts were taken with the help of a magnifying glass.

RESULTS

Table 1 show that the mucin interacted with the chitosan; changing the properties of the mucin in terms of zeta potential, which was also affected by the concentration of the mucin in the system. This is also supported by Figures 1-3. The results of the binding studies of *Staphylococcus aureus* onto the bovine mucin are shown in Figure 4. At mucin concentrations above 800 mg all the cells were bound; that is no cell count could be taken. From the graph, it is clear that the extent of binding is concentration dependent. The result, therefore, shows that mucin has the ability to prevent microbial pathogens such as *Staphylococcus aureus* from penetrating mucosal surface of the intestinal tract. This demonstrates the usefulness of the mucin as a barrier against many organisms.

Table 1. *Various parameters of the mucin used in the studies and its interaction with chitosan*

Record	Type	Sample Name	T(°C)	ZP(mV)	Mob (mcm/Vs)	Cond (mS/cm)
2	Size	Mucin	37			
3	Size	Mucin 5 mg/ml	37			
4	Zeta	Mucin 5mg/ml	37	-1.045	-0.1003	44.34
5	Zeta	Mucin 5mg/ml	37	-0.6283	-0.06029	42.54
6	Size	Mucin 4mg/ml	37			
7	Zeta	Mucin 4mg/ml	37	2.295	0.2202	35.99
8	Size	Mucin 3mg/ml	37			
9	Zeta	Mucin 3mg/ml	37	-1.352	-0.1297	42.25
10	Size	Mucin 2.5 mg/ml	37			
11	Zeta	Mucin 2.5 mg/ml	37	-1.433	-0.1375	41.79
12	Size	Mucin 2 mg/ml	37			
13	Zeta	Mucin 2 mg/ml	37	-2.438	-0.2339	44.49
14	Size	Mucin 1 mg/ml	37			
15	Zeta	Mucin 1 mg/ml	37	-0.3029	-0.02906	45.15
16	Size	Chitosan:Mucin 1:4	37			
17	Zeta	Chitosan:Mucin 1 mg/ml	37	6.968	0.6686	39.77
18	Zeta	Chitosan:Mucin 1 mg/ml	37	10.55	1.012	40.45
19	Size	Chitosan:Mucin 1:1	37			
20	Zeta	Chitosan:Mucin 1:1	37	14.64	1.405	37.01
21	Size	Chitosan:Mucin 2:3	37			
22	Zeta	Chitosan:Mucin 2:3	37	1.534	0.1472	1.695
23	Zeta	Chitosan:Mucin 2:3	37	26.78	2.569	38
24	Size	Chitosan:Mucin 3:2	37			
25	Zeta	Chitosan:Mucin 3:2	37	23.72	2.276	36.91
26	Size	Chitosan:Mucin 4:1	37			
27	Zeta	Chitosan:Mucin 4:1	37	20.76	1.992	35.74
28	Size	Chitosan 1 mg/ml	37			
29	Zeta	Chitosan 1 mg/ml	37	29.5	2.83	42.22
30	Size	Chitosan 2mg/ml	37			
31	Zeta	Chitosan 2mg/ml	37	31.28	3.002	36.78
32	Size	Chitosan 2.5 mg/ml	37			
33	Zeta	Chitosan 2.5mg/ml	37	34.25	3.286	40.62
34	Size	Chitosan 3 mg/ml	37			
35	Zeta	Chitosan 3mg/ml	37	30.61	2.937	36.47
36	Size	Chitosan 4mg/ml	37			
37	Zeta	Chitosan 4 mg/ml	37	42.89	4.115	32.51
38	Size	Chitosan 5mg/ml	37			
39	Zeta	Chitosan 5mg/ml	37	53.94	5.176	33.95

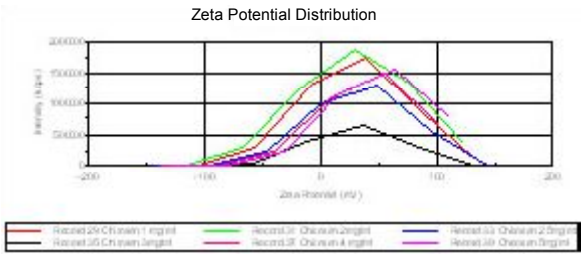

Fig. 1: *Zeta potential distribution for chitosan used in the studies*

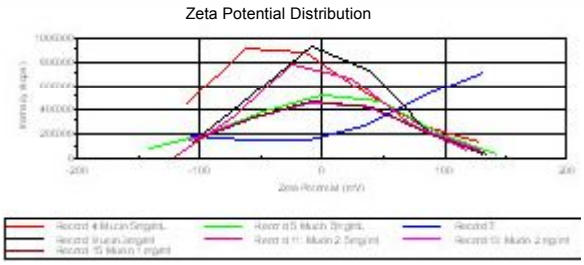

Fig. 2: *Zeta potential of the mucin used in the studies*

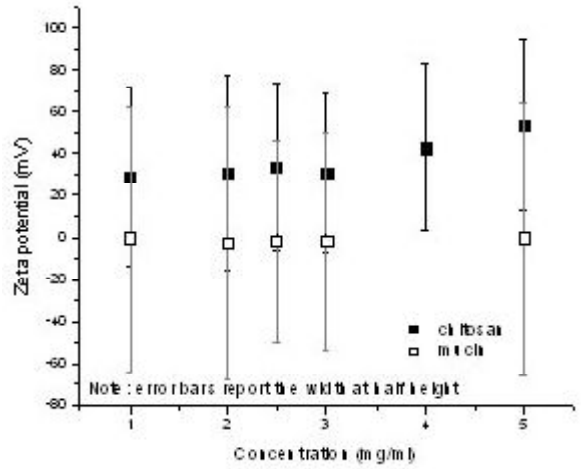

Fig. 3: *Dependence of zeta potential of 250*10^3 g/mol chitosan (black squares) and mucin type II (open squares) on concentration*.

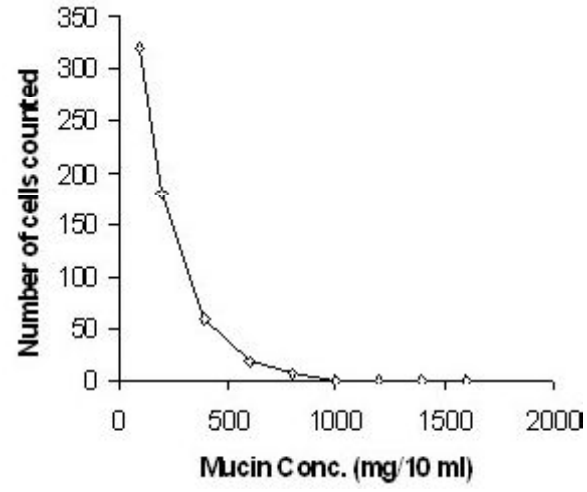

Fig. 4: *Effect of concentration of mucin on the binding of Staphylococcus aureus*

DISCUSSION

Among the specific adhesion-receptor interactions described for *Staphylococcus aureus,* different domains of the protein component of mannoprotein have been shown to specifically recognize *L*-fucose of the disaccharide component. Such a mechanism could be postulated since mucin contains fucose and sialic acid and other monosaccharides as sugar moieties of the *O*-linked glycan[13]. Interestingly, the terminal glycopeptides of intestinal mucin which bind *Staphylococcus aureus* is rich in hydrophobic amino acids.

Previous attempts to characterize the interactions of *Staphylococcus aureus* with mucin have been focused on human nasal mucin[13]. Scanning electron microscopy of *Staphylococcus aureus* incubated with human nasal mucosal tissue showed minimal binding to ciliated respiratory epithelium. Binding of pathogenic micro organisms to mucin is recognized as a critical initial step in successful colonization of host mucosal surfaces[14].

The study reported describes the first example of binding of a microbial pathogen to the protein backbone of the 118-kDa C- terminal glycopetide mucin. *Escherichia coli* also binds to the glycopeptide, but to the *N*-linked oligomannoside side chain. *O*-Glycans in the tandem repeats of mucin represent a far more ubiquitous recognition site for pathogenic bacteria, either through specific receptor-lignand interactions or by hydrophobic-bonding[15]. The production of mucin-degrading proteases, mucinsase, sialidase have been implicated as a virulence factor in the breakdown of mucus and penetration of the mucin barrier by a number of enteropathogenic bacteria[9].

CONCLUSION

The results demonstrate that pathogenic fungus and bacteria may both adhere to, and degrade mucins in the small intestine and both properties may act to modulate microbial populations in the gastrointestinal tract thereby explaining the major role mucin play in protecting mucosal or intestinal surfaces and decrease its susceptibility to infective microorganisms.

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