REVISTA ESPAÑOLA DE FISIOLOGIA, 40, 227-230. 1984

Toxicity of an Algal Mucopolysaccharide for *Escherichia coli* and *Neisseria meningitidis* Strains

M. T. Criado and C. M. Ferreirós

Departamento de Microbiología
Facultad de Medicina y Farmacia
Universidad de Santiago de Compostela (Spain)

(Received on September 13, 1983)

M. T. CRIADO and C. M. FERREIROS. *Toxicity of an Algal Mucopolysaccharide for* Escherichia coli *and* Neisseria meningitidis *Strains.* Rev. esp. Fisiol., **40**, 227-230, 1984.

Several bacterial strains of clinical significance have been tested to assess the toxic effect of a lectin-like algal mucopolysaccharide from *Fucus vesiculosus* on their growth.

The toxic effect of the mucopolysaccharide has been found to be exerted only on *Escherichia coli* and *Neisseria meningitidis* strains. The degree of toxicity, measured by the effect on the growth of the bacteria, is variable depending on the strains of *E. coli* tested, whereas with *N. meningitidis* the results obtained indicate homogeneity between the strains, without significative variations among different serotypes even in the same serogroup.

Key words: Mucopolysaccharide, *Escherichia coli, Neisseria meningitidis.*

Lectins are proteins or glycoproteins which specifically bind to sugars, (more or less complex), a peculiarity which enable them to bind to cell surfaces and in this way make a usefull contribution to their study (10). The role of lectins in nature is not well known, although in some cases it has been stated that they can mediate the association between bacteria and root nodules in leguminous plants or the attachment of gametes during fecundation in certain algae (11). Some of the lectins are toxic to normal and/or neoplasic cells and can interact with many different mammalian cells, but little is known about their interaction with bacterial strains, specially those of clinical significance.

The mucopolysaccharide extracted from the brown alga *Fucus vesiculosus* has been purified to homogeneity and characterized in our laboratory. We have determined that it is a high molecular weight (over 2×10^6) acidic mucopolysaccharide which specifically binds to complex carbohydrates which posess terminal oligosaccharide sequences constituted by ...Man-Gal-NANA (6). It agglutinates erythrocytes from several mammalian species and has an stimulatory effect *in vitro* and *in vivo* to Balb/c mice lymphocytes (3). All

these effects can be neutralized or reversed by the presence of molecules containing carbohydrates with the specific sugar sequence above mentioned (i.e. fetuin, thyroglobulin, etc.). All these facts suggest that this mucopolysaccharide can be considered a lectin-like molecule.

In a previous paper we have shown the specific interaction of the *F. vesiculosus* lectin-like mucopolysaccharide with several *Candida* species and its effect on the growth of the yeasts (2). In this paper we present the study of its action on clinically significative bacterial strains.

Materials and Methods

The following strains were used for this study: *Escherichia coli* JE2571 (Leu$^-$, Thr$^-$, Fla$^-$, Pil$^-$, Str^r), *E. coli* 339 (O15, H11), *E. coli* J53 (Pro$^-$ Lac$^+$, Met$^-$), *E. coli* DF110 (Met$^-$, Lac$^-$, Str^r), *E. coli* 637 (O64, K99), *E. coli* B2C (O6, H16), *E. coli* TD225 (O75, H9), and *Neisseria meningitidis* M136, M981, M986 and B16B6 (all are different serotypes of serogroup B). When not indicated, the O and H antigens of the *E. coli* strains correspond to those of *E. coli* K12.

For all the assays, the strains were cultured in tubes with 5 ml of Trypticase Soya Broth (TSB) inoculated with 10^4 bacteria/ml from 18 h old preinocula in the same medium at 37° C. Culture conditions were 37° C for 24 h, statically for the *E. coli* strains and with a 50 rpm constant shaking for the *N. meningitidis* strains.

For the agglutination assays, the cultures were centrifuged at 8,000 × *g* for 15 min at 4° C, the bacterial cells being then washed three times in phosphate-buffered saline (PBS) and finally suspended at 10^{12} bacteria/ml in the same buffer. The agglutination assays were done in V-shaped Microtiter plates by mixing equal volumes (50 μl) of twofold serial dilutions of a *F. vesiculosus* mucopolysaccharide (FVM) solution in PBS (10 mg/ml initial concentration) with the bacterial suspensions.

The toxicity assays were done by adding different concentrations of FVM (from 40 μg/ml to 1.25 μg/ml) to the culture tubes before their inoculation. Growth was measured spectroscopically at 545 nm each two hours during a period of 24 h. In the tubes with no growth after the incubation time, the bactericidal or bacteriostatic effect of the FVM was determined by spreading 100 μl of the cultures on plates with TSB plus agar and incubating them at 37° C for 24 or 48 h. All experiments were made with five replicates.

Results and discussion

Lectin-cell interactions have been used frequently to obtain structural information about the cell surfaces and cell-wall polymers in several microorganisms (4). Among them are the lipopolysaccharides from several serotypes of *N. gonorrhoeae* and *E. coli* and the surfaces of several enterobacteriaceae (1, 5, 9).

Contrary to the results obtained in studies of the interaction of the *Pseudomonas aeruginosa* lectin with some enterobacteriaceae (8), in which bacterial agglutination was produced but with no effect on the bacterial growth rate, the FVM does not agglutinate any of the bacteria tested and it only affects the growth of those of the genera *Escherichia* and *Neisseria* (in a preliminary screening of the FVM action we tested several clinical isolates of the genera *Citrobacter, Proteus, Serratia, Pseudomonas, Klebsiella, Escherichia* and *Neisseria*; data not shown).

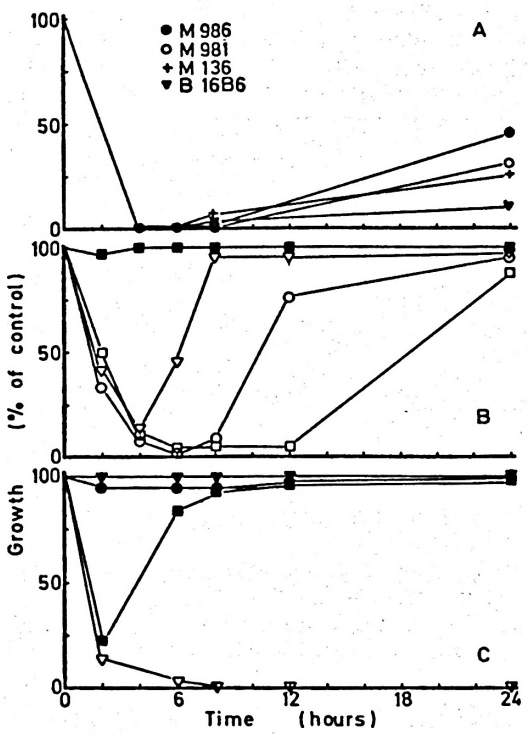

Fig. 1. *Effect of the FVM on the growth of* Neisseria meningitidis *and* Escherichia coli *strains.* Inhibition on the growth of *N. meningitidis* strains in the presence of FVM at 5 μg/ml (A). Effect on the *E. coli* strain 637 (similar plots are obtained with the strains B2C and TD225). Effect on the *E. coli* strain DF110 (similar plots are obtained with the strains J53, JE2571 and 339) (C). FVM concentrations in μg/ml are (□) 40, (o) 20, (△) 10, (■), (●) 2.5 and (▲) 1.25.

The FVM has a bactericidal effect on all the *N. meningitidis* strains at concentrations over 10 μg/ml. At 5 μg/ml it produces an inhibition of the final growth after 24 h of culture. The inhibition values are different for each strain and vary between 55 % and 95 % (figure 1A). At lower concentrations it produces a delay on the lag-phase duration but with no effect on the final growth yield.

With *E. coli*, the FVM at concentrations of 10 μg/ml produces a visible growth inhibition on the strains J53, JE2571, DF110 and 339, whereas the same concentrations produce only a delay in the lag-phase on the other strains. Figures 1B and 1C show the growth-versus-time plots of the most representative experiments.

The effect of the FVM on the *E. coli* strains is therefore variable, depending on the strain and the FVM concentration (table I) whereas on the other enterobacteriaceae the FVM has a null effect on their growth.

With the *N. meningitidis* strains, the FVM has a more homogeneous effect, with no dependence on the bacterial serotypes tested (experiments are under way in order to determine if the serogroup is relevant to the FVM effect). ALLEN *et al.* (1) showed no differences in the agglutinability of several serotypes of *N. gonorrhoeae* (somewhat similar to those of *N. meningitidis*) with different lectins.

The effect of FVM on erythrocytes and other mammalian cells is exerted through its interaction with carbohydrate chains present on the surface of the cells. If the FVM action on bacteria is through a similar mechanism, interacting with surface carbohydrates or glucoproteins, this could indicate that the FVM receptors on *E. coli* and *N. meningitidis* are somewhat similar to those of the above mentioned cells. To this respect, it has been shown that certain *E. coli* strains have surface oligosaccharides immunologically equal to those of the antigenic determinants of human blood groups, reacting with group-specific anti-human erythrocytes antisera (7).

The FVM toxicity mechanisms are not known until now. Experiments are under way in order to determine if its action is through the interaction with the surface of the bacteria or due to antibiotic-like actions.

Table I. *Effect of FVM on the growth of several* Escherichia coli *strains.*
The bacteria were cultured in the presence of the FVM concentrations indicated and the growth was assessed by measuring the absorbance of the cultures (at 545 nm) periodically. The numbers indicate the delay (hours) produced in the lag-phase. Bs = bacteriostatic; Bc = bactericidal.

FVM (μg/ml)	*Escherichia coli* strain						
	JE2571	339	J53	DF110	637	B2C	TD225
0	0	0	0	0	0	0	0
1.25	2	2	0	0	0	0	0
2.50	2	2	0	2	0	0	0
5	2	2	2	2	0	0	0
10	Bs	Bs	Bs	Bs	4	8	4
20	Bs	Bc	Bs	Bs	8	12	4
40	Bc	Bc	Bs	Bc	12	12	12

Resumen

Se estudia el efecto tóxico de un mucopolisacárido de alto peso molecular, con características similares a las lectinas, extraído del alga parda *Fucus vesiculosus*, sobre el crecimiento de diferentes especies bacterianas con significación clínica.

Los resultados obtenidos hasta la fecha indican que este efecto tiene lugar solamente frente a cepas de *Escherichia coli* y *Neisseria meningitidis*, variando la toxicidad según la cepa en estudio, aunque con *N. meningitidis* los resultados obtenidos son más homogéneos que con *E. coli*, sin que haya variaciones significativas entre los diferentes serotipos (dentro del mismo serogrupo).

References

1. Allen, P. Z., Conelly, M. C. and Apicella, M. A.: *Can. J. Microbiol.*, **26**, 468-474, 1980.
2. Criado, M. T. and Ferreirós, C. M.: *Ann. Microbiol. (Inst. Pasteur)*, **133 A**, 149-154, 1983.
3. Criado, M. T. and Ferreirós, C. M.: *IRCS Med. Sci.*, **11**, 286-287, 1983.
4. Boyle, R. J. and Birdsell, D. C.: *J. Bacteriol.*, **109**, 652-657, 1972.
5. Ebisu, S., Sonngren, J. and Goldstein, I. J.: *Carbohydrate Res.*, **58**, 187-191, 1977.
6. Ferreirós, C. M. and Criado, M. T.: *Rev. esp. Fisiol.*, **39**, 51-60, 1983.
7. Garber, N., Glick, J., Gilboa-Garber, N. and Heller, A.: *J. Gen Microbiol.*, **123**, 359-363, 1981.
8. Gilboa-Garber, N. and Mizhari, L.: *Microbios*, **26**, 31-34, 1979.
9. Goldstein, I. J., Murphy, L. A. and Ebisu, S.: *Pure Appl. Chem.*, **49**, 1095-1103, 1977.
10. Sharon, N. and Lis, H.: *Science*, **177**, 949-958, 1972.
11. Sharon, N. and Lis, H.: *Chem. Eng. News*, **59**, 21-44, 1981.