The differential attachment of antibiotics to glycoprotein and blood lymphocytes

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SYNOPSIS The differential attraction *in vitro* of antibiotics to blood lymphocytes and salivary glycoprotein was studied in an attempt to determine the distribution of antibiotics in sputum. Results from studies *in vivo* on sputum antibiotic levels are also presented.

Purulent sputum is a complex mixture of glycoprotein, pus cells, bacteria, and their degradative products. Siggers and Lawson (1969) have shown that bacteria pathogenic in the respiratory tract have an affinity for glycoprotein, and the work of May and Delves (1965) and of Siggers and Lawson (1968) indicates that antibiotics are not randomly distributed within this complex mixture. It is hoped from the knowledge obtained from our later experiments, together with the results on the affinity of bacteria for glycoprotein, that a more specific antibiotic therapy for respiratory infections will be evolved.

Materials and Methods

GLYCOPROTEIN

This was prepared from mixed saliva by the method of Siggers and Lawson (1969).

BLOOD LYMPHOCYTES

These were prepared by the method of Glen (1967) from freshly drawn venous blood from antibiotic-free patients. The lymphocytes were washed and stored in ice-cold 0.15M NaCl before use. Blood lymphocytes, when prepared by the above method, contained 5% polymorphonuclear leucocytes. It was decided to use this system instead of one of pure polymorphs because large numbers of lymphocytes could be

readily obtained in an antibiotic-free state, and the available patients whose polymorph counts were high were invariably on antibiotic therapy. It was also considered that the phagocytic action of the polymorphs would be an uncontrollable variable due to the ingestion of antibiotic-containing particles in the test. We were also only interested in the binding of antibiotics to the cell membrane, the antigenic structure of which is genetically determined and, as far as it is known, is the same for all nucleated cells in an individual.

TEST ANTIBIOTICS

Antibiotics commonly used in the treatment of respiratory infections in cystic fibrosis were chosen for study, namely, ampicillin, benzylpenicillin, carbenicillin, cephaloridine, chlor-tetracycline, cloxacillin, desoxytetracycline, erythromycin, fusidic acid, gentamicin, lincomycin, methicillin, neomycin, oxtetracycline, and tetracycline HCl.

ATTACHMENT OF ANTIBIOTICS TO GLYCOPROTEIN

One millilitre of the prepared glycoprotein was added to 3 ml of the test antibiotic solution in sterile Ringer's saline to give a final antibiotic concentration of 5 µg/ml. The mixture was incubated at 37°C for three hours and then centrifuged at 2,000 rpm in a bench centrifuge for 10 minutes. After the removal of the supernatant, the glycoprotein was washed in 3 ml

of ice-cold Ringer's saline and recentrifuged. The resulting glycoprotein plug was dissolved in 1 ml buffered pancreatin (Oxoid) and assayed for antibiotic content by agar diffusion assay. Antibiotic standards used in the assays were made up in sterile Ringer's saline at 10 µg/ml, 5 µg/ml, and 0.5 µg/ml. The assay organisms used were those recommended by those in charge of the National Collection of Type Cultures.

ATTACHMENT OF ANTIBIOTICS TO BLOOD LYMPHOCYTES

Two million blood lymphocytes were placed into a sterile centrifuge tube and centrifuged for 10 minutes at 2,000 rpm. The supernatant was removed and 3 ml of the test antibiotic solution at 5 µg/ml in Ringer's saline was added to the button of lymphocytes and mixed. The mixture was incubated for three hours at 37°C and the lymphocytes were removed by centrifugation at 2,000 rpm for 10 minutes. The lymphocytes were then washed in 3 ml of ice-cold Ringer's saline, centrifuged, digested in 0.5 ml of buffered pancreatin, and assayed by the agar diffusion technique.

Results

The results of the differential attraction of antibiotics to salivary glycoprotein and blood lymphocytes are shown in Table I. The penicillin

Test Antibiotic at 5 µg/ml	Amount of Antibiotic Attracted to	
	1 ml Glycoprotein	2 × 10 ⁶ Lymphocytes
Ampicillin	Trace	0.12
Benzyl penicillin	0.03	0.14
Carbenicillin	Trace	0.06
Cloxacillin	Trace	0.09
Methicillin	Trace	0.16
Cephalosporin	Nil	0.12
Lincomycin	0.4	0.6
Fucidin	0.5	Trace
Erythromycin	0.12	0.1
Gentamicin	2.2	Nil
Neomycin	1.4	0.4
Chlortetracycline	1.1	Nil
Desoxytetracycline	5.0	0.16
Oxytetracycline	1.5	Nil
Tetracycline HCl	3.5	Trace

Table I *Differential attraction of antibiotics to glycoprotein and blood lymphocytes at pH 7.0*

group of antibiotics showed a marked attraction for lymphocytes, whereas the tetracycline group was strongly attracted to the glycoprotein. The attraction of the tetracyclines to glycoprotein was confirmed by using solutions at 500 µg/ml when the glycoprotein removed the orange-yellow colour from the solution. The

other antibiotics tested showed varied affinities for the two systems.

In view of these results, antibiotic assays were done to determine the antibiotic content of cystic fibrosis sputum before and after washing three times in ice-cold Ringer's solution. Microscopic examination of the washed sputum showed that only mucus plugs remained after washing. The results of these pilot experiments for cloxacillin and gentamicin are shown in Table II.

Patient	Antibiotic Levels (µg/ml)			
	Cloxacillin		Gentamicin	
	Whole Sputum	Washed Sputum	Whole Sputum	Washed Sputum
1	0.35	Nil	—	—
2	0.4	Nil	—	—
3	0.9	Trace	—	—
4	3.6	0.7	—	—
5	—	—	1.8	1.2
6	—	—	0.24	0.23
7	—	—	0.3	0.29

Table II *Studies in vivo on the differential attachment of cloxacillin and gentamicin to glycoprotein and pus cells*

Discussion

May and Delves (1965) have shown that the levels of ampicillin obtained in bronchitic sputum are dependent on the purulence of the sample. A similar relationship between purulence and penicillin level was also noted by Saggars and Lawson (1968) in one case of cystic fibrosis and cloxacillin. These findings, together with the results of this series of experiments, indicate that the penicillin group of antibiotics enters the respiratory tract via the lymphocytes.

The occurrence of a fully cloxacillin-sensitive *Staphylococcus aureus* in the mucopurulent sputum of a cystic fibrosis patient on prophylactic cloxacillin (Lawson, 1969) can therefore be explained by the lack of cloxacillin present in the sputum, especially the glycoprotein to which the staphylococcus would be strongly attracted (Saggars and Lawson, 1969). Any available cloxacillin in the sputum would be found in the pus cells and debris, thus enabling the organism to survive without a pathogenic response, as its penetration of the surrounding lung tissue would be prevented by the adequate cloxacillin tissue and blood levels maintained by prophylaxis.

Since this report, another case has occurred in which serum precipitins to ribitol teichoic acid were absent (Burns and May, 1968). In both cases the *Staph. aureus* was eradicated by the use of lincomycin which is distributed between glycoprotein and lymphocytes.

The use of tetracyclines in the treatment of respiratory infections due to *Haemophilus in-*

fluenzae should be more successful than using ampicillin as both the tetracyclines and *H. influenzae* are attracted to mucus. Millard and Batten (1963), however, did not find any difference between ampicillin and tetracycline therapy in bronchitis, possibly due to the bactericidal effect of ampicillin overcoming the advantages of a bacteriostatic antibiotic being attached to the glycoprotein.

Saggars and Lawson (1966) also noted that binding of the tetracyclines to hog gastric mucin could be reversed to some extent by pretreatment with ethylene diamine tetracetic acid which indicated that some chelation reaction was occurring, thus affecting tetracycline activity.

The frequent failure of gentamicin to eradicate sensitive strains of *Pseudomonas aeruginosa* from sputum may be attributed to its strong mucus binding as demonstrated by our experiments *in vitro* and *in vivo*. The removal of gentamicin from the complex system of purulent sputum would make it ineffective against *Ps. aeruginosa* which has been shown to be unattracted to glycoprotein (Saggars and Lawson, 1969). The suggestion that gentamicin may be inactivated in this way has already been made by Pines, Raafat, and Plucinski (1967).

Consideration of the relative distribution of antibiotics and bacteria in sputum will in some cases help in the choice of the antibiotic therapy. If the information is considered, together with the maximum sputum antibiotic levels obtainable through the blood/bronchial barrier and the bactericidal or bacteriostatic nature of the antibiotic, a more effective chemotherapeutic control of respiratory infections should result.

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