Sensitivity of *Haemophilus influenzae* to Antibiotics

J. D. WILLIAMS, J. ANDREWS

British Medical Journal, 1974, 1, 134-137

Summary

The use of many different antibiotics to treat chest infection has led us to test the sensitivity of 68 strains of *Haemophilus influenzae* to 15 different compounds. These included established compounds such as ampicillin and tetracycline and newer agents such as cephalosporins and clindamycin. The minimum inhibitory concentrations of the compounds for *H. influenzae* were then compared with blood levels attained after the usual dose regimens. There has been a significant increase in tetracycline resistance in the last few years, but all strains were sensitive to ampicillin, chloramphenicol, sulphamethoxazole, and trimethoprim. Several antibiotics were found to be microbiologically unsuitable for treating *H. influenzae* infections.

Introduction

Haemophilus influenzae is a common respiratory pathogen which is the most frequent infecting agents in both the acute and chronic forms of bronchitis (May, 1968).¹ It is also an important pathogen in other parts of the upper respiratory tract, in meningitis (Mathies *et al.*, 1966), and in osteomyelitis (Taylor and Fallon, 1966). Less commonly it may cause bacterial endocarditis. Because of its demanding growth requirements antibiotic sensitivity tests with this organism are more difficult to perform than with staphylococci or Gram-negative bacilli. For this reason there have been some areas where differing results have been found—for example, with lincomycin. (Zinnemann and Frazer, 1970; Phillips, *et al.*, 1970). Several new antibiotics produced in the past few years have been recommended for chronic bronchitis, often with only sparse information on their activity against *H. influenzae*.

This survey of sensitivity of current strains of *H. influenzae* was undertaken to discover the incidence of strains resistant to agents such as ampicillin and tetracycline, which have

been used for many years in chronic bronchitis, to examine the areas of discrepancy in reported sensitivity findings, and to test the sensitivity of this organism to several recently introduced antibiotics.

Methods

A total of 68 strains of *H. influenzae* isolated from respiratory tract infections were studied. Identity of the strains was confirmed by cultural characteristics, morphology, Gram stains, and growth requirements. The sensitivity of the organisms was determined by incorporating dilutions of antibiotic into Levinthal agar. A phage multiple inoculator was used to inoculate the plates, the final number of organisms added in each inoculum being 1,000. The minimum inhibitory concentrations (M.I.C.s) were read after overnight incubation.

The antibiotics tested were chloramphenicol, ampicillin, amoxycillin, tetracycline, five cephalosporins, two erythromycins, lincomycin, clindamycin, rifamide, trimethoprim, and sulphamethoxazole.

When the sensitivity of the strains had been determined the blood levels attained after a standard dosage of the antibiotics were drawn on the same graph. The blood levels given are those generally accepted (Garrod and O'Grady, 1972; Kucers, 1972). Our own data are used for the amoxycillin and cephalosporin curves.

Results

The M.I.C.s of the 16 compounds tested are shown in the table. The compounds were divided into four groups.

Group 1. Standard Agents and Amoxycillin.—This group includes three agents that have been used against *H. influenzae* for several years—chloramphenicol, tetracycline, and ampicillin—together with amoxycillin, a new analogue of ampicillin. All strains tested were inhibited by 1 µg chloramphenicol per ml and all but six by 0.5 µg/ml. The M.I.C.s of chloramphenicol for all strains were below normally attainable blood levels after a standard dose. With tetracycline 61 strains (90%) were sensitive to 0.5 µg/ml; seven strains (10%) were not inhibited by 4 µg tetracycline per ml and were considered resistant. Ampicillin was highly active against *H. influenzae*, almost all strains being inhibited by 0.12 µg/ml,

Dudley Road Hospital, Birmingham Y18 7QH

J. D. WILLIAMS, M.D., M.R.C.PATH., Consultant Microbiologist
J. ANDREWS, F.I.M.L.T., Chief Technician, Chemotherapy

Antibiotic Sensitivity of 68 Strains of *H. influenzae* to 15 Antibiotics

M.I.C. (µg/ml):	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32 or More
Chloramphenicol							7	45	10	6					
Tetracycline							14	39	8				6	1	
Ampicillin							67	1							
Amoxycillin							7	59	2						
Cephalexin										5	16	15	26	6	
Cephalothin							14	11	19	21	3				
Cephaloridine									4	9	8	16	26	5	
Cephmandole							22	27	18	1					
Cephacetrile										13	32	11	3	5	4
Erythromycin															
Rifamide						34	17	13	4	15	9				
Lincomycin								15	29	1	16	19	23	9	
Clindamycin								9	30	18	11				
Sulphamethoxazole								8	36	5		7	10	1	1
Trimethoprim						39	12	11	3	3					
Trimethoprim/sulphamethoxazole	1	8	21	21	10	4	2	1							
Sulphamethoxazole/trimethoprim					1	8	21	21	11	4	2				

with a few strains inhibited by 0.25 µg/ml. These concentrations were well within peak blood levels of ampicillin. Amoxycillin was found to be always slightly less active than ampicillin, almost all strains being inhibited by 0.25 µg/ml and three strains requiring 0.5 µg/ml. The M.I.C.s were, however, well within the peak blood levels attained from standard doses of amoxycillin (fig. 1).

FIG. 1—Sensitivity of *H. influenzae* to chloramphenicol, tetracycline, ampicillin, and amoxycillin. Hatched area represents range of sensitivity. Blood level curve is shown with vertical time scale after standard dosage of the antibiotic.

Group 2. Cephalosporins.—The results of M.I.C. determinations for three well known cephalosporins—cephalexin, cephalothin, and cephaloridine—together with two new agents—cephamandole (73949 Lilly) and cephacetrile (Ba 36278 Ciba)—are shown in fig. 2. M.I.C.s of cephalexin for *H. influenzae* ranged from 1 to 16 µg/ml, with most strains inhibited by 8 µg/ml, but peak blood levels of cephalexin after an oral dose barely exceed 8 µg/ml. Cephalothin inhibited almost all strains at a concentration of 1 µg/ml, with some strains sensitive to 0.12 µg/ml. Peak blood levels of cephalothin greatly exceeded the M.I.C.s. *H. influenzae* was more resistant to cephaloridine than to cephalothin, the M.I.C.s ranging from 1 to 16 µg/ml; peak blood levels after 500 mg cephaloridine given parenterally exceeded the M.I.C.s. Cephamandole was the most active cephalosporin, with almost all strains of *H. influenzae* being inhibited by 0.5 µg/ml—a figure well within blood level curves obtained with this compound. The last cephalosporin, cephacetrile, inhibited most strains of *H. influenzae* at 4 µg/ml, but some strains were resistant to 16 µg/ml. Peak blood levels after a standard dose again exceeded the M.I.C.s.

Group 3. Macrolides and Related Antibiotics.—These antibiotics were grouped together because of some similarities in their general antibacterial spectrum. The group contained erythromycin base and erythromycin estolate, lincomycin and its 7-chloro derivative clindamycin, and one of the rifamycin antibiotics, rifamide. Erythromycin base was highly active, all strains of *H. influenzae* being inhibited by 0.5 µg/ml. The blood levels reached by erythromycin were not high, however, and some strains were not included by the blood level curve. Erythromycin estolate sensitivity was practically identical with erythromycin, and most strains of *H. influenzae* would be sensitive to the levels attainable in the blood if enough estolate

FIG. 2—Sensitivity of *H. influenzae* to five cephalosporins. Hatched area represents range of sensitivity. Blood level curve is shown with vertical time scale after standard dosage of the antibiotic.

were to be hydrolysed to the base (see Discussion). Lincomycin was the least active agent in this group, with M.I.C.s ranging from 1 to 16 µg/ml. Blood levels of lincomycin after oral dosage did not exceed the M.I.C.s of lincomycin for *H. influenzae*. Clindamycin showed an eightfold increase of activity against *H. influenzae* compared with lincomycin, with sensitivities ranging from 0.25 to 2 µg/ml. Blood levels of clindamycin after normal doses exceeded the M.I.C.s. Rifamide showed similar activity to clindamycin, with M.I.C.s ranging from 0.25 to 2 µg/ml. Nevertheless, peak blood levels of rifamide after 300 mg intramuscularly barely exceeded M.I.C.s of the more resistant strains (fig. 3).

FIG. 3—Sensitivity of *H. influenzae* to erythromycin and its estolate, lincomycin, clindamycin, and rifamide. Hatched area represents range of sensitivity. Blood level curve is shown with vertical time scale after standard dosage of the antibiotic.

Group 4. Trimethoprim and Sulphamethoxazole.—These two compounds were tested alone and in combination. With sulphamethoxazole two groups of organisms were found, one of 49 strains with M.I.C.s ranging from 0.25 and 1 $\mu\text{g/ml}$ and another of 19 strains with M.I.C.s ranging from 4 to 32 $\mu\text{g/ml}$. Thus 28% of the strains showed increased resistance to the sulphonamide. Trimethoprim was highly active, 39 strains (57%) being inhibited by 0.06 $\mu\text{g/ml}$ and 62 strains (91%) being inhibited by 0.25 $\mu\text{g/ml}$. In a combination of 20 parts sulphamethoxazole to one part trimethoprim an eight-to-ten-fold increase in sensitivity was seen, all strains of *H. influenzae* being inhibited by 0.25 $\mu\text{g/ml}$. A combination of 20 parts trimethoprim to one part sulphamethoxazole, however, was not quite as active, and the M.I.C.s ranged from 0.03 to 2 $\mu\text{g/ml}$. (fig. 4).

FIG. 4—Sensitivity of *H. influenzae* to sulphamethoxazole and trimethoprim alone and in combination. Hatched area represents range of sensitivity. Blood level curve is shown with vertical time scale after standard dosage of the antibiotic.

Discussion

Chronic bronchitis is common and acute exacerbations are frequently treated with antibiotics. Several factors, such as the variation in clinical presentation, the wide variety of pathogens (and non-pathogens) isolated from sputum, and the technical difficulties in determining sensitivity, make assessment of therapy difficult. We have examined only *H. influenzae*, but as this is one of the most important respiratory pathogens the findings of the study may be relevant to treatment of chest infections.

Chloramphenicol is recognized as being effective against *H. influenzae* infections and all strains were sensitive to it. The blood levels of chloramphenicol vary widely (Prescott and Nimmo, 1971) but most preparations give blood levels which exceed the M.I.C.s found by us. Many resistant strains to tetracycline have now appeared, and in Birmingham these account for 12% of isolates. Ampicillin has not changed in its activity against *H. influenzae*, our results being identical with those of Stewart *et al.* (1961) and Rolinson and Stevens (1961). A comparison of ampicillin and tetracycline in the treatment of acute bronchitis (Malone *et al.*, 1968; Ayliffe and Pride, 1962) failed to show any advantage of one over the other, but a similar study might now be influenced by the presence of tetracycline-resistant strains. Amoxycillin is almost as active as

ampicillin against haemophilus and the pharmacological advantages of this compound both in its absorption (Kosmidis *et al.*, 1972; Sutherland, *et al.*, 1972) and in its sputum penetration (May and Ingold, 1972) suggest that it is likely to supersede ampicillin.

The cephalosporins showed less activity against *H. influenzae* than one might expect from the wide use of these compounds in chest infections. Cephamandole showed the highest activity against *H. influenzae*, inhibiting over half of the strains at a concentration of 0.25 $\mu\text{g/ml}$ and almost all at 0.5 $\mu\text{g/ml}$. Cephacetrile showed activity intermediate between cephaloridine and cephalothin. Clinical results reported with cephaloridine have been variable (Howard, 1967; Walker *et al.*, 1970). The reported M.I.C.s of cephalexin for *H. influenzae* also vary widely from those of Griffith and Black (1970), who found 50% to have a M.I.C. of 3.1 $\mu\text{g/ml}$, to those of Braum *et al.* (1968), who found the M.I.C. range to be 12.5–100 $\mu\text{g/ml}$. Since completing these tests a further oral cephalosporin, cephadrine, has been introduced which showed similar activity to cephalexin against *H. influenzae* (Williams and Geddes 1973).

Erythromycin showed high activity but is often disappointing clinically in treatment of infections due to haemophilus because of the low and variable blood levels obtained with this compound (fig. 3). The estolate of erythromycin is better absorbed than the base (Griffith and Black, 1964; Bell, 1971) but antimicrobial activity is dependent on the hydrolysis *in vivo* to release the free base; this is a variable factor and the active levels may be only one-fifth to one-third that of the estolate level (Ryden, 1973). Rifamide shows useful activity but with a relatively low blood level, and Citron and May (1969) reported disappointing results in treating acute chest infections with rifamide.

The figures for lincomycin indicate that blood levels do not reach the M.I.C. for haemophilus, but clindamycin shows much improvement *in vitro*. Though our M.I.C. results agree with those of Zinnemann and Frazer (1970) the results of Phillips *et al.* (1970) suggest that haemophilus is resistant to clindamycin.

The results of tests for M.I.C.s of sulphamethoxazole showed a group of *H. influenzae* with increased sulphonamide resistance, and though blood levels of sulphonamide exceed the recorded M.I.C.s sulphonamide sensitivity tests are well known to be highly inoculum-dependent and clinical studies would be needed to determine variation in response to treatment. Trimethoprim was highly active and our results with trimethoprim alone and in combination with sulphonamides were similar to those of Darrell *et al.* (1969). Though most strains were sensitive to 0.06 $\mu\text{g/ml}$ there was a tail of more resistant organisms, with three strains inhibited by 0.5 $\mu\text{g/ml}$ and three by 1 $\mu\text{g/ml}$. May and Davies (1972) regard strains with a "trailing end-point" up to 10 $\mu\text{g/ml}$ as resistant and found 52% of strains from a highly selected group of patients resistant to these levels using a different technique from ours. We are not certain where the borderline of resistance should be placed, but all our strains were inhibited by the expected blood levels. The concentrations of trimethoprim in sputum exceed the blood level by a factor of 2.5 (Reeves, 1971). With the synergistic effect of sulphonamides added to trimethoprim the organisms were about 10 times more sensitive, and clinically, excellent results have been recorded with co-trimoxazole (Hughes, 1969; Pines, 1969).

It is difficult to extrapolate from laboratory results to clinical indications for therapy but there are some conclusions which may be drawn. We found that all strains were sensitive to ampicillin, amoxycillin, trimethoprim, and chloramphenicol. Though there will be some geographical variations in resistance tetracycline was found to be active against 88% of isolates. The existing cephalosporins show only marginal activity against haemophilus and the oral cephalosporins are not indicated. Cephamandole shows high activity against haemo-

philus but can be given only parenterally. Erythromycin, rifampicin, and lincomycin do not appear suitable for treating haemophilus infections and the value of clindamycin remains in doubt.

References

- Ayliffe, G. A. J., and Pride, N. B. (1962). *British Medical Journal*, 2, 1641.
 Bell, S. M. (1971). *Medical Journal of Australia*, 2, 1280.
 Braun, P., Tillotson, J. R., Wilcox, C., and Finland, M. (1968). *Journal of Applied Microbiology*, 16, 1684.
 Citron, K. M., and May, J. R. (1969). *Lancet*, 2, 982.
 Darrell, J., Garrod, L. P., and Waterworth, P. M. (1969). *Journal of Clinical Pathology*, 21, 202.
 Garrod, L. P., and O'Grady, F. W. (1972). *Antibiotic and Chemotherapy*. London, Churchill Livingstone.
 Griffith, R. S., and Black, H. R. (1964). *American Journal of the Medical Sciences*, 247, 69.
 Griffith, R. S., and Black, H. R. (1970). *Medical Clinics of North America*, 54, 1229.
 Howard, P. (1967). *British Medical Journal*, 1, 573.
 Hughes, D. T. D. (1969). *British Medical Journal*, 4, 470.
 Kosmidis, J., Williams, J. D., Andrews, J., Goodall, J., and Geddes, A. M. (1972). *British Journal of Clinical Practice*, 26, 7.
 Kucers, A. (1972). *Use of Antibiotics*. London, Heinemann.
 Malone, D. N., Gould, J. C., and Grant, I. W. B. (1968). *Lancet*, 2, 594.
 Mathies, A. W., et al. (1966). *Antimicrobial Agents and Chemotherapy*, 5, 610.
 May, J. R. (1968). *Chemotherapy of Chronic Bronchitis and Allied Disorders*, p. 26. London, English University Press.
 May, J. R., and Davies, J. (1972). *British Medical Journal*, 3, 376.
 May, J. R., and Ingold, A. (1972). *British Journal of Diseases of the Chest*, 66, 185.
 Pines, A., Greenfield, J. S. B., Raafat, H., Rahman, M., and Siddiqui, A. M. (1969). *Postgraduate Medical Journal*, 45, Nov. Suppl., p. 89.
 Phillips, I., Fernandes, R., and Warren, C. (1970). *British Medical Journal*, 2, 89.
 Prescott, L. F., and Nimmo, J. (1970). *Acta Pharmacologica et Toxicologica*, 29, Suppl. No. 3, p. 288.
 Reeves, D. S. (1971). *Journal of Clinical Pathology*, 24, 430.
 Rolinson, G. N., and Stevens, S. (1961). *British Medical Journal*, 2, 191.
 Ryden, R. (1973). Personal communication.
 Stewart, G. T., Coles, H. M. T., Nixon, H. H., and Holt, R. J. (1961). *British Medical Journal*, 2, 200.
 Sutherland, R., Croydon, E. A. P., and Rolinson, G. N. (1972). *British Medical Journal*, 3, 13.
 Taylor, J. C., and Fallon, R. J. (1966). *Lancet*, 1, 715.
 Walker, A. N., Bailey, A., Hart, S., Hadley, A., and James, D. G. (1970). *Postgraduate Medical Journal*, 46, Oct. Suppl., p. 146.
 Williams, J. D., and Geddes, A. M. (1973). *British Medical Journal*, 2, 613.
 Zinnemann, K., and Frazer, J. (1970). *British Medical Journal*, 2, 481.

Progesterone Deficiency and Premature Labour

A. I. CSAPO, O. POHANKA, H. L. KAIHOLA

British Medical Journal, 1974, 1, 137-140

Summary

Plasma oestradiol 17 β and progesterone levels in 11 patients admitted to hospital for threatened premature labour of unknown aetiology were compared with those of women at similar stages of gestation whose pregnancy was normal. Oestradiol levels in the study group were slightly higher than in the normal controls but their progesterone levels were significantly lower. This progesterone deficiency increased the oestradiol/progesterone ratio in the study group patients, and it increased still more as the progesterone withdrawal continued during premature labour.

Since uterine activity during pregnancy is regulated by a balanced action of several factors a deficiency in progesterone, an opponent of uterine activity, creates a regulatory imbalance which, if uncorrected, provokes premature labour. An increase in uterine volume stimulates uterine activity, and the present study reinforced our previous conclusion that the uterine-volume/plasma-progesterone ratio is a more accurate measure of the state of regulatory balance than the progesterone level alone.

The cause of the progesterone deficiency in these cases remains unexplained, but we suggest that placental growth and function are contributory factors. We are investigating ways of correcting the resulting imbalance in the regulatory mechanism.

Introduction

Agreement is still lacking on the exact nature of the physiological processes that control the onset of labour. A uterine stimulatory mechanism is known to build up during pregnancy (Csapo, 1969), and prostaglandin has been identified as the intrinsic myometrial stimulant. Synthesis of prostaglandin is promoted by the increase in uterine volume (Csapo, 1972) and by oestradiol (Caldwell *et al.*, 1972), and the action of the intrinsic stimulatory mechanism is amplified by oxytocin and by fetal factors (Perry, 1972). That pregnancy is normally maintained despite this stimulus to uterine activity may be explained if the gestational rise in progesterone output is regarded as a fetoplacental effort to counterbalance the action of the stimulatory mechanism, and the terminal fall in progesterone as the end of that effort. Thus pregnancy is maintained by a regulatory balance of opposing forces. If that is so then, provided the potential of the stimulatory mechanism is undiminished, progesterone withdrawal during pregnancy should provoke premature labour. The present study aimed to test this theory and also to re-examine the contention (Csapo *et al.*, 1970) that the uterine-volume/plasma-progesterone ratio is the controlling factor in myometrial activity and that this ratio is a more accurate indicator of the state of uterine regulatory balance than the plasma progesterone level alone.

Patients and Methods

A study group was chosen consisting of 11 patients admitted to hospital because of threatened premature labour. Their average age was 23 years and their average stage of gestation 34 weeks (table I). Criteria for admission to the study were: (1) the presence of uterine activity and more effacement and dilatation of the cervix than expected at 30 to 36 weeks' pregnancy and more than observed at previous examinations; (2) the absence of any clear clinical cause of premature labour, such as hydramnios, fetal death, twin pregnancy, or incom-

Department of Obstetrics and Gynaecology, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

A. I. CSAPO, M.D., Professor

Department of Obstetrics and Gynaecology, Debrecen University, Hungary

O. POHANKA, M.D., Assistant Professor

Department of Obstetrics and Gynaecology, Turku University, Finland

H. L. KAIHOLA, M.Sc., Research Associate