Serum Unbound Levels of Cloxacillin and Erythromycin in Cystic Fibrosis

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Antibiotics are considered to be of vital importance in the improved prognosis of children with cystic fibrosis (Shwachman et al., 1959). However, staphylococci were eliminated from the respiratory tracts of only 20% of 46 patients by oral antibiotics (Shwachman et al., 1959). At the Queen Elizabeth Hospital for Children, London, experience with prophylactic long-term oral antibiotics has been similar (W. F. Young, 1968, personal communication). This may have been due either to an inadequate blood level resulting from an insufficient dose or from malabsorption due to pancreatic dysfunction, or to a failure of the antibiotic to penetrate to the site of infection due to the presence of the characteristic micro-abscesses. Infection could therefore progress in an apparently well child causing further lung damage.

The object of this study was to determine whether malabsorption of cloxacillin or erythromycin occurred in children with cystic fibrosis and whether the dosage given was adequate. We investigated the serum total levels of cloxacillin and erythromycin after oral administration in 40 patients with cystic fibrosis. We measured the extent of binding of the antibiotics by serum proteins in a small number of the patients. These values were used to calculate the serum protein unbound levels from the total levels in the rest of the patients.

The serum protein unbound levels, the biologically active fractions, were compared with the levels of the antibiotics required to inhibit strains of Staphylococcus pyogenes isolated from these patients.

Patients and Methods

We studied 21 patients receiving sodium cloxacillin and 19 receiving erythromycin aged between 5 months and 16 years, who were having continuous antibiotic therapy. All had abnormal sweat electrolytes and evidence of pancreatic insufficiency. 22 had no sputum and in 28 the chest x-ray showed no localized or generalized changes other than thickened bronchial wall pattern. Thus the majority were in a clinically quiescent phase. 28 were out-patients and the rest in-patients. The standard antibiotic dosage used in the unit for many years has been 250 mg. four times daily, but infants below 9 kg. received a relatively higher dosage of 110 mg./kg. in each 24 hours divided into four doses, and children after puberty 500 mg. three times daily. Children under 7 years who needed erythromycin received erythromycin stearate suspension and those over 7 years erythromycin base tablets. Aerosols and sulphonamides were omitted on the morning of the test. Half an hour after the usual therapeutic dose of the antibiotic, breakfast, including the normal amount of pancreatin, was given and venous blood was taken after one and a half hours and four hours in the case of cloxacillin, and after two hours and four hours in the case of erythromycin. The separated serum was kept at −15 °C until assayed.

The levels of total cloxacillin in the sera were determined by the cup method, using nutrient agar seeded...
with *Sarcina lutea* (Rolinson and Sutherland, 1965). This method has an error of ± 15% (R. Sutherland, 1969, personal communication). The erythromycin levels were determined by a disc filter paper method using similar seeded agar (Grove and Randall, 1955).

For estimation of protein binding, the serum protein with its bound antibiotic was separated from the unbound antibiotic by ultra-filtration, and the filtrate was assayed (Rolinson and Sutherland, 1965). Sputum or a cough swab from each patient was cultured. The minimum inhibitory concentrations of cloxacillin and erythromycin for seven strains of *Staphylococcus pyogenes* isolated from these cultures were determined by serial double dilution in nutrient broth, the inoculum being one drop of an overnight broth culture.

**Results**

**Protein binding of antibiotics.** Since 10 ml. serum is required to determine the protein binding of cloxacillin by our methods and there is no significant variation in the extent of serum binding between different normal adults (Rolinson and Sutherland, 1965), we carried out only one estimation. The patient had a serum protein unbound percentage of 7-4%, which did not differ significantly from the normal adult control value of 6.9% or previous results with normal adult sera (Rolinson and Sutherland, 1965).

Smaller volumes of serum were needed for the erythromycin assay and the protein binding was carried out on 4 of our patients' sera. The mean percentage of the serum erythromycin levels, which was protein unbound, was 10.7 (range 10.3-11.0) and did not differ significantly from the normal adult value (R. G. Wiegand and A. H. C. Chun 1966 unpublished data). We have used these results to calculate the unbound levels from the serum total levels in our patients.

**Cloxacillin.** We have expressed the results as the serum unbound levels which have been calculated from the serum total levels using the conversion factor noted above. The mean serum unbound level at 1$\frac{1}{2}$ hours was 0.46 μg./ml. (Fig. 1) (range 0.07-1.31) and at 4 hours 0.21 μg./ml. The heavier children tended to have the lowest levels and this was statistically significant ($p < 0.001$). There was no significant difference in the serum levels between those with purulent sputum, 0.45 μg./ml., and those without, 0.49 μg./ml. The average level in 11 patients with localized or generalized lesions on the chest x-ray was 0.61 μg./ml. and in 9 without, it was 0.36 μg./ml.

**Erythromycin.** The serum unbound levels have been calculated from the total levels using the conversion factor of 10.7%. The mean serum unbound level 2 hours after erythromycin stearate was 0.2 μg./ml. (Fig. 2) (range 0.046-0.5) and erythromycin base 0.15 μg./ml. (range 0.006-0.36). As the base is given to the heavier children, these differences are probably not significant. However at 4 hours it was 0.1 μg./ml. with either preparation. The mean serum unbound level in patients with purulent sputum was 0.2 μg./ml. and with no sputum 0.16 μg./ml. These differences are probably not significant. The chest x-rays of only 3 patients showed localized or generalized lesions, and no conclusions can be drawn about the serum levels in relation to the chest x-rays. There was poor correlation of serum levels with the weights of individual patients.
Antibiotic sensitivity of strains of staphylococcus pyogenes. Seven strains of Staphylococcus pyogenes were isolated from the 40 patients. The levels of cloxacillin and erythromycin required for inhibition of these strains is shown in Fig. 3. All were sensitive to cloxacillin but 4 were resistant to erythromycin.

**Discussion**

Absorption of antibiotics. The mean serum total level of cloxacillin at 90 minutes in our patients was 6.2 µg./ml. which was similar to the levels in normal adults after 500 mg. (Knudsen, Brown, and Rolinson, 1962) and in children without cystic fibrosis who were receiving 250 mg. (Stewart, 1962). Two hours after erythromycin stearate the mean serum total level in our patients was 2.1 µg./ml. This does not differ significantly from the level after 500 mg. in adults (Wiegand and Chun, 1966, unpublished data) or 200 mg. in normal children (Sylvester and Josselyn, 1953). This suggests that there was no malabsorption of the antibiotics in the majority of our patients.

Serum unbound levels and minimum inhibitory concentrations of antibiotics. Both erythromycin and cloxacillin are largely bound to plasma proteins. The protein-bound fractions of drugs are biologically inactive (Davis, 1943; Goldstein, 1949; Rolinson and Sutherland, 1965) and only the protein unbound fraction diffuses freely into the extracellular and intracellular fluids (Goldstein, 1949; Dettli, 1961; Scholtan and Schmid, 1962; Verwey and Williams, 1962). Since these are probably the sites of activity of an antibiotic in the earliest stages of an infection, the serum unbound antibiotic level gives a good indication of the active antibiotic level in the lung. However, the level at the site of chronic infection may be lower due to fibrin or fibrous tissue barriers. Before a therapeutic effect can occur, the serum unbound level of an antibiotic must be higher than the minimum concentration of the antibiotic required to inhibit the growth of the pathogen in vitro (Rolinson, 1967). This observation was derived from average serum levels and is confirmed by our results in individual patients (Table). The serum unbound level did not significantly exceed the minimum level required for inhibition of the patient’s own pathogen in vitro in any patient from whom a staphylococcus was isolated. This suggests that if a staphylococcus is isolated during long-term chemotherapy the patient may have a serum level in the lower part of the range (Fig. 1 and 2), or the pathogen may require a relatively high level of antibiotic for inhibition. Knudsen et al. (1962) showed that the serum level was directly proportional to the dose, and therefore an adequate level could probably be achieved in the majority of these patients by doubling or trebling the dose.

The serum unbound levels after oral administration in several patients were close to the minimum inhibitory concentration of the antibiotics for the pathogens. This means that in these patients there is a narrow safety margin between the serum level required for a therapeutic effect and the level obtained. Therefore, in any acutely ill infant these drugs should be given intramuscularly as this doubles the serum level for the same dosage (Knudsen et al., 1962), and variability of intestinal absorption is eliminated. The small number of strains of staphylococci isolated may be due to our policy of prescribing an antibiotic aerosol in addition to oral antibiotics when staphylococci are cultured persistently from the respiratory tract, or because only strains cultured at the time of the serum sample were included in the investigation.

**TABLE**

Minimum Inhibitory Concentrations of Antibiotics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cloxacillin</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (µg./ml) Unbound Serum Level (µg./ml)</td>
<td>MIC (µg./ml) Unbound Serum Level (µg./ml)</td>
</tr>
<tr>
<td>A</td>
<td>5.0</td>
<td>0.6</td>
</tr>
<tr>
<td>B</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>C</td>
<td>1.25</td>
<td>1.24</td>
</tr>
<tr>
<td>D</td>
<td>0.5</td>
<td>0.45</td>
</tr>
<tr>
<td>E</td>
<td>0.5</td>
<td>0.28</td>
</tr>
<tr>
<td>F</td>
<td>1.25</td>
<td>1.31</td>
</tr>
<tr>
<td>G</td>
<td>0.23</td>
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Dosage of antibiotics. Nayler et al. (1962) showed that 97% of 230 penicillin-resistant strains of *Staphylococcus pyogenes* required 0·125–0·5 \( \mu \)g./ml. of cloxacillin for inhibition. This suggests that for treatment of an infection due to *Staphylococcus pyogenes* by cloxacillin an attempt should be made to attain a serum unbound level of at least 0·5 \( \mu \)g./ml. Our results (Fig. 1) show that this level is usually obtainable by a dose of 250 mg. in children below 20 kg. (about the age of 5 years) and 500 mg. for those over 20 kg. Finland, Hirsch, and Wallmark (1960) found that 75% of 228 strains of *Staphylococcus pyogenes* were inhibited by 0·2–0·4 \( \mu \)g./ml. of erythromycin, and for the reasons mentioned previously it is desirable that the serum unbound level should reach 0·4 \( \mu \)g./ml. Our results (Fig. 2) show a considerable range which is not related to weight, and it is impossible to predict which patients would reach this level with an increase in dose. However, if staphylococci are persistently isolated or the patient does not improve clinically, the pathogens may be eliminated by increasing the dose of either cloxacillin or erythromycin.

Conclusions

There is no malabsorption of either cloxacillin or erythromycin in the majority of patients with cystic fibrosis. Only a small percentage of the antibiotic serum level is protein unbound and biologically active. A relatively high oral dosage regimen (see patients and methods) produces a wide range of serum levels. In some patients the antibiotic serum unbound levels provide little or no safety margin. If staphylococci are isolated despite long-term chemotherapy, increasing the dose may eliminate them.

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References


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