Short reports

In vitro assessment of combined antibiotic and mucolytic treatment for Pseudomonas aeruginosa infection in cystic fibrosis

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SUMMARY The minimal inhibitory concentration of azlocillin for Pseudomonas aeruginosa is appreciably reduced when combined with the mucolytic agent mesna (Mistabron) because of an independent bacteriostatic effect of mesna. Bactericidal activity of azlocillin is unaltered by mesna. Mesna inhalations alone or combined with azlocillin may benefit cystic fibrosis patients with pseudomonas lung infections.

Sodium 2-mercaptoethanesulphonate, mesna (Mistabron) is a mucolytic agent shown to have a beneficial therapeutic effect when given as an intermittent inhalation in selected patients with cystic fibrosis (CF). Many of these patients have chronic lung infections with Pseudomonas aeruginosa and recent work by Hodson et al. suggested that they benefit from long term inhaled antibiotics. An earlier report examined the interaction between N-acetylcysteine (NAC), another mucolytic agent, and the penicillan group of antibiotics. It suggested that NAC reduced the antibacterial effect of the penicillins and warned against their combined use. A more recent study, however, showed that 1% NAC actually potentiated the antipseudomonal activity of carbenicillin in vitro. We measured the antipseudomonal activity of azlocillin when combined with mesna and determined if mesna alone had any antipseudomonal activity.

Method

Twenty isolates of Ps. aeruginosa were tested with azlocillin, mesna, and a mixture of azlocillin and mesna. Fourteen were mucoid strains isolated from CF patients, four were matt strains isolated from CF patients, and two were matt strains isolated from non-CF patients. Suspensions were obtained by incubating one colony of Ps. aeruginosa in 20 mls of antibiotic broth No 3 (Oxoid) for 6 hours at 37°C. One drop of this suspension was added to each antibiotic and antibiotic/mucolytic test solution.

Azlocillin, supplied as a powder, has 90.3% purity. Stock solutions ranging from 200 — 0.8 mg/l were prepared in distilled water. Test solutions, prepared by diluting the stock solutions 1:10 with antibiotic broth No 3, had an azlocillin concentration ranging from 20 — 0.08 mg/l and were used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for azlocillin alone.

Mesna (Mistabron) is supplied in 20% concentration vials and to define the MIC and MBC of azlocillin combined with mesna, 1% and 0.5% mesna were added to the test solutions of azlocillin. To determine if mesna alone had any bacteriostatic or bactericidal activity, stock solutions ranging from 20% — 0.3% were prepared in sterile distilled water. Test solutions ranging from 2% — 0.03% mesna were prepared by diluting stock solutions 1:10 with antibiotic broth. Test solutions were inoculated with one drop of Ps. aeruginosa suspension and incubated at 37°C for 18 hours. Solutions showing no growth were subcultured (using 0.1 ml volume by sterile pipette) onto blood agar and incubated for a further 18 hours at 37°C. MIC’s and MBC’s were determined as the lowest dilution showing no growth and a fourfold or greater change in MIC or MBC was taken as significant.

Results

The minimum inhibitory concentrations for azlocillin alone and for azlocillin combined with 1% mesna are shown in Table 1. All the strains of Ps. aeruginosa were considered sensitive to azlocillin when tested by disc method, but MIC’s varied from 0.08 mg/l — 20 mg/l. When 1% mesna was combined with azlocillin all strains of Ps. aeruginosa showed inhibition of growth, even at very low concentrations of the antibiotic, and on average there was a 32 fold reduction in MIC compared with azlocillin alone. This effect was not seen, however, if a 0.5% con-
concentration of mesna was combined with azlocillin. We then tested the effect of mesna alone and found that it inhibited the growth of all strains of *Ps. aeruginosa*, both matt and mucoid types, at a concentration of 1% and greater. Table 2 shows the MBC for azlocillin alone and for azlocillin combined with 1% mesna. For three strains of *Ps. aeruginosa* there was an appreciable reduction in the MBC when azlocillin and mesna were combined, compared with the results for azlocillin alone, but for the other 17 strains there was no significant alteration in MBC. Furthermore, mesna showed no bactericidal activity of its own when tested up to a maximum concentration of 2%.

**Discussion**

These results show that mesna has a notable inhibitory effect on *Ps. aeruginosa* growth, both alone and when combined with azlocillin. This effect is lost when concentrations of 0.5% mesna or less are used. Roberts et al. showed a reduction in MIC when 1% NAC was combined with carbenicillin and concluded that NAC potentiated the activity of carbenicillin. We conclude that mesna has an independent antipseudomonal activity and that the reduction in MIC seen when mesna is combined with azlocillin is caused by its direct effect on pseudomonal growth and not by its potentiation of azlocillin activity.

Earlier studies showed that mucolytic agents may inhibit antibiotic activity when used in combination. We have shown that mesna, despite inhibiting pseudomonal growth, does not reduce the bactericidal activity of azlocillin, and for three strains of *Ps. aeruginosa* actually potentiates this. We suggest that these drugs are safe to use in the same treatment regimen but do not know if they can be safely mixed together before administration.

How mesna affects *Ps. aeruginosa* growth is not known. In a 20% solution it is hyperosmolar but when diluted to a 1% solution with distilled water it is unlikely to have much osmotic effect. Sodium 2-mercaptoethanesulphonate (mesna) may affect the outer membrane porin proteins of *Ps. aeruginosa*. A related compound with similar disulphide bond disrupting properties, 2-mercaptoethanol, has been shown to modify the outer membrane porin proteins of *Ps. aeruginosa* in vitro and this may inhibit bacterial growth and allow penetration of certain antibiotics.

Mistabron alone has been clinically shown to improve the chronic sputum production of cystic fibrosis patients. As many of these patients have chronic pseudomonas lung infections both the antipseudomonal and mucolytic actions of mesna may be of benefit. Further studies are required to look at the antipseudomonal effect of mesna in vivo as this drug used alone or combined with azlocillin may have an important role in the treatment of chronic pseudomonal lung infection.

**References**

Endotoxaemia in cystic fibrosis: response to antibiotics

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SUMMARY In a study of 6 children with cystic fibrosis receiving intravenous antibiotics for Pseudomonas aeruginosa lung infection, serum endotoxin values were monitored by a modification of the limulus lysate technique. The values fell with treatment, reflecting a response that was not always apparent on clinical assessment. Endotoxin concentrations may offer a more precise way of monitoring the effects of antibiotic treatment in CF patients.

As the respiratory tract of children with cystic fibrosis (CF) becomes colonised with *Pseudomonas aeruginosa* there is usually an associated clinical deterioration. It is because of this association that attempts are often made to control *Ps. aeruginosa* infection by courses of intravenous antibiotics, although it is questionable whether their effect is valuable or prolonged. It is difficult, using routine bacteriological methods, to assess the true extent of the infection since quantitative sputum bacteriology is imprecise. Moreover, the extent of lung damage produced by *Ps. aeruginosa* and the effect of antibacterial treatment can only be assessed indirectly.

All Gram negative organisms produce endotoxin, a part of their cell wall. It is a high molecular weight complex of lipid, polysaccharide, and protein and the bioactive part of the molecule is remarkably conserved among most Gram negative organisms. This means that the biological effects of endotoxin from most of these organisms are very similar despite species differences.

We have developed a rapid, sensitive assay for endotoxin which depends upon its biological activity, and wished to see whether endotoxin was detectable in the serum of patients with exacerbation of CF, and if so, whether the value changed in response to treatment.

**Patients and methods**

As part of a wider study of bacterial antigenaemia in CF, 6 children with CF (4 girls and 2 boys) with ages ranging from 6–19 years were studied. They were admitted to hospital for antibiotic treatment either for an acute exacerbation or in an attempt to improve respiratory function before a new school term. They received their usual diet and physiotherapy. Antibiotic treatment consisted of gentamicin (3 mg/kg/8 hourly, IV) and carbenicillin (50 mg/kg/8 hourly, IV) for 10–14 days.

A clinical scoring system, grading symptoms 0–3 for cough, shortness of breath, and sputum production was devised. Peak expiratory flow rate was measured daily and the growth of *Ps. aeruginosa* from sputum was classed as absent, light, moderate, or heavy.

Blood for endotoxin assay was taken with routine specimens before treatment, after 5–7 days treatment, and where possible after completion of the antibiotic course. Specimens were collected by clean venepuncture into endotoxin free glass bijou bottles, allowed to clot at room temperature, and separated within one hour. Serum was then stored at $-20^\circ\text{C}$ until assayed.

The assay was a modification of the limulus assay, using the chromogenic substrate S2222 (Kabi Diagnostics). The chromogenic substrate increases the sensitivity of the limulus assay and enables quantitative results to be obtained. Before assay the samples were diluted 1:4 with pyrogen free water and heated to 68°C for 30 minutes to destroy any enzyme activity. Ten μl samples and dilutions of the WHO