Stability of Antibiotics and Amino Acids in Two Synthetic L-Amino Acid Solutions Commonly Used for Total Parenteral Nutrition in Children

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The stability and interaction at 29°C of ampicillin, carbenicillin, gentamicin, and polymyxin B were examined in a common electrolyte solution, invertebrate darrow, and in two synthetic L-amino acid solutions, one commercial (vamin with fructose; Vitrum) and the other a neonatal preparation modified for use in newborn infants. The concentration of amino acids was measured before and after the addition of these antibiotics. The concentration of antibiotics was measured over a 24-h period with a microbiological method. The concentration of ampicillin in invertebrate darrow fell 52%, and in vamin with fructose it fell 69%, whereas in the neonatal preparation the fall was only 22%. The concentration of carbenicillin in vamin with fructose fell 37%, and in the neonatal preparation it fell 31%. The combination of ampicillin or carbenicillin with gentamicin or polymyxin B did not influence the activity of the penicillins. The concentration of gentamicin and polymyxin B was unchanged in all solutions over a 24-h period. With the exception of cysteine, the concentration of all amino acids remained constant after 24 h in the neonatal preparation with and without the different combinations of antibiotics. For cysteine there was a fall of 20 to 30%.

Since the appearance of intravenous solutions containing synthetic amino acids and lipid, it has become possible to cover patients' needs for protein, energy, electrolytes, vitamins, and essential fatty acids by so-called total parenteral nutrition (TPN). Within the last few years, this treatment has been used successfully even in small newborn infants. To avoid contamination of the solutions during infusion, it is necessary to use a closed infusion system. Addition of antibiotics to the solutions before the start of the infusion would therefore be preferable to intravenous injection via the system or intramuscular injections, which may often cause problems, particularly in very small newborns with reduced muscle mass. Furthermore, by adding antibiotics to the fluids, which are infused via a pump, it is possible to maintain a steady-state concentration higher than the minimal inhibitory concentration for most bacterial strains. Aminoglycosides administered in such a way have been shown to result in a high cure rate in adults (1).

The addition of antibiotics to the solutions used in TPN raises the question of the stability of antibiotics over longer periods, e.g., 24 h, in such solutions. The stability of antibiotics for 24 h in solutions containing electrolytes and carbohydrate and in protein hydrolysate solutions has been quite extensively investigated (5, 6, 12), whereas the stability and compatibility of antibiotics in synthetic amino acid solutions has only been studied by one group and not with antibiotics in combination (2). The aim of the present study has been to assess the stability of ampicillin, carbenicillin, gentamicin, and polymyxin B, added alone or in therapeutically relevant combinations to synthetic amino acid solutions and to an electrolyte and carbohydrate solution commonly used in newborn infants. In addition, the concentration of amino acids has been measured before and 24 h after the addition of these antibiotics.

MATERIALS AND METHODS

Intravenous solutions. The electrolyte and carbohydrate solution used in the study was invertebrate darrow, which contains (per liter): glucose, 37.5 g; fructose, 37.5 g; K, 9 mmol; Na, 31 mmol; Cl, 26 mmol; and lactate, 14 mmol. As a typical example of a synthetic amino acid solution, the commercially available L-amino acid solution, vamin with fructose (Vitrum AB, Stockholm) was employed. For TPN of newborns, the vamin with fructose was modified by the hospital pharmacy to contain optimal amounts of electrolytes, amino acids, carbohydrates, and vitamins. This solution is here called the neonatal preparation. The com-
position of vamin with fructose and the neonatal preparation is given in Table 1. Bottles that hold 100 ml were used rather than 300- to 500-ml bottles to save solutions and antibiotics.

**Antibiotics and procedures.** The stability of ampicillin (Astra), carbenicillin (Astra), gentamicin (Schering), and polymyxin B (NOVO), added alone or in therapeutically relevant combinations, was tested in the three parenteral solutions. The 0-h concentration was: 1,500 μg of ampicillin per ml, 5,000 μg of carbenicillin per ml, 50 μg of gentamicin per ml, and 40 μg of polymyxin B per ml. The concentrations of antibiotics were chosen to approximate what is normal in neonatal practice for a newborn weighing 3 to 4 kg and receiving an infusion of about 100 ml/kg per 24 h. The antibiotics were separately reconstituted with sterile water just before being added to the bottles. One gram of ampicillin or carbenicillin was reconstituted with 10 and 2 ml of sterile water, respectively, and 1.5 and 1.2 ml of these solutions, respectively, were added to the bottles. Gentamicin was received as a solution containing 10 mg/ml, and 0.5 ml of the solution was added to the bottle. Fifty milligrams of polymyxin B was reconstituted with 5 ml of sterile water, and 0.4 ml was added to the bottle. Portions of the solutions were analyzed before the antibiotics were added, immediately after (zero time), and 3 and 24 h after addition. At the same time, changes in color and turbidity and the appearance of precipitation or gas formation were noted. The pH of each portion was measured with a Radiometer (model 28) pH meter.

**Antibiotic assays.** The concentration of antibiotics was measured microbiologically using a paper disk method (9). *Bacillus subtilis* (a laboratory strain) was used as a test organism for ampicillin and gentamicin, *Pseudomonas ellsworth* (NCTC 10490) was used for carbenicillin, and *Bordetella bronchiseptica* (ATCC 4617) was used for polymyxin B. Ampicillin and carbenicillin were assayed on Bacto antibiotic medium no. 4, gentamicin was assayed on Bacto antibiotic medium no. 5, and polymyxin B was assayed on Bacto antibiotic medium no. 10. When assaying ampicillin and carbenicillin, inactivation of gentamicin and polymyxin B was controlled on Bacto antibiotic medium no. 4 as previously described (8). When assaying gentamicin or polymyxin B in the mixtures, the penicillins were inactivated by penicillinase.

The standard curve for each antibiotic was determined by using standard solutions prepared in the respective intravenous solutions. It was necessary to make a 1:300 or 1:100 dilution of the specimens when the stability of ampicillin or carbenicillin was investigated due to the high concentration of these drugs. Assays were performed in quadruplicate in two bottles of the parental solutions, and the concentration was expressed as a mean of the eight results. All the concentrations were measured immediately after the samples had been taken from the solution investigated.

**Amino acids.** Amino acids were determined in the Technicon Sequential Multisampler Amino Acid Analyzer at zero time and 24 h after the addition of antibiotics to the neonatal preparation. All measurements of antibiotics and amino acids were made at 29°C, which is a common room temperature of a neonatal department.

**RESULTS**

The 0-h determination was given a value of 100% for all antibiotics, since the deviation from the theoretical concentration was below 10%. In Fig. 1, changes in ampicillin and carbenicillin concentrations in the synthetic L-amino acid solutions and invertose darrow are given. At 24 h the mean concentration of ampicillin was 78% of the initial concentration in the neonatal preparation, 31% in vamin with fructose, and 48% in invertose darrow. The concentration of carben-
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The present study has shown that there is a loss of activity of ampicillin of about 50% after 24 h when added alone or in combination with gentamicin or polymyxin B to the carbohydrate-, lactate-, and electrolyte-containing solution, invertose darrow, commonly used in newborns. This confirms the results of previous investigators who studied the deterioration of ampicillin in various carbohydrate solutions (3, 5, 11, 12). Gentamicin and polymyxin B were found to be stable in invertose darrow and might be administered as a continuous intravenous infusion. Gentamicin has been shown by others to be inactivated when incubated with very high concentrations of ampicillin (7); however, in this study, gentamicin was stable in combination with ampicillin.

The stability of four penicillins has earlier been investigated at room temperature in protein hydrolysate solutions for TPN. Thus, in aminosol (Vitrum), there was a fall in ampicillin concentration of about 20% over a 24-h period. Carbenicillin was stable for 24 h (6). It is, however, considered undesirable to mix penicillins with proteinaceous materials before infusion, since immunogenic and allergenic conjugates could be formed.

In the present study, the average fall in ampicillin concentration in the neonatal preparation was 22%, which is of the same order of magnitude as described by Feigin et al. (2), who, in a crystalline amino acid solution (FreAmine), found a fall in ampicillin activity of 28% after 24 h at 25°C. In vamin with fructose, the fall was 69% after 24 h. This difference was not anticipated, because pH changes were only minimal, and total carbohydrate concentrations were the same. Vitamins cannot be incriminated, since the addition of pancebrin did not alter the stability of the different antibiotics (unpublished data). It is probably the differences in electrolyte and amino acid concentrations that are responsible for the better stability in the neonatal preparation. The concentration of carbenicillin in the neonatal preparation fell 31%, and in vamin with fructose, fell 37%. In FreAmine, Feigin et al. found a fall in carbenicillin activity of 19%.

Gentamicin and polymyxin B added alone or in combination with ampicillin or carbenicillin were stable in the two amino acid solutions. However, the origin of gentamicin seems to play a role in the stability of gentamicin in combination with carbenicillin. In two measurements (gentamicin batch no. 5 AMK 222), the concentration of gentamicin fell 44% in combination with carbenicillin. In six subsequent and consec-
utive measurements (gentamicin batch no. 76 K 3003) gentamicin activity did not change after 24 h in combination with carbenicillin.

The fall in cystine concentration after 24 h when ampicillin and carbenicillin had been added to the amino acid solution is probably caused by disulfide binding of cystine to the breakdown products of these two antibiotics, which both have an S-group in the side chain. Since cystine is essential for premature newborns, addition of extra cystine must be considered if these two antibiotics are added to the solution and used in premature infants.

It is concluded that gentamicin and polymyxin B can be administered as a continuous intravenous 24-h infusion added to the three solutions investigated. Carbenicillin should not be given in amino acid solutions. Ampicillin should not be given in invertose darrow or in vamin with fructose as a continuous 24-h infusion. Whether ampicillin can be infused in the neonatal preparation over 24 h is open to discussion. From a microbiological point of view, a 22% fall in activity is acceptable, since the serum concentration would still be much higher than the minimal inhibitory concentration for most bacterial strains. From an immunological point of view, however, a 22% activity fall might lead to the formation of immunogenic and allergenic breakdown products, which may be important in the allergic reactions caused by penicillins (4, 10).

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LITERATURE CITED