Stability of heparin and physical compatibility of heparin/antibiotic solutions in concentrations appropriate for antibiotic lock therapy

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Objectives: The purpose of this study was to determine the biological stability of heparin and to test for physical compatibility in heparin/antibiotic solutions in concentrations suitable for antibiotic lock therapy.

Methods: Solutions were prepared with heparin 5000 U/mL or heparin 10 U/mL and cefazolin 10 mg/mL, ampicillin 10 mg/mL, or piperacillin 40 mg/mL. Solutions of vancomycin 2.5 mg/mL with heparin 5000 U/mL and vancomycin 2 mg/mL with heparin 10 U/mL were also prepared. The ability of each solution to elevate the activated partial thromboplastin time (APTT) of pooled normal plasma and the physical compatibility of the solutions were assessed for 14 days.

Results: The APTT levels never varied by more than 16.4% from baseline. Physical incompatibility never occurred before day 14 in any of the solutions.

Conclusions: Mixing of antibiotics in the concentrations chosen for the study had no clinically significant effect on biological heparin activity, and all solutions were physically compatible for at least 14 days.

Keywords: stability, cefazolin, ampicillin, piperacillin, vancomycin, heparin

Introduction

The Infectious Disease Society of America guidelines for management of infected central venous catheters recommend liberal use of heparin/antibiotic lock therapy (ALT) for intravascular catheter-related bacteraemia. Although it is not clear if heparin prevents clotting of intravascular catheters, it remains standard practice in most centres to use varying concentrations of heparin in central venous catheters. However, there are no published guidelines on the concentration of heparin or antibiotics that should be used in ALT, and minimal published data on the stability of various combinations of heparin with antibiotics, particularly at the temperature present in an intravascular catheter. The purpose of this study was to analyse the biological stability of heparin and to test for evidence of physical incompatibility when heparin is combined with antibiotics in concentrations that would be appropriate for ALT.

Materials and methods

Preparation of solutions

To study the biological stability of heparin, commercially available ampicillin (500 mg, NovoPharm Limited), cefazolin (1 g, NovoPharm Limited), vancomycin (500 mg, PharmaScience), and piperacillin (3 or 4 g, Mayne Pharma Inc.) were reconstituted with sterile water for injection according to the manufacturers’ instructions (except for cefazolin in solutions tested for heparin stability which was reconstituted to 95 mg/mL) and then further diluted with commercially available sodium heparin 10 000 U/mL (mean molecular weight 15 000 Da, Leo Pharma Inc.) and 0.9% sodium chloride (NS) (Baxter, Toronto) to achieve final concentrations of ampicillin 10 mg/mL, cefazolin 10 mg/mL, piperacillin 40 mg/mL, or vancomycin 2.5 mg/mL in heparin 5000 U/mL. These antibiotic concentrations were chosen because they are the concentrations that result when standard...
administration concentrations of intravenous antibiotic solutions are mixed 1:1 with commercially available or pharmacy-prepared heparin solutions and so would simplify the preparation of solutions for ALT. The combined solutions and control solutions of heparin 5000 U/mL, ampicillin 10 mg/mL, cefazolin 10 mg/mL, piperacillin 40 mg/mL, and vancomycin 2.5 mg/mL were incubated in the dark for a total of 14 days at 4°C (to determine stability while being stored in a refrigerator) and at 37°C (to determine stability at the approximate temperature of an intravascular catheter). The procedure was repeated using heparin 1000 U/mL (mean molecular weight 15 000 Da, Leo Pharma Inc.) diluted with NS and the same concentration of antibiotic (except for vancomycin, where a 2 mg/mL concentration was substituted) to make a final heparin concentration of 10 U/mL. These concentrations of heparin were chosen because 5000 U/mL is a common concentration used in haemodialysis lines and 10 U/mL is the concentration used in central venous lines in the paediatric and adult haematology patients in our centre.

To study physical compatibility, the same solutions used to study the biological stability of heparin and additional solutions that would be considered for ALT (heparin 10 U/mL and heparin 5000 U/mL with either vancomycin 1 mg/mL, vancomycin 0.5 mg/mL, piperacillin 20 mg/mL, or piperacillin 10 mg/mL) were prepared by adding 9 mL of a twofold concentration of antibiotic solution to 9 mL of a twofold concentration of heparin solution in NS and stored at 37°C. Solutions were prepared in this manner as immediate precipitation problems had been noted when higher concentrations of vancomycin were added to dilutions of heparin in NS. Control solutions were prepared in the same manner with saline in place of either antibiotic or heparin.

Outcome measures

Solutions were tested for biological heparin stability by measuring the activated partial thromboplastin time (APTT) of the solution diluted in pooled normal plasma (Precision Biologic, Dartmouth, Nova Scotia, Canada) at 0, 24, 48 and 72 h, and then at 14 days. The APTT assays were performed on a STA Compact coagulation analyser (Diagnostica Stago, Asnieres, France). To assess physical compatibility, a single blinded observer visualized 18 mL of each solution under normal room lighting in a sterile clear colourless glass vial against both a black and a white background daily for 10 days and then again on day 14. Physical incompatibility was defined as the presence of any visible precipitation or cloudiness.

Results

Heparin stability

At 4°C with heparin 5000 U/mL, there was a gradual increase in the APTT with time in all solutions except for the heparin/piperacillin solution, where there was an initial increase at 48 h, but then a levelling off at 72 h (Figure 1). The largest increase in APTT was 16.4% in the heparin/cefazolin solution at 14 days (versus 14.1% in...
the heparin control). At 37°C with 5000 U/mL heparin, APTT values fell and then rose again, with the largest deviation from baseline being a decrease of 12.4% in the heparin/vancomycin solution at 24 h (although APTT was then above baseline at 72 h and at 14 days in this solution). With heparin 10 U/mL, at both temperatures APTT levels tended to fall and then rise. The largest deviation from baseline at 4°C was a decrease of 7.2% in the heparin/ampicillin solution at 48 h. The largest deviation from baseline at 37°C was a decrease of 12.7% in the heparin/piperacillin solution at 48 h.

The baseline APTT values were higher for the heparin 10 U/mL and piperacillin solution than for any of the other solutions with heparin 10 U/mL at both temperatures. Further testing was performed to determine whether piperacillin itself might increase the APTT. Pooled normal plasma had an APTT of 31.9 s and pooled normal plasma with a piperacillin final concentration of 2 mg/mL had a negligibly higher APTT of 34.1 s.

**Physical incompatibility**

Physical incompatibility was not detected up to day 14. However, a yellow colour was noted in many of the ampicillin, piperacillin and cefazolin heparin solutions prior to colour change in the control solutions, with the earliest colour change being in the ampicillin 10 mg/mL and piperacillin 40 mg/mL in heparin 5000 U/mL solutions on day 3.

**Discussion**

Biological stability of heparin and physical compatibility were demonstrated in all solutions. The largest drop in APTT from baseline was 12.7%, which is unlikely to be of clinical relevance. In fact, piperacillin appears to exhibit a synergic effect with unfractonated heparin. This lack of effect on the biological stability of heparin fits with previous studies that looked at various concentrations of heparin with benzylpenicillin, ampicillin, methicillin, vancomycin, cefepime, cefazidime, ciprofloxacin, meropenem, teicoplanin, trovafloxacin, and tobramycin.

Because of the high incidence of Gram-positive infections in intravascular catheter-related bacteraemia, vancomycin is the most commonly used antibiotic for ALT. This study demonstrated that vancomycin concentrations ranging from 0.5 to 2.5 mg/mL in heparin 10 U/mL or 5000 U/mL were physically compatible when mixed 1:1 with heparin at a twofold concentration. Previous studies have not reported precipitation when higher concentrations of vancomycin were used with high concentrations of heparin (vancomycin 3 mg/mL in heparin 10 000 U/mL and vancomycin 10 mg/mL in heparin 5000 U/mL). However, one study reported precipitation when a high concentration of vancomycin (10 mg/mL) was mixed 1:1 with a low concentration of heparin (100 U/mL). In ALT studies, vancomycin 1–3 mg/mL in heparin 100–2500 U/mL, vancomycin 0.5 mg/mL and 2 mg/mL in heparin 100 U/mL, and vancomycin 2.5 mg/mL in heparin 2500 U/mL have been used but the absence of precipitation and the methods used to determine physical compatibility were not consistently reported.

The duration of chemical stability of antimicrobials in ALT is an important consideration in determining how often ALT solutions need to be changed. At 25°C in NS and stored in glass, ampicillin 10 mg/mL is stable for 24 h, cefazolin 10 mg/mL for 48 h, piperacillin 40 mg/mL for 48 h, and vancomycin 5 mg/mL for 17 days but there is limited data on the stability of antibiotics at 37°C. There was minimal loss (9% decrease in absorbance values as measured spectrophotometrically) of cefazolin when combined at 10 mg/mL with heparin 5000 U/mL in glass for 72 h at 37°C, but significant loss (27.4% reduction in absorbance values) in a polyurethane catheter.6 Future stability studies of ALT solutions should be carried out at body temperature in polyurethane rather than in glass vials.

There is a need for further randomized, controlled trials to demonstrate if ALT is effective for intravascular catheter-related bacteraemia. It appears that solutions containing vancomycin 2 mg/mL, cefazolin 10 mg/mL, ampicillin 10 mg/mL, or piperacillin 40 mg/mL in heparin 5000 U/mL or heparin 10 U/mL would be appropriate ALT solutions to study.

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**Transparency declarations**

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