Intravenous infusions of heparin and penicillins

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SYNOPSIS The chemical stability and anticoagulant activity of heparin (20 U/ml) were studied in five intravenous fluids at room temperature. Heparin remained stable and active for 24 hours in normal saline, but there was a rapid inactivation of 40 to 55% in solutions containing dextrose or lactate, as measured by chemical and biological methods.

High concentrations of benzylpenicillin, ampicillin, or methicillin had no effect on heparin activity in normal saline or dextrose 5%: nor was the stability of the penicillins in these fluids affected to any marked extent by the presence of heparin. Ampicillin was, however, found to be unstable in dextrose 5%, and it would be preferable for it not to be added to dextrose infusions. It is concluded that heparin may be given intravenously in normal saline with benzylpenicillin, ampicillin, or methicillin but several other antibiotics were found to be unsuitable for concurrent infusion with heparin.

Heparin is frequently given by intravenous infusion, either alone or in conjunction with other agents. A survey of drugs administered by this route in the Hadassah University Hospital showed that heparin sodium was involved in 11% of all instances where one or more drugs were added to intravenous bottles (Jacobs, 1972). It may be used either as a primary therapeutic agent in the prophylaxis and treatment of thromboembolic disorders, or incorporated as a secondary agent to prevent blockage of the intravenous needle due to local clotting. As such infusions may be administered over periods as long as 24 hours, it is essential that the vehicle chosen and any drug added should not significantly reduce the anticoagulant activity of the heparin. It is also important that the heparin should not adversely affect the activity of the other drug.

Despite many years of clinical usage, data concerning the stability of heparin in intravenous fluids are both scarce and conflicting, and there is little information about its physicochemical interaction with other agents. Antibiotics, particularly benzylpenicillin and the semi-synthetic penicillins, are among the agents most often mixed with heparin in intravenous infusions. Indeed, intravascular

coagulation has been reported as a complication of Gram-negative septicaemia (Preston, Malia, Sworn, and Blackburn, 1973). Joint research was therefore undertaken by Beecham Research Laboratories and the Department of Pharmacy Services of the Hadassah University Hospital to determine the stability profile of heparin in a range of common intravenous fluids, and to assess the compatibility and stability of mixtures of heparin with benzylpenicillin, ampicillin, or methicillin in normal saline and 5% dextrose solution. The suitability of several other antibiotics for infusion with heparin was also investigated.

Materials and Methods

HEPARIN ASSAYS

Heparin sodium 147 U/mg (Organon), derived from mammalian mucosa, was used as the standard and for stability testing. The biological activity of heparin was measured by the method of Jaques and Charles (1941), which utilizes the anticoagulant effect of heparin *in vitro*. Tubes containing serial dilutions of heparin were made up to 0.4 ml with saline. To each tube was added 0.5 ml of bovine plasma, followed by 0.1 ml of a thrombin solution containing 1.75 U/ml in normal saline. The endpoint was judged as the minimum concentration of heparin which prevented clotting in an inverted tube at 25°C.

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The chemical stability of heparin was determined by metachromatic measurement of the light absorbance of a heparin/Azure-A complex. Two ml of pH 7.3 phosphate buffer and 1 ml of a solution containing 85 μ g/ml Azure-A were added to tubes containing heparin in varying dilutions in normal saline. Readings were made using a no. 54 filter in a model 800-3 Klett colorimeter.

HEPARIN STABILITY IN INTRAVENOUS INFUSIONS

Heparin sodium was dissolved at a final concentration of 20 U/ml (10 000 U/500 ml) in either sodium chloride 0.9% (pH 6.8), dextrose 5% (pH 3.9), dextrose 4.3% with sodium chloride 0.18% (pH 3.9), compound sodium lactate injection (Hartmann's solution, Ringer-lactate solution, pH 6.3), or sodium lactate 1/6 M (pH 6.3). Samples of the solutions were assayed for anticoagulant activity and chemical stability at one, four, eight, and 24 hours, during storage at room temperature (20–23°C).

HEPARIN STABILITY IN INTRAVENOUS INFUSIONS CONTAINING ANTIBIOTICS

Assays were repeated using the same concentrations of heparin sodium in solutions of sodium chloride 0.9% and dextrose 5% to which were added either sodium benzylpenicillin 1 mega unit/500 ml, sodium ampicillin 5 g/500 ml, or sodium methicillin 5 g/500 ml. Control experiments showed that the

penicillins did not interfere with the heparin assays.

The addition of erythromycin, cephaloridine, gentamicin, kanamycin, tetracycline, or oxytetracycline to solutions of heparin in sodium chloride 0.9% or dextrose 5% produced visible changes varying from cloudiness to heavy precipitation, and no further biological or chemical testing of these solutions was carried out.

PENICILLIN ASSAYS

Sodium benzylpenicillin (Purapen G, Beecham Research Laboratories) 10 mega units/500 ml, sodium ampicillin (Penbritin, Beecham Research Laboratories) 5 g/500 ml, or sodium methicillin (Celbenin, Beecham Research Laboratories) 5 g/500 ml was dissolved in either sodium chloride 0.9% (pH 5.2) or dextrose 5% (pH 4.0–4.2). Samples were then taken for initial measurements of pH and optical rotation and for iodometric assay of penicillin (British Pharmacopoeia, 1963). The assay method used involves a penicillin concentration of approximately 0.1% w/v, and thus a 1 in 10 dilution of the sample was necessary. Solutions were stored at room temperature (20–25°C) and sampling was repeated at six, 16, and 24 hours. One blank and two test estimations were made at each time interval. The mean initial assay value was taken to represent 100%, and the six-, 16-, and 24-hour assay results could therefore be expressed as percentages of penicillin remaining. Iodine and thiosulphate reagents were freshly prepared daily

Fig 1 Anticoagulant activity of heparin (10 000 U per 500 ml) in five intravenous solutions over a 24-hour period.

and standardized using the appropriate penicillin reference substance. The entire series of experiments was performed in duplicate.

The above procedure was repeated with the incorporation of 10 000 U/500 ml heparin sodium before the addition of the penicillin. Control determinations showed that heparin did not affect the iodometric assay of the penicillins.

Results

HEPARIN STABILITIES

Heparin remained stable and maintained full anticoagulant activity for 24 hours in normal saline at 20 to 23°C. In comparison, a reduction of over 50% in anticoagulant effect was noted at initial assay in dextrose 5% and sodium lactate 1/6M (fig 1). Similarly, there was more than 40% reduction in anticoagulant activity at one hour in dextrose saline and Hartmann's solution (fig 1). Thereafter, the level of activity in each solution was constant for the remainder of the 24 hours. Results for chemical stability matched those for anticoagulant activity.

Assays of heparin in normal saline and dextrose 5% in the presence of benzylpenicillin, ampicillin, or methicillin gave values for stability and degree of activity similar to those obtained in the absence of these compounds. Heparin was therefore unaffected by the addition of the penicillins. In contrast, the other antibiotics tested all showed visible signs either of incompatibility or of limited stability in admixture with heparin. The results of these tests are summarised in the table.

PENICILLIN STABILITIES

Mean values for percentages of benzylpenicillin,

ampicillin, and methicillin remaining at the various time intervals in normal saline and dextrose 5%, both with and without the addition of heparin, are shown graphically in figures 2, 3, and 4. It can be

Fig 2 Stability of sodium benzylpenicillin (10 mega units per 500 ml) in sodium chloride 0.9% and dextrose 5% (a) without heparin and (b) with heparin sodium 10 000 U/500 ml.

seen that benzylpenicillin and ampicillin have good stability in normal saline, and both antibiotics maintain more than 90% of their activity for 24 hours at 20 to 25°C. Heparin did not have a very marked effect. Ampicillin appeared to be slightly more stable in saline in the presence of heparin (the differences were statistically significant at six and 16 hours), whereas decomposition of benzylpenicillin was somewhat higher at 24 hours in the heparin-containing solution. Methicillin was stable for six hours in normal saline whether or not heparin was present, with inactivation of less than 5% at this time. Thereafter, however, the solutions

Antibiotic Added	Concentration	Solution (Dextrose 5% (D) or Sodium Chloride 0.9% (S) Containing Heparin Sodium 20 U/ml)
Erythromycin lactobionate iv (Erythrocin iv)	1.5 mg/ml (300 mg/200 ml)	D Slight precipitate S Precipitate
Cephaloridine (Ceporan)	2 mg/ml (1 g/500 ml)	D Precipitate S Precipitate
Gentamicin sulphate (Garamycin)	1 mg/ml	D Opalescence S Opalescence
Kanamycin sulphate (Teva Labs)	2 mg/ml (1 g/500 ml)	D Precipitate S Precipitate
Tetracycline iv (Tevacycline iv)	1 mg/ml (500 mg/500 ml)	D Heavy precipitate S Clear for 6 hours only
Oxytetracycline iv (Terramycin iv)	1 mg/ml (500 mg/500 ml)	D Slight turbidity after 4 hours S Slight turbidity after 4 hours

Table Effect of mixing heparin with various antibiotics in dextrose or normal saline

Fig 3 Stability of sodium ampicillin (5g per 500 ml) in sodium chloride 0.9% and dextrose 5% (a) without heparin and (b) with heparin sodium 10 000 U/500 ml.

Fig 4 Stability of sodium methicillin (5g per 500 ml) in sodium chloride 0.9% and dextrose 5% (a) without heparin and (b) with heparin sodium 10 000 U/500 ml.

became turbid, with 40 to 45% inactivation at 16 hours.

The most striking finding in the 5% dextrose solutions was the instability of ampicillin. Decomposition was at least 25% at six hours and approximately 45% at 16 hours, though the rate of decomposition then became slower. Heparin had a significant stabilizing effect on the antibiotic but the loss of potency was still unacceptable at six hours. Because of the marked degree of decomposition at six hours, it was decided to investigate the stability of ampicillin in 5% dextrose before this time. These further studies showed that inactivation averaged about 5% at one hour, 12% at two hours, 19% at four hours, and 25% at six hours. Subsequent work using lower concentrations of ampicillin in 5% dextrose (1 or 2 g/500 ml) has shown only marginally slower inactivation of the antibiotic (Lynn, 1973).

Benzylpenicillin retained more than 90% activity for 16 hours in 5% dextrose. Inactivation at 24 hours, however, was about 15% higher in dextrose

than in normal saline. Heparin had a stabilizing effect on benzylpenicillin which was significant at six and 24 hours.

One of the three batches of methicillin tested in 5% dextrose solution showed an average decomposition of just over 10% at six hours, but the remaining two retained 95% or more of their activity at six hours. All three batches showed at least 95% activity at four hours, and infusions would certainly be satisfactory for this period. As in the saline solutions, precipitation took place between six and 16 hours (when the pH had fallen below about 4). Heparin did not have a significant effect on methicillin stability.

During analysis of the results, the significance of an effect was determined by the appropriate form of the *t* test, at the 5% level.

Discussion

Conflicting statements on the stability of heparin in intravenous infusions appear in the literature. Pritchard (1964) noted that heparin solutions underwent rapid loss of potency when autoclaved at below pH 5, and suggested that the drug might lose activity at the relatively low pH of dextrose if mixed with this solution for any length of time. On the other hand, Parker (1969) reported that heparin was stable for 24 hours in 5% dextrose or normal saline. The choice of 5% dextrose as a vehicle for heparin administered by clockwork syringe pump (Handley, 1970) was criticized by O'Riordan and MacGowan (1970), who found heparin therapy very difficult to control when this solvent was used. The latter recommend the use of normal saline or Hartmann's solution. Stock and Warner (1971) found little or no loss of activity in 24 hours at room temperature when heparin was dissolved in 5% dextrose (pH 4.4). A lower limit of pH 3.5 is, however, allowed for dextrose injection BP.

In a recent report of a within-patient cross-over trial comparing dextrose 5% (pH 3.8-4.5) and sorbitol 5% (pH 6.2) as diluents for heparin, Chessells, Braithwaite, and Chamberlain (1972) found little variation in plasma heparin levels at three hours and 12 hours, although there was wider variation in the partial thromboplastin time with kaolin (PTTK) over this period.

In the tests *in vitro* reported here, there was more than a 50% fall in anticoagulant activity of heparin in 5% dextrose solution and about 40% reduction in activity in dextrose saline. Both intravenous solutions had an initial pH of 3.9 and the addition of heparin sodium raised this, on average, to just below 6.0. These losses of activity were noted at

one hour and the levels of activity then remained constant throughout the rest of the 24-hour period of observation. This could account for the fact that the assays of Chessells *et al* (1972) failed to show any marked differences in plasma heparin level between three and 12 hours. Our results may also explain the clinical observations of O'Riordan and MacGowan (1970). Low pH of the vehicle is apparently not the only fact influencing the stability of heparin, however, since solutions containing lactate ions at pH 6.3 were also found to lose about 50% activity. The present results show that heparin is most stable in normal saline of almost neutral pH. The reason for the rapid initial decrease in heparin activity in intravenous fluids containing dextrose or lactate is unknown and should be the subject of further work.

According to the widely accepted criterion that intravenous infusion solutions containing an antibiotic should be used within a period which ensures that loss of activity does not exceed 10%, this study has shown that normal saline infusions of benzylpenicillin and ampicillin are satisfactory for 16 hours and 24 hours, respectively, at room temperature. However, factors such as the serum concentration achieved, in relation to the site and nature of the infection and the sensitivity of the infecting organism, must also be taken into account in deciding upon the method, rate, and frequency of infusion (Lynn, 1971). Methicillin is less stable in normal saline, and in view of the results of this study and earlier work (Lynn, 1970), it should be infused within six hours.

It is clear that the stability of ampicillin in dextrose and other carbohydrate solutions is very limited (Lynn, 1970, 1971). Decomposition was even more rapid than had been indicated by previous work using microbiological assay techniques (Lynn, 1970; Jacobs, Nathan, Superstine, and Sacks, 1970) but the present results agree with other recently published findings (Hiranaka, Frazier, and Gallelli, 1972). Ampicillin is an amino acid with marked buffering properties, and solutions of the sodium salt are alkaline. It was noted that addition of 1.5 g sodium ampicillin to 500 ml dextrose 5% raised the pH from 4.1/4.3 to 8.5/8.9: at 24 hours the pH was still in the region of 8.0. In this pH range, decomposition of penicillins in carbohydrate solutions is accelerated (Simberkoff, Thomas, McGregor, Shenkein, and Levine, 1970). The results of this study indicate that solutions of sodium ampicillin in 5% dextrose should be infused within one to two hours (preferably within one hour) of preparation. Since this period would tend to be exceeded in practice, it would seem preferable for the antibiotic to be given by bolus injection into

the infusion tubing, and not to be added to the fluid in the bottle. Benzylpenicillin and methicillin should be infused within 16 hours and four hours, respectively, when dissolved in dextrose fluids.

Apart from the three penicillins, all of the antibiotics tested were unsuitable for simultaneous administration with heparin in dextrose or saline infusions. In contrast, no important interactions took place between heparin and benzylpenicillin, ampicillin, or methicillin. In heparin/benzylpenicillin mixtures in normal saline both drugs were stable at room temperature for 16 hours, while in heparin/ampicillin mixtures both agents were stable for 24 hours. The limiting factor with mixtures of heparin and methicillin in normal saline is the instability of the antibiotic, and solutions should be infused within six hours.

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