Inhibitory Effect of Heparin on Gentamicin Concentrations in Blood

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In monitoring gentamicin concentrations in the blood of patients with renal insufficiency, the assayed antibiotic concentration was found to be lower when the sample was drawn as heparinized plasma rather than as serum. This lowering of gentamicin concentrations by heparin was studied further by adding increasing doses of heparin and various amounts of gentamicin to human serum. With a range of 2 to 100 units of heparin per ml, gentamicin concentrations in the serum were lowered by 9 to 14%; with higher heparin concentrations, an even greater and increasing inhibition was noticed, reaching 56% for 1,000 units/ml. This inhibitory effect of heparin on gentamicin was reversible by dilution, indicating that it was not due to degradation or to formation of an inactive chemical complex. Underestimation by the laboratory of gentamicin concentrations in blood is likely to be greatest with capillary tubes, with which the concentration of heparin is especially high. With clinical heparinization, the amount of active heparin in the blood does not exceed 10 units/ml and is for the most part under 3 units/ml; thus, therapeutically significant inhibition of the antibiotic is unlikely in patients receiving anticoagulation.

The importance of measuring gentamicin concentrations in the blood of patients with renal insufficiency is well established. In following serial determinations clinically, we observed that gentamicin concentrations were lower when specimens collected for assay consisted of heparinized plasma rather than serum. This study was undertaken to quantitate the effect of heparin on gentamicin, and to assess its importance in the clinical monitoring of gentamicin concentrations in blood.

MATERIALS AND METHODS

To demonstrate the influence of heparin on gentamicin concentrations in blood, 8-ml blood samples were drawn in sterile syringes from a uremic patient at various time intervals after an 80-mg intramuscular dose of gentamicin. The samples were divided equally between tubes containing 143 units of dried heparin and tubes with no anticoagulant (Vacutainer, green and red stoppered, respectively, Becton, Dickinson, and Co.). The specimens were obtained before, during, and after hemodialysis and were assayed for gentamicin by the method to be described below.

To quantitate the effect of heparin on gentamicin, sodium heparin (Lipo-Hepin, 1,000 units of heparin per ml, lot number 410-01, Riker Laboratories) was diluted with sterile water to obtain solutions containing 10, 100, and 500 units/ml. From these solutions, 0.2- to 1.0-ml amounts were pipetted into small sterile vials to give 2, 5, 10, 20, 50, 100, 200, 500, and 1,000 units of heparin. The vials were put into an incubator at 60 C overnight. This permitted evaporation of the water, and the heparin was left as a powder coating the glass wall. A 10-μg amount of gentamicin sulfate standard powder (lot GMC-8-M-65-1, Schering-White Corp.) was diluted in 5.79 ml of buffered saline to give a solution containing 1,000 μg of gentamicin/ml with a pH of 7.2. From this stock solution, dilutions were made in pooled human serum from healthy volunteers to provide concentrations of 1, 2, 4, 8, and 16 μg of gentamicin/ml in greater than 99% serum. From each of these five solutions of gentamicin in serum, 1 ml was then pipetted into the vials containing various amounts of dried heparin powder. Controls consisted of vials containing serum without gentamicin for each heparin concentration, and of vials containing gentamicin in serum but no heparin powder.

Gentamicin concentrations in each of the 60 vials (see Table 1) were determined with an agar-well diffusion assay in which 1.5% Nutrient Agar (Difco) seeded with spores of Bacillus subtilis ATCC 6633 was used (1). The agar wells had an internal diameter of 5.5 mm, and zone sizes were read after overnight incubation at 30 C to 0.1 mm with Vernier calipers. Each determination was made with five or six replications, and the mean zone size was used to read the gentamicin concentration from a standard curve made with solutions containing 0.5, 1, 2, 4, 8, and 16 μg of gentamicin/ml in pooled human serum without heparin (1). To test the reversibility of the inhibition of gentamicin activity by heparin, a vial containing 500 units of dried heparin and 16 μg of gentamicin in 1 ml of pooled human serum was prepared according to the
method described above. Four serial twofold dilutions were made in vials each containing 1 ml of pooled human serum, and the contents of the vials were then assayed for gentamicin by the same agar-well diffusion method.

RESULTS

In the blood of the patient used to illustrate the influence of heparin, gentamicin concentrations were 10 to 20% lower when the specimens to be assayed were collected as heparinized plasma rather than as serum (Fig. 1).

Table 1 summarizes the study designed to quantitate the loss of gentamicin activity in the presence of increasing amounts of heparin for different concentrations of the antibiotic. As little as 2 units of heparin per ml of serum had an inhibitory effect. With amounts of heparin varying from 2 to 100 units/ml, an average of 88% of the expected concentrations of gentamicin were assayable in the vials containing antibiotic concentrations of 1 to 16 μg/ml. The average losses of gentamicin activity for the six concentrations of heparin increasing from 2 to 100 units/ml were 11.4, 10.9, 12.6, 9.4, 11.9, and 14.4%, respectively. An even greater inhibition of gentamicin was noted with heparin concentrations higher than 100 units/ml. With all five gentamicin concentrations, the loss of antibiotic activity averaged 24, 44, and 56% in vials containing 200, 500, and 1,000 units of heparin/ml, respectively. Heparin itself had no antibacterial activity. Figure 2 illustrates the above points graphically and emphasizes the progressive increase in the inhibition of gentamicin activity with concentrations of heparin greater than 100 units/ml.

The inhibitory effect of heparin on gentamicin
was readily reversible by dilution (Table 2). Diluting a solution containing 500 units of heparin/ml and 16 μg of gentamicin/ml serially in serum was associated with a progressive increase in measurable gentamicin activity from 54% of the expected value up to 90% in the final tube that contained 31 units of heparin/ml and 1 μg of gentamicin/ml. These figures are comparable to those obtained in the first experiment.

**DISCUSSION**

These studies clearly demonstrate that the antibacterial activity of gentamicin is inhibited by heparin, and help to clarify the extent and characteristics of the inhibition. Gentamicin activity was decreased by 9 to 14% with heparin concentrations up to 100 units/ml, and inhibition increased progressively with higher concentrations, reaching 56% with 1,000 units/ml. The interaction between the drugs was readily reversible by dilution, indicating that the inhibition of gentamicin was not due to degradation or to the formation of an inactive chemical complex. Although the details are not described, we also studied other commonly used anticoagulants (ethylenediaminetetraacetate, citrate, and oxalate) and found that they did not affect the antibacterial activity of gentamicin. Sabath et al. (8) observed that gentamicin activity was decreased by more than 80% when gentamicin was added to outdated bank blood, but a number of unknown factors, including the anticoagulant used, made their results difficult to compare with ours.

An important practical implication of this phenomenon is the fact that the laboratory will underestimate the concentration of gentamicin when blood submitted for assay is drawn in a heparinized tube since standard curves are usually made in serum. The level reported is likely to be 10 to 15% too low when the correct amount of blood (6 to 7 ml) is added to commercially obtainable tubes containing dried heparin (see the example in Fig. 1), since the final concentration of heparin is well under 100 units/ml. However, if only 1 ml of blood is obtained, about 150 units of heparin/ml is present, and the gentamicin concentration reported may be at least 20% too low. A large error is especially likely to occur with infants and small children, from whom blood specimens are usually obtained in capillary tubes that contain relatively large amounts of heparin. It would be possible, of course, to obviate the error by constructing the standard curves for gentamicin assays in heparinized plasma, but there would still be the problem that the amount of heparin in the samples to be assayed might be quite different from that in the standards. It is evident that whenever possible, blood should be drawn in tubes without heparin, and both the unknowns and standards should be assayed in serum.

Also of interest is the possibility that, in patients receiving heparin, there might be a significant lowering of gentamicin levels in the blood, and that this might actually interfere with the response to therapy. Heparin is a mixture of sulfated mucopolysaccharides that are rapidly eliminated from the body, partially through renal excretion but mainly by metabolism in the liver (5). Clinically effective anticoagulation is achieved by 1 to 2 units of active heparin/ml (7) in blood, and a rapid intravenous injection of 100 units/kg gives a peak blood concentration of about 2.10 units/ml (6). A maximal plasma concentration of 7.74 units/ml is obtained when 400 units/kg is administered intravenously over a 5-min period.
(6); the blood concentration then falls rapidly ($T^{1/2} = 1$ to 2.5 hr; 2, 4) so that, even if the dose is repeated every 6 hr, a level under 3 units/ml is present for at least 90% of the time (3, 6). The results presented in Table 1 make it apparent that the maximal decrease in gentamicin activity to be anticipated would be about 10%. Thus, it can be concluded that a therapeutically significant lowering of the gentamicin concentration is unlikely in the blood of patients receiving heparin.

LITERATURE CITED


