Inhibition of Aminoglycoside Activity by Heparin

LENNART NILSSON,¹* ROLF MALLER,² AND STEFFAN ANSEHN

Departments of Clinical Bacteriology¹ and Infectious Diseases,² Linköping University, Regionsjukhuset, S-581 85 Linköping, Sweden

Received 20 November 1980/Accepted 18 May 1981

Measurements of aminoglycosides by an agar disk diffusion assay are inhibited by heparin in a dose-dependent way. When assayed by a homogeneous immunoassay, this was only evident for tobramycin. This indicates that specimens for aminoglycoside measurement should not be obtained in heparinized tubes. When heparin is used clinically as an anticoagulant, the amount in blood does not reach levels that affect the aminoglycoside activity.

Heparin has been reported to inhibit the measurement of gentamicin concentrations by an agar diffusion procedure (6). This was confirmed, and it was also shown that heparin inhibited gentamicin measurements with a newly developed rapid luciferase method (1, 2). In this report the effects of heparin on gentamicin, amikacin, netilmicin, and tobramycin were studied, and the effect of sampling in clinical practice with heparinized capillaries compared with non-heparinized tubes was evaluated.

MATERIALS AND METHODS

Antibiotic standards. Stock aqueous solutions of 1,000 μg of active aminoglycosides per ml were prepared from amikacin base (910 μg/mg; Bristol Laboratories, Syracuse, N.Y.), gentamicin sulfate (656 μg/mg; Schering Corp., Bloomfield, N.J.), netilmicin sulfate (646 μg/mg; Schering Corp.), and tobramycin sulfate (942 μg/ml; Eli Lilly & Co., Indianapolis, Ind.). From these stock solutions standards of 16, 8.0, 4.0, 2.0, and 1.0 μg of gentamicin, netilmicin, and tobramycin per ml and standards of 32, 16, 8.0, 4.0, and 2.0 μg of amikacin per ml were prepared in pooled human serum.

Effect of heparin on test strain. Escherichia coli LU14 was exposed to 1,000, 100, and 10 IU of heparin per ml in nutrient broth (Difco) for 16 to 18 h, and growth was recorded.

Effect of heparin on aminoglycoside activity. To quantitate the effect of heparin on amikacin, gentamicin, netilmicin, and tobramycin, a sterile water solution of heparin (Vitrum, Stockholm, Sweden) was made containing 1,000 IU/ml. This solution was diluted in twofold steps in sterile water to a final concentration of 1.95 IU/ml. From these solutions, 0.25 ml each was pipetted into 4-ml sterile glass tubes, which were held at 60°C overnight to evaporate water and give a coating of heparin on the tube walls. Each aminoglycoside at concentrations of 16.0, 8.0, and 4.0 μg/ml (0.25 ml) was pipetted into tubes containing each amount of heparin.

To study the reversibility of the inhibitory effect of heparin on aminoglycoside activity, samples from the tubes containing 500 IU of heparin per ml and 16.0 μg of amikacin per ml were diluted in twofold steps in serum and assayed by the agar disk diffusion assay.

The effect of heparin on aminoglycoside activity was also studied by sampling 8.0, 4.0, and 4.0-μg/ml concentrations of each aminoglycoside in pooled human serum with commercially available heparinized capillary tubes containing no preservative (Drummond Scientific Co., Broomall, Pa.).

Clinical samples. Eleven patients with severe infections were treated with gentamicin or amikacin. Most patients also received a second antibiotic, usually a penicillin. The initial doses were either 3 mg of gentamicin per kg of body weight, divided in three daily doses, or 15 mg of amikacin per kg of body weight, divided in two daily doses, given as intramuscular injections. The aminoglycoside concentrations were monitored, usually on day 2 before dosage and after 1, 2, 4, and 8 h.

Samples of venous blood in nonheparinized tubes and skin puncture samples in heparinized capillaries (Drummond Scientific Co.) were taken simultaneously.

Determination of aminoglycosides. The aminoglycoside concentrations were determined by an agar disk diffusion assay with E. coli LU14 as the test strain and antibiotic medium no. 2 (Oxoid) (pH 7.9) as the assay medium. E. coli LU14 is resistant to most antibiotics encountered in combination with aminoglycosides (3).

The aminoglycoside concentrations in the clinical samples were determined only by the agar disk diffusion assay, whereas in all other samples they were also determined by a homogeneous enzyme immunoassay (EMIT; Syva Corp., Palo Alto, Calif.). Netilmicin was determined by an EMI gentamicin assay kit. It is not possible to determine amikacin by a commercially available EMI kit. All assays were performed in duplicate.

RESULTS

The growth of E. coli LU14 was not affected by heparin itself. The inhibitory effect of heparin on the recovery of amikacin, gentamicin, netilmicin, and tobramycin (8 μg/ml) in the presence...
of increasing amounts of heparin in pooled human serum, as determined by the agar disk diffusion assay, is shown in Fig. 1. The inhibition increased quantitatively at 62.5 IU of heparin per ml. Similar results were obtained with 16 and 4.0 μg of the aminoglycosides per ml. The inhibition of tobramycin (8 μg/ml) when assayed by EMIT was more pronounced, and started at a lower concentration of heparin, whereas gentamicin and netilmicin were less affected (Fig. 1). The results presented in Fig. 1 are the mean values of five experiments with the agar disk diffusion assay and two experiments with EMIT. The standard deviation ranged between 0.9 and 10.1% (mean, 5.0%) for the agar disk diffusion assay and between 0.7 and 8.6% (mean, 3.7%) for EMIT.

The results from sampling aminoglycosides (8 μg/ml) with heparinized capillaries are given in Table 1. The recovery of aminoglycosides with EMIT was higher than with the agar disk diffusion assay, except for tobramycin, where the recovery was much lower.

The inhibitory effect of heparin on the aminoglycoside activity was reversible by dilution (Table 2).

The mean concentrations of gentamicin (six patients) and of amikacin (five patients) in samples of venous blood (serum) and in simultaneously taken blood in heparinized capillaries (plasma) are given in Fig. 2. All gentamicin concentrations in heparinized plasma were lower than corresponding concentrations in serum, whereas 3 of 25 amikacin plasma concentrations were higher than corresponding serum concentrations.

When the aminoglycoside plasma concentration was expressed as a percentage of the aminoglycoside serum concentration, the mean recovery of gentamicin was 78.2% (range, 64.7 to 90.5%), and that of amikacin was 91.3% (range, 66.7 to 111.1%).

In six cases, fingertip skin punctures were sampled with both heparinized and nonheparinized capillaries, and all of the former samples had lower gentamicin concentrations than the latter. The mean recovery of gentamicin in the heparinized samples was 77.7% (range, 70.1 to 87.5%). Furthermore, none of six fingertip skin punctures sampled in nonheparinized capillaries gave lower gentamicin concentrations than simultaneously taken venipuncture samples.

**DISCUSSION**

The observation by Regamey et al. (6) that heparin reversibly inhibited the gentamicin activity in a dose-dependent way was recently confirmed by Nilsson (2). In the present study similar results were obtained with amikacin, netilmicin, and tobramycin when the drug concentrations were determined by the agar disk diffusion assay. However, when assayed by EMIT the dose-dependent inhibition was evident only for tobramycin. Heparin is highly anionic (7), and the aminoglycosides exist as positively charged molecules at the pH range 5 to 8 (8). The masking of the aminoglycoside activity by heparin is probably an inhibition by ionic interaction, rather than an inactivation, since the effect may be reversed by dilution. This might explain why gentamicin and netilmicin are less affected when assayed by EMIT than when assayed by the agar disk diffusion assay. In the

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**Table 1. Recovery of aminoglycoside activity after sampling in serum with heparinized capillaries**

<table>
<thead>
<tr>
<th>Assay technique</th>
<th>Amikacin</th>
<th>Gentamicin</th>
<th>Netilmicin</th>
<th>Tobramycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar disk diffusion</td>
<td>89.9 (84.4-101.5)</td>
<td>81.3 (69.5-87.5)</td>
<td>77.1 (72.2-82.3)</td>
<td>91.1 (81.0-98.7)</td>
</tr>
<tr>
<td>EMIT</td>
<td>ND</td>
<td>90.0 (88.1-91.7)</td>
<td>96.0 (93.3-98.2)</td>
<td>52.1 (49.7-60.6)</td>
</tr>
</tbody>
</table>

*Mean percentage; parentheses indicate range of 10 samplings. ND, Not done. Aminoglycosides were at 8 μg/ml in serum.*

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**Fig. 1. Recovery of amikacin (□), gentamicin (○), netilmicin (△), and tobramycin (×) (8 μg/ml) in tubes containing increasing amounts of heparin, determined by the agar disk diffusion assay (——) and EMIT (– – –).**

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**Fig. 2.**
HEPARIN INHIBITION OF AMINOLYCOSIDES

TABLE 2. Reversibility by dilution of the inhibitory effect of heparin on assayable concentrations of aminoglycosides

<table>
<thead>
<tr>
<th>Heparin concn (IU/ml)</th>
<th>Aminoglycoside concn (µg/ml)</th>
<th>Amikacin</th>
<th>Gentamicin</th>
<th>Netilmicin</th>
<th>Tobramycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Assayable concn (µg/ml)</td>
<td>Recovery (%)</td>
<td>Assayable concn (µg/ml)</td>
<td>Recovery (%)</td>
</tr>
<tr>
<td>500</td>
<td>16</td>
<td>10.2</td>
<td>63.7</td>
<td>8.5</td>
<td>53.1</td>
</tr>
<tr>
<td>250</td>
<td>8</td>
<td>6.8</td>
<td>84.4</td>
<td>5.3</td>
<td>66.2</td>
</tr>
<tr>
<td>125</td>
<td>4</td>
<td>3.6</td>
<td>90.0</td>
<td>3.1</td>
<td>77.5</td>
</tr>
<tr>
<td>62.5</td>
<td>2</td>
<td>1.9</td>
<td>96.0</td>
<td>1.6</td>
<td>80.0</td>
</tr>
<tr>
<td>31.2</td>
<td>1</td>
<td>1.1</td>
<td>110.0</td>
<td>1.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The amount in blood does not exceed 10 IU/ml (4). Our results show that aminoglycoside activity is not affected at these concentrations of heparin. Thus heparin treatment is unlikely to interfere with aminoglycoside therapy.

In vitro sampling and clinical sampling with heparinized capillaries lowered the aminoglycoside activity to the same degree, as determined by the agar disk diffusion assay. The smallest amount of heparin in the capillary tubes was 3 IU. The tubes contained 60 µl, which gave a heparin concentration of 50 IU/ml. The amount of heparin in the tubes differs, and the manufacturer cannot predict the highest amount of heparin in the tubes. The sample volume obtained in the capillary also affects the concentrations of heparin reached after centrifugation of the tube. Furthermore, the progressive inhibition of aminoglycoside activity at rising concentrations of heparin (Fig. 1) indicates that the lowest degree of inhibition is achieved in heparinized capillaries (50 IU/ml). The sample volume of heparinized capillaries shows that the level of aminoglycosides will be underestimated when samples are submitted for assay in heparinized tubes or capillaries.

The fact that heparin and not sampling differences accounts for the inhibition of aminoglycoside activity in the heparinized clinical specimens is evident from the results presented. Thus the outcome of the clinical samples and the in vitro sampling with heparinized capillaries shows that the level of aminoglycosides will be underestimated when samples are submitted for assay in heparinized tubes or capillaries.

**LITERATURE CITED**


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