Experimental Bacteriophage Protection against Staphylococcus aureus Abscesses in a Rabbit Model

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In a rabbit model of wound infection caused by Staphylococcus aureus, 2 × 10⁹ PFU of staphylococcal phage prevented abscess formation in rabbits when it was injected simultaneously with S. aureus (8 × 10⁹ CFU) into the same subcutaneous site. Phage multiplied in the tissues. Phages might be a valuable prophylaxis against staphylococcal infection.

Studies (2, 4, 5, 10–14) have shown substantial efficacy of bacteriophage therapy for experimental infections by gram-negative bacteria, but for gram-positive bacteria the efficacy of phage has been limited and has been demonstrated only in a few recent studies (3, 7, 8). Staphylococcus aureus, a cause of wound and soft-tissue infection, is often resistant to all β-lactam antibiotics, and strains resistant to vancomycin occur (15): surgical infections may become untreatable. We describe a model for wound infection in rabbits by a strain of S. aureus that caused infection on a rabbit farm (9) and protection by a phage.

S. aureus strain 2698 from a rabbit was kindly provided by E. Espun˜a of Laboratorios HIPRA, Girona, Spain. Phage LS2a was isolated from sewage. Nutrient broth (no. 2), agar (no. 1), brain heart infusion, and nutrient agar were obtained from Oxoid.

Adult New Zealand White rabbits of 1.5 to 2.5 kg in weight were housed in separate cages. S. aureus, phage, and control suspensions (0.1 ml in each case) were injected subcutaneously into a shaved area on the flank of each rabbit. Where more than one injection was given, they were given at the same site, and when given together, bacteria were injected first followed by a phage or control suspension. The model was established, and then pilot protection studies were done with up to four rabbits per group. In three main studies, three groups of rabbits received the following: bacteria and phage, bacteria and control suspension, and phage-only controls (i.e., no bacteria), which also received a liquid product by subjecting brain heart infusion to the procedure that had been used to purify the bacterial suspensions. Rabbits were examined daily for 4 to 6 days and then killed. Skin and fascia (2 by 2 cm) were removed from around the injection site, including any abscess present.

Bacterial inocula were prepared by culturing S. aureus in brain heart infusion for 14 h overnight and centrifuging it at 2,000 × g for 5 min, washing, recentrifuging it twice, and then resuspending it in saline.

Phage and control suspensions were prepared as follows. 2698 (2 × 10⁹ CFU) and 2 × 10⁸ PFU of LS2a were shaken together in 20 ml of nutrient broth at 37°C for 3.5 h. Phage was purified by filtration ( pore size, 0.45 μm), ultracentrifugation, resuspension in saline, and refiltration. The control suspension was prepared by lysing 14-h broth cultures of 2698 containing 10⁶ CFU/ml by agitating them with 0.1-mm silica beads for 3 min, using the Fastprep system (www.qbiogene.com). The resulting suspensions were then processed in the same way as the phage lysates.

Bacteria were counted by incubating 0.1-ml portions of serial 10-fold dilutions of homogenates on nutrient agar. Phages were counted on nutrient agar using the overlay method (1), with the overlays consisting of 0.5% agar. Low numbers of phages in the homogenates of livers and spleens were sought by incorporating 10-h broth enrichment cultures (with S. aureus) into agar overlays seeded with S. aureus.

Data were analyzed by Fisher’s exact and Mann Whitney U tests.

In a prophylaxis study, rabbits each received 8 × 10⁹ CFU of S. aureus 2698 and either control suspension or 2 × 10⁹ PFU of LS2a. After 4 days, one of the eight phage-treated rabbits had an abscess (area, 64 mm²), whereas all eight of the untreated rabbits had abscesses (median area, 106; range, 32 to 144 mm²) (P = 0.001). Bacterial CFU from the injection sites were lower (P < 0.003) for treated rabbits (median, 330; range, 0 to 11,000) than for the untreated controls (median, 2.3 × 10³; range, 1,800 to 100,000). Numbers of PFU of phage from injection sites of infected rabbits that had received phage ranged from 240 to 600,000 (median, 6,800), while 1,200 and 340 phage PFU were cultured from two rabbits that had received 2 × 10⁹ PFU of LS2a and no bacteria. No bacteria were isolated from the livers or spleens of any of the rabbits, and no phages were isolated from the spleens of the untreated rabbits. Phage (60 PFU) was isolated from the liver of only one treated rabbit. Phages were isolated from the spleens of all but one of the phage-treated rabbits and from five of the rabbits treated by enrichment only. In the remaining two, the counts were 20 and 1,400 PFU, while counts in the spleens of the phage-only controls were 700 and 5,700.

In a dose response study, rabbits each received 8 × 10⁹ CFU of S. aureus 2698 and either 6 × 10⁷, 6 × 10⁶, or 6 × 10⁵ PFU of LS2a or control suspension. One phage-only control was included for each dose group, receiving the same dose of phage but no bacteria. After 4 days, all but one of the 12 rabbits that had received bacteria produced abscesses; the exception had received the highest dose of phage. The median areas and bacterial counts in the abscesses (Table 1) increased consistently as the dose of phage decreased, with the largest abscesses being those of the control group, which contained higher bacte-
achieved against gram-negative bacteria, in which protection was more convincing results, protecting fish from lactococcal infections, though one study by Nakai et al. (8) has yielded slightly more convincing results, protecting fish from lactococcal infection with a phage PFU/bacterial CFU ratio of 1/40.

For three rabbits, we recovered more phages than were administered; this, to our knowledge, is the first direct evidence of administered phage multiplying in the tissues infected by gram-positive bacteria. Many previous models of S. aureus infection have studied animals that do not normally suffer from S. aureus infection (e.g., mice) by using either large infecting doses (14, 7) or foreign bodies (6). Since rabbits, like humans, readily suffer from S. aureus infections, they are more appropriate, and infections can be produced without foreign bodies and with only moderate bacterial doses. The use of strains derived from rabbits makes the model close to naturally occurring infection and the results applicable to farmed rabbits. With concerns about future untreated strains, phage prophylaxis for human surgery might be appropriate.

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REFERENCES


TABLE 1. Abscess area and bacterial and phage countsa

<table>
<thead>
<tr>
<th>No. of rabbits</th>
<th>Dose (10^7 PFU)</th>
<th>Abscess area (mm²)</th>
<th>Count of 2698 in abscess (10^7 CFU)</th>
<th>Count of LS2a in abscess (10^7 PFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>4</td>
<td>60,000</td>
<td>58–0–120</td>
<td>6</td>
<td>3–10</td>
</tr>
<tr>
<td>4</td>
<td>6,000</td>
<td>64–36–120</td>
<td>20</td>
<td>2–42</td>
</tr>
<tr>
<td>5</td>
<td>600</td>
<td>80–80–168</td>
<td>66</td>
<td>28–1,600</td>
</tr>
<tr>
<td>0</td>
<td>120</td>
<td>60–120</td>
<td>60</td>
<td>54–840</td>
</tr>
</tbody>
</table>

a Counts were made 4 days after 21 rabbits received (subcutaneously) 8 × 10^7 CFU of S. aureus 2698 and either control suspension or one of three doses of phage LS2a. Where 0 is quoted as a count, this means <10 CFU or PFU.

b Control suspension.

TABLE 2. Abscess area and bacterial and phage countsa

<table>
<thead>
<tr>
<th>No. of rabbits</th>
<th>Time before dose (h)</th>
<th>Abscess area (mm²)</th>
<th>Count of 2698 in abscess (10^7 CFU)</th>
<th>Count of LS2a in abscess (10^7 PFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
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<td>6</td>
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<td>475</td>
<td>190–4,000</td>
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<td>116–116–288</td>
<td>865</td>
<td>450–1,100</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>160–80–256</td>
<td>550</td>
<td>120–760</td>
</tr>
</tbody>
</table>

a Counts were made 4 days after 21 rabbits received (subcutaneously) 5 × 10^7 CFU of S. aureus 2698 bacteria and either control suspension or 3 × 10^7 PFU of LS2a phage at the times shown. Where 0 is quoted as a count this means <10 CFU or PFU.

b Control suspension.

This table provides data on the abscess area and bacterial and phage counts for different times after treatment. The table includes columns for the number of rabbits, time before dose, abscess area, count of 2698 in abscess, count of LS2a in abscess, median, and range. The data indicate the progression and spread of the infection, as well as the effectiveness of the treatment.