Influence of Inflammation on the Efficacy of Antibiotic Treatment of Experimental Pyelonephritis

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An acute exudative Escherichia coli pyelonephritis rat model was used to study the influence of progressive pyelonephritis on the efficacy of antibiotic treatment. In this model, transient ureteral obstruction after E. coli bladder inoculation induces early bacterial multiplication in the kidney parenchyma, and the bacterial counts peak by 48 h. The inflammatory response (assessed by the increase in kidney weight) is somewhat delayed, starting 36 h after inoculation and peaking by 72 h. Groups of rats received 4 doses over 48 h of saline, ceftriaxone (100 mg/kg), or ceftriaxone (100 mg/kg) plus gentamicin (4 mg/kg). These treatments were initiated 24, 36, 48, or 72 h after bladder inoculation. Antibiotic treatment started at 24 h was significantly more effective in reducing bacterial counts in the kidney parenchyma than at any later therapy onset. Only when started 24 h after inoculation was the synergistic combination of ceftriaxone plus gentamicin more effective in reducing bacterial counts than ceftriaxone alone. Ceftriaxone and ceftriaxone plus gentamicin regimens started at 24 h reduced significantly (by 42 and 55%, respectively) the incidence of acute exudative pyelonephritis when compared with the incidence in saline-treated controls. Early therapy onset (24 h) strikingly reduced the development of the inflammatory response. This reduction was less marked when antibiotic therapy was started at 36 h and no longer apparent when therapy onset was delayed up to 48 or 72 h. In conclusion, the efficacy of antibiotics in eradicating bacteria from the kidney parenchyma and in preventing acute exudative pyelonephritis was markedly hampered by the development of pyelonephritis.

In previous studies, we have been using a rat model of acute exudative pyelonephritis (AEP) that permits us to investigate the relationship between the presence of bacteria in the kidney parenchyma (infection), the inflammatory processes in response to infection, and permanent kidney damage (4). This model mimics complicated urinary tract infection in humans (21) in that renal infection is acquired by the retrograde route and leads to AEP after temporary obstruction has been applied to urinary flow. Later in the course of the disease, chronic pyelonephritis ensues with scarring and loss of renal parenchyma (6). Careful observation of this model reveals that after ascending infection, there is first an early stage of rapid bacterial multiplication in the kidney parenchyma, followed by a burst of exudative response, while bacterial numbers remain rather constant. Because of this sequence of events, this model offers the unique opportunity to investigate the influence of both the growth state of bacteria and the magnitude of the inflammatory response on antibiotic efficacy. We have previously shown that antibiotic treatment, when delayed, does not prevent the development of kidney scars, suggesting that the lesions of chronic pyelonephritis are a consequence of the acute exudative processes occurring early during AEP in response to the multiplication of bacteria in the kidney parenchyma (3, 13). Similar observations have been made by others in rats (19, 28), piglets with infected reflux (24), and primates (25). We now report on the influence of the stage of bacterial multiplication and of the exudative response on the efficacy of antibiotic treatment, as measured by the reduction of bacterial counts in the kidney parenchyma.

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MATERIALS AND METHODS

Production of pyelonephritis. Ascending unilateral pyelonephritis was produced in male Wistar rats weighing 200 to 250 g (Madorin, Füllinsdorf, Switzerland) as previously described (4), with slight modifications (13).

Experimental design of antibiotic therapy. To study the influence of the development of AEP on the effect of antibiotic treatment, antibiotic therapy was started 24 h after operation, when no detectable inflammatory response had developed yet; at 36 h, when the inflammatory response began to develop and scattered tiny abscesses became visible over the renal cortex; or at 48 and 72 h, during the full development of the inflammatory response.

Rats received four doses of either ceftriaxone (100 mg/kg) alone (Hoffmann-La Roche, Basel, Switzerland) or a combination of ceftriaxone (100 mg/kg) plus gentamicin (Essex Chemie, Luzern, Switzerland) (4 mg/kg). The Escherichia coli pyelonephritogenic strain (serotype O6:K5:H1 [5]) used in this study was susceptible to both antibiotics (MICs, 0.125 µg/ml for ceftriaxone and 1.2 µg/ml for gentamicin [11]). Each antibiotic was diluted to a final volume of 0.1 ml and was administered intramuscularly into the thigh every 12 h over a 36-h period. For combined treatment, the antibiotics were administered separately into each thigh. Control rats received 4 intramuscular doses of 0.1 ml of saline. Ceftriaxone was chosen because it has a relatively long half-life allowing twice-a-day administration. As previously shown (11), 100 mg of ceftriaxone per kg achieved a peak serum level of 155 µg/ml 30 min after injection. Four hours after injection, the serum level was still 10.6 µg/ml. Gentamicin administration resulted in a serum level of 8 µg/ml at 30 min and 1.9 µg/ml 2 h after an intramuscular injection of 4 mg/kg [11]. Although these serum gentamicin levels were below the MIC for the E. coli strain during approximately 10 h before the next injection, it has been shown that the 12-h dosage intervals afford
substantial therapeutic efficacy in the kidney due to the parenchymal accumulation of the drug (2, 11, 13, 14).

For each therapy onset experiment, groups of approximately 30 rats were randomly selected into four subgroups. One subgroup of rats was sacrificed immediately before the therapy onset to determine the incidence and severity of AEP at that time. The remaining animals were equally divided into the three therapeutic subgroups: saline subgroup (control rats), the ceftriaxone subgroup, and the ceftriaxone-gentamicin subgroup. All treated animals were sacrificed 12 h after the last injection. For each time of therapy onset, two to four experiments were performed. These repeated experiments gave reproducible results which were pooled for analysis.

Sacrifice of animals and evaluation of antibiotic efficacy.
The rats were killed with a pentobarbital overdose. The abdominal wall was opened, and the kidneys were excised aseptically and weighed. The left kidney was homogenized in 2 ml of sterile saline. Serial 1/10 dilutions of the homogenate were plated onto MacConkey agar (Difco Laboratories, Detroit, Mich.). To prevent the effect of antibiotic residue in the homogenates, the plates were incubated under anaerobic conditions, which inactivate gentamicin (30), or supplemented with a broad-spectrum beta-lactamase mixture (Whatman, Maidstone, Kent, England), which rapidly destroys ceftriaxone, or both. The colonies were counted after incubation for 48 h at 37°C. The results were expressed as the log_{10} of the number of viable bacteria (CFU) per gram of tissue. Histological studies. In each experiment, three kidneys from randomly selected animals in each subgroup were processed for histologic examination. The kidneys were cut in half and weighed. One half was processed for colony counting. The other half was fixed in 10% formaldehyde. After routine processing and cutting, sections were stained with hematoxylin-eosin.

Statistical analysis. In this model of ascending unilateral pyelonephritis, severe infection and subsequent AEP develop in only three of four of the rats (12, 13). Twenty-four hours after the injection of the refluxing bacterial inoculum, two groups of kidneys can be distinguished upon culture: (i) a majority (65 to 84%, depending on the experiments) of left kidneys with high bacterial numbers (>10^{7} log_{10} CFU of E. coli per g of kidney) and (ii) a minority of left kidneys (16 to 35%) with low bacterial counts (<10^{5} log_{10} CFU/g). There is no overlap in bacterial counts between these two groups. When infection is allowed to develop, macroscopic signs of AEP appear on the cortex surface as early as 36 h after operation in the left kidneys harboring high bacterial counts, whereas kidneys with low bacterial counts will invariably become sterile and remain free of detectable lesions. Thus, only those kidneys with high bacterial counts at 24 h and those with macroscopic evidences of AEP later on were used to evaluate the efficacy of antibiotic treatment.

In each experiment, the following parameters were evaluated: (i) Bacterial counts in the kidney parenchyma (log_{10} CFU per gram of tissue) were determined. (ii) Evaluation of the intensity of the exudative response, i.e., the extent of kidney lesions that had developed in response to infection, was made in rats that developed macroscopic AEP (or with bacterial counts of >10^{7} CFU/g when sacrificed at 24 h) by comparing the weight of the left diseased kidney with that of the right kidney. This parameter provides a quantitative measure for the severity of exudative response (13). During the acute phase of AEP, the left kidney weight increases in proportion to the extent of kidney surface affected by abscesses (6). To minimize variation of kidney weight among animals, the ratio of the left kidney weight over the right kidney weight (L/R kidney weight ratio) was used (13). This ratio equals 1/1 in normal rats and increases up to 2/1 during AEP. (iii) The incidence of gross, macroscopic pyelonephritis is expressed as the ratio of the number of rats with left macroscopic kidney lesions over the total number of rats. In animals sacrificed 24 h after operation, pyelonephritis was defined merely by high bacterial counts.

Comparison of the incidence of pyelonephritis. The comparison of the incidence of AEP between antibiotic-treated and saline-treated rats was performed by the chi-square test with the Yates correction.

Comparison of the bacterial counts and of the severity of the exudative response in the different treatment groups. The bacterial counts (log_{10} CFU per g of tissue) and the intensity of the exudative response (L/R kidney weight ratio) were submitted to a two-dimensional variance analysis with a biomedical computer program (9). In addition, multiple comparisons were performed, with the contrasts test of Scheffe (20) and a biomedical computer program (10), to assess (i) the influence of time of therapy onset on the antibacterial efficacy of the various antibiotic treatments (as measured by the reduction of bacterial counts during treatment) and (ii) the effect of the various antibiotic treatments on the severity of AEP (as measured by a reduction of the L/R weight ratio). This statistical analysis was chosen because the Student t test does not allow multiple comparisons without a considerable lack of accuracy (18). Furthermore, the contrast test allows us to compare not only means but also differences of means (Table 1).

RESULTS

Natural history of AEP. The natural history of AEP was assessed in sacrificing subgroups of control rats at each time of therapy onset.

Sixty-five percent (13 of 20) of the rats sacrificed 24 h after bladder inoculation already had high bacterial counts in the left kidney (7.11 to 8.44 log_{10} CFU per g of tissue). These kidneys were enlarged and pale, but macroscopic examination displayed only tubular dilatation and no inflammatory infiltrate. In contrast, the remaining 35% (7 of 20) of the animals had low bacterial counts in the left kidney (<1.5 to 3.52 log_{10} CFU per g of tissue). Macroscopically however, these kidneys were indistinguishable from their highly infected counterparts. Indeed, rats with either high or low bacterial counts in the left kidney had similar mean L/R kidney weight ratios (1.42 ± 0.11 versus 1.31 ± 0.23, P = 0.16, unpaired Student t test).

Seventy-five percent (9 of 12) of the control rats sacrificed 36 h after bladder inoculation had macroscopic lesions of left AEP. The mean bacterial count in the left kidney of these animals was about five times that observed in the 24 h group (8.32 versus 7.57 log_{10} CFU; Table 1), and the mean L/R kidney weight ratio had increased (1.56 versus 1.42). This increase in left kidney weight was accompanied by the appearance of scattered tiny abscesses over the left kidney surface. Microscopic examination disclosed scattered focal polymorphonuclear leukocyte infiltrates, tubular polymorphonuclear leukocyte casts, and surrounding tubular epithelial necrosis. Rats without AEP (25%, 3 of 12) had low bacterial counts in their left kidney (3.0 to 3.72 log_{10} CFU per g of tissue), and their mean L/R kidney weight ratio was 1.19 ± 0.41. Both macroscopic and microscopic findings of these latter kidneys appeared normal.

Seventy-nine percent (11 of 14) of the control animals
TABLE 1. Efficacy of antibiotic therapy started at various times during the development of AEP

<table>
<thead>
<tr>
<th>Time of therapy onset (h)</th>
<th>Therapy regimen</th>
<th>Time of sacrifice (h)</th>
<th>Total no. of rats</th>
<th>No. (%) of rats with pyelo-nephritis</th>
<th>log_{10} CFU/g of tissue</th>
<th>L/R kidney weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before therapy</td>
<td>After therapy</td>
</tr>
<tr>
<td>24</td>
<td>Saline</td>
<td>24</td>
<td>20</td>
<td>13 (65)</td>
<td>7.57 ± 0.41</td>
<td>1.42 ± 0.11</td>
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<td></td>
<td>Ceftriaxone</td>
<td>72</td>
<td>26</td>
<td>18 (69)</td>
<td>9.10 ± 1.01</td>
<td>1.94 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone-gentamicin</td>
<td>72</td>
<td>35</td>
<td>14 (40)</td>
<td>2.83 ± 1.12</td>
<td>-6.29 ± 0.01</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.28 ± 0.81</td>
<td>1.28 ± 0.14/1.92</td>
</tr>
<tr>
<td>36</td>
<td>Saline</td>
<td>36</td>
<td>12</td>
<td>9 (75)</td>
<td>8.32 ± 0.52</td>
<td>1.56 ± 0.10</td>
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<td>Ceftriaxone</td>
<td>84</td>
<td>13</td>
<td>11 (85)</td>
<td>8.63 ± 0.82</td>
<td>2.34 ± 0.27</td>
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<td></td>
<td>Ceftriaxone-gentamicin</td>
<td>84</td>
<td>18</td>
<td>11 (61)</td>
<td>5.38 ± 1.22</td>
<td>-2.94 ± 0.02</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>5.00 ± 1.40</td>
<td>1.60 ± 0.32/1.92</td>
</tr>
<tr>
<td>48</td>
<td>Saline</td>
<td>48</td>
<td>14</td>
<td>11 (79)</td>
<td>8.85 ± 1.38</td>
<td>1.62 ± 0.26</td>
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<tr>
<td></td>
<td>Ceftriaxone</td>
<td>96</td>
<td>15</td>
<td>10 (67)</td>
<td>8.31 ± 1.55</td>
<td>2.01 ± 0.45</td>
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<td>Ceftriaxone-gentamicin</td>
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<td>18</td>
<td>12 (67)</td>
<td>5.87 ± 1.08</td>
<td>-2.98 ± 0.02</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>5.61 ± 1.20</td>
<td>2.02 ± 0.27/1.92</td>
</tr>
<tr>
<td>72</td>
<td>Saline</td>
<td>72</td>
<td>11</td>
<td>7 (64)</td>
<td>8.49 ± 1.17</td>
<td>1.98 ± 0.32</td>
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<tr>
<td></td>
<td>Ceftriaxone</td>
<td>120</td>
<td>14</td>
<td>12 (86)</td>
<td>7.76 ± 0.55</td>
<td>2.09 ± 0.47</td>
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<td>Ceftriaxone-gentamicin</td>
<td>120</td>
<td>18</td>
<td>13 (72)</td>
<td>6.78 ± 1.81</td>
<td>-1.71 ± 0.02</td>
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<td></td>
<td>6.12 ± 1.25</td>
<td>1.78 ± 0.43/1.92</td>
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</table>

* Hours after inoculation.
* Pyelonephritis was defined by bacterial counts >10^7 log_{10} CFU per g of kidney at 24 h and by macroscopic evidence of AEP from 36 h on (see Materials and Methods).
* Mean ± standard deviation of kidneys with high bacterial counts (when sacrificed at 24 h) or with macroscopical lesions of AEP (when sacrificed from 36 to 120 h).
* Difference between the mean log CFU in rats sacrificed at therapy onset and in rats after therapy completion. For comparisons of antibiotic efficacy in relation to therapy onset, see text.
* p < 0.05 compared with value for saline-treated rats.
* p < 0.001 compared with value for saline-treated rats.
* P = 0.0025 compared with value for ceftriaxone-treated rats.
* P is not significant compared with value for ceftriaxone-treated rats.
* P is not significant compared with value for saline-treated rats.

sacraficed at 48 h and 64% (7 of 11) of those sacrificed at 72 h had AEP. The bacterial counts were the highest in the animals sacrificed 48 h after bladder inoculation, whereas the L/R kidney weight ratio was the highest at 72 h (Table 1). The pyelonephritic kidneys were greatly enlarged and displayed numerous small abscesses over the surface. Microscopic examination revealed heavy focal polymorphonuclear leukocyte infiltration with tubular necrosis and leukocyte casts. The left kidney of animals which did not show AEP lesions when sacrificed at 48 (21%, 3 of 14) or 72 (36%, 4 of 11) h had low bacterial counts (<1.5 to 4.22 log_{10} CFU per g of tissue). Neither macroscopic nor microscopic examination revealed traces of inflammatory response in these kidneys, and the mean L/R kidney weight ratio remained low (1.25 ± 0.19 and 1.19 ± 0.20 at 48 and 72 h, respectively).

Effect of time of therapy onset on the reduction of bacterial counts. The effect of the three regimens (saline, ceftriaxone alone, and ceftriaxone plus gentamicin) was assessed by comparing bacterial counts in the kidneys of treated animals with those in the kidneys of rats sacrificed at therapy onset (Table 1). First, within each therapy onset group antibiotic treatment (ceftriaxone alone or ceftriaxone-gentamicin) was superior to saline in reducing the bacterial counts. Second, both antibiotic treatments started at 36 h were significantly less effective in reducing the bacterial counts than the same treatments started at 24 h (P = 0.01 for the ceftriaxone treatment, P = 0.0001 for the combined ceftriaxone-gentamicin treatment). A longer delay in starting treatment further reduced the effect of antibiotics on bacterial counts, but the differences between these late therapy onset groups were not statistically significant. Third, the in vitro synergistic combination of ceftriaxone plus gentamicin (11%) was significantly superior to ceftriaxone alone in reducing the bacterial counts only in the 24-h therapy onset group (P = 0.0025). When therapy onset was delayed up to 36 h or later, there was no longer any benefit of the combined therapy over ceftriaxone alone.

Effect of antibiotic treatment on the incidence of AEP. When antibiotic treatment was started early (24 h) after bladder inoculation, it was able to prevent the development of AEP in a substantial number of animals (Table 1). Among saline-treated rats, 18 of 26 (69%) displayed pyelonephritic lesions at the time of sacrifice (72 h), in contrast to only 14 of 35 (40%) ceftriaxone-treated rats (P = 0.045 compared with controls) and 9 of 29 (31%) ceftriaxone-gentamicin-treated rats (P = 0.01). This represented a 42% reduction of the incidence of AEP after ceftriaxone treatment and a 55% reduction of AEP after combined treatment. In contrast, when therapy onset was delayed until 36, 48, or 72 h after bladder inoculation, neither ceftriaxone alone nor ceftriaxone plus gentamicin reduced the incidence of AEP.

Effect of antibiotic treatment on the intensity of the inflammatory response. Table 1 shows the effect of antibiotic treatment on the inflammatory response as measured by the L/R kidney weight ratio. In those rats which developed AEP, ceftriaxone and ceftriaxone plus gentamicin started at
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24 h reduced by 65 and 70%, respectively, the inflammatory response compared with that in saline-treated rats. In the 36-h therapy onset subgroups, the reduction was of a lesser magnitude (53% and 65%, respectively). When therapy onset was delayed for more than 36 h, no significant reduction was detectable. The effect of ceftriaxone alone on the development of the inflammatory response was not different at any time of therapy onset from the effect of ceftriaxone plus gentamicin. Thus, these detailed observations parallel and extend our previous observations on the effect of early treatment and prevention of inflammatory processes (13).

DISCUSSION

In previous experiments, we investigated the effect of antibiotics on the prevention of acute and chronic pyelonephritis (13). The aim of the present experiments was to determine the efficacy of antibiotics in eradicating bacteria from the kidney parenchyma under various conditions of bacterial growth and inflammatory response. Ceftriaxone was chosen because it has a relatively long serum half-life in rats and could therefore be administered at 12-h intervals in doses that produced peak serum levels similar to those in humans given standard intravenous doses (11). Gentamicin was given at 12-h intervals despite its short half-life, because its intrarenal accumulation has been related to therapeutic effectiveness in experimental pyelonephritis (2, 11, 13, 14).

Two new interesting observations were made regarding the relation of AEP to antibiotic effectiveness in the present experiments.

The first observation concerns the effect of the progression of AEP on the efficacy of antibiotic treatment in eradicating bacteria from kidney tissue. Treatment with ceftriaxone alone started at 24 h after inoculation was more effective in reducing the bacterial counts in the kidney parenchyma than at any of the later therapy onsets. The addition of gentamicin significantly enhanced the efficacy of ceftriaxone alone only when combined treatment was started at 24 h. Several mechanisms may account for this reduced efficacy of antibiotics with increasing development of pyelonephritis. First, although bacteria were rapidly growing at 24 h, bacterial multiplication apparently ceased later during the course of AEP. Many cell wall-active antibiotics that are highly active against exponentially growing bacteria are less effective against stationary-phase bacteria (8, 31). This might in part explain the reduced killing effect observed in the delayed-therapy groups. Second, the reduction in the ability of antibiotics to eradicate bacteria from kidney tissue paralleled the development of the inflammatory response in the kidney parenchyma. Although no lesions were detectable 24 h after inoculation, abscesses appeared and enlarged from 36 h on and peaked by 72 h. The necrotic and avascular nature of abscesses may impair the diffusion of drugs from capillaries into the abscesses (1, 22, 23). Anaerobiosis and low pH, which prevail in suppurative foci (17, 26), may dramatically reduce both the efficacy of aminoglycoside antibiotics (27, 30) and the lytic effect of beta-lactam antibiotics (16). In addition, purulent exudates have been shown to inactivate gentamicin by a binding mechanism to lysed neutrophils (7, 29). Whether due to reduced bacterial growth, increasing inflammatory response, or both, the diminished efficacy of antibiotics which paralleled the development of AEP confirms quantitatively the clinical observation that antibiotic therapy is often suboptimal for the treatment of supplicative foci.

The second new finding of interest is that very early (24 h) antibiotic therapy reduced the incidence of AEP. Previous observations have shown the invariable development of AEP in rats with high bacterial counts in the left kidney 24 h after operation (12). In the present experiments, antibiotic treatment started 24 h after bacterial inoculation significantly reduced the incidence of AEP, whereas delayed therapy did not. A similar reduction has been observed in rats given indomethacin early during the course of AEP (12). This was interpreted as evidence that if the early inflammatory response to severe infection of the kidney could be mitigated by indomethacin, the kidney was able to get rid of bacteria without developing AEP once urinary flow was restored (12). A similar result was observed in the present experiments, although it was probably due to other mechanisms. In all likelihood, early antibacterial treatment reduced the incidence of AEP by stopping the bacterial multiplication, thus suppressing the stimulus for the inflammatory response and preventing the development of AEP.

A third finding of the present experiments confirms and extends previous observations (13) that early antibiotic therapy diminished the severity of AEP, whereas delayed treatment had no effect. In the delayed-therapy groups, the ability of antibiotics to reduce the severity of AEP decreased in parallel with the appearance of suppurative lesions. Thus, only antibiotic therapy administered early during the development of AEP is likely to diminish the severity of the ensuing kidney scars (13, 19, 24, 28).

In conclusion, the present experiments quantify the progressively reduced effectiveness of antibiotic treatment in eradicating bacteria in tissues in which severe infection and inflammatory processes develop.

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LITERATURE CITED