GENTAMYCIN REDUCES BACTEREMIA AND MORTALITY RATES ASSOCIATED WITH THE TREATMENT OF EXPERIMENTAL PERITONITIS WITH RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR

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BACKGROUND: Recombinant tissue plasminogen activator (rtPA), administered intraperitoneally, reduces intra-abdominal abscess formation in rats with fecal peritonitis at the costs of increased mortality and early Escherichia coli bacteremia. It was determined whether or not mortality and bacteremia could be prevented by gentamycin in these rats.

STUDY DESIGN: Fecal peritonitis was induced by intraperitoneal injection of sterile feces contaminated with $10^8$ (experiment 1) or $10^4$ (experiment 2) colony forming units (cfu) E. coli and $10^4$ cfu Bacteroides fragilis. Male Wistar rats were randomly assigned to receive either methyl hydroxy propyl cellulose (MHPC) gel alone (M) or 0.5 mg/mL rtPA dissolved in MHPC gel (M-tPA). Three hours after inoculation, one-half of the rats in each of these groups received 6 mg/kg gentamycin sulfate (G) intramuscularly (group M-G and M-tPA-G). At one, three, six, 12, and 24 hours after inoculation, blood cultures were taken. At five days after inoculation, intra-abdominal abscess formation was assessed and abscesses were cultured (experiment 2).

RESULTS: All rats in groups M and M-tPA in experiment 1 developed bacteremia and died within 24 hours. Bacteremia occurred significantly earlier in group M-tPA compared with group M (p<0.05). Gentamycin significantly reduced the number of rats with bacteremia, the bacterial concentration in the blood, and mortality rates. Although in experiment 2 none of the rats developed bacteremia, gentamycin prevented mortality associated with the use of rtPA. The number of abscesses in groups M-tPA and M-tPA-G was significantly lower than in those in groups M and M-G (p<0.01). Gentamycin did not influence the number of abscesses.


INTRA-ABDOMINAL ABScessES are an important cause of morbidity and mortality in patients with generalized peritonitis (1). Formation of intra-abdominal abscesses is mediated by fibrin clots, which are colonized by bacteria (2). Several experimental studies have been attempted to prevent the formation of intra-abdominal abscesses by inhibition of fibrin formation with heparin or by activation of fibrinolysis with plasminogen activators (3, 4). In a previous experiment, we demonstrated a reduction of intra-abdominal abscesses with the use of a viscous gel that contained recombinant tissue plasminogen activator (rtPA) in rats with generalized peritonitis (5). However, rats treated with rtPA had an increased mortality rate, and early Escherichia coli (E. coli) bacteremia was observed more frequently, probably as a result of early degradation of fibrin. In the present study, it was determined whether or not mortality and bacteremia could be prevented by the administration of gentamycin in rats with generalized peritonitis treated with rtPA intraperitoneally.

MATERIALS AND METHODS

Design of the study. Experiment 1.—Male Wistar rats, weighing between 255 and 290 g were used. Generalized peritonitis was induced by intraperitoneal injection of 2 mL fecal suspension containing $10^8$ colony forming units (cfu)/mL E. coli and $10^4$ cfu/mL Bacteroides fragilis (B. fragilis). After one hour, a midline laparotomy was performed under general anesthesia (halothane-ni-
trous oxide-oxygen mixture). The abdomen was debrided (including partial omentectomy) and rinsed with normal saline solution. Before closure of the abdomen, the rats received either 2.5 mL methyl hydroxy propyl cellulose (MHPC) (Clinical Pharmacy, Groningen, The Netherlands) gel (n=14) or 2.5 mL MHPC gel containing 0.5 mg/mL human rtPA (Boehringer Ingelheim, Alkmaar, The Netherlands) (n=20) intraperitoneally. Methyl hydroxy propyl cellulose gel was used as a vehicle. After operation, 5 mL of normal saline solution were administered subcutaneously for resuscitation. At three hours after induction of peritonitis, one-half of the rats in each group received gentamycin sulfate, 6 mg/kg body weight, intramuscularly (IM) in a single dose. Thus, four experimental groups were formed: MHPC gel alone (M), MHPC gel plus rtPA (M-tPA), MHPC gel plus gentamycin (M-G), and MHPC gel plus rtPA plus gentamycin (M-tPA-G). At one, three, six, 12, and 24 hours after inoculation, blood samples (0.45 mL) were taken by cardiac puncture while rats were under general anesthesia to assess bacteremia. Lost blood volume was compensated by subcutaneous administration of 2 mL of normal saline solution. Five days after inoculation, the surviving rats were sacrificed by intracardial pentobarbital (Nembutal®, Abbott Laboratories, North Chicago, IL) injection and inspected for intra-abdominal abscess formation.

Experiment 2.—In a similar experiment, published previously (5), we inoculated $10^4$ instead of $10^8$ cfu/mL *Escherichia coli*. It was found that mortality rates in rats treated with rtPA were significantly higher but bacteremia was not observed. In the present study, this experiment was repeated, including a group of rats receiving gentamycin IM.

The same model of intra-abdominal infection and treatment was used as already described. Fecal suspension contained $10^4$ cfu/mL *E. coli* and *B. fragilis*. At laparotomy, the rats received either 2.5 mL of MHPC gel (n=16) or 2.5 mL of MHPC gel containing 0.5 mL/mg rtPA (n=24). Half of the rats in each group received gentamycin sulfate (6 mg/kg body weight IM, three hours after inoculation). Thus, analogous experimental groups to those in experiment 1 were obtained. Blood cultures were taken at three, six, 12, and 24 hours after inoculation. Five days after inoculation, the abdomens of surviving rats were re-
Mortality rate in rats after inoculation of sterile feces contaminated with $10^9$ *Escherichia coli* and $10^4$ *Bacteroides fragilis*. Mortality is significantly (p<0.05) reduced by gentamycin, *M*, Methyl hydroxy propyl cellulose gel; *tPA*, tissue plasminogen activator; and *G*, gentamycin sulfate.

opened under sterile conditions and abscesses were counted. If present, abscesses on different spots were excised for culture. During the experiments, the rats had free access to water and standard rat chow (MH-B 1010, Hope Farms, Woerden, The Netherlands).

**Bacterial fecal suspension.** *Escherichia coli* strain American Type Culture Collection (ATCC) 25922 and *B. fragilis* from a clinical isolate were used. Bacteria were cultured according to standard methods. The mean inhibitory concentration of gentamycin for this *E. coli* strain was 0.5 mg/L as measured by the E-test (AB Biodisk, Solna, Sweden).

Fresh feces of Wistar rats were prepared as described previously (5). Prior to intraperitoneal injection, the sterile fecal suspension was vigorously mixed with the bacterial suspensions.

** Cultures of abscesses.** The excised abscesses were cultured semiquantitatively using standard aerobic and anaerobic bacteriologic techniques.

**Blood cultures.** Blood (0.45 mL) was collected in sterile tubes containing 0.05 mL buffered citrate. Serial dilutions in brain-heart infusion broth were made and plated onto both aerobic (MacConkey, blood agar; Oxoid, Hampshire, United Kingdom) and anaerobic (Bacteroides Bile Esculin agar, Brucella Blood agar; Becton-Dickinson, Baltimore, USA) plates. After 24 and 48 hours of incubation at 37 degrees C, bacteria were counted and identified. Bacterial concentration was expressed in $10^{10}$ cfu/mL whole blood.

**Statistics.** Statistical analysis was performed using chi-square test, Yates and Cochran or Kruskall Wallis rank analysis, when appropriate. When the p value was less than 0.05, a difference between groups was considered significant.

## RESULTS

**Experiment 1.** At one hour after inoculation, none of the rats had bacteremia. At 12 hours after inoculation, all rats in group M and M-tPA had bacteremia, and all died within 24 hours. Bacteremia occurred earlier in rats in group M-tPA in comparison with those in group M, as reflected by a significantly (p<0.05) higher number of rats with bacteremia at six hours after inoculation (Fig. 1). Gentamycin significantly reduced the number of rats with bacteremia: in the M-tPA-G group at six and 12 hours and in the M-G group at 12 hours after inoculation. Also, the mortality rate was significantly (p<0.05) reduced by gentamycin in these groups of rats (Fig. 2).

At 12 hours after inoculation, blood cultures revealed a median bacterial concentration of $10^5$, ranging from $10^2$ to $10^7$, in rats in group M and M-tPA. The bacterial concentration was significantly (p<0.05) reduced in rats treated with gentamycin to a median concentration of zero (range, zero to $10^7$). However, the bacterial concentration in the one rat in group M-G and the three rats in group M-tPA-G that had bacteremia did not differ significantly from those in group M and M-tPA: $10^4$ (range, $10^2$ to $10^5$) compared with $10^5$ (range, $10^2$ to $10^7$). Only *E. coli* ATCC 25922 was isolated from the blood.

At 24 hours after inoculation, all blood cultures available from surviving rats revealed no growth. Three of the six surviving rats in group M-tPA-G were free of intra-abdominal abscesses at day 5, whereas all five surviving rats in group M-G had multiple intra-abdominal abscesses.

**Experiment 2.** When inoculated with $10^4$ *E. coli*, five out of 12 rats (42 percent) in group M-tPA died compared to zero out of eight rats (zero percent) in group M. Rats died between 12 and 24 hours after inoculation and none of them had abscesses at autopsy. Positive blood cultures were not found in any rat during this experiment. Surprisingly, gentamycin prevented mortality: 0/12 in group M-tPA-G compared with 5/12 in group M-tPA; p<0.05. None of the eight rats in group M-G died.
TABLE I.—BACTERIOLOGY OF INTRA-ABDOMINAL ABSCESSES IN SURVIVING RATS WITH POSITIVE CULTURES

<table>
<thead>
<tr>
<th>Group</th>
<th>Rats with positive cultures, n</th>
<th>Rats with E. coli ATCC 25922, n</th>
<th>Rats with other species*, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>8</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>M-G</td>
<td>6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>M-tPA</td>
<td>6</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>M-tPA-G</td>
<td>9</td>
<td>7</td>
<td>67</td>
</tr>
</tbody>
</table>

*Other species are Enterococci, Staphylococci, Proteus, and Candida, which were not sensitive to gentamycin.

Five days after inoculation, intra-abdominal abscesses were found in all rats in group M and M-G, in six of seven surviving rats in group M-tPA, and in 11 of 12 rats in group M-tPA-G. In four rats (two in group M-G, two in group M-tPA-G), no bacteria were isolated from the excised abscesses. In rats with positive cultures of intra-abdominal abscesses and treated with rtPA, the median number of abscesses was significantly lower (p<0.01) than in those not treated with rtPA: three (range, 1 to 4) in group M-tPA and four (range, 1 to 8) in group M-tPA-G compared with 11 (range, 8 to 15) in group M and ten (range, 7 to 13) in group M-G. Gentamycin did not influence the number of abscesses. Escherichia coli ATCC 25922 was cultured from abscesses in most cases (Table I). In contrast, B. fragilis was not found. Other species seemed to be more frequently isolated from abscesses in rats treated with gentamycin compared with those not treated with gentamycin.

**DISCUSSION**

The present study demonstrates that antibiotic treatment markedly reduces early bacteremia and reduces the mortality rate in rats with generalized peritonitis that are treated with rtPA to prevent the formation of residual abscesses. Fibrin entraps bacteria, thus preventing early bacteremia and mortality in generalized peritonitis (2, 6). However, these colonized fibrin clots are the nidus for intra-abdominal abscesses. It is expected that intra-abdominal application of rtPA to prevent these abscesses will cause early bacteremia. In concordance with previous findings, early bacteremia occurred in association with the use of rtPA (4, 5).

A single dose of 6 mg/kg gentamycin sulfate, administered intramuscularly three hours after onset of peritonitis, reduced bacteremia and mortality rates in experiment 1, and the mortality rate in experiment 2. Recombinant tissue plasminogen activator appeared to be the main factor reducing abscess formation in contrast to gentamycin. Several authors have reported the inability of antibiotics to reduce abscess formation (6–8). Hau and associates have demonstrated, using a fibrin clot model, that gentamycin, like several other antibiotics, does not influence abscess formation mainly because inhibitory levels within the fibrin clot are not reached (7). However, in the same model, a reduction of colony count per abscess by gentamycin sulfate has been reported (8). We did not quantitate bacteria in abscesses. Qualitatively, the use of gentamycin seemed to have altered the bacterial flora of the abscesses. The absence of B. fragilis in the abscesses was remarkable. This might be due to the peritonitis model used, as was pointed out previously (5).

From this and previous studies (4, 5, 9), it emerges that intra-abdominal administration of rtPA may be a valuable adjunct to the standard treatment of patients with generalized peritonitis in order to prevent intra-abdominal abscess formation. Antibiotics directed against the causal bacteria are an essential part of this treatment. With the use of appropriate antibiotics, the risk of bacteremia associated with the use of rtPA in the clinical situation is expected to be minor. Bleeding, another potential risk of the use of rtPA clinically, has so far not been observed in experiments wherein rtPA has been administered into the abdominal cavity (5, 10).

**REFERENCES**

5. van Goor, H., de Graaf, J. S., Kooi, K., and others. Effect of recombinant tissue plasminogen activator (rtPA)


