Role of Intestinal Excretion in the Effect of Subcutaneously Administered Sedecamycin on Cecal Infection Caused by Treponema hyodysenteriae in Mice

TOSHIKATSU HAYASHI,¹,* JUNYA OKADA,¹ SADAO KONDO,¹ AND TOSHIYUKI YAMAZAKI²

Animal Health Research Laboratories, Agro Division,¹ and Biology Research Laboratories, Research and Development Division, Pharmaceutical Affairs,² Takeda Chemical Industries, Ltd., 17-85, Juso-honnachi, 2-chome, Yodogawa-ku, Osaka 532, Japan

Received 17 December 1990/Accepted 6 June 1991

The therapeutic effects of subcutaneously administered sedecamycin on experimental Treponema hyodysenteriae infection in mice were evaluated. Sedecamycin was more active than tiamulin and lincomycin. The efficacy of sedecamycin upon subcutaneous administration was similar to that upon oral administration. Sedecamycin given subcutaneously provided similar degrees of protection in bile duct-ligated and intact mice. Pharmacokinetic studies utilizing a liquid chromatographic technique were carried out to determine the concentration of sedecamycin in the cecum, the site of T. hyodysenteriae infection in mice. Little sedecamycin was found; however, lankacidinol, a major metabolite of sedecamycin, was found in the cecal contents of intact mice after subcutaneous or oral administration of sedecamycin. Lankacidinol was also found in the cecal contents of bile duct-ligated mice, although the concentration found after subcutaneous administration of sedecamycin was much lower than that found after subcutaneous or oral administration to intact mice. These results indicate that sedecamycin is excreted directly into the intestinal tract as an active metabolite by a route other than the bile duct. It is suggested that this intestinal excretion plays an important role in the efficacy of subcutaneously administered sedecamycin against cecal infection of mice by T. hyodysenteriae.

MATERIALS AND METHODS

Antimicrobial agents. Sedecamycin was prepared by Takeda Chemical Industries, Ltd. (Osaka, Japan). Tiamulin (Squibb Japan Inc., Tokyo, Japan) and lincomycin (Sigma Chemical Co., St. Louis, Mo.) were used as reference drugs. These drugs are marketed for treatment of swine dysentery in Japan.

Animals. Four- to six-week old Ta:CF#1 female mice (Takeda Chemical Industries, Ltd., Takatsuki, Japan) (24) were used. The animals were housed in standard cages, and food and water were available ad libitum.

Microorganism. T. hyodysenteriae DJ70P3 (11), which is pathogenic in mice, was used. The inoculum was prepared as described previously (23). Mice were inoculated orally with 0.5 ml of an inoculum containing approximately 10⁶ CFU of T. hyodysenteriae per ml.

Therapeutic test. Procedures previously reported (11) were used for the therapeutic test. Drugs were administered subcutaneously to mice once a day for 4 days, starting 7 days postinoculation with T. hyodysenteriae. The mice were necropsied on postinoculation day 14. Groups of five mice each were used.

Protection test. Sedecamycin was administered subcutaneously once a day for 2 days, postinoculation days 1 and 2. The mice were sacrificed on postinoculation day 3. Groups of 10 mice each were used.

Assessment of results. The therapeutic and protective effects of the drugs were evaluated on the basis of colonization of T. hyodysenteriae in the cecum at necropsy. Cecal homogenate samples were streaked on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 400 μg of spectinomycin per ml and 5% horse blood (21) to isolate T. hyodysenteriae selectively. The plates were incubated at 42°C for 72 h in an aerobic glove box (11). The 50% effective dose (ED₅₀) was calculated by

* Corresponding author.

Treponema hyodysenteriae, the etiologic agent of swine dysentery, is characterized by mucohemorrhagic diarrhea (9, 25). In general, severely infected animals are reluctant to consume the medicated feed. In such cases, parenteral administration of effective drugs, lincomycin (8) and tiamulin (1, 2), is used for treatment. Swine dysentery is infection with T. hyodysenteriae in the large intestine (5, 10); therefore, a sufficient amount of a drug must be distributed to the large intestine. When a drug is administered parenterally, the route by which it is distributed to the large intestine is thought to be biliary and intestinal excretion. Recently, intestinal excretion has become a well-recognized phenomenon (3, 4, 7, 13, 22). However, little is known about the pharmacokinetics of injectable drugs for swine dysentery in relation to efficacy.

Experimental infection of mice and rabbits with T. hyodysenteriae had failed until Joens and Glock (14) reported that CF₃ mice inoculated with T. hyodysenteriae for 2 consecutive days after a 72 h of fasting developed cecal and colonic lesions. Later, Suegata and Yamazaki (24) reported that they examined mouse strains and found that Ta:CF#1 mice developed gross cecal lesions after a single oral administration of T. hyodysenteriae without fasting. By using this animal model, we have shown that orally administered sedecamycin, a 17-member macrocyclic antibiotic (Fig. 1), shows good efficacy in mice (11).

The objective of this study was to evaluate the efficacy of subcutaneous administration of sedecamycin against experimental infection of mice with T. hyodysenteriae and to explain the mechanisms of the efficacy by the concentration of sedecamycin and its active metabolites in the ceca of mice.

1601
the probit method (17) from the rate at which \( T. \) hydysenteriae was eradicated from the cecum.

**Pharmacokinetic analysis.** Sedecamycin and its metabolites in the cecal contents were analyzed after 40 mg of sedecamycin per kg was subcutaneously administered to mice. Samples were analyzed by the liquid chromatographic method described previously, with slight modification (18). The cecal contents were diluted with 0.1 M phosphate buffer (pH 4.5) and extracted three times with ethyl acetate. The extract was appropriately concentrated for determination of lankacidinol. The ethyl acetate solution was washed with the buffer twice to make a sample solution for determination of sedecamycin. The mean recoveries ± standard deviations at the fortification level of 10 ppm were 87.5 ± 4.6% for sedecamycin and 80.9 ± 10.2% for lankacidinol. Groups of three mice each were used for analyses of sedecamycin in the cecal contents.

**Bile duct ligation.** Bile duct ligation was performed as follows. Each mouse received an intraperitoneal injection of 1 mg (0.2 ml) of pentobarbital sodium (Dainippon Pharmaceuticals, Co. Ltd., Osaka, Japan). The animal was placed dorsally on an operating table, and a 10-mm incision was made along the upper abdomen. The bile duct was exposed and ligated with silk (JIS 1; Shirakawa Shigyo, Tokyo, Japan), and subsequently the peritoneum and the skin of the abdomen were closed. The closed abdomen was then pasted with a surgical bond (Alon Alpha A; Sankyo, Co. Ltd., Tokyo, Japan). This operation was performed on postinoculation day 1 for the protection test. Sedecamycin was subcutaneously administered after the mice had recovered from the anesthesia.

**RESULTS**

**Therapeutic effect upon subcutaneous administration.** The therapeutic effect of subcutaneously administered sedecamycin in mice infected with \( T. \) hydysenteriae DJ70P3 was compared with those of tiamulin and lincomycin. The therapeutic effect of sedecamycin (ED\(_{50}\), 4.9 mg/kg) was twice that of tiamulin and seven times that of lincomycin (\( P < 0.05 \)) (Table 1). The control mice were all positive for isolation of \( T. \) hydysenteriae from the cecum. The results obtained here for subcutaneous administration are similar to the previously reported results for oral administration (11); however, the potency of subcutaneously administered lincomycin was lower than that of orally administered lincomycin.

**Protective effect in bile duct-ligated mice.** The protective effect of subcutaneously administered sedecamycin in mice in which the bile duct was ligated for the prevention of biliary excretion after infection with \( T. \) hydysenteriae DJ70P3 was compared with that in intact mice. In this experiment, animals were treated once a day on days 1 and 2 postinoculation and necropsied on day 3. Sedecamycin given subcutaneously in bile duct-ligated mice was effective; the ED\(_{50}\) was 5.1 mg/kg, and the 95% confidence limit was 3.8 to 7.0 mg/kg. The ED\(_{50}\) of sedecamycin in intact mice was 4.4 mg/kg (95% confidence limit, 3.3 to 5.9 mg/kg).

**Cecal concentration of sedecamycin in mice.** Sedecamycin and its metabolites in the cecal contents after a single subcutaneous or oral dose of 40 mg/kg were analyzed to investigate the relationship between the protective effect of the drug administered subcutaneously and the amount of the drug distributed in the cecum. No sedecamycin and only negligible levels of lankacidin C (19) and lankacidinol A (19) were found in the cecum. However, lankacidinol, a major metabolite of sedecamycin (Fig. 1) (18), was found (Table 2). The maximum concentration of lankacidinol in the cecal contents was 74.9 ± 18.8 \( \mu \)g/g (mean ± standard deviation) 4 h after subcutaneous administration of sedecamycin, while the peak was 185.7 ± 30.9 \( \mu \)g/g 2 h after oral administration. In addition, the concentration of lankacidinol in the cecal contents after oral administration was consistently higher than that after subcutaneous administration.

**Cecal concentration in bile duct-ligated mice.** The cecal contents of sedecamycin and its metabolites were determined in bile duct-ligated mice 4 h after subcutaneous administration of sedecamycin at a dose of 40 mg/kg. Only lankacidinol was detected in the cecal contents. The concentration of lankacidinol in the cecal contents of the mice was 23.7 ± 2.9 \( \mu \)g/g. The lankacidinol concentration in the ceca of sham-operated mice was 91.8 ± 27.2 \( \mu \)g/g.

**DISCUSSION**

The present study was designed to test the hypothesis that intestinal excretion plays an important role in the efficacy of

**TABLE 1.** Therapeutic effects of subcutaneously administered sedecamycin, tiamulin, and lincomycin on experimental infection of mice with \( T. \) hydysenteriae DJ70P3

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC(_b) (( \mu )g/ml)</th>
<th>( ED_{50}) (mg/kg/day)(_d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedecamycin</td>
<td>6.25</td>
<td>4.9 (2.4–8.6)</td>
</tr>
<tr>
<td>Tiamulin</td>
<td>0.1</td>
<td>8.3 (4.1–15.8)</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>50</td>
<td>37.6 (20.7–67.0)(_c)</td>
</tr>
</tbody>
</table>

\(_a\) Animals were treated once a day from days 7 to 10 postinoculation and necropsied on postinoculation day 14.

\(_b\) MICS were determined by the agar dilution method.

\(_c\) The numbers in parentheses are 95% confidence limits.

\(_d\) The oral ED\(_{50}\) data shown have been previously reported (11) and are included for comparison.

\(_e\) Significantly different from the value for sedecamycin treatment at \( P < 0.05 \).

**TABLE 2.** Concentration of lankacidinol in the cecal contents of intact mice after subcutaneous or oral administration of 40 mg of sedecamycin per kg

<table>
<thead>
<tr>
<th>Time after administration (h)</th>
<th>Mean lankacidinol concn (( \mu )g/g) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.6 ± 2.3</td>
</tr>
<tr>
<td>2</td>
<td>33.1 ± 3.0</td>
</tr>
<tr>
<td>4</td>
<td>74.9 ± 18.8</td>
</tr>
<tr>
<td>6</td>
<td>41.9 ± 19.0</td>
</tr>
<tr>
<td>8</td>
<td>18.2 ± 4.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oral</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28.9 ± 18.2(_d)</td>
</tr>
<tr>
<td>2</td>
<td>185.7 ± 30.9(_c)</td>
</tr>
<tr>
<td>4</td>
<td>168.0 ± 82.4</td>
</tr>
<tr>
<td>6</td>
<td>127.4 ± 65.9</td>
</tr>
<tr>
<td>8</td>
<td>63.7 ± 16.2(_c)</td>
</tr>
</tbody>
</table>

\(_a\) A group of three mice was sacrificed at each point.

\(_b\) Significantly different (\( P < 0.05 \); \( t \) test) from the value for subcutaneous administration.

\(_c\) Significantly different at \( P < 0.001 \).

\(_d\) Significantly different at \( P < 0.01 \).
subcutaneously administered sedecamycin against experimental *T. hyodysenteriae* infection in mice. This hypothesis is based upon the finding that similar therapeutic effects were observed following subcutaneous and oral administrations of sedecamycin (Table 1), despite the higher cellular concentrations of lankacidinol, an active metabolite of sedecamycin, observed after oral administration (Table 2).

It is thought that when a drug is administered subcutaneously, the main route of delivery to the intestine is the bile duct. Thus, we examined the protective effect of subcutaneously administered sedecamycin in mice in which the bile duct had been ligated to prevent biliary excretion. It is interesting that the protective effects of sedecamycin in bile duct-ligated mice and intact mice were almost equal. Analysis of drug concentrations in the ceca of bile duct-ligated mice revealed only lankacidinol when sedecamycin was administered subcutaneously. The results of the present study demonstrate that a metabolite of sedecamycin is excreted into the gastrointestinal tract by a means other than bile.

Intestinal excretion has been reported for erythromycin. When erythromycin was infused into the jugular veins of anesthetized rabbits, the antibiotic was secreted into the midjeunal segments (12). Haass et al. (6) reported that after intravenous infusion of digitoxin or digoxin, metabolites were present in the gut contents of guinea pigs, although the bile duct had been cannulated. For some compounds, intestinal excretion is reported to be an important process (3). However, according to Israeli and Dayton (13), intestinal excretion has largely been ignored by pharmacologists and toxicologists, probably because the absorption process masks the reverse phenomenon of excretion into the intestine.

The in vitro activity of lankacidinol against *T. hyodysenteriae* DJ70P3 is less than that of sedecamycin. The MICs for lankacidinol and sedecamycin are 25 and 6.25 μg/ml, respectively. A single subcutaneous administration of sedecamycin at a dose of 40 mg/kg resulted in mean peak cecal lankacidinol levels in mice of 74.9 μg/ml 4 h after administration (Table 2). In this case, the peak concentration exceeded the MIC. However, when sedecamycin was given subcutaneously and orally, the ED50 for sedecamycin were 4.9 and 6.4 mg/kg, respectively (Table 1). With this dose of approximately 5 mg/kg, it can be assumed that the concentration of lankacidinol in the cecum is below the MIC. Therefore, there is a discrepancy between the MIC and the cecal concentrations of lankacidinol.

The cecal lankacidinol concentration was 23.7 μg/g in bile duct-ligated mice 4 h after administration, and this drug concentration was 91.8 μg/g in intact mice. The difference indicates that 30% of the lankacidinol was not derived via bile. Despite the difference in the cecal concentrations of lankacidinol between bile duct-ligated and intact mice, the efficacy of sedecamycin was almost the same in both. These data suggest that concentration of the drug in the cecal contents alone does not determine the efficacy of the drug, but rather that intestinal excretion of an active metabolite is important for efficacy.

*T. hyodysenteriae* has been observed within the crypts of Lieberkühn and goblet cells in mice (14) and pigs (5, 15, 16). It has also been shown that the crypts of Lieberkühn are the site of cyclic AMP-mediated fluid and electrolyte secretion in the large intestine (26). Therefore, when sedecamycin is given subcutaneously, the drug and its metabolites are distributed by the biliary route and may be excreted by the crypts, which *T. hyodysenteriae* colonizes. In this way, *T. hyodysenteriae* is probably exposed to a higher concentration of the drug and its metabolites in the crypts than in the cecal contents. Thus, *T. hyodysenteriae* would be eradicated from the crypts. Our results support the suggestion by Olson and Rodabaugh (20) that the drug which was not secreted in the mucus in the colon did not eliminate *T. hyodysenteriae* from swine, although the drug caused cessation of diarrhea.

In the present experiment, sedecamycin given subcutaneously showed a protective effect against experimental infection with *T. hyodysenteriae* in bile duct-ligated mice. Moreover, lankacidinol, a sedecamycin metabolite, was found in the ceca of bile duct-ligated mice. Therefore, intestinal excretion proved to play an important role in the efficacy of sedecamycin against infection of mice by *T. hyodysenteriae*.

ACKNOWLEDGMENTS

We thank M. Matsui and I. Suenaga for valuable advice and suggestions. We also thank K. Morishima, Y. Matsubara, and Y. Yamaguchi for technical assistance.

REFERENCES


