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

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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 10^7 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

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the probit method (17) from the rate at which T. hyodysenteriae 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 Pharmaceutical 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 no. 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. hyodysenteriae 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. hyodysenteriae 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. hyodysenteriae 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 lankacidinol 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 μg/g (mean ± standard deviation) 4 h after subcutaneous administration of sedecamycin, while the peak was 185.7 ± 30.9 μg/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 μg/g. The lankacidinol concentration in the ceca of sham-operated mice was 91.8 ± 27.2 μg/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. hyodysenteriae DJ70P3 |
|---------------------------|---------------------------|---------------------------|
| **Compound**              | **MIC** (μg/ml)           | **ED_{50}** (mg/kg/day)    |
|                           |                           | Subcutaneous              | Oral                  |
| Sedecamycin               | 6.25                      | 4.9 (2.4–8.6)             | 6.4 (3.6–11.1)        |
| Tiamulin                  | 0.1                       | 8.3 (4.1–15.8)            | 12.1 (6.9–21.6)       |
| Lincomycin                | 50                        | 37.6 (20.7–67.0)          | 12.8 (7.3–22.1)       |

* Animals were treated once a day from days 7 to 10 postinoculation and necropsied on postinoculation day 14.
* MICs were determined by the agar dilution method.
* The numbers in parentheses are 95% confidence limits.
* The oral ED_{50} data shown have been previously reported (11) and are included for comparison.
* 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 |
|---------------------------|---------------------------|
| Time after administration (h) | Mean lankacidinol concn (μg/g) ± SD |
|                           | Subcutaneous              | Oral                  |
| 1                         | 4.6 ± 2.3                 | 28.9 ± 18.2            |
| 2                         | 33.1 ± 3.0                | 185.7 ± 30.9           |
| 4                         | 74.9 ± 18.8               | 168.0 ± 82.4           |
| 6                         | 41.9 ± 19.0               | 127.4 ± 65.9           |
| 8                         | 18.2 ± 4.1                | 63.7 ± 16.2            |

* A group of three mice was sacrificed at each point.
* Significantly different (P < 0.05; t test) from the value for subcutaneous administration.
* Significantly different at P < 0.001.
* Significantly different at P < 0.01.
subcutaneously administered sedecamycin against experi-
mental *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 central concentra-
tions of lankacidinol, an active metabolite of sedecamycin,
observed after oral administration (Table 2).

It is thought that when a drug is administered subcutane-
ously, the main route of delivery to the intestine is the bile
duct. Thus, we examined the protective effect of subcutane-
ously 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. Anal-
ysis 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
midjejunal segments (12). Haass et al. (6) reported that after
intravenous infusion of digoxin or digoxin, metabolites
were present in the gut contents of guinea pigs, although the
bile duct had been cannulated. For some compounds, intes-
tinal excretion is reported to be an important process (3).
However, according to Israili 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 intes-
tine.

The in vitro activity of lankacidinol against *T. hyodysen-
teriae* DJ70P3 is less than that of sedecamycin. The MICs
for lankacidinol and sedecamycin are 25 and 6.25 μg/mL, respec-
tively. A single subcutaneous administration of sedecamycin
at a dose of 40 mg/kg resulted in mean peak cecal lankaci-
dinol levels in mice of 74.9 μg/g at 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 lankac-
dinol 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 secre-
tion 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 concentra-
tion 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 subcutane-
ously showed a protective effect against experimental infec-
tion with *T. hyodysenteriae* in bile duct-ligated mice. More-
over, 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*.

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REFERENCES

1. Blaha, T. W., E. Erler, and D. G. S. Burch. 1987. Swine dysen-
tery control in the German Democratic Republic and the suitability
of injections of tiamulin for the programme. Vet. Rec. 121:416-
419.
injection for the treatment of swine dysentery. Vet. Rec. 113:236-
237.
Elimination of drugs by passive diffusion from blood to intesti-
nal lumen: factors influencing nonbiliary excretion by the intesti-
4. De Vries, M. H., C. M. A. Rademaker, C. Geerlings, A. V. Dijk,
of activated charcoal on the intestinal secretion of theophylline,
using the isolated vascularly perfused rat small intestine. J.
Pharm. Pharmacol. 41:528–533.
of spirochetes with the structural characteristics of *Treponema
hyodysenteriae* in the lesions of swine dysentery. Infect. Immun.
of some cardiac glycosides and portal blood flow. Eur. J. Phar-
of parental administration of lincomycin on experimentally
1972. Swine dysentery. I. Isolation of pigs with *Treponema
hyodysenteriae* (new species) and reproduction of the disease.
1972. Isolation and propagation of spirochetes from the colon of
1988. In vitro and in vivo activities of sedecamycin against
32:458–461.
otic elimination: role of intestinal excretion. Drug Metab. Rev.
15:1123–1159.
mice with *Treponema hyodysenteriae*. Infect. Immun. 25:757–
760.


