Pharmacokinetics of amoxicillin after oral administration in recently weaned piglets with experimentally induced Escherichia coli subtype O149:F4 diarrhea

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Objective—To measure the effect of Escherichia coli subtype O149:F4-induced diarrhea on the pharmacokinetics of orally administered amoxicillin in affected piglets relative to that of uninfected piglets.

Animals—22 healthy 4-week-old recently weaned Danish crossbred piglets.

Procedure—12 piglets were orally inoculated through gastric intubation with $10^9$ CFUs of an E coli O149:F4 strain and responded by developing diarrhea 12 to 16 hours later. Piglets were dosed with amoxicillin trihydrate solution (20 mg/kg) by gastric intubation. A control group of 10 age-matched piglets without signs of diarrhea was dosed similarly. Blood samples were obtained before amoxicillin administration and at 0.5, 1, 1.5, 2, 3, 6, 12, and 24 hours after amoxicillin administration. The plasma concentration of amoxicillin was analyzed by high-performance liquid chromatography.

Results—A significant 39% decrease in the area under the plasma concentration versus time curve of amoxicillin was observed in piglets with diarrhea relative to that of control piglets. The maximum plasma concentration ($C_{max}$) was significantly (52%) lower in piglets with diarrhea, compared with control piglets, while the elimination rate constant, time to reach $C_{max}$ and elimination half-life were unchanged.

Conclusions and Clinical Relevance—Escherichia coli-induced diarrhea may decrease systemic bioavailability of amoxicillin. Escherichia coli bacteria attach to the intestinal epithelial cells. Because it is assumed that the concentration of the antimicrobial at the site of infection reflects the systemic concentration, higher doses of amoxicillin in the treatment of piglets with E coli O149:F4-induced diarrhea may be appropriate.


Escherichia coli O149:F4-induced diarrhea is a common enteric disease in piglets, especially in the first week after birth and weaning, causing a reduction in welfare and substantial economic costs. Escherichia coli O149:F4-induced diarrhea has often been treated with amoxicillin given orally, which has proven active against E coli O149:F4.

Infectious diseases may result in altered pharmacokinetics of antibiotics, possibly as a result of changes in blood supply in the intestine, tissue permeability, or distribution between plasma and tissue. It has been shown that humans and rats with Giardia lamblia infections have a decrease in intestinal absorption of amoxicillin following oral administration. Intestinal absorption of amoxicillin and E coli O149:F4-induced secretion may share the same physiologic transport mechanisms. Intestinal absorption of amoxicillin has been shown to result from passive diffusion and a saturable active sodium-dependent transport mechanism.

Secretory diarrhea may alter the bioavailability of amoxicillin by changing the intestinal absorption without affecting hepatic first pass metabolism or excretion. Although oral bioavailability of amoxicillin is known in healthy piglets, it may well be different during E coli O149:F4-induced diarrhea. The purpose of the study reported here was to measure the effect of E coli O149:F4-induced diarrhea on the pharmacokinetics of amoxicillin in affected piglets relative to that of uninfected piglets.

Materials and Methods

Animals and housing—The Danish Animal Experiments Inspectorate approved the study. Twenty-two clinically healthy 32- to 34-day-old Danish crossbreed piglets were used. Piglets came from 4 different litters and were housed 1 to 8 piglets/pen to obtain a minimum floor area of 0.3 m²/piglet. Sawdust was used as bedding and room temperature kept at 20°C to 24°C. Light was both natural and artificial. Ventilation was maintained with a total room air exchange of 7 exchanges/h.

Experimental design—All chosen piglets had no history of intestinal diseases or medication administration for at least 3 weeks. At the time of arrival to the animal facility, piglets weighed 6.0 to 10.1 kg and were housed 1 to 8 piglets/pen to obtain a minimum floor area of 0.3 m²/piglet. Sawdust was used as bedding and room temperature kept at 20°C to 24°C. Light was both natural and artificial. Ventilation was maintained with a total room air exchange of 7 exchanges/h.

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Diarrhea-group piglets were inoculated for the first time on the day of arrival (day 0). Administration of amoxicillin and blood sample collection was initiated in diarrheal diarrheagroup piglets when diarrhea was first observed. Administration of amoxicillin and blood sample collection were initiated in control-group piglets the day after arrival (day 1). Both groups of piglets were observed clinically until the last blood sample was taken. To prevent contamination between groups, boot covers, gloves, and overalls were changed.

Amoxicillin trihydrate crystallized into water (10 mg/mL) was orally administered through gastric intubation as a single dose of 20 mg/kg to diarrhea-group piglets. Control-group piglets were dosed similarly; food was withheld from both groups of piglets from 10 hours before until 2 hours after amoxicillin administration.

Blood samples were collected from the jugular vein by use of 5-mL evacuated glass tubes that contained heparin as an anticoagulant. Blood samples were obtained prior to drug administration and at 0.5, 1, 1.5, 2, 3, 6, 12, and 24 hours after administration of amoxicillin and kept at 5°C for a maximum of 3 hours before centrifugation (1,200 g; 10 minutes) to separate plasma. Plasma was kept at −80°C until analysis within 2 months.

Inoculum—The challenge strain of E. coli O149:F4ac was isolated at the Danish Veterinary Institute from the intestinal content of a piglet with postweaning diarrhea.13 The challenge strain harbored the genes for enterotoxins STb, LT, EAST1,12 and fimbria F4ac13 and was hemolytic when grown on blood agar. The challenge strain was grown at 37°C for 6 to 7 hours in 1 L of veal infusion broth that was shaken constantly. Six hundred milliliters of this culture was centrifuged (17,700 × g; 30 minutes), the supernatant discarded, and the pellet resuspended in 300 mL of PBS solution.

Infection model—Each piglet in the experimentally inoculated diarrhea group received 25 mL of inoculum suspension through orogastric intubation at 5 hours after arrival (day 0) and every morning and afternoon thereafter until diarrhea developed for a maximum of 3 days; the median daily dose per piglet was 1.8 × 10⁸ CFUs (range, 5.0 × 10⁶ CFUs to 1.9 × 10⁸ CFUs). After each inoculation, the gastric tube was flushed with 30 mL of 10% NaHCO₃ to ensure that no inoculum was left in the tube and that gastric pH was increased to extend bacterial survival. Control-group piglets were not sham gavaged with bicarbonate because antibiotic treatment was not initiated until at least 12 hours later. It was presumed that the normal stomach pH was fully restored at this point. Ordinary weaning food without antibiotics was offered ad libitum.

Microbiologic analysis—At the day of arrival and before inoculation, fecal samples were collected from the rectum of piglets and examined for the presence of common porcine pathogenic E. coli, rotavirus, and coccidia. For detection of E. coli, swabs were streaked onto blood agar plates and grown at 37°C overnight. Two colonies from each plate were chosen for serotyping in a panel of rabbit O-antisera, which is used for routine typing of common porcine pathogenic E. coli organisms. For fecal samples from piglets with diarrhea, 3 colonies were tested by the same method. Isolates with positive results in this panel were further examined for the presence of virulence factor genes by use of a 5' nuclease PCR assay.12 The presence of rotavirus was tested by use of an ELISA.14 The presence of coccidia was tested by use of a flotation technique followed by determination of oocyte count under light microscopy.15

Clinical examination—Clinical examinations included observation of fecal consistency; rectal temperature measurement, physical examination for cyanosis, and evaluation for possible behavioral disturbance. Fecal consistency was visually judged at the day of arrival before inoculation (day 0) and each morning and evening thereafter for the development of diarrhea. Piglets with feces without shape (forming a pool) or watery feces were considered to have diarrhea. Piglets with feces that were fully shaped were considered not to have diarrhea. Fecal consistency was evaluated by determining the percentage of fecal dry matter, which was calculated by weighing feces before and after drying to a constant weight at 100°C for 18 to 24 hours.

Rectal temperature was measured at the day of arrival and subsequently once a day in the morning. Piglets were observed for signs of behavioral disturbances and cyanosis on the day of arrival and subsequently every morning and afternoon. Disturbed behavior was defined by slow reactions, an unsteady and slow gait at the walk, and inattentive responses when encouraged to move. Undisturbed behavior was characterized by immediate reactions, a fast and steady gait at the walk, and attentive responses when encouraged to move. Cyanosis was defined as blue discoloration of the ears or limbs.

High-performance liquid chromatography analysis—The plasma concentration of amoxicillin was determined by use of a high-performance liquid chromatography method adapted from Miyazaki et al.16 The system was equipped with a fluorescence detector, which separated at excitation and emission wavelengths of 335 and 435 nm, respectively. Separation was achieved on a C18 guard column17 fitted with a C18 column,18 which was maintained at 30°C. The mobile phase consisted of a mixture of methanol and water (40:60). Flow rate was set at 1.0 mL/min. The product eluted at 5.0 minutes.

Pharmacokinetics—Data were fitted to an open 1-compartment model. The area under the plasma concentration versus time curve (AUC) for amoxicillin was calculated by use of the trapezoidal rule from time 0 to the last experimental time point (ie, AUC₀₋₃₆₅).17 Relative bioavailability was assessed as the AUC ratio between diarrhea and control group piglets. The elimination rate constant was calculated by regression analysis of the linear part of the descending slope of log-transformed plasma concentrations curve. The elimination half-life (T₁/₂) was calculated as the natural logarithm (base 2)/elimination rate constant (kₑ).

Statistical analysis—Analysis of the plasma amoxicillin concentration was performed by use of a repeated measure ANOVA on ranks, and results are reported as estimated means (± SE). Pharmacokinetic parameters were analyzed by use of a Mann-Whitney rank sum test, and results are reported as median and range. Percentages of fecal dry matter were analyzed by use of a repeated measure ANOVA, and results are reported as estimated means (± SE). Values of P < 0.05 were considered significant.

Results

Mean plasma amoxicillin concentrations of infected diarrhea- and control-group piglets were determined (Figure 1). A significant (P < 0.001) effect of diarrhea on plasma amoxicillin concentrations was found throughout the following time course: 0.5, 1, 1.5, 2, 3, and 6 hours. Diarrhea-group piglets had a significant decrease in the mean (± SD) time that plasma concentrations of amoxicillin were > 1.0 mg/L, compared with control-group piglets; diarrhea-group piglets had a mean time of 9.9 ± 3.3 hours, and control-group piglets had a mean time of 13.4 ± 3.6 hours. Pharmacokinetic

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parameters were calculated from individual plasma concentration curves (Table 1). Diarrhea-group piglets had a 39% significantly lower AUC for amoxicillin, compared with control-group piglets. The maximum plasma concentration ($C_{\text{max}}$) significantly ($P < 0.001$) decreased by 52% among diarrhea-group piglets, compared with control-group piglets. No effect of diarrhea was observed on the time to reach $C_{\text{max}}$ and $T_{1/2}$.

For all inoculated piglets in the diarrhea group, the onset of diarrhea was observed in the morning of day 1 at 12 to 16 hours after the first inoculation and lasted for 12 to 48 hours (Table 2). The percentage of fecal dry matter was significantly ($P < 0.001$) different between days 0 and 1 and between days 0 and 2 in diarrhea-group piglets. The percentage of fecal dry matter was also significantly ($P < 0.001$) different between diarrhea- and control-group piglets on days 1 and 2. Rectal temperatures ranged in diarrhea-group piglets from 38.1°C to 39.4°C, except for 1 piglet with a rectal temperature of 40.1°C on day 0. Rectal temperatures ranged in control-group piglets from 36.4°C to 38.7°C. The hyperthermic piglet did not have signs of any other disease, and the rectal temperature returned to within the reference range within a few hours. Signs of systemic infection that included behavioral disturbances and cyanosis were not observed.

Escherichia coli O149:F4 was found in fecal samples from all piglets in the diarrhea group on days 1 or 2 after infection and was indistinguishable from the E coli used for inoculation. In control-group piglets, only nonhemolytic E coli organisms were detected in fecal samples. Rotavirus was found in most of the fecal samples of piglets from the control group and in 1 fecal sample of a piglet from the diarrhea group. Coccidia were detected in 1 fecal sample of a piglet from the diarrhea group.

### Discussion

In our study, we hypothesized that intestinal absorption of amoxicillin in piglets may be changed by E coli O149:F4-induced diarrhea. Indeed, our results indicate that oral bioavailability and $C_{\text{max}}$ of orally administered amoxicillin are significantly decreased by diarrhea in piglets relative to that of uninfected piglets. Similar findings for amoxicillin were reported in a study with human subjects and rats infected with Giardia lamblia.

Amoxicillin absorption and E coli O149:F4 secretion could presumably share the same physiologic mechanisms. Amoxicillin absorption is mediated through an active transport process involving a sodium exchange mechanism and through passive diffusion that is dependent on a large mucosal surface area. The E coli enterotoxin-induced fluid secretion is partly mediated through a sodium-chloride secretion mechanism and partly through a reduction of the intestinal mucosal surface area caused by bacterial adherence to the epithelia. Thus, it is possible that E coli O149:F4-induced diarrhea reduces intestinal absorption of amoxicillin by molecular mechanisms. The role

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**Table 1**—Median (range) values of amoxicillin pharmacokinetic parameters following gastric intubation of a single dose (20 mg/kg) in 4-week-old piglets with (diarrhea group) and without (control group) experimentally induced diarrhea caused by inoculation with Escherichia coli O149:F4.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Diarrhea (n = 12)</th>
<th>Control (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (mg·h/L)</td>
<td>28 (40)*</td>
<td>46 (59)</td>
</tr>
<tr>
<td>AUC/AUC&lt;sub&gt;control&lt;/sub&gt;</td>
<td>0.61</td>
<td>1.00</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (mg/L)</td>
<td>3.6 (4.8)*</td>
<td>7.5 (5.7)</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>$k_e$ (mg/L·h)</td>
<td>0.14 (0.10)</td>
<td>0.17 (0.11)</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>5.0 (4.8)</td>
<td>4.2 (3.6)</td>
</tr>
</tbody>
</table>

*Significantly ($P < 0.01$) different from control group.
†Significantly ($P < 0.001$) different from control group.

**Table 2**—Mean (± SE) clinical values during 3 consecutive days in 4-week-old newly weaned piglets with (diarrhea group) and without (control group) experimentally induced diarrhea caused by inoculation with E coli O149:F4.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control-group piglets</th>
<th>Diarrhea-group piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea ratio</td>
<td>Day 0</td>
<td>Day 1</td>
</tr>
<tr>
<td>Fecal dry matter (%)</td>
<td>0:10</td>
<td>0:10</td>
</tr>
<tr>
<td>Diarrhea group Day 0</td>
<td>ND</td>
<td>30 ± 3 (n = 4)*</td>
</tr>
</tbody>
</table>

Day 0 represents 5 hours before E coli O149:F4 inoculation. Day 1 and day 2 represent 1 and 2 days, respectively, after E coli O149:F4 inoculation. *Significantly ($P < 0.001$) higher value in the control group, compared with the diarrhea group, on day 1. †Significantly ($P < 0.001$) higher value in the control group, compared with the diarrhea group, on day 2. Diarrhea ratio = Number of piglets with diarrhea to number of piglets without diarrhea. ND = Not determined.
of passive and active absorption has been studied with respect to secretory diarrhea; findings indicate that active absorption is mainly decreased in instances of secretory diarrhea. In our study, E coli O149:F4-induced diarrhea decreased amoxicillin AUC and C max most likely as a result of a decrease in intestinal absorption. Moreover, diarrhea decreased the time above plasma concentrations of 1.0 mg/L. In instances of therapeutic use, time above bacterial \textit{minimum inhibiting concentration} (MIC, i.e., \( t > \text{MIC} \)) is the major determinant of bacterial elimination rate for amoxicillin.\textsuperscript{22–24} For MIC values exceeding corresponding plasma concentrations of 1.0 mg/L, diarrheic piglets may have plasma amoxicillin concentrations with a decreased time above MIC and hence a less effective bacterial elimination rate. This suggests that a higher dose of amoxicillin may be appropriate in the treatment of piglets with diarrhea caused by \( E \) \textit{coli}. Because intestinal tissue antibiotic concentration mainly reflects the systemic concentration and \( E \) \textit{coli} organisms attach to the mucosal epithelial cells while causing diarrhea, it is assumed that the drug concentration at the site where the bacteria are located also mainly reflects the systemic drug concentration.

\textsuperscript{5}Boesen, Ballrup, Denmark.
\textsuperscript{6}Clamoxyl vet 31%, Pfizer, Ballrup, Denmark.
\textsuperscript{1}ID 9910045-1, Danish Veterinary Institute, Frederiksberg, Denmark.
\textsuperscript{2}Columbia agar (Oxoid) supplemented with 5% calf blood, Danish Veterinary Institute, Frederiksberg, Denmark.
\textsuperscript{3}Difco, Detroit, Mich.
\textsuperscript{4}Waters, Milford, Mass.
\textsuperscript{5}Sperisorb S50ODs2, C18 column, Mikrolab Aarhus, Denmark.
\textsuperscript{6}Security Guard, Phenomenex, Torrance, Calif.

References