Pharmacokinetics and tissue distribution of amoxicillin in healthy and Salmonella Typhimurium-inoculated pigs

Henrik Agersø, DVM, PhD; Christian Friis, DVM, PhD; Jens Peter Nielsen, DVM, PhD

Objective—To determine pharmacokinetics and tissue distribution of amoxicillin in healthy and Salmonella Typhimurium-inoculated pigs.

Animals—12 healthy pigs and 12 S Typhimurium-inoculated pigs.

Procedure—Concentration of amoxicillin in tissue was measured by use of high-performance liquid chromatography 4, 8, 12, and 24 hours after IM administration. Pharmacokinetic values of amoxicillin in plasma were assessed by use of a 1-compartment model with first-order absorption.

Results—Inoculation caused diarrhea and increased rectal temperature and WBC count. Absorption half-life was shorter in inoculated pigs (0.26 hours) than in healthy pigs (0.71 hours), and inoculated pigs had longer elimination half-life. Distribution ratios in healthy pigs ranged from 0.31 to 0.56 and in inoculated pigs ranged from 0.14 to 0.48. Ratios for distribution to intestinal mucosa ranged from 0.34 to 1.16 in healthy pigs and from 0.22 to 0.36 in inoculated pigs.

Conclusions and Clinical Relevance—Salmonella Typhimurium inoculation altered absorption of amoxicillin from the injection site and prolonged elimination half-life. However, distribution of amoxicillin to intestinal tract tissue was only affected to a minor degree. (Am J Vet Res 2000;61:992–996)

Infection with Salmonella Typhimurium is a concern to veterinarians and swine producers as a cause of septicemia and diarrhea in pigs and as a source of foodborne salmonellosis in humans. The mechanisms by which Salmonella spp induce diarrhea are not fully understood, but enterotoxins are important factors. Another important factor is invasiveness of the bacteria; only invasive Salmonella strains induce an intestinal fluid response. Following penetration of the intestinal mucosa, Salmonella organisms may spread to the mesenteric lymph nodes and other tissues. High concentrations of amoxicillin have been detected throughout the small intestine following oral administration to calves, indicating that oral administration is an effective method of achieving high concentrations in the gastrointestinal tract. However, elimination of Salmonella organisms in the intestinal lumen alone may not be satisfactory when treating salmonellosis.

Generally, limited information is available for the distribution of drugs during pathologic conditions, although it is known that pathophysiologic changes induced by inflammation may influence drug disposition. The objective of the study reported here was to determine pharmacokinetics and tissue distribution of amoxicillin following IM administration to healthy and S Typhimurium-inoculated pigs.

Materials and Methods

Pigs—Twenty-four healthy female pigs (Landrace X Yorkshire) weighing 25 to 41 kg and obtained from a herd known to be free from salmonellosis were included in the study. Pigs were randomly allocated into 2 groups of 12 pigs. Pigs were fed pelleted commercial feed twice daily and had free access to drinking water. Pigs were housed in individual pens. Experiments were performed in 2 different locations and periods to separate infected pigs from healthy pigs.

Drug administration and blood sampling—Amoxicillin sodium was supplied by the manufacturer. Concentration of amoxicillin in the preparation was determined by use of high-performance liquid chromatography (HPLC), using an amoxicillin standard containing 97.6% amoxicillin. All other chemicals were obtained from commercial sources and were of analytic grade.

For all pigs, amoxicillin (15 mg/kg of body weight) was administered IM in the cervical region, 10 cm caudal to the ear. Blood samples were collected into tubes containing heparin before and 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 hours after drug administration.

For Salmonella-inoculated pigs, rectal temperatures were recorded, and supplementary blood samples for differential cell counts were collected into tubes containing EDTA before and 6, 12, and 24 hours after inoculation and just before pigs were euthanized. Serum and plasma was separated and stored at –20°C until assayed.

Experimental design—For healthy pigs, tissue samples and intestinal contents were collected serially from groups of 3 pigs 4, 8, 12, and 24 hours after drug administration. At sampling time, pigs were killed by use of a bolt pistol and bled by cutting the vena jugularis externa. The intestine was removed intact, and specimens of intestinal wall tissue and mucosa as well as contents were collected at dilated jejunal loops, cecum, and the flexura centralis of colon. Specimens were also collected from the mesenteric lymph nodes.

For Salmonella Typhimurium-inoculated pigs, an inoculation model was used, as described. Briefly, Salmonella strain NVL810 was prepared from a stock culture kept at –80°C and subcultured on call blood agar for 24 hours at 37°C. One colony was inoculated in a 10-ml aliquot of real infusion broth (VB) and incubated at 37°C for 16 hours, without shaking. From this broth culture, 100-ml aliquots were inoculated into twelve 100-ml vials of
trifuged at 2,000 g for 10 minutes at 4 C. The pellet was washed 2 times and centrifuged at 2,000 × g for 10 minutes at 4 C. The pellet was resuspended in saline (0.9% NaCl) solution. Viable count in saline (0.9% NaCl) solution was determined, using a standard dilution technique. Each pig received 20 ml of this suspension intragastrically by use of a stomach tube. Amoxicillin was administered 24 hours after inoculation.

Dry matter content—Samples of intestinal contents (jejunum, cecum, and colon) of approximately 1 to 3 g wet weight were obtained for determination of dry matter content. Dry weight of samples was recorded after dehydration for 24 hours at 100 C. Percentage dry matter was calculated as: dry weight × 100/wet weight.

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Amoxicillin analysis—Concentration of amoxicillin in plasma, tissue, and mucosa was determined by a HPLC method adapted from Miyazaki et al.21 and described in detail elsewhere.21 Limit of quantification (LOQ) was 0.05 µg/ml in plasma and 0.1 µg/ml in tissue and mucosa. All values lower than LOQ were excluded from the calculations.

Pharmacokinetic analysis of data—Pharmacokinetic analysis of amoxicillin concentration versus time in plasma was performed individually for 6 pigs in each group, by use of a least-squares nonlinear regression program.1 Data were fit to 2 models: a 1-compartment model with equal first-order input and first-order output and a 1-compartment model with first-order input and first-order output. Best fit was determined by application of Akaike and Swartz criteria.

The area under the concentration versus time curves (AUC) in tissues and plasma were calculated by use of the trapezoid rule. The infinite part of the curve was determined as Ct last /λz, where Ct last is the last concentration and λz is the slope of the last phase, calculated on the basis of the last 4 sampling points in plasma. The infinite part of the curve was not included in the AUC for tissues. Mean residence time (MRT) was calculated by use of the trapezoid rule.

Distribution ratio was calculated as:

\[
\text{Distribution ratio} = \frac{AUC_{\text{tissue}(0-t)}}{AUC_{\text{plasma}(0-t)}}
\]

where t was the last sampling time. Statistical evaluations were made by use of a nonparametric Mann-Whitney test, using commercially available software. A value of P < 0.05 was considered significant.

Results

Clinical response to inoculation—Inoculation with S Typhimurium elicited a significant febrile response within 6 hours (Fig 1), and inoculated pigs became anorectic. After inoculation, a small decrease in WBC count developed, reaching a minimum value of 17 × 10^9 WBC/L 6 hours after inoculation. During the following 24 hours, WBC count increased significantly. Inoculation induced a significant decrease in dry matter content 8 and 12 hours after inoculation in all 3 segments of the intestine (Fig 2).

Pathologic changes were characterized by catarrhal enterocolitis, hyperemia of the intestinal mucosa, and in 12 of pigs, fibrinonecrotic typhlitis and colitis. The associated mesenteric lymph nodes were greatly enlarged and moist.

Pharmacokinetics—Disposition of amoxicillin after IM administration to pigs was best described by a 1-compartment model with first-order input and first-order output, as indicated by the equation:

\[
\frac{F-D}{C_t} = \frac{k_1}{V} (e^{k_1 t} - e^{k_2 t})
\]

where C_t was the plasma concentration at time t, F was bioavailability, D was dose administered, V was volume, k_1 was the absorption rate constant, and k_2 was the elimination rate constant.

Inoculation with S Typhimurium resulted in a change in the plasma concentration-time profile, chara-
Characterized by significantly faster absorption from the injection site and an increase in the elimination half-life (Fig 3), compared with healthy pigs. Peak concentration ($C_{\text{max}}$) was not altered in inoculated pigs, whereas time to reach peak concentration ($t_{\text{max}}$) was shorter in inoculated pigs, although not significantly ($P = 0.09$; Table 1).

Volume of distribution (V) and clearance cannot be calculated directly from the estimated parameters. Assuming bioavailability (F) was the same in the 2 groups, alteration in the V/F value (Table 1) indicated a significant increase in volume of distribution in the inoculated pigs.

Disposition to tissue and mucosa—The distribution of amoxicillin to the intestinal wall tissue was lower in inoculated than in healthy pigs, although not significantly (Fig 4–6). At certain sampling points, tissue concentrations were lower than LOQ and were included in the calculations as 0. Likewise, the tissue to plasma ratio of amoxicillin for cecum, colon, and lymph nodes was lower in infected pigs, compared with healthy pigs, although not significantly, whereas the distribution ratio to jejunum was comparable for

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**Table 1—Pharmacokinetic values (mean ± SD; n = 6 for each group) for amoxicillin after IM administration to healthy and Salmonella Typhimurium-inoculated pigs**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy pigs</th>
<th>S Typhimurium-inoculated pigs</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V/F (mg/L)</td>
<td>2.3 ± 0.6</td>
<td>3.9 ± 1.5</td>
<td>0.04</td>
</tr>
<tr>
<td>$k_{\text{abs}}$ (1/h)</td>
<td>1.04 ± 0.24</td>
<td>3.1 ± 1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$k_{\text{el}}$ (1/h)</td>
<td>0.21 ± 0.07</td>
<td>0.12 ± 0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>$t_{\frac{1}{2}}$ (abs) (h)</td>
<td>0.71 ± 0.24</td>
<td>0.26 ± 0.13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$t_{\frac{1}{2}}$ (el) (h)</td>
<td>3.6 ± 1.1</td>
<td>11.2 ± 11.3</td>
<td>0.06</td>
</tr>
<tr>
<td>$\lambda_{\text{c}}$ (h)</td>
<td>0.20 ± 0.04</td>
<td>0.08 ± 0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>3.9 ± 1.6</td>
<td>12 ± 10</td>
<td>0.02</td>
</tr>
<tr>
<td>AUC (mg-h/L)</td>
<td>35 ± 4</td>
<td>54 ± 32</td>
<td>0.18</td>
</tr>
<tr>
<td>AUC$_{0-t}$ (mg-h/L)</td>
<td>33 ± 5</td>
<td>31 ± 6</td>
<td>0.39</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>6.3 ± 1.7</td>
<td>17 ± 13</td>
<td>0.03</td>
</tr>
<tr>
<td>MRT$_{0-t}$ (h)</td>
<td>5.3 ± 1.3</td>
<td>6.2 ± 1.9</td>
<td>0.24</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>4.7 ± 1.2</td>
<td>3.7 ± 1.0</td>
<td>0.24</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>2.1 ± 0.5</td>
<td>1.3 ± 0.5</td>
<td>0.09</td>
</tr>
</tbody>
</table>

V/F = Volume divided by bioavailability, $k_{\text{abs}}$ = Absorption rate constant, $k_{\text{el}}$ = Elimination rate constant, $t_{\frac{1}{2}}$ (abs) = Half-life, $t_{\frac{1}{2}}$ (el) = Half-life calculated by use of $\lambda_{\text{c}}$, $\lambda_{\text{c}}$ = Slope for the last phase of the curve, $t_{\text{max}}$ = Half-life calculated by use of $\lambda_{\text{c}}$, AUC = Area under the curve, AUC$_{0-t}$ = Area under the curve from zero to the last sampling point, MRT = Mean residence time, MRT$_{0-t}$ = Mean residence time from zero to the last sampling point, $C_{\text{max}}$ = Peak plasma concentration of amoxicillin, $t_{\text{max}}$ = Time to reach $C_{\text{max}}$. 

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**Figure 3**—Amoxicillin concentration (mean ± SD; n = 6 for each group) in plasma after administration (15 mg/kg, IM) to healthy and Salmonella Typhimurium-inoculated pigs.

**Figure 4**—Amoxicillin concentration (mean ± SD; n = 3 for each group) in jejunal intestinal wall tissue and mucosa in healthy and Salmonella Typhimurium-inoculated pigs. No significant differences between groups were observed.

**Figure 5**—Amoxicillin concentrations (mean ± SD; n = 3 for each group) in cecal intestinal wall tissue and mucosa in healthy and Salmonella Typhimurium-inoculated pigs (bar missing for tissue in inoculated pigs 4 hours after inoculation because concentrations were less than limit of quantification value). No significant differences between groups were observed.

**Figure 6**—Amoxicillin concentrations (mean ± SD; n = 3 for each group) in colonic intestinal wall tissue and mucosa in healthy and Salmonella Typhimurium-inoculated pigs (bar missing for mucosa in inoculated pigs 4 and 8 hours after inoculation because concentrations were less than limit of quantification value). No significant differences between groups were observed.
healthy pigs (Table 1); this variation may have been
the cecum and colon, although not significantly.
were not significant, whereas this value was lower in
haps caused by a rebound effect), although differences
was higher in inoculated pigs than in healthy pigs (per-
inoculation, dry matter concentration in the jejunum
was delayed. At the last sampling point, 24 hours after
maximum response in the cecum and colon
mum response to inoculation (lowest dry matter con-
centration to healthy and S Typhimurium-inoculated pigs

Table 2—Pharmacokinetic values describing the disposition of amoxicillin in the
intestinal wall of the jejunum, cecum, and colon after IM administration to healthy
and S Typhimurium-inoculated pigs

<table>
<thead>
<tr>
<th>Tissue</th>
<th>AUCP,t (mg·h/L)</th>
<th>AUCPlasma(0–t)</th>
<th>MRT(0–t) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>Healthy 15.56</td>
<td>Inoculated 14.85</td>
<td>0.47</td>
</tr>
<tr>
<td>Cecum</td>
<td>Healthy 18.49</td>
<td>Inoculated 4.36</td>
<td>0.56</td>
</tr>
<tr>
<td>Colon</td>
<td>Healthy 15.63</td>
<td>Inoculated 9.89</td>
<td>0.48</td>
</tr>
<tr>
<td>Lymph node</td>
<td>Healthy 10.50</td>
<td>Inoculated 7.00</td>
<td>0.32</td>
</tr>
</tbody>
</table>

AUCPlasma(0–t) = Area under the curve for plasma, from zero to the last sampling point.
See Table 1 for remainder of key.

As described for intestinal wall tissue, lower amoxicillin concentrations in mucosal tissues of in-
oculated pigs were detected, compared with healthy pigs, although differences between these groups were not
significant (Fig 4–6, Table 3). At certain sampling
points, mucosal concentrations were lower than LOQ
and were included in the calculations as 0. The MRT
was greater for inoculated pigs, compared with healthy
pigs, but not significantly (Table 3).

**Discussion**

Clinical response to inoculation with S Typhimurium was characterized by diarrhea and an
increase in rectal temperature, as reported elsewhere. White blood cells were initially high, declined to
lower values, and then increased substantially. High
WBC counts at the first sampling point may be explained by stress caused by the inoculation pro-
cEDURE, whereas the subsequent increase may be ascribed to the presumed Salmonella infection.
Analysis of dry matter content in the intestines sug-
gested progression of infection. In the jejunum, maxi-
mum response to inoculation (lowest dry matter con-
tent) was observed at the first 2 sampling points,
whereas maximum response in the cecum and colon
was delayed. At the last sampling point, 24 hours after
inoculation, dry matter concentration in the jejunum
was higher in inoculated pigs than in healthy pigs (per-
haps caused by a rebound effect), although differences
were not significant, whereas this value was lower in
the cecum and colon, although not significantly.

Pharmacokinetic values in inoculated pigs com-
monly had greater variation (ie, SD) than those of
healthy pigs (Table 1); this variation may have been
caused by differences in response to inoculation.
However, although variation was high, the absorption
rate constant and the elimination half-life were influ-
enced by the inoculation. The significant increase in
absorption rate constant in the infected pigs may be
ascribed to the increase in body temperature and the
accompanying muscle shivering, which caused
increased blood flow at the site of injection. This
finding is in contrast to previous observations in calves, in
which experimental Escherichia coli endotoxemia
caused reduced absorption of amoxicillin. The dis-
crepancy may be explained by a different response to
inoculation between studies; the febrile response in the
calves was not as distinct as in the pigs reported here.
Intramuscular administration of penicillin G to Streptococcus suis-infected pigs resulted in a significant
decrease in the plasma Cmax and a prolonged elimina-
tion half-life, which is in agreement with our results.
The observed significant increase in the elimina-
tion half-life (Table 1) may be explained by a con-
comitant increase in V (assuming F was the same in
the 2 groups). The change in V may reflect a shift in
blood flow away from heat loss tissues (eg, skin) to
heat production tissues (eg, shivering muscle). A sim-
ilar shift in V of amoxicillin has been described in Actinobacillus pleuropneumoniae-infected pigs.

Distribution of amoxicillin to the intestinal tis-
sues and mucosa decreased in inoculated pigs, com-
pared with healthy pigs, although not significantly.
The limited number of pigs evaluated at each time
point may explain the failure to detect statistical dif-
fences between groups. In theory, infection is
expected to cause dilation of capillaries and increased
vascular supply to the infected region, which should
facilitate entry of antimicrobials to the inflamed
area. However, several factors may influence the
concentration of amoxicillin in the intestinal tissues
and mucosa. First, the AUC for amoxicillin in plasma
were not equal for healthy and inoculated pigs, con-
centrations in tissues will indirectly reflect the concentration in plasma. Second, healthy pigs retained a normal appetite, whereas inoculated pigs became anorectic, which may have affected blood flow to the intestinal tissues. Third, fever reduces blood flow to the gastrointestinal tract; endotoxin-induced fever causes a 20 to 40% decrease in blood flow to the stomach and the small and large intestines. Decreased blood flow may also explain the prolonged MRT we observed in intestinal wall tissue and mucosa. It seems that distribution of drugs to infected tissues may depend on a number factors: the type of infection, the infection site, and the drugs used for treatment of the infection. Results of previous experiments in our laboratory indicated an improved distribution of amoxicillin to the lung mucosa in Actinobacillus pleuropneumoniae-infected pigs. Results of the study reported here indicated lower tissue and intestinal mucosa concentrations in infected pigs, although statistical differences from healthy pigs were not detected. Inflammation per se does not seem to be decisive for the distribution kinetics. Other factors such as changes in blood flow in the inflamed areas may be of greater importance.

References