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.

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.

Materials and Methods

Pigs—Twenty-four healthy female pigs (Landrace × 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 veal infusion broth (VB) and incubated at 37°C for 16 hours, without shaking. From this broth culture, 100-µl aliquots were inoculated into twelve 100-ml vials of...
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 X 100/wet weight.

Amoxicillin analysis—Concentration of amoxicillin in plasma, tissue, and mucosa were determined by a HPLC method adopted from Miyazaki et al.11,12 and described in detail elsewhere.13 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.14 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 Clast/λz, where Clast 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:

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

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 \( 17 \times 10^9 \) 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:

\[ \frac{F \cdot D}{V} \cdot k_p = \frac{\left( e^{k_e \cdot t} - e^{k_a \cdot t} \right)}{k_e - k_a} \]

where \( C_t \) was the plasma concentration at time t, F was bioavailability, D was dose administered, V was volume, \( k_p \) was the absorption rate constant, and \( k_e \) was the elimination rate constant.

Inoculation with S Typhimurium resulted in a change in the plasma concentration-time profile, charac-
terized by significantly faster absorption from the injection site and an increase in the elimination half-life (Fig 3), compared with healthy pigs. Peak concentration (Cmax) was not altered in inoculated pigs, whereas time to reach peak concentration (tmax) 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...
the 2 groups of pigs (Table 2). The MRT for cecum, colon, and lymph nodes of inoculated pigs was greater than for healthy pigs, although not significantly (Table 2).

As described for intestinal wall tissue, lower amoxicillin concentrations in mucosal tissues of inoculated 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 cell counts 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 procedure, whereas the subsequent increase may be ascribed to the presumed *Salmonella* infection. Analysis of dry matter content in the intestines suggested progression of infection. In the jejunum, maximum response to inoculation (lowest dry matter content) 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 (perhaps 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 commonly 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 influenced 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 discrepancy 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 *C*<sub>max</sub> and a prolonged elimination half-life, which is in agreement with our results. The observed significant increase in the elimination half-life (Table 1) may be explained by a concomitant 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 similar shift in V of amoxicillin has been described in *Actinobacillus pleuropneumoniae*-infected pigs.

Distribution of amoxicillin to the intestinal tissues and mucosa decreased in inoculated pigs, compared with healthy pigs, although not significantly. The limited number of pigs evaluated at each time point may explain the failure to detect statistical differences between groups. In theory, infection is expected to cause dilatation 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;
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