THE INFLUENCE OF DISEASE ON FEED AND WATER CONSUMPTION AND ON PHARMACOKINETICS OF ORALLY ADMINISTERED OXYTETRACYCLINE IN PIGS

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ABSTRACT

In the present study the feed and water consumption and pharmacokinetic parameters of orally administered oxytetracycline were compared in clinically healthy pigs and in the same pigs following a challenge with Actinobacillus (Haemophilus) pleuropneumoniae toxins. Endobronchial challenge with A. pleuropneumoniae toxins was accompanied by anorexia, increased lassitude, labored breathing, fever, and increased white blood cell counts. Pleuropneumonia was evident in all pigs on autopsy. Following the challenge, both feed and water consumption were markedly reduced. In contrast to recommendations in the literature, it is concluded that drugs should not be administered to pneumonic pigs via water. In healthy pigs the oral bioavailability of oxytetracycline (50 mg/kg), given on an empty stomach, was 4.8% and the elimination half-life (t₁/₂β) was 5.92 h. After challenge, the pigs showed great variation in oxytetracycline plasma concentrations. In addition, the mean computed elimination rate constant (β), t₁/₂β, the area under the plasma concentration-time curve (AUC), and clearance in pneumonic pigs differed significantly (P < .05) from the values found in healthy pigs. The elimination half-life (t₁/₂β), AUC, and volume of distribution (Vd) were increased. In diseased pigs the mean of maximum plasma concentrations (87 µg/ml) was reached after 7 h, in contrast to 1.74 h (1.87 µg/ml) in the healthy pigs.

Key Words: Actinobacillus pleuropneumoniae, Appetite, Water Intake, Oxytetracycline, Pigs


Introduction

Mass medication is widely used in the swine industry. In particular, tetracyclines are administered via drinking water and as medicated feed to treat respiratory tract infections (Kunesch, 1986). Several authors suggest that water medication is preferred because sick pigs may drink but frequently will not eat (Blood et al., 1981; Kunesch, 1986). Anorexia during febrile conditions has been described in several species (Baile et al., 1981; McCarthy et al., 1984; van Miert et al., 1986). However, information quantifying feed and water intake of swine during disease is scarce.

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Febrile conditions may also influence the pharmacokinetics of drugs, as described for goats (Anika et al., 1986), calves (Groothuis et al., 1980), rabbits (Ladefoged, 1979a), and pigs (Ladefoged, 1979b; Pijpers et al., 1991). This phenomenon is of special interest with regard to orally administered drugs, due to potential for absorption changes. In particular, the pharmacokinetic profile of orally administered oxytetracycline (OTC) in diseased pigs is not addressed in the literature, although information is available on the pharmacokinetics of orally administered tetracyclines in healthy pigs (Mercer et al., 1978; Black and Gentry, 1984; Mevius et al., 1986; Hall et al., 1989; Kniffen et al., 1989). Therefore, OTC pharmacokinetics was studied in pigs using a pleuropneumonia model (van Leengoed, 1988).

The aim of this study was to investigate a) the feed and water consumption and b) the pharmacokinetics of oxytetracycline following oral administration to pigs challenged with *Actinobacillus pleuropneumoniae* toxins and to compare these to the healthy-state parameters of the same pigs prior to challenge.

**Materials and Methods**

**Animals.** Seven Great Yorkshire × Dutch Landrace castrated male pigs, weighing 35 to 46 kg BW, were obtained from the University of Utrecht's breeding farm. These clinically healthy pigs were housed in individual metabolic pens provided with separate urine and feces collection facilities. The pigs were placed in their pens 7 d before the start of the experiment to acclimate them. Two days before the start of the experiment, catheters were placed in the jugular vein according to an earlier described method (Pijpers et al., 1989) to facilitate frequent blood sampling. The pigs were given ad libitum access to drinking water and were fed .75 kg of antibiotic-free, pelleted feed twice daily.

**Drugs.** Oxytetracycline-HCl (OTC) was intravenously injected into the pigs via the catheter as a 10% solution at a dose of 10 mg/kg. After the administration of OTC the catheter was flushed with 10 ml of .9% NaCl. Oxytetracycline was orally administered as a 12.5% suspension at a dose of 50 mg/kg (Table 1). Within 1 h after preparing the suspension, the drug was administered by syringe directly into the mouth of the pigs, which had fasted for 20 h.

**Experimental Disease Model.** Six clinically healthy pigs were endobronchially inoculated with *A. pleuropneumoniae* toxins as described by van Leengoed et al. (1988). A seventh pig served as a control and was endobronchially inoculated with pyrogen-free saline. Rectal body temperatures were measured two times a day before and at 0, 1, 2, 3, 4, 6, 9, 12, 18, and 24 h after the challenge, respectively. Blood samples were collected at 0, .5, 1, 2, 4, 6, 9, 12, 18, and 24 h after challenge. White blood cell (WBC) counts were determined using methods described earlier (van Miert et al., 1982). Three days after challenge all pigs were killed, dissected, and examined for pneumonia. Lung lesions were weighed and gross visual and histological examination of pneumatic lesions were performed. The pneumatic lesions were examined bacteriologically.

**Experimental Procedure.** Six pigs were used to study the pharmacokinetics of OTC. First, OTC was administered intravenously at a dose of 10 mg/kg. Two days later the pigs were dosed orally with the OTC suspension at a dosage of 50 mg/kg. To prevent oxytetracycline from complexing with feed components, pigs were fasted for 20 h before oral dosing. Five days later the animals were challenged with *A. pleuropneumoniae* toxins. Three hours after the challenge OTC was given orally to fasted pigs at a dose rate of 50 mg/kg.

**Feed and Water Consumption.** Daily at 0800 and 1700, 750 g of pelleted feed was given to the pigs. Feed consumption of each pig was determined and recorded. Water intake was measured and recorded daily at 0800 and 1700.

**Sampling Procedure.** After intravenous administration of OTC, heparinized blood samples were collected at 0, 1, 1.5, 2, 4, 6, 9, 12, 15, 18, 21, 24, 28, and 32 h. After the oral administration of the OTC-suspension,

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**Table 1. Contents of the Oxytetracycline Suspension**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline-HCl&lt;sup&gt;*&lt;/sup&gt;</td>
<td>25 g</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>32.5 g</td>
</tr>
<tr>
<td>Syrup</td>
<td>60 g</td>
</tr>
<tr>
<td>Methylparaben</td>
<td>200 mg</td>
</tr>
<tr>
<td>Carboxymethylcellulose-Na 3% up to</td>
<td>200 ml</td>
</tr>
</tbody>
</table>

<sup>*</sup>Oxytetracycline-HCl, Pfizer, Brussels, Belgium.

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<sup>6</sup>Terramycin 100<sup>®</sup>, Pfizer, Brussels, Belgium.
Urine samples were collected at 0, 4, 8, 12, 16, 24, 28, 32, 40, and 48 h. The plasma was collected after centrifugation and stored at -20°C. Urine samples were collected at 0, 4, 10, 16, 24, 32, and 48 h during the experiment to measure the renal excretion of OTC. Urine samples were stored at -20°C.

Assays. All plasma samples were analyzed for OTC by bioassay; the plasma samples collected at 9, 16, and 24 h after drug administration were also analyzed by HPLC. All urine samples were analyzed for OTC by HPLC.

Bioassay. The plasma OTC-concentrations were carried out by bioassay using an agar-gel diffusion method employing Bacillus cereus var. mycoides ATCC 11778 as the test organism (Dombush and Abbey, 1972). The limit of detection was .1 μg/ml. Standard solutions were prepared in pig plasma and all samples were analyzed in duplicate.

High-Performance Liquid Chromatography. The HPLC method used was modified from that described by de Leenheer and Nelis (1979). The method comprises extraction of OTC from plasma or urine with ethyl acetate, evaporation to dryness, and reconstitution in eluent. Analyses were performed on RP 8 packing using acetonitrile-citric acid as eluent.

Pharmacokinetic Analysis. Pharmacokinetic analysis of the data were performed with the aid of NONLIN, a computer program for nonlinear regression analysis (Metzler, 1969). The data were analyzed using a two-compartment open model (Figure 1). The bioavailability (%) was calculated by the formula [(AUCoral/AUCiv) × (Doseiv/Doseoral)] × 100, where AUC = area under the curve, which is the integral of drug blood level over time from zero to infinity.

The volume of distribution (Vd area) was calculated by the equation Vd area = (D × f)/ (AUC × β), where D = dose administered, f = fraction of dose absorbed, and β = elimination rate constant.

The mean pharmacokinetic parameters were obtained by averaging the parameters calculated for drug disposition in individual pigs. The curves shown in Figure 3 are drawn by using the average plasma concentration data.

Statistical Procedures. Significance of differences was tested with Student's paired t-test or independent t-test, where appropriate. The null-hypothesis was rejected at the 5% level.

Results

A. pleuropneumoniae Disease Model. The response to the endobronchially inoculated A. pleuropneumoniae toxins was marked by a depression and labored breathing coupled with an increase of rectal temperature and WBC counts (Figure 2). The febrile response was rapid in onset and persisted above 40°C for at least 24 h. One animal got a more severe dyspnea 32 h after the challenge and died about 12 h later. After instillation of the saline the control animal showed only a slight, brief increase in rectal temperature and WBC count. Autopsy revealed that the six challenged pigs had a fibrinous pleuropneumonia. The pig that died during the experiment was suffering from an extensive pleuropneumonia on both sides. The five remaining pigs showed a one-sided pleuropneumonia. In four animals the lesion was located in the caudal part of the diaphragmatic lobe of the right lung. In one pig the lesion was located in the diaphragmatic lobe of the left lung. The lesions had a diameter of approximately 6 to 8 cm. Histological alterations consisted of alveolar and interstitial hemorrhage with edema and fibrin formation and a cellular infiltrate of macrophages and neutrophils. In five of the six pigs A. pleuropneumoniae could be determined by bacteriological examination. The lungs of the control pig showed no abnormalities.

Feed and Water Consumption. Before the challenge all pigs ate 750 g of feed twice daily and consumed 3 to 7 liters of water per pig per day. After the challenge both the feed and
water consumption were much reduced and increased slowly (Figure 3). The control pig consumed normal amounts of feed and water after the endobronchial instillation of the saline.

Oxytetracycline Administration and Pharmacokinetic Data. The intravenous administration of OTC did not cause any visually noticeable pain or discomfort to the pigs. The pharmacokinetic parameters of OTC following intravenous and oral administration of a single dose in healthy pigs are shown in Table 2. In Figure 4 the serum OTC concentration vs time profiles in clinically healthy pigs are shown after intravenous (10 mg/kg) and oral (50 mg/kg) administration. The bioavailability of OTC following oral administration after a 20-h fast was 4.8%. The elimination half-life ($t_{1/2\beta}$) was nearly 6 h after both methods of administration.

Pharmacokinetic data of OTC in pneumonic pigs after a single oral dose of 50 mg/kg are also shown in Table 2. A comparison of the serum OTC concentrations vs time profile after a single oral administration of 50 mg/kg before and after induction of pneumonic actinobacillosis is shown in Figure 5. Pneumonic pigs showed great variation in OTC plasma concentrations. Two pigs had a lag time of 6 h and had maximum OTC plasma levels of .4 μg/ml. Moreover, four pigs had an almost constant OTC level between 6 and 32 h following oral administration. In addition, the mean computed β, $t_{1/2\beta}$, AUC, clearance, maximum plasma OTC level (C-max), and time of peak concentration (T-max) in pneumonic pigs differed significantly ($P < .05$) from those in healthy ones. The mean elimination rate constant (β), clearance, and C-max were decreased, whereas

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**Figure 2.** Rectal temperatures (mean ± SE) and white blood cell counts (mean ± SE) following endobronchial inoculation of *A. pleuropneumoniae* toxins in six pigs (○). Three hours after challenge oxytetracycline (OTC) was administered. One pig served as a control (○).
the AUC and V_d area increased. In healthy and diseased pigs the mean maximum plasma concentrations were reached after 1.74 h and 7.00 h, respectively. Remarkable pharmacokinetic differences in the absorption and in the distribution phase were observed between healthy and pneumonic pigs. In the control pigs, instillation of the saline did not markedly influence serum OTC concentrations. No differences in OTC recovery were determined between healthy and pneumonic pigs. Both in healthy pigs and after A. pleuropneumoniae challenge, OTC was recovered in urine for 3 to 7% of the administered dose after 48 h.

**Figure 3.** Water (●) and feed intake (○) before and after endobronchial challenge (↓) with A. pleuropneumoniae toxins in six pigs (—). One pig served as a control (— —). The intake of feed and water were measured twice daily at 0800 and 1700.

**TABLE 2. A COMPARISON OF PHARMACOKINETIC PARAMETERS OF OXYTETRACYCLINE (+ SD) IN PIGS (N = 6) AFTER INTRAVENOUS ADMINISTRATION OF 10 mg/kg AND 50 mg/kg PER OS PRIOR TO AND AFTER INDUCTION OF PNEUMONIA BY ACTINOBACILLUS PLEUROPNEUMONIAE TOXINS**

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Intravenous 10 mg/kg</th>
<th>Oral 50 mg/kg</th>
<th>Oral 50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>control</td>
<td>pneumonia</td>
</tr>
<tr>
<td>B, mg/liter</td>
<td>5.0 ± 0.32</td>
<td>1.36 ± 0.46</td>
<td>1.66 ± 0.55</td>
</tr>
<tr>
<td>β, per h</td>
<td>1.17 ± 0.012</td>
<td>1.12 ± 0.026</td>
<td>0.048* ± 0.030</td>
</tr>
<tr>
<td>t_1/2B, h</td>
<td>5.99 ± 0.63</td>
<td>5.92 ± 1.09</td>
<td>14.1* ± 5.8</td>
</tr>
<tr>
<td>V_d area, liters/kg</td>
<td>1.46 ± 0.12</td>
<td>— —</td>
<td>1.90 —</td>
</tr>
<tr>
<td>V_d AREA, liters/kg</td>
<td>1.49 —</td>
<td>1.44 —</td>
<td>1.90 —</td>
</tr>
<tr>
<td>A, mg/liter</td>
<td>24.8 ± 6.2</td>
<td>2.01 ± 0.55</td>
<td>0.000ᵇ —</td>
</tr>
<tr>
<td>α, per h</td>
<td>1.52 ± 0.15</td>
<td>0.37 ± 0.11</td>
<td>7.86ᵇ —</td>
</tr>
<tr>
<td>t_1/2α, h</td>
<td>0.46 ± 0.05</td>
<td>2.86 ± 1.23</td>
<td>0.09ᵇ —</td>
</tr>
<tr>
<td>AUC, mg/liter/kg</td>
<td>57.5 ± 5.3</td>
<td>13.7 ± 3.3</td>
<td>26.2* ± 12.7</td>
</tr>
<tr>
<td>Cl, liters/kg</td>
<td>0.173 ± 0.016</td>
<td>0.0030 ± 0.0006</td>
<td>0.0011* ± 0.0004</td>
</tr>
<tr>
<td>C-max, mg/liter</td>
<td>1.87 ± 0.29</td>
<td>0.87* ± 0.45</td>
<td>7.00* ± 1.50</td>
</tr>
<tr>
<td>T-max, h</td>
<td>1.74 ± 0.53</td>
<td>3.05 ± 0.47</td>
<td>1.74ᵇ —</td>
</tr>
<tr>
<td>C (O), mg/liter</td>
<td>3.05 ± 0.47</td>
<td>1.89 ± 0.29</td>
<td>2.3ᵇ —</td>
</tr>
<tr>
<td>Ka, per h</td>
<td>0.38 ± 0.06</td>
<td>1.46 ± 0.12</td>
<td>3.03ᵇ —</td>
</tr>
</tbody>
</table>

*Bi* = zero-time plasma drug concentration intercept of the elimination phase, β = elimination rate constant, t_1/2B = elimination half-life, V_d = volume of distribution during β-phase, V_d area = volume of distribution by the area method, A = zero-time drug concentration intercept of the distribution phase, α = distribution rate constant, t_1/2α = distribution half-life, AUC = area under the plasma concentration-time curve from zero to infinity, Cl = clearance, C-max = peak concentration, T-max = time of peak concentration, C (O) = hypothetical concentration at time 0, Ka = absorption rate constant, t_1/2abs = absorption half-life.

ᵇCalculated from Figure 4.

*P < .05 (paired t-test).
Pleuropneumonia was induced by A. pleuropneumoniae toxins in all challenged pigs, as evidenced by clinical signs, increased rectal body temperatures and WBC counts, and the extent of the lung lesions at autopsy. In comparison with earlier experiments, the response to the A. pleuropneumoniae toxins was severe (Pijpers et al., 1991). In addition, A. pleuropneumoniae was isolated from the lungs of five of the six pigs. Apparently, concurrent infection by A. pleuropneumoniae bacteria was present as well. Nevertheless, the observed respiratory disease was comparable with the findings reported by van Leengoed (1988).

It is well known that disease, in particular fever, may have a negative influence on feed intake (McCarthy et al., 1984; van Miert et al., 1986). As expected, we found anorexia during the febrile episodes in the challenged pigs. Blood et al. (1981) and Kunesch (1986) suggested that diseased pigs still drink. However, quantitative data about water consumption during disease could not be found in the literature. The present study showed that both feed and water consumption were significantly reduced during disease. Whether the reduced water consumption was caused by the same mechanisms as appetite suppression should be studied. In conclusion, drugs should preferably be administered to pneumonic pigs parenterally, and not through feed or water.

The pharmacokinetic parameters of OTC following intravenous administration of 10 mg/kg to healthy pigs were similar to those reported earlier (Pijpers et al., 1991). In the present study the elimination rate constant (β) and distribution rate constant (α) were .117 (t1/2β: 5.99 h) and 1.52 (t1/2α: .46 h), whereas in the above-mentioned experiments β and α amounted to .118 (t1/2β: 5.86 h) and 1.59 (t1/2α: .49 h), respectively.

After oral administration of 50 mg/kg of OTC as a suspension to fasted, healthy pigs, β and α were .122 (t1/2β: 5.92 h) and .37 (t1/2α: 2.86 h). The AUC was small, which resulted in a bioavailability of 4.8%. Mevius et al. (1986) determined a mean bioavailability of 9% in three pigs weighing 14 to 17 kg after oral administration of 20 mg/kg of OTC as a drench. For tetracycline hydrochloride Kniffen et al. (1989) reported a bioavailability of 23% in four gilts weighing 31 to 33 kg; however, in one gilt it was 11%. So, oxytetracycline and tetracycline must be considered poorly and variably absorbed when administered orally to swine. Remarkably, in preeminant veal calves Schifferli et al. (1982) found a mean bioavailability of 46.35% of OTC after oral administration of 50 mg/kg given with a milk replacer. It should be noted that they determined the bioavailability in different groups of calves and not in a cross-over study. Moreover, for tetracycline they calculated a better mean relative bioavailability than for oxytetracycline. In the present study, the maximum plasma OTC level (C-max) was computed to be 1.87 μg/ml at 1.74 h following oral administration of 50 mg/kg, whereas Black and Gentry (1984) and Mercer et al. (1978) determined a C-max of 6.3 and 8.26 μg/ml, respectively, at 2 h after the administration of this dosage. Unfortunately, further pharmacokinetic comparisons between the studies are difficult. Kniffen et al. (1989) described no pharmacokinetic parameters because the resulting plasma concentrations of tetracycline hydrochloride were too variable to be fitted. Black and Gentry (1984) and Mercer et al. (1978) did not compute pharmacokinetic parameters. Mevius et al. (1986) administered a smaller dose (20 mg/kg) and used three pigs weighing 14 to 17 kg.

After oral administration on an empty stomach, plasma OTC concentrations of the six pneumonic pigs showed great variation, probably due to the induced “acute phase response.”
During such febrile conditions gastric function may be inhibited by a decreased gastric emptying rate and an increased pH of the gastric juice, through which tetracyclines will precipitate and the drug absorption out of the gastro intestinal tract will be reduced (Leenen and van Miert, 1969; van Miert and de la Parra, 1970; van Miert, 1980; Dorrestein, 1986). This is of special interest for tetracyclines because the duodenum is the primary absorption site (Brander et al., 1982). Moreover, changed blood flow and other factors can be of influence. Interestingly, Groothuis et al. (1980) described that intramuscularly administered drugs showed a retarded and decreased absorption during fever also.

We did not statistically compare the parameters of absorption and distribution of the diseased pigs with those before the challenge, because they were calculated from the mean observations. For the pneumonic pigs the mean absorption half-life \( (t_{1/2\text{abs}}) \) was computed to be 3.03 h. However, two pigs had no measurable absorption during the first 6 h. Logically, the mean C-max and T-max were lowered and delayed in the diseased pigs. The volume of distribution \( (V_{d\text{area}}) \) tended to be higher in diseased pigs. This is in agreement with the findings of Ladefoged (1979b) with trimethoprim and antipyrene in pigs with endotoxin-induced fever. Moreover, in rabbits and calves a greater volume of distribution was observed during febrile episodes (Ladefoged, 1979a; Groothuis et al., 1980). The mean elimination half-life \( (t_{1/2\beta}) \) increased significantly from 5.9 h before challenge to 14.1 h in diseased pigs. Conversely, following intravenous administration of OTC, \( t_{1/2\beta} \) was decreased in pneumonic pigs (Pijpers et al., 1991). It seems likely that the computed \( t_{1/2\beta} \) was strongly influenced by a retarded absorption caused by a decreased gastric emptying rate. In four pigs the absorption and elimination were almost equal to each other after 6 h. As a consequence, plasma OTC levels remained fairly constant during 26 h.

The AUC of the diseased pigs was enlarged. The apparent bioavailability was proportionally increased. In earlier experiments the AUC did not change in A. pleuropneumoniae-diseased pigs following intravenous administration of OTC (Pijpers et al., 1991). Possibly, the increased AUC was caused by a decreased gastric emptying rate with much more time available for drug absorption from the intestinal tract.

It seems clear that only healthy pigs should be administered OTC via feed or water. If pigs become ill, despite oral consumption of OTC, then it should be considered that the OTC elimination changes, which could have consequences for the appropriate length of the preslaughter withdrawal period.
Implications

After pleuropneumonia was induced by A. pleuropneumoniae toxins both feed and water consumption were significantly decreased. Whether the reduced water consumption was caused by the same mechanisms as appetite suppression should be studied. It seems clear that only healthy pigs should be administered drugs via feed or water. In healthy pigs the bioavailability of oxytetracycline was small (4.8%) and the elimination half-life was 5.92 h. After the endobronchial challenge with A. pleuropneumoniae toxins both the mean bioavailability and elimination half-life increased significantly. If pigs become ill, despite oral consumption of oxytetracycline, it can be expected that the appropriate length of the preslaughter withdrawal period will be prolonged.

Literature Cited


