Bioavailability and disposition of sodium and procaine penicillin G (benzylpenicillin) administered orally with milk to calves

J. M. B. MUSSER &
K. L. ANDERSON

Department of Farm Animal Health and Resource Management, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606, USA

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Eighteen 1-week-old Holstein calves were randomly assigned to one of three groups: (a) sodium penicillin G administered intravenously, (b) sodium penicillin G administered orally, or (c) procaine penicillin G administered orally. All calves were dosed with penicillin G at 4.0 mg/kg BW. At 5 weeks of age, the calves were dosed again. Blood samples were taken serially for 24 h after both dosings. Plasma was assayed for penicillin G by high performance liquid chromatography (HPLC). For i.v. administration, the area under the concentration–time curve (*AUC*), 7456 and 5508 ng/mL h, and systemic clearance, 0.54 and 0.73 L/kg h, were significantly different ($P < 0.05$) at 1 and 5 weeks of age, respectively. There were no significant differences between orally administered sodium and procaine penicillin G within the same age groups. Following oral (p.o.) administration, there were significant differences ($P < 0.01$) at 1 and 5 weeks of age in the *AUC*, 760 and 409 ng/mL h, terminal half-life, 2.1 and 1.6 h, time of maximum concentration (T_{MAX}), 3.0 and 2.3 h, and maximum plasma concentration (C_{MAX}), 85 and 58 ng/mL, respectively. Bioavailability was 10.2 and 7.4% at 1 and 5 weeks, respectively.

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Jeffrey M. B. Musser, Department of Veterinary Pathobiology, Texas Veterinary Medical Center, Texas A & M University, 4467 TAMU, College Station, TX 77843-4467, USA. E-mail: jmusser@cvm.tamu.edu

INTRODUCTION

Despite the use of penicillin G for many decades to treat lactating cows and the feeding of the withheld milk to calves, no definitive studies could be found on the bioavailability or disposition of penicillin G in the calf after ingestion of milk containing subtherapeutic concentrations of penicillin G. Research has been published on the bioavailability of other β-lactams in the calf (Larkin, 1972; Palmer *et al.*, 1977; Ziv *et al.*, 1977; Ziv & Horsey, 1979; Palmer *et al.*, 1983; Soback *et al.*, 1987), but not penicillin G. Penicillin G is degraded in the stomach by gastric acid resulting in limited absorption, however, the reported percentage of the dose absorbed (bioavailability) is quite varied (Huber, 1982; Knifton, 1982; Riviere *et al.*, 1991; Nathwani & Wood, 1993; Rang *et al.*, 1995; Mandell & William, 1996). The possibility of penicillin G causing drug residues when calves are fed waste milk has been suggested (Kesler, 1981). A few studies (Yndestad & Helmen, 1980; Prange *et al.*, 1984; Musser & Anderson, 1998; Musser *et al.*, 2001) investigated the presence of penicillin G in urine, tissue, and blood, but the authors are unaware of studies examining the pharmacokinetics of penicillin G when administered concurrently with milk. This study investigated the age related disposition kinetics and bioavailability of penicillin G when administered intravenously and orally with milk.

MATERIALS AND METHODS

Animals

Eighteen Holstein calves were used. The calves were housed in individual hutches on a North Carolina State University dairy farm (Dairy Educational Unit of the Lake Wheeler Field Laboratories). Calves were determined to be clinically normal by physical examination and were tested for evidence of passive transfer of colostral antibodies using a refractometer; a measurement of >5.5 g/dL was used as the threshold concentration to suggest adequate passive transfer. Animals were fed whole milk from untreated cows twice daily. Milk from cows was tested for the presence of β-lactam antibiotics by a milk screening test with a concentration of detection of 5 ng/mL (SNAP™ Beta-Lactam Test Kit, Idexx Laboratories, Westbrook, ME, USA). Only milk that tested negative was used in the study. Also, calves were fed a pelleted calf starter (Bartlett Calf Starter; Bartlett Milling Co., Statesville, NC, USA), hay and water.

Experimental protocol

Calves were randomly assigned to one of three treatment groups, with six calves per group. Treatment groups consisted of:

Treatment group A – At the morning feeding, sodium penicillin G (Penicillin G sodium; Apothecon Inc., Princeton, NJ, USA) was administered as a single intravenous (i.v.) bolus at a dosage of 4.0 mg penicillin G per kilogram body weight (kg BW);

Treatment group B – at the morning feeding, whole milk was fed at an amount approximating 5% of body weight. Sodium penicillin G was added at 4.0 mg penicillin G per kg BW to the milk;

Treatment group C – at the morning feeding, whole milk was fed at an amount approximating 5% of body weight. Procaine penicillin G (Penicillin-G Procaine Salt; Sigma Chemical Co., St Louis MO, USA) was added at 4.0 mg penicillin G per kg BW to the milk.

Both formulations of penicillin G were stored in desiccators at the manufactures' recommended temperature. All products were used prior to the labelled expiration date.

In treatment group B and C, the antibiotic was thoroughly mixed with 1 L of warm whole milk in a feeding bucket. The calves voluntarily consumed all this milk and then a volume of milk was added to total an amount equal to 5% BW. Using this technique, all the milk, first litre and subsequent addition, was consumed within approximately 5 min.

Dosing for all groups occurred at 7 days of age and was repeated at 35(±2) days of age (day of birth being day 0). Calves remained in the same assigned treatment group throughout the study. Calves were weighed prior to the morning feeding and treatment.

The calf's urine was collected prior to dosing and tested for the presence of antimicrobials using the Live Animal Swab Test (LAST) (Live Animal Swab Test, Editek Inc., Burlington, NC, USA). If positive, the calf was not included in the study.

For the i.v. trials, samples were collected prior to, and at 6, 12, 20, 30, 45, 60, 90 min and 2, 3, 4, 5, 6, 8, 9, 10.5, 14 and 24 h after drug administration. Early sampling times were selected to characterize a possible distribution phase. For the oral (p.o.) trials, samples were collected prior to, and at 20, 40, 60 min and 2, 3, 4, 5, 6, 8, 9, 10, 14 and 24 h after drug administration. The sampling times for the p.o. trials were chosen with the knowledge that peak plasma concentrations for similar β-lactam antibiotics occurred approximately 1.5–3 h after administration (Ziv *et al.*, 1977; Thompson & Black, 1978; Palmer *et al.*, 1983; Soback *et al.*, 1987).

Blood samples (10 mL/sample) were collected by jugular venipuncture, using the contralateral vein if drug was administered intravenously. Blood was collected into tubes containing the anticoagulant EDTA. Blood samples were immediately placed on ice and transported to the laboratory within 3 h for processing. The blood samples were centrifuged ($2200 \times \boldsymbol{g}$) for 10 min; the plasma was decanted and immediately stored at –20 °C until assayed. Analysis was completed within 2 days of sample collection.

Analytical method for penicillin G

Plasma samples were assayed for penicillin G concentrations using the high performance liquid chromatographic method described by Boison *et al.* (1992) with modifications. The assay was validated and used to determine penicillin G residues in calf plasma. Sample preparation consisted of adding a constant amount of a penicillin V standard solution, as an internal standard, and 4 mL 2% sodium chloride solution to 1.5 mL of plasma, then loading it onto a solid phase extraction cartridge (Oasis HLB, 1 cc, Waters Corporation, Milford, MA, USA). The retained penicillins were eluted with 0.5 mL of a 60% acetonitrile elution solution. The eluted penicillins were reacted with 0.5 mL of the derivatizing reagent, 2.0 M 1,2,4 triazole (pH 6.8) with 0.01 M mercuric chloride, in a 55 °C hot water bath for 90 min to form their mercaptide derivatives. The samples were cooled for 5 min, removed, and filtered for high performance liquid chromatography (HPLC) analysis. Using an injection volume of 0.2 mL, reversed-phase HPLC analysis was carried out isocratically with a mobile phase that consisted of 0.1 M phosphate buffer containing 0.0157 M thiosulphate: acetonitrile (69:31, v:v) (adjusted to pH 5.5) at a flow rate of 0.7 mL/min on an Inertsil 5, ODS (250 mm × 4.6 mm i.d). The chromatograms were acquired at 325 nm (Model 510 pump, WISP 712 autosampler, Lambda Max Model 481 UV detector, Waters Corporation, Milford, MA, USA).

With-in day precision was determined by examining plasma samples fortified with penicillin G to achieve concentrations of 40, 70, 100, 400 and 1000 ng/mL. Analysis was performed on five aliquots of the fortified plasma sample, which were processed and assayed by the same technician on the same day. Day-to-day precision was determined by examining plasma samples fortified with penicillin G to concentrations of 20, 100, 500, 1000 ng/mL. Analysis was performed on five aliquots of the fortified plasma sample.

Accuracy was determined by examining plasma samples fortified with penicillin G to achieve concentrations of 20, 100, 500 and 1000 ng/mL. Analysis was performed on five aliquots of the each concentration. Accuracy was reported as the percent difference between the mean of the measured concentrations and the actual concentration of the sample.

Recovery was determined by comparing area under the peak for penicillin G to area under the peak for penicillin V (*AUC*penG/*AUC*penV) ratio of the fortified samples to the *AUC*penG/*AUC*penV ratio of corresponding reference standards. Recovery was examined at 40, 100 and 1000 ng/mL. Four samples at each concentration were processed and assayed.

Stability

The stability of penicillin G in calf plasma was determined in fortified samples stored frozen at –20 and –79 °C for 1 and 4 weeks. Plasma samples were fortified with penicillin G to achieve concentrations of 100 and 500 ng/mL. Three samples of each concentration were assayed immediately. Three samples of

each concentration from the two different storage temperatures were assayed on weeks 1 and 4.

Pharmacokinetic analysis

Analysis was assisted by the use of a pharmacokinetic data analysis computer program (PKAnalyst; MicroMath Scientific Software, Salt Lake City, UT, USA). Polyexponential equations were fitted to the plasma concentration–time data by means of non-linear weighted least-squares regression technique. All i.v. data were weighted with the inverse of the squares of the observed drug concentration ($1/Y^2$), and all p.o. data were weighted with the inverse of the observed drug concentration ($1/Y$). Parameters collected directly from the software for i.v. administration were half-life of distribution ($t_{½}\lambda_1$), extrapolated zero-time intercept of the distribution phase (A_1), distribution rate constant (λ_1), half-life of elimination ($t_{½}\lambda_{2\ \text{i.v.}}$), extrapolated zero-time intercept of the elimination phase (A_2), elimination rate constant ($\lambda_{2\ \text{i.v.}}$), and the intercompartmental rate constants (K_{12} and K_{21}). Parameters collected directly from the software for p.o. administration were half-life of absorption ($t_{½}\lambda_3$), absorption rate constant (λ_3), half-life of elimination ($t_{½}\lambda_{2\ \text{p.o.}}$), and elimination rate constant ($\lambda_{2\ \text{p.o.}}$), time to maximal concentration (T_{MAX}), and maximal plasma concentration (C_{MAX}). Also, collected from the software were area under the plasma concentration–time curve from zero to infinite time (*AUC*), area under the first moment curve (*AUMC*), and mean residence time (*MRT*).

Initial plasma concentration ($C_{(0)}$) after i.v. administration was calculated as $C_{(0)} = A_1 + A_2$. Clearance (*Cl*) for i.v. administration was calculated as $Cl = D/AUC$, where D is the dose administered. Clearance following p.o. administration (Cl/F) was collected by $Cl/F = D/AUC{\cdot}F$, where F is the determined percent bioavailability. Volume of distribution at steady-state (Vd_{SS} was calculated as $Vd_{SS} = D/C_{(0)}\ [(K_{12} + K_{21})/K_{21}]$. The observed mean absorption time (*MAT*) for each of the orally dosed groups was calculated as the difference between the median *MRT* for individual p.o. administration group and the i.v. administration group, matched by age. For p.o. administration, the bioavailability (F) was calculated as $F = (AUC_{\text{p.o.}}/AUC_{\text{i.v.}}) \cdot 100$, where $AUC_{\text{p.o.}}$ and $AUC_{\text{i.v.}}$ are the median *AUC* for the p.o. and i.v. dosing groups matched by age, respectively. The value was expressed as a percentage compared with intravascular administration.

Statistical analysis

The pharmacokinetic parameters of the individual calves in each group were used to determine the median and the first and third quartile values. Using a computerized statistical package (Proc NPAR1WAY, SAS Institute Inc, Cary, NC, USA) the parameters from the treatment groups were compared by the Wilcoxon's rank sum test with an acceptable probability of α error as $P < 0.05$ in a two-tailed test. The stability of penicillin G over time was compared using a paired *t*-test ($P < 0.05$).

Detection of outliers

The 'box plot' method was used to identify outliers (Ostle & Malone, 1988). Using the equations lower limit $= Q_1 - 3(Q_3 - Q_1)$ and upper limit $= Q_3 + 3(Q_3 - Q_1)$, where Q_1 is the first quartile and Q_3 is the third quartile, any observation that was less than the lower limit or greater than the upper limit was determined an outlier and omitted from data analysis.

RESULTS

Analytical method for penicillin G and stability

No interfering peaks were present in blank (unfortified) plasma (pooled plasma) in the analytical window (15–20 min) of either the penicillin G or penicillin V. The mean (±SD) recoveries of penicillin G from calf plasma spiked with 40, 100 and 1000 ng/mL were 105 (±7), 97 (±5) 96 (±4)%. With-in day precision and day-to-day precision and accuracy did not exceed 10% for the concentration range tested. For within day precision, the mean (±SD) concentrations determined for plasma fortified to concentrations of 40, 70, 100, 400 and 1000 ng/mL were 38.3 (±2.6), 65.9 (±4.5), 94.7 (±6.9), 399.2 (±22.4) and 1016.4 (±38.6), respectively. For day-to-day precision, the mean (±SD) concentrations determined for plasma fortified to concentrations of 20, 100, 500 and 1000 ng/mL were 22.4 (±2.0), 96.3 (±4.9), 476.1 (±33.7) and 996.4 (±31.1), respectively. With regards to accuracy, the mean (±SD) concentrations determined for plasma fortified to concentrations of 20, 100, 500 and 1000 ng/mL were 21.2 (±0.5), 96.1 (±6.0), 488.0 (±21.7), and 1010.8 (±34.6), respectively. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 4 and 12 ng/mL, respectively. Penicillin G in plasma was stable when stored at –20 and –79 °C for 1 week and –79 °C for 4 weeks. However, penicillin G concentrations determined in samples stored at –20 °C for 4 weeks were significantly different ($P < 0.02$) from the original concentrations measured. For plasma samples spiked at 100 and 500 ng/mL, initially determined to be 96.0 and 487.4 ng/mL, the mean (±SD) concentrations determined after 4 weeks of storage at –20 °C were 85.4 (±1.6) and 426.6 (±3.7) ng/mL, respectively.

Detection of outliers

One calf in treatment group B at 1 week of age was determined to be an outlier. The calf had an *AUC* of 2429 ng/mL h and a C_{MAX} of 425.5 ng/mL. The upper limits of *AUC* and C_{MAX}, using all the calves' data in group B at 1 week of age, were 2200 ng/mL h and 302.9 ng/mL, respectively. Because of this, all data from this calf at 1 week of age were not included in the analysis.

Pharmacokinetic analysis

Antimicrobials were not detected in any urine sample taken prior to dosing. Penicillin G was not detected in any age or

treatment group in plasma samples collected prior to treatment and 24 (±2) h after drug administration.

Mean plasma concentration–time curves for 1 and 5-week-old calves were prepared from the compilation of individual calf data following i.v. administration for both the 1 and the 5-week-old calves (Fig. 1). Disposition of sodium penicillin G following i.v. administration was best described by a biexponential equation of the form $C_{(t)} = A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t}$, where $C_{(t)}$ is the plasma drug concentration at time (t).

Mean plasma concentration–time curves for 1 and 5-week-old calves were prepared from the compilation of individual calf data following p.o. administration of sodium penicillin G (Fig. 2) or procaine penicillin G (Fig. 3) for both 1 and 5-week-old calves. A biexponential equation of the form $C_{(t)} = A_2e^{-\lambda_2 t} - A_3e^{-\lambda_3 t}$, where A_2 and A_3 are the extrapolated zero-time intercept for elimination and absorption, respectively, best described the concentration curves. Selection of the appropriate equation used a modified Akaike's Information Criterion that is independent of

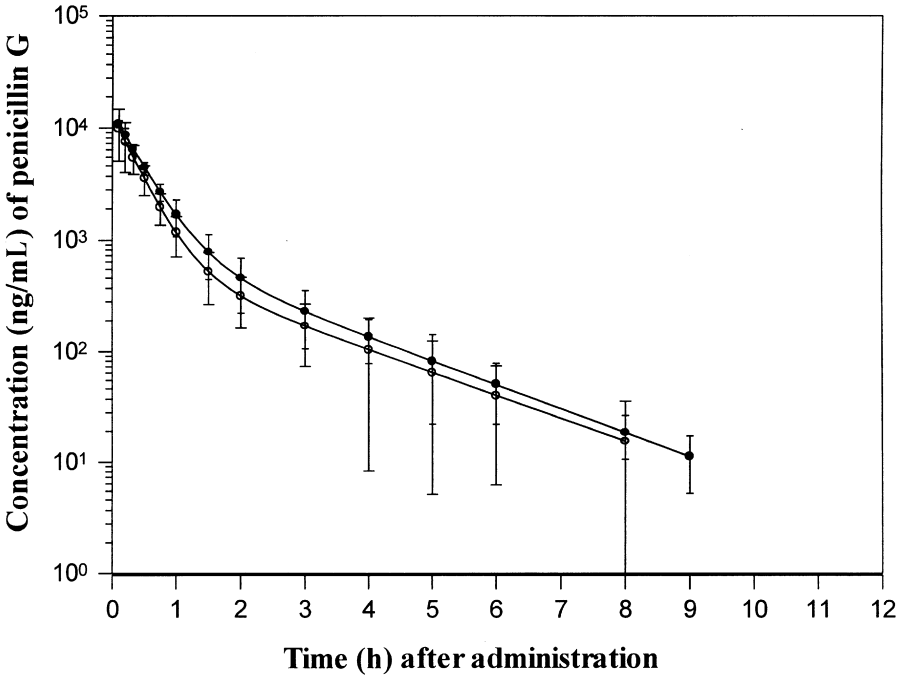

Fig. 1. Mean (±SD) plasma concentration-time curve following IV administration of sodium penicillin G at 4.0 mg penicillin G per kg BW to calves at 1 week of age (●) and 5 weeks at age (○) ($n = 6$).

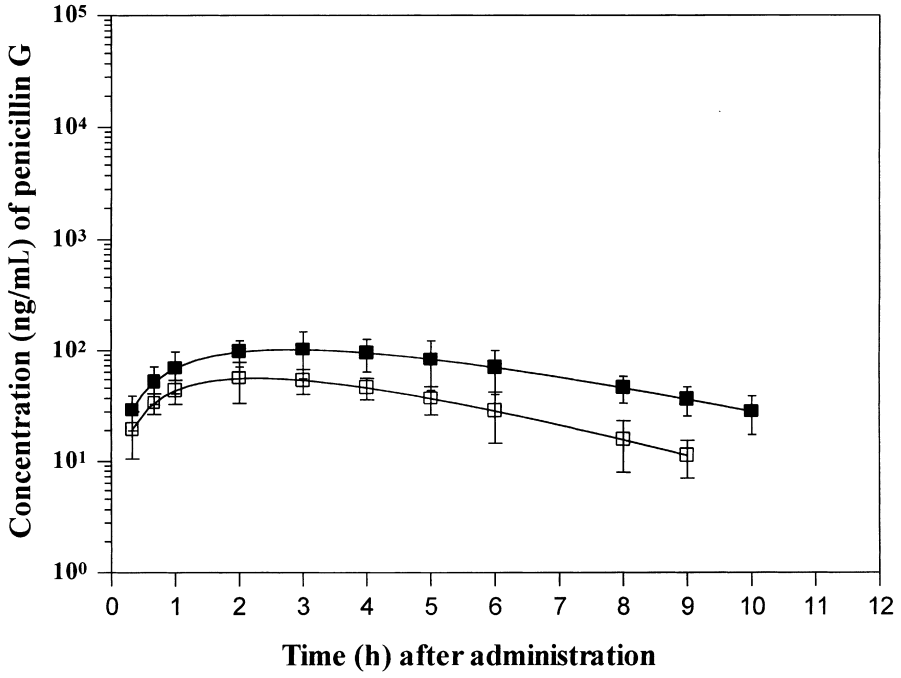

Fig. 2. Mean (±SD) plasma concentration-time curve following p.o. administration of sodium penicillin G at 4.0 mg penicillin G per kg to calves at 1 week of age (■) and 5 weeks at age (□). $n = 5$ at 1 week of age and $n = 6$ at 5 weeks of age.

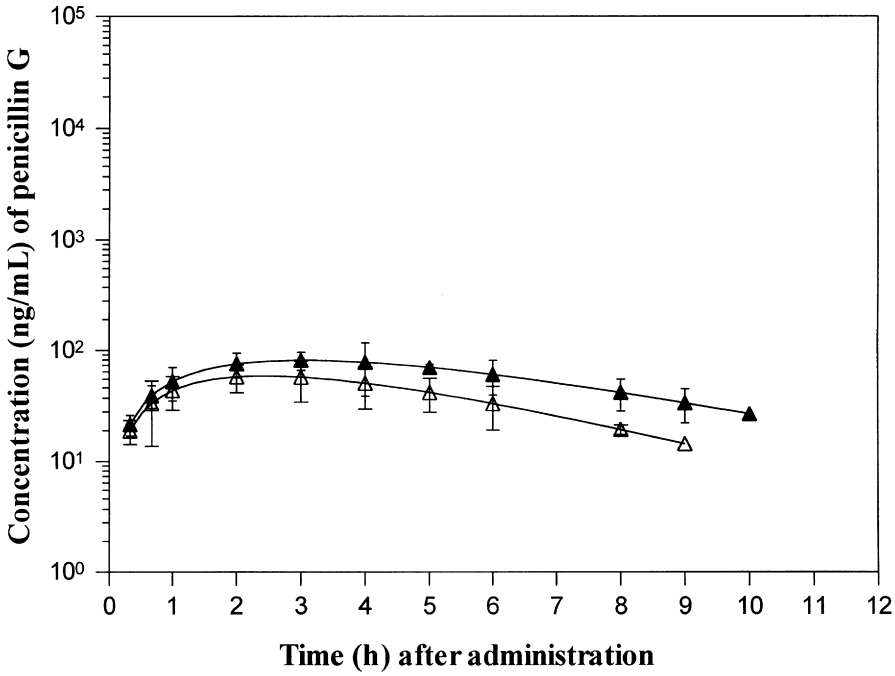

Fig. 3. Mean (±SD) plasma concentration-time curve following p.o. administration of procaine penicillin G at 4.0 mg penicillin G per kg to calves at 1 week of age (▲) and 5 weeks at age (△) ($n = 6$).

the scaling of the data points (PKAnalyst Handbook, MicroMath Scientific Software, UT, USA: 1995).

Mean plasma concentration (±SD) for the various sample times are presented in Table 1. Following i.v. administration to 1 and 5-week-old calves, penicillin G concentrations were below the LOD in all calves at the 14 h sample time. Following the p.o. administration of sodium penicillin G to 1 and 5-week-old calves, penicillin G concentrations were below the LOD in all calves 24 and 14 h following administration, respectively. Following the p.o. administration of procaine penicillin G to 1 and 5-week-old calves, penicillin G concentrations were below the LOD in all calves 14 h following administration.

Pharmacokinetic parameters (median, first and third quartile) for p.o. treatment groups B and C are summarized in Table 2. There were no significant differences between values for treatment groups B and C parameters within the same age of calves. The pharmacokinetic data for the treatment groups B and C, in the same age, were combined in Table 3. The parameters (median, first and third quartile) for the i.v. administration are summarized in Table 3.

DISCUSSION

The i.v. data were best explained by a biexponential equation; distribution and elimination were first order processes. This was expected and consistent with previous studies (Ziv *et al.*, 1973; Volner *et al.*, 1987; Bengtsson *et al.*, 1997).

A biexponential equation best explained the plasma concentration–time curve for p.o. administration of both penicillin G formulations. The $t_{½}\lambda_{2\ \text{p.o.}}$ and $t_{½}\lambda_3$ were similar following p.o. administration, which was consistent with earlier studies on ampicillin and amoxicillin (Ziv *et al.*, 1977). Examining data from a study which administered penicillin V, 10 mg/kg, orally to 2–3-week-old calves, the half-lives of absorption and elimination were also similar (Soback *et al.*, 1987).

After i.v. administration of sodium penicillin G, 5-week-old calves had a significantly ($P < 0.05$) larger *Cl* than 1-week-old calves, 0.73 and 0.54 L/kg h, respectively. Unlike neonates of other domesticated species (Horster & Lewy, 1970; Horster & Valtim, 1971; Aperia *et al.*, 1974; Friis, 1979, 1983), calves have well developed renal secretory elimination function (Dalton, 1968) and a considerable ability to eliminate penicillin G at 2 days of age (Short *et al.*, 1984). As penicillins have minimal hepatic elimination (Chesa-Jimenez *et al.*, 1994), the results of this study suggest renal functions (i.e. glomerular filtration rate, active tubular secretion, and effective renal plasma flow) involved in penicillin elimination continually developed with age from 1 to 5 weeks of age. This finding is consistent with other reports indicating continued development of renal elimination processes in calves (Short *et al.*, 1984; Clarke *et al.*, 1985; Volner *et al.*, 1987). The other kinetic parameters of sodium penicillin G were similar to cattle following i.v. administration (Soback *et al.*, 1987; Volner *et al.*, 1987; Bengtsson *et al.*, 1997) and displayed the typical characteristics of the penicillin family of antibiotics. These characteristics include a rapid distribution phase, short half-life of elimination, and Vd_{SS} indicative of distribution to the vascular and the extracellular fluid (Riviere *et al.*, 1991).

A parallel design for comparing the different routes of administration, with each treatment group remaining constant over the time period studied, was used in this study. In this manner, the intrasubject variability because of maturation, which would be present in a two-period crossover design using neonates, was corrected for. However, as the study was a parallel design, bioavailability (*F*) could not be determined by

Table 1. Mean (±SD) plasma concentration (ng/ml) of penicillin G in 1 and 5-week-old calves following (A) single i.v. bolus of sodium penicillin G at 4.0 mg penicillin G per kg BW (treatment group A) and (B) p.o. administration with milk of sodium penicillin G (treatment group B) and procaine penicillin G (treatment group C) at 4.0 mg penicillin G per kg BW

(A)	i.v. administration of sodium penicillin G			
Time (h)	1-week-old calves		5-week-old calves	
0.1	11890.6	(2942.3)	10979.9	(4293.2)
0.2	8364.7	(1314.7)	7234.1	(2367.4)
0.33	6043.1	(878.8)	4702.1	(1117.3)
0.5	4353.7	(347.0)	3392.8	(891.4)
0.75	2856.3	(485.4)	2192.8	(463.6)
1	1870.3	(355.6)	1319.3	(501.4)
1.5	992.93	(333.9)	569.1	(210.2)
2	630.1	(194.1)	370.8	(118.8)
3	311.8	(110.6)	185.8	(108.5)
4	169.9	(61.9)	126.0	(113.3)
5	102.3	(59.9)	69.6	(52.4)
6	68.8	(48.3)	38.7	(33.4)
8	20.2	(12.5)	17.2	(14.9)
9	11.8	(7.1)	<12	
10.5	<12		<12	
14	<4		<4	
24	<4		<4	

(B)	1-week-old calves p.o. administration		5-week-old calves p.o. administration	
Time (h)	Sodium penicillin G*	Procaine penicillin G	Sodium penicillin G	Procaine penicillin G
0.33	23.5 (11.1)	15.1 (8.4)	16.0 (8.1)	16.0 (8.3)
0.67	40.7 (19.5)	33.0 (7.8)	30.2 (6.3)	31.1 (18.6)
1	57.4 (28.7)	47.3 (18.4)	40.5 (11.0)	40.9 (16.0)
2	107.1 (32.6)	97.9 (12.1)	75.4 (18.2)	68.0 (13.3)
3	117.7 (34.3)	105.4 (15.4)	75.3 (11.8)	75.8 (23.3)
4	113.0 (31.4)	101.8 (16.0)	63.5 (14.6)	64.2 (20.6)
5	99.8 (28.8)	86.0 (5.2)	49.1 (15.4)	45.2 (15.1)
6	92.7 (26.5)	71.5 (22.0)	40.8 (18.5)	37.8 (13.3)
8	51.8 (11.0)	47.0 (14.3)	17.6 (5.8)	16.6 (6.7)
9	33.7 (8.1)	24.8 (4.7)	<12	<12
10	27.7 (6.8)	11.2 (0.9)	<12	<4
14	<12	<4	<4	<4
24	<4	<4	<4	<4

*n = 5.

$AUC_{p.o.}/AUC_{i.v.}$ within the same animal, but was calculated as the ratio of the median *AUC* for the p.o. group to the median *AUC* for the i.v. group. Consequently, statistical comparison of the bioavailability between the p.o. routes could not be accomplished. As *AUC* is the key term in estimating bioavailability (Baggot, 1992; Riviere, 1999), the *AUC* can provide an estimate of the relative (comparative) bioavailability. The difference in the relative bioavailability of sodium penicillin G and procaine penicillin G was determined by comparing the *AUC*s of the two formulations given orally to the same aged calves (Blanchard & Sawchuk, 1979). The relative bioavailability of sodium penicillin G and procaine penicillin G was not significantly different.

The *AUC* is one measure of the extent of absorption, whereas T_{MAX} is a factor of the rate of absorption and C_{MAX} is a factor of the rate and extent of absorption (Baggot, 1992). There were no significant differences in the T_{MAX} and C_{MAX} between orally administered sodium penicillin G and procaine penicillin G in the same aged calves. These findings suggest that the extent and rate of p.o. absorption are similar for both penicillin salts. Likewise, there were no significant differences between the other parameters, within age groups, between the two salts of penicillin G. These findings connote that sodium and procaine penicillin G are bioequivalent when administered orally with milk, unlike intramuscular (i.m.) administration.

Combining the data from treatment group B and C, within the same ages, the bioavailability of penicillin G given orally with milk was 10.2 and 7.4% at 1 and 5 weeks of age, respectively (Table 3). The age-related increase in clearance contributed to this 46% decrease in the *AUC*, along with a shorter T_{MAX}, and lower C_{MAX} in the 5-week-old calves. However, the total decrease in *AUC* could not be accounted for by the 35% increase in clearance.

Table 2. Estimation of pharmacokinetic parameters for sodium penicillin G administered orally (treatment group B) and procaine penicillin G administered orally (treatment group C) at 4.0 mg/kg BW at 1 and 5 weeks of age. The values are the median (first and third quartile limits) values of the calves in each group

	1-week-old calves p.o. administration		5-week-old calves p.o. administration	
Parameter (units)	Sodium penicillin G*	Procaine penicillin G†	Sodium penicillin G†	Procaine penicillin G†
$t_{½}\lambda_{2\ p.o.}$ (h)	1.9 (1.9, 2.2)	2.1 (2.0, 2.2)	1.6‡ (1.4, 1.7)	1.7‡ (1.5, 1.9)
$\lambda_{2\ p.o.}$ (h)	0.36 (0.32, 0.36)	0.33 (0.31, 0.35)	0.44‡ (0.41, 0.49)	0.41‡ (0.37, 0.46)
$t_{½}\lambda_3$ (h)	1.9 (1.9, 2.2)	2.1 (2.0, 2.2)	1.6‡ (1.4, 1.7)	1.7‡ (1.5, 1.9)
λ_3 (h)	0.36 (0.32, 0.36)	0.33 (0.31, 0.35)	0.44‡ (0.41, 0.49)	0.41‡ (0.37, 0.46)
AUC (ng/ml h)	870 (650, 888)	710 (604, 781)	362‡ (340, 406)	423‡ (410, 450)
AUMC (ng/ml h^2)	4721 (3975, 5461)	4439 (3726, 4839)	1638‡ (1560, 1849)	2220‡ (2104, 2270)
MRT (h)	5.6 (5.5, 6.3)	6.0 (5.7, 6.5)	4.5‡ (4.1, 4.9)	4.8‡ (4.3, 5.4)
MAT (h)	4.7	5.1	3.8	4.1
Cl/F (L/kg h)	0.54 (0.53, 0.72)	0.54 (0.49, 0.63)	0.73 (0.65, 0.78)	0.73 (0.68, 0.75)
T_{MAX} (h)	2.8 (2.8, 3.1)	3.0 (2.9, 3.2)	2.3‡ (2.0, 2.4)	2.4‡ (2.2, 2.7)
C_{MAX} (ng/mL)	102 (85, 123)	81 (73, 99)	56‡ (51, 68)	58‡ (55, 71)
F (%)	11.7	9.5	6.6	7.7

*n = 5; †n = 6; ‡significantly different from 1 week values. $t_{½}\lambda_{2\ p.o.}$ = half-life of elimination after p.o. administration; $\lambda_{2\ p.o.}$ = elimination rate constant after p.o. administration; $t_{½}\lambda_3$ = half-life of absorption; λ_3 = absorption rate constant; *AUC* = area under the plasma concentration-time curve from zero to infinite time; *AUMC* = area under the first moment curve; *MRT* = mean residence time; *MAT* = observed mean absorption time; *Cl/F* = clearance corrected for bioavailability; T_{MAX} = time of maximal plasma concentration; C_{MAX} = maximal plasma concentration; *F* = bioavailability.

Table 3. Estimation of pharmacokinetic parameters for sodium penicillin G administered as a single IV bolus at 4.0 mg penicillin G per kg BW (treatment group A) to 1 and 5-week-old calves. The p.o. values are the combined sodium and procaine penicillin G values when administered at 4.0 mg penicillin G per kg BW (treatment group B and C) to 1 and 5-week-old calves. The values are the median (first and third quartile limits) values of the calves in each group

	1-week-old calves		5-week-old calves	
Parameter (units)	i.v. administration*	p.o. administration†	i.v. administration*	p.o. administration‡
$t_{½}\lambda_1$ (h)	0.29 (0.24, 0.33)		0.25 (0.21, 0.37)	
λ_1 (h)	2.46 (2.08, 2.90)		2.81 (1.91, 3.28)	
$t_{½}\lambda_{2\ i.v.}$ (h)	1.4 (1.4, 1.5)		1.5 (1.2, 2.1)	
$\lambda_{2\ i.v.}$ (h)	0.50 (0.47, 0.51)		0.48 (0.34, 0.57)	
$t_{½}\lambda_{2\ p.o.}$ (h)		2.1 (1.9, 2.2)		1.6¶ (1.5, 1.8)
$\lambda_{2\ p.o.}$ (h)		0.34 (0.31, 0.36)		0.43¶ (0.39, 0.47)
$t_{½}\lambda_3$ (h)		2.1 (1.9, 2.2)		1.6¶ (1.5, 1.8)
λ_3 (h)		0.34 (0.31, 0.36)		0.43¶ (0.39, 0.47)
K_{12} (h)	0.37 (0.29, 0.66)		0.52 (0.27, 0.54)	
K_{21} (h)	0.63 (0.59, 0.77)		0.62 (0.48, 0.72)	
AUC (ng/mL h)	7456 (6899, 7785)	760§ (636, 838)	5508¶ (4979, 6311)	409§,¶ (340, 429)
AUMC (ng/mL h^2)	6930 (6092, 8252)	4440§ (3816,5178)	4618 (3849, 5223)	1990§,¶ (1595, 2216)
$C_{(0)}$ (ng/mL)	13628 (11857, 14794)		12637 (8345, 16059)	
Vd_{ss} (L/kg)	0.51 (0.48, 0.54)		0.49 (0.43, 0.61)	
Cl (L/kg h)	0.54 (0.51, 0.58)		0.73¶ (0.64, 0.81)	
Cl/F (L/kg h)		0.54 (0.47, 0.66)		0.73¶ (0.69, 0.87)
T_{MAX} (h)		3.0 (2.8, 3.2)		2.3¶ (2.1, 2.6)
C_{MAX} (ng/ml)		85 (74, 104)		58¶ (52, 72)
F (%)		10.2		7.4

**n = 6; †n = 11; ‡n = 12; §Significantly different from the IV administration in the same age group; ¶Significantly different from 1 week age group of the same treatment group; $t_{½}\lambda_1$ = half-life of i.v. distribution; λ_1 = i.v. distribution rate constant; $t_{½}\lambda_{2\ i.v.}$ = half-life of i.v. elimination; $\lambda_{2\ i.v.}$ = i.v. elimination rate constant; K_{12} and K_{21} = intercompartmental micro-rate constants; $C_{(0)}$ = initial plasma concentration; Vd_{SS} = volume of distribution at steady-state; *Cl* = clearance; see Table 2 for other abbreviation key.

Age related changes in the presystemic effects probably contributed to the lower bioavailability, *AUC*, T_{MAX}, and C_{MAX} in older calves as there should be little hepatic first pass metabolism of β-lactam antibiotics (Chesa-Jimenez *et al.*, 1994). The presystemic effects on the β-lactam antibiotics include acid-catalysed degradation, enzymatic degradation by rumen and

intestinal microflora and intestinal membrane bound enzymes, metabolism by intestinal cells, and decreased intestinal permeability to β-lactam antibiotics.

Penicillin G is hydrolysed at the low pH found in the abomasum (Huber, 1982). The extent of penicillin G degradation by rumen microflora has yet to be fully elucidated. In a review on rumen microflora degradation of xenobiotics, Prins (1987) reported that penicillin G was rapidly inactivated. Research by Akkad and Hobson (1966) emphasized the effect culture media had on the inactivation of penicillin G by the rumen microflora. Further mechanisms that may limit intestinal absorption of β-lactam antibiotics are metabolism by intracellular and membrane bound intestinal cytochrome P450 (Chesa-Jimenez *et al.*, 1994; Suzuki & Sugiyama, 2000); intestinal epithelial efflux transporters such as P-glycoprotein (Saitoh *et al.*, 1996) and energy-dependent intestinal peptide transporters predominantly in the brush border membrane of intestinal epithelial cells (Saitoh *et al.*, 1997; Skowronski *et al.*, 2000). However, to the authors' knowledge, these mechanisms have not been fully researched and elucidated in the bovine. Additionally confounding this issue is an age related change in the permeability of intestinal mucosa to β-lactam antibiotics (Morita *et al.*, 1992).

When penicillin V was administered orally to 6-week-old weaned calves, the drug had very low absorption and minimal bioavailability (Soback *et al.*, 1987). As penicillin V resists hydrolysis in an acidic environment, the authors attributed the destruction to inactivation and degradation in the gastrointestinal tract of the calves. Because of the uncertain extent to which rumen microflora degrades penicillin G (Akkad & Hobson, 1966) and that bucket feeding warm milk in calves stimulates esophageal groove closure resulting in most of the milk being delivered to the omasum and abomasum (Abe *et al.*, 1979), ruminal degradation may be of limited consequence. Therefore, the increase in presystemic inactivation of penicillin G may feasibly be a result of increased intestinal degradation and decreased absorption across the intestinal mucosa. However, this needs to be researched further to fully ascertain the relative contributions.

The results reported in this study provide information on the bioavailability and pharmacokinetics of penicillin G and can be used in estimating withholding times to avoid violative drug residues.

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