RESEARCH REPORT
PORK SAFETY

Title: Pharmacokinetics and tissue residues of procaine penicillin G in sows after administration of 33,000 IU/kg intramuscularly and by needle-free injection - Part A - Live Animal Work – **NPB #07-235**

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Industry summary

Forty sows were utilized in a study to determine the plasma concentration profile and tissue residues of procaine penicillin G after injection by two methods (20 sows each method). A target dose of 33,000 IU/kg (5 ml/100 lbs) of a 300,000 IU/ml commercially available procaine penicillin G suspension[1] was injected by either conventional intramuscular injection with a needle (CI) or injection by needle-free device[2] (NF) in the hip. Live animal work including treatment and tissue collection was conducted at Iowa State University and is reported here. Sample analysis and pharmacokinetic work was conducted a Kansas State University and is reported elsewhere.

For the plasma concentration study, 15 plasma samples were taken over 24 hours from 8 pigs in each of the two treatment groups. In the tissue residue study, samples of kidney, liver, fat, muscle, and injection site were collected at 2, 4, 6, and 8 days post-injection. In swine tissues, the accepted tolerance is no detectable concentration in the United States and 50 PPB in many of the export markets. There were no statistically significant differences in the injection sites by microscopic examination. Gross evaluation of muscle, fat, kidney and liver did not reveal significant differences in the frequency or severity of lesions. When tissues were evaluated microscopically, there were no statistically significant differences in the frequency or type of lesion.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

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[1] Pen Aqueous, Durvet, Blue Springs, MO
[2] Pulse NeedleFree Systems, Inc., Lenexa, KS

Keywords
Penicillin, injection, lesions, needle free, carcass defects

Scientific Abstract

Forty sows were utilized in a study to determine the plasma concentration profile and tissue residues of procaine penicillin G after injection by two methods (20 sows each method). A target dose of 33,000 IU/kg (5 ml/100 lbs) of a 300,000 IU/ml commercially available procaine penicillin G suspension[3] was injected by either conventional intramuscular injection with a needle (CI) or injection by needle-free device[4] (NF) in the hip. Live animal work including treatment and tissue collection was conducted at Iowa State University and is reported here. Sample analysis and pharmacokinetic work was conducted a Kansas State University and is reported elsewhere.

For the plasma concentration study, 15 plasma samples were taken over 24 hours from 8 pigs in each of the two treatment groups. Plasma concentrations were determined by Ultrahigh-Pressure Liquid Chromatography coupled with a triple quad mass spectrometer at Kansas State University and reported elsewhere. No further analysis was conducted as part of the live animal work at Iowa State University.

In the tissue residue study, samples of kidney, liver, fat, muscle, and injection site were collected at 2, 4, 6, and 8 days post-injection. There were no statistically significant differences in histological examination of the injection sites.

Introduction: Procaine penicillin G (PPG) residues are an important issue to the swine industry. Specific information related to PPG residues in sows supports residue-free marketing of sows after treatment. In addition, the description of plasma pharmacokinetics specific to sows contributes to treatment regimen construction that balances practicality of administration, economics, animal welfare, and food safety. Another recently added factor is the growth of options in methods of administration. It is important to understand the pharmacokinetics and tissue residues associated with conventional administration using a needle in comparison to new technologies such as needle-free injection systems. The research question is "how does PPG move in the bodies of sows injected by conventional needle injection and by needle-free injection, and for what amount of time does the drug persist in tissues following injection'?

Objectives: There are three main objectives to this study. Objectives 1 and 2 were conducted at Kansas State University and are reported elsewhere. Objective 3 was completed at Iowa State University and is reported here.

1. Describe and mathematically model the concentrations of procaine penicillin in the plasma of swine after injection by conventional needle and needle-free methods.

2. Describe and mathematically model concentrations in muscle, fat, kidney, liver, and the injection site after injection by the same two methods.

3. Evaluate injection sites from both injection methods by histology.

[3] Pen Aqueous, Durvet, Blue Springs, MO
[4] Pulse NeedleFree Systems, Inc., Lenexa, KS

Materials and Methods:

This study was approved by the committee on animal use and care at Iowa State University

Animals and housing: Study animals consisted of 40 healthy cull sows obtained from commercial sources. None of the sows had records of previous antimicrobial treatment prior to the study. A pre-study acclimation period of a minimum of 5 days was observed. The sows were housed in small groups in a light and temperature controlled environment and fed a typical commercial sow ration during the acclimation period. The number of sows necessitated conducting the in-life phase of the study in two phases with the first beginning with receipt of sows on June 20th and final sample collection on July 3rd and the second beginning with receipt of sows on July 9, and final sample collection on July 22nd, 2008

The sows selected for the plasma pharmacokinetic portion of the study were moved to gestation crates for the plasma collection phase and then returned to group housing until euthanized for tissue harvest.

Treatments and treatment assignment: Each sow received only one treatment. The sows were blocked by weight prior to random allocation to the treatment groups. The procaine penicillin G suspension contained 300,000 IU/kg (Pen Aqueous, Durvet, Blue Springs, MO).

> Treatment 1: (N = 20) Intramuscular procaine penicillin G at 33,000 IU/kg in the hip. The total dose was split between multiple sites using a 16 gauge, 1 inch needle observing a maximum volume per site of 10 ml.
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> Treatment 2: (N = 20) Needle-free injection of procaine penicillin G (Pulse NeedleFree Systems, Inc, Lenexa, KS). The dose was determined by calculating injection volume based on 33,000 IU/kg and then rounding down to the nearest 5 ml increment to reflect the maximum single injection volume of the needle-free system of 5 ml. This was the amount per injection site. Due to this actual dose variation in the needle-free group, the pharmacokinetic data from the needle-free injection treatment group was normalized to 33,000 IU/kg for analysis.

Tissue sample collections were scheduled at 2, 4, 6, and 8 days after injection with 5 sows from each treatment group assigned to each time point. Eight sows were then selected from each of the treatment groups for the pharmacokinetic study by random selection of 2 from each group at 5 assigned to each of the 4 tissue collection times.

To facilitate injection site recovery, all injections were placed within square areas delineated by permanent marker. The sows were restrained during injection by means of a snare.

Pharmacokinetic Study Sample Collection:

Samples were collected by jugular venipuncture. The gestation crate was opened from the front and the sow was restrained with a snare.

Blood samples of 3 ml each were collected at time zero (preadministration), and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, and 24 hours postadministration. Collection was by vacutainer needle and hub assembly combined with a 5 ml draw, heparin tube. The tubes were placed on ice until spun, after which the supernatant was immediately placed into tubes suitable for ultra-low freezer storage, cataloged, and frozen at -80º C until analysis.

Tissue residue study sample collection:

Tissue sample collection for each treatment consisted of 4 evenly spaced sample collection times with 5 sows per time for each treatment group. The sample times, 2, 4, 6, and 8 days post-injection, were calculated with a goal of all sows at the last sample time being at the U.S. safe concentration and Japanese MRL of 0.00 ppm (undetectable). Euthanasia was by captive bolt followed by exsanguination, timed to be as close as possible to the original time of administration on the sample collection day.

Samples collected for residue assays included muscle (semimembranosus/ semitendinosis), liver, kidney, pelvic fat, and injection site. The tissues were stored at -80° C until analysis.

Two injection sites were collected per sow and divided into 2 parts. Tissue collection at necropsy included both kidneys; capsule removed and bagged separately, 200 g of transected liver, 200 g of pelvic fat, 200 g of semi-membranosus or semi-tendinosus muscle, and both injections sites excised around the visual markings externally and to the depth of the bone.

A 1 cm^3 sample of the injection site was removed and placed in 10% buffered neutral formalin. The remaining injection site sample was weighed and all collected tissues from each sow were bagged collectively and frozen at -70°C until testing.

The kidney, liver, pelvic fat and muscle tissue were forwarded to Kansas State University for residue testing by the primary investigators referred to above. The injection site formalin fixed tissue was submitted to Iowa State University Veterinary Diagnostic Laboratory for microscopic analysis.

Sample Testing:
Tissues were prepared for histological analysis using standard laboratory operating procedures. Histological analysis of the muscle fibers immediately surrounding the visible injection sites was performed to determine the type and extent of microscopic damage present in the tissue. The muscle fibers were examined for presence of hemorrhage, edema, inflammation, fibrosis (tissue under repair) and foreign material. Findings were reported on a categorical scale based on lesions seen by the pathologist. Table 1 describes the categorical classification scheme for histological analysis.

Data analysis:

Injection-site histology data were analyzed using the Pearson Chi-square test comparing the number of counts in each description category for the two treatment groups within each post-injection day for both muscle and connective tissue

Results

Results of histology examination of the injection sites are reported in Table 1. No comparison of histology score distributions resulted in a P value ≤ 0.05. The 10 comparisons that resulted in P values < 0.1 are highlighted in Table 9.

Table 1: Histology results by tissue (connective or muscle) by injection treatment and days post-injection. Results are reported by the number of observations for each score by day. The scoring key is presented below. Cells in the table with values of zero were left blank. Scores of zero are not reported in the table, but would equal 10 minus reported occurrences for scores of 1, 2, and 3 for each tissue-day-category combination.

SCORE	Muscle Inflammation
0	None
1	Mild scattered or clusters of lymphocytes and macrophages; mild perivascular cuffs
2	Moderate focal or multifocal nonsuppurative inflammation
3	Severe multifocal or diffuse granulomatous inflammation with active macrophages

SCORE	Connective Tissue Inflammation
0	None
1	Mild scattered mixed inflammatory cells
2	Moderate scattered to locally extensive mixed inflammation
3	Severe diffuse granulomatous inflammation

SCORE	All tissues - Hemorrhage
0	None
1	Focal
2	Multifocal
3	Diffuse

SCORE	All tissues - Edema or Fibrosis
0	None
1	Mild
2	Moderate
3	Severe

Conventional Needle Injection										
		Connective Tissue					Muscle			
Days PI	Score	Hemorrhage	edema	fibrosis	foreign material	Inflammation	Hemorrhage	fibrosis	foreign material	Inflammation
2	1	3	2		1	2	6			3
	2	1					1			
	3									
4	1		1			1	4	1		5
	2								1	1
	3									
6	1	2	1			2	2	3	1	3
	2				2	2			3	4
	3									1
8	1	2	2	1	1	2	5	3	6	4
	2			1	1	1			3	6
	3									
Needle-Free Injection										
		Connective Tissue					Muscle			
Days PI	Score	Hemorrhage	edema	fibrosis	foreign material	Inflammation	Hemorrhage	fibrosis	foreign material	Inflammation
2	1	7					2			1
	2	2	1				1			
	3									
4	1	1	2		3	2	5	1	4	3
	2	2	3			2	1		1	4
	3	1								2
6	1	2	3	2	3	3	4	7	2	2
	2	3	3		3	3			7	4
	3					1				3
8	1	3	3	2	1	1	3	4	1	3
	2	1	1	1	6	6	1		3	2
	3					1			1	3

Table 2 Focus on injection histology score comparisons where the probability < ChiSquare is <0.1000. These data were extracted from Table 6 for comparisons where the P value was <0.1. Blank cells represent a value of zero.

Muscle					
Days PI	**Category**	**Score**	**Needle-Free**	**Needle**	**P-value**
4	Foreign Material	1	4		0.0764
		2	1	1	
		3			
6	Foreign Material	1	2	1	0.0638
		2	7	3	
		3			
8	Foreign Material	1	1	6	0.0647
		2	3	3	
		3	1		
6	Fibrosis	1	7	3	0.0736
		2			
		3			
8	Inflammation	1	3	4	0.0675
		2	2	6	
		3	3		

Connective Tissue					
Days PI	**Category**	**Score**	**Needle-Free**	**Needle**	**P-value**
2	Hemorrhage	1	7	3	0.0638
		2	2	1	
		3			
4	Foreign Material	1	3		0.0603
		2			
		3			
8	Foreign Material	1	1	1	0.0538
		2	6	1	
		3			
6	Edema	1	3	1	0.0517
		2	3		
		3			
8	Inflammation	1	1	2	0.0530
		2	6	1	
		3	1		

Discussion and Summary

Injection site histology: This study provided relatively low power to detect differences due to confining analysis to within post-injection day. An interesting observation is the numerical trend towards more counts (more histological observations) in the needle-free injection group as compared to the conventional needle injection group in comparisons where the P value was < 0.1 (Table 2). This is opposed to the observation of the conventional needle injection group having more prolonged injection site residues.