PENICILLIN CONCENTRATIONS IN SERUM AND MILK FOLLOWING ADMINISTRATION OF PROCAINE PENICILLIN G BY DIFFERENT ROUTE AT DIFFERENT DOSAGE.

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INTRODUCTION

The aim of the clinician when using antimicrobials is to provide effective treatment while minimizing residues in milk and meat. Among all antibiotics used in food animals, procaine penicillin G (PPG) is one of the oldest and is still one of the most widely used. Over the years, the recommended dosage has increased from 7,500 IU/kg I.M. (the label dosage) to the recommended, but not approved dosage of 11,000 to 22,000 IU/kg administered I.M. or S.C. (1). The subcutaneous route is easier to use when large volumes need to be administered.

Because of the discrepancy between the approved dosage of PPG and the dosages currently used in clinical practice, the purposes of the present study were twofold: firstly to determine whether increasing doses of PPG would provide corresponding increases in efficacy, as measured by the peak serum concentration (Cmax) value and the length of time during which the serum concentration of PPG would exceed the minimum inhibitory concentration (MIC); secondly to determine whether the two routes of administration are comparable as true alternatives.

MATERIALS AND METHODS

Animals:

Eight healthy lactating Holstein cows in their 3rd or 4th lactation were used, with body weights ranging from 557 to 682 kg. The animals were kept indoors and milked twice daily. The average daily milk production was 20±5 kg. The study was conducted during months of May, June and July. Before the beginning of the study the animals had an acclimatation period of 3 weeks.

Drugs:

Procaine penicillin G at the concentration of 300,000 IU/mL was used. (Ethacillin^{R}, Pfizer Rogar/STB, Pointe-Claire, Quebec, Canada).

Experimental Procedures:

The 8 experimental animals were divided into 2 groups of 4 cows. The 4 cows in Group 1 were treated by intramuscular injections and the 4 cows in Group 2 were treated by subcutaneous injections. Within both groups, the individual cows received four different doses over four different periods. Each of the four different doses (7,000, 14,000, 21,000 and 28,000 IU/kg, 20 mL/site) of PPG was administered once daily for 5 consecutive days. Samples of milk were taken before the administration of the antibiotic and every 12 hours for 6 consecutive days. Blood samples were taken before and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20 and 24 hours following administration of the first and fifth injection.

Milk samples were taken from pooled quarter milk after each milking. Blood samples were allowed to clot then the sera was harvested and frozen (-70 C) until analyzed. Pooled serum and milk standards were frozen along with the samples.

There was a period of 16 days between the last injection of one series of doses and the first injection of another series.

The concentration of penicillin in serum was measured using a biological assay (2) with Bacillus subtilis as the test organism. The sensitivity of the test was 0.12 IU/mL. Standard concentrations from pooled serum were from 16, 8, 4, 2, 1, 0.5, 0.25, 0.12 IU/mL.

Penicillin milk concentrations were measured by a disk assay method using Bacillus stearothermophilus as test organism (3). Standard milk concentrations ranged from 0.064 IU/mL to 0.008 IU/mL. When concentrations were expected to be greater than the standard, the milk samples were diluted 1:3 or 1:10. All milk samples taken from day 1 (12 hour sample) to day 5 were diluted.

Statistical Analysis:

The area under the curve (AUC) was calculated using a trapezoidal summation method. The peak serum concentration (Cmax), the time of the peak (Tmax), the AUC and the time duration that the serum penicillin concentrations remained above the MIC of 0.4, 1.6 and 3.3 IU/mL were compared for the four different doses using the GLM Procedure of SAS.

RESULTS

Tables 1 and 2 report the Cmax, Tmax and AUC following IM or SC injection of PPG after the first and the fifth injections. Following the IM injections, a dose-dependent increase of the penicillin AUC was observed on both days of sampling; however a dose-dependent increase of the Cmax was observed only after five days of injection. On both days, no dose effect of the PPG was observed in the time of obtention of the peak. On the first day of SC injection, no dose-response effect was observed and after five days, a dose-relation effect was observed on the Cmax and AUC parameters.

Results from Table 3a and 3b show that doses of 14,000, 21,000 and 28,000 IU/kg by both routes give serum penicillin concentrations above a MIC of 0.4 IU/mL for more than 20 h. However only the two highest doses maintained serum penicillin concentration above MIC 1.6 IU/mL and for a MIC of 3.3 IU/mL no dose in both routes maintained serum penicillin concentration for more than 6 hours.

A dose-dependent increase in milk penicillin concentration was observed independently of the route of injection; however no effect of repetitive injection was observed over time. (Table 4).

TABLE 1. PHARMACOKINETICS PARAMETERS FOLLOWING THE FIRST AND FIFTH DAY OF INTRAMUSCULAR INJECTION OF PROCAINE PENICILLIN G AT FOUR DIFFERENT DOSES IN FOUR LACTATING COWS

	DOSE (IU/KG)					CONTRAST[e]		
	7000	14000	21000	28000	SEM[a]	7000 vs 14000	14000 vs 21000	21000 vs 28000
FIRST DAY								
C max[b]	0.98	1.58	2.81	3.42	0.22	N.S.	0.007	N.S.
T max[c]	3.25	7.13	4.38	3.50	1.19	N.S.	N.S.	N.S.
AUC[d]	12.0	23.3	36.5	46.1	1.21	0.0006	0.0003	0.0013
FIFTH DAY								
C max[b]	1.32	2.47	3.52	4.88	0.14	0.0014	0.0021	0.0005
T max[c]	2.00	2.00	2.75	2.13	1.65	N.S.	N.S.	N.S.
AUC[d]	14.5	31.8	44.7	56.8	2.1	0.0012	0.005	0.007

[a] Standard error of the mean
[b] Peak serum concentrations (IU/mL)
[c] Time of the peak (hours)
[d] AUC (IU.hour/mL)
[e] P value for the contrast between doses
N.S. Non significant

TABLE 2. PHARMACOKINETIC PARAMETERS FOLLOWING THE FIRST AND FIFTH DAY SUBCUTANEOUS INJECTIONS OF PROCAINE PENICILLIN G AT FOUR DIFFERENT DOSES IN FOUR LACTATING COWS

	DOSE (IU/KG)					CONTRAST[e]		
	7000	14000	21000	28000	SEM[a]	7000 vs 14000	14000 vs 21000	21000 vs 28000
FIRST DAY								
C max[b]	1.29	2.33	3.09	3.56	0.32	N.S.	N.S.	N.S.
T max[c]	5.63	4.63	2.63	3.75	2.32	N.S.	N.S.	N.S.
AUC[d]	13.8	29.0	39.3	47.0	3.56	0.02	N.S.	N.S.
FIFTH DAY								
C max[b]	1.53	3.45	4.13	4.86	0.15	0.0001	0.02	0.01
T max[c]	2.88	3.75	4.13	3.25	1.26	N.S.	N.S.	N.S.
AUC[d]	17.5	36.4	49.6	58.6	2.2	0.001	0.005	0.03

a Standard error of the mean
b Peak serum concentrations (IU/mL)
c Time of the peak (hours)
d AUC (IU.hour/mL)
e P value for the contrast between doses
N.S. Non significant

TABLE 3a. PERIOD OF TIME DURING WHICH SERUM PENICILLIN CONCENTRATIONS WERE OVER THE MINIMAL INHIBITORY CONCENTRATION OF 0.4, 1.6 AND 3.3 IU/mL FOLLOWING THE FIRST PENICILLIN INJECTION

ROUTE	DOSES (IU/KG)	DURATION ABOVE 0.4 IU/mL (H)			DURATION ABOVE 1.6 IU/mL (H)			DURATION ABOVE 3.3 IU/mL (H)		
		Mean[c]	Min[d]	Max[d]	Mean[c]	Min[d]	Max[d]	Mean[c]	Min[d]	Max[d]
IM	7000	12.5[a]	12	14	0[a]	0	0	0[a]	0	0
	14000	21.0[b]	16	24	0.75[a]	0	3	0[a]	0	0
	21000	24.0[b]	24	24	10.9[b]	7.5	14	0[a]	0	0
	28000	24.0[b]	24	24	13.5[b]	10	16	1.5[a]	0	3.5
SC	7000	13.0[a]	10	14	0[a]	0	0	0[a]	0	0
	14000	21.0[b]	16	24	5.75[b]	0	9	0[a]	0	0
	21000	22.0[b]	20	24	10.0[b]	8	12	0.9[a]	0	3.5
	28000	24.0[b]	24	24	14.5[b]	12	20	2.1[a]	0	4

[c] Values are mean of four cows. Values in the same column with different superscripts are significantly different ($P < 0.05$).
[d] Range value which includes all animals.

TABLE 3b. PERIOD OF TIME DURING WHICH SERUM PENICILLIN CONCENTRATIONS WERE OVER THE MINIMAL INHIBITORY CONCENTRATION OF 0.4, 1.6 AND 3.3 IU/mL FOLLOWING THE FIFTH PENICILLI INJECTION

ROUTE	DOSES (IU/KG)	DURATION ABOVE 0.4 IU/mL (H)			DURATION ABOVE 1.6 IU/mL (H)			DURATION ABOVE 3.3 IU/mL (H)		
		Mean[c]	Min[d]	Max[d]	Mean[c]	Min[d]	Max[d]	Mean[c]	Min[d]	Max[d]
IM	7000	14.5[a]	12	16	0[a]	0	0	0[a]	0	0
	14000	22.0[b]	16	24	9[b]	7	12	0[a]	0	0
	21000	23.0[b]	20	24	13.0[c]	10	14	1.4[b]	0	3.5
	28000	23.0[b]	20	24	14.5[c]	10	16	4.8[c]	.5	7.5
SC	7000	16.0[a]	16	16	2.3[a]	0	5	0[a]	0	0
	14000	22.0[a]	16	24	10.3[b]	7	14	0[a]	0	0
	21000	24.0[b]	24	24	14.5[c]	12	16	3[b]	2	4.5
	28000	24.0[b]	24	24	15.5[c]	14	16	5.6[c]	4	6.5

[c] Values are mean of four cows. Values in the same column with different superscripts are significantly different ($P < 0.05$).

[d] Range value which includes all animals.

TABLE 4. MILK PENICILLIN CONCENTRATIONS (IU/mL) TAKEN 12 HOURS APART DURING THE 5 CONSECUTIVE DAYS OF INTRAMUSCULAR (IM) OR SUBCUTANEOUS (SC) INJECTIONS OF INCREASING DOSES OF PROCAINE PENICILLIN G

ROUTE	DOSES (IU/KG)	↓ 0	12	↓ 24	36	↓ 48	TIME (H) ↓ 60	72	84	↓ 96	108	120
IM	7000	0	.071[a]	.043	.088	.055	.115	.056	.085	.072	.090	.045
			.015	.005	.013	.008	.016	.013	.017	.021	.014	.005
	14000	0	.113	.093	.118	.120	.198	.098	.140	.106	.176	.093
			.016	.003	.016	.027	.026	.005	.027	.022	.027	.021
	21000	0	.245	.135	.244	.170	.337	.179	.252	.153	.352	.153
			.016	.008	.022	.011	.039	.020	.053	.024	.033	.014
	28000	0	.257	.164	.277	.201	.386	.159	.312	.228	.473	.169
			.047	.032	.045	.039	.066	.011	.042	.036	.140	.012
SC	7000	0	.054	.029	.066	.040	.056	.066	.047	.109	.069	.036
			.009	.004	.012	.004	.005	.034	.006	.075	.011	.007
	14000	0	.091	.123	.086	.070	.139	.083	.086	.057	.129	.057
			.009	.070	.011	.010	.018	.014	.014	.007	.008	.013
	21000	0	.171	.097	.104	.112	.179	.109	.163	.112	.197	.115
			.032	.017	.024	.019	.030	.025	.032	.010	.026	.012
	28000	0	.154	.151	.164	.160	.243	.169	.214	.131	.252	.141
			.034	.045	.019	.023	.044	.026	.021	.022	.015	.019

[a] Values are mean ± SE of 4 cows. Samples were taken prior to injection.

↓ Indicates time of penicillin injection.

DISCUSSION

The Cmax and AUC measured for the 4 doses were almost all significantly different; however the clinical effectiveness might not be different for all dosages. In a review by Dalhoff (4), the time that serum concentrations exceed MIC was the most significant parameter that determined efficacy of the B-lactams. In the present study, doses of 14,000, 21,000 and 28,000 IU/kg would be effective against organism with MIC less than 0.4 IU/mL (0.25 μg/mL). The lowest dose (7,000 IU/kg) provided serum concentrations greater than 0.4

IU/mL for periods ranging from 12 to 16 hours. For organisms requiring higher inhibitory concentrations (Pasteurella sp between 1.6 and 3.3 IU/mL equivalent to 1 and 2 μg/mL), the inhibitory concentrations were reached in only 50% of the time for the two largest doses (for 1.6 IU/mL) and only the highest provided levels of penicillin greater than MIC 3.3 IU/mL and this time period was minimal (5).

This study was designed to compare the 2 routes of administration and although the SC group had values slightly higher than the IM group these small differences are probably of minor importance. The fact that serum penicillin concentrations following SC administration were as high or higher than IM administration was not in agreement with other studies (5, 6). The milk penicillin concentrations were much lower than in serum. In fact the highest mean milk penicillin concentration over the five days of injection reached a maximum of 0.473 IU/mL as previously reported (7). The small diffusion of the antibiotic in the mammary gland might explain the low efficacy of this antimicrobial agent when administered systemically (8).

Seemingly, the doses of 14,000 21,000 and 28,000 IU/kg given at 24 hour intervals would be equally effective against sensitive organisms since there is practically no difference between the time that the concentrations are maintained over 0.4 IU/mL. For a MIC of 1.6 IU/mL, the doses of 21,000 and 28,000 IU/kg appeared effective while at MIC 3.3 IU/mL none of the therapeutic regimen used would seem effective. In conclusion the route of administration does not seem to affect serum concentrations of penicillin and in order to minimize residue in milk and meat a dose of 21,000 IU/kg of PPG should preferably be used.

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ABSTRACT

The purpose of this study was to determine if increasing doses of procaine penicillin G (PPG) administered by two routes would increase in the same fashion the serum and milk penicillin concentrations. Using two 4x4 latin square design experiments, two groups of lactating dairy cows (n=4 per group) received 4 different doses (7,000, 14,000, 21,000 and 28,000 IU/kg, < 20 mL/site of a 300,000 IU/mL suspension) of PPG. Each dose was administered once daily for 5 consecutive days by either the intramuscular (square 1) or subcutaneous (square 2) route. Every 3 weeks a new dose was administered. Samples of milk were taken before administration and every 12 hours for 6 consecutive days. Blood samplles were taken serially at day 1 and day 5 for a 24 hour period following the injection. Penicillin concentrations were measured using a microbiological assay with Bacillus subtilis as the test organism. The sensitivity of the test was 0.12 IU/mL. Milk penicillin concentrations were measured with a quantitative Bacillus stearothermophilus disk assay method and the sensitivity of the assay was 0.016 IU/mL. The maximal concentration (Cmax),

the time of the peak (Tmax), the area under the curve (AUC) in serum and the time duration the penicillin concentration remained above MIC's of 0.4, 1.6 and 3.3 IU/mL were compared for the different dosages. The milk penicillin concentrations were much lower than in the serum. For a given dose the 2 routes of administration were similar for the variables evaluated. The Cmax and AUC were significantly increased in a dose-dependent manner at day 5. The Tmax was not different between any doses. The time duration above the MIC of 0.4 IU/mL was smaller for the 7,000 IU/kg than for the other 3 doses. The time duration above the MIC 1.6 IU/mL was smaller for the 7,000 and 14,000 IU/kg doses than for the 21,000 and 28,000 IU/kg doses. The results indicate that serum concentrations increase with the injected dose but the clinical effectiveness may not differ between the 21,000 and 28,000 IU/kg doses.

RÉSUMÉ

Le but de cette étude était de vérifier si des doses croissantes de pénicilline G procaïnique (PGP) injectées par voies sc ou im provoquent des augmentations des concentrations de pénicilline dans le sérum et le lait. Un dispositif expérimental en carré latin 4x4 (4 animaux recevant 4 différentes doses pendant 4 périodes). Chaque dose (7 000, 14 000, 21 000 et 28 000 UI/kg; 20 mL/site d'une suspension contenant 300 000 UI/mL) fut injectée durant 5 jours consécutifs par voies sc (carré 1) ou im (carré 2). Une période de trois semaines fut respectée entre chaque série d'injections. Des échantillons de lait furent prélevés à toutes les 12 heures pour une période de 6 jours. Des échantillons de sang furent prélevés de façon séquentielle aux jours 1 et 5 sur une période de 24 heures. Les concentrations de pénicilline dans le lait et le sérum furent mesurés par essai microbiologique en utilisant Bacillus stearothermophilus pour le lait et Bacillus subtilis pour le sérum. Les concentrations maximales (Cmax), le temps d'obtention du pic (Tmax) et l'aire sous la courbe (SSC) ainsi que la période de temps durant laquelle les concentrations minimales inhibitrices (CMI) dépassaient les seuils de 0,4, 1,6 et 3,3 UI/mL furent analysées. Aucune différence importante n'a été notée entre les deux voies d'administration. Les concentrations de pénicilline dans le lait étaient moins élevées que celles du sérum. La Cmax et la SSC ont augmenté en relation avec la dose au jour 5, tandis que le Tmax n'a pas été affecté. La période de temps excédant la CMI de 0,4 UI/mL était inférieure pour la dose de 7 000 UI/kg comparativement aux 3 autres doses. Pour la période excédant la CMI de 1,6 UI/mL, les doses de 21 000 et 28 000 UI/kg ont été supérieures aux deux autres doses. Ces résultats suggèrent que les concentrations sériques de pénicilline augmentent avec les doses mais l'importance clinique entre les doses de 21 000 et 28 000 UI/kg semble minime.

RESUMEN

El objectivo del estudio es determinar si dosis crecientes de penicilina G-procaínica administradas por dos vías distintas traen incrementos producen similares de concentración de penicilina en la sangre y en la leche. Empleando 2 Planos de Cuadrado Latino de 4x4 dos grupos de vacas lecheras lactantes (n=4 en cada grupo) recibieron 4 dosis distintas (7 000, 14 000, 21 000 y 28 000 U.I./kg) de PPG. Cada dosis fue administrada una vez al dí durante 5 días seguidos o por la vía intramuscular (cuadrado 1) o por la vía subcutánea (cuadrado II). Muestras de leche fueron tomadas antes de administrar el antibiótico, así como a intervalos de 12 horas durante 6 días consecutívo. Muestras de sangre se tomaron en serie el primero y quinto días (día 1 y día 5) durante un periodo de 24 horas después de la injeccion. Las concentraciones de penicilina fueron medidas empleando el test microbiológico con Bacillus searothemophilus para la leche y el Bacillus subtilis para el serum. La concentración máxima (Cmax) el tiempo que se tarda en conseguir esa concentración (Tmax) y la superficie bajo la curva (AUC) fueron comparadas con dosis de 0.4, 1.6 UI/mL. No se apreció ninguna diferencia entre las dos vías de administración. Las concentraciónes de penicilina en la leche fueron menos elevadas que del serum. La Cmax y la superficia bajo la curva (AUC) aumentaron en relación a la dosis al quinto día; no se apreció ninguna diferencia en el Tmax.

Estos resultados sugieren que la concentraciónes sanguíneas de penicilina aumentar con la dosis, paro la eficacia clinica entre las dosis de 21,000 y 28,000 UI/kg para mínima.