PHARMACOKINETICS OF PENICILLIN-G IN PREGNANT AND NON-PREGNANT EWES AND COWS.

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INTRODUCTION.

Penicillin-G (Pen-G) is an antibacterial agent widely used in treating bacterial infections in ruminants. The pharmacokinetics of the drug in cattle and sheep have been studied (1,2,3,4,5) but if pharmacokinetics are affected by pregnancy has to our knowledge not been investigated. The objective of the present study was to compare pharmacokinetics of Pen-G in pregnant and non-pregnant cows and ewes.

MATERIALS AND METHODS.

Five Swedish Red and White dairy cows, 5-8 years old, and eight Swedish Landrace Pelt Sheep ewes, 2-5 years old, were used in the experiments. Each animal was given an intravenous injection of an aqueous solution of potassium-penicillin-G (10 mg/kg) (Novocillin vet.® Novo Industri A/S Denmark) before and after parturition. The cows were given the first injection in the period 2-3 weeks before parturition and the second in the period 2-7 weeks after parturition. Ewes were dosed 1-4 weeks before and 1-3 weeks after parturition

Whole blood was sampled by puncture of the jugular vein not used for injection of Pen-G at intervals after the injection as indicated in Fig 1 and 2. Samples were allowed to clot and serum collected. Serum samples were stored at -70 C° and analysed within five weeks after sampling.

Penicillin-G in serum was analysed by the agar-well-diffusion method using *Bacillus stearothermophilus* var. *calidolactis* ATCC 10142 and *Micrococcus luteus* ATCC 9341 as test organisms (6,7). Standard solutions were prepared from pooled serum sampled prior to the experiments. The assays were performed on 24 x 24 cm trays containing double sets of standard and samples, the means of which were used to calculate the concentration of the samples from regression lines according to the least squares method.

Pharmacokinetic parameters were estimated by iterative non-linear regression analysis using a computer program (PCNONLIN). Bi- and tri-exponential pharmacokinetical models were fitted to individual serum data. In the analysis, data were given the weighting $1/Ycalc^2$, where Ycalc is the model-predicted concentration. The best fit of model to data was chosen based on the least weighted sum of squared residuals and the lowest standard error of estimates. The most appropriate model was chosen using the F-ratio test (8). The parameters estimated as primary in the regression analysis were for a bi-exponential model A, B, α and β. For a tri-exponential model primary parameters also included C and γ. From these estimated parameters total body clearance (Cl_T), volume of distribution at steady state (Vd_{ss}), volume of distribution by the area method ($Vd_{(area)}$), volume of the central compartment (Vc) and total area under curve ($AUC_{0-\infty}$) were calculated according to conventional methods (9).

Pharmacokinetic parameters were also calculated using model independent methods. Overall elimination constant (K_{el}) was determined by the least-squares method using the last five ln serum vs. time points. Elimination half-life was calculated as $t_{1/2} = Ln2/K_{el}$. Total area under curve ($AUC_{0-\infty}$) and area under curve of the product of concentration and time (AUCM) were calculated by the trapezoidal rule and extrapolated to infinity. Total body clearance was calculated from $Cl_T = dose/AUC_{0-\infty}$, mean residence time from $MRT = AUCM/AUC_{0-\infty}$ and volume of distribution at steady state from $Vd_{ss} = dose \times MRT/AUC_{0-\infty}$. Volume of distribution by the area method was calculated as $Vd_{(area)} = dose/AUC_{0-\infty} \times K_{el}$.

A paired *t*-test was used for statistical calculations. A difference was considered statistically significant at $p<0.05$.

RESULTS.

Concentrations of penicillin-G in serum were higher in pregnant than in non-pregnant animals throughout the sampling period (Figs 1 and 2). In Ewes the difference was statistically significant at all sampling times, except at 4 and 8 hours but only in the first hour in cows.

In cows a three-compartment model gave the best fit to experimental data in all pregnant and in four non-pregnant animals. A two compartment model gave the best fit in one non pregnant cow. In ewes the three-compartment model was superior in six pregnant animals but a two-compartment model gave a better fit in two pregnant and all non-pregnant ewes. Parameters derived from both two- and three-compartment models are given in Table 1. As no single model could be used in all pregnant and non-pregnant animals, in neither cows nor ewes, the calculated parameters were not suited for comparison of kinetics before and after parturition.

However, using parameters derived by model independent methods it could be demonstrated that in both cows and ewes Cl_T and Vd_{ss} were lower and $AUC_{0-\infty}$ higher in pregnant than in non-pregnant animals (Table 2). These differences were statistically significant. In ewes also MRT was significantly longer in pregnant than in non-pregnant animals.

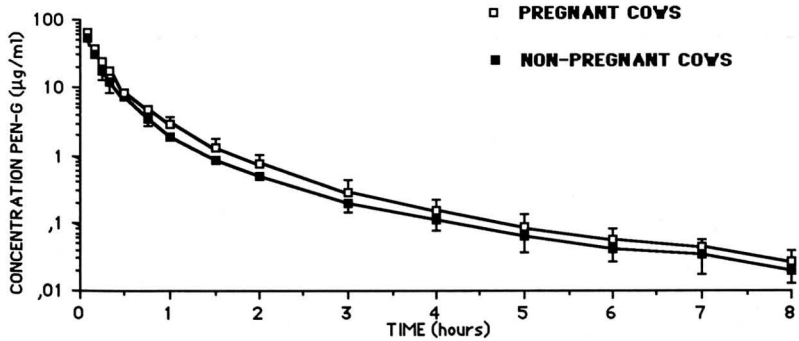

FIGURE 1. Concentration of penicillin-G in serum from pregnant and non-pregnant cows after a single intravenous injection of potassium-penicillin-G (10 mg/kg). Means ±SD., n=5.

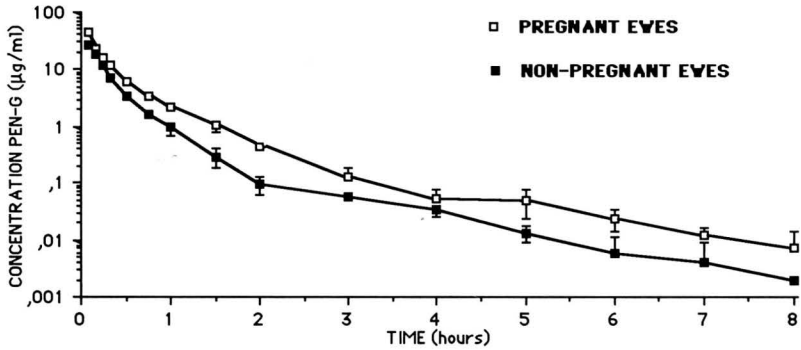

FIGURE 2. Concentration of penicillin-G in serum from pregnant and non pregnant ewes after a single intravenous injection of potassium-penicillin-G (10 mg/kg). Means ±SD., n=8.

TABLE 1. Pharmacokinetic values for penicillin-G in pregnant and non-pregnant sheep (n=8) and cows (n=5). Values obtained by fitting two- and tri-exponential models to experimental data by computerized iterative non-linear regression analysis. Means ±SD.

	EWES				COWS			
	2-COMP		3-COMP		2-COMP		3-COMP	
	PREG	NOT PREG	PREG	NOT PREG	PREG	NOT PREG	PREG	NOT PREG
A	**40.88** *	**32.99**	**64.44** *	**39.37**	**66.05**	**58.13**	**93.39**	**85.89**
(µg/ml)	(5.53)	(3.89)	(9.38)	(11.84)	(6.15)	(2.61)	(11.57)	(35.99)
B	**1.23** *	**0.51**	**13.23**	**13.92**	**1.84**	**1.22**	**13.72**	**10.60**
(µg/ml)	(0.68)	(0.28)	(3.02)	(6.75)	(0.86)	(0.22)	(5.68	(3.35)
C			**0.44**	**0.35**			**0.63**	**0.48**
(µg/ml)			(0.29)	(0.16)			(0.41)	(0.23)
α	**3.25** *	**4.06**	**8.63**	**9.67**	**3.68**	**3.95**	**7.29**	**7.68**
(h^{-1})	(0.74)	(0.49)	(1.19)	(4.25)	(0.50)	(0.38)	(1.06)	(3.21)
β	**0.61**	**0.66**	**1.89** *	**2.99**	**0.55**	**0.53**	**1.68**	**1.84**
(h^{-1})	(0.10)	(0.19)	(0.21)	(0.40)	(0.05)	(0.04)	(0.34)	(0.31)
γ			**0.44**	**0.59**			**0.38**	**0.38**
(h^{-1})			(0.07)	(0.16)			(0.08)	(0.10)
Vc	**241** *	**303**	**131** *	**196**	**149** *	**169**	**94**	**113**
(ml/kg)	(29)	(41)	(15)	(44)	(14)	(7)	(11)	(30)
$Vd_{(area)}$	**1174** *	**2017**	**1532** *	**2060**	**857** *	**1121**	**1222**	**1665**
(ml/kg)	(273)	(1184)	(361)	(1168)	(94)	(82)	(361)	(475)
Vd_{ss}	**327** *	**403**	**273** *	**335**	**238** *	**280**	**208**	**246**
(ml/kg)	(23)	(69)	(28)	(70)	(25)	(14)	(33)	(40)
Cl_T	**681** *	**1140**	**653** *	**1054**	**470** *	**587**	**447** *	**553**
(ml/kg/h)	(40)	(123)	(31)	(122)	(33)	(41)	(30)	(44)
$AUC_{0-\infty}$	**14.72** *	**8.88**	**15.36** *	**9.62**	**21.35** *	**17.11**	**22.48** *	**18.16**
(µg/ml/h)	(0.84)	(1.02)	(0.71)	(1.15)	(1.61)	(1.10)	(1.57)	(1.35)

* = statistically significant difference ($p<0.05$) between parameter value in pregnant and non-pregnant animal.

Table 2. Pharmacokinetic values for penicillin-G in pregnant and non-pregnant sheep (n=8) and cows (n=5). Values calculated by model independent methods. Means ±SD.

	EWES			COWS		
	PREGNANT		NOT PREGNANT	PREGNANT		NOT PREGNANT
K_{el}	**0.47**		**0.63**	**0.43**		**0.42**
(h^{-1})	(0.06)		(0.23)	(0.05)		(0.10)
$t_{1/2}$	**1.49**		**1.41**	**1.65**		**1.78**
(h)	(0.24)		(1.08)	(0.21)		(0.58)
$AUC_{0-\infty}$	**14.43**	*	**8.70**	**21.34**	*	**17.00**
(µg/ml/h)	(0.85)		(0.91)	(1.66)		(1.12)
MRT	**0.44**	*	**0.35**	**0.49**		**0.46**
(h)	(0.06)		(0.06)	(0.09)		(0.04)
Vd_{ss}	**303**	*	**405**	**227**	*	**269**
(ml/kg)	(28)		(94)	(27)		(12)
$Vd_{(area)}$	**1503**		**2434**	**1124**		**1513**
(ml/kg)	(281)		(2116)	(181)		(466)
Cl_T	**695**	*	**1160**	**471**	*	**590**
(ml/kg/h)	(40)		(121)	(34)		(40)

* = statistically significant difference ($p<0.05$) between parameter value in pregnant and non-pregnant animal.

DISCUSSION.

The values of the pharmacokinetic parameters calculated by the bi-exponential model in non-pregnant cows agree with previous reports where the same model was used for calculation (2,5). In previous reports of kinetics in ewes, however, the values of α, β, are higher and Cl_T, Vc, Vd_{ss} and $Vd_{(area)}$ are lower than the values reported here (4,10).

In pregnant animals Cl_T and Vd_{ss} were lower than in non-pregnant animals. The changes were numerically smaller in cows than in ewes. In ewes the longer MRT in pregnant animals indicate that the elimination of Pen-G was slower than in non-pregnant animals. These findings are in contrast to the observations in humans where cefazolin and cephradine had a higher clearance, larger volume of distribution and shorter elimination half-life in pregnant than in non pregnant women (11). The authors concluded that these changes were caused by an increase in renal clearance due to an increase in renal blood flow, glomerular filtration and tubular secretion during pregnancy. Wether the changes in kinetics observed in the present investigation are caused by a decreased renal clearance or by a primary change in volume of distribution needs further investigation.

The practical importance of the change in kinetics of Pen-G in pregnant animals is probably small, at least in cows. In sheep, however, using the same dose in pregnant and non pregnant animals results in slightly higher and longer lasting serum concentrations in pregnant animals as illustrated in the simulation in Fig. 3.

SUMMARY.

The pharmacokinetics of penicillin-G after a single intravenous injection (10 mg/kg) were studied in pregnant and non-pregnant cows and ewes. Pharmacokinetic parameters calculated by model dependent and model independent methods are reported. Total body clearance was lower, and volume of distribution smaller in pregnant than in non-pregnant animals.

RESUMEN.

La farmacocinética de la penicilina G después de una inyección intravenosa sola (10 mg/kg) fue estudiada en vacas y ovejas preñadas y no preñadas. Parámetros farmacocinéticos fueron calculados por métodos dependientes e independientes del modelo. La eliminación total del cuerpo era más baja y el volumen de distribución más pequeño en hembras preñadas que en las no preñadas.

SOMMAIRE.

La pharmacocinétique de la pénicilline G après une seule injection intraveneuse (10mg/kg) a été étudiée chez les vaches et brébis gestantes et non-gestantes. Des paramètres pharmacocinétiques ont été calculés par des méthodes dépandantes et indépandantes du modèle. L´élimination totale du corps était plus basse et le volume de distribution plus petit chez les femelles gestantes que chez les non-gestantes.

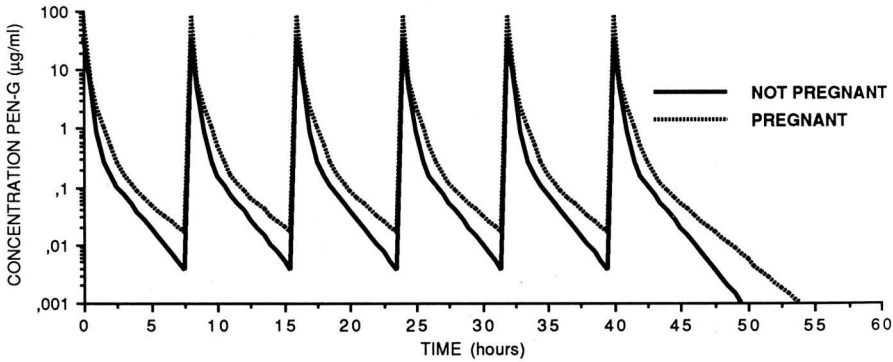

FIGURE 3. Simulated serum concentrations of Pen-G in pregnant and non-pregnant ewes after six intravenous doses of 10 mg/kg with eight hour intervals. A bi-exponential model was used for non pregnant ewes and a tri-exponential model for pregnant ewes.

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