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Influence of the Local Tolerance on the Pharmokinetics of two Penicillin G Preparations in Cattle and Swine

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Luthman J., V. Dall, S. O. Jacobsson, B. Bengtsson and C. Korpe: Influence of the local tolerance on the pharmokinetics of two penicillin G preparations in cattle and swine. Acta vet. scand. 1988, 29, 199–206. – The addition of citrate buffer to a penicillin G preparation for injection was in a preliminary study found to improve the local tolerance in rabbits. In the present study 2 penicillin G preparations with different citrate buffer content was tested in the target species swine and cattle. The results of the local tolerance studies indicated that the preparation with the highest citrate content caused less tissue damage. In swine this preparation gave 100 % higher maximum levels in serum than the more tissue irritating preparation. In calves the most irritating preparation showed significantly longer half-life. The results indicated that the local tolerance may influence the serum levels in a way that may influence the penetration of penicillin.

Introduction

The local tolerance of the most used antibacterial drug preparations intended for intramuscular injection is rather well known. The early research in this field was summarized by *Rasmussen* (1980). Tetracyclines, sulfonamides and trimethoprim are considered to cause severe tissue damage, while penicillins are rather well tolerated.

In a preliminary study (*Dall & Luthman* 1988) it was shown that the local tolerance of a potassium penicillin G preparation was significantly improved when the citrate buffer content was increased. When tested in rabbits the improved preparation caused significantly less tissue damage than the original.

The absorption of drugs from the injection site is controlled by a number of factors e. g. the absorptive surfaces in contact with the injected volume, the physico-chemical properties of the drug and the vehicle, the anatomical localization of the injection site. A comprehensive review on this topic was earlier published by *MacDiarmid* (1983). There are very few publications discussing the influence of local tissue irritation on the pharmacokinetics of drugs. A comparison of several 20 % oxytetracycline preparations was published by *Mevius et al.* (1986). The most tissue irritating products were found to be absorbed slower from the injection site and to give lower maximum levels in serum.

The aim of the present investigation was to study the local tolerance of 2 penicillin preparations in the target species swine and cattle and to compare their pharmacokinetic properties.

Material and methods

Drug preparations

Novocillin 6.3 g, Novo (potassium penicillin G 6.3 g, dibasic sodium citrate 36 mg, sodium citrate 190 mg) and Novocillin 6.3 g added 300 % more of citrate buffer (the improved preparation). Both preparations were dissolved in 16 ml of sterile water giving a solution with a penicillin G concentration of 315 mg/ml.

Local tolerance in cattle and swine

Four heifers and 2 cows of the Friesian breed were injected intramuscularly with a therapeutic dose (10 mg/kg) of the 2 drug preparations. The weight of the heifers varied from 320 to 375 kg and the weights of the cows were 620 and 650 kg. The injections were given in M. triceps. A 1.4 × 50 mm needle was inserted at half the distance between the lowest point of the scapula and the elbow. The injection was given slowly avoiding pressure on the injection site. The original Novocillin-preparation was injected on the right side and the improved preparation on the left.

The pigs, 3 castrates and a female of the danish landrace breed weighing between 70 and 80 kg, were treated in the same way. A 1.2 × 40 mm needle was used. Only 1 pig was given the whole volumen of the original preparation at 1 site. The injection caused a violent pain reaction. In the remaining 3 animals the volume was divided equally and injected at 2 sites. The improved preparation was injected at 1 site in all animals. The animals were inspected daily for local reactions. The pigs were slaghtered after 3 days and the cattle after 6 days.

The injected muscle was free dissected and the injection site was localized over a light cabinet. The muscle was sliced for examination of macroscopic damages. Necrotic parts were carefully isolated from surrounding tissue and weighed. This technique was earlier described by *Svendsen et al.* (1979) and *Svendsen* (1983).

Pharmacokinetic studies

Six calves of the Swedish Red and White breed and 6 pigs of the Swedish landrace breed were used. The calves weighed about 150 kg and the pigs showed a mean weight of 60 kg at the start of the experiments. Each calf carried 6 subcutaneously implanted tissue cages of a model described earlier (*Bengtsson et al.* 1984). The cages were implanted about 8 weeks before the experiments. In all pigs a jugular vein was catheterized for blood sampling as described by *Karlbom et al.* (1981). The dose of penicillin used was 10 mg/kg. The original Novocillin preparation was given intravenously to 4 of the calves and 5 calves received the improved preparation. The interval between the injections was 5 days.

In a cross-over study all calves and pigs were given the drug preparations intramuscularly. The injections were given in M. triceps. The original Novocillin preparation was injected on the right side and the improved preparation on the left. The dose, 10 mg/kg was injected at 1 site in all animals. The interval between the injections was 5 days. Blood was sampled at intervals shown in tables and figures. Tissue cage fluid (TCF) was sampled by puncturing the skin and the unperforated cage wall as described by *Bengtsson et al.* (1984).

Penicillin in serum and TCF was analyzed by the well diffusion technique. Sarcina lutea and Bacillus stearothermophilus var. calidolactis were used as test organisms.

A semi-logaritmic plot of the concentration-time curve was used for calculation of the half-life, $t_{1/2}$. The last part of the curve where concentration decreased exponentially was used. Half-life was calculated ac-

cording to the method of least squares (*Baggot* 1977). Area under curve (AUC) was determined according to the trapezoidal rule. Student's t-test was used for statistical evaluation. The values given in text and figures are mean ± standard error of the mean.

Results

Local tolerance

The results of the local tolerance studies in cattle are shown in Table 1. The original Novocillin preparation caused well defined necrotic areas with focal hemorragies in all animals except 1. In this animal there was a less demarcated, very irregular necrosis which was impossible to isolate and weigh. As expected the weight of the necrotic tissue was higher in the cows which were injected with a greater volume. The improved preparation caused well demarcated necrosis in only 2 animals, 1 cow and 1 heifer. In these 2 animals the weight of the damaged tissue was about 50 % less after the improved than after the original preparation.

Table 2 shows the results of the local tolerance studies in the pigs. Well defined necrotic areas could be demonstrated in all animals. The fact that 3 pigs receiving the original preparation were injected at 2 sites makes the comparison of the 2 preparations difficult.

Pharmacokinetics

The serum concentrations of penicillin in calves after intravenous injection of the 2 preparations are shown in Table 3. The se-

Table 1. Findings at the injection site 6 days after intramuscular injection of 2 penicillin preparations in cattle.

Preparation	Animals and injected volume (ml)		Macroscopic findings	Necrotic area (g)
Novocillin 6.3 g	Heifer no			
Diluted in 16 ml	25	9.5	All heifers except no 70	25.8
of sterile water	71	9.5	showed well defined grey	25.3
	70	10.0	necrosis with focal	–
	48	11.5	hemorrhages	15.3
	Cow no			
	72	19.5	The same picture as in the	47.4
	76	18.5	heifers. The necrosis was irregular in cow 72	47.1
Novocillin 6.3 g	Heifer no			
added 300 % more	25	9.5	Only animal 25 showed a	10.2
of citrate buffer	71	9.5	well defined necrotic area.	–
and diluted in 16 ml	70	10.0	In the other animals the	–
of sterile water	48	11.5	damaged areas were very irregular with focal hemorrhages and grey stripes	–
	Cow no			
	72	19.5	Irregular necrosis with focal	–
	76	18.5	hemorrhages and grey stripes in non-necrotic tissue	24.0

Table 2. Findings at the injection site 3 days after intramuscular injection of 2 penicillin preparations in pigs.

Preparation	Animal no and injected colume (ml)		Macroscopic findings	Necrotic area (g)	
Novocillin 6.3 g	1*	2.4	Well-defined necrotic	3.2	3.5
diluted in 16 ml	2*	2.5	areas with focal	0.3	11.7
of sterile water	3	2.4	hemorrhages	9.5	
	4*	2.4		3.9	8.2
Novocillin 6.3 g	1	2.4	Well-defined necrotic	7.0	
added 300 % more	2	2.5	areas with focal hemorrhages	9.0	
of citrate buffer	3	2.4		4.4	
and diluted in 16 ml	4	2.4		8.3	
of sterile water					

* The volume equally divided at 2 injection sites.

rum levels were very similar and the elimination half-life did not differ. The half-life of the original preparation was 1.18 ± 0.06 h (range 1.04–1.35 h) and of the improved preparation 1.21 ± 0.12 h (range 1.01–1.56).

The serum levels after intramuscular injection in calves are shown in Table 4. There were no significant differences during the first 90 min, but from 2 to 6 h the levels were significantly higher after the original preparation. The half-life of the original preparation was 1.82 ± 0.16 h and of the improved preparation 1.50 ± 0.13 H. The difference was statistically significant ($p < 0.05$, $t = 2.639$). AUC from zero to infinity (AUC_0^{∞}) was significantly higher after the original preparation ($p < 0.01$, $t = 6.387$).

The penicillin concentrations were significantly lower in TCF than in serum (Table 4), and maximum was reached much later (4–6 h). The levels did not differ significantly after injection of the 2 preparations.

The serum concentrations after intramuscular injection in pigs are shown in Fig. 1. The improved preparation gave 100 % higher maximum levels (after 10 and 20 min). From 1.5 h the levels were higher after the original preparation, the differences were

Table 3. Serum concentrations (μg/ml) of penicillin G in calves after intravenous injection of 2 penicillin preparations. Dose 10 mg/kg. $\bar{x}$ ± S.E.M.

Preparation	Time after injection (min) 5	10	20	30	60	90	120	180	240	360	420
Novocillin 6.3 g diluted in 16 ml of sterile water. n = 4	51.8 ±2.80	31.4 ±3.20	17.9 ±1.29	11.98 ±0.447	3.24 ±0.41	1.62 ±0.20	0.90 ±0.11	0.42 ±0.06	0.18 ±0.05	0.05 ±0.01	0.031 ±0.00
Novocillin 6.3 g added 300 % more of citrate buffer and diluted in 16 ml of sterile water. n = 5	51.8 ±2.80	29.8 ±0.93	17.9 ±2.59	9.4 ±0.80	2.9 ±0.21	1.30 ±0.11	0.80 ±0.10	0.31 ±0.04	0.13 ±0.01	0.05 ±0.00	0.027 ±0.00

Table 4. Penicillin concentrations (μg/ml) in serum and tissue cage fluid (TCF) in calves after intramuscular injection of 2 penicillin preparations in calves. $\bar{x} \pm$ s.e. n = 6.

Preparation	Minutes after injection											
	10	20	30	40	50	60	90	120	180	240	360	480
Serum												
A	3.47	3.71	4.45	3.80	3.92	3.92	2.87	2.28	1.99	1.32	0.60	0.29
	±0.34	±0.53	±0.67	±0.46	±0.57	±0.57	±0.25	±0.14	±0.21	±0.12	±0.07	±0.02
								**	***	***	*	*
B	3.50	4.03	3.84	3.95	3.34	3.06	2.32	1.85	1.28	0.84	0.33	0.13
	±0.41	±0.56	±0.14	±0.27	±0.16	±0.24	±0.17	±0.13	±0.18	±0.63	±0.03	±0.01
TCF												
A						0.16		0.34	0.52	0.50	0.54	0.50
						±0.04		±0.11	±0.09	±0.11	±0.10	±0.07
B						0.26		0.28	0.37	0.46	0.39	0.32
						±0.15		±0.09	±0.08	±0.06	±0.03	±0.04

A = Novocillin 6.3 g diluted in 16 ml of sterile water
B = Novocillin 6.3 g added 300 % more of citrate buffer and diluted in 16 ml of sterile water.

* $p < 0.05$
** $p < 0.01$

statistically significant after 2 and 4 h. Half-lifes and AUC's did however not differ.

Some pharmacokinetic parameters for the 2 preparations in pigs and calves are summarized in Table 5.

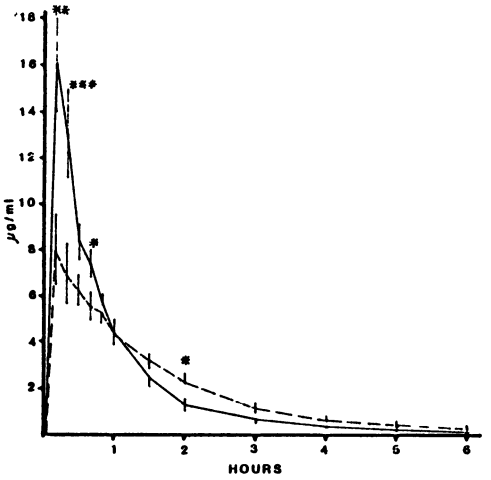

Figure 1. Serum concentrations of penicillin after intramuscular injection of two penicillin G preparations. Novocillin – – – –, and Novocillin added 300 % more of citrate buffer ——. Dose 10 mg/kg, $\bar{x} \pm$ s.e.

Discussion

In the preliminary rabbit study it was shown that addition of 300 % more citrate buffer to the original Novocillin preparation significantly reduced the local toxicity. Well demarcated necrotic areas, easy to isolate, were induced by both preparations. The rabbit therefore seemed to be a suitable species for studies of that kind. In the present study the improved preparation caused a well demarcated necrosis in only 2 of 6 cattle. The difference in reaction pattern may be a species difference, since *Diness* (1985) found rabbits to be more sensitive than pigs to strongly tissue irritating preparations.

As discussed earlier the original preparation was injected at two sites in three of four pigs, which reduces the validity of the tolerance study. The aim of the study was to document the tissue damage which occur after a therapeutic dose. In comparison with some other antibiotics e.g. tetracyclines the local toxicity of penicillins is considered as slight (*Rasmussen & Høgh* 1971). The results from the study of *Diness* (1985) and from

Table 5. Pharmacokinetic data of 2 penicillin preparations after intramuscular injection of 10 mg/kg in calves (n = 6) and pigs (n = 6). $\bar{x} \pm$S.E.M.

	Novocillin 6.3 g			Novocillin 6.3 g added 300 % more of citrate buffer		
	Pigs		Calves	Pigs		Calves
Cmax, µg/ml	7.91 ± 1.82	N.S.	4.03 ± 0.56	15.96 ± 2.06	***	4.45 ± 0.67
Tmax, h	0.27 ± 0.04	N.S.	0.42 ± 0.06	0.20 ± 0.03	N.S.	0.36 ± 0.07
β, h^{-1}	0.435 ± 0.050		0.393 ± 0.031	0.498 ± 0.051		0.478 ± 0.036
$t_{1/2}$ β, h	1.71 ± 0.19		1.82 ± 0.16	1.46 ± 0.14		1.50 ± 0.13
AUC_0^{∞} µg/ml · h	12.41 ± 0.95		14.35 ± 0.79	13.71 ± 1.09		10.24 ± 0.31

Cmax = maximum serum levels
Tmax = time when maximum serum level occurs
β = The slope of the regression line on a semilogarithmic plot
$t_{1/2}$ β = half-life during elimination phase
AUC_0^{∞} = area under curve from zero to infinity

N.S. = Not significant
*** = $p < 0.001$

the present study show that penicillin causes a measurable necrosis in pigs. *Diness* (1985) found the tissue damage to be directly proportional to the injected volume when 0.5–3 ml was injected in M. longissimus dorsi of pigs weighing 19–60 kg. Larger volumes tended to cause less damage.

The maximum serum levels (Cmax) of penicillin after intramuscular injection of the two preparations in calves did not differ. From 2 to 6 h the original preparation gave however significantly higher levels. The difference between the 2 preparations were probably due to their different local toxicity, since half-life did not differ after intravenous injection (Table 3).

The serum levels obtained in calves after intramuscular injection are lower than was reported earlier (*Luthman & Jacobsson* 1986). The serum levels may vary with the injection site (*Marshal & Palmer* 1980). The injections were given in M. triceps in the present study, while the neck musculature was used in the previous investigation.

The tissue cage technique was developed as a method to obtain serial samples from interstitial fluid. The technique has been widely used in pharmacokinetic studies. There is experimental evidence that the clinical counterparts to tissue cages are abscesses or other encapsulated foci (*Barza & Weinstein* 1974, *Bengtsson et al.* unpublished). As shown in Table 4 the penicillin concentrations in TCF were much lower than in serum and maximum was reached later. The concentration-time profile is typical for a cage model with a small open surface (*Holm et al.* 1978, *Luthman et al.* 1984). The results showed that the penicillin concentration in TCF after intramuscular injection of 10 mg/kg was sufficient to inhibit sensitive bacteria also in such deep compartments as represented by the cage.

The improved preparation gave 100 % higher maximum levels than the original in pigs (Fig. 1). A difference of this order may have clinical importance, since it was shown earlier that the peak, rather than AUC, is of

major importance for the extravascular penetration of penicillin (*Luthman & Jacobsson* 1986). In this study significantly higher TCF levels were obtained after intramuscular injection of benzylpenicillin than after procaine penicillin.

The total tolerance studies in pigs were difficult to interprete and the results shown in Table 2 do not give a clear picture of the difference in local toxicity between the 2 preparations. Differences in local toxicity seemed however to be the most plausible explanation to the different serum levels.

Cmax was much higher in pigs than in calves after injection of both preparations, while the half-lifes did not differ (Table 5). The cause to the different serum levels is difficult to explain. It can however not be excluded that the preparations caused more severe tissue damages in calves than in pigs when injected in M. triceps.

Addition of citrate buffer to the original Novocillin preparation appeared to improve the local tolerance also in cattle and pigs. the results from the pharmacokinetic studies showed that the local tolerance of the preparations influenced their pharmacokinetic properties.

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Sammanfattning

Inverkan av den lokala toleransen hos två penicillin G beredningar på farmakokinetiken hos svin och nötkreatur.

En ökning av innehållet av citratbuffert i en penicillin G beredning för injektion visades i preliminära försök förbättra den lokala toleransen hos kanin. I föreliggande arbete undersöktes effekterna av två penicillin G beredningar med olika citratinnehål hos svin och nötkreatur. Resultaten av toleransstudierna indikerade att en ökad mängd citrat reducerade vävnadsskadorna efter intramuskulär injektion. Hos svin gav den mest vävnadsvänliga beredningen 100 % högre maximumkoncentration än den mera vävnadsirriterande. Hos kalv var halveringstiden signifikant längre för den mest vävnadsirriterande beredningen. Resultaten visar att den lokala toleransen efter intramuskulär injektion påverkar farmakokinetiken på ett sätt som kan ha betydelse för penetrationen av penicillin.

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