J. vet. Pharmacol. Therap. **13**, 179–185, 1990.

The effect of phenylbutazone on the plasma disposition of penicillin G in the horse

E. C. FIRTH, J. F. M. NOUWS,* W. R. KLEIN & F. DRIESSENS

Department of General and Large Animal Surgery, Faculty of Veterinary Medicine, State University, Utrecht, Yalelaan 12, de Uithof, 3508 TD, Utrecht and *RVV, District 6, Postbus 40010, 6504 AA Nijmegen, The Netherlands

Firth, E.C., Nouws, J.F.M., Klein, W.R. & Driessens, F. The effect of phenylbutazone on the plasma disposition of penicillin G in the horse. *J. vet. Pharmacol. Therap.* **13**, 179–185.

A pilot study in two ponies showed that the plasma concentrations of intramuscularly administered procaine penicillin were higher if phenylbutazone was administered concurrently. In two other trials, each involving five horses, intravenous sodium penicillin was administered with and without concurrent intravenously injected phenylbutazone, and procaine penicillin was injected intramuscularly with and without oral phenylbutazone. In both cases the plasma concentrations of penicillin were higher when phenylbutazone was given. The pharmacokinetic parameters indicated that the effect was probably due to a lower peripheral distribution because the penetration of penicillin into the tissues was greatly reduced.

E. C. Firth, Veterinary Surgery and Companion Animal Health, Department of Clinical Sciences, Faculty of Veterinary Science, Massey University, Palmerston North, New Zealand.

INTRODUCTION

Antibacterial chemotherapeutic agents and non - steroidal anti - inflammatory agents (NSAIDs) are two of the most commonly used classes of drugs in equine practice. The possible interaction between these two groups of drugs, however, has received little attention. If there was an effect of NSAIDs on plasma penicillin disposition, or on the pharmacokinetics of any commonly used antibacterial agent, it would be important that the interaction be described so that guidelines for the use of antibacterial agents concurrently with NSAIDs could be supplied. Sullins *et al.* (1984) conducted a study on various regimens of procaine penicillin, and concluded that the effect of phenylbutazone (PBZ) on the bioavailability of penicillin was negligible in the horse. This paper presents the results of a study in which horses were given penicillin G on two occasions, on one of which PBZ was administered concomitantly.

METHODS

Animals

The studies were conducted on two groups of horses. A pilot study (Trial I) was performed on two female mixed-breed ponies, weighing 290 and 282 kg. Trials II and III were performed on four adult horses and one pony, as described in a previous publication describing the effect of injection site on the bioavailability of procaine penicillin (Firth *et al.*, 1986).

Treatments and sampling

Trial I. Intramuscular procaine penicillin G (Depocilline®, Gist-brocades NV, Delft, the

Netherlands) was administered with concomitant intravenous phenylbutazone (PBZ) (Phénylarthrite®, Vétoquinol SA, Lure, France; containing 200 mg/ml PBZ) at two different dose rates. Procaine penicillin G (20 000 iu/kg) was injected into the biceps femoris muscle of both ponies on three occasions. On the three occasions different concurrent PBZ dose rates were administered: (a) no PBZ administered; PBZ was given intravenously 15 min before and at 7.75, 15.75 and 23.75 h after procaine penicillin injection; at doses of (b) 0.8 mg/kg and (c) 2.2 mg/kg. Five days elapsed between (a) and (b), and 9 days between (b) and (c).

Venous blood samples were taken at 0, 15, 30, 45, 60 and 90 min, and at 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 26, 28, 30 and 48 h. The ponies were restrained standing on a steel grate, beneath which a large tray was placed to catch urine. Using sterile latex catheters, the bladder was catheterized and emptied at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h. The urine that had been voided onto the tray in each intervening period was collected and added to the catheterized sample. The volume was measured and recorded and a 10-ml sample taken for analysis.

Trial II. Intravenous sodium penicillin G (Gist-brocades NV) with and without concurrent intravenous PBZ administration. The five animals were housed in 3.5 × 3.5-m boxes and allowed freedom of movement except when samples were being taken. Sodium penicillin G was administered intravenously into the right jugular vein at a dose rate of 20 000 iu/kg on two occasions. On the first occasion the penicillin was administered alone, on the second occasion PBZ was administered at a dose rate of 10 mg/kg 10–15 min before penicillin injection. Blood samples were taken through a left jugular venous catheter at 0, 10, 20, 30, 45, 60 and 90 min, and at 2, 3, 4, 5, 6, 7, 8, 10, 12, 22 and 24 h after antibiotic administration.

Trial III. Intramuscular procaine penicillin G was administered with and without concurrent oral PBZ treatment. Procaine penicillin G was injected at a dose rate of 20 000 iu/kg into the serratus ventralis muscle, as previously described (Firth *et al.*, 1986). The volume of antibiotic solution was divided into 20-ml aliquots and the remaining volume, and was injected in separate sites at least 7 cm apart in the serratus ventralis muscle. Five to seven days later, this was repeated while the horses were maintained on orally administered PBZ (10 mg/kg) divided into two doses daily. Oral PBZ treatment commenced 12 h before procaine penicillin administration. The horses were not fasted, and water and hay were constantly available. Blood samples were taken as in Trial I up to 24 h, and thereafter at 28, 32, 48, 56 and 70 h following procaine penicillin-G injection.

In Trial I the samples were taken in heparinized syringes, and the plasma was harvested immediately after centrifugation. In Trials II and III the blood samples were allowed to clot, and the serum harvested. Urine samples were not centrifuged. All samples and standards were stored at −20°C until bioassay, which was never longer than 14 days after collection of samples.

Bioassay and protein binding

Antibiotic concentrations were determined by standardized bioassay procedures (*M. luteus* ATCC 9341 test plate at pH 6.0) (Nouws *et al.*, 1976) and protein binding (Nouws *et al.*, 1975) was determined in a total of 122 samples in Trials II and III, as previously described (Firth *et al.*, 1986). The degree of significance between means was evaluated by the paired Student's *t*-test. There was no interaction in the bioassay between PBZ and penicillin G at PBZ concentrations less than 500 μg/ml (above these concentrations the PBZ dissolved poorly but there was still no interaction observed).

Pharmacokinetic analyses

The plasma penicillin concentrations of each horse were analysed with NONLIN for the best fit to the two- and three-compartment pharmacokinetic model by weighted least-squares regression analysis. The area under the curve (*AUC*) was calculated by the trapezoidal rule. The $V_{d(area)}$, the total body

clearance (Cl_b), and renal penicillin clearance were calculated employing standard procedures. The pharmacokinetic parameters after intramuscular administration of procaine penicillin with and without concomitant phenylbutazone administration were calculated according to the one-compartment model (Baggot, 1977).

RESULTS

The intravenous penicillin concentration–time data from treatments with and without concomitant PBZ administration could be adequately described by a two-compartment open pharmacokinetic model. In the case of concomitant PBZ administration, the data of two of the four horses could be slightly better described with a three-compartment model. For reasons of comparison, the kinetic data derived from the two-compartment model are used and presented.

Pharmacokinetic data are shown in Tables I, II and III. The serum and plasma penicillin concentration curves for the three experiments are shown in Figs 1–3. In Trial I, there was a higher C_{max} and higher AUC when PBZ was injected concurrently with intramuscular procaine penicillin administration. The effect

TABLE I. Pharmacokinetic parameters of intravenous sodium penicillin G injection with (+) and without (−) concurrent administration of intravenous PBZ (Trial II)

Parameter	Unit	−PBZ	+PBZ	Significance*
A°	iu/ml	58.55 ± 9.10	78.87 ± 18.45	$P < 0.1$
α	h^{-1}	1.823 ± 0.182	1.042 ± 0.178	$P < 0.005$
$t_{1/2}$	h	0.383 ± 0.039	0.680 ± 0.110	$P < 0.005$
B°	iu/ml	1.517 ± 1.284	0.366 ± 0.203	$P < 0.05$
β	h^{-1}	0.190 ± 0.028	0.120 ± 0.047	$P < 0.025$
$t_{1/2\beta}$	h	3.717 ± 0.601	6.45 ± 2.24	$P < 0.025$
C°_p	iu/ml	60.72 ± 7.86	79.17 ± 18.43	$P < 0.1$
k_{12}	h^{-1}	0.256 ± 0.079	0.031 ± 0.01	$P < 0.005$
k_{21}	h^{-1}	0.232 ± 0.056	0.125 ± 0.05	$P < 0.005$
$k_{12/21}$		1.09 ± 0.271	0.256 ± 0.046	$P < 0.005$
k_{el}	h^{-1}	1.524 ± 0.256	1.007 ± 0.116	$P < 0.025$
$AUC_{0-\infty}$	iu.h/ml	40.05 ± 6.52	80.0 ± 14.66	$P < 0.005$
Cl_b	ml/min/kg	8.5 ± 1.4	4.4 ± 0.9	$P < 0.005$
$V_{d(area)}$	l/kg	0.718 ± 0.161	0.332 ± 0.082	$P < 0.005$
$V_{1(central)}$	l/kg	0.337 ± 0.045	0.262 ± 0.058	$P < 0.05$
$V_{2(peripheral)}$	l/kg	0.373 ± 0.13	0.068 ± 0.025	$P < 0.005$

*Paired Student's t-test.

TABLE II. Pharmacokinetic parameters of intramuscular procaine penicillin G administration with (+) and without (−) oral PBZ (Trial III)

Parameter	Unit	−PBZ	+PBZ	Significance
B°	iu/ml	4.80 ± 2.68	4.49 ± 2.66	$P < 0.05$
$t_{1/2}$	h	10.0 ± 1.6	12.8 ± 5.1	NS
C_{max}	iu/ml	3.31 ± 1.56	4.18 ± 1.06	NS
t_{max}	h	4.6 ± 1.3	4.8 ± 2.7	NS
AUC_{0-12}	iu.h/ml	26.0 ± 10.2	34.2 ± 8.7	$P < 0.01$
AUC_{0-32}	iu.h/ml	39.9 ± 8.2	61.5 ± 17.7	$P < 0.025$
AUC_{0-72}	iu.h/ml	44.9 ± 5.7	73.6 ± 20.0	$P < 0.01$

TABLE III. Renal clearance (+SD) of unbound penicillin (ml/min/kg) after administration together with variable doses of PBZ given intravenously to two ponies, E and B (Trial I): *n* is the number of time intervals in which urine was collected and clearance calculated

Dose	Pony B	Pony E
Control (n = 7)	8.77 ± 3.75 (n = 8)	4.06 ± 1.98 (n = 8)
Low-dose PBZ	12.22 ± 7.51 (n = 6)	6.28 ± 3.96 (n = 6)
High-dose PBZ	7.98 ± 6.88 (n = 8)	7.78 ± 3.37 (n = 7)

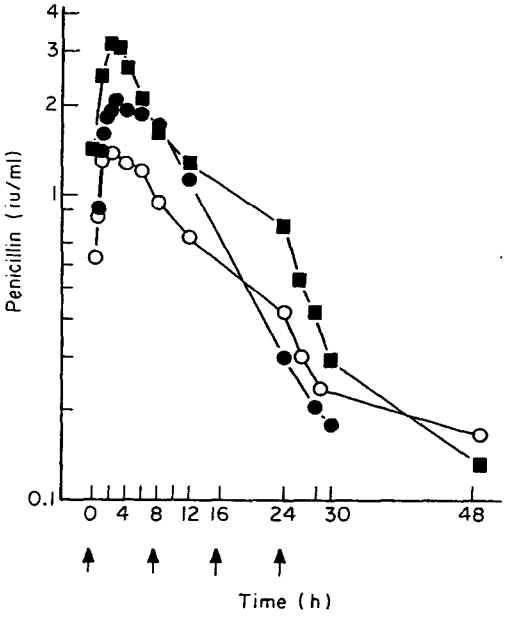

FIG. 1. Semilogarithmic mean plasma penicillin concentration curves of two ponies (Trial I) injected once intramuscularly with procaine penicillin G (20 000 iu/kg), after intravenous phenylbutazone at two different dosages: (○) control, i.e. no PBZ; (●) 0.8-mg/kg PBZ t.i.d.; (■) 2.2-mg/kg PBZ t.i.d.

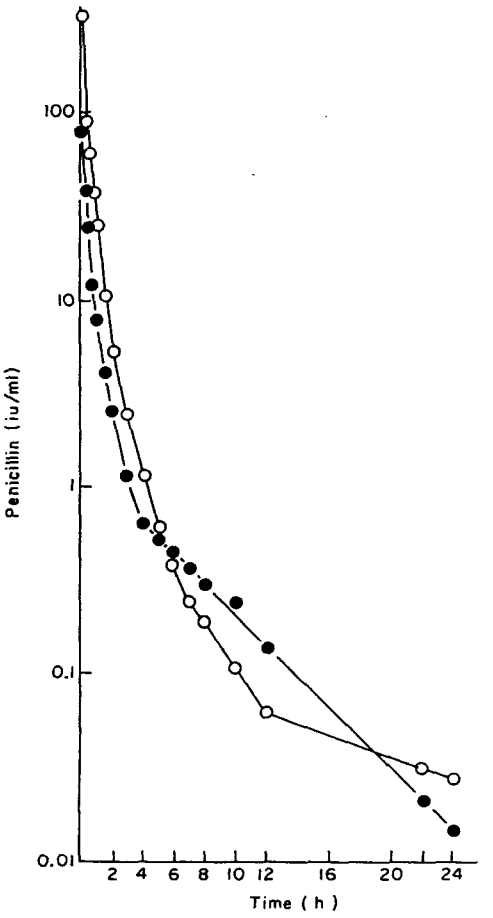

FIG. 2. Semilogarithmic mean serum penicillin concentration time curves of five horses (Trial II) administered intravenous sodium penicillin G (20 000 iu/kg), with (○) and without (●) concurrent intravenous administration of 10-mg/kg phenylbutazone.

appeared to be PBZ dose related. In Trial II, PBZ administration was associated with a significant increase in $t_{1/2}$ and AUC, and a decrease in Cl_b and $V_{d(area)}$ of penicillin. Further reference to Table I indicates that there were significant effects of PBZ on k_{12} and k_{21}. The penetration rate of penicillin into the tissues (k_{12}) was 8.2 times slower when PBZ was administered a few minutes before the sodium penicillin G, and the penicillin diffusion (k_{21}) out of the tissue was 1.86 times slower with PBZ treatment than in the control situation. In the control situation (i.e.

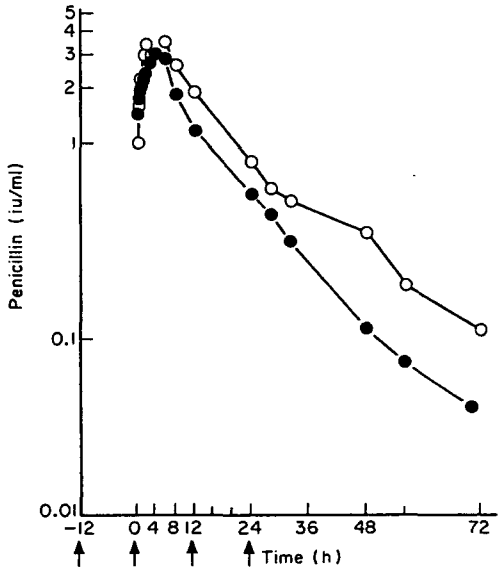

FIG. 3. Semilogarithmic mean serum penicillin concentration time curves of five horses (Trial III) injected once with intramuscular procaine penicillin G (20 000 iu/kg). Phenylbutazone (10 mg/kg) was administered orally on four occasions (↑), beginning 12 h before antibiotic injection: with PBZ (○); without PBZ (●).

sodium penicillin without PBZ), k_{12} and k_{21} were almost equal. However, with PBZ treatment, back diffusion exceeded diffusion into the tissues approximately fourfold, as is shown by the k_{12}:k_{21} ratio. There was a significantly lower $V_{d(area)}$ peripheral distribution (V_2) of penicillin associated with PBZ administration.

In Trial III there was a significant increase in *AUC* and B^0 associated with PBZ treatment. Table III indicates that in Trial I there were no significant differences in renal clearance of penicillin associated with concurrent PBZ administration, and Table IV indicates that in Trials II and III, concurrent PBZ treatment was not associated with significant differences in the degree of serum protein binding of penicillin.

DISCUSSION

Apart from the study of Sullins *et al.* (1984), no reference to the interaction of antibacterial agents and PBZ in the horse could be found in the recent literature. Information concerning other species is also not abundant. Zarfin *et al.* (1985) found a possible interaction between indomethacin and aminoglycosides in preterm infants. The increased peak-and-trough aminoglycoside concentrations were associated with the effect of indomethacin in reducing glomerular filtration rate, urine flow rate, free water clearance, and electrolyte excretion. In rabbits, indomethacin caused no change in protein binding of ceftazidime, but did reduce excretion of the antibiotic by decreasing the fraction cleared by glomerular filtration (Carbon *et al.*, 1984). The same authors found that PBZ did compete with another cephalosporin, cefazolin, for plasma protein binding sites *in vitro*, and that PBZ altered the extravascular disposition of this drug in rabbits: higher extravascular concentrations of the antibiotic were detected when PBZ was administered concurrently (Carbon *et al.*, 1981).

We used two ponies in a pilot study to

TABLE IV. Protein binding of penicillin (+SD) in 122 serum samples from Trials II and III

	−PBZ	+PBZ	Probability
Trial II intravenous (concentration range iu/ml)	57.3 ± 7.2 (n = 24) (0.08–18.4)	56.7 ± 7.6 (n = 58) (0.23–28.8)	NS
Trial III intramuscular (concentration range iu/ml)	63.8 ± 6.7 (n = 16) (0.087–2.89)	55.2 ± 9.0 (n = 24) (0.45–5.03)	NS

n = number of samples.

determine whether or not concurrent intravenous PBZ administration affected the disposition of intramuscularly injected procaine benzyl penicillin. The C_{max} and *AUC* appeared to be affected in a dose-dependent fashion. We then used a much higher dose of PBZ prior to intravenous sodium penicillin G (Trial II) to reduce the variability associated with absorption of intramuscularly administered penicillin, and to attempt to define more clearly the changes in the distribution of penicillin associated with PBZ. Finally, we investigated whether or not orally administered PBZ would also affect penicillin distribution (Trial III).

Our pharmacokinetic data indicate that penicillin concentrations were elevated in serum or plasma after administration of PBZ, when compared to concentrations achieved after penicillin injection without concurrent use of PBZ. There was considerable variation in the results, for instance in Trials I and III, and this may be due to the small number of animals used in the pilot study (Trial I). PBZ is thought to be absorbed onto food material (Maitho *et al.*, 1986), and considerable variation in absorption after oral administration has been previously demonstrated (Rose *et al.*, 1982; Sullivan & Snow, 1982; Maitho *et al.*, 1986); such an effect may account for the variation in Trial III. These latter authors also showed a delay in the attainment of peak PBZ concentrations after oral administration to non-fasted horses, and this may explain the continued elevation of serum penicillin concentrations after withdrawal of oral PBZ in our horses (Trial III), which had access to roughage at all times.

The elevated serum concentrations of penicillin were not due to differences in its renal clearance or protein binding, as shown in Trials I and III, respectively. The very small differences in t_{max} in Trial I, and the presence of clear differences in penicillin concentration after intravenous administration of penicillin (Trial II), indicate that differences in absorption of the drug are probably not the reason for the effect of PBZ. The explanation possibly lies in the relative change in the penetration and back diffusion rates of penicillin after PBZ injection. The predominant effect of PBZ was a dramatic decrease in penetration of penicillin into the tissues, and this resulted in the elevated intravascular penicillin concentrations measured and lower peripheral distribution calculated. The limitation of penicillin distribution caused the increased plasma *AUC* following PBZ treatments, and the mathematically derived parameters Cl_b (= Dose/*AUC*) and $V_{d(area)}$ (= Cl_b/β) were therefore diminished. The apparent discrepancy between a diminished total body clearance (Cl_b) and an unaltered renal penicillin clearance may be explained by the fact that the Cl_b is a mathematically derived parameter and renal clearance a measured parameter. The therapeutic consequences are that despite elevated plasma penicillin concentrations, limited tissue penetration may reduce the therapeutic efficacy. On the other hand, it may be anticipated that PBZ administration after the time of achievement of maximum tissue penicillin concentration (about 3–4 h) would induce prolongation of high tissue concentrations. In similar experiments conducted in dairy cows, a combination of PBZ and isopyrin did not affect the rate constants of drug transfer (k_{12}:k_{21} ratio) or V_1 (Hekman *et al.*, 1982). In calves, a reduced volume of distribution, lengthening of the elimination half-life, and reduced total body clearance were also found when PBZ was administered intravenously concurrently with sodium penicillin (Volner *et al.*, 1988).

The mechanism of the interaction reported in this study may be competition of two acidic drugs for the same cellular binding sites in the membrane transport process. Alternatively, because inhibition of a process by PBZ implies the involvement of mediators derived from the cyclo-oxygenase pathway in that process, penicillin transport across the cell membrane may be, at least partly, eicosanoid regulated. The other study conducted in horses on the effect of PBZ of penicillin (Sullins *et al.*, 1984) found no effect of PBZ. This may be accounted for by the lower dose (4.4 mg/kg) of PBZ, in the form of a paste, fed to unfasted horses. We used a higher dose rate, only slightly higher than the loading dose in a clinically effective and safe dose regimen (Taylor & Verrall, 1983) to demonstrate more clearly the nature of the effect. Our study indicates that plasma penicillin concentrations were higher when PBZ was given, among other possible reasons, because the volume of

distribution of penicillin was decreased. The strategic use of phenylbutazone in altering perfusion out of the tissues may be of therapeutic interest and warrants further study.

ACKNOWLEDGMENTS

The authors are particularly grateful to Messrs P. Schmaetz, K. Peperkamp and A. Klarenbeek for their assistance in the trials, and to Mr A. J. de Wit and his staff for care of the animals.

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