

Clinical signs and etiology of adverse reactions to procaine benzylpenicillin and sodium/potassium benzylpenicillin in horses

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Case reports of 59 horses reacting adversely to procaine benzylpenicillin or to sodium or potassium benzylpenicillin in Sweden in 2003–2005 were obtained through contacts with horse-owners. For the assessment of the reports, various parameters were evaluated, such as the times to the reactions, information on previous penicillin treatment, the clinical signs and the actions taken in the reacting horses. Among the reports, two horses had received sodium or potassium benzylpenicillin intravenously, whereas the remaining 57 horses had been treated with procaine benzylpenicillin intramuscularly. Allergy may underlie the adverse reactions in the horses given sodium and potassium benzylpenicillin, and in a few of the horses given procaine benzylpenicillin. However, in most horses in the latter group, the clinical signs may be due to the toxic effects of procaine. In these horses, the dominating clinical signs were locomotor and behavioral changes. Some risk factors may enhance the probability that horses react to procaine. One is repeated injections, which increase the likelihood of intravascular administration and also may increase the sensitivity to procaine due to neuronal sensitization (kindling). Procaine is rapidly hydrolyzed by plasma esterases to nontoxic metabolites. When high amounts of procaine enter the circulation, the hydrolyzing capacity may be exceeded and toxicity occurs. Analyses of plasma esterases from reacting horses showed lower activity than in nonreacting control horses. Low esterase activity may increase the possibility of procaine toxicity and constitute another risk factor.

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INTRODUCTION

Benzylpenicillin is the most frequently used penicillin in equine therapy. It is used as highly water-soluble sodium or potassium salts or as a procaine salt with low water-solubility. The sodium and potassium salts are usually injected intravenously (i.v.), whereas procaine salt is used as an intramuscular (i.m.) depot preparation. The latter must be dissolved into free procaine and benzylpenicillin before absorption in the blood, implying that therapeutic benzylpenicillin-concentrations will be maintained for at least 24 h (Uboh *et al.*, 2000).

Penicillin has a wide safety margin, and is usually well tolerated. Yet, adverse reactions are sometimes observed. Thus, it is well known that acute adverse reactions may occur following

i.v. or i.m. injections of benzylpenicillin in horses. Penicillin, or its metabolites, have an affinity to proteins and may form hapten conjugates, which participate in immunological reactions. In human medicine, hypersensitivity is the most common cause of negative reactions to penicillin. Hypersensitivity reactions to penicillin have been reported to be rare in veterinary medicine, although their number and occurrence are not known in detail (Wilcke, 1986; Davis, 1987).

As regards procaine benzylpenicillin, additional mechanism may contribute to toxicity. Thus, inadvertent intravascular administration of the procaine benzylpenicillin may result in a rapid dissociation of the salt into procaine and benzylpenicillin when diluted in the large blood volume. The clinical signs may then be due to an effect of free procaine on the central nervous

system (CNS). Procaine benzylpenicillin was previously used to a considerable extent in human medicine. Acute adverse reactions were described in connection with such treatments (Hoigné's syndrome). The patients suddenly expressed extreme fear and had hallucinations and muscle tremors, but there was no blood pressure decrease or circulatory collapse. The clinical signs appeared during or immediately following the administration of procaine benzylpenicillin and are probably caused by inadvertent intravascular penetration from the muscular injection site (Björnberg & Selstam, 1960; Downham *et al.*, 1978; Schreiber & Krieg, 2001).

Following entry into the systemic circulation, procaine is hydrolyzed by plasma esterases to the nontoxic metabolites *para*-aminobenzoic acid (PABA) and diethylaminoethanol (Tobin *et al.*, 1976). It has been reported that a group of human patients who experienced systemic toxic reactions to procaine benzylpenicillin had significantly lower plasma esterase activity compared with a group of nonreacting patients. It was proposed that procaine toxicity may occur when the rate of absorption exceeds the hydrolyzing capacity of the plasma esterases (Downham *et al.*, 1978).

Although the acute reactions to benzylpenicillin in horses are well known there are only a few reports in the literature which describe these events. Most reports concern adverse reactions to procaine benzylpenicillin (Marshall, 1980; Allpress & Heathcote, 1986; Nielsen *et al.*, 1988; Tjälve, 1997). There are also a few reports of anaphylaxis and death in horses treated with water-soluble salts of benzylpenicillin (Tjälve, 1991, 1997).

One aim of the present investigation was to collect and describe case reports in horses reacting acutely to benzylpenicillin in Sweden in 2003–2005. Various parameters were evaluated, such as the time to the reactions, the clinical signs and the actions taken in the reacting horses. Another aim was to assess if the horses reacting acutely to procaine benzylpenicillin have lower plasma esterase activity compared with nonreacting control horses.

MATERIALS AND METHODS

Horses

To assess the problem with adverse reactions to benzylpenicillin in horses in Sweden, we searched for horses with a history of an adverse reaction to benzylpenicillin within a 6-month period prior to the study. The horses were found via advertisements in horse magazines and in the research home page of the Swedish University of Agricultural Sciences (<http://hippocampus.slu.se>). Fifty-nine clinical cases were retrieved. The horses ranged in age from 8 months to 29 years, and comprised 34 mares and 25 geldings. Within this group it was possible to get blood samples of 29 horses (14 mares and 15 geldings aged 2–24 years), which had shown adverse reactions to procaine benzylpenicillin. A prerequisite for inclusion in this part of the study was that the reactions had appeared immediately after or during the injections. Seventeen horses

(eight mares and nine geldings aged 2–20 years) which had been treated with procaine benzylpenicillin without problem were used as controls.

Sample collection

Blood samples were taken from a jugular vein and collected into heparin vacutainer tubes and placed in an ice bucket. They were centrifuged at 1500 *g* at +4 °C for 15 min and the plasma was collected and stored at –70 °C until analyses. The study protocol was approved by the Local Ethics Committee for Animal Experiments, Uppsala, Sweden.

Chemicals and reagents

All chemicals (including PABA and procaine from Sigma-Aldrich Sweden AB, Tyresö, Sweden) were of the reagent grade and all solvents were of the high-performance liquid chromatography (HPLC) grade.

Procaine hydrolysis in equine plasma

To measure the esterase activity, horse plasma was incubated with procaine (20 µg/mL of plasma) in a water bath at 37 °C for 12 min, and the amount of PABA formed was measured by HPLC.

Samples were then taken and deproteinised with acetonitrile and centrifuged at 12 000 *g* for 15 min. For determination of PABA, 20 µL of the supernatant were injected onto the HPLC. The endogenous PABA-levels in the plasma were determined separately and subtracted from the incubated samples.

To evaluate the linearity of the reaction and optimize the method in relation to incubation period and procaine concentration, the amount of procaine and the incubation period were varied from 1 to 60 µg/mL and 1 to 90 min, respectively. The stability of the esterases in plasma was evaluated in samples stored at +4 °C and at –70 °C. There was no decrease in activity at used storing periods. Previous studies in our laboratory have shown that there is no marked variation in the esterase activity depending on the season of the year or the time of the day.

Analysis of PABA in horse plasma

High-performance liquid chromatography was performed on a Beckman System Gold Module 126 (Beckman Instruments Inc., San Ramon, CA, USA), equipped with a spectrometric detector (Shimadzu SPD-2A Shimadzu Corporation, Kyoto, Japan) and an Ultra Sphere column (250 × 4.6 mm, 5 µm particles Beckman Instruments Inc). The detection wavelength was set at 300 nm, the mobile phase was 0.01% trifluoroacetic acid in acetonitrile:water (1:9) and the flow rate was 1 mL/min. The method, with some adjustments, was adopted from Murtaza *et al.*, 2001.

Plasma samples spiked with PABA were used as standards. The standard curve was calculated using the least-square linear regression. The relative concentration residual for quality

control, using samples containing 1, 2, 4, 8, 16 and 32 µg/mL ranged between 0.4% and 9.3%.

Data analysis

Median and range or mean and standard deviation (SD) values were calculated for all investigated parameters. All plasma data were tested for normality with the Anderson–Darling Normality Test before further analyses ($P = 0.51$). The plasma data were subjected to statistical analyses using Student's *t*-test. The software was Minitab®, release 14 (Minitab Ltd, Coventry, UK).

RESULTS

Case reports

Reports of adverse reactions to benzylpenicillin were obtained from 59 horses. Among these two horses had received water-soluble benzylpenicillin i.v. – one potassium benzylpenicillin (Novocillin® vet.; Boehringer Ingelheim Vetmedica, Malmö, Sweden; 320 mg/mL); the other sodium benzylpenicillin (Geopenil vet.; Orion Pharma AB Animal Health, Sollentuna, Sweden; 300 mg/mL). All the remaining 57 horses had been treated i.m. with procaine benzylpenicillin (300 mg/mL). Two formulations of procaine benzylpenicillin had been used: Ethacillin® vet. (Intervet AB, Stockholm, Sweden) (17 horses) and Penovet vet. (Boehringer Ingelheim Vetmedica) (40 horses). The most common prescription of procaine benzylpenicillin was a course of daily injections of 20 mg/kg body weight for 5–7 days.

Most reports were obtained for Warmblood Riding Horse followed by English Thoroughbred, Standardbred Trotter and Pony (Fig. 1). It appears that the percentage of reacting horses in relation to the distribution of the horse population in Sweden is highest for Warmblood Riding Horse and English Thoroughbred. The benzylpenicillin was administered to the horses according to

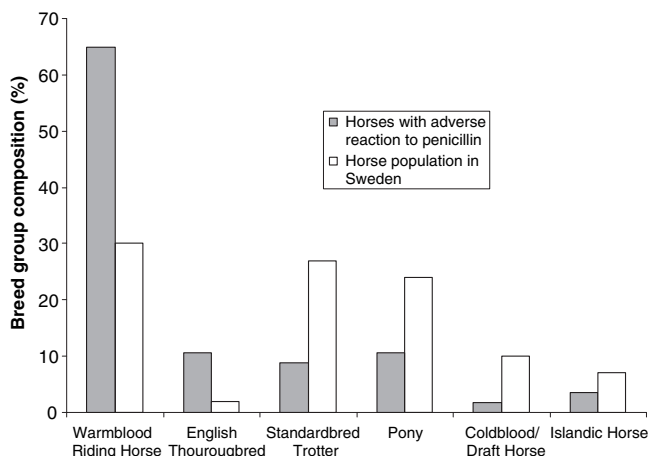


Fig. 1. Breed group composition (%) of 59 horses with adverse reactions to procaine benzylpenicillin or sodium/potassium benzylpenicillin. The breed group composition (%) of the horse population in Sweden is also shown.

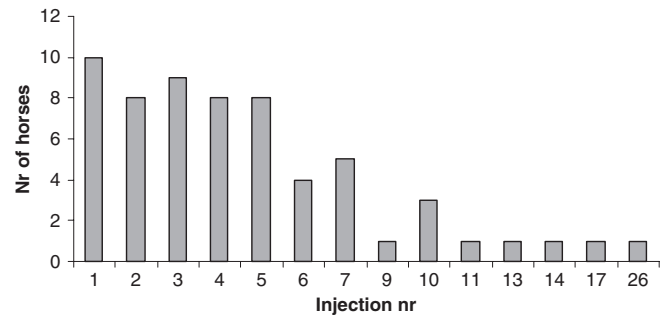


Fig. 2. Number of injections to the appearance of the adverse reactions in 59 horses treated with procaine benzylpenicillin or sodium/potassium benzylpenicillin. The courses of benzylpenicillin treatments are generally 5–7 days.

veterinary instructions to treat a range of infections. The number of injections prior to the adverse reactions varied from 1 to 26 (Fig. 2). Most reactions occurred on days 1–7.

An overview of the clinical signs, the time to reaction, previous penicillin treatment and actions taken are presented in Table 1. The horse given potassium benzylpenicillin developed urticaria, whereas the one given sodium benzylpenicillin became recumbent, struggled and showed dyspnea. Both these horses were Warmblood Riding Horses and the adverse reactions occurred after the first injections of the treatment courses. Both horses recovered within 24 h. None of these horses received continued treatment with benzylpenicillin.

Among the 57 horses given procaine benzylpenicillin, two developed skin reactions: one had rash and swollen neck and legs; the other had palm-sized watery spots with itch and dandruff at the injection sites. The remaining 55 horses showed various CNS clinical signs. These initially included startled behavior, sudden backing and rearing, loss of coordination and muscle tremors. Some horses were groaning, gasping and snorting. Additional clinical signs included staggering, sagging, tachypnea, seizures, nystagmus, tenseness, sweating and tachycardia. Several horses became frightened and showed evidence of terror, described by the horse-owner as fearfulness (e.g. as if they had seen a ghost or a snake). Others were described as being transiently blind. A number of horses became recumbent after showing initial in-coordination. One horse fell to the ground, galloped while lying down and died after approximately 20 min. The other horses recovered from the acute reaction within periods ranging from a few minutes to about 1 h. Several horses showed behavioral changes and fearness (e.g. sensitivity to sound and light) for one or few days. In one horses, these clinical signs were seen as long as 2 weeks after the acute reaction.

Fifty-three of the horses injected with procaine benzylpenicillin showed adverse reactions during the injections or within approximately 1 min after the injections. Of the remaining horses within this group, one horse was found in the box after 15 min and another showed a reaction which started 25 min after the injection. In the two horses showing skin reactions the clinical signs appeared at the end of the treatment periods.

In nine horses marked swelling and pain had been observed at the sites of the injections prior to acute adverse reactions.

Table 1. Overview of clinical signs, time to reaction, previous penicillin treatment and actions taken in 59 horses with adverse reactions to potassium or sodium benzylpenicillin or to procaine benzylpenicillin. In the horses reacting to procaine benzylpenicillin the clinical signs have been ordered in relation to the frequency at which they were observed. Individual horses may have shown more than one of the indicated clinical signs

Substance	Time to reaction	Previous penicillin treatment	Clinical signs	Actions taken
Potassium benzylpenicillin (one horse)	During the injection	Unknown	Urticaria	Corticosteroid treatment: no additional antimicrobial treatment
Sodium benzylpenicillin (one horse)	After approximately 3 min	Previous treatment with procaine benzylpenicillin	Recumbency, struggling and dyspnea	Fluid supplementation; new treatment with trimethoprim-sulfadiazine and enrofloxacin
Procaine benzylpenicillin (57 horses)	During the injection: 12 horses Within approximately 1 min: 41 horses	Previous treatment with procaine benzylpenicillin: 27 horses Previous treatment with potassium or sodium benzylpenicillin: four horses No previous treatment: 12 horses Unknown: 10 horses	Galloping, striding and/or crashing into stable walls: 45 horses Restlessness and/or being absent-minded: 29 horses Sudden backing, rearing and kicking: 25 horses Ataxia, loss of coordination, staggering and/or sagging: 16 horses Recumbency: 16 horses Startled behavior: 13 horses Fear and evidence of terror: 12 horses Gasping, groaning and/or snorting: 11 horses Tachypnea: 11 horses Seizures: 10 horses Nystagmus: eight horses Tenseless: seven horses Tremor: seven horses Appearing blind: five horses Sweating: four horses Tachycardia: two horses Death: one horse	Continued treatment with procaine benzylpenicillin: five horses; among these three horses showed new reactions, whereas two horses showed no reactions Interruption of treatment with procaine benzylpenicillin: 48 horses; among these new treatment was performed with sodium benzylpenicillin in five horses and with trimethoprim-sulfadiazine in 12 horses; none of these horses showed any further reactions Note: several horses in the procaine benzylpenicillin group were in addition treated for injuries caused during the acute reactions
	One horse was found in the box after 15 min One horse showed a reaction after approximately 25 min	Unknown	Sagging, nystagmus, bradycardia and hypotension Tachycardia, staggering and restlessness	Interruption of treatment
	In two horses showing skin reactions (only) the clinical signs appeared after a few days treatment	Previous treatment with procaine benzylpenicillin	One of these horses had rash and swollen neck and legs; the other had palm-sized watery spots with itch and dandruff at the injection sites	Interruption of treatment In these two horses continued treatment was carried out with procaine benzylpenicillin

In seven horses showing acute adverse reactions the treating person observed that there was some blood leakage when the needle was retracted from the injection site, in spite of the fact that the plunger had been drawn back before the dose was given.

It appears from Table 1 that twelve of the horses reacting acutely to procaine benzylpenicillin had not been treated previously with penicillin, and that in eleven horses it was unknown if previous penicillin treatment had occurred. Among the remaining horses, 30 had previously been treated with procaine benzylpenicillin and four with sodium or potassium benzylpenicillin. Among these horses two had previously presented skin reactions and one a previous minor nervous reaction.

In 50 of the horses reacting to procaine benzylpenicillin the treatment course with this drug was interrupted. Five of these horses, instead, received sodium benzylpenicillin (Geepenil vet.) i.v., whereas 12 were treated orally with trimethoprim-sulfadiazine (Hippotrim vet. Bayer AB, Göteborg, Sweden). None of these horses showed any further reaction. In seven horses, the procaine benzylpenicillin course was continued after the first reaction. Two of these horses showed no further adverse reactions during the treatment course, whereas in three of the horses new adverse reactions occurred 1, 2 and 3 days later, respectively. In the two horses showing skin reactions the treatment course was continued despite the clinical signs. Following a therapy-free interval of 6 weeks, one horse had a new course of procaine benzylpenicillin without signs of adverse reactions. One horse which had a new course of procaine benzylpenicillin following a therapy-free interval of 5 weeks developed abscesses at the sites of the injections.

Esterase activity in plasma

Determination of the amount of PABA formed at the procaine hydrolysis in the group of reacting horses was $7.13 \pm 1.54 \mu\text{g/mL}$ (mean \pm SD), whereas in the control group the amount of PABA formed was $8.31 \pm 1.22 \mu\text{g/mL}$. The difference between the two groups was statistically significant ($P = 0.003$) (Fig. 3).

DISCUSSION

In the present compilation of clinical case reports, two concern horses injected i.v. with water-soluble sodium or potassium benzylpenicillin, whereas 57 concern horses injected i.m. with water-insoluble procaine benzylpenicillin. The dominance of reactions to procaine benzylpenicillin over the water-soluble salts of benzylpenicillin correlates with previous reports in the literature (Marshall, 1980; Allpress & Heathcote, 1986; Nielsen *et al.*, 1988; Tjälve, 1997). In Sweden 25 750 L of procaine benzylpenicillin (Ethacillin[®] vet. and Penovet vet.) were sold during 2003–2005, whereas 2774 L of potassium and sodium benzylpenicillin (Novocillin[®] vet. and Geepenil vet.) were sold during the same period. These benzylpenicillin-preparations are, in addition to horse, registered for other species, such as cattle and swine, and the proportions of these drugs used in horses and

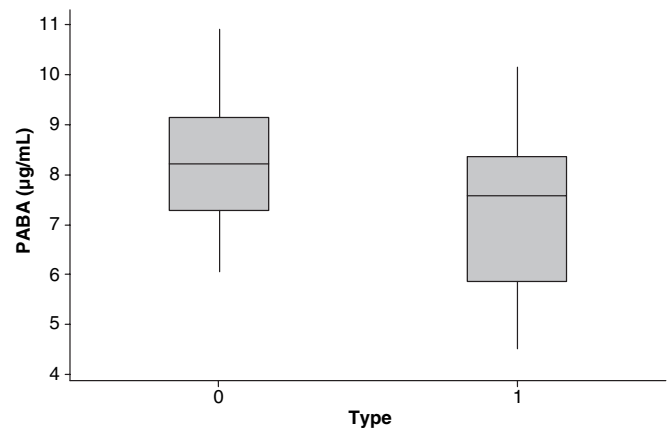


Fig. 3. Formation of PABA ($\mu\text{g/mL}$ plasma) from procaine by esterases in horse plasma. Plasma-samples from horses having shown acute reactions to procaine benzylpenicillin and from nonreacting control horses were incubated with procaine for 12 min at 37°C . The boxes represent the middle half of the data with whiskers extending to the extreme values and the lines inside the boxes representing the medians. The values differ significantly (Student's *t*-test, $P = 0.003$). 0, control horses ($n = 17$), 1, horses which have shown adverse reactions to procaine benzylpenicillin ($n = 29$).

in the other species are unknown. Therefore, it is not possible to calculate the relative incidence of the adverse reactions in horse (i.e. the number of reactions in relation to the number of treated horses).

Apart from anaphylaxis and milder hypersensitivity reactions the water-soluble benzylpenicillin salts are remarkably free of negative effects even at doses grossly in excess of those recommended. Thus, the negative effects in horses given i.v. injections of water-soluble benzylpenicillin salts are probably due to hypersensitivity reactions. The manifestations of immediate hypersensitivity are urticaria, angioedema and anaphylaxis. The clinical signs associated with anaphylaxis in horses include systemic hypotension and circulatory collapse and varying degrees of bronchoconstriction, leading to dyspnea (Wilcke, 1986; Davis, 1987).

In our study, one of the horses given water-soluble benzylpenicillin showed urticaria. The other horse fell to the ground, struggled and had dyspnea. There are a few reports in the literature of anaphylaxis and death in horses treated with water-soluble salts of benzylpenicillin, reactions which thus can be assumed to have an allergic origin. Clinical signs reported in such horses include ataxia, recumbency and seizures; autopsies have shown acute circulatory failure (Tjälve, 1991, 1997).

It is likely that the adverse reactions in the horses given i.m. injections of procaine benzylpenicillin can be mediated by more than one mechanism. Allergy-related reactions may account for some cases. This may apply to the horse indicated in Table 1, which after 25 min reacted with tachycardia, staggering and restlessness, and to the two horses, which showed skin reactions. However, it is likely that most of the reactions to procaine benzylpenicillin are due to causes other than anaphylaxis. Several horses showing reactions to procaine benzylpenicillin received continued treatment with the same drug or with a

water-soluble benzylpenicillin salt without problems. It appears that penicillin allergy can be excluded in these cases.

In a majority of the horses given procaine benzylpenicillin, the dominating clinical signs were locomotor and behavioral changes, which occurred during the injections or within 1 min after the injections. The clinical signs observed in these horses include a spectrum of different CNS-related manifestations, which fit into those expected at procaine toxicity. This was also concluded by Nielsen *et al.* (1988), who analyzed the clinical signs in 11 horses reacting to procaine benzylpenicillin. The recommended dose of procaine benzylpenicillin for horses is 20 mg/kg b.w. This corresponds to a procaine-dose of 8.3 mg/kg b.w. Experimental studies have shown that i.v. injections of procaine in horses at doses of 2.5–10 mg/kg b.w. give similar neurological signs as those observed in the horses in the present study (Tobin *et al.*, 1977a; Chapman *et al.*, 1992).

It can be assumed that for procaine to exert its toxic effects it must enter the circulation rapidly after the i.m. administration of procaine benzylpenicillin. This can be achieved by accidental intravascular administration. It has been proposed that increased vascularization may take place at the sites of the injection following repeated injections of procaine benzylpenicillin and that this might increase the likelihood for inadvertent intravascular injection (Nielsen *et al.*, 1988). Thus, repeated injections at the same site may lead to a highly vascularized granulomatous inflammation. Muscle soreness is common after repeated injection of procaine benzylpenicillin and several of the horses in our clinical case collection showed evidence of this. In several cases in our study blood was seen at the injection site.

Horses are more sensitive to procaine than humans (Tobin *et al.*, 1977a). This implies that some horses may be highly responsive to elevated plasma concentrations of procaine. Following systemic administration, procaine is a relatively specific stimulant of the limbic system, which in man can result in a range of emotional and somatic clinical signs similar to those observed in Hoigné's syndrome (Servan-Schreiber *et al.*, 1998). It is known that the limbic system is susceptible to neuronal sensitization, implying that there is a gradually increasing response after repetitive stimulation over time of sub threshold stimuli, which initially are without effects (kindling) (Adinoff *et al.*, 2001). It has been proposed that a kindling mechanism, related to repeated injections of procaine benzylpenicillin, may increase the risk for Hoigné's syndrome (Araszkiewicz & Rybakowski, 1996–1997). It is possible that a kindling mechanism may increase the susceptibility to procaine benzylpenicillin also in horse. In the present study, 30 of the horses reacting to procaine benzylpenicillin had previously been treated with the same drug. Procaine is a local anesthetic and it has been proposed that the mechanism of kindling may involve cross sensitization to other local anesthetics (Araszkiewicz & Rybakowski, 1996–1997). It is possible that anesthesia with, e.g. lidocaine in connection with previous surgical therapy or clinical examination for lameness might increase the sensitivity to procaine benzylpenicillin.

The present study showed that the group of horses suffering from adverse reactions to procaine benzylpenicillin had a

significantly lower procaine hydrolyzing capacity compared with the control group. There were large individual variations in the plasma esterase activity, but reduced plasma-esterase activity may be a factor which increases the likelihood of reaction by delaying the hydrolysis of procaine.

Our results showed that calculation of the percentage of horses reacting to procaine benzylpenicillin in relation to the horse population in Sweden gives an over-representation for Warmblood Riding Horse and English Thoroughbred. This could be attributed to the sampling method (i.e. owners to riding horses may read and answer to our advertisements to a higher extent than other horse owners). Alternatively, some genetic variations within the horse population might contribute to high procaine sensitivity. As regards the plasma esterases, it is known that esterases in plasma hydrolyzing procaine also hydrolyze the muscle relaxant succinylcholine. It is well known that among humans some individuals have atypical forms of plasma esterases, which implies that succinylcholine causes prolonged muscle relaxation and apnea (Li *et al.*, 2005). It is not known whether a similar variation in plasma esterase activity may exist between different horses and/or between different breeds of horses, but individuals with atypical plasma esterases might be highly sensitive to procaine. Further studies are needed to explore this issue.

It has been proposed that inadvertent injection of a large dose of procaine benzylpenicillin may lead to vascular emboli, resulting in pulmonary embolic shock and that this may be one explanation for the acute reactions to this drug. However, severe respiratory distress was not a prominent clinical sign among the horses of our study. In addition, pulmonary embolism would have been expected to result in residual respiratory clinical signs following recovery and neither was this seen in our study. We assume that procaine benzylpenicillin is rapidly dissociated following inadvertent intravascular injection. This assumption is supported by the results of Tobin *et al.* (1977b)), which indicate that procaine benzylpenicillin is rapidly dissociated into procaine and benzylpenicillin in equine blood *in vivo* as well as under various *in vitro* conditions. In addition, in a study by Chapman *et al.* (1992) one horse which was injected i.v. with procaine benzylpenicillin responded with locomotor and behavioral changes consistent with the excitatory effects of procaine. There was no clinical or pathological evidence of possible microemboli lodging in the lungs.

A collection of clinical case reports sent to the Swedish Medical Products Agency by Swedish veterinarians during 1987–2005 has shown seven reported adverse reactions to water-soluble benzylpenicillin salts and 80 adverse reactions to procaine benzylpenicillin (L. Olsén & H. Tjälve, unpublished data). These data confirm that reactions to procaine benzylpenicillin are more common than reactions to water-soluble benzylpenicillin salts. However, among the reports to the Swedish Medical Products Agency there were 24 deaths, compared with only one death in the present study. It is probable that the reports to the authority constitute a selection of the most serious cases, whereas in the present collection information on a higher proportion of less serious cases has been achieved.

In conclusion, several mechanisms may contribute to the adverse reactions of procaine benzylpenicillin in horse. Low plasma esterase activity may increase the likelihood of procaine toxicity and constitute one risk factor.

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REFERENCES

- Adinoff, B., Devous, M.D., Best, S.M., George, M.S., Alexander, D. & Payne, K. (2001) Limbic responsiveness to procaine in cocaine-addicted subjects. *The American Journal of Psychiatry*, **158**, 390–398.
- Allpress, R.G. & Heathcote, R. (1986) Adverse reactions in the horse to intramuscular penicillin. *The Veterinary Record*, **116**, 411–412.
- Araszkievicz, A. & Rybakowski, J.K. (1996–1997) Hoigne's syndrome, kindling, and panic disorder. *Depression and Anxiety*, **4**, 139–143.
- Björnberg, A. & Selstam, J. (1960) Acute psychotic reaction after injection of procaine penicillin. *Acta Psychiatrica Scandinavica*, **35**, 129–139.
- Chapman, C.B., Courage, P., Nielsen, I.L., Sitaram, B.R. & Huntington, P.J. (1992) The role of procaine in adverse reactions to procaine penicillin in horses. *Australian Veterinary Journal*, **69**, 129–133.
- Davis, L.E. (1987) Adverse drug reactions in the horse. *The Veterinary Clinics of North America. Equine Practice*, **3**, 153–179.
- Downham, T.F., Cawley, R.A., Salley, S.O. & Dal Santo, G. (1978) Systemic toxic reactions to procaine penicillin G. *Sexually Transmitted Diseases*, **5**, 4–9.
- Li, B., Sedlacek, M., Manoharan, I., Boopathy, R., Duysen, E.G., Masson, P. & Lockridge, O. (2005) Butyrylcholinesterase, paraoxonase, and albumin esterase, but not carboxylesterase, are present in human plasma. *Biochemical Pharmacology*, **70**, 1673–1684.
- Marshall, A.B. (1980) Penicillin: suspected adverse reaction. *The Veterinary Record*, **106**, 207–208.
- Murtaza, R., Jackman, H., Alexander, B., Lleshi-Tali, A., Winnie, A. & Iqic, R. (2001) Simultaneous determination of mepivacaine, tetracaine and *p*-butylaminobenzoic acid by high-performance liquid chromatography. *Journal of Pharmacological and Toxicological Methods*, **46**, 131–136.
- Nielsen, I.L., Jacobs, K.A., Huntington, P.J., Chapman, C.B. & Loyd, K.C. (1988) Adverse reaction to procaine penicillin G in horses. *Australian Veterinary Journal*, **65**, 181–184.
- Schreiber, W. & Krieg, J.C. (2001) Hoigne syndrome. Case report and current literature review. *Der Nervenarzt*, **72**, 546–548.
- Servan-Schreiber, D., Perlstein, W.M., Cohen, J.D. & Mintun, M. (1998) Selective pharmacological activation of limbic structures in human volunteers: a positron emission tomography study. *The Journal of Neuropsychiatry and Clinical Neurosciences*, **10**, 148–159.
- Tjälve, H. (1991) Adverse reactions to veterinary drugs reported in Sweden during 1990. *Svensk Veterinärtidning*, **43**, 626–629 [in Swedish].
- Tjälve, H. (1997) Adverse reactions to veterinary drugs reported in Sweden during 1991–1995. *Journal of Veterinary Pharmacology and Therapeutics*, **20**, 105–110.
- Tobin, T., Blake, J.W., Sturma, L. & Arnett, S. (1976) Pharmacology of procaine in the horse: procaine esterase properties of equine plasma and synovial fluid. *American Journal of Veterinary Research*, **37**, 1165–1170.
- Tobin, T., Blake, J.W., Sturma, L., Arnett, S. & Truelove, J. (1977a) Pharmacology of procaine in the horse: pharmacokinetics and behavioural effects. *American Journal of Veterinary Research*, **38**, 637–647.
- Tobin, T., Tai, C.Y., O'Leary, J., Sturma, L. & Arnett, S. (1977b) Pharmacology of procaine in the horse: evidence against the existence of a 'procaine–penicillin' complex. *American Journal of Veterinary Research*, **38**, 437–442.
- Uboh, C.E., Soma, L.R., Luo, Y., McNamara, E., Fennel, M.A., May, L., Telesis, L.M., Rudy, J.A. & Watson, A.O. (2000) Pharmacokinetics of penicillin G procaine vs. penicillin G potassium and procaine hydrochloride in horses. *American Journal of Veterinary Research*, **61**, 811–815.
- Wilcke, J.R. (1986) *Allergic Drug Reactions. Current Veterinary Therapy IX: Small Animal Practice*, pp. 444–448. W.B. Sanders Company, London.