Appendix F

Thresholds For High Potency Drugs: Plasma or Urinary Thresholds?

Thomas Tobin, J. Daniel Harkins, Scott D. Stanley, and George D. Mundy
Maxwell H. Gluck Equine Research Center
The Department of Veterinary Science
University of Kentucky
Lexington, KY 40506

Truesdail Laboratories
Tustin, California
The Kentucky Racing Commission
4063 Iron Works Pike, Lexington, Ky 40511

Published as #198 from the Equine Pharmacology and Experimental Therapeutics Program at the Maxwell H. Gluck Equine Research Center and the Department of Veterinary Science, University of Kentucky.

Published as Kentucky Agricultural Experiment Station Article with the approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station.

Supported by grants entitled "Development of a test for procaine in horses" and "Thresholds and clearance times for therapeutic medications in horses" funded by The Equine Drug Council and The Kentucky Racing Commission, Lexington, KY and by research support from the National Office of the Horsemen's Benevolent and Protective Association, New Orleans, LA.

Send correspondence to:

J. Daniel Harkins
108 Gluck Equine Research Center
Dept. of Veterinary Science
University of Kentucky
Lexington, KY 40506-0099
Telephone: (606) 257-4173
Fax: (606) 257-5169

SUMMARY

Administration of 80 mg of procaine HCl subcutaneously produced plasma procaine concentrations of about 9 ppb at one hour after dosing. This concentration approaches the detection limit for the analytical method we used. We next determined the highest no-effect dose (HNED) for the local anesthetic (LA) effect of procaine: this dose is about 2.5 mg/site, a 32 fold smaller dose than our originally selected dose of procaine. This low HNED for procaine effectively precludes blood from being a satisfactory biological fluid in which to monitor quantitative thresholds for this drug.

We therefore determined the concentrations of procaine found in equine urine after subcutaneous administration of a 5 mg dose of procaine HCl to standing horses. The peak urinary concentration of procaine was about 25 ng/ml at four hours after drug administration. This concentration is therefore the maximum urinary concentration of procaine likely to be associated with no pharmacological effect of this agent. Since this threshold was determined in basic urines (mean pH 8.3), this threshold applies only to

urines of about this pH.

Preliminary investigations directed at determining the concentrations of procaine likely to be found in acidic post race urines yielded equivocal results.

INTRODUCTION

Procaine is one of the most commonly detected drugs in post-race urine samples[1]. Many of these identifications appear to be inadvertent, resulting from therapeutic administration of procaine as procaine penicillin. Procaine penicillin is a legitimate therapeutic agent widely used by veterinarians and is well known to persist in urine for longer periods (up to 30 days) after the last administration of procaine penicillin.

Inadvertent identifications of procaine from procaine penicillin are a major problem for equine veterinarians, horsemen and regulatory officials. These identifications are also a public relations problem for the industry. This is because the popular press distinguishes poorly between innocuous identifications of legitimate therapeutic agents such as procaine and identifications of illegal agents such as cocaine.

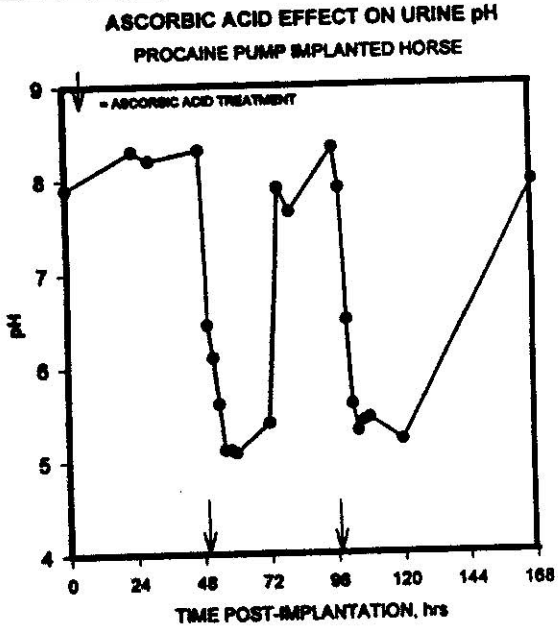
One proposed approach to the problem of trace residues of legitimate therapeutic medications is to identify concentrations of these agents unlikely to be associated with a pharmacological effect of these agents. These concentrations then become "No Effect Thresholds" and concentrations of therapeutic agents below these "No Effect Threshold" concentrations are of no forensic significance[2]. In this report we describe studies aimed at identifying such a "No Effect Threshold" for procaine in racing horses.

MATERIALS AND METHODS

Horses:

Experimental horses were drawn from a pool of about 15 Thoroughbred mares weighing 413 to 602 kg. All mares were kept at grass and brought into the experimental stalls 24 hours prior to each experiment. All drug solutions and control solutions were administered in sterile solution by intravenous (IV) or subcutaneous (SQ) injection. Blood samples were collected by venipuncture from the opposite jugular vein and all urine samples were collected by bladder catheterization.

Figure 1 *Changes in urinary pH following oral administration of 1 kg ascorbic acid at 50 and 98 hr.:*



Urinary Acidification

Ascorbic acid was infused intravenously over a 30 min period to mimic the brief (1-2 hour) decline in urinary pH seen after strenuous exercise in horses. Different concentrations (100, 300, and 400 g) of ascorbic acid were infused to rapidly reduce urinary pH. Following intravenous infusion of 300 g ascorbic acid, the pH decreased to ~5.5 within 2 hours and then began to rise toward control values.

Osmotic Pumps:

An osmotic pump (Alzet, Model 2M11, Palo Alto, CA) was used for continuous administration of procaine HCl for 1 week. The osmotic pumps were sterile spheres 1.3 cm in diameter and 4.5 cm in length that can hold 2 ml of solution.

The hair on the anterior chest was clipped, the skin was scrubbed with Betadine scrub and washed with alcohol following routine surgical procedures. The skin was anesthetized with 2% Procaine HCl and a 1 cm incision was made through the skin. Blunt dissection was used to open up a small area under the skin for placement of the osmotic pump. The skin

Figure 2 *Urinary procaine concentrations from implanted infusion pump following oral administration of 1 mg ascorbic acid at 58 and 90 hours:*

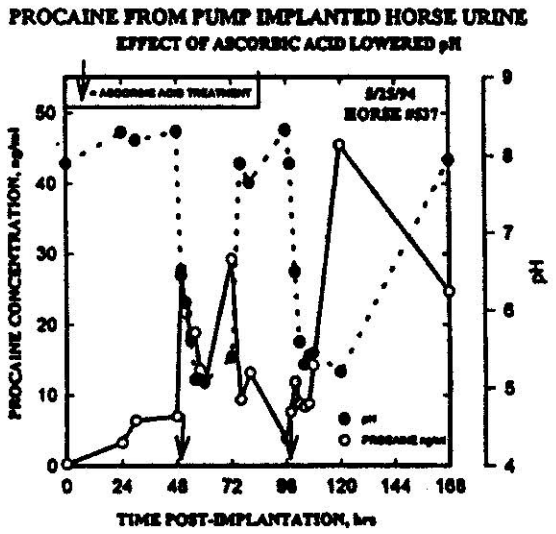
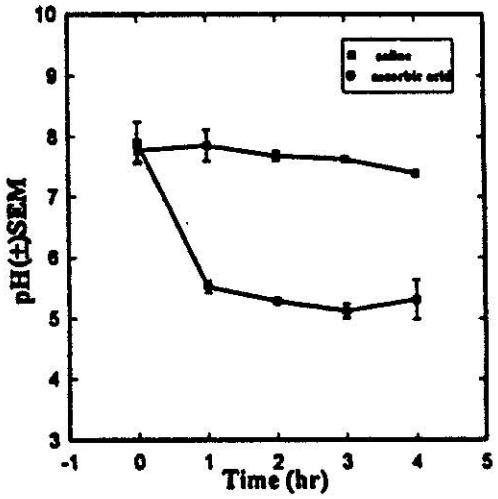


Figure 3 *Induced acidosis in 4 horses following intravenous infusion of 300 mg ascorbic acid dissolved in 2 liters sterile water or a matching volume of normal saline:*



was closed with 1-2 sutures.

Analytical methods:

The samples (4ml/sample urine or plasma) were placed in screw-top culture tubes. To each urine sample, standard and blank was added 4ml 50% ammonium hydroxide and 5ml dichloromethane (DCM), and a known amount of procainamide (20ml of 5mg/ml methanol solution) as internal standard. To each plasma sample, standard and blank was added 5ml saturated sodium tetraborate solution and 5ml DCM, and a known amount of tetracaine (5ml of 10mg/ml methanol solution) as internal standard. The samples were mixed by rotation 20 min and then centrifuged 30 min to reduce the emulsions. The upper (aqueous) layer was discarded by aspiration, and the DCM phase was evaporated to dryness under a stream of N_2.

Authentic procaine, procainamide and tetracaine standards were purchased from Sigma Chemicals (St. Louis, MO).

The instrument employed was a Beckman System Gold High Pressure Liquid Chromatography (HPLC) System (Beckman Instruments, Fullerton, CA) with two 110B Solvent Delivery Pumps and a 167 Scanning Detector. The column was a Regis Workhorse Octadecyl, 4.6mm x 300mm with 10m particle size (Regis Technologies, Morton Grove, IL). The mobile phase consisted of acetonitrile: 0.0165M triethylamine pH 3.0 (86:14) at a flow rate of 1 ml/min. The UV detector wavelength was set at 288nm. All injections were made onto a 20ml loop.

Each sample was redissolved in 200ml methanol. An aliquot of each sample (20ml) was injected on the HPLC. The height of the peaks corresponding to procaine and internal standard were measured. The internal standard peak heights were used to normalize the procaine peak heights. The procaine standard curve was used to calculate an estimated procaine concentration for each sample.

RESULTS AND DISCUSSION

Our preliminary work was based on the hypothesis that between 50 and 80 mg of procaine was a minimum (threshold) dose for a local anesthetic effect of this agent[3]. Although this dose had not been rigorously established as a no effect dose, it served as a useful starting point. We therefore injected horses with 80 mg of procaine HCl subcutaneous-

ly over the sesamoid bones and followed the disposition of procaine in plasma.

As will be reported elsewhere, administration of 80 mg subcutaneously yielded mean serum procaine concentrations of about 14 ppb at one hour after dosing, declining to about 9ppb by one hour after dosing. We also reviewed the data on procaine disposition in the horse published recently by Canadian researchers[4], and unpublished data generated by Dr. Scott Stanley of Truesdail Laboratories, Tustin, CA, and Dr. Robert Jack (personal communication with Dr. Jack), , the Equine Medical Director for the state of California. Based on these data we suggested a 5ppb concentration of procaine in equine plasma as an interim plasma threshold for procaine in racing horses.

This proposal was not warmly received by regulators. It was received in this manner because Kentucky does not routinely draw blood samples from racing horses. Beyond this, the introduction of a procaine program based on a plasma concentration of procaine would require the use of special blood sampling tubes for procaine containing esterase inhibitors. Our proposed plasma threshold for procaine was never seriously considered for implementation.

We next determined the Highest No Effect Dose (HNED) for procaine as described previously[2]. After administration of doses of 10.0, 20.0, and 40.0 mg procaine HCl, there was a significant difference between control and procaine values up to 30 min after injection of the anesthetic. The injected dose had to be reduced to 5.0 mg/ site before there was no statistically significant LA effect. There was still a non-significant increase in hoof withdrawal reflex latency (HWRL) observable at 5.0 mg, and only after injection of 2.5 mg procaine was there no apparent LA effect. Therefore, the highest no-effect dose (HNED) for procaine was determined to be 2.5 mg/site.

These data suggest that the apparent plasma threshold for no pharmacological effect of procaine was about 80/2.5 or about 1/32 of our previous estimate, or about 1 ng/ml or less. It is not practical to routinely quantify such low plasma concentrations of procaine; we therefore abandoned all thought of developing a plasma threshold for procaine and elected to concentrate on developing a urinary threshold for this agent.

To establish a urinary threshold for procaine v dosed five horses with 5mg of procaine HCl subcu neously and followed urinary concentrations of p ent procaine. As will be reported elsewhere, urina concentrations of procaine peaked at about 25 ng/ at four hours after dosing and declined to beco undetectable by about 24 hours after dosing. The data suggest that urinary concentrations of procai as high as 25 ppb are unlikely to be associated w pharmacological effects of this agent and may the fore be suggested as a reasonable urinary "No Eff Threshold" for procaine.

A major concern when evaluating the significan of urinary thresholds is the effect of pH on the co centration of the drug in a urine sample[5,6]. Becau of the very large range in urinary pH (pH = 4.5 - 9 in post race urines from Thoroughbred horses (t not apparently in Standardbred horses), urinary pH likely to be a dominant factor in determining the co centration of drugs in post-race urines. This is esp cially important since basic drugs such as procai and other local anesthetics may be expected to co centrate in acidic urines. Therefore, it is necessary develop an experimental model in which the pH the urine of a horse can be rapidly and reproducil lowered (to about pH=5.0) to investigate the effect such changes in urinary pH on drug disposition a concentration in post-race urine.

Previous work in our lab[7] has shown that adm istration of 1 kg of ascorbic acid by stomach tu rapidly reduces the pH of horse urine to about 5 over a 12 hour period (Figure 1). We elected to us this model to simulate the changes in urinary pH f lowing a race as follows in Figure 1.

An experiment was therefore designed in whi an infusion pump (Alza) was placed subcutaneou and calibrated to release procaine at a rate that wo yield a steady state plasma concentration of this dr of about 1 ng/ml. We then administered 1 Kg ascorbic acid/ horse to these horses by stomach tu and followed urinary concentrations of procaine urine pH decreased over the next 12 hours. Figur shows that, initially, urinary concentrations of p caine rose rapidly as urinary pH decreased, but th stopped rising and fell back to control concentrati This unexpected decline in urinary procaine conce trations, in the face of decreasing urinary pH, h caused us to re-evaluate the utility of the oral asc bic acid model. In particular, we were concern

about the possibility of non-physiological changes occurring in the kidneys of horses treated with large doses of ascorbic acid.

In an attempt to reduce the amount of ascorbic acid administered and to reduce the duration of the ascorbic acid induced acidosis, we have infused ascorbic acid (300 g) dissolved in 2 liters of sterile water intravenously over a shorter period (30 min) to more closely mimic the brief (1-2 hour) decline in urinary pH seen after strenuous exercise in horses. Figure 3 shows the change in urinary pH following ascorbic acid induced acidosis which reached peak acidity at 60 minutes after the start of infusion.

In summary, therefore, it appears from recent work in our laboratory that the Highest No Effect Doses (HNED) of local anesthetics are likely to be very low indeed, with carefully placed doses of potent local anesthetic agents of less that 10 mg/site producing clearcut local anesthetic effects. These very low doses of the local anesthetics are likely to be very difficult to detect in plasma; we therefore had no choice but to investigate the practicality of urinary thresholds for these agents.

Experiments with procaine suggest that 25 ppb of parent procaine is a reasonable threshold for this agent in alkaline or basic urines. Preliminary work on developing a useful model for the acidic post-race urine seen in Thoroughbred horses met with little success. A satisfactory laboratory model of the acute post-race urinary acidosis seen in many Thoroughbred horses remains to be developed.

LITERATURE CITED

1. Tobin T., Blake J.W. The pharmacology of procaine in the horse: Relationships between plasma and urinary concentrations of procaine. *J Equine Med Surg* 1977;1:188*f*194.

2. Harkins J.D., Mundy G.D., Stanley S., et al. Determination of anesthetic and highest no*f*effect doses (HNEDs) of procaine, cocaine, and bupivacaine following abaxial sesamoidean nerve block and topical administration of benzocaine in Thoroughbred mares. *Equine Vet J* 1995. (In Press)

3. Agriculture Canada .Race Track Division Schedule of Drugs. Ottowa, Ont., *Agriculture Canada*, 1991;

4. Stevenson A.J., Weber M.P., Todi F., et al.Plasma elimination and urinary excretion of procaine after administration of different products to Standardbred mares. *Equine Vet J* 1992;24:118*f*124.

5. Houston T., Chay S., Woods W.E., et al. Phenylbutazone and its metabolites in plasma and urine on Thoroughbred horses: Population distributions and the effects of urinary pH. *J Vet Pharmacol Therap* 1985;8:136*f*149.

6. Gerken D.F., Sams R.A., McKeever K.H., et al. Urinary pH effects on the renal clearance of lidocaine and phenylbutazone in exercising horses. *The Toxicologist* 1991; 297. (Abstract)

7. Wood T., Weckman T.J., Henry P.A., et al. Equine urine pH: normal population distributions and methods of acidification. *Equine Vet J* 1990; 22:118*f*121.