Pharmacology

Invited Review*

PRE-PURCHASE TESTING FOR DRUGS IN HORSES: A REVIEW

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SUMMARY

The use of medications to mask defects of wind, limb or temperament in horses offered for sale can be a problem for purchasers, and for veterinarians performing pre-purchase examinations. Drugs which may be used in this way include the phenylbutazone-like drugs, cotricosteroids, local anaesthetics, and tranquilizers. If skillfully used, no signs suggestive of medication by these agents will be detectable by the examining veterinarian. In the absence of information on the medications status of an animal, the usefulness of a pre-purchase examination may be limited.

These limitations can be circumvented by the analysis of blood or more expecially blood and urine samples from such horses. Current analytical methodology allows the detection of many medications in blood, and of virtually all significant medications if blood and urine samples are available. Such analysis, therefore, allows the veterinary practitioner to offer a significantly better pre-purchase test, and protects both the veterinarian and the purchaser.

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*See Author's Guidelines page 50.

INTRODUCTION

The principal problem associated with the pre-purchase examination of a horse is to determine whether or not the horse is sound for its projected use. While the uses to which horses may be put are varied, it is rarely that a purchaser requires an animal that is not sound in wind and limb and whose temperament is such that the horse is reasonably easy to manage and safe to be around. Unfortunately, not all horses are sound in wind, limb and temperament, and failure to meet these requirements may, indeed, be the primary reason for sale of certain horses. In this regard, one of the strategies that a seller may use to make such an animal marketable is to use specific medications to mask the animal's unsoundness.

The Medications

The potential for modification of the appearance, behavior or performance of an animal by the use of medications is limited only by the range of medications available to the seller. There are about 4,000 drugs in common use in human and veterinary medicine, and perhaps an equivalent number of drugs approved for use outside North America, any of which may show up in horses. Beyond this, there is an unknown number of experimental drugs.[4] Because of this very large range of drugs, the analytical methodology use in any pre-purchase testing scheme must be of very broad scope. Beyond this, however, the basic ways in which these drugs may be used may be categorized by the pharmacological actions of these medictions. For example, one of the commonest defects that a seller may want to mask is simple soft tissue inflammation, and Non-Steroidal

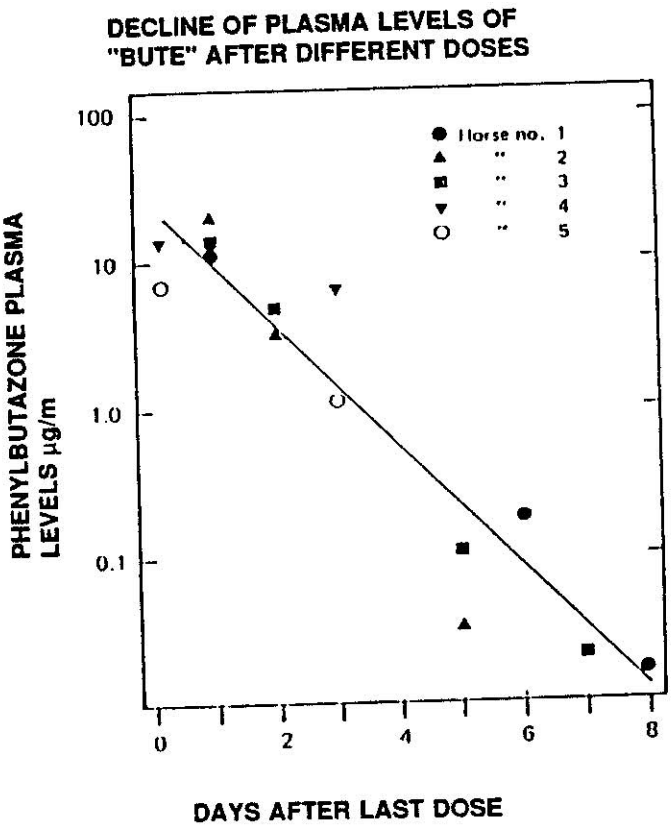

Figure 1. The symbols show plasma levels of phenylbutazone in horses that had been dosed with 2.4, and 6 gm phenylbutazone for up to three days before measurement of plasma levels. Phenylbutazone was detectable in plasma for eight days and in urine for up to nine days. After Norheim et al. (1978). Reproduced with permission from Tobin, T. Drugs and the Performance Horse, Charles C. Thomas, Springfield, Illinois, 1981.

Anti-Inflammatory drugs (NSAID's) such as phenylbutazone are the most popular approach to this problem.

Phenylbutazone and other NSAID Drugs

The treatment of choice for localized soft tissue inflammation (bruises, muscle soreness, tendon and joint pain) is phenylbutazone or one of the related NSAID drugs. These agents are effective against sore feet (Pedal osteitis), jacks (cunean tendon bursitis) sprains, minor sprains, muscle soreness, splints, muscular disease and ring bones. These drugs begin to act within 3-6 hours of administration, are effective for about 24 hours after a single dose, and longer after repeated doses.[5] Because of the wide spread availability of phenylbutazone, it is the medication most commonly used to "touch up" a sore horse before a pre-purchase examination, or before a sale.

In general, the phenylbutazone-like drugs are relatively easy to detect either blood, or more particularly, in urine samples. Phenylbutazone is detectable in the blood for about 48 hours after a clinically effective dose, and for longer if the dose is large or if a urine sample is used (Figure 1). If a sample is taken at the time of examination, or indeed at the time that lameness appears in the horse, phenylbutazone should be readily detectable in both blood and urine samples.

The problem with NSAID drugs is that there are at least 40-50 other NSAID drugs (Flunixen, Naproxen, Equiproxen, Ibuprofen, Zomax and so forth) which are broadly equivalent to phenylbutazone. In general, however, these drugs are fairly readily detectable in blood or urine samples, and detection of the improper use of these agents is technically quite feasible.[6]

The Corticosteroids

The Corticosteroids are a group of drugs that also have potent anti-inflammatory effects. For this reason, they are also effective in the treatment of soft tissue inflammation. Unlike the NSAID drugs, however, they can be injected directly into the inflammed area. They are very powerful drugs, and small amounts of them produce good anti-inflammatory responses, especially when administered locally. In particular, they can be injected directly into inflammed joints, to reduce swelling and lameness in the joint, and restore normal action in the joint.[5] The corticosteroids take longer to act than the phenylbutazone-like drugs, and when given directly into a joint, they restore normal function for a period of weeks. With this group of drugs, therefore, it is important that the sample be taken at the time of the pre-purchase examination, as by the time that the effect has worn off the drug is likely to be relatively difficult to detect in blood or even urine. For these drugs, therefore, because of the small amounts of drug that can be effective when administered directly into a joint, it is important for detection of this group of drugs to obtain a urine sample if at all possible.

Tranquilizers

Another major way in which a horse can be manipulated pharmacologically is by the use of agents affecting the central nervous system. To calm a hyperactive horse or a horse that is difficult to ride or manage, a tranquilizer such as reserpine or one of the phenothiazine tranquilizers may be administered. Reserpine has a reputation among horsemen as the "three-week-tranquilizer." Whether or not its action lasts for three weeks is not clear, but work in our laboratory has shown that reserpine affects the demeanor of horses in subtle ways for at least ten days.[3] Reserpine, therefore, is the type of drug that one can give to a horse and expect to have the behaviour of that horse influenced for up to at least one week after a single administration. On the other hand, reserpine is unusual in that it is most readily detected in blood, and a blood sample is all that is needed for detection of this drug.[7]

The phenothiazine tranquilizers such as acepromazine have often been used by horsemen to influence the

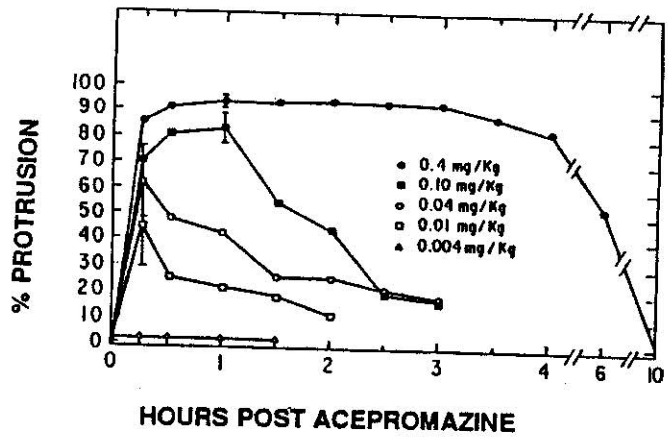

Figure 2. Effect of acepromazine on penile protrusion in geldings. Acepromazine at 0.4 mg/kg was administered to four geldings and the maximal length of penile protrusion measured. The symbols show the penile protrusion measured after each dose of acepromazine, expressed as a percentage of the maximal protrusion seen in each horse. Reproduced with permission from Tobin T, Drugs and the Performance Horse. Charles C. Thomas, Springfield, Illinois, 1981.

demeanor of a horse. These agents have the advantage that they are relatively short acting, and can, therefore, be used to produce subtle but short lived effects on a horse. In general, their effects appear within ten minutes after administration, peak within thirty minutes to one hour, and then decline. It is rare for these drugs to have much observable effect after four to six hours, unless the dose of drug administered is very large indeed.[1]

The phenothiazine-like tranquilizers are commonly used to render young horses easy to handle. They are used to load them for shipping to a sale, but they may also be used to make an unruly horse appear calm and manageable. One of their most clearcut side effects in the male horse is that they cause extension of the penis (Figure 2). If you see a young horse presented for sale with its penis extended, one of the possibilities is that this horse has been treated with acepromazine a short time previously.

The phenothiazine tranquilizers are very potent drugs, and a total dose of 5 mg can markedly effect the behavior of a horse. The small dose of acepromazine is virtually impossible to detect in blood. For successful detection of a small dose of one of the phenothiazine tranquilizers, a urine sample is an absolute requirement. However, although acepromazine and other members of this group are very difficult to detect in blood at clinically effective doses, it's metabolites are easily detectable in urine and for two to three days after administration of quite small doses.[1]

Other Agents

A variety of other agents may be administered to horses to make the animal more presentable to a purchaser. At one time in England the use of anabolic steroids was widespread in the preparation of yearlings for sale. Administration of an anabolic steroid has very clearcut effects on horses, especially fillies and prepubescent males. Anabolic steroids act to increase appetite, to build up muscle and bone, to make animals more alert and aggressive, and to improve their coats. These actions all lead to the development of a more marketable yearling, and also serve to add to the appearance of an adult horse, particularly a mare or gelding.[8]

On the other hand, when given to fillies, or to young brood mares, the masculinizing effects of the anabolic steroids can give rise to subsequent problems with breeding. If sufficient anabolic steroid has been administered, estrus and even ovulation can be suppressed. These affects can last for months after administration of the drug ceases, and such fillies can be significantly more difficult to get in foal. All in all, depending on the amounts of drug administered, and the time since their administration, the effect of the anabolic steroids can be of considerable significance for the subsequent performance of the horse, expecially for its breeding performance.

Analysis for the anabolic steroids is difficult. To date, their detection has tended to depend on radioimmunoassay screening, which does not "positively" identify specific drugs. However, depending on the amounts of anabolic steroid administered to the animal, positive confirmation by mass spectrometry may be possible.

Drug Detection

When a drug is administered to a horse, its detectability depends on the concentrations of the drug or its metabolites present in the body fluids submitted for examination, and the availability of a sufficiently sensitive method to detect and unequivocally identify the drug in the sample submitted. As a general rule, urine is virtually always the superior medium for detection of a drug or drug metabolite.[9] This is because most drugs, and more especially drug metabolites, are found in higher concentrations in urine than in blood. As a rule of thumb, it may be assumed that the concentrations of any drug or drug metabolite in urine will be at least 50 times higher than the corresponding blood level. While this rule does not always hold, it is a very rare drug indeed (e.g. Reserpine) that is detectable only in blood and not at all in urine. For this reason, if at all possible, the submission of samples for pre-purchase testing should include urine samples.

Urine Samples

Urine samples may be obtained relatively easily from mares by bladder catheterization. One simply runs a catheter into the bladder, applies a little negative pressure to the catheter (suck gently on it), and one can usually syphon out

a good urine sample. One should try to obtain at least 200 ml of urine.

Obtaining a sample from a male horse, however, is more difficult. While administration of furosemide virtually guarantees that the horse will produce a urine sample within ten minutes, it also acts to dilute out at least some of the concentrating effect that renal excretion has added to the urine sample. So a urine sample obtained with the help of furosemide is vastly better than no urine sample at all, but not nearly as useful as a "normal" urine sample.

While it is easy to detect drugs or drug metabolites in urine, it is difficult to draw conclusions from a urine sample about when the drug was administered or whether or not there was significant blood level of drug present in the horse. This is because there is, as a general rule, no direct relationship between blood and urinary levels of drugs or drug metabolites. Therefore, while a urinary concentration can tell you, with virtual certainty, that an animal was recently administered a certain drug, estimates of the time of administration of that drug tend to be extremely speculative.[2]

Blood Samples

Blood samples are easy to obtain, and at least twenty ml of blood should be provided for useful analysis. For best results, the sample should be drawn into tubes containing oxalate or fluoride, to poison the esterase enzymes found in blood, which can break down certain drugs. Alternatively, the blood sample may be drawn into serum tubes, but samples are best not drawn into tubes that contain separating agents for serum. This is because the separating agents contain poorly described chemicals that could lead to inadvertent contamination of the sample, thereby confusing the interpretation of the test and its forensic validity.

For most Non-Steroidal Anti-Inflammatory Drugs, such as phenylbutazone, blood levels of this drug are readily detectable for up to two days after administration of the drug. This means that if the animal is under the pharmacological effect of a drug at the time of sale, then the blood will contain easily detectable levels of the drug. On the other hand, a urine sample will contain both the parent or unchanged drug, and also drug metabolites, likely for a much longer period than the drug is detectable in blood. The upshot of this is that urine is the superior sample from the point of view of maximizing the probability of drug detection, but that better and forensically more useful results can be drawn from blood level data, if these can be obtained.[10]

Shipping and Storage

Urine samples are best stored frozen, as any changes likely to occur during storage are prevented by storing the sample frozen. Plasma samples can also be stored frozen, and, if necessary, blood samples may also be stored in the same way. The next most satisfactory storage method is refrigeration, which will hold most samples for several days. On the other hand, if samples are allowed to stand room temperature for much more than one day, their quality as forensic samples tends to be markedly reduced.

The Testing Process

When samples are received at the laboratory, their receipt is recorded and the quality of the sample noted. In addition, if the sample is of urine, its pH and specific gravity are noted. Then a portion of the sample is taken for analysis.

The initial screening analysis of a sample is by High Performance Thin Layer Chromatography (HPTLC). The sample is taken, extracted under acidic or basic conditions, and then subjected to High Performance Thin Layer Chromatography. As soon as the chromatograms are developed, they are treated with a sequence of oversprays, which demonstrates the presence of different drugs. These methods are very sensitive, indeed, and can detect concentrations of drugs as low as 20 nanograms/ml under optimal conditions. In addition, the scope of these tests is very wide, which makes HPTLC the method of choice for screening for a wide range of drugs. On the other hand, if the search is to be limited to a specific drug or class of drugs, tests known to be optimal for detection of that class of drug will be used.[5]

While a drug can be tentatively identified by HPTLC this kind of identification is only tentative. If necessary, the presence of the drug in the sample is confirmed by mass spectrometry. Mass spectrometry yields evidence which constitutes virtually unequivocal identification of the drug. With good mass spectral evidence, the probability of effective challenge of the validity of the analytical findings is greatly reduced.

Utility of Pre-purchase Testing

When used in this way, pre-purchase testing can be of great assistance to the veterinarian in advising his client as to the suitability or soundness of a horse for purchase. There is virtually no other way for a veterinarian to determine at the time of examination whether or not a horse is under the influence of a specific medication. Simple drawing of a blood, or better still a blood or urine sample, can go a long way towards protecting a veterinarian and his client from the effects of medication on the appearance, demeanor and performance of a horse at the time of a pre-purchase examination, or, indeed, at any other time.

REFERENCES

1. Ballard S, Shults T, Kownacki AA, Blake JW, Tobin T: The pharmacokinetics, pharmacological responses, and behavioral effects of acepromazine in the horse. *J Vet Pharmacol Therap*, 5:21-31, 1982.

2. Houston T, Tobin T, Blake JW: Effect of urine pH on urine levels of phenylbutazone, oxyphenbutazone and y-hydroyphenyl-utazone in racing horses. *Proc 5th Intn'l Conf, Cont use of drugs in race horses*, Toronto, Canada, June 1983, pp. 26-33.

3. Shults T, Combie J, Dougherty J, Tobin T: Variable interval conditioning in the horse: A sensitive measure of behavior. in Tobin T, Blake JW, Woods WE (eds) *Proceedings of the Third Internationa*

Symposium on Equine Medication Control, Lexington KY. pp 367-379, 1979.

4. Tobin T: *Drugs and the Performance Horse*. Charles C. Thomas, Springfield, Illinois, 1981.

5. Tobin T: Pharmacology Review: The non-steroidalanti-inflammatory drugs. I Phenylbutazone. *J Eq Med Surg*, 3:253-258, 1979.

6. Tobin T: Pharmacology Review: The non-steroidal anti-inflammatory drugs. II. Equiproxen, meclofenamic acid, flunixin and others. *J Eq Med Surg*, 2(7:298-392, 1979.

7.Tobin T, White CB, Wood WE: The pharmacology of reserpine in the horse. II Effects of reserpine on platelet serotonin uptake and behavior. *J Eq Med Surg*, 3:446-451, 1979.

8. Tobin T: The anabolic steroid androgen group of drugs. *J Eq Med Surg*, 2:163-165, 1978.

9. Tobin T, Combie J. Nugent T: Drug "clearance times" and detection times" in horses: A reappraisal. *J Vet Pharmacol Ther*, 5:195-197, 1982.

10. Tobin T: Plasma and urinary drug concentrations, drug "clearance times," and pharmacological effects. *J Eq Med Surg*, 2(6):262-267, 1978.