Review of a Consequence of Highly Sensitive Drug Testing: The Need for Data on Analytical–Pharmacological Relationships for Therapeutic Medications

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Modern analytical tests, including those for legitimate therapeutic medications, can be extremely sensitive. Because of this, ineffective traces or residues of a number of legitimate therapeutic medications can be detected for significant periods after the pharmacological effects of the medications have dissipated. This review outlines both the mechanisms and the information base that the performance horse industry is developing to cope with the increasing sensitivity of analytical testing for legitimate therapeutic medications. Authors' address: The Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546. © 1997 AAEP.

1. High-Sensitivity Testing

The increasing detection capabilities of analytical chemists and the advent of high-sensitivity drug testing equipment and techniques have substantially increased the analytical capabilities of racing chemists. With current analytical techniques, chemists can routinely detect concentrations of drugs, medications, and metabolites in the low parts per billion (ppb) concentration range. The ability to quantify small amounts of drugs and therapeutic medications remains critically important as a research and regulatory tool as, for example, in drug development (pharmacokinetics) and regulatory work. However, in the field of performance horse testing, new approaches have to be developed to cope with this technical capability. The problem revolves around the large number of drug molecules injected when we treat the horse and, to some extent, the large number of agents detectable by current technology.

2. Historical Background

To put this problem in historical perspective, let us look at progress in this field during this century. At the beginning of this century, in about 1905, all foreign substances administered to a horse were, by definition, administered in contravention of the rules of racing. In 1905, however, analytical chemistry was not much of an art; in those days a chemist was unlikely to produce an overwhelming number of unequivocal chemical identifications of foreign substances for his authority.[1] Indeed, at that time the

NOTES

Table 1. Number of Molecules Administered per Day/Dose

Drug	Number
Naproxen	10^{22}
Furosemide	10^{20}
Fentanyl	10^{18}
Etorphine	10^{16}
Hyaluronic Acid	10^{16}

number of chemical substances known to exist was relatively small—a condition that certainly does not exist today.

In contrast, in the closing years of the twentieth century, we see an entirely different picture. Today many agents are readily detectable, and a large number of agents (those altering performance, legitimate therapeutic medications, and dietary, environmental, and endogenous substances) can be detected at very low concentrations, for significant periods of time after administration, and long after the pharmacological or therapeutic effect is over.

The greater sensitivity of testing is due in part to the advent of enzyme-linked immunosorbent assay testing and also to improved mass spectral confirmation techniques. However, what these techniques have also brought to the forefront is the not clearly understood fact that most drugs and medications are retained by horses and other animals for very long periods after most analytical methods cease to detect them.

3. Numbers of Drug Molecules Administered

To bring this point home, let us calculate the actual number of drug molecules injected into a horse with a clinical dose of a therapeutic medication and then follow its clearance from the horse. In this regard, the number of drug molecules injected can be as high as $\sim 10^{20}$, a number of molecules similar to all the stars in the known (pre-Hubble?) universe, or all the grains of sand on the beaches of the world (Table 1). This is a very large number of molecules indeed, and it brings home to us the ability of some drugs, medications, and agents to be retained at low but detectable concentrations in horses for relatively long periods.

4. Specific Example: Phenylbutazone

Let us look at a very common therapeutic medication, phenylbutazone. If we assume that phenylbutazone has a half-life of 7.22 h, then it turns out that 90% of the dose administered, or one log unit of the amount remaining in the horse, is eliminated each day (Figs. 1 and 2). Now a typical dose of phenylbutazone in a horse contains $\sim 10^{21}$ molecules, and by simple arithmetic we see that it will take 21 days to eliminate this entire amount of drug from the horse. However, the pharmacological effect of phenylbutazone is lost within 1–2 days, but it will take another 19 days for the drug to be completely eliminated.[3]

In more practical terms, we can look at the experience of our colleagues in Hong Kong,[a] who at one

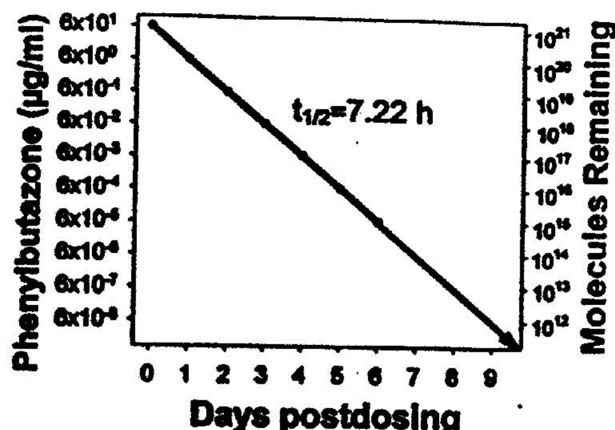

Fig. 1. Elimination of phenylbutazone after a single administration.

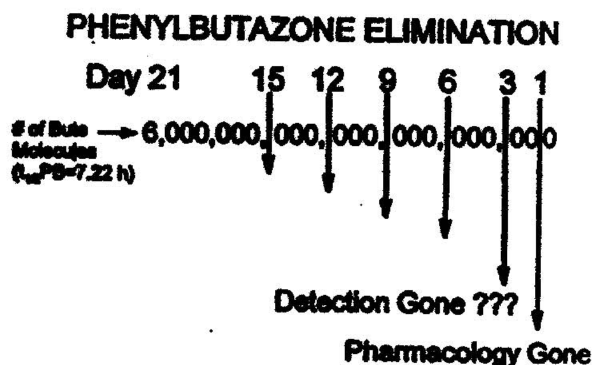

Fig. 2. Daily elimination of phenylbutazone molecules.

Table 2. Elimination of Phenylbutazone

Assume a half-life of 7.22 h
90% of dose eliminated/day
10^{21} molecules administered
21 days to eliminate
Pharmacology gone in 1–2 days
Must have limit on sensitivity of testing

point had in place a test that detected phenylbutazone for ~1 week after the last dose. There then came a time when a more sensitive test was put in place, and this test was found to detect phenylbutazone for ~2 weeks after the last dose. However, the Hong Kong authorities eventually concluded that the more sensitive test served no useful purpose, and they chose to return to the original 1-week withdrawal time test. The Hong Kong authorities had, at least for this particular test, chosen to arbitrarily limit the sensitivity of their test (Table 2).

5. Summary of the Withdrawal-Time Problem

As with the phenylbutazone example described above, all medications are retained in the horse for long periods and are not completely eliminated by the horse until long after the pharmacological effects are over. Therefore, for many drugs and medications, it

is possible to detect traces of the agents that are not associated with pharmacological effects. On one hand, if the drug in question is an illegal performance-altering agent, this is not a problem. These drugs have no place in horse racing, and their detection at any concentration should be and is vigorously pursued.

On the other hand, if the agent is one of the 50 or so legitimate therapeutic medications that is administered to a horse to promote the health and welfare of the horse, then the position is much different. For such agents, the horseman and the industry need guidelines on where to set the sensitivity of testing so that horses run on their merits and not on the direct effects of the medication. However, the sensitivity should be set so that horsemen are not penalized by the detection of ineffective residual traces of these agents in postrace urine samples. For therapeutic agents, we need to set limits on the sensitivity of testing so that withdrawal-times research can commence and specific withdrawal-time guidelines can be determined.

6. Bringing the Problem Down to Manageable Size

It is sometimes suggested that the problem of withdrawal times is too big or too difficult or too complicated to tackle successfully. Individuals taking these positions point to the 63,000 known chemicals and the 4000 common prescription agents and note that it would be impossible to develop the necessary data on more than a fraction of this number of agents. However, if one carefully reviews the problem, as was done in Lexington at a workshop entitled Testing for Therapeutic Medications, Environmental and Dietary Substances in Racing Horses in 1994, it is evident that all one has to do is to develop data on a relatively small fraction of these agents.

7. AAEP List of Therapeutic Medications

Because we are only concerned with developing thresholds or withdrawal-time data for legitimate therapeutic medications, the list of candidate agents is immediately limited to the 57 legitimate therapeutic medications listed by the American Association of Equine Practitioners.[3] If we further review this list and compare it with the chemical identifications made by chemists, we find that approximately nine agents give rise to more than 50% of the chemical identifications reported in the racing industry. These agents include, in no particular order, (1) procaine, (2) isoxsuprine, (3) methocarbamol, (4) dexamethasone, (5) flunixin, (6) prednisolone, (7) acepromazine, (8) promazine, and (9) pyrilamine. This is a very manageable list of agents and one on which we have focused our research attention.

8. How Do We Identify the Point at Which We Limit the Sensitivity of Testing?

In our research program in Kentucky, we use various testing methods to identify the point at which the pharmacological activity of an agent is lost. After that point any trace remaining in blood (preferably) or urine (if there is no other choice) is likely to be an ineffective residue of the agent. To do this we have developed a systematic approach to this problem, which we now briefly review.

9. Critical Pharmacological Effect

Before we can develop a database on analytical–pharmacological relationships, we need to identify the specific pharmacological effect of concern to the racing industry. At times this is a straightforward process; for example, for the local anesthetics, local anesthesia is clearly the pharmacological action of concern.[4] With other agents it is sometimes not so easy; for example, after we have orally administered isoxsuprine, we have been unable to identify any pharmacological responses whatsoever. However, identification of the specific pharmacological effect of concern to racing is a critical part of this process and one on which we have an active research program underway.

10. Highest No Effect Dose

Once we have identified the critical pharmacological effect, we can then identify the highest no effect dose (HNED) for the agent in question. For example, using our heat lamp–local anesthesia–abaxial sesamoid block model, we have successfully developed a family of dose-response curves and identified the HNED's for (1) bupivacaine, (2) mepivacaine, (3) lidocaine, (4) procaine (Fig. 3), (5) cocaine, (6) benzocaine,[4] (7) sarapin,[5] and (8) fentanyl. This research has shown some of these agents to be highly potent, some to be of intermediate potency, and some to be pharmacologically inactive in this model.

11. Critical Metabolites

A further complication in this process is that the residue or metabolite found in horse urine after the administration of some of these agents, and on which the chemical identification is made, is often not the parent drug but a metabolite specific and sometimes unique to the horse. To solve this problem, we have synthesized, purified, characterized, and authenticated a series of these metabolites, including 3-OH-mepivacaine, 3-OH-lidocaine, hydroxyethylpromazine sulfoxide, O-desmethylpyrilamine, and 3-OH-promazine (Table 3). These metabolites serve many purposes: as authentic standards for forensic identifications, as reference standards for metabolite quantification, and as specific qualitative and quantitative spikes (supplemental proficiency or double blind samples) for quality assurance work.

12. Putting the Package Together

Once we have all these parts in place, we can then assemble the product. For example, with procaine we have identified the HNED, which is ~5 mg/site subcutaneously. We have administered this agent to horses and quantified free procaine and its glucuronide metabolite in postadministration urine. Be-

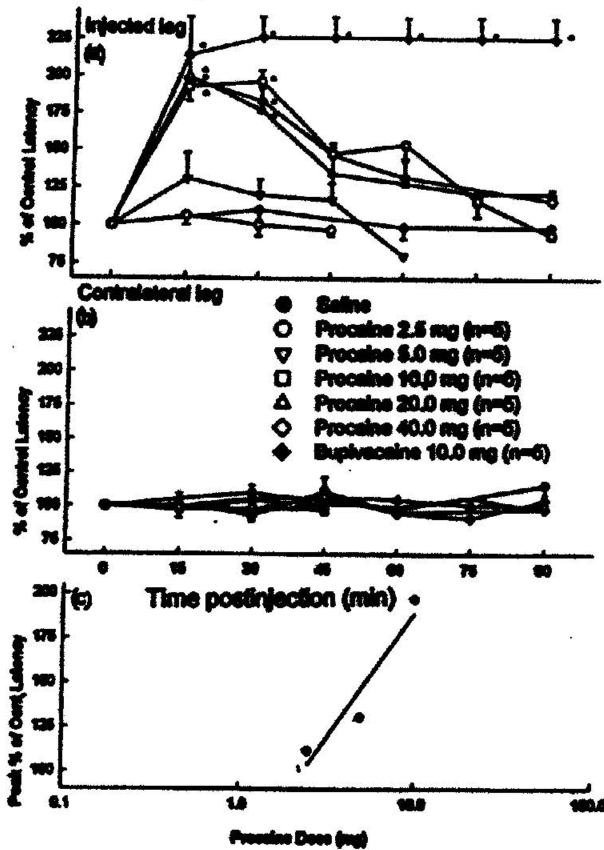

Fig. 3. (a) Hoof withdrawal reflex latency % increase following injection of procaine doses; (b) % change in contralateral leg following saline injection; (c) procaine dose-response curve. Asterisks indicate a significant difference from control values.

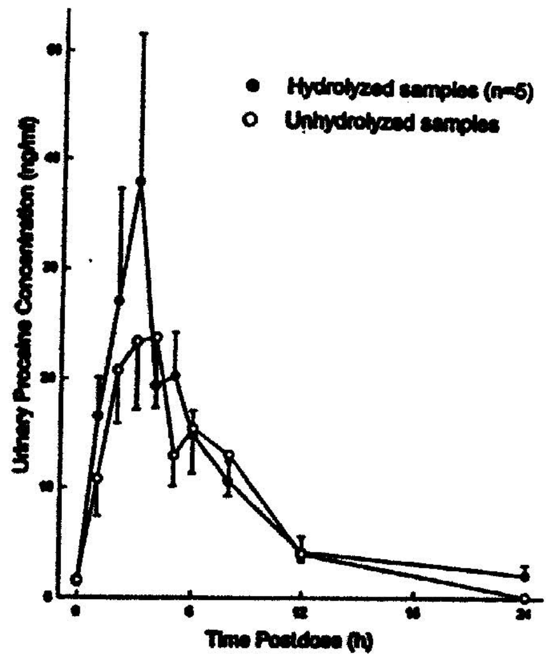

Fig. 4. Urinary procaine concentrations of hydrolyzed and unhydrolyzed samples following procaine HCl 5 mg SQ.

Table 3. Critical Metabolites that Have Been Synthesized

Parent Drug	Metabolite/Analog
Lidocaine	3-OH-lidocaine
Mepivacaine	3-OH-mepivacaine
Pyrilamine	O-desmethylpyrilamine
Acepromazine	2-(1-hydroxyethyl)promazine sulfoxide
Acepromazine	2-(1-hydroxyethyl)promazine
Acepromazine	2-(1-trimethylsilyloxyethyl) promazine
Promethazine	promethazine sulfoxide
Propionylpromazine	2-(1-hydroxypropyl)promazine sulfoxide
Propiopromazine	2-(1-hydroxypropyl)promethazine sulfoxide
Promazine	3-OH-promazine
Tripelennamine	3-OH-tripelennamine
Phenylbutazone	phenylbutazone D_9
Propanolol	4-OH-propanolol
Mazindol	mazindol metabolite

cause these horses were producing alkaline (pH 8.3) urine, this concentration of procaine is as low as one is going to find in equine urine. The peak concentrations found in these urine samples were ~28 ng/ml of free procaine and 45 ng/ml of total procaine (Fig. 4). If the concentrations of procaine metabolites found in a postrace urine sample are smaller than these,

Table 4. Summary of Thresholds Project

Agent	Metabolite Synthesis	HNED Dose Determination	Threshold Determination Status
Benzocaine	not required	no effect	ineffective; published
Sarapin	not required	no effect	ineffective; in press
Procaine	not required	5 mg/site	30–50 ppb; published
Cocaine	available commercially	>5 mg/site	ms. in draft; published
Lidocaine	synthesized	4 mg/site	in progress
Mepivacaine	synthesized	2 mg/site	in progress
Bupivacaine	not synthesized	<1 mg/site	in progress
Acepromazine	synthesized	1 mg/1000 lb	in progress
Isoxsuprine	not required	no activity orally	2 papers in press
Detomidine	available (?)	determined	in progress
Pyrilamine	synthesized	—	—
Promazine	synthesized	—	—

then the procaine concentrations are unlikely to have been associated with a significant local anesthetic effect of procaine at the time of racing.

13. Research in Progress

Table 4 summarizes our progress to date in this area; the research is ongoing. The limitation of testing methods has been attempted and is successfully in place in both Canada and Australia. In Canada, at least, all laboratories participating in postrace urine

drug analysis are required to use the same testing method, which promotes higher trainer–veterinarian confidence in the established withdrawal times for each particular agent. However, these methods are only applied to therapeutic substances or dietary or environmental contaminants; illicit substances are actively pursued without limitation by using more specific tests.

In the U. S., we are actively seeking an answer to this problem. A conference was held in 1994 to discuss the testing for therapeutic medications and environmental and dietary substances in racing horses at the University of Kentucky. The workshop endorsed the Canadian approach to testing problems, and a quote from Mr. Clinton Pitts, a Jockey Club steward, summed up the problem extremely well: racing's problem is that "racing has 50's rules and 90's testing technology."

As we approach the 21st century, this quote becomes even more significant. Changes have to occur on many different levels, including (1) use outreach programs to educate horsemen on withdrawal-time data, (2) announce changes in testing for therapeutic medications and explain what this means, and (3) focus on the tail end of therapeutic medications. The end result is that chemists will have the chance to use their equipment to focus on the nontherapeutic medications, and horsemen and veterinarians will be encouraged to provide proper care to horses who are in need of veterinary assistance.

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