The Dietary Anthelmintic Pyrantel Tartrate: An Analysis of its Detection Times in Geldings, Mares and Stallions

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Summary

Three groups (n=6) of performance bred (Thoroughbred or Standardbred) geldings, mares, and stallions were fed the recently developed dietary anthelmintic pyrantel tartrate daily for a period of 21 days to achieve steady state conditions. The effective "clearance times" of pyrantel tartrate in each of these groups was then determined to allow guidelines to be established for veterinarians, horse trainers, farm managers, and analytical chemists in jurisdictions where a "positive" test for pyrantel tartrate might violate existing rules or regulations.

Analysis of these urine samples was conducted by standard racing chemistry thin layer chromatographic (TLC) techniques in the University of Kentucky Equine Drug Research Laboratory. Pyrantel tartrate was readily detected in equine urine samples. Significant levels were detected in urine samples collected on days 7 and 21 during the administration portion of the study and on day +1 (24 hr) of the post-administration time period. Analysis of the urine samples by direct probe-mass spectroscopy confirmed the presence of pyrantel tartrate or its metabolites. No confirmable level of pyrantel tartrate was detected in samples taken at 48 or 72 hr post dosing in these experiments.

Introduction

In most equine deworming programs the horse is administered anti-parasite medications every 30 or 60 days depending on the particular program or manager's/trainer's preference. The newly developed anthelmintic pyrantel tartrate, however, is designed to be supplemented in the normal daily ration formulation. The rationale behind this treatment schedule is to prevent the horse from becoming reinfected with parasites in the intervening time periods between medications, thereby protecting the animals digestive tract from parasite damage. Administering pyrantel tartrate on a daily basis should protect the horse against reinfections

and will therefore carry a significantly lower level of parasite infestation.

A potential problem with this approach is that many racing or show horse jurisdictions forbid the equine athlete from competing with medications in their system. Problems can therefore arise if a veterinarian or trainer is not aware of the clearance times for a medication in attempting to decide when to discontinue treatment prior to an event.

To aid officials, veterinarians and trainers in establishing guidelines for withdrawal times for pyrantel tartrate, we conducted an analysis of the "clearance times" for pyrantel tartrate after administration of clinical doses of pyrantel tartrate to horses.

Pyrantel tartrate is recommended for use in mares, geldings, and stallions and there are concerns about possible differences in the clearance times for pyrantel tartrate between these groups. We therefore evaluated the clearance times of pyrantel tartrate in groups of six each of mares, geldings, and stallions.

Analysis of the data shows that all horses tested cleared pyrantel tartrate within 24 hr of dosing, with only trace and essentially unconfirmable levels visible at 48 hr after dosing in some animals.

Materials and Methods

Horses

Six purebred (Thoroughbred or Standardbred) geldings, mares, and stallions, approximately 500 kg in body weight, that were routinely maintained at pasture were used as the test animals. The horses were brought daily to the research barn and placed in individual box stalls with free access to hay and water. Feed tubs containing the feed mixture (either the control feed or the control feed plus the pyrantel tartrate) were then placed in the stalls with the horses, allowing them sufficient time to consume the feed and associated pyrantel tartrate. All animals' feed tubs were examined each day to ensure that each animal consumed all the pyrantel tartrate offered them.

Drugs

Strongid C® (pyrantel tartrate), a commercially available orally administered equine anthelmintic, was supplied by Pfizer Inc., Animal Health Division, New York, NY, and used as supplied.

Feed Mixture

The control feed mixture consisted of approximately 100 g of a commercial sweet feed complete ration (Omaleac 100®, Ralston Purina). The test ration consisted of the control ration plus pyrantel tartrate at the manufacturer's recommended dose rate of 1 ounce of Strongid C® per 250 lb of body weight.

Experimental Protocol

For a period of 7 days the geldings, mares, and stallions were brought to the research barn and allowed to acclimate to the general study conditions (control feed, daily handling and sampling procedures). At the end of this 7 day period, the horses were fed the control ration plus pyrantel tartrate (in the form of Strongid C®) daily for 21 days. After the 21st day the horses were fed the control ration for an additional 6 days. Urine samples were collected on days -1, 21, +1, +2, +3, +4, +5, and +6. Urine samples from the mares were obtained by bladder catheterization, aliquoted, and frozen. Urine samples from the geldings and stallions were collected by fitting each animal with a leather harness fashioned with a collection reservoir. This harness remained in place until an adequate sample volume was obtained. These samples were also aliquoted and stored frozen until assayed for pyrantel tartrate. In addition to the samples described above, samples were drawn at 7 days from three horses to allow us to prepare the analytical method.

Analysis of Urine Samples

All samples were thawed out prior to analysis and subjected to the "drug screen" as performed by the Kentucky Equine Drug Research Laboratory in its routine testing of post-race samples. The method used was an alkalization of the sample with buffer and a base extraction of the urine into solvent followed by thin layer chromatography (TLC), optimized for detection of pyrantel tartrate. Preparative TLC plates (silica gel 60 F_{254}, 20 x 20 cm, 0.5 mm thickness) were purchased from Analtech, Newark, Delaware. Analytical TLC plates (silica gel 60 F_{254}, 5 x 10 cm, 0.25 mm thickness) were purchased from EM Science, Inc., Cherry Hill, New Jersey.

"Special," or Non-routine, Base Extraction

To a 16 mm x 125 mm tube were added 3 ml urine sample, 1.0 ml isopropanol/ammonia (1:1), and 7 ml dichloromethane (DCM). The sample was extracted, the extract evaporated to dryness under a stream of nitrogen, and the residue spotted on a silica gel TLC plate using DCM. The plate was developed 5 cm in chloroform ($CHCl_3$) methanol (MeOH)/acetic acid (AcOH) 70/25/5, viewed under short-(254 nm), medium-(302 nm), and long-(365 nm) wave UV light, and sprayed with Lady Tenger's spray. In this system, pyrantel tartrate has an R_f of 0.35.

Mass Spectrometric Confirmation of Pyrantel Tartrate

Urine samples were extracted by the "special base" method described above. The residue from 12 - 15 ml of urine was applied to a TLC plate in a streak, developed, and the silica from the region corresponding to pyrantel tartrate were visualized under UV light and then collected with a Kontes 3.0 ml zone collector and placed into a small glass culture tube. A few drops of 0.2 N sulfuric acid (H_2SO_4) were added, the sample was vortexed, a few drops concentrated ammonium hydroxide (NH_4OH) and 1.0 ml ethyl acetate (EtOAc) (GC/MS grade) were added, vortexed, and the sample was centrifuged. The EtOAc layer was transferred to a clean tube and evaporated to dryness under a stream of nitrogen.

The resulting residue was taken up in 10 - 20 µl EtOAc and 1 - 2 µl was placed on the filament of a direct exposure probe. The sample was introduced into the source region of a Hewlett Packard 5890 mass spectrometer and the filament heated at 20°C/sec. Mass spectra were collected in electron impact mode over the range 40 to 400 amu at a scan rate of greater than 1 scan/sec.

Figure 1 shows a direct probe mass spectrum of this material along with a mass spectrum of pyrantel tartrate.

Results

All pre-dose samples were found to be negative for pyrantel tartrate by TLC. The screening methods applied to samples from the treatment period showed no unusual spots and were indistinguishable from the corresponding control samples with the exception of the spot for parent pyrantel tartrate. Pyrantel tartrate was found only on the TLC plates from basic extracts of urine samples.

Each individual result observed and characterized is presented in Tables 1, 2, and 3. These tables also present the laboratory ID numbers used on each TLC plate. All urine samples taken during the treatment period were clearly positive for pyrantel tartrate. The presence of parent pyrantel tartrate in selected samples was confirmed by direct probe/mass spectrometry.

Review of the data sheets shows that the urine samples from day +1, collected 24 hr after the last dose, yielded a much-diminished spot for pyrantel tartrate as compared to samples from the treatment period. Attempts at mass spectrometric confirmation of the 1-day (24 hr) post-dose samples resulted in poor quality spectra. Therefore, many samples from this time period would likely be labeled "suspicious" rather than "positive" in a post-race drug testing setting.

All samples collected two or more days after the last dose were negative by TLC; thus pyrantel tartrate was rapidly cleared by all horses in each of the three groups of horses tested in this experiment; the data therefore suggest, and are consistent with suggestions, that pyrantel tartrate is rapidly cleared by mares, geldings, and stallions.

In other work we demonstrated that a non-routine analytical TLC system ($CHCl_3$/MeOH/AcOH 70/25/5) was better able to identify the presence of pyrantel tartrate. Still, viewing under UV light was critical to distinguish pyrantel tartrate from another substance that was colorized by the Lady Tenger's spray. The R_f of pyrantel tartrate in this system is variable, depending in part on the pyrantel tartrate concentration, but also on the presence of other extractable material in the urine. Pyrantel tartrate-spiked urine consistently showed a spot at an R_f greater than that seen for the pyrantel tartrate standard. Rechromatography of material isolated from these plates showed it to be identical with pyrantel tartrate.

At times a second pyrantel tartrate-related spot was observed at a slightly lower R_f. This spot was observed in the control as well as in some older

standard solutions, which were prepared in urine. Preparative TLC followed by direct probe-mass spectrometry gave rise to mass spectra very similar to those obtained for pyrantel tartrate. This product is probably the (4-cis-2) isomer, since pyrantel tartrate, (4-trans-2) is readily photoisomerized.

Discussion

These results show that pyrantel tartrate has a relatively short (approximately 24 hr) clearance time, appears only under basic extraction procedures, and has a relatively low R_f value in the "special" non-routinely used TLC system for base extracts. No major metabolites were seen in this TLC screening system. Simple hydroxylated metabolites would be expected to appear "under" pyrantel tartrate (have a lower R_f) in the base-extract system. Any further degradation products, such as thiophene-acrylic acids or N-methyl propanediamine, if detected would be unlikely to be associated by the analyst with drug use in the equine athlete. Additionally, since pyrantel tartrate is not eluted from methyl silicone gas chromatography columns, it may be difficult to confirmed by routine GC/MS methods, and in our hands required a direct probe or liquid chromatographic interface.

These data are broadly similar to those reported for pyrantel tartrate in mares by Wood and his co-workers (1991). Wood only found pyrantel tartrate in his basic extracts, and pyrantel tartrate had a very low R_f in his test systems. Wood also reported the presence of the apparent cis isomer of pyrantel tartrate, and he reported rapid clearance of pyrantel tartrate in his test mares. Wood also reported that his pyrantel tartrate residues did not cross-react with any of seven ELISA tests that he tested for cross reactivity with pyrantel tartrate.

These results therefore confirm and extend to stallions and geldings the findings of Wood, *et al.*, who reported that the use of pyrantel tartrate in racing horses is unlikely to interfere with either ELISA or TLC testing if the drug is withdrawn 24 to 48 hr prior to post.

It also appears that the forensic significance of trace level residues of pyrantel tartrate in equine urine is likely to be small. Reviewing and categorizing medications detected in racing horses, the Uniform Classification Guidelines of The Foreign Substances Document (developed by Racing Commissioners International, Drug Testing and Quality Assurance Program), which did not classify anthelmintics, considering them to be of no forensic significance. While this classification system is not incumbent on analytical chemists, it clearly suggests the level of forensic interest that anthelmintics such as pyrantel tartrate should attract.

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Figure 1 *Mass spectrum - dosed horse urine sample.*

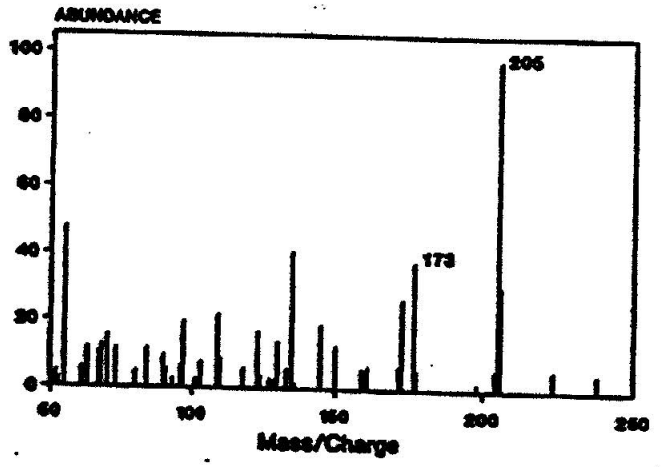

Table 1 *TLC results of mares urine for presence pyrantel tartrate.*

	#1	#2	#3	#4	#5	#6
Control	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
21 Days	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
+ 24 Hr	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
+ 48 Hr	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
+ 72 Hr	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

Table 2 *TLC results of geldings urine for presence pyrantel tartrate.*

	#7	#8	#9	#10	#11	#
Control	Neg.	Neg.	Neg.	Neg.	Neg.	Ne
21 Days	Pos.	Pos.	Pos.	Pos.	Pos.	Po
+ 24 Hr	Pos.	Pos.	Pos.	Pos.	Pos.	Po
+ 48 Hr	Neg.	Neg.	Neg.	Neg.	Neg.	Ne
+ 72 Hr	Neg.	Neg.	Neg.	Neg.	Neg.	Ne

Table 3 *TLC results of stallions urine for presence pyrantel tartrate.*

	#13	#14	#15	#16	#17	#1
Control	Neg.	Neg.	Neg.	Neg.	Neg.	Ne
21 Days	Pos.	Pos.	Pos.	Pos.	Pos.	Po
+ 24 Hr	Pos.	Pos.	Pos.	Pos.	Pos.	Po
+ 48 Hr	Neg.	Neg.	Neg.	Neg.	Neg.	Ne
+ 72 Hr	Neg.	Neg.	Neg.	Neg.	Neg.	Ne