Detomidine Clearance in the Horse as Determined by ELISA

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Abstract

Detomidine [4(5)-(2,3-dimethylbeazyl) imidazole] is a newly developed non-narcotic drug which has been approved for equine use in the United States. Detomidine is a potent α-2 adrenoreceptor agonist, and is thus pharmacodynamically related to clonidine and the widely used animal sedative, xylazine. For veterinary clinical use it is classified as a sedative, analgesic and muscle relaxant.

Four horses were administered detomidine at four dose levels in a cross over design and the clearance times for detomidine determined by enzyme-linked immunosorbent assay (ELISA).

Four performance bred mares were used as the test animals. The mares were acclimated to the study conditions for 3 days and were then administered detomidine at the dose rates of 2.0, 8.0, 20, and 40 µg/kg body weight. Blood and urine samples were obtained at the -24, +2, +4, +8, +12, +24, +48, +72, +96, and +120 hr time points.

Administration of detomidine at a level of 40 µg/kg was no longer detectable by ELISA at 72 hr post-dosing, in all four horses. At all other dosing rates, Detomidine was no longer detectable by ELISA at time points earlier than 72 hr. The ELISA used was developed at the University of Kentucky and incorporated an antibody raised against a metabolite of detomidine.

Introduction

Detomidine is a newly developed non-narcotic drug which the manufacturers (Farmos Group Ltd., Turku, Finland) have targeted for the large animal veterinary pharmaceutical market. Detomidine is a potent alpha-2 adrenoreceptor agonist, and is thus pharmacodynamically related to clonidine and the widely used animal sedative, xylazine (1). For

veterinary clinical use it is classified as a sedative, analgesic and muscle relaxant (2). Chemically, detomidine is described as 4(5)-(2,3-dimethyl-benzyl) imidazole as shown in Figure 1.

Recently, several studies of the pharmacology of detomidine have been conducted in Finland as well as the United States using both the intravenous and intramuscular routes of administration (3,4). Administration of detomidine to the horse by either route will result in effective and relatively long lasting sedation and analgesia (3). The drug is highly lipophilic and as such is rapidly absorbed and possesses a high affinity for the central nervous system (5).

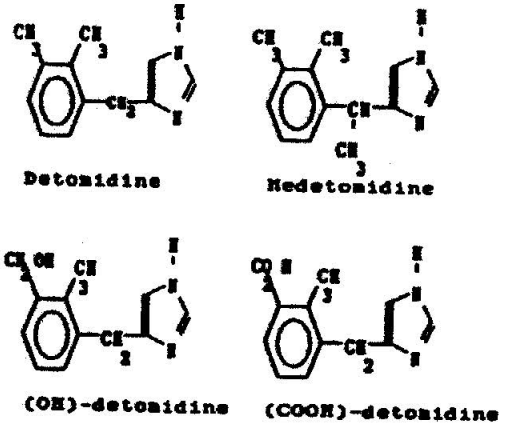
The sedation and analgesia produced in the horse by detomidine allows for a wide range of manipulations. The fact that these effects can be produced without rendering the animal recumbent allows for numerous procedures to be performed in the standing horse, a feature of importance to practicing veterinarians. The major signs of sedation include head drop, lack of reaction to environmental stimuli, drooping eyelids or lips and, in some cases, an excessive amount of frothy saliva (2).

The analgesic response to detomidine is thought to be a consequence of its potent and specific α_2-adrenoreceptor agonist effects in the central and peripheral nervous systems (3). This analgesic effect has been evaluated in the equine using the electrode shock generator and balloon-induced colic models, reaction to pin pricks and the use of noxious thermal stimuli (1). Decreases in reaction to pain following injection of detomidine were found to occur in a dose dependant manner in all of these models (3). The latter have shown that the initial signs of analgesia occur as soon as 2 to 4 min post-injection and can last as long as 4 hr following doses of up to 160 µg/kg. Since detomidine has also been reported to decrease gut motility, it would appear to be a drug of choice in cases of visceral pain or colic (6).

Preliminary metabolic studies indicate that the metabolism of detomidine is complex and extensive, involving both phase I and phase II mechanisms (7,8). The primary phase I compound formed in the horse appears to result from the oxidation of the methyl group located at the ortho position of the benzene moiety (7). Salonen's work with detomidine has shown that this compound is most likely the primary metabolite in the horse (8). The methylol metabolite may be excreted in the urine unchanged, or it may be conjugated with glucuronic acid and excreted, or undergo a second Phase I reaction to form the carboxylic metabolite. The carboxylic metabolite is then eliminated unchanged, or conjugated and eliminated. The structures of the methylol and carboxylic metabolites as well as a newly developed analog, medetomidine, are presented in Figure 1. Salonen's studies with radiolabeled detomidine indicate that the majority of administered detomidine, whether conjugated or not, is eliminated in the urine of the horse (4).

It appears from published reports that detomidine offers great promise as a veterinary medication which can provide a stable level of moderate to long lasting sedation and analgesia in the horse while not rendering the animal recumbent. These characteristics of detomidine establish its potential usefulness for minor surgery, examination or manipulation of fractious animals, sedation of animals prior to and during transportation, and in the management of certain types of colic. Consequently, detomidine is likely to have widespread use in the United States. Therefore, it is necessary to establish the characteristics of detomidine's clearance from the horse to allow trainers and veterinarians to make informed decisions about withdrawal times for this medication prior to any competitive event.

Figure 1 *Chemical structures of detomidine, medetomidine, (OH)-detomidine and (COOH)-detomidine.*



Materials and Methods

Materials

Horses. Thoroughbred or Standardbred horses approximately 500 kg in body weight were used in all experiments. The horses were maintained at pasture until such time as they were needed for experimental procedures. At that time they were housed in standard box stalls with free access to hay and water.

Drugs. Injectable detomidine was generously supplied by SmithKline Beecham, Exton, PA. Injectable medetomidine, detomidine standard, methylol-detomidine and carboxy-detomidine were generously supplied by Orion Corporation Farmos, Turku, Finland.

Methods

Pharmacokinetic Analysis. Detomidine was administered to a total of four horses at dose levels of 2.0, 8.0, 20, and 40 µg detomidine per kilogram body weight. Blood samples were collected at 0, 2, 4, 8, 12, 24, 48, 72, 96, and 120 hr post-administration and immediately centrifuged at 2000 x g for 10 min. The serum fraction of the samples were aliquoted and frozen at -20°C until assayed. Urine samples from each horse were collected at 0, 2, 4, 8, 12, 24, 48, 72, 96, and 120 hr post-administration, aliquoted and frozen at -20°C until assayed.

Experimental Procedures

Dosing and Sampling. Injectable detomidine, as well as all drug treatments, were administered by rapid IV bolus injection into the jugular vein from the right side of the horse. Blood samples (whole or serum) were collected into vacuum tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ) from the left side. All urine samples were collected by bladder catheterization.

Blood Collection. At least three serum or whole blood samples were obtained at each sampling time point. The samples from each horse's time points were pooled, and aliquoted to ensure that each sample accurately represented its respective time point.

Detomidine Extraction Procedure. This procedure required mixing a 1 ml urine sample with 250 µl 1 N NaOH and 6 ml chloroform/isopropyl alcohol (3:1) for 15 min. The resulting mixture was then centrifuged to reduce emulsions. Following centrifugation the top layer was then aspirated off and discarded. The remaining lower organic phase was tranfered to a clean tube. The sample (organic phase) was then evaporated to dryness under a stream of N_2. The residue was then dissolved in 10 µl methanol and brought to a final volume of 200 µl in assay buffer. For sample analysis 20 µl of the final extract volume was used for evaluation.

Detomidine ELISA Test Method. The ELISA tests developed by our research group are similar to the ELISA formats previously described (9). The anti-detomidine antibody was coated to flat bottom wells (Costar®, Cambridge, Massachusetts) as described in earlier works (10). Detomidine was linked to horse radish peroxidase (HRP) to give rise to a covalently linked detomidine-HRP complex (11). All assays were performed at room temperature. The assay was started by adding 20 µl of the standard, test, or control samples to each well, along with 180 µl of the detomidine-HRP solution. During the test, the presence of detomidine in the sample competitively prevented the binding of detomidine-HRP complex to the antibody. Since the HRP enzyme was responsible for the color-producing reaction in the ELISA, the concentration of detomidine in the sample was inversely related to the optical density of the test well. The optical density (OD_{650}) of the test wells was read at a wavelength of 650 nm with an automated microplate reader (Bio-Tek Instruments, Winooski, Vermont) approximately 30 min after addition of substrate. This detomidine test is now commercially available from WTT ELISA TESTS Inc.[1]

Results

A calibration curve for the detomidine ELISA test indicates that an addition of 3.0 ng detomidine/ml to the system produced about 50 percent inhibition (Fig. 2). Increasing concentrations of detomidine increased the inhibition in a sigmoidal manner, with essentially complete inhibition of the ELISA test occurring at 100 ng/ml of detomidine. The antibody also cross-reacted well with medetomidine, (I_{50} 4 ng/ml), and detomidine metabolite. Cross-reactivities for epinephrine, xylazine, and acepromazine were also evaluated (Fig. 2), and this antibody did not have any significant cross reactivity affinity with these drugs.

The ability of this ELISA test to detect detomidine or its metabolites in the urine of horses dosed with a number of clinically relevant doses of this drug was evaluated. Detomidine was administered intravenously at 2.0 µg/kg to four horses and

apparent detomidine levels peaked at about 100 ng/ml and decayed to less than 1.0 ng/ml by 12 hr after administration of the drug (Fig. 3). After administration of 8.0 µg/kg urinary concentrations of the drug peaked at about 500 ng/ml and had declined to about 10 ng/ml at 12 hr after adminstration of the drug (Fig. 4). When detomidine was administered at a rate of 20 µg/kg peak urinary concentrations from the four horses ranged from 400 to 1200 ng/ml at two hr post-dosing. These levels remained readily detectible for 12 hr and stayed above baseline levels through 24 hr (Fig. 5). The highest level received by the four horses was 40 µg/kg. Following this dose peak urinary concentrations ranged from 500 to 1500 ng/ml at two hr post-dosing. These levels remained readily detectible for 24 hr and stayed slightly above baseline levels through 48 hr (Fig. 6).

The ELISA reaction in urine was essentially complete from 1 hr through 24 hr post-dose. By 8 hr after dosing however, the reaction was only about 20% inhibited with inhibition still detectable at 24 hr post-dose. The inhibition profile for the 4.0 mg/horse and 1.0 mg/horse doses were similar, with the only difference being that inhibition had returned to base line by 8 hr after the 1.0 mg/horse dose.

When ELISA tests are used for post-race screening in race horse urines, unknown substances in horse urine gives variable levels of background or matrix effect that interfere with the assay. To evaluate this endogenous background activity, we added 20 µl aliquots of about 40 post-race urines[2] to the ELISA system and plotted a frequency distribution of the levels of "apparent drug" due to these background materials. We then compared the highest level of "apparent drug" with the I_{20} of the test for the drug. If, as was the case for detomidine, the level of apparent detomidine in any of the 40 samples is above the I_{20} for detomidine, extraction or dilution of the samples prior to assay is recommended. As shown in Figure 7, dilution of horse urines prior to assay reduced the highest apparent background in these samples to less than 5.0 ng/ml of apparent detomidine. This level of apparent background is sufficiently below the apparent I_{20} of this test that background interferance is not a problem. When reading this test using extracted urines, therefore, a positive is readily discernable as a clear "whiteout" well represented as "dosed horse urine" on Figure 7 against a panel of blue negative tests.

Discussion

The ELISA test for detomidine reported here is both rapid and sensitive and as such it represents a marked improvement over previously described radioimmunoassay (RIA) methods. Sensitivity is a crucial factor in a screening method. In this regard, regulatory control of equine medication faces problems similar to those in clinical and forensic screening for drugs of abuse in humans. The difficulty with all such testing is that insufficiently sensitive screening methods yield false negatives by failing to detect drugs which are actually present in test samples.

The development of immunoassay based drug screening technology has substantially improved the ability of racing chemists to detect drugs in racing horses. The ELISA system reported here is very sensitive to detomidine with half-maximal inhibition occurring at about 3.0 ng/ml detomidine. After a dose of detomidine as low as 1.0 mg/horse, this test detected the presence of detomidine or medetomidine in horse urine for at least 8 hr. Moreover, this level of detection sensitivity corresponds well with the previously determined threshold dose for the depressant/sedative effects of detomidine in the horse, it seems clear that this test has the ability to detect detomidine when this drug is used at threshold doses for pharmacological effect.

One of the reported characteristics of the illegal use of detomidine in performance horses is that this drug is administered to horses at doses in the order of about 2.0 µg/kg (about 1.0 mg/horse or less). Detection of any drug adminstered to horse at a dose of less than 1.0 mg/horse is analytically challenging and detomidine is no exception to this rule. Nevertheless, as shown in Figure 3, this dose of detomidine is readily detected by for at least eight hr after administration of detomidine. The data and review of this data suggests that lower doses of detomidine, in the order of 1.0 mg/horse are also detectable for several hr after their administration.

The ELISA technique is also rapid, requiring minimal pre-assay preparation for horse urine specimens. ELISA testing thus satisfies the requirement for speed of analysis, and represents a substantial saving of time and effort over older RIA-based procedures for pre- and post-race testing. However, despite the sensitivity and efficacy of this test and the suggested widespread use, there

have to date been no reported positives for detomidine based on this or any test for detomidine.

In summary therefore, we have developed a fast and sensitive ELISA test for detomidine. The test readily detected detomidine in urine after dosing with relatively small (1.0 mg/horse) doses of this drug. Based on the widespread use of detomidine, the small doses of this drug that are effective in horses, and the ease with which this drug can be administered to horses, a simple, inexpensive and sensitive test for detomidine such as the one described here is required for effective control of detomidine abuse in racing horses.

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[1]WIT ELISA TESTS INC., 311 Codell Dr., Lexington, KY.
[2]Urine specimens were provided by the Analytical Toxicology Laboratory of The Ohio State University, Columbus, OH.

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Figure 2 *Cross-Reactivity Plot. The ELISA test activity for the anti-detomidine antibody as a function of added drug concentration is shown. Half-maximal inhibition at 3.0 ng/ml for detomidine, 4.0 ng/ml for medetomidine, 3.0 ng/ml (COOH)-detomidine, and 1.0 ng/ml for (OH)-detomidine.*

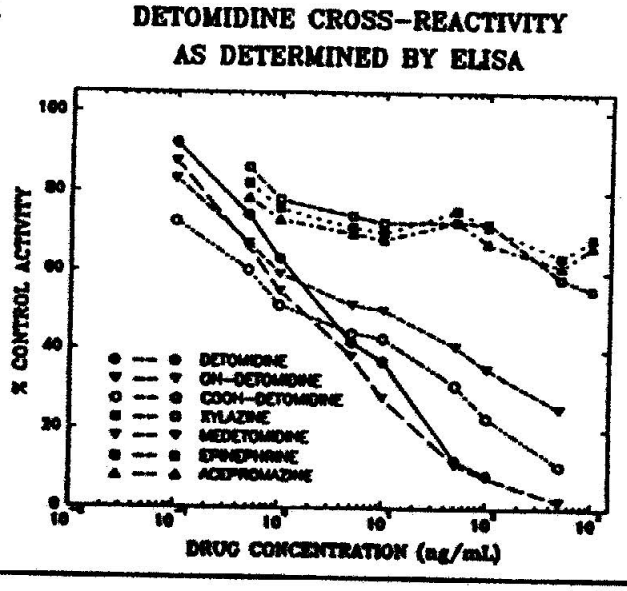

Figure 3 *Detomidine clearance in four horses administered 2.0 µg/ml detomidine HCl. Urine concentrations approach baseline (>1 ng/ml) 12 hr post-dosing.*

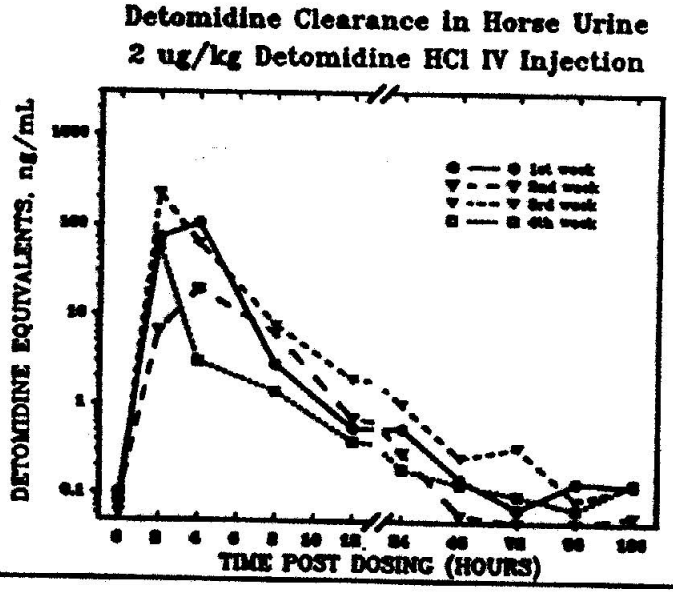

Figure 4 *Detomidine clearance in four horses administered 8.0 µg/ml detomidine HCl. Urine concentrations approach baseline (>1 ng/ml) 24 hr post-dosing.*

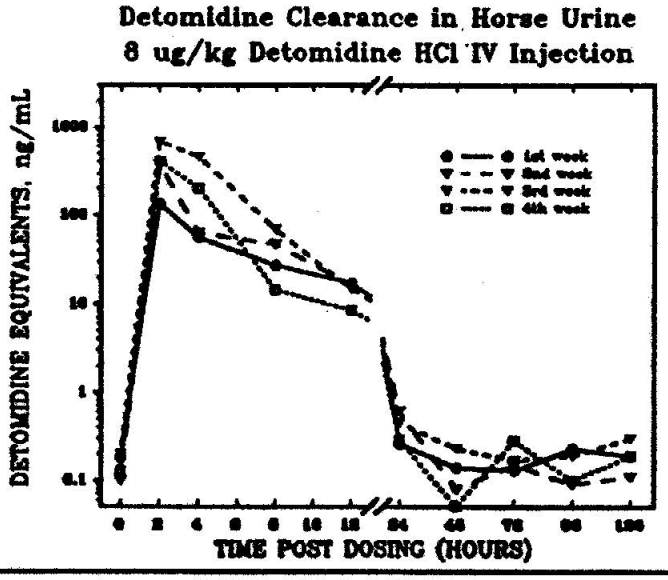

Figure 5 *Detomidine clearance in four horses administered 20 µg/ml detomidine HCl. Urine concentrations approach baseline (>1 ng/ml) 48 hr post-dosing.*

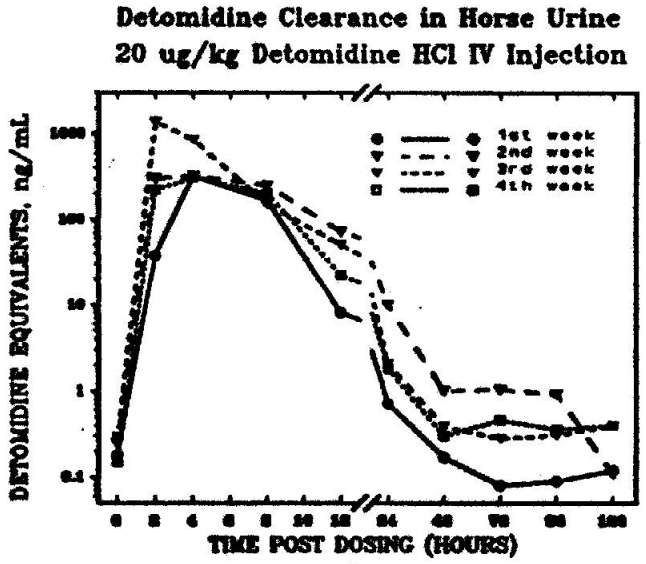

Figure 6 *Detomidine clearance in four horses administered 40 µg/ml detomidine HCl. Urine concentrations approach baseline (>1 ng/ml) 72 hr post-dosing.*

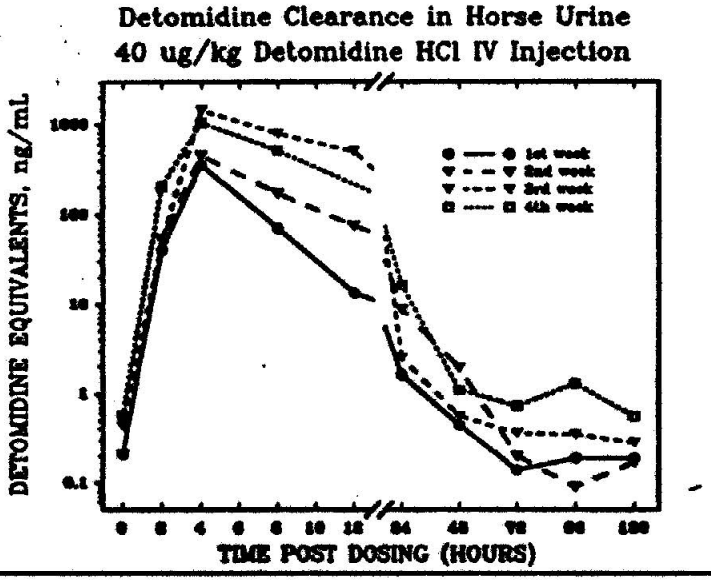

Figure 7 *The distribution of apparent detomidine concentration for 40 post-race urine samples is shown.*