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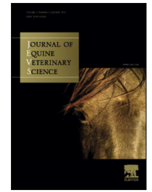

Short Communication

Disposition of Stanozolol in Plasma After Intra-articular Administration in the Horse

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ABSTRACT

The purpose of this study was to provide data on the disposition of stanozolol after bilateral intra-articular injection in the tarsal joints, to discover the length of time for which the drug can be detected in plasma after administration. Fourteen horses were included in the study. After aseptically preparing the injection site, 1 mL of an aqueous suspension containing 5 mg of stanozolol was injected into both the right and left tarsal joints of 12 horses; the two remaining animals were not treated and were used as a control group. Five milliliters of blood was collected immediately before stanozolol administration (t0) and at 1, 2, 4, 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, and 168 hours after injection. The plasma concentration was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) after solid phase extraction. The maximum plasma concentration was 1.7 ng/mL (range, 0.5–3.0 ng/mL), measured at 6 hours (range, 4–12 hours). The plasma elimination half life varied between 4 and 12 hours, whereas the plasma clearance per fraction of dose absorbed was in the 257.85–820.88 L/h range. The results of the present study make a preliminary contribution toward understanding the elimination profile of intra-articularly administered stanozolol in the horse. The drug passes rapidly into the systemic circulation, is eliminated rapidly, and is detected in plasma for no more than 36 hours after local administration.

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1. Introduction

Stanozolol is a potent anabolic androgenic steroid. This drug is prescribed for short-term treatments. Prolonged use can produce side effects, particularly on the reproductive system in both human and horses [1,2]. The Association of Racing Commissioners International listed stanozolol as a class III drug that may or may not have generally accepted medical use in the racing horse [3].

In equine practice, the intra-articular (IA) administration of corticosteroids is commonplace especially in racing horses, for the treatment of exercise-induced joint damage and to minimize the damage caused by intensive training [4]. For most corticosteroids, drug concentrations are detected for a longer period of time in plasma after IM versus IA administration. Recently, the results of clinical IA use of 5 mg of stanozolol in horse joint diseases have been reported, and its disease-modifying effects in equine osteoarthritis have been emphasized [5]. The disposition of stanozolol in the horse after IM injection of a standard dose (0.55 mg/kg body weight) has been described in two different studies [6,7]. To our knowledge, no information has yet been reported regarding the disposition of

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stanozolol after IA administration. The purpose of this study was to provide data on the disposition of stanozolol after bilateral IA injection of an aqueous suspension containing 5 mg of the drug, which could be useful to discover the length of time for which the drug can be detected in plasma.

2. Materials and Methods

2.1. Study Design

In the present study, the plasma kinetics of stanozolol was investigated in 14 horses. The inclusion criteria were horses aged between 11 and 14 years; stallion, mare, or gelding; the animals had to be healthy, with no lameness in their hindlimbs, and must not have received any pharmacological treatment in the previous month.

The animals were cared for and handled in accordance with Directive 86/609/EEC, adopted by the Italian Government with Italian Law 116/1192. Horse owners signed an informed consent form for inclusion in the study and undertook to adhere strictly to the rules of the study. The study was conducted following authorization by the Italian Ministry of Health, Decree no 166/2006 B.

2.2. Treatment

The tarsal joints were aseptically prepared, and joint centesis was performed by an equine orthopedic surgeon (AS). After withdrawing 1 mL of joint fluid from each side, 1 mL of aqueous suspension containing 5 mg of stanozolol was injected into both the right and left tarsal joints of 12 horses. The two remaining animals were not treated and were used as a control group.

Each vial (1 mL) of stanozolol suspension was prepared as follows: sodium chloride and di-sodium phosphate dodecahydrate were dissolved in the 90% of the total water for injection. The solution was made up to pH 7.5 with phosphoric acid (buffering system). Polysorbate 80 (surfactant) was dissolved in the remaining water for injection. The two solutions were mixed to obtain the suspending medium. The stanozolol was added into the suspending medium and suspended by a turbine. Once the product was homogenous, the final containers were filled. The concentration of the active principle was assessed instrumentally before each use.

2.3. Blood Sample Collection and Plasma Stanozolol Determination

A catheter in the right jugular vein was used for blood sampling. Five milliliters of blood was collected immediately before stanozolol IA administration (t0) and at 1, 2, 4, 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168 hours after injection. To avoid contamination with the venous flushing solution, 2 mL of blood was collected before each sampling and re-injected thereafter. All samples were immediately transferred to heparinized tubes and centrifuged (1500× *g*) for 20 minutes at room temperature. After centrifugation, the plasma was separated from the RBCs, transferred into clean tubes, and stored at −80°C until analysis, which was performed within 2 months.

Stanozolol plasma concentration was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) after solid phase extraction. Two millliliters of plasma was diluted with 2 mL of water and loaded onto an Oasis HLB (6 cc, 500 mg) cartridge (Waters, Milford, MA). This was then washed with water followed by a water/acetone (4/1) solution and dried by applying a soft vacuum pump. Elution was performed firstly with methanol and then with ethyl acetate. The extract was dried under a gentle nitrogen stream and finally reconstituted in a water/acetonitrile (1/1) solution containing 0.2% formic acid.

Quantitative determination was performed on a UPLC-MS/MS system by Waters, including an Acquity UPLC binary pump, equipped with an Acquity UPLC BEH C18 (50 × 2.1 mm, 1.8 μm) reversed-phase column (Waters, Milford, MA), maintained at 40°C. The mobile phase consisted of water and acetonitrile, both containing 0.1% formic acid. Analyses were conducted under programmed conditions at a constant flow rate of 0.450 mL/min, and 5 μL of the sample was injected. The LC system was coupled to a Waters Quattro Premier XE mass spectrometer (Waters, Milford, MA), operating in positive electrospray ionization (ESI+) mode. The capillary voltage was 3.6 kV, whereas the cone voltage was set at 70 V. Source and desolvation gas temperature were 120°C and 450°C, respectively. Two transitions were monitored: 329.6 > 121.2 m/z (collision energy: 37 eV) and 329.6 > 107.1 m/z (41 eV). Data acquisition and processing were carried out using TargetLynx 4.1 software (Waters, Milford, MA).

The method was fully validated according to EMA guidelines (EMEA/CHMP/EWP/192217/2009). Plasma calibrators were prepared by adding stanozolol solutions to drug-free equine plasma, obtaining final concentrations of 0.2, 0.5, 1, 1.5, 5, 10, and 15 ng/mL. A linear regression model was applied to calibration curves, proving the linear response of the analyte and giving correlation coefficients (R2) that were always >0.99. The method was optimized to provide a lower limit of quantification (LLOQ) of 0.2 ng/mL, and specificity was assessed by the absence of interferences at the specific retention time of stanozolol in 20 blank plasma samples. Recovery and precision were evaluated with samples fortified at three different concentrations (0.5, 1.5, and 5 ng/mL) and analyzed in six replicates per day on three different days. Mean recoveries for the three concentrations ranged between 77% and 81%, whereas intraday and interday precision (percentage relative standard deviation) were below 11% and 15%, respectively. Matrix-matched calibration curves and quality control samples were freshly prepared on each day of analysis to ensure that the method continued to perform well. The technique used was found to be suitable for the quantitative determination of stanozolol plasma concentration after IA injection.

2.4. Statistical Analysis

The pharmacokinetic data are reported as median values and ranges. Nonlinear least square regression was performed on plasma concentration versus time data using

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