Preliminary Pharmacokinetics of Diclazuril and Toltrazuril in the Horse

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This pilot study suggests that diclazuril and toltrazuril are absorbed after oral administration and have longer (40–55 h) plasma half-lives. These kinetic characteristics suggest readily maintained steady-state plasma concentrations. These are useful characteristics for therapeutic agents; however, therapeutic specifics for these agents in the horse remain to be determined. Authors' address: Maxwell H. Gluck Equine Research Center and Dept. of Veterinary Science, University of Kentucky, Lexington, KY 40506.

1. Introduction

Diclazuril and toltrazuril are triazine-based coccidiocidal agents that are effective against coccidia in birds and mammals. In unpublished preliminary *in vitro* research from our lab, toltrazuril was active against *Sarcocystis neurona*. Similarly, there is limited preliminary evidence that may suggest that diclazuril has the potential to be active against *S. neurona*.[1] This report describes analytical detection methods and preliminary pharmacokinetics for toltrazuril and diclazuril in the horse.

2. Materials and Methods

A. Experimental Approach

Five horses were dosed orally with 10 mg/kg of toltrazuril and four horses were dosed with 5 mg/kg of diclazuril. In a second experiment, two horses were dosed intravenously with 1.0 g of toltrazuril. Samples were taken for up to 7 days in both experiments and all samples were stored at −20 °C prior to analysis.

Toltrazuril (Baycox[a]) was provided in both its pure form and as an oral formulation. Diclazuril (Clinacox[b] 0.5%) was obtained in its pure and oral forms.

B. Pharmacokinetic Analysis

Pharmacokinetic analysis was determined by using a nonlinear regression program, NONMEM.[c] The area under the curve was measured by linear trapezoidal approximations with extrapolation to infinity, and the slope of the log of the terminal half-life was determined by the method of least-squares regression.

C. Diclazuril–Toltrazuril Extraction and Detection

Toltrazuril was recovered on a Varian Bond Elut column and eluted with 4 ml of methylene chloride. The solvent was evaporated under a stream of nitrogen gas at 40°C, and the residue was reconstituted in 150 µl of ethyl acetate for mass spectral analysis by using a Hewlett-Packard 3890 GSMS. A GC/MS analysis of toltrazuril yielded sharp peaks and provided good linearity in the 10–5000 ng/ml standard range.

NOTES

Diclazuril and a methylated diclazuril internal standard were recovered from plasma samples by using solid phase extraction. The samples were washed and eluted with 95% methanol:5% HCl. A high-performance liquid chromatography analysis of diclazuril and its internal standard using a Beckman C18 column and 280-nm UV detection produced a linear standard curve from 5 to 400 ng/ml.

3. Results

The oral administration of single doses of toltrazuril 10 mg/kg yielded a mean peak plasma concentration of 4.5 µg/ml with no signs of toxicity. The apparent plasma half-life of toltrazuril was approximately 55 h. The oral administration of a single dose of diclazuril 5 mg/kg yielded a peak plasma concentration of 1 µg/ml after 24 h and declined with an apparent plasma half-life of ~50 h.

A preliminary pharmacokinetic analysis suggests that these agents are well absorbed after oral administration. Daily dosings of toltrazuril 10 mg/kg and diclazuril 5 mg/kg are projected to yield steady-state plasma concentrations of 30 and 5 µg/ml, respectively, with up to 10 days required to attain a steady-state plasma concentration.

4. Discussion

Both diclazuril and toltrazuril are selectively toxic for apicomplexans, and this activity appears to include *S. neurona*.[2] Toltrazuril and diclazuril appear to be absorbed after oral administration and to have long plasma half-lives. The pharmacokinetics described in this report are consistent with a *potential* for therapeutic efficacy of members of the benzene acetonitrile group in certain selected circumstances. However, further critical studies on the therapeutic window, potential for therapeutic efficacy, and potential for adverse reactions in this group of agents are clearly required.

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References and Footnotes

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2. Lindsay DS, Blagburn BL. Activity of diclazuril against *Toxoplasma gondii* in cultured cells and mice. *Am J Vet Res* 1994;55:530–532.

[a]Bayer Pharmaceuticals, Leverkusen, Germany.
[b]Janssen Pharmaceuticals, Beerse, Belgium.
[c]Micromath Scientific Software, Salt Lake City, UT 84121.