348 04-14-21

# Cyanide in the Horse: Normal Blood Concentrations and their Elevation, Monitoring and Manipulation

CG Hughes, L Dirikolu, AF Lehner, JD Harkins, M Sebastian, J Crutchfield, T Tobin

Equine Pharmacology, Therapeutics and Toxicology Laboratory, Department of Veterinary Science, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546-0099

**Abstract**

In the Dubai Equine Grass Sickness Project we have focused on providing the required analytical tools and the pharmacokinetic and toxicokinetic knowledge which will allow safe and humane administration of cyanide to experimental horses. In cooperation with the US Army Medical Research Institute and our colleagues at the Livestock Disease Diagnostic Center and the Department of Agronomy at the University of Kentucky, we have developed a highly sensitive, reliable, and economical semi-automated analytical method for the spectrophotometric determination of cyanide ($CN^-$) concentrations in equine blood, as well as thiocyanate ($SCN^-$) in equine serum. The analytical method is capable of quantifying blood cyanide concentrations down to 2 ng/ml and serum thiocyanate concentrations down to 200 ng/ml. Using this method we have readily detected and quantitated cyanide and thiocyanate after administration of sodium cyanide to horses. We have also defined the safe working range for blood cyanide concentrations in the horse as well as the toxicokinetics of cyanide in the horse. We have also established the range of normal blood cyanide concentrations found in horses at pasture in Kentucky. Horses on fall pasture in Kentucky had blood cyanide concentrations between 3 and 18 ng/ml, with significant differences in blood cyanide concentrations from pasture to pasture. Hay-fed horses in January had blood cyanide concentrations of 2-7 ng/ml. Blood cyanide concentrations in a small number of cattle at pasture showed similar concentrations. Serum thiocyanate concentrations from horses sampled in the spring were in the lower ranges of concentrations reported in the literature as normal for livestock and humans.

Intravenous infusions of sodium cyanide solutions were performed in order to evaluate the distribution and elimination kinetics of cyanide. Data suggested a two-compartment, open model with distribution (alpha) phase half-life of 0.74 hours and terminal (beta) phase half-life of 16.16 hours. The mean residence time was 12.4 hours. Steady-state volume of distribution was 2.21 L/kg and the mean systemic clearance was 0.0182 L/h/kg. Cyanide was approximately 98% associated with the red cells with about 2% in plasma. With the goal of experimentally manipulating blood cyanide concentrations in the horse, we showed that oral administration of amygdalin and apricot seed amygdalin can be used to increase blood cyanide concentrations in horses. We have defined the pharmacokinetics and toxicokinetics of cyanide in the horse after its administration by various routes and as associated oral precursors. At higher doses of cyanide, consistent with those found in most human forensic cases of toxicity, cyanide appears to be rapidly distributed to the CNS where it produces its primary toxic effects. The acute toxic effects of cyanide when administered as a high, sub-lethal, bolus dose are rapidly terminated by

redistribution. At lower or subacute exposures to cyanide, the metabolic phase of detoxification appears to be dominant. Under these circumstances, the toxicokinetics of cyanide are characterized by a large volume of distribution and long plasma half-life, on the order of 16 hours. Under these circumstances, it is clear that it may require as much as 60 hours to achieve steady-state blood concentrations of cyanide in the horse. Thus, changes in steady-state blood concentrations of cyanide after exposure to low doses may be relatively gradual, and it is likely that the low concentration toxic effects of cyanide will be due to subtle effects on specific metabolic pathways.

We have transferred the analytical technology which underpins this experimental work to our colleagues in Dubai, along with the pharmacokinetic and toxicokinetic data developed during this work. This work constitutes the first detailed analysis of normal blood cyanide concentrations and the pharmacokinetics and toxicokinetics of cyanide in the horse.

This work has been reported in the following papers:

Hughes C, Lehner F, Dirikolu L, *et al.* A Simple and Highly Sensitive Spectrophotometric Method for the Determination of Cyanide in Equine Blood. *Toxicology Mechanisms and Methods,* 13:129-138, 2003.

Dirikolu L, Hughes C, Harkins D, *et al.* The Toxicokinetics of Cyanide and Mandelonitrile in the Horse and Their Relevance to the Mare Reproductive Loss Syndrome. *Toxicology Mechanisms and Methods*, 13:199-211, 2003.

Harkins JD, Dirikolu L, Sebastian M, *et al.* Cherry Trees, Plant Cyanogens, Caterpillars and Mare Reproductive Loss Syndrome: Toxicological Evaluation of a Working Hypothesis. *Proceedings of the First Workshop on Mare Reproductive Loss Syndrome*, 68-74, 2003.

Hughes C, Lehner A, Crutchfield J, *et al.* Analytical Detection and Normal Population Data for Cyanide and Thiocyanate in Equine Blood and Serum. *Proceedings of the 14th International Conference of Racing Analysts and Veterinarians*, Orlando, FL 2002, pp. 376-382.

This work would not have been possible without the input, cooperation and expertise of the following researchers, listed alphabetically.

Steven I. Baskin, Jeff Boyles, Jim Crutchfield, Levent Dirikolu, Terrence Fitzgerald, J. Daniel Harkins, Lenn Harrison, Jimmy Henning, Charlie Hughes, Fritz Lehner, Wayne Long, Karen McDowell, Manu Sebastian, Amy Troppmann.

The support from the Director of the Maxwell H. Gluck Equine Research Center and the Dubai Millennium Research Foundation is also gratefully acknowledged.

Publication #348 from the Equine Pharmacology, Therapeutics and Toxicology Program of the Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY.