CLINICAL PHARMACOLOGY REVIEW

Division of Hematology
Office of Blood Review & Research

STN 125335
Product: Anascorp, Centruroides (Scorpion), Immune $F(ab)_2$ intravenous (equine)
Sponsor: Instituto Bioclon
Indication: Treatment of clinically important signs of scorpion venom
Date Received: January 22, 2009
Reviewer: Iftekhar Mahmood, Ph. D.
RPM: Debra Cordaro
Through: Basil Golding, M.D.

TABLE OF CONTENTS

Introduction

Scorpion envenoming in Mexico is a serious health problem with an incidence of 213,458 cases in 2004. The effectiveness of antivenom treatment depends on the potency of the antivenom, its activity spectrum, the time that elapses from the envenomation to the treatment onset, as well as the antivenom pharmacokinetics (PK). The ideal antivenom must adequately reach the different tissues in which venom produces its toxic effect and, once bound to toxin, the complex must be rapidly eliminated. Adverse events associated with the use of antivenoms are related to their purity and constitution.

Fabotherapics are used as specific antidotes in envenomation from scorpion stings, the bites of spiders and different species of snakes. With recent $F(ab)_2$ purification techniques the risks of allergies, anaphylactic shock or serum sickness are reduced.

In their Biologic License Application (BLA), the sponsor (Instituto Bioclon) submitted a pharmacokinetic study report for Antivenom Crotalinae (pit viper) Equine Immune $F(ab)_2$. The sponsor however, did not submit a detailed PK report of Anascorp, Centruroides (Scorpion),

Immune $F(ab)_2$ intravenous (equine), the product for which the sponsor is seeking FDA's approval. Rather than submitting a detailed PK report for Anascorp, the sponsor submitted a published manuscript (Vazquez H, et al. Pharmacokinetics of a $F(ab)_2$ scorpion antivenom in healthy human volunteers. Toxicon 46 (2005) 797–805). Therefore, the following review is based on the manuscript submitted by the sponsor. The sponsor however, provided FDA with plasma concentration-time data of Anascorp. Although, in the submitted manuscript, PK parameters were estimated, the plasma concentration-time data of Anascorp was re-analyzed by the PK reviewer in the FDA just to ensure the accuracy of the sponsor's PK analysis. The PK parameters information in the labeling and the review is based on the FDA's analysis of the PK data.

CLINICAL PHARMACOLOGY LABELING COMMENTS

CLINICAL PHARMACOLOGY

12.1 Mechanism of Action[1]

Anascorp is a venom-specific $F(ab)_2$ fragment of immunoglobulin G (IgG) that binds and neutralizes venom toxins, facilitating redistribution away from target tissues and elimination from the body.

12.2 Animal Studies (delete the entire section from clinical pharmacology. This section should be placed under non clinical studies)

~~Anascorp Centruroides (Scorpion) immune $F(ab)_2$ intravenous (Equine) is produced using the venoms of 4 different Centruroides scorpions. Studies using ---------(b)(4)---------- have shown high cross-reactivity of the Anascorp $F(ab)_2$ to toxins from eight different Centruroides species, including *C. exilicauda*. In addition, complete neutralization of *C. sculpturatus/exilicauda* venom in mice was demonstrated using Anascorp.~~

~~Anascorp venom-specific antigen binding fragments bind to scorpion venom thereby preventing or reversing the systemic effects of scorpion envenomation. When venom-specific $F(ab)_2$ is injected into rabbits pre-treated with Centruroides venom, serum venom area under the curve is increased by 2 orders of magnitude. This indicates Centruroides immune $F(ab)_2$ binds to the scorpion toxins, removes the toxins from their site of action, and sequesters them in the vascular space.~~[1]

12.3 Clinical Pharmacokinetics (delete because this is not the PK of the product). Please see the FDA's clinical pharmacokinetics labeling recommendation.

~~No controlled pharmacokinetic studies were conducted using Anascorp, nevertheless a well-controlled pharmacokinetic study in healthy volunteers was conducted with an antisnake $F(ab)_2$ that has essentially the same physicochemical characteristics. Analysis included determination of the pharmacokinetic parameters of AUC, Vc, Vss, mean residence time, elimination half-life, distribution half-life, and total clearance.~~

~~Table 2. Pharmacokinetic Parameters in Human Plasma for Antisnake $F(ab)_2$~~

~~(n=13)~~	**~~Units~~**	**~~Mean~~**	**~~SD~~**
~~$AUC_{0-\infty}$~~	~~µg·hr/mL~~	~~4144~~	~~669.77~~
~~Vc~~	~~L~~	~~3.54~~	~~0.61~~
~~Vss~~	~~L~~	~~6.37~~	~~1.65~~
~~Mean residence time~~	~~hr~~	~~157.42~~	~~39.78~~
~~Elimination $T_{1/2}$~~	~~hr~~	~~133.01~~	~~52.53~~
~~Distribution $T_{1/2}$~~	~~hr~~	~~16.03~~	~~8.98~~
~~CL~~	~~L/hr~~	~~0.04~~	~~0.01~~
~~C_{max}~~	~~µg/L~~	~~47.84~~	~~8.84~~

~~A separate pharmacokinetic study was performed in healthy volunteers using Anascorp. The results demonstrated comparable pharmacokinetics for Anascorp compared to the antisnake $F(ab)_2$.~~[1,4]

FDA's clinical pharmacokinetics labeling recommendation:

Eight clinically healthy volunteers (6 males and 2 females, age: 17 to 26 years) received a bolus intravenous dose of 47.5 mg of scorpion antivenom Alacramyn. Blood samples were collected till 504 hours (21 days) and pharmacokinetic parameters were estimated by non-compartmental analysis which are summarized in Table 2.

Table 2

Pharmacokinetic parameters of scorpion antivenom Alacramyn

Parameters	Mean ± sd
$AUC_{(0-\infty)}$ (µg*hr/mL)	706 ± 352
Clearance (mL/hr)	83.5 ± 38.4
Half-life (hrs)	159 ± 57
V_{ss} (liters)	13.6 ± 5.4

RECOMMENDATION

The review is based on a published manuscript by Vazquez H, et al. From the manuscript, the method for PK study appears to be acceptable. However, it is not known if the study meets the "Good Laboratory Practice" standard. The sponsor provided plasma concentration-time data of the PK study that was analyzed by the PK reviewer in the FDA. This analysis is the basis of the PK parameters shown in the clinical pharmacology labeling section as well as in this review.

Iftekhar Mahmood, Ph. D.
Senior Clinical Pharmacology Reviewer
Division of Hematology
Office of Blood Review & Research

Basil Golding, MD
Division Director, Division of Hematology
Office of Blood Review & Research

Study Title: Pharmacokinetics of a $F(ab)_2$ scorpion antivenom in healthy human volunteers.

Eight clinically healthy volunteers (6 males and 2 females, age: 17 to 26 years) from the Antiscorpion Center of the Mexican Red Cross, Leon branch, Guanajuato, Mexico, were enrolled. The subjects received a bolus intravenous dose of 47.5 mg of scorpion antivenom Alacramyn. One ampoule of the product was able to neutralize 150 mice lethal doses 50% equivalent to 0.75 mg protein of Centruroides limpidus scorpion venom. One ampule of Alacramyne is the dose used clinically in 76.9% of patients. The Alacramyne used was all from Lot. BOJ04, and had the following composition determined by triplicate using FPLC on a Zorbax GF250 column (4.6 x 250 mm):

Dimeric (aggregated) $F(ab)_2$ = 1.9%
$F(ab)_2$ = 87.7%
F(ab) = 4.8%
Low molecular weight compounds = 5.6%

Blood samples for PK study were collected at time 0, 5, 15, 30, 45, 60, 90, 120, 180 and 360 min, and at 24, 48, 72, 96, 240, and 504, hours.

Antivenom measurement in serum:

A highly specific sandwich immunoassay was developed for measuring horse $F(ab)_2$ fragments in human serum. The immunoassay uses the specific chicken antibodies and a commercial conjugate to detect Alacramyn. Polystyrene micro plates (Maxisorp, Nunc, Inc., USA) were coated overnight at 4 ^{o}C with 100 ml/well of 5 mg/mL immunopurified chicken IgY anti-Alacramyn in 100 mM carbonate/bicarbonate buffer, pH 9.5. The plates were then washed four times with washing buffer (50 mM Tris/HCl pH 8, 150 mM NaCl and 0.05% Tween 20). The remaining binding sites were blocked with Tris/HCl pH 8, containing 0.5% gelatin and 0.05% Tween 20 for at least 2 h at 37 ^{o}C. The plates were then washed four times with washing buffer. Alacramyn standards were prepared in 10% of pool human sera from healthy donors in vehicle buffer (50 mM Tris/HCl pH 8, 500 mM NaCl and 0.1% gelatin, 0.05% Tween 20); serum samples were also diluted in the same buffer. Samples and antivenom standards (100 mL/well) were added to the plates and incubated 1 h at 37 ^{o}C. Plates were washed four times with washing buffer and then 100 ml/well of affinity purified goat anti-horse $F(ab)_2$ conjugated to peroxidase (Rockland, Gilbertsville, PA) were added and incubated 1 h at 37 ^{o}C. After washing, 100 mL/well ABTS solution (Roche) were added and incubated 15 min at 25 8C; after this time the reaction was stopped with 25 ml/well of 20% sodium dodecyl sulfate. Absorbance was read at 405 nm in a Microplate Reader Model 550 (BIO-RAD). To measure the concentrations of antivenom, serum samples were diluted 1:10 and 1:50 and incubated in triplicate. Alacramyn calibration curves were run in triplicate on each plate using a range of antivenom concentrations from 1.5 to 30,000 ng/mL, as determined by a Coomassie Protein Assay Kit (Pierce). Sigmoidal curves were generated using a non-linear regression program (Prism, GraphPad). The useful

range was found to be between 15 and 1100 ng/ml. High sample dilutions (1:50) were used to quantitate antivenom concentration in samples obtained within 6 h after antivenom administration whereas a lower dilution (1:10) was used for samples collected after 24 h.

Pharmacokinetic Analysis and Results:

The pharmacokinetic parameters of Alacramyn were estimated by non-compartmental analysis and are summarized in Table 1. The results of the analysis indicates that Alacramyn has a long half-life and slow clearance.

Table 1

Pharmacokinetic parameters of scorpion antivenom Alacramyn

Parameters	Mean ± sd
$AUC_{(0-\infty)}$ (μg*hr/mL)	706 ± 352
Clearance (mL/hr)	83.5 ± 38.4
Half-life (hrs)	159 ± 57
V_{ss} (liters)	13.6 ± 5.4

Conclusions

Based on its pharmacokinetic characteristics, Alacramyn can be described as a long half-life and slow clearance drug.