



Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Biologics Evaluation and Research

To: BLA STN 125488/0

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Applicant: Instituto Bioclon S.A. de C.V.

Product: Crotalidae Immune Fab2 Equine Injection Anavip®

Proposed indication: Management of patients with pit viper envenomation and prevention of late or recurrent coagulopathies

Subject: Memo, Nonclinical Pharmacology/Toxicology

Table of Contents

Conclusions and Recommendation.....	2
Complete Review.....	3
<i>Assessment of Excipients and Impurities</i>	3
Glycine	4
Sucrose	5
Cresol	5
Borates and Boron.....	6
<i>Animal studies</i>	6
Pharmacology Study, Toxicon 41, (2003) 357-365	6

Conclusions and Recommendation

There are no preclinical issues to prevent this application from being approved.

The formulation of Anavip® contains cresol at a higher concentration than Anascorp®. There is potential for adverse reactions due to cresol such as generalized myalgias. A label warning should be included (b) (4)

The other impurities and excipients present in Anascorp® final product are safe when product is used according to the PI.

The GLP animal study submitted (#1299-001, Acute Toxicology Study in Rats) cannot be used to derive a NOAEL of Anavip® in animals for label use as the sponsor failed to demonstrate that intended systemic exposure with the biologic was achieved in this study.

Complete Review

Assessment of Excipients and Impurities

This assessment was performed based on the Anavip® dose that is being sought, i.e. 10 vials, up to a maximum of 24 vials. Specifications for Anavip® are shown in Table 1. With the exception of cresol and borates, all the other compounds are commonly found in IGIV products. The exposure for all excipients and impurities following a typical (10 vials) and maximal (24 vials) dosing of Anavip®, is shown in Table 2.

Table 1 Specifications for Anavip® (from submission)

Test Description	Test Method(s)	Specifications / Limit(s)
Appearance (Lyophilized)	Visual SOP M-FQ-078	(b) (4)
Appearance (Reconstituted)	Visual SOP M-FQ-078	Yellow-green, opalescent liquid
Identification	(b) (4) - SOP M-CB-011	Meets requirements
Potency	SOP M-CB-016	BF: NLT 780 LD ₅₀ neutralized/vial CF: NLT 790 LD ₅₀ neutralized/vial
Purity ((b) (4))	SOP M-CB-027	F(ab) ₂ NLT 85% Fab NMT 7% (b) (4)
Purity ((b) (4))	SOP M-CB-001	(b) (4) IgG NMT 5%
(b) (4)	(b) (4)	(b) (4)
Protein Content	(b) (4) SOP M-CB-005	NMT 120 mg / vial
Sulfate	(b) (4)	NMT 1.7 mg / vial
Cresol	SOP M-FQ-019	NMT 0.99 mg / vial
Sterility	(b) (4)	Meets requirements
Pyrogens	(b) (4)	Meets requirements
Glycine	SOP M-FQ-091	16.2 – 51.8 mg / vial
(b) (4)	(b) (4)	(b) (4)
Sodium Chloride	SOP M-FQ-092	25.2 – 56.8 mg/vial
Borates	Instituto Bioclon	NMT 1.0 mg/vial

Sucrose	SOP M-FQ-093	18.2 – 85.8 mg/vial
Safety	21 CFR 610.11	Meets requirements
Moisture Content	(b) (4)	(b) (4)
Reconstitution	SOP M-FQ-038	(b) (4)
Leak Test	SOP M-FQ-030	(b) (4)

Table 2 Potential Patient Exposure to Excipients and Impurities in Anavip

Compound	Amount Anavip	Exposure After 10 Vials, mg	Exposure After 24 Vials, mg
Glycine	NMT 51.8 mg/vial	518	1243
Sucrose	NMT (b) (4) mg/vial	(b) (4)	(b) (4)
Sodium Chloride	NMT (b) (4) mg/vial	(b) (4)	(b) (4)
Borates	NMT 1.0 mg/vial	10	24
Sulfate	NMT 1.7 mg/vial	17	40.8
Cresol	NMT 0.99 mg/vial	9.9	23.76

Sodium chloride and sulfate are inorganic salts commonly used in pharmaceutical formulations, including IgIV products, thus represent no safety risk to patients. An analysis of the safety of all the other excipients in Anascorp® is presented below.

Glycine

Final specification of Glycine (FW 75.07 g/mol) is set at NMT 51.8 mg/vial. This corresponds to a maximum exposure to glycine of 518 mg following a dose of 10 vials of Anavip®, or 1243 mg after 24 vials.

Table 3 shows concentration of glycine in different approved IGIV products. Given the volumes of these products used during IGIV therapy for PIDD i.e. several hundred mL, the amount of glycine in Anavip® will result in exposures that are smaller than those routinely obtained in clinical practice with approved IGIV therapies. Thus, glycine in Anavip® formulation does not represent a safety risk to patients.

Table 3 Glycine in IGIV Approved Products and Anavip®

Product Name/Concentration (Sponsor)	Glycine Concentration According to Label	Glycine Dose
Gamunex (Talecris)	0.24 M (18 mg/mL)	8,100 mg
Gammagard Liquid/10% (Baxter)	0.25 M (18.7 mg/mL)	8,415 mg
Gammagard S/D/5% (Baxter)	0.30 M (22.5 mg/mL)	20,250 mg
Anavip®	NMT 51.8 mg/vial	518 mg

Calculated assuming an IGIV dose of 600 mg/kg and a 75 kg patient

Sucrose

Sucrose is present in the final formulation of Anavip® at a total amount of NMT (b) (4) mg/vial. Sucrose from IGIV products has been associated with Acute Renal Failure (ARF) when resulting in sucrose exposure of 1 g/kg, perhaps smaller for susceptible populations. If receiving a dose of 10 vials of Anavip®, a patient's exposure to sucrose would be (b) (4) mg (Table 2). This, for a 75 kg patient, corresponds to (b) (4) and for a 2.5 kg neonatal patient would be (b) (4). Even after a 24 vial dose, the amount of sucrose would be smaller than 1 g/kg. As such, taking Anavip® would result in an exposure to sucrose smaller than the amount associated with kidney damage in adults and newborns, respectively. Thus, the amount of sucrose present in the final formulation of Anavip® is unlikely to cause kidney damage when the product is used according to PI.

Cresol

Cresol is used (b) (4) during manufacturing steps and is present in final Anavip® product at a specification NMT 0.99 mg/vial resulting in a total patient exposure of 9.9 mg (0.13 mg/kg) cresol from 10 vials or 23.8 mg (0.3 mg/kg) from 24 vials. The specification for Anascorp® is set at NMT (b) (4), which corresponds to NMT (b) (4).

Cresol is present in many insulin and insulin analogs, as well as growth hormone preparations where it is used a (b) (4). For example, Apidra (insulin glulisine [rDNA origin] injection) contains 3.15 mg/ml cresol or 3.15 mg for each 100 U of insulin. Given the typical dose of Apidra of 0.5-1 U/kg/day, this amount corresponds to 0.015-0.03 mg/kg/day.

Cresol at these doses when used as an injectable has been associated with myalgia and elevated creatine kinase activity, and malignant hyperthermia. For this reason, the following warning is included in both classes of the injectables mentioned: "Localized reactions and generalized myalgias have been reported with the use of cresol as an injectable excipient".

The exposure to cresol from Anavip® could be up to 20 times higher than the one resulting from daily dosing of Apidra. Given for this high potential exposure it is recommended that the (b) (4)

National Toxicology Program (NTP) has evaluated the potential genetic toxicology of cresols in cell culture and *in vitro* as well as potential carcinogenicity *in vivo* in 2 year studies in rats and mice. All *in vitro* tests did not show a signal whereas in 2 year studies cresol showed "equivocal evidence" of carcinogenetic activity in male F344/N rats (marginally increased incidence of renal tubule adenoma at a dose of 15,000 ppm or at least 750 mg/kg) and "some evidence" of carcinogenic activity in female mice (increased incidence of forestomach squamous cell papilloma at 10,000 ppm or at least 1,429 mg/kg).

Additional non-neoplastic lesions were also seen at all doses used, starting at 230 mg/kg in rats and 300 mg/kg in mice. Both studies determined a NOAEL for carcinogenicity in the chronic studies at 70 and 100 mg/kg cresol. Base on this study, a human equivalent dose (HED) can be calculated using appropriate conversion factors (Table 4).

Based on the indication of the product, i.e. not intended for chronic use, the lack of mutagenic signal during *in vitro* tests, the human exposure being similar to HED of NOEL in the animal studies, and the regulatory precedent i.e. its presence in insulin and growth hormone products, the use of cresol in Anascorp® is not likely to pose a carcinogenicity risk to patients.

Table 4 Safety Margins for Chronic Cresol Exposure

Carcinogenic (NOAEL) Chronic Animal dose	Carcinogenic (NOAEL) Chronic HED ^a	Safety margin ^b (NOEL/Dose ^c)
Rats, 750 (70) mg/kg	120 (11) mg/kg	36.7
Mice, 1429 (100) mg/kg	116 (8.1) mg/kg	27

^a Human Equivalent Dose

^b Calculated as NOEL/Dose

^c Cresol dose is calculated using the exposure resulting from maximal (24 vial) dose of Anavip®

Borates and Boron

Borates are an impurity in the final formulation of Anavip® with a specification set at NMT 1 mg/vial, resulting in a patient exposure of NMT 10 mg after taking 10 vials of the product. Borates are found at small amounts in vaccines such as gardasil and recombinant HAV, in ophthalmic solutions, topical irrigant solutions, in OTC vitamin preparations and dietary supplements, and are part of a normal diet.

Borates are rapidly absorbed from oral intake and readily available systemically (6 and references within). They are not metabolized by humans or animals and are excreted unchanged⁷. For example, ~ 99% of the administered dose was excreted in urine over a 120-hr period following intravenous administration of doses of 570 to 620 mg boric acid to healthy adult human volunteers⁶.

EPA has determined an RfD for boron (a dose likely to be safe for chronic exposure) ⁸ equal to 0.16 mg/kg/day when taken orally, i.e. ~12 mg/day for a 75 kg human. This dose was calculated from data obtained in dietary studies in pregnant rats where the adverse effect observed at higher doses was a decrease of fetal body weight. Taking into account the formula weight of boron (B, 11 g/mol) and borate (BO₃⁻, 59 g/mol), ~12 mg/day boron corresponds to ~12x59/11 = 64 mg/day borate.

The exposure to borate following administration of 10 vials of Anavip® would be 10 mg, i.e. more than 6x smaller than RfD. As such, borates do not represent a safety risk for patients when Anavip® is used according to PI.

Animal studies

There are no GLP animal studies performed with the preparation. There are two pharmacology studies published in the literature evaluating the efficacy of the preparation in mice.

Pharmacology Study, Toxicon 41, (2003) 357-365

Title: The efficacy of two antivenoms against the venom of North American snakes, Sanchez EE, Galan JA, Perez JC, et al, Toxicon 41, (2003) 357-365

Aim: The study aimed to evaluate the efficacy of two antivenom preparations: Instituto Bioclon equine F(ab')₂ preparation and an ovine Fab preparation that is likely Cro-Fab, and not subject of this BLA.

Performing Laboratories: Natural Toxins Research Center, Texas A&M University and University of Venezuela, Department of Tropical Medicine

Test model: the study evaluated different outcomes in three different models 1) rabbit (strain and source not specified), 2) BALB/c mice (source not specified) and 3) in vitro glass bead activated coagulation test (gbACT).

Study design: venom from 15 species of North American vipers was used in the study. All, (excepting *Crotalus adamanteus*, purchased from Sigma) were extracted and lyophilized. Pooling of different specimens such as juvenile, adults and both sexes was performed, included if possible, to account for variability in composition of venom. The venom mixtures were characterized via HPLC and UV absorption at 280 nm.

To assess the effect of the antivenoms on venom induced hemorrhage, anti-hemorrhagic dose (AHD) was defined as the concentration of antivenom that neutralized 50% of minimal hemorrhagic dose (MHD) in rabbits. MHD was defined as the amount of venom protein that causes a 10 mm hemorrhagic spot in the dorsal subcutis of the rabbit.

To assess the effect of the antivenoms against venom pro-coagulant effect, *in vitro* gbACT was used to monitor percent reduction in human blood coagulation in the presence of venom without or with antivenom, respectively.

To assess the effect of the antivenoms in survival, serum protection test (ED_{50}) was performed in BALB/c mice. For this, six groups of eight mice received a mixture of one LD_{50} of each venom pre-mixed with six different concentrations of antivenom, or saline control. The mice were observed for 48 h and the percent survival and ED_{50} was calculated. [Lethal dose (LD_{50}) for each of the 15 venoms was also determined in BALB/c mice.]

Results

$F(ab)_2$ antivenom neutralized the hemorrhagic activity of all the hemorrhagic venoms, while the ovine Fab (CroFab) neutralized 11 out of the 14 hemorrhagic venoms. $F(ab)_2$ was effective in neutralizing the LD_{50} of all the venoms used in this study while the ovine Fab was effective in neutralizing all the venoms with the exception of *C. m. molossus* venom. Ovine Fab antivenom was more potent than $F(ab)_2$ preparation as measured by the ED_{50} value. $F(ab)_2$ neutralized the hemorrhagic venoms better than the ovine Fab, but the opposite was true for the pro-coagulant venoms.

Conclusions

This study shows that the product can neutralize all the venoms tested, however, its potency appears lower than the existing ovine Fab product for 7/15 venoms as measured by ED_{50} in mice and marginally better for the rest (Table 5).

There is some indication that Anavip is more effective in neutralizing anti-hemorrhagic effect of the most, but not all hemorrhagic venoms (Table 6). As such it can be useful for the treatment of recurrent coagulopathy that is sometimes seen with ovine Fab (CroFab).

Table 5: ED_{50} of 15 snake venoms by two different antivenoms. In gray are the venoms neutralized better by the ovine product (Modified from publication).

Venom ^a	$F(ab)_2$ ED_{50} ^b	Fab Ovine ED_{50}	Ratio ^c
<i>C. s. scutulatus</i> type A	140.5 (11)	21 (4)	6.7
<i>C. h. horridus</i>	111.6 (8)	20.9 (3)	5.3

<i>C. h. atricuadatus</i>	58.9 (3)	8.9 (1)	6.6
<i>C. v. viridis</i>	93.6 (7)	17.7 (2)	5.2
<i>Sistrurus catenatus edwardsii</i>	140 (10)	226 (12)	0.6
<i>C. adamanteus</i> ^c	34.9 (1)	70 (6)	0.50
<i>C. v. helleri</i>	46.7 (2)	70 (6)	0.67
<i>C. v. oreganus</i>	114.1 (9)	121 (10)	0.94
<i>S. c. tergimus</i>	83.1 (4)	78.4 (8)	1.05
<i>A. p. leucostoma</i>	186.8 (12)	55.2 (5)	3.3
<i>C. m. molossus</i>	93.1 (6)	NP (15)	
<i>C. atrox</i>	295 (14)	310 (14)	0.95
<i>C. s. scutulatus type B</i>	88.4 (5)	278 (13)	0.31
<i>A. c. contortrix</i>	331.6 (15)	93.7 (9)	3.5
<i>A. c. laticinctus</i>	293 (13)	140.5 (11)	2.1

Number in parenthesis indicates the rank order in which the antivenom neutralized 3 x LD₅₀.

^aPooled venom obtained for the NTRC serpentarium.

^bExpressed as mg of antivenom/kg of mouse body weight; ED₅₀ values were determined against 3 x LD₅₀ of venoms.

^cED₅₀ of Fab₂ antivenom/ED₅₀ of the Fab Ovine antivenom.

C. adamanteus was purchased from Sigma-Aldrich, Co.

Table 6

MHD for 15 snake venoms and the antihemorrhagic dose (AHD) of two antivenoms. In gray are the venoms neutralized better by the ovine product.

Venom ^a	MHD ^b (mg)	F(ab') ₂ AHD (mg) ^c	Ovine Fab AHD (mg)	Ratio ^d
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<i>C. adamanteus</i> ^e	0.3	1	4	0.25
<i>C. v. viridis</i>	0.7	4.4	4.4	1.0
<i>C. v. helleri</i>	2.25	3.3	13.3	0.25
<i>Sistrurus catenatus tergimimus</i>	2.4	8.8	13.3	0.66
<i>C. atrox</i>	2.5	27	7	3.85
<i>S. c. edwardsii</i>	3.5	26.6	141.7	0.19
<i>C. h. horridus</i>	5.6	4.4	6.5	0.67
<i>C. s. scutulatus-B</i>	12.2	283	35.4	7.9
<i>C. m. molossus</i>	12.5	35.4	283	0.12
<i>A. p. leucostoma</i>	29	70.8	141.7	0.49
<i>C. h. atricaudatus</i>	37.5	212	- ^f	
<i>C. v. oreganus</i>	43	425	- ^f	
<i>A. c. laticinctus</i>	67	283	- ^f	
<i>A. c. contortrix</i>	143	26.5	70.8	0.37
<i>C. s. scutulatus-A</i>	- ^g			

^a Pooled venom obtained for the NTRC serpentarium.

^b MHD: the amount of venom protein injected into the back of depilated rabbit causing a 10 mm hemorrhagic spot in diameter.

^c Antivenoms were at a starting concentration of 8.5 mg/ml. AHD: the amount of antivenom (mg) that neutralizes 50% of 1 MHD of venom.

The AHD is calculated by dividing the starting concentration of antivenom by the antihemorrhagic titer that neutralizes 50% of 1 MHD and then multiplying by the amount of volume injected into the back of a depilated rabbit.

^d Fab2H AHD/FabO AHD.

e C. adamanteus venom was purchased from Sigma-Aldrich, Co.

f Indicates that the MHD was not neutralized with equal volume of antivenom at a concentration of 8.5 mg/ml.

g Venom contains no hemorrhagic activity.

Pharmacology Study, Toxicon 41 (2003) 315–320

Title: Cross reactivity of three antivenoms against North American snake venoms, Sanchez et al. Toxicon 41 (2003) 315–320

Performing Laboratories: Natural Toxins Research Center, Texas A&M University and University of Venezuela, Department of Tropical Medicine

Aim: To measure neutralization of hemorrhagic, fibrinolytic, gelatinase and hide powder azure activities in eight snake venoms with three different antivenoms: 1) Antivipmyn [equine F(ab')₂], 2) Crotalidae Polyvalent Immune Fab (Ovine) (CroFab) and 3) UCV (FabV) that is an equine origin produced at the Universidad Central de Venezuela in Caracas, Venezuela by the Department of Pharmacy.

Outcome Measures:

Antihemorrhagic assay, as described. The strain of rabbit is specified as being New Zealand White rabbit.

Antifibrinolytic assay: The antifibrinolytic dose is defined as the amount (mg) of antivenom inhibiting the degradation of fibrin by one minimal fibrinolytic dose MFD. The lower the number the more efficient the antivenom. MFD was determined as the amount of venom protein (mg) that will clear a 5 mm area in the fibrin clot made by mixing fibrinogen and thrombin.

Antigelatinase assay: The antigelatinase dose (AGD) is defined as the amount (mg) of antivenom inhibiting the clearance of the gelatin on the X-ray film by one minimal gelatinase dose, MGD. MGD is the minimal amount of venom that causes a clear zone on a Kodak X-OMAT scientific imaging film.

Hide powder azure assay was used to test proteolytic activity. Antihide powder azure assay measured the antihide powder azure dose (AHPD), defined as the amount (mg) of antivenom inhibiting an absorbance reading of 0.1 of one minimal hide powder dose, MHPD .

Results and Conclusions

Antivipmyn reduced the hemorrhagic activity of all the eight venoms tested, while CroFab and FabV only neutralized half of the venoms. It also better inhibited fibrinolytic activity, gelatinase activity and hide powder activity than other antivenoms.