Toxicology Review of a Two Dose Intramuscular Toxicity Study of Agrippal in -b(4)----------

BLA: STN 125297

Sponsor: -b(4)--

Product: experimental test article - influenza cell culture subunit vaccine, 45 ug haemagglutinin (inactivated purified influenza virus surface glycoprotein from 3 influenza virus strains with 15 ug per strain) in 0.5 ml; reference test article – influenza subunit vaccine (Agrippal) 45 ug haemagglutinin (inactivated purified influenza virus surface glycoprotein from 3 influenza virus strains with 15 ug per strain) in 0.5 ml

Cross references: IND -b(4)-

Proposed use: immunization for seasonal influenza virus. The vaccine predominately contains the purified outer membrane proteins, hemagglutinin and neuraminidase, from each of the 3 influenza virus strains in accordance with various regulatory bodies.

Précis: A general toxicity study was conducted of Agrippal using ----b(4)---------- rabbits. The chief objectives of the toxicity study were the detection and evaluation of potential local and/or systemic toxicities. Rabbits were given 2 intramuscular injections

Introduction: Agrippal is a seasonal flu vaccine containing hemagglutinin and neuramidinidase. The vaccine is presented as a liquid for injection using a pre-filled syringe.

Proposed clinical study: a number of clinical studies were conducted in support of the BLA. Please see the clinical review for the BLA.

Toxicology Study Review

Title and study number: Two Dose Intramuscular Toxicity Study of Influenza Vaccine Formulations in --b(4)---------- Rabbits; document number 191-44
Performing laboratory: ----------b(4)----------
Study initiation date: April 15, 2002
Final Report date: August 28, 2002
Test article batch/lot: --b(4)---------- for influenza cell culture subunit vaccine; No.-b(4)- for reference test article influenza subunit vaccine (Agrippal)
Animal species and strain: ---------b(4)---------- rabbits
Breeder/supplier: ----------b(4)----------
Number of animal per group and sex: 36 (18 males and 18 females)
Age: approximately 13 weeks
Body weight range: 2.47 to 3.62 kg

Route and site of administration: intramuscular into hind limbs with 1st injection to left side; 2nd to right side
Volume of injection: 0.5 ml
Frequency of administration and study duration: 2 injections on study days (SDs) 1 and 8; 15 days which includes recovery period
Dose: 45 ug haemagglutinin per single injection
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use vials (one vial per dose). The influenza cell culture subunit vaccine was reported to be stable through Sept 20, 20002 and the Agrippal through June 2002. The experimental phase of the study did not continue beyond June 2002. Thus the stability of the test articles was established by reference to CoA.
Means of administration: needle and syringe
Report status: final

Experimental design

Group	Treatment	Number of Animals (#/sex/group)	
		Treatment phase	Recovery phase
1	Placebo (phosphate buffered saline)	3	3
2	Cell culture subunit vaccine	3	3
3	Agrippal	3	3

Methods: differential blood count by microscopic examination; RBC parameters; -b(4)---------- hematology analyzer; clinical chemistry by -----b(4)---------- serum electrophoresis (-----b(4)----------); coagulation parameters by --b(4)----------, Quick-test; pathology data collected offline and transferred into the --b(4)------ system.
Randomization procedure: performed, but not indicated
Statistical analysis plan: T-test for mean body weights, mean food consumption and mean (groups 1 & 2; 2 & 3) absolute organ weights. Wilcox's Test for mean clinical pathology endpoints.

Toxicity related endpoints were measured in accordance with the table below.

Parameters	Frequency of Testing
Cageside observation[1]	After each injection and twice daily
Clinical observations[2]	Daily
Body weight	Prior to treatment, SDs 8 and 15
Food consumption	Weekly
Body temperature	Prior to injection, 24 and 48 hours after; prior to necropsy
Ophthalmologic exam	Before treatment and at necropsy

[1] Cageside observations include mortality, morbidity, general health and signs of toxicity.
[2] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Clinical chemistry*	Prior to treatment, 48 hours after injection and SD 22
Hematology*	Prior to treatment, 48 hours after injection and SD 22
Coagulation*	Prior to treatment, 48 hours after injection and SD 22
Immunological response	SD 1 before treatment; prior to 2nd injection; SD 15, before necropsy
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Prior to treatment, 24 and 48 hours post and prior to necropsy
Necropsy	SD 10 for males and SD 11 for females for main treatment group; SD 14 for males and SD 15 for females for recovery group
Tissues for histopathology	As above

*blood collection site not indicated and refers to SOP VT 18. (NC = not collected)

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Organ/Tissue	Collected	Not collected
Adrenal glands	X	
Aorta	X	
Bone (femur including knee joint capsule)	X!	
Bone marrow (sternum)	X!	
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	X!	
Cervix		
Cecum	X	
Colon	X	
Duodenum	X	
Epididymides	X	
Esophagus	X	
Eyes (optic nerve)	X!	
Fallopian tubes (oviduct)		
Gall bladder	X	
Gross lesions (if any)		
Harderian gland (if applicable)		
Heart	X!	
Ileum	X	
Injection site	X!	

Jejunum	X	
Kidneys	X!	
Lacrimal glands		X
Larynx		X
Liver	X!	
Lung (main-stem; bronchi)	X!	
Lymph nodes (cervical)	X!	
Lymph nodes (mandibular)		X
Lymph nodes (ileum, mesenteric)	X!	
Mammary glands	X	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		X
Ovaries	X	
Pancreas	X	
Peyer's patch (if applicable)		X
Pituitary gland	X	
Prostate	X	
Rectum	X	
Salivary glands (mandibular)	X	
Sciatic nerve	X	
Skeletal muscle (femur)	X	
Skin	X	
Spinal cord (cervical, lumbar, thoracic)	X	
Spleen	X!	
Stomach (squamous and glandular)	X	
Testes	X!	
Thymus	X!	
Thyroid (w/ parathyroid glands)	X	
Tongue		
Trachea	X	
Ureters		
Uterus (w/ cervix)	X	
Urinary bladder	X!	
Vagina		X
Zymbal's gland (if applicable)		X

Table of Histology

Results:
Morbidity and mortality: All animals **survived** to their scheduled termination.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids
B) HEPATOBILIARY		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein, fibrinogen (also under coagulation),
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin (A) Globulin (G, calculated) or A/G Ratio Total Cholesterol Cholinesterase Total protein Creatine kinase Fasting Triglycerides

Table of Clinical Chemistry Results

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.5^3, i.e., ≥1.6 or ≤ 1.6	NOT OF NOTE
RED BLOOD CELLS	Reticulocytes per cent age SDs 3, 10, 22; all groups, M & F,↑ ≥1.6	Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC)
WHITE BLOOD CELLS	Neutrophil count Total leukocytes (WBC) SDs 3 & 10, male, G3 ↓>2.8 lymphocyte count SD 3, 10 & 22, male, G3 ↓≥2.8	Basophils, Eosinophils count Macrophage/Monocyte count Large Unstained Cells (LUC)
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

Table of Hematology Results.

Systemic toxicity:
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, clinical chemistry, gross anatomy or organ weight were found.

There were no treatment-related effects on organ weights or weight rations.

There were no significant differences in the levels of food consumption or body weight during the course of the study.

There was no treatment-related clinical pathology in treated animals. However, a sharp and significant increase in the per cent recticulocytes was observed across all groups and sexes relative to initial values on day 1. Treated groups of animals exhibited greater increases in per cent reticulocyte count as compared to control animals. The per cent recticulocytes decreased during the recovery period but did not return to baseline levels. Additionally, a decrease in the per cent of neutrophils with concomitant increase in the

per cent of lymphocytes was observed across all groups including the control without recovery at the end of period of observation (SD22) The causative factor resulting in the increased number of recticulocytes was not established but is not considered to be test article related as it occurred across all groups and did not correlate with any changes in RBC indexes or histopathology. Furthermore, no explanation is offered for the alteration in WBC populations. The sponsor reported a protocol deviation for WBC as a discrepancy was found in the results of the automatic differentiation relative to the results being confirmed by a manual count using light microscopy. Again, given that the changes in WBC populations occurred across all groups it is not related to treatment.

SEX	MALES				FEMALES			
GROUPS	1 (CONTROL)	2	3		1 (CONTROL)	2	**3**	
NUMBER OF ANIMALS	3/3	3/3	3/3		3/3	3/3	3/3	
BODY WEIGHT (terminal)	2731.7; 2927.7	2772.0; 2945.3	2823.3; 2998.0		3006.0; 3283.7	3049.3; 3360.7	2995.0; 3194.3	
BRAIN	9.80; 10.29	9.53; 10.15	10.15; 10.15		10.36; 10.01	9.86; 10.18	9.65; 9.97	
ADRENALS	0.149; 0.227	0.162; 0.255	0.180; 0.238		0.236; 0.266	0.214; 0.186	0.239; 0.205	
EPIDIDYMIDES								
HEART	8.95; 8.28	7.04; 8.09	8.43; 9.19		7.61; 9.32	7.13; 8.85	6.89;7.95	
KIDNEYS	15.44; 15.41	14.67; 18.26	15.51; 16.22		17.43; 18.56	18.18; 18.74	17.64; 18.58	
LIVER	69.78; 67.01	64.09; 76.45	69.30; 78.53		90.32; 93.85	83.58; 102.0	88.22; 83.46	
SPLEEN	0.922; 1.01	0.985; 0.886	0.865; 1.06		1.40; 1.55	1.46; 1.66	1.58; 1.09	
LUNG	10.47; 10.74	10.11; 11.10	9.62; 11.65		12.49; 12.50	9.73; 11.75	10.59; 11.82	
TESTES	4.62; 5.24	3.71; 4.60	4.10; 4.64					
THYROID and PARATHYROID								
THYMUS	3.53; 2.91	2.64; 3.20	2.64; 4.33		3.60; 3.52	2.92; 4.28	2.64; 4.30	
OVARIES					0.302; 0.351	0.312; 0.556	0.308; 0.371	

Table of organ weights in means (expressed in grams). Absolute weights are expressed as mean in grams. Statistical differences were not notated in the study report by the sponsor. Organ weighed for the main group are separated from recovery group values by a semicolon.

Gross Pathology:

Group	Findings
1M	NF
2M	Underdeveloped/missing kidney and testicle with epididymis (#2001);
3M	Undeveloped testis with epididymis (#3002); discolorations in testes (#3003);
1F	Emphysema in 1 animal; cysts in a single female as well as hemorrhagic thymus due to puncture of the heart in a single animal
2F	Reddened area in skeletal muscle right leg (#2008)
3F	Renal cysts in kidneys with discoloration and enlarged iliac lymph node (3012)

NF = no findings

Microscopic finding are listed below:

Groups	Findings
1, 2, 3	Injection site exhibited a low incidence of slight focal necrosis with lymphohistiocytic cells and in rarely slight hemorrhage either at the time of necropsy in the main and recovery groups.
1, 2	Iliac lymph - low incidence of slight to moderate hemorrhage in both the main and recovery groups
1, 2, 3	Lungs – frequent slight to moderate interstitial inflammatory foci
1, 2, 3 females	Kidneys – tubular degeneration and/or dilation in both main and recovery groups; low incidence of cortical cysts found in group 1 and 3 females
1, 2, 3	Liver – frequent minimal to slight inflammatory foci; focal fatty change in 1 male in group 1 in the main group1 and 1 male group 3 at recovery
2, 3	Mesenterial lymph node – low frequency of minimal to slight hemorrhage

Findings relative to the kidney, liver and lung appeared evenly distributed between treated and control groups of animals. No increased incidence of histological findings indicative of potential adverse events was observed in the treated groups relative to the controls. Histological changes found at the site of injection are typical of trauma induced by intramuscular injections and represent an expected and acceptable degree of toxicity.

Body temperature: The number of excursions in body temperature ≥40º C which were observed during the study is noted below.

Group	Males	Females
Control	0	0
1	0	0
2	0	0

Table of occurrences for body temperature ≥40º C

Local toxicity: Draize scoring of the injection site revealed no evidence of local reactogenicity.

Erythema / Edema

Treatment group	1st dose					2nd dose				
	0	1	2	3	4	0	1	2	3	4
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0

Test article related effects	Effects considered incidental*
none	Increase in reticulocyte per cent age Reciprocal changes in WBC in terms of per cent neutrophils and lymphocytes

Assessment: There were no clear treatment-related effects on clinical pathology parameters. Changes were observed in the number of recticulocytes, neutrophils and lymphocytes relative to pre-dose levels; however, no treatment effects were observed as the changes were also found in the control group. Similarly there were no treatment-related effects on body weights, food consumption, ophthalmology, organ weight or ratios, or gross pathology. Other than evidence of inflammation at the site of injection which was attributable to recovery of trauma due to injection, there were no treatment-related effects on histopathology. Any histopathology findings were considered to be incidental to the study and not related to the test article. Adverse gross or microscopic alterations that could be indicative of an unacceptable systemic or local toxicity were not observed.

No immunological assessment was reported for this study; thus, no independent verification was made that an active dose was administered in the toxicity study. The sponsor indicates that a nonGLP subsection to the study indicates immunogenicity occurred. No quantitative findings are cited.

GLP study deviations or amendments: No significant deviations or amendments were recorded that significantly influenced the quality, integrity or interpretation of the results.

Conclusions: There are no toxicological issues precluding approving the BLA.

Communications: No wording is proposed for inclusion in the product label.