

Toxicology Review - Rotarix

Toxicology Review of Rotarix, an Oral Human Rotavirus Vaccine

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Re: Submission STN 125265

Product: live, attenuated monovalent human rotavirus given orally (Rotarix) - lyophilized in one vial containing amino acids, dextran, Dulbecco's Modified Eagle Medium, sorbitol and sucrose to be used with a vaccine diluent of calcium carbonate (CaCO_3) suspended in sterile water and formulated with xanthan gum (----%). The oral vaccine is supplied as a vial of lyophilized vaccine and is reconstituted with the liquid diluent which is supplied in a prefilled oral applicator. One dose of Rotarix is 1 ml of vaccine that contains at least $10^{6.0}$ median Cell Culture Infective Dose (CCID₅₀) of live, attenuated human retrovirus, which was produced on Vero cells.

Sponsor: GlaxoSmithKline (GSK)

Performing toxicology laboratory:-----

Study number: 802/534

Indication: immunization of infants who are 5 weeks or older to gastroenteritis due to G1 and non-G1 types of Rotavirus (including G2, G3, G4 and G9).

Dosing schedule: two dose series during the first 6 months of life (6 to 24 weeks of age) with 3 weeks between doses

Vaccine dose: $10^{5.5}$ focus forming units (ffu) and at least $10^{6.0}$ rotavirus titer that is a cell culture infectious dose 50% (CCID)

Background

The product is derived from strain 89-12 which was obtained from the stool of a 15-month old child with a mild case of rotavirus diarrhea. The 89-12 strain belongs to the serotype G1 and genotype P[8]. The attenuated rotavirus is combined with CaCO_3 as an antacid to protect the virus against inactivation in the acid environment of the stomach. Initially, the clinical development of the vaccine was undertaken by AVANT and was later assumed by GSK. They developed at passage -- a lyophilized product containing the cloned --- 89-12 HRV strain which was named RIX4414.

A previous rotavirus vaccine, RotaShield, was approved and later withdrawn because of an apparent increase in the incidence of intussusception as an adverse effect.

RotaShield was developed using a tetravalent rhesus-human rotavirus and is therefore unlike the present vaccine. Nevertheless, the potential for intussusception is a significant issue.

The present submission for toxicology consists of one repeat dose toxicity study which incorporated a single dose study as a subcomponent. No other studies were submitted in this application. The validity of the animal model was established in the study titled, "Preliminary study of human rotavirus strains in Rats -----" (study number PIMS 20010595). In this study, 5-day and 21-day old ----- rats were given two different human rotavirus strains (Wa and RIX 4414) intragastrically on days 0 and 14 of the study. Anti-rotavirus antibodies, diarrhea and viral shedding in the stools as measured by ELISA were measured. Antibody levels were measured after the second dose of

vaccine whereas diarrhea and shed virus were monitored after the first dose during a four day period. Although no specific antibody response was detected to human rotavirus in 5-day old rats, a specific antibody response was found in 21-day rats (see the sponsor's table below).

Age at first vaccination	Strain Wa Bleeding		Strain 89/12 Bleeding	
	14 PII	28 PII	14 PII	28 PII
5 days	-	ND	-	-
21 days	+ (weak)	+ (weak)	+++	+++

ND: not done -: negative +: weak positive (less than 2 fold)
 +++: positive (more than 2 fold)

Since no evidence of viral shedding was observed using the ELISA method a RT-PCR method was developed for this purpose and utilized in the repeat dose toxicity study.

Study Review

Title: Toxicity study after four administrations by the oral route in the rat (study number 802/534)

The repeat dose toxicology study used 21-day old ----- rats (----- supplier) as a model animal on the basis of scientific publication by Ciarlet et al, 2002 (J Virology 76(1): 41 -57) which determined that rotavirus infected 5-day old, but not ≥21-day ----- rats. The ----- rats were caged in isolators and developed a rotavirus infection accompanied by diarrhea for up to 10 days post-inoculation. This animal model of the rat was initially studied in a preliminary pharmacology study to investigate the vulnerability to different human rotavirus strains by measuring seroconversion, viral shedding by classical viral titration methods and diarrhea. In the preliminary pharmacology study, no evidence of diarrhea or viral shedding by ELISA was observed regardless of the age of the rat or rotavirus strain given. Later when the repeat dose toxicity study was conducted a RT-PCR assay with improved sensitivity for viral shedding in the stools was utilized and demonstrated that 21-day old -----rats were susceptible to the RIX 4414 strain. However, a robust response was not observed. Additionally, the effect of CaCO₃ was investigated in 21-day old ----- rats in terms of seroconversion and viral shedding. In this study, rats were given RIX 4414 alone or as one of two different varieties of CaCO₃ (-- um (small granularity, --- mg) or --- um (large granularity, --- mg). Viral shedding peaked at day 5 after vaccination with RIX 4414/CaCO₃. When viral shedding was measured by RT-PCR, peaks were observed between day 4 and day 6 and were present in the stools of rats. Seroconversion was observed in 20% of the rats receiving 10⁶ ffu of RIX 4414 and --- um of CaCO₃ and in 80% of the rats given ---- um CaCO₃.

Methods

In the repeat dose GLP toxicity study, 21-day old ----- rats were given 4 doses of rotavirus vaccine orally (intragastic administration). The four doses used in the repeat dose toxicity study exceed the number of administrations that are intended for use in the clinic. The dosing interval was 2-weeks; thus, dosing occurred on days 0, 14, 28, and 42. The full human dose of vaccine of 0.5 ml was used in the toxicity study. The study was design with the following 4 groups: saline (group 1); antacid alone, ---- mg of CaCO₃ (group 2); RIX 4414, 10^{6.7} ffu and ---- mg of CaCO₃ (group 3); or RIX 4414,

10^{6.1} (group 4). Groups were composed of N = 15/sex with animals killed on days 5, 47 and 70 of the study.

Immunogenicity for antibodies against Rotavirus was measured on days 27 (13 days post the second dose of vaccine) and 70 (28 days post the fourth dose of vaccine) from a subset composed of 10 rats. Stools specimens were taken from the same subset on days 4, 5 and 6 after the first dose and days 18, 19 and 20 after the second dose of vaccine. Viral shedding was determined by a reverse transcriptase-PCR method. Batch DRVC201A48PL was used for CaCO₃ in group 2. Batch DRVC200A48 was used for CaCO₃ in group 3 and batch DRVCO13A48 was used for RIX4414 in group 4.

Results

No treatment-related effects were observed on the following endpoints: clinical signs, mortality, body weight, food intake, ophthalmology, body temperature, coagulation, macroscopic findings upon necropsy, histopathology and clinical chemistry. Of note, no histopathological changes were found in the intestinal villi such as epithelial syncytia and no intracytoplasmic eosinophilic inclusions in the ileum.

Low seroconversion rates were observed in the test population as illustrated in the sponsor's table below. To determine seroconversion rates, a cut-off value was determined as the average of the titers measured from the sera of non-vaccinated rats +3 standard deviations. The seroconversion rate was defined as the percentage of animals with titers above the cut-off.

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In terms of viral shedding, no positive response was found in the control groups and waning and variable response was observed for the rats given

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Combined virology and serology analysis demonstrated an 80% response in rats given RIX4414 and CaCO₃ --- mg and 40% in rats given the vaccine alone (see sponsor's table below). The vaccine take was defined as the percentage of rats that either seroconverted and/or shed virus in their stools.

Table 2 Vaccine take for each treatment group

<i>Formulation</i>	<i>Gender</i>	<i>Vaccine take</i>	<i>Vaccine take</i>
Group 1 : <i>saline</i>	Males	0/5	0 %
	Females	0/5	
Group 2 : <i>CaCO₃</i>	Males	0/5	0 %
	Females	0/5	
Group 3 : <i>HRV 10^{6.7} ffu</i> <i>(candidate)</i>	Males	3/5	80 %
	Females	5/5	
Group 4 : <i>HRV 10^{6.1} ffu</i> <i>(reference)</i>	Males	1/5	40 %
	Females	3/5	

Although no treatment related deaths occurred during the course of the study, 4 animals died (one in group 1, one in group 2 and two in group 3) after blood sampling on day 70 and were necropsied shortly after their deaths. The cause of death was thought to be associated with the blood sampling procedure.

Assessment

Rotarix was administered orally in the course of a repeat dose toxicity study and found to be immunogenic to 21-day old ----- rats. These animals were given 4 doses at 2-week intervals (days 0, 14, 28, and 42) with the full human dose. The vaccine did not exhibit evidence of toxicity as measured by a number of endpoints including histopathology data of the gut including the intestinal villi of ileum as measured by epithelial syncytia or intracytoplasmic eosinophilic inclusions. Reproductive and developmental toxicity studies were not performed because the target population does not include women of child bearing potential or involve male fertility as the clinical population is composed of infants. Although the toxicity study did not reveal evidence of intussusception, the validity of the model is uncertain and should not be used as a tool in assessing the risk.

Action Required

A review of the proposed label revealed that a pregnancy category was not proposed by the sponsor. To be consistent with the labeling provisions of the CFR (201.57) the labeling of this product should be 'Pregnancy category C' as no animal studies of reproduction were conducted. Additionally, wording as specified in the CFR for pregnancy category C should be added such as the following:

"Pregnancy. Pregnancy category C. Animal reproduction studies have not been conducted with Rotarix. It is also not known whether Rotarix can cause fetal harm when

administered to a pregnant women or can affect reproductive capacity. Rotarix should be given to a pregnant woman only if clearly needed."

The following section was submitted by the sponsor as amendment 0006 on 10/18/2007 (DATS #427569) and fulfils the requirement cited in §201.57 regarding pregnancy labeling.

[NOTE TO REVIEWER: Section 8.1 was added per 06Aug2007 CBER communication.]

8.1 Pregnancy

Pregnancy Category C

ROTARIX is not indicated for women of child-bearing age and should not be administered to pregnant females. Animal reproduction studies have not been conducted with ROTARIX. It is not known whether ROTARIX can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity.
