

New and improved recombinant hepatitis B vaccine

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Hepatitis B virus (HBV) infection is a major health problem which can result in acute and/or chronic liver disease. Infection may result in a chronic carrier state which may be the cause of up to 40% of cases of hepatocellular carcinoma and is probably acquired vertically from mother to neonate, either at birth or subsequently during close maternal contact. Approximately 10% of patients contracting hepatitis B as adults and 98% of those infected as neonates will not clear HBsAg from the serum within 6 months of infection, resulting in a carrier state which is likely to persist.

The only currently feasible means of preventing the spread and consequences of chronic HBV infection is active immunization. The classical approach to producing an attenuated or inactivated viral vaccine failed due to the fact that HBV cannot be propagated in tissue culture. The 22nm non-infectious HBsAg particles, produced in the natural infection process, provided a unique solution. The first effective consists of 22nm HBsAg particles formulated in an alum adjuvant. The efficacy of this type of vaccine was demonstrated both in high risk adult populations and

in newborn infants of HBsAg positive mothers.

While still in use, the plasma-derived Hepatitis B vaccine has limitations with respect to a universal vaccination program. These include (1) limited availability of healthy chronic carrier sera, (2) the need for elaborate purification and virion inactivation procedures, and (3) concern about contamination with other potential human pathogens associated with blood. These limitations make the plasma-derived vaccines highly expensive and beyond financial reach of developing countries where HBV has high endemicity and where universal early childhood vaccination is desirable.

Production of HBsAg by expression of the surface proteins in heterologous hosts via recombinant DNA technology has overcome many of the problems cited above for plasma-derived vaccines. The choice of hosts is dictated by the absolute requirement that the recombinant proteins be assembled in the correct particulate conformation and tertiary structure in order to retain immunogenicity in humans. Vaccines produced using eukaryotic systems such as yeast

(*Saccharomyces cerevisiae*) or mammalian cells, capable of correctly expressing and assembling recombinant HBsAg, have been shown to be protective against HBV infection and are being widely used for immunization.

However, high manufacturing of these vaccines are still limiting factors in the development of a new and affordable Hepatitis B vaccine which is both safe and inexpensive compared with currently available recombinant vaccines.

Korea Green Cross Corporation has developed a proprietary S containing RDNA HB vaccine, Hepavax-GeneTM, by expression in other yeast (*Hansenula polymorpha*) cells than *Saccharomyces cerevisiae*. The expression system for Hepavax-GeneTM is based on *H. polymorpha*. This yeast can grow on methanol as a sole energy and carbon source. In cells grown on methanol the key enzymes of the methanol metabolisms.

MOZ (methanol oxidase), FMD (formate dehydrogenase) and DHAS (dihydroxy acetone synthase) may contribute up to 40% of the total cell protein.

The abundance of MOX, FMD and DHAS indicates that the respective genes are controlled by very strong promoters. We have used these promoters to control the expression of foreign proteins in *H. polymorpha*. The promoters are depressed on glucose and fully induced on methanol. The strong depression is a special feature of *H. polymorpha*. Some other methylotrophic yeast, such as *P. pastoris*, do not show this phenomenon.

A unique feature of *H. polymorpha* is the spontaneous integration of up to 100 copies of the expression vector

into the genome. Mostly the vector is integrated in form of multimers in a head to tail arrangement. The integrated foreign DNA is mitotically stable as shown for several recombinant strains. Additionally this high copy integration phenomenon contributes to the productivity potential of the system.

Standard vectors consist of a marker complementing an auxotrophic mutation, an autonomously replicating sequence (HARS) and an expression cassette where the foreign gene can easily be inserted. Two strong promoters, MOX or FMD can be used alternatively. These two promoters exhibit some differences in their regulatory properties. The choice of promoter depends on the requirements of the production process and on the type of protein to be expressed. In general, the use of FMD promoter allows the establishment of a single-carbon-source fermentation process. In this case, growth on glycerol to high cell density is followed by a production phase characterized by growth on a limiting concentration of glycerol.

Auxotrophic mutants of *H. polymorpha* are used as standard host cells such as Ura 3 and /or Leu 2. Such strain is currently used as host for the construction of several industrial recombinant strains. In all cases, very high productivity and ease of scale-up is preserved.

A favorable property of *H. polymorpha* is the ability to grow efficiently on glycerol-almost no ethanol is produced from this carbon source. Thus, the negative effects of ethanol on the fermentation process, known for some other yeast, are avoided.

Furthermore, the high mitotic stability of *H. polymorpha*, due to integrated genome, can provide

growth on non-selective, simple, and low-cost media without affecting the productivity.

Using this *H. polymorpha* production system, production yields are very high and the purification procedure yields a homogeneous preparation which is over 98% pure. Development of a scale-up production process has been completed.

Pre-clinical analysis in mice and chimpanzees has indicated that the efficacy of KGCC's RDNA HB vaccine exceeds the seroconversion rates and anti-HBs titer levels of currently available commercial RDNA HB vaccines. KGCC has also completed the characterization and safety evaluation of the vaccine, as outlined in the U.S. FDA guidelines.

A clinical trial was performed at Leicester Clinical Research Centre in U.K. during the period April 10, 1995 - February 5, 1996 in order to assess the safety and immunogenicity of Hepavax-GeneTM for 20 healthy male and female volunteers. Hepavax-GeneTM was administered as single 2Oug injection to the volunteers on Days 1(Visit 1), 30(Visit 2), and 180 (Visit 4).

Two 9ml serum samples were collected at Visits 2-6, one of which was used for HBV marker testing and the other stored at -70°C in case of future analysis. One 9ml serum sample for storage was collected at Visit 1. HBV marker screen analyses were performed by Medical Diagnostics Limited(MDL).

Total Hepatitis B core antibody(HBcAb/anti-HBc) was undetectable for all subjects throughout the study,

indicating no infection with Hepatitis B.

Hepatitis B surface antibody levels(HBsAb/anti-HBs) were greater than 10mIU/ml in 2 subjects at Visit 2; in 9 subjects at Visit 3(detectable in 11 subjects); in 16 subjects at Visit 4(detectable in 17 subjects); in all 20 subjects at Visit 5; and in 19 subjects at Visit 6(detectable in all 20 subjects). Nineteen subjects of 20 had therefore seroconverted by the end of this study, where the criteria for seroconversion was defined as Hepatitis B surface antibody levels(HBsAb/anti-HBs) greater than 10mIU/ml.

Hepatitis B surface antibody levels(HBsAb/anti-HBs) were greater than 100mIU/ml in one subject at Visits 2 and 3, in 12 subjects at Visit 4, in 19 subjects at Visit 5, and in 18 subjects at Visit 6.

Blood and urine samples were taken for safety laboratory at pre-study screening and at Visits 2(Day 30), 3(Day 60), 4(Day 180) and 6(post-study screening).

Pulse rate and blood pressure were recorded at each visit prior to vaccination and 30 minutes post-dose on Visits 1(Day 1), 2(Day 30) and 4(Day 180).

No significant changes were noted in clinical or laboratory test results during the course of the study.

The three doses of 2Oug HB vaccine were well tolerated. A total of 64 adverse events were reported, of which only 8 events were considered to be possibly or probably related to the test compound and none were unexpected or life-threatening.

In order to assess and confirm the safety and immunogenicity of Hepavax-GeneTM in Vietnamese infants, another clinical trial was performed at Hung Vuong Hospital in Ho Chi Minh City, Vietnam. A total of 400 infants born to HBV(-) mothers was enrolled and single 10ug injection was given to all the subjects at months 0, 1 and 2. No adverse event was

observed after 1,189 doses were administered.

The immune response of 124 eligible infants were assessed at 3 months.

Seroconversion rates was 100%, geometric mean titer (GMT) was 446.5 mIU/mL.