PROGRESS IN DIARRHEAL DISEASE RESEARCH AT NAMRU-2 IN COLLABORATION WITH BADAN LITBANGKES AND RSPI

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ABSTRAK

KEMAJUAN PENELITIAN MENGENAI PENYAKIT DIARE OLEH NAMRU-2, BADAN LITBANGKES DAN RSPI

Penyakit diare termasuk kolera masih merupakan masalah kesehatan yang penting untuk Indonesia. Kejadian luar biasa (KLB) "muntah-berak" masih terus dilaporkan dari berbagai daerah di Indonesia. Salah satu upaya yang dilakukan untuk mengatasi keadaan ini adalah menemukan suatu vaksin yang aman, praktis dan terjangkau harganya. CVD-103 adalah strain V. cholerae yang sebagian besar gen penyebab diare, toksin subunit A, telah dibuang.

Pada penelitian dengan peserta anak-anak di Jakarta, CVD 103-HgR ternyata memberikan kenaikan antibodi dan dapat diterima baik oleh anak-anak Indonesia berusia 5-9 tahun dan 25-59 bulan. Di samping itu pemeriksaan sampel bakteriologik dari lingkungan dekat tempat kediaman peserta dengan menggunakan teknik yang peka untuk mendeteksi V cholerae, tidak menemukan adanya strain vaksin.

Suatu penelitian lapangan dengan tujuan untuk menilai kemanjuran dosis tunggal vaksin oral kolera CVD 103-HgR untuk pencegahan kolera secara klinis selama tiga tahun, telah dimulai didaerah Jakarta Utara pada tahun 1993. Selain itu penelitian ini juga bertujuan untuk menetapkan kemanjuran vaksin terhadap berbagai kelompok umur, terhadap kolera berat, serta membandingkan kemanjurannya pada peserta dengan golongan darah O. Jumlah penduduk yang ikut dalam penilaian vaksin ini adalah 67.000 peserta sukarela. Hasil penelitian ini diharapkan dapat diketahui pada akhir tahun 1996.

Di samping penelitian tentang vaksin kolera, NAMRU dan Badan Litbangkes juga telah ikut serta dalam berbagai penelitian WHO, misalnya tentang larutan garam oralit (ORS) yang dilakukan di RS Karantina/RS Penyakit Infeksi Prof. Dr. Sulianti Saroso. Salah satu penelitian terakhir adalah manfaat larutan ORS dengan osmolaritas rendah pada tahun 1994. Hasil penelitian ini telah mendorong WHO untuk melaksanakan penelitian dengan larutan yang sama di berbagai pusat penelitian (multicenter) pada tahun 1995 sebelum mengusulkan suatu perubahan formulasi larutan ORS secara global.

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INTRODUCTION

Diarrheal diseases including cholera continue to be an important cause of dehydration and death among residents of Indonesia. Cholera occurs in endemic form in rural areas and cities, including Jakarta, and occurs sporadically in epidemic form. The history of the seventh pandemic begins with the detection of the El Tor biotype of *Vibrio cholerae* 01 in south Sulawesi in 1961. From Indonesia, this strain spread throughout the developing world over the next 30 years. This included a major outbreak of cholera in 1991 in South America, involving over 550,000 individuals with thousands of deaths[1].

During 1994 and 1995, outbreaks of cholera have been documented in many areas of Indonesia, including Ciamis, Bekasi, and Garut in Java, Pulau Panggang (Kepulauan Seribu), Biak, Bali, Palembang, Pontianak, and West Timor. CDC continue to maintain active surveillance for cholera in outbreak situations, however, NAMRU-2 and NIHRD were involved frequently. In addition, the introduction of the 0139 strain of *V. cholerae* is being monitored in several sites in Indonesia, through the collaboration of NAMRU with local hospitals in various cities and Badan Litbangkes.

Cholera pathogenesis begins with attachment of the cholera bacteria to the small intestinal mucosa. Mucosal cells are then stimulated by the A subunit of the cholera toxin to secrete sodium into the intestinal lumen; water and potassium follow. *V cholerae* 01 may cause asymptomatic colonization of the intestine, mild diarrhea, or the severe form with massive fluid loss and dehydration. Death can result if fluid replacement via the oral or intravenous route is not instituted promptly. Prevention of cholera involves provision of clean water, proper sewage disposal, and avoidance of food contamination, but these measures are difficult to implement in many developing areas with scarce resources. Cholera illness provides immunological protection to those who survive the diarrhea, but it is often of short duration. Because of the presumption that natural immunity occurs in populations in whom cholera disease is endemic, vaccine development has been pursued.

The parenteral inactivated whole cell cholera vaccine has largely been abandoned as a public health tool for the control of cholera, due o low efficacy for only about 6 months[2] The oral killed vaccine (inactivated *V. cholerae* 01) with or without the non-toxic B subunit of cholera toxin, displayed some efficacy in trial in Bangladesh in the 1980s[3]. This vaccine requires a 3-dose immunization schedule. Demonstrated efficacy of about 50% was an improvement over the previous vaccines, but because of low efficacy in children under age 6, and the need for 3 doses, other vaccines have been under development[4]. In addition, the oral inactivated vaccine provided lower protection to individuals of blood group O, who are at greater risk than others for severe disease[5,6].

It is the hope of the World Health Organization that a vaccine can be found which provides protection against cholera with a high efficacy rate after a single dose in a susceptible population, especially young children and persons with blood group O. If affordable, such a cholera vaccine could be useful in the control of endemic and possibly epidemic cholera.

ORAL CVD103-HgR VACCINE TRIAL

The University of Maryland Center for Vaccine Development produced the oral vaccine CVD103-HgR by genetic engineering, as a possible candidate for the prevention of cholera.

This is a live vaccine which is derived from a virulent strain by deletion of most of the genes for the toxic A subunit of cholera toxin, while the B subunit which stimulates the immune response is preserved. A mercury resistance gene was inserted to enable researchers to distinguish the vaccine strain from wild *V. cholerae* 01.[7]

Challenge studies in North American volunteers showed protection from severe or moderate diarrhea by one oral dose of CVD103-HgR when challenged with *V. cholerae* 01.[8, 9, 10]. The protection lasted for at least six months, and began eight days after taking the vaccine[10].

Studies among volunteers in developed and developing countries demonstrated that the CVD 103-HgR strain is safe, with no more adverse reactions than placebo. These studies also demonstrated the immunogenicity of one oral dose of CVD 103-HgR, as measured by high serum vibriocidal antibody responses, which correlate with protection[11-13].

Trials of the CVD 103-HgR vaccine in Jakarta investigated the reactogenicity, immunogenicity and transmissibility of CVD 103-HgR when given to children ages 2-4 years and 5-9 years. The vaccine was found to be highly immunogenic and well-tolerated in these Indonesian children. In only < 0.5% of vaccinees was the vaccine strain recovered from stool cultures, and only minimal evidence of transmissibility to family members was found[14,15].

Data from prospective surveillance for cholera at the Infectious Disease Hospital (formerly the Rumah Sakit Karantina, now named Rumah Sakit Penyakit Infeksi Professor Sulianti Saroso) during 1992-93, revealed that cholera continues to be endemic in North Jakarta, with incidences sufficiently high to justify an efficacy trial of the CVD 103-HgR vaccine in this area.

In collaboration with the Indonesian Ministry of Health, the Indonesian CDC and Litbangkes, and with the advice and support of the WHO, the Swiss Serum and Vaccine Institute, and the University of Maryland Center for Vaccine Development, a large-scale efficacy trial of the vaccine was initiated in April, 1993 The objectives of the trial are:

1. To assess the effectiveness of one dose of the oral cholera vaccine CVD 103-HgR in preventing cholera of a severity that leads the patient to seek medical care at a hospital or clinic, over a period of three years.

2. To determine vaccine efficacy according to age, severity of illness, and/or blood group.

The study is currently in progress, with over one year of surveillance for cholera illness completed among the over 67,000 vaccine participants. Efficacy data will be available after completion of the 3 years of surveillance. Included in the study is long-term safety monitoring at the hospital and community level, assessment of vaccine immunogenicity, and evaluation of side effects. Close cooperation with hospital staff, community leaders, and vaccination participants is maintained, and has been accompanied by excellent assistance at all levels.

The institutions collaborating in this study include the National Institute of Health Research and Development (Badan Penelitian dan Pengembangan Kesehatan Indonesia, Litbangkes), the Naval Medical Research Unit-Two (NAMRU-2), the Directorate General of CDC, Ministry of Health Republic of

Indonesia *(Direktorat Jenderal P2M&PLP Depkes RI)*, and the University of Indonesia. The hospitals are critical to the success of the project, specifically the Rumah Sakit Penyakit Infeksi Prof. Sulianti Saroso (RSPI-SS), Rumah Sakit Atmajaya, Rumah Sakit Koja, and Rumah Sakit Islam in Sukapura, all North Jakarta hospitals. International organization which are supporting and advising the study are the World Health Organization, the Swiss Serum and Vaccine Institute, and the University of Maryland.

OTHER STUDIES ON DIARRHOEAL DISEASES

In addition to this vaccine trial, NAMRU and Litbangkes have been involved in studies evaluating the efficacies of various oral rehydration solutions, in the pursuit of improved ORS to treat dehydration and diarrhea. These studies have been conducted at the Rumah Sakit Penyakit Infeksi Prof. Dr. Sulianti Saroso. The latest trial of this kind was the evaluation of a solution with a lower osmolarity than standard ORS, by a reduced amount of sugar and sodium content. This solution appears to be an improvement in the rehydration of children and for rehydration when non-cholera illness is seen. This study, was conducted in 1994 in Jakarta and in Dhaka. Supported by encouraging data from the trials, the WHO is sponsoring multicenter trials to confirm the results and assess whether a change in the recommended composition of ORS should be adopted.

REFERENCES

1. Swerdlow DL, Mintz ED, Rodriguez M, Tejada E, Ocampo C, Espejo L, Greene KD, Saldana W, Seminario L, Tauxe RV, Wells JG, Boan NH, Rios AA, Pollack M, Vertiz B, Blake PA. (1992). Waterborne transmission of epidemic cholera in Trujillo, Peru: lessons for a continent at risk, Lancet; 340;28-33

2. Levine MM, Pierce NF. (1992). Immunity and vaccine development. In: Greenough WB III & Barua D, eds. Cholera. Plenum press, New York. pp. 285-327.

3. Clemens JD, Sack DA, Harris Jr, et al. (1990). Field trial of oral cholera vaccines In Bangladesh: results from three year follow-up. Lancet; 335:270-273.

4. Clemens JD, Vari Loon F, Sack DA, Rao MR, Ahmed F, Chakraborty J, Kay BA, Khan MR, Yunus M, Harris Jr, Svennerholm A-M, Holmgren J. (1991). Biotypes as determinant of natural immunising effect of cholera. Lancet; 337:883-884.

5. Clemens JD, Sack DA, Harris J, Chakrabofly J, Khan M, Huda S, Ahmed F, Gomes J, Rao M, Svennerhom A-M, Holmgren J. (1989). ABO blood groups and cholera: new observations on specificity of risk and modifications of vaccine efficacy. J Infect Dis; 159:770-773.

6. Levine MM, Barua D, Nalin DR, Rennels MB, Hornick RB, Sotman S, Van Blerk G, Hughes TP, O'Donnell S. (1979). Genetic susceptibility to cholera. Ann Human Biol , 6:369-374.

7 Kaper JB, Levine MM, (1990). Recombinant attenuated *Vibrio cholerae* strains used as live oral vaccines, Res Microbiol , 141:901-906.

8. Levine MM, Kaper JB, Horrington D, et al. (1988). Safety, immunogenicity and efficacy of recombinant liveoral cholera vaccine CVD 103 and CVD 103-HgR. Lancet , II:467-470.

9. Tacket CO, Losonsky G, Nataro J, Cryz SJ, Edelman R, Kaper JB, Levine MM. (1992). Rapid onset and duration of protective immmunity in challenged volunteers after vaccination with live oral cholera vaccine CVD 103-HgR. J Infect Dis , 166:837-841

10. Levine MM, Kaper JB. (1993). Live oral vaccines against cholera: an update. Vaccine; 11:207-212.

11. Mosley WH, Ahmad S, Beneson AS, Ahmed A. (1968). The relationship of vibriocidal antibody titer to susceptibility to cholera in family contacts of cholera patients. Bull WHO; 38:777-785.

12. Glass RM, Svennerholm A-M, Khan MR, Huda S, Huq Mi, Holmgren J. (1985). Seroepidemiological studies of El Tor cholera in Bangladesh: association of serum antibody levels with protection. J Infect Dis; 151:236-242.

13. Clemens JD, Van Loon F, Sack DA, et al. (1991). Field trial of oral cholera vaccines in Bangladesh; serum vibriocidal and antitoxic antibodies as markers of the risk of cholera. J Infect Dis ; 163:1235-1242.

14. Suharyono, Simanjuntak CH, Witham N, Punjabi N, Heppner DG, Losonsky GA, Clemens J, Totosudirjo H, Rifai A, Lin YL, Burr D, Wasserman SS, Kaper J, Sorensson K, Cryz S, Levine MM. (1992). Safety and immunogenicity of single-dose live oral cholera vaccine CVD 103-HgR in 5-9-year-old Indonesian children. Lancet ; 340:689-694.

15. Simanjuntak CH, O'Hanley P, Punjabi N, et. al. Studies of the safety, immunogenicity, and transmissibility of single-dose live oral cholera vaccine CVD 103-HgR in 24 to 59 month old Indonesian children. Journal of Infectious Diseases 168:1169-1176.