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STABILIZATION OF THE ATTENUATED POLIOVIRUS TYPE 3 VACCINE STRAIN BY SUCROSE

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Summary. — Highly temperature-sensitive and attenuated poliovirus type 3, strain Leon $12a_1b$, was stabilized at 37 °C by 15% sucrose without 1 mol/l $MgCl_2$. The finding will contribute to poliovaccination in tropical countries.

Key words: attenuated poliovirus; stabilization; sucrose

Introduction

Both inactivated (Salk, 1955, 1984) and live-attenuated (Sabin, 1959, 1985) poliovaccines have been proved to be safe and effective to decrease the incidence of paralytic poliomyelitis in developed countries. In tropical countries, however, localized epidemics of paralytic poliomyelitis have been reported even after vaccination with the live poliovaccine (LaForce *et al.*, 1980; John, 1981; Albert, 1987). Thermal inactivation of vaccine viruses was accounted for the factors of such vaccine failures (John, 1984; Albert, 1987), even when the vaccine contained 1 mol/l $MgCl_2$, which was used as the best stabilizer so far tested (Wallis and Melnick, 1961).

In this paper we report on the effect of sucrose in stabilizing the live-attenuated poliovirus type 3 vaccine strain.

Materials and Methods

Virus and cells. Attenuated poliovirus type 3 Sabin vaccine strain Leon $12a_1b$ without $MgCl_2$ was obtained from Hoechst Co., West Germany, and was passaged once in human rhabdomyosarcoma (RD) cells. The RD cells were grown in Eagle's minimal essential medium (MEM, Eagle, 1959) with 10 % heat-inactivated calf serum at 37 °C.

Infectivity assay. Virus specimens were serially diluted in 10-fold steps with physiological saine, pH 7, at room temperature. Growth medium was removed from RD cell cultures in 6 cm in diameter Petri dishes and 0.1 ml of diluted virus was inoculated to each dish. After 1 hour of adsorpt on at 37 °C, the cells were covered by 5 ml of overlay medium with 1 % agarose and 2 % cal fserum in MEM with neutral red. When agarose was solidified, the dishes were incubated at 37 °C for 4—5 days to form plaques. The virus infectivity was expressed in plaque-forming units (PFU) per ml.

Results

Sucrose (Lachema, Brno, Czechoslovakia) was spread on a sheet of aluminium foil and sterilized by overnight irradiation of ultraviolet light from 4 lamps of 15 Watts at distance of 30 cm. The sterilized sucrose was dissolved in physiological saline, pH 7, to make 90 % (w/v) and 30 % (w/v) solutions.

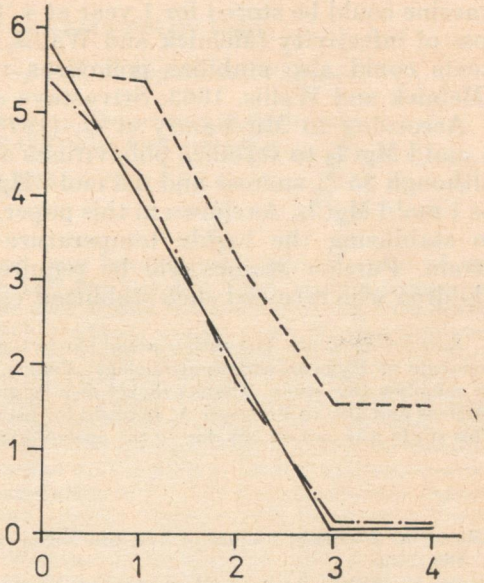

Fig. 1.
Effect of sucrose on the stability of attenuated poliovirus vaccine strain Leon $12a_1b$ in the absence of 1 mol/l $MgCl_2$ at 37 °C
Full line (———): 45 % sucrose; dashed line (— — —): 15 % sucrose; dotted line (.—.—.—.): no sucrose.

These solutions were mixed with equal volumes of the virus diluted 1 : 10 in physiological saline to final concentrations of 45 % and 15 % sucrose and virus titre of 3.75×10^5 PFU/ml. Each mixture was divided into several tubes, tightly stoppered, and incubated at 37 °C up to 4 weeks. As a control, the virus specimen was mixed with an equal volume of physiological saline and run in the same way. From each series, the tubes were taken out at 1 week interval and kept at —20 °C until infectivity was assayed.

The result in Fig. 1 shows that the virus infectivity in the control (dotted line) as well as in 45 % sucrose solution (full line) was exponentialiy inactivated until it became undetectable after 3 weeks of incubation. In contrast, the virus infectivity in 15 % sucrose solution was almost stable for the first week of incubation, then its inactivation rate became similar to the control until 3 weeks and leveled off until 4 weeks of incubation (dashed line). This finding shows that 15 % sucrose has some stabilizing effect on the attenuated strain of poliovirus type 3 even without $MgCl_2$.

Discussion

Live attenuated poliovaccines have widely been used to control the epidemics of paralytic poliomyelitis (Sabin, 1959; 1985; Melnick, 1978), but their efficacy was sometimes disappointing in tropical countries where cold-chain system is not always sufficient (LaForce *el al.*, 1980; John, 1981; Albert, 1987). Various cations and organic substances (Wallis and Melnick, 1961; Wallis *et al.*, 1962) were tested to stabilize poliovirus vaccine strains and $MgCl_2$ at a molar concentration was found to be the best. All the types of poliovirus

vaccine could be stored for 1 year at 4 °C or for 3 weeks at 25—28 °C without loss of infectivity (Melnick and Wallis, 1963). Several organic and inorganic acids could also stabilize poliovirus vaccine strains at high temperature (Melnick and Wallis, 1963; Srivastava *et al.*, 1987).

According to Mirchamsy *et al.* (1978), 70 % sucrose was as effective as 1 mol/l $MgCl_2$ to stabilize polioviruses at 4 °C but less effective at 22—25 °C, although 35 % sucrose and 0.5 mol/l $MgCl_2$ could stabilize the virus similarly as 1 mol/l $MgCl_2$. As shown in this paper, sucrose at around 15 % was effective in stabilizing the highly temperature-sensitive type 3 poliovirus vaccine strain. Further studies will be required to see the seroconversion rate in children who received such stabilized vaccines in the tropical countries.

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References

Albert, M. J. (1987): Failure of live, oral virus vaccines in developing countries. *J. infect. Dis.* **155**, 1350.

Eagle, H. (1959): Amino acid metabolism in mammalian cell cultures. *Science* **130**, 432—437.

John, T. J. (1981): How shall we control poliomyelitis in India? *Indian J. Pediatr.* **48**, 565—568.

John, T. J. (1984): Poliomyelitis in India: prospects and problems of control. *Rev. infect. Dis.* **6** (Suppl), S438—441.

LaForce, F. M., Linchnevski, M. S., Keja, J., and Henderson, R. H. (1980): Clinical survey techniques to estimate prevalence and annual incidence of poliomyelitis in developing countries. *Bull. World Hlth. Org.* **58**, 609—620.

Melnick, J. L. (1978): Advantages and disadvantages of killed and live poliomyelitis vaccines. *Bull. World Hlth. Org.* **56**, 21—38.

Melnick, J. L., and Wallis, C. (1963): Effect of pH on thermal stabilization of oral poliovirus vaccine by magnesium chloride. *Proc. Soc. exp. Biol. Med.* **112**, 894—897.

Mirchamsy, H., Shafyi, A., Mahinpour, M., and Nazari, P. (1978): Stabilizing effect of magnesium chloride and sucrose on Sabin live polio vaccine. *Develop. biol. Standard* **41**, 255—257.

Sabin, A. B. (1959): Present position of immunization against poliomyelitis with live virus vaccine. *Brit. Med. J.* **1**, 663—680.

Sabin, A. B. (1985): Oral poliovirus vaccine: history of its development and use and current challange to eliminate poliomyelitis from the world. *J. infect. Dis.* **151**, 420—436.

Salk, J. E. (1955): A concept of the mechanism of immunity for preventing paralysis in poliomyelitis. *Ann. N.Y. Acad. Sci.* **61**, 1023—1038.

Salk, J. E. (1984): One-dose immunization against poliomyelitis using a noninfectious vaccine. *Rev. infect. Dis.* **6** (Suppl), S444—450.

Srivastava, A. K., Matyasova, I., and Koza, J. (1987): Effect of hydrochloric acid concentration on the stabilization of poliovirus at high temperature. *Trop. Med.* **29**, 55—56.

Wallis, C., and Melnick, J. L. (1961): Stabilization of poliovirus by cations. *Texas Rep. Biol. Med.* **19**, 683—700.

Wallis, C., and Melnick, J. L. (1962): Effect of organic and inorganic acids on poliovirus at 50 °C. *Proc. Soc. exp. Biol. Med.* **111**, 305—308.

Wallis, C., Melnick, J. L., Ferry, G. D., and Wimberly, I. (1962): An aluminium marker for the differentiation and separation of virulent and attenuated polioviruses. *J. exp. Med.* **115**, 763—775.