SEVENTEEN YEARS OF APPLICATION OF HERPES VACCINES IN BULGARIA

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Summary. – This study reports on our experience with whole herpes simplex vaccines in Bulgaria for a period of 17 years. More than 1 500 immunized patients with *herpes ophtalmicus* showed a reduction of the recovery period, reduced number of the recurrences and reduced risk of visual damage. More than 14,000 patients suffering from other forms of herpes diseases for a longer period showed reduced recovery period and recurrence frequency in over 95% of the cases. Whole vaccines were well tolerable with no side effects. This report emphasizes the usefulness of whole herpes vaccines.

Key words: herpes vaccines; vaccination; efficiency

Introduction

Herpetic diseases play an important role in human pathology. Investigation in different regions of Bulgaria have indicated an average incidence of more than 60% of different forms of herpetic diseases among the population. During the last 25 years an increase in both frequency and severity has been established (Neshev, 1980; Tomov, 1982; Dundarov *et al.*, 1982*b*; Dundarov *et al.*, 1983; Antonova, 1986). The prolongation and increased frequency of herpes diseases requires effective therapy. Bulgaria is a small country with well organized health care and benefits from certain advantages in the diagnosis and the therapy of herpetic diseases, namely (a) well trained teams of specialists involved in studies on this problem (Bakalov, 1978; Hadjieva, 1980; Neshev, 1980; Tomov, 1982; Antonova 1986), following up the effectiveness in 42 major hospitals throughout Bulgaria, (b) a production base and clinical experience, namely vaccines, a number of various herpes inhibitors, specific immunoglobulins and interferons (Dundarov *et al.*, 1983), and (c) free vaccines paid for by the Ministry of Health since 1975.

Materials and Methods

Vaccines. In 1975 NCIPD as a manifacturer licensed 3 types of whole herpes simplex vaccines – type 1, type 2 and a combined 1 + 2 vaccine. The first two vaccines contained 5 different strains of HSV-1 and HSV-2 and the vaccine 1 + 2 was a combination of both. The vaccinal strains were selected after cross-neutralization studies from 400 newly isolated HSV strains with different properties (Dundarov *et al.*, 1975; Andonov *et al.*, 1975*a*; Bakalov, 1978; Andonov *et al.*, 1979; Dundarov *et al.*, 1980). The viral antigens were prepared in primary rabbit kidney cells. The virus was inactivated with formaldehyde and the vaccine was lyophilized in ampoules. One vaccinal dose contained at least 10^8 viral particles.

The innocuity of the vaccines was confirmed as follows: (a) the vaccinal strains did not show transforming activity on diploid human lung cell cultures even after 20 passages (100 days of observation) (Andonov *et al.*, 1979); (b) formalin-inactivated vaccinal strains did not lead to changes in chromosomal apparatus of the same culture (Varadinova, 1976); (c) the three vaccines did not show oncogenic properties after inoculation into newborn white mice for 1 year (Andonov *et al.*, 1975*b*); (d) no chromosomal deviations in cell cultures and in the lymphocytes of the patients were established by chromosomal banding (Mincheva *et al.*, 1984); (e) no side effects after immunization were observed in more than 15,000 patients during a follow-up period of 1 to 17 years (Dundarov *et al.*, 1982*a*; Kavaklova *et al.*, 1986; Dundarov *et al.*, 1988; Dundarov *et al.*, 1993). The quality of every batch of herpes vaccine was controlled and approved by the National Institute of Drugs, Bulgaria.

Patients. All patients were over 14 years of age suffering from frequent and severe HSV-1 or HSV-2 recurrences. Most of the immunized patients were followed up annually by more than 50 specialists, including dermatologists and ophtalmologists.

Two immunization schedules were used: (a) six subcutaneous injections at an interval of 20 – 30 days; (b) patients with *herpes*

ophthalmicus or with anamnesis of allergic disease were given increasing daily doses for a period of two weeks (Dundarov *et al.*, 1982*b*). The type of vaccine used corresponded to the virus type involved in the disease.

Vaccine effectiveness was estimated by the frequency and average decrease of the number of the days during which the patients had herpetic lesions per year in comparison with the frequency and duration of the recurrences before immunization. "Very good" effectiveness was recorded when there were no new reccurencies or they were reduced more than 5 times, "good" corresponded to 2 – 5-fold reductions, and "limited" to 1.5 – 2-fold reductions.

Results

Whole herpes vaccines were used in more than 15,000 patients. 6,324 of whom were regularly followed up for not less than one year and 92 – for 15 years. In more than 1,500 patients with *herpes ophthalmicus* the type 1 vaccine was used after the 2nd – 3rd reccurence without limitation in their age, 827 patients were under observation for more than one year. The control group of 160 patients with *herpes ophthalmicus* was treated with chemotherapy and symptomatic drugs. The advantages of immunotherapy are clear (Fig. 1): (a) the reccurence rate in non-immunized patients (the control group) was 30 – 33%, while reccurency rate amongst immunized patients was reduced down to 5 – 20%; (b) there was a systematic improvement of the state of immunized patients during the next years; (c) the average period of hospitalization in these patients was reduced by 11 days; (d) the risk of injured vision was reduced more than 10 times.

Patients with other clinical forms of herpes simplex diseases were immunized only if the recurrences lasted for more than 60 days annually. The immunizations were done in 3-month cycles with 6 injections every 20 days. Further immunizations were done if indicated, e.g. in the case of an insignificant clinical response or disappearance of the response. The effectiveness of the type 1 vaccine on patients with labial or facial herpes is compared to that of type 2 or of the combined vaccine on patients with genital or gluteal herpes is displayed on Fig. 2. The summarized results obtained through detailed registration of 5,337 patients followed up for more than one year indicated (a) the effectiveness of the vaccines in term of clinical response which increased with the period of observation and reached more than 95%, and (b) that in the first 2 – 3 years the effectiveness of the HSV-1 vaccine was slightly higher than that of HSV-2, but after more immunizations the results became equal. Five years after immunization the general effectiveness was stable in over 95% of the case.

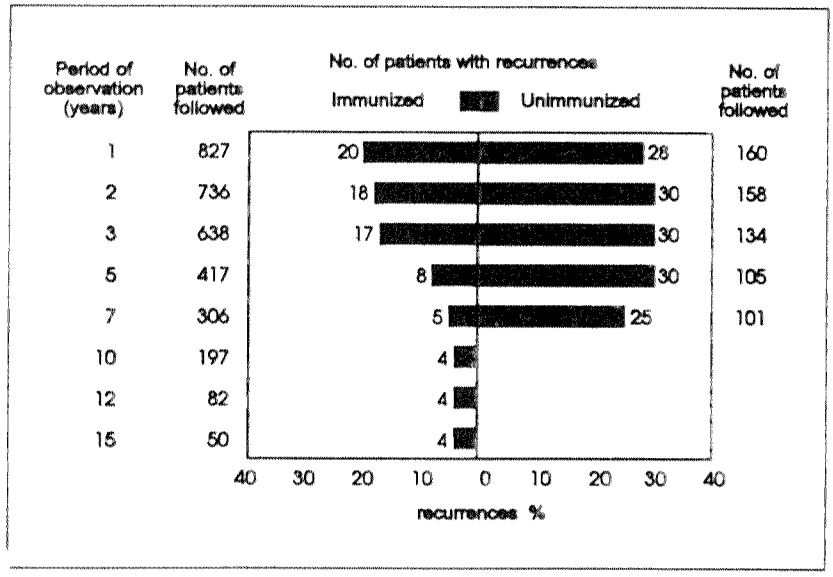

Fig. 1
Incidence of herpes keratitis recurrences after immunization

Discussion

There are differing views about the usefulness and the innocuousness of the whole herpes vaccines with a prevailing scepticism (Melnick, 1989; Roizman, 1991). According to Meigner (1985) herpes vaccines containing DNA have been proved to be risky and their use should be discontin-

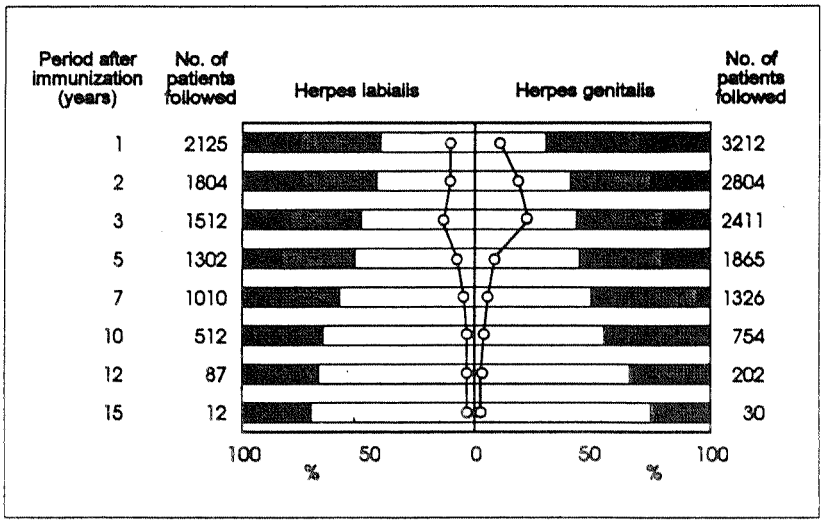

Fig. 2
Effectiveness of herpes immunization after different time
Effectiveness: very good (white columns), good (light gray columns), limited (dark gray columns), without effect (black columns).
Percentage of patients requiring new immunization during the period observed (o).

ued; but he admits himself that this view point is not sufficiently supported by scientific arguments. Skinner *et al.* (1991) claimed that Bulgarian whole herpes vaccines are completely harmless. Perkins (1982) decleared that there are no requirements formulated by WHO limiting the application of whole herpes vaccines.

This discussion has been perpetuated due to the lack of publicity on a continuous observation of clinical effectiveness of herpes vaccines. Preliminary positive results with different subunit herpes vaccines on animals and limited number of volunteers, more than 10 years ago (Kutinova *et al.*, 1982; Hilfenhaus *et al.*, 1982; Cappel *et al.*, 1982) have not been confirmed and applied into practice later on. The live attenuated and recombinated herpes vaccines are still at a preclinical stage (Stanberry *et al.*, 1987, 1988; Burke *et al.*, 1989, 1991; Meigner, 1991; Skinner *et al.*, 1991) and their reliability needs to be proved in clinical trials.

Our comparative studies of whole and DNA-free herpes vaccines type 1 and type 2 in "double blind trial" on many animals and 150 volunteers have showed undoubtedly higher and more durable effectiveness of the whole herpes vaccines (Kavaklova *et al.*, 1986; Dundarov *et al.*, 1988). The "placebo" data have proved that the improvement of status of the patients after immunization was due to the immunospecific factors.

A good vaccine must be effective, cheap and safe. Our whole herpes vaccines have proved their effectiveness on a large number of patients after long and well-controlled observation in Bulgaria and in limited groups of patients in Russia (Golubev *et al.*, 1993) and Great Britain (Skinner *et al.*, 1991). The vaccines are safe and not oncogenic from the following reasons: (a) our vaccines are used only in patients with very frequent recurrences. The amount of viral DNA given with the vaccine is a hundred times less than the amount patients receive nearly every month during recurrences and nobody has proved that people with frequent herpetic recurrences have a higher incidence of cancer; (b) whole herpes vaccines have been used for more than 20 years in many countries without any evidence of oncogenicity. In Bulgaria, nobody among the immunized patients has developed cancer in the long follow-up period; (c) there are proofs that formalin-inactivated herpes viruses lose their oncogenic properties and can prevent the primary infection with live oncogenic animal herpes virus (Laufs and Steinke, 1974).

Out of more than 15,000 immunized patients in our country no side effects have been observed even after many immunizations. The local reaction at the place of the injection on the next day - reddening and slight swelling up to 5 – 7 cm in diameter, disappears usually after 1 – 2 days. According to Tomov (1982) a better clinical effect is achieved in patients with stronger local reaction.

We do not find clear correlation between immune response and clinical effectiveness in immunized patients. About 80% of them had an increased titer of herpes antibodies by neutralization and complement-fixation tests (Bakalov, 1978; Hadjieva, 1980). An increase in cell-mediated immunity by the migration-inhibition test and immunoblast-transformation test were noted in the same experiments (Hadjieva, 1980). After application of herpes vaccine in guinea pigs, lymphocytes accumulate in the regional lymph knots, the lungs of the animals become quickly and actively involved in the immune process (Zvetanov *et al.*, 1991). Our preliminary study does not suggest the impor-

tance of certain HLA antigens for development of herpetic diseases or for a favourable results after immunization.

Our experience in the use of whole herpes vaccines is encouraging and gives us grounds to continue their application. We think. it is unreasonable to restrict their general application at a world-wide scale.

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