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ORIGINAL ARTICLE

Multicentre clinical trial with herpes simplex virus vaccine in recurrent herpes infection

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Summary: The aim of this work was to confirm our preliminary clinical and immunological evaluation of the protective effects of a herpes simplex virus (HSV) vaccine derived from killed virus in the treatment of relapsing facial or genital herpes simplex infection. A total of 142 patients were treated with the HSV vaccine and a control group of 50 were treated with intermittent oral acyclovir (ACV). The vaccine reduced annual active disease days in vaccinees to 11.59 (±15.3) after treatment (65.11±31.64 before treatment) compared to 30.4±17.49 days after treatment of the control group patients (71.86±32.5 before treatment).

Keywords: Herpes infection, vaccine, acyclovir

INTRODUCTION

Since 1924 over 50 vaccines against herpes simplex virus (HSV) have been tested. None has been shown to be effective in prevention of HSV infection in humans because of failure to promote an adequate or prolonged immune response, given that primary HSV exposure may have taken place some years earlier[1]. Conversely, many vaccines have been successfully shown to prevent or drastically reduce recurrences[2,3]. The live attenuated HSV vaccines have been abandoned because of the risk of mutant virus or viral recombinant particles replicating in the central nervous system and theoretical oncogenic risk[4]. Herpes simplex virus type 2 (HSV2) has previously been considered as a possible causal agent for cervical carcinoma[5]. Subsequent studies have shown that HSV DNA is incapable of coding 'transforming proteins' and is only able to cooperate in an oncogenic sense with particular human papillomaviruses (HPVs)[6]. Several papers have shown[7–14] that maximum safety, efficacy and antigenic specificity is achieved by using a subunit vaccine, with glycoproteins D (gD) and B (gB) or a combination of both (natural or purified), as both subunits have a high degree of homology between HSV1 and HSV2 (86% homology at the level of DNA and amino acid sequence). The conservation of gD and gB protein sequences between different strains of the same virus is even higher (>99% homology)[11]. Other authors[4,15,16–21] maintain that, as not all immune responses thus induced are relevant for protection against infection, only pure preparations containing a major part of glycoproteins of the viral envelope are immunogenic. This is because they stimulate a large variety of immune responses. These vaccines are among the few to have been tested on humans[22–30]. These preparations demonstrated a partial capacity to protect the seronegative partner from exposure to herpes genital infection[24,29], yet in 65% to 80% of cases the vaccines significantly reduced the frequency and severity of herpetic manifestations[27]. In the light of these data we have conducted a multicentre clinical trial of HSV vaccine to examine the clinical course of recurrent infection in a large number of patients.

MATERIALS AND METHODS

The study was conducted in 9 Departments of Dermatology in Italy*, all of which followed an agreed protocol which was approved by the hospital ethical committee.

Patients admitted to this study all had recurrent facial or genital herpes, with a disease-free period not exceeding 70 days. Diagnosis was confirmed

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with the isolation and/or identification of the virus (EIA, DIF, ISH) from the lesions. The patients were screened for the presence of HSV1 and 2 antibodies. This blood test was repeated at relapse or during follow-up visits to monitor changes in antibody titre. Those patients who had already been exposed to similar vaccination, with positive clinical history of exanthem, drug-induced erythema multiforme or purpura, atopic dermatitis, pregnant women and patients receiving immunotherapy with interferons or steroids were excluded from the study. The subjects were randomly matched from each centre in two groups with a 3 : 1 Vaccine group to Control group ratio.

The vaccine we used has been previously described[27]. In summary, it consists of chick embryo chorioallantoic membrane growing HSV1 and HSV2, rendered inactive by heating at 56°C for 2 h. No intact, disrupted virus or viral DNA fragments were detected by polymerase chain reaction (PCR) although it does contain gB and gD[31]. Type H and type G vaccine respectively for HSV1 and HSV2 infection were administered subcutaneously to the deltoid region.

Table 1. Patients and disease characteristics

	HSV vaccine group $n=142$ (70 F, 72 M)	Control group $n=50$ (20 F, 30 M)
Mean age	32.21 ± 5.4	27.15 ± 4.8
HSV infection	69 facial (64 F, 5 M) 65 genital (55 F, 10 M) 8 facial & genital (6 F, 2 M)	24 facial (20 F, 4 M) 26 genital (26 M)
Site of HSV-1 infection	Lip 43 Perioral region 5 Zygomatic region 5 Oral mucosa 7 Nasal mucosa 10 Cornea 12	Lip 18 Perioral region 5 Zygomatic region 3 Oral mucosa 3 Nasal mucosa 1
Site of HSV-2 infection	Minor & major labia 6 Perineum 8 Perianal region 5 Cervix uteri 4 Gluteal region 9 Foreskin 28 Glans 21 Penis skin 12 Urethra 2	Minor & major labia 4 Vulva 2 Perianal region 4 Foreskin 13 Glans 9 Penis skin 4

Dose schedule

The dose schedule was as follows: month 1–3, 1 ml (100.000 EIC50) per week; month 4–5, 1 ml every 2 weeks; month 6–9, 1 ml per month; month 12, 1 ml (21 vials in all). At the appearance of eventual relapse, 1.2–2 g of oral acyclovir was administered daily until the resolution of the episode.

Controls

The control group patients were treated with 1.2–2 g of oral acyclovir per day only in cases of relapse during a follow-up period of one year. To assess the efficacy of the therapeutic treatment, we considered specific clinical parameters related to the past 12 months of disease, the 12 months of actual study and a 3-month follow-up. We recorded the frequency, intensity, duration, and location of each HSV episode. Patients with no outbreaks were examined at 3-monthly intervals. Written informed consent was obtained from all subjects.

Statistical analysis

The Mann-Whitney U-test was used for the statistical analysis of the comparability of the 2 groups. The Wilcoxon test for paired data was used to study the clinical therapeutic effects in each group following specific evaluation criteria (number of recurrences a year, average duration, active disease days per year, and disease-free time). The Mann-Whitney U-test was used for the comparison of therapeutic results. In order to demonstrate the homogeneity of the 2 groups, age and duration of episode was compared using the Student's 't'-test for independent data, herpetic infection, facial or genital, and sex by the 'Chi' squared test. All evaluations were conducted with the Systat ver. 5.2 computer program.

RESULTS

All subjects in the study tested positive for HSV. When it was not possible to subtype the virus, only the clinical criteria were considered for administration of the vaccine. A total of 142 subjects were treated with the HSV vaccine, 72 males and 70 females, aged 10–70 years (mean age 32.21) of whom 69 had facial herpes infections, 65 had genital herpes infections, and 8 had both (Table 1). In 6 of 8 patients with both facial and genital recurrences we chose to use the vaccine type for the most severe clinical manifestation. In the other 2 patients, both types of vaccine were used simultaneously.

The control group consisted of 50 patients, 30 males and 20 females aged 16–63 years (mean age 27.15), of whom 24 had facial and 26 had genital herpes (Table 1). The 2 groups of patients had overlapping clinical characteristics. At time of enrolment patients assigned to receive HSV vaccine showed a mean number of recurrences per year of 8.55 and an average of 65.11 active disease days per year (Table 2). Those in the control group had a mean number of 8.84 manifestations a year and 71.86 active disease days per year (Table 2). The results obtained (Table 3) demonstrate an improvement in both the frequency and duration of relapse in the patients treated with vaccine. The mean number of recurrences a year, in fact, was reduced from 8.55 (±3.24) to 2.92 (±2.95) with an increase of disease-free time from a mean of 40.23 (±15.41) to 205.64 (±135.92) days, and the duration

Table 2. Clinical parameters: comparability of the two groups before treatment

	HSV vaccine group	Comparability°	Control group
Recurrence (number/year*)	8.55±3.24	No significant difference $P=0.165$	8.84±2.37
Disease free time (days)*	40.23±15.41	No significant difference $P=0.127$	36.38±12.86
Recurrence duration (days)* #	7.74±3.05	No significant difference $P=0.222$	8.12±2.78
Active disease (days/year)*	65.11±31.64	No significant difference $P=0.124$	71.86±32.5

*Mean±SD
°Mann-Whitney's 'U' test
Student's 't'-test

of the episodes from a mean of 7.74 (±3.05) to 2.85 (±2.62) days. The annual number of active disease days dropped from a mean of 65.11 (±31.64) to 11.59 (±15.3).

In the control group the improvement of clinical parameters was less remarkable. The annual mean number of recurrences decreased from 8.84 (±2.37) to 6.22 (±2.05). Disease-free time increased from a mean of 36.38 (±12.86) to 61.58 (±25.38) days. Single episode duration dropped from 8.12 (±2.78) to 4.78 (±1.98) days. The annual active disease days were reduced from 71.86 (±32.5) to 30.4 (±17.49) (Table 3).

The statistical analysis of the clinical results (Table 4) between the 2 groups is significant. It is important to underline that 37 subjects (26%) treated with vaccine, who at the time of enrolment had a mean of 7 recurrences per year and 45 annual active disease days, did not have any relapses during the treatment period. Among all vaccinees no significant differences between the results of the facial and genital herpes treatments were found. All the control group patients continued to experience recurrent herpes.

There was no increase in IgG titre immediately after vaccination and we found a decreased antibody level related to a parallel decrease of relapses. We did not observe any general or local side effects in the vaccinees. Only six (4.2%) complained of pain and/or intense short-lasting burning at time of injection.

DISCUSSION AND CONCLUSION

HSV1 and HSV2 can cause human infection involving mucocutaneous surfaces, the eyes and the brain, and occasionally other tissues and organs[32]. A 'definitive pharmaceutical' which would be capable of eradicating the disease does not yet exist, therefore much attention is devoted to the study of vaccines. The ideal vaccine should be able to prevent the primary infection in seronegative subjects and neonatal infection when given to seronegative mothers[33].

Table 3. Clinical parameters: pre/post-treatment

	HSV vaccine group Pre-treatment	Post-treatment	Control group Pre-treatment	Post-treatment
Recurrence (number/year)*	8.55±3.24	2.92±2.95	8.84±2.37	6.22±2.05
Disease-free time (days)*	40.23±15.41	205.64±135.92	36.38±12.86	61.58±25.38
Recurrence duration (days)*	7.74±3.05	2.85±2.62	8.12±2.78	4.78±1.98
Active disease (days/year)*	65.11±31.64	11.59±15.30	71.86±32.5	30.4±17.49

*Mean±SD

Table 4. Clinical parameters: pre- and post-treatment statistical comparison

	HSV vaccine group		Pre/post-vaccine treatment comparison°	Control group		Pre/post-antiviral treatment comparison°	Vaccine/antiviral treatment comparison #
	Pre-treatment*	Post-treatment*		Pre-treatment*	Post-treatment*		
Recurrence (number/year)	8.55±3.24	2.92±2.95	$P<0.001$	8.84±2.37	6.22±2.05	$P<0.001$	$P<0.001$
Disease-free time (days)	40.23±15.41	205.64±135.92	$P<0.001$	36.38±12.86	61.58±25.38	$P<0.001$	$P<0.001$
Recurrence duration (days)	7.74±3.05	2.85±2.62	$P<0.001$	8.12±2.78	4.78±1.98	$P<0.001$	$P<0.001$
Active disease (days/year)	65.11±31.64	11.59±15.3	$P<0.001$	71.86±32.5	30.4±17.49	$P<0.001$	$P<0.001$

*Mean±SD
°Wilcoxon's test
Mann-Whitney's 'U' test

The HSV2 gD vaccine has been shown[7,12–14,34] to confer a high degree of protection against latent infection due to the presence of an elevated level of neutralizing antibodies and cytotoxic T-lymphocytes (CTL)[14]. These lymphocytes are T4+, T3+, T8− and HLA class II DR-1 antigen restricted[35]. The gB vaccine produces similar effects[16,36,37] as does a combination of these 2 glycoproteins. Many authors[23,35,38] stress the irrelevant role of neutralizing antibody, and the primary role of macrophages, natural killer cells (NK) and helper or suppressor CTL.

Our previous experience[27] shows that HSV vaccine can both decrease or eliminate HSV recurrences and clinical symptoms in 67% and 32% of cases respectively. In vaccinees a selective stimulation of the CD3+ (64%), CD2+ (48%), CD4+(56%) lymphocytes and NK cells (40%) was observed suggesting as a probable mechanism the specific increase of the CMI response.

The results of this larger study confirm that the vaccinees experienced clinical benefits in terms of decrease of both annual mean number and severity of recurrences, and a disease-free time increase greater than the control group patients. The HSV vaccine produced a significant decrease of annual active disease days in vaccinees to 11.59 (±15.3) after treatment (65.11±31.64 before treatment), compared with 30.4 (±17.49) days after treatment of the control group patients (71.86±32.5 before treatment) (Table 4). This agrees with the results of a previous trial[27].

These results are also comparable to those of Mertz and colleagues[29] which demonstrate that antibodies against the HSV vaccine specific glycoproteins develop slowly and are not always detectable after vaccination. Furthermore, the total antibody level was lower compared with subjects who had naturally acquired disease and 5% lower than in subjects with recurrences[29]. The clinical results obtained confirm that selective stimulation of CD3+, CD2+, CD4+ and NK cells is the basis of clinical improvement.

Further studies will be necessary to clarify the relationship of the humoral and cellular immune responses to vaccines. These may be effective in preventing recurrence in primates as well as humans, but prove ineffective in preventing the infection. The dose and type of glycoprotein used would be essential for the induction and amplification of a specific immune response and above all a CTL biphasic lymphoproliferative response[14,19,30].

Finally, the treatment was well tolerated by the patients and has shown itself free of negative side effects and/or overdosage.

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