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Combined antiviral effects of acyclovir or bromovinyldeoxyuridine and human immunoglobulin in herpes simplex virus-infected mice

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Summary

Suboptimal dosage regimens of antivirals (acyclovir or bromovinyldeoxyuridine) and human immunoglobulin have been combined in the treatment of hairless mice infected with herpes simplex virus type 1. The aim of this study was to establish how late after infection human immunoglobulin could be given to still be effective and for how long would the protective effect last. The combination of acyclovir or bromovinyldeoxyuridine with passive immunization proved additive or even synergistic depending on the time of immunoglobulin administration and the observation period after infection. When the survival rate of the mice was followed for up to 20 days postinfection, synergistic action seemed to occur with immunoglobulin given as late as 2 or 3 days after infection. When the mice were followed for up to 100 days after infection, however, it turned out that only the immunoglobulin given 4 h after infection led to a synergistic effect with the antivirals. Most of the mice subjected to combined treatment, in contrast to mice treated with the antivirals only, did not develop anti-HSV antibodies. This lack of a specific humoral immune response possibly reflects the rapid inhibition of virus replication early after challenge by the combined treatment, thus preventing the production of a sufficient amount of viral antigen in the body needed for a measurable antibody induction.

Chemotherapy; Immunization, passive; HSV

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Introduction

From previous studies in animal models it is well known that passive immunization with antisera or immunoglobulins shortly after infection may protect the host from the sequelae of a primary herpes simplex virus (HSV) infection [9,14]. On the other hand, chemical substances have also been shown to protect HSV-infected animals, provided treatment was again started shortly after infection [3–5,11]. Since passive immunization alone or chemotherapy alone often conferred only partial protection, the efficacy of combined treatment has been studied by several investigators [1,2,15]. In HSV-infected immunocompetent mice a synergistic effect of passive immunization and adenine arabinoside or acyclovir therapy was observed [1,2] and even in immunocompromised mice a synergistic effect of acyclovir and human immunoglobulin could be obtained [15]. The effect of combined immuno- and chemotherapy was less pronounced in immunocompromised mice than in immunocompetent mice, which may not be unexpected in view of the role of cellular immunity, particularly of T lymphocyte, in the control of HSV infection [10]. In the combined immuno- and chemotherapy studies antibodies were administered 1–3 h after infection. The effect of antibody administered at several days after infection has so far not been investigated as part of a combined immuno- and chemotherapy protocol. Moreover, most of the previous studies were based on observation periods of about 3 wk postinfection, for all animals of the untreated infected protocol groups died within about 2 wk. From our own studies on the protective effect of passive immunization we know, however, that HSV-infected mice treated with a single dose of human immunoglobulin (HIG) may eventually die up to 12 wk after infection. We have now investigated in immunocompetent HSV-infected mice the combined effects of acyclovir or bromovinyldeoxyuridine and passive immunization. In these studies we wanted to establish: (1), how late after primary infection passive immunization would still be effective as part of such a combined immuno- and chemotherapy regimen, and (2), how long the protection offered by such combination therapy may last.

Materials and Methods

Virus and mouse model

Immunocompetent hairless mice were infected intradermally with the HSV-1 strain Wal as described previously [6]. Briefly, mice were infected intradermally with 50 μl containing approximately 10^3 ID_{50} of the challenge virus at 2 different sites each adjacent to the lumbar part of the spine.

Chemotherapeutics

(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (bromovinyldeoxyuridine, BVDU) was synthesized by R. Busson and H. Vanderhaeghe (Rega Instituut, Katholieke Universiteit Leuven, Belgium) following a modification of the procedure described in [7]. For acyclovir (ACV), commercial preparations, purchased as Zovirax® in the F.R.G., were used.

Human immune globulin

Beriglobin®, the commercial HIG of Behringwerke, was dialysed against 0.15 M NaCl, then diluted to 50 mg HIG/ml and sterile filtered. The preparation (lot no. 401222) had an ELISA antibody titer of 1:8000 and a neutralizing antibody titer of 1:220 when assayed against 100 ID_{50} of HSV-1.

Therapeutic treatment

Infected mice were treated intraperitoneally with either BVDU of ACV (each at 40 mg/kg body weight per day) dissolved in phosphate-buffered saline. Treatment was initiated 3 h after infection followed by a second administration 5 h later and then continued twice daily for 4 consecutive days. A single dose of HIG (1.0 ml per mouse) was administered intraperitoneally at either 4, 24, 48 or 72 h after infection. Combined immuno- and chemotherapy consisted of either the BVDU or the ACV treatment protocol combined with a single HIG dose administered at either 4, 24, 48 or 72 h after infection. Infected, but not treated mice were included as virus control group. Any controls regarding the toxicity of the antivirals were omitted, because of the well-documented low toxicity of these drugs [3].

ELISA antibody determination The ELISA method was performed exactly as described previously [12].

Statistical analysis The significance of differences between the results of different groups was examined by use of Fisher's exact test.

Results

From previous studies we knew that a single administration of a potent HIG preparation 4 h after HSV-1 infection completely protected hairless mice for a period of 20 days (data not shown). For the present series of experiments we selected a HIG preparation which conferred only partial protection at 20 days postinfection. As can be seen from the results of 2 separate experiments, the protection achieved by HIG continued to decline after the 20th postinfection day (Fig. 1A). Apparently, single HIG treatment did not completely eliminate infectious HSV but merely reduced the virus burden and thus delayed the development of disease in most of the mice treated. In HSV-1 infected mice treated with suboptimal doses of ACV or BVDU (40 mg/kg/day) for 5 days (Fig. 1B), mortality rates did not change beyond the 10th postinfection day (except for a single mouse in the BVDU group which died at the 51st day). The sub-optimal dosages of HIG or the chemotherapeutics as used in Fig. 1 were also used in the combined therapy experiments described below.

Table 1 summarizes the results of an experiment in which mice were treated repeatedly with ACV or BVDU alone or combined with a single dose of HIG given 4, 24, 48 or 72 h postinfection, respectively. At 20 days after infection combined treatment resulted in an at least additive effect for all groups, including the group

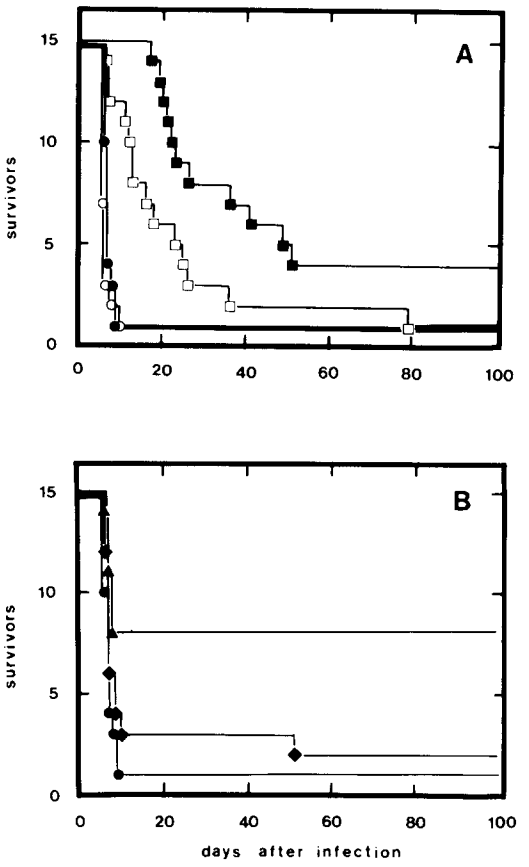

Fig. 1. Efficacy of passive immunization (A) or chemotherapy (B) in hairless mice infected intradermally with HSV-1. (A) HIG was injected intraperitoneally at 4 h postinfection; the results of 2 independent experiments are shown. Exp. 1: untreated, virus-infected mice (●); HIG-treated, virus-infected mice (■). Exp. 2: untreated, virus-infected mice (○); HIG-treated, virus-infected mice (□). (B) Virus-infected mice were treated with ACV or BVDU (see Materials and Methods), beginning 3 h postinfection: untreated, virus-infected mice (●); ACV-treated, virus-infected mice (▲); BVDU-treated, virus-infected mice (◆).

treated with HIG at 72 h postinfection. However, because of some variation in protection observed 20 days postinfection it would be difficult to decide whether synergistic effects really occurred. Furthermore, the results of different groups on postinfection day 20 seem to be difficult to interpret. For example, the protection rate in the group treated only with HIG at 48 h postinfection was higher than in the group treated only with HIG at 24 h postinfection. Also, protection was higher in the group treated with BVDU plus HIG at 24 h postinfection than in the group treated with BVDU plus HIG at 4 h postinfection. These variations, however, may be attributed to the delayed development of the disease under HIG treatment and thus to the too short observation period (20 days). The situation became much

TABLE 1

Protection rate on the 20th day postinfection of HSV-1-infected mice subjected to chemotherapy with ACV or BVDU and/or passive immunization.

Chemotherapy	Rate of protection (survivors/mice infected)				
	No HIG treatment	time of postinfection treatment with HIG			
		4 h	24 h	48 h	72 h
None	0/20	7/20	2/20	6/20	0/20
40 mg ACV/kg/day*	7/20	17/20	17/20	13/20	11/20
40 mg BVDU/kg/day*	2/19	10/20	16/20	10/20	4/20

* For 5 days; see Materials and Methods.

clearer when the observation period was extended to 100 days postinfection.

The results for a 100-day observation period are presented in Fig. 2 showing those of combined treatment of ACV plus HIG administered 4 h or 24 h after infection in Fig. 2A and those of combined treatment of BVDU plus HIG in Fig. 2B. The data for the combination of chemotherapy with HIG administered at 48 or 72 h postinfection are not shown, because these combinations did not confer better protection than ACV or BVDU alone when followed over a 100-day observation period. From the data presented in Fig. 2A and B it is clear that in our study only the combination of ACV or BVDU with HIG administered at 4 h postinfection caused a long-term protection, while in the case of combined therapy with HIG administered at 24 h postinfection, the protection rate remained for several weeks higher than that of ACV, BVDU or HIG alone, but eventually the results of the combined treatment approximated that of ACV or BVDU alone.

Since none of the HSV-1 infected mice treated only with HIG survived postinfection day 100, the question of whether combined treatment resulted in a synergistic effect is reduced to the question of whether combined treatment is significantly better than chemotherapy alone. Thus the significance of the difference between the 100 day mortality rates of mice (data shown in Fig. 2) subjected to chemotherapeutic or combined treatment was assessed using Fisher's exact test, resulting in $P < 0.05$ for HIG treatment 4 h postinfection combined with ACV, or in $P < 0.1$ for HIG treatment 4 h postinfection combined with BVDU. Similar results were obtained in three independent experiments, showing that combination of chemotherapy with HIG administration 4 h postinfection resulted in an unequivocally reproducible synergistic effect. In 2 of these experiments (data not shown) the survival curves of the group ACV plus HIG at 24 h postinfection resembled that of the group BVDU plus HIG at 24 h postinfection as shown in Fig. 2B, i.e. decline of protection during the first 50 days after infection. A number of very late deaths (80–100th day postinfection) as observed in the group ACV plus HIG at 24 h postinfection of the experiment shown here (Fig. 2A) was rather a rare event.

From the delay in death, however, the question arose as to whether the survivors had developed antibodies to the challenge virus. Therefore, anti-HVS-1 an-

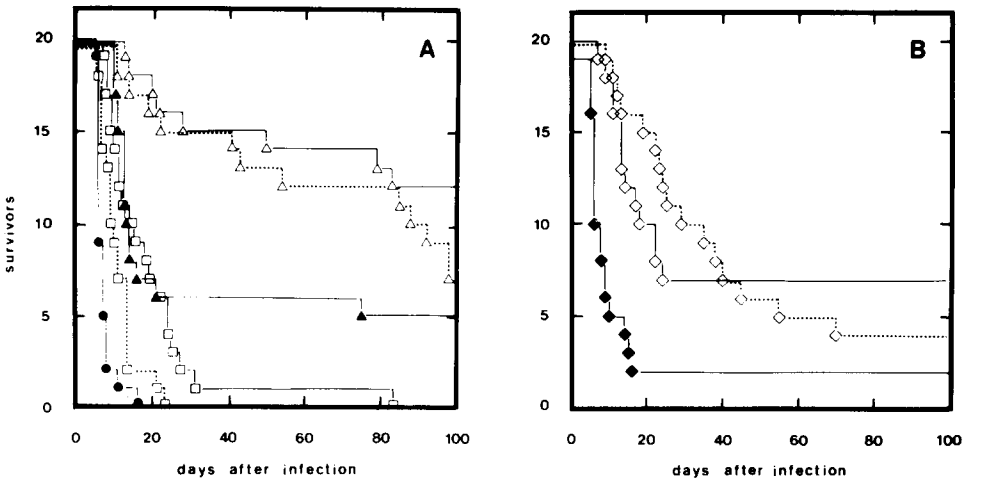

Fig. 2. Combined immuno- and chemotherapy of HSV-1 infected hairless mice, consisting of (A) ACV treatment plus a single dose of HIG at either 4 h or 24 h after infection or (B) BVDU treatment plus a single dose of HIG at either 4 h or 24 h after infection. All combinations were evaluated in the same experiment. The results for the virus-infected control group and the groups treated only with HIG at 4 h or 24 h postinfection are shown only in graph A. Graph A: untreated, virus-infected mice (●); virus-infected mice treated with HIG at either 4 h (□——□) or 24 h (□·····□) after infection; virus-infected mice treated with ACV (▲); virus-infected mice treated with ACV plus HIG at 4 h (△——△) or with ACV plus HIG at 24 h postinfection (△·····△). Graph B: BVDU treatment only (◆); BVDU combined with a single HIG injection at 4 h postinfection (◊——◊); BVDU combined with a single HIG injection at 24 h postinfection (◊·····◊).

tibody titers were determined by ELISA in the sera of the survivors for the experiment shown in Fig. 2A and B. From the data presented in Table 2, it is clear that 6 out of 7 animals submitted to chemotherapy alone (ACV or BVDU) had developed antibodies to HSV-1. On the contrary 30 of 37 mice surviving a combined chemo- and immunotherapy did not develop detectable amounts of antibodies. Those 7 of 37 which developed antibodies, however, had titers of the same range as the mice treated with ACV or BVDU alone. The rate of antibody-positive mice treated with a chemotherapeutic only was significantly different from that of mice subjected to combined treatment ($P < 0.005$).

Discussion

In this study we show that combination of ACV or BVDU and passive immunization (HIG) may have a synergistic effect in immunocompetent mice infected intradermally with HSV-1. ACV or BVDU were administered twice daily for 5 days and HIG was administered as a single dose at 4, 24, 48 or 72 h postinfection. At 20 days postinfection, all combinations resulted in a higher protection rate than any single treatment. When followed for a longer observation period of 100 days,

TABLE 2

Anti-HSV-1 ELISA antibody titers in the sera of mice on the 100th day after infection[a].

Treatment	Numbers of survivors[b] (100 days postinfection)	Reciprocals of anti-HSV-1 ELISA antibody titers	No. of seropositive to total no. of survivors
ACV	5	<10, 80, 640, 1600, 3200	4/5[c]
ACV + HIG 4 h postinfection	12	<10 (11)[d], 400	1/12
ACV + HIG 24 h postinfection	7	<10 (7)	0/7
ACV + HIG 48 h postinfection	5	<10 (4), 320	1/5
BVDU	2	800/800	2/2[c]
BVDU + HIG 4 h postinfection	7	<10 (5), 160, 1100	2/7
BVDU + HIG 24 h postinfection	4	<10 (3), 1600	1/4
BVDU + HIG 48 h postinfection	2	400, 800	2/2

[a] Blood was taken from the surviving mice presented in Fig. 2A and B.
[b] Number of mice treated was 20 in each group.
[c] In total 6 of 7 mice treated with a chemotherapeutic only were seropositive.
[d] The number of sero-negative mice is indicated in parentheses.

however, HIG administered at 48 or 72 h postinfection, did not appear effective in increasing the protective effects of BVDU or ACV, whereas the combination of ACV or BVDU with a single HIG dose administered at 4 h postinfection considerably increased the protection rate resulting in a synergistic effect.

HIG given at 24 h postinfection did not significantly enhance the protection achieved by chemotherapy, as based on the survival rates at the 100th postinfection day. However, HIG administered at 24 h postinfection caused a marked delay in mortality during the 100-day observation period. The deaths which eventually occurred in this group may be due to the lack of anti-HSV-1 antibodies and the failure of combined chemo- and immunotherapy to completely eradicate infectious HSV-1 in the host. Mice which became seropositive may have been protected, since our previous studies on active immunization with an experimental HSV-1 glycoprotein vaccine in the same mouse model have indicated that an ELISA antibody titer of at least 1:160 at the time of virus challenge is fully protective [6].

Although the majority of surviving mice treated with ACV or BVDU and HIG at 4 h postinfection did not develop anti-HSV-1 antibodies, these mice survived presumably because chemotherapy plus early passive immunization supported by non-specific immune factors may have completely eliminated the challenge virus.

From the results presented here we therefore conclude that: (1), as already shown by others for short-term observation periods [1,2,15], combination of chemotherapy and passive immunization 4 h after infection has a synergistic protective effect on primary HSV infection even over an observation period of 100 days; (2), combination of chemotherapy with a late passive immunization is effective over a 20-day, but not over a 100-day observation period; (3), because of the short duration of this effect and the half-life of human immunoglobulins (about 20 days in the homologous host [13]) repeated immunoglobulin administration might be superior to a single dose; and (4), combined chemo- and immunotherapy started immediately after primary HSV infection is the most effective procedure, although it prevents seroconversion in most of the infected animals.

Although the present results were obtained in a primary HSV infection model, they argue in favor of the combined use of chemotherapy and passive immunization in humans infected with HSV, including those patients that suffer from recurrent HSV infections. In patients suffering from recurrent HSV infections such combined chemo- and immunotherapy will, of course, not eliminate latent virus or prevent further recurrences [8], but probably will efficiently inhibit virus replication and thus mitigate the clinical symptoms of the acute outbreak of the disease. Furthermore, in case of primary HSV infection such combined therapy may be effective in completely eradicating the virus when started sufficiently early after a primary HSV infection. Although the consequence of a rapid and complete eradication of any infectious HSV could be that no specific immune response will develop, the advantage of such a therapy would be that no latent HSV infection will establish in the infected host. To find out whether this hypothesis is true, we will investigate in the near future whether mice protected by combined therapy and lacking any specific immune response are also free of any latent HSV.

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