Antiviral Research, 10 (1988) 99–106
Elsevier

AVR 00312

Therapeutic effect of pyrophosphate analogues on cutaneous herpes simplex virus type 1 infection in guinea pigs

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(Received 24 April 1988; accepted 3 August 1988)

Summary

We investigated the influence of disodium phosphonic formate (PFA-Na_2) and trisodium thiophosphonic formate (TPFA-Na_3), in comparison with acyclovir (Zovirax ®) and trisodium phosphonic formate (PFA-Na_3) (Triapten ®) ointment, on the course of primary cutaneous herpes simplex virus infection in a guinea pig skin model. PFA-Na_2 at 3.0%, TPFA-Na_3 at 0.5% and PFA-Na_3 at 0.5% as well as Triapten ointment (2.0% PFA-Na_3) completely inhibited virus infection. Zovirax cream (5.0% acyclovir), applied five times (15 min., 4, 20, 24, and 28 h) after virus inoculation did not prevent virus infection. Similarly, application of Zovirax cream 5 times daily for 5 days did not prevent a vesicle formation following cutaneous herpes simplex virus infection of the guinea pig.

Herpes simplex virus; Guinea pig skin; Antiherpetic substance; Pyrophosphate analogue; Acyclovir

Introduction

The development of selective antiviral agents permits a causal treatment of human herpes simplex virus infections. In addition to different nucleoside analogues such as iododeoxyuridine (IDU), (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) and acyclovir (ACV), pyrophosphate analogues have proved effective as topical antiviral modalities. In recent years the status of pyrophosphate analogues as an-

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tiviral substances has been repeatedly reviewed (Eriksson et al., 1980; Henkler et al., 1982; Hutchinson et al., 1985; Öberg, 1983). Phosphonoformic acid and derivatives of 1,1-diphosphonic acid, and their sodium salts, i.e. trisodium phosphonic formate (PFA-Na_3), emerged as the most effective compounds against herpes simplex virus (HSV) type 1 and type 2.

The intention of the present work was dual: firstly, to investigate the effects of PFA-Na_3 and two pyrophosphate analogues, disodium phosphonic formate (PFA-Na_2) and trisodium thiophosphonic formate (TPFA-Na_3), developed by Issleib et al. (1985a,b), on cutaneous HSV type 1 infection in guinea pigs; secondly, to compare, in the same animal model, the efficacy of Triapten ointment (2.0% PFA-Na_3) and Zovirax cream (5.0% ACV).

Materials and Methods

Animals

Female and male albino guinea pigs weighing 250 to 450 g served as test animals (G. Halangk, breeding farm for medical test animals, Liebschütz, G.D.R). They were held at 20–25°C in single cages and were given *ad libitum* standardized pellet food and water containing 500 mg/l ascorbic acid.

Virus

The virus strain HSV-1 Kupka isolated by Benda (Prague, CSSR) from a patient with herpes labialis in 1962 was used. The virus stock was propagated in primary rabbit testes cells. After freezing and thawing twice the cell-free virus suspension was harvested by centrifugation and the virus stock was stored in liquid nitrogen until use.

Antiviral substances and ointment bases

Disodium phosphonic formate (PFA-Na_2), supplied from VEB Leipziger Arzneimittelwerk (Leipzig, G.D.R.); MW 226 for PFA-$Na_2 \cdot 4H_2O$.

Trisodium thiophosphonic formate (TPFA-Na_3), supplied from VEB Leipziger Arzneimittelwerk (Leipzig, G.D.R.); MW 316 for TPFA-$Na_3 \cdot 6H_2O$. Trisodium phosphonic formate (PFA-Na_3, VEB Chemiekombinat Bitterfeld, Bitterfeld, G.D.R.), present at 2.0% in Triapten ointment (VEB Leipziger Arzneimittelwerk, Leipzig, G.D.R.); MW 300 for PFA-$Na_3 \cdot 6H_2O$. The structures of PFA-Na_2, TPFA-Na_3 and PFA-Na_3 are depicted in Fig. 1.

9-(2-Hydroxyethoxymethyl)guanine (Acyclovir, ACV), The Wellcome Foundation Ltd., London, U.K., present at 5% in Zovirax cream.

Ointment base I (prepared according to the supplied dispensing of the VEB Leipziger Arzneimittelwerk, Leipzig, G.D.R.) for Triapten ointment, PFA-Na_2, TPFA-Na_3, and ACV: propylene glycol, 8.00%, emulsifying alcohols, 6.00%, glycerol stearate, 3.00%, stearyl alcohol, 2.50%, aqua ad 100.00%.

Ointment base II (The Wellcome Foundation Ltd., London, U.K.) for Zovirax cream contains, amongst others: propylene glycol, 40.00%, poloxamer, 1.00%, cetylstearyl alcohol, 6.75%, aqua, 30.00%.

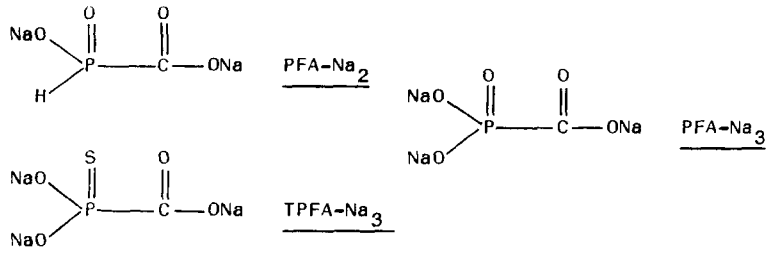

Fig. 1. Structural formulas of disodium phosphonic formate (PFA-Na_2), trisodium thiophosphonic formate (TPFA-Na_3), and trisodium phosphonic formate (PFA-Na_3).

Virus inoculation

The symptoms of a cutaneous herpes simplex virus infection in the guinea pig were induced with the aid of a simple inoculation device described recently (Klöcking et al., 1986; Helbig et al., 1987). After mechanical depilation of the back between shoulder blades and pelvis, the guinea pigs were treated chemically with a depilatory cream (Eva-Creme®, VEB Kolloid-chemie Leipzig, Leipzig, G.D.R.). The hair-free skin was divided into 4 areas with a marking pen. On each area 40 µl of virus suspension (10^6 $TCID_{50}$) were spread. The inoculation apparatus was pressed 9 or 16 times onto each area.

Treatment

For each area and treatment 0.4 g ointment with antiviral compound was spread over the skin of the guinea pigs. The treatments were as follows: (1) Five-fold treatment, 15 min, 4, 20, 24 and 28 h after virus inoculation. PFA-Na_2 and TPFA-Na_3 were incorporated into ointment base I at concentrations of 0.1, 0.5, 1.0 and 3.0% and ACV at a concentration of 5.0%. In the ointment base II only ACV was incorporated at a concentration of 5.0% (Zovirax cream). Control areas were treated with 2.0% PFA-Na_3 in ointment base I (Triapten ointment) and with ointment base I without antiviral substances. (2) Twenty-five-fold treatment 5 times daily for 5 days beginning 15 min after virus inoculation. Zovirax cream (5.0% ACV in ointment base II) and Triapten ointment (2.0% PFA-Na_3 in ointment base I) were compared. Control areas were treated with ointment base I without antiviral substances. For each concentration of the antiviral substances at least 10 animals were used.

Scoring

At 24-hourly intervals the antiviral effectiveness of treatment was evaluated following a scoring system based on the symptoms of the infection (Alenius and Öberg, 1978).

Infection score (IS). 0, no appearance of skin lesions; 1, redness (and swelling) of the skin; 2, vesicles at the inoculation spots; 3, additional vesicles, denoted as satellites.

Healing score (HS). 2, incrustation of vesicles; 2, incrustation of satellites; 1, falling off of the crusts.

Cumulative score (CS). Sum of the score values of symptoms. Without treatment the CS amounted to 11 = 1 + 2 + 3 for IS and 2 + 2 + 1 for HS (total: 11 for CS). A complete prevention of infection by treatment results in a CS of 1.

Results

Symptoms of infection without treatment

After intracutaneous virus application, redness and swelling were observed as a consequence of tissue damage at the inoculation sites. This redness and swelling subsided after a few hours. On the third day after virus inoculation, 1 to 2 mm-large vesicles appeared at all inoculation sites. Incrustation began on the 5th day after inoculation. Five to six days after virus application the infection flared up by appearance of new vesicles which were denoted as satellites within the inoculated areas. The incrustation of the satellites began on day 7 after virus inoculation. In general, all lesions healed after 13 days. This infection course resulted in a CS of 11 (see above).

Treatment with PFA-Na_2 and TPFA-Na_3

PFA-Na_2 ointment at 0.1% or 0.5% did not prevent clinical signs of virus in-

TABLE 1

Effects of disodium phosphonic formate (PFA-Na_2), trisodium thiophosphonic formate (TPFA-Na_3) and trisodium phosphonic formate (PFA-Na_3) in ointment base I on cutaneous herpes simplex virus type 1 infection of guinea pigs (n = 10)

Concentration %	Substance	Animals with vesicles	Animals with satellites	Cumulative score (CS)
3	PFA-Na_2	0	0	1
	TPFA-Na_3	0	0	1
	PFA-Na_3	0	0	1
1	PFA-Na_2	10	0	6
	TPFA-Na_3	0	0	1
	PFA-Na_3	0	0	1
0.5	PFA-Na_2	10	10	11
	TPFA-Na_3	0	0	1
	PFA-Na_3	0	0	1
0.1	PFA-Na_2	10	10	11
	TPFA-Na_3	10	0	6
	PFA-Na_3	10	1	6.5
0	Control (ointment base I)	10	10	11

TABLE 2

Effect of acyclovir (ACV) and trisodium phosphonic formate (PFA-Na_3) on cutaneous herpes simplex virus type 1 infection of guinea pigs (n = 10)

Substance	Number of applications	Animals with		Cumulative score (CS)
		Vesicles	Satellites	
ACV 5% in ointment base I	5	10	10	11
Zovirax (5% ACV)	5	10	10	11
Triapten (2% PFA-Na_3)	5	0	0	1
Control 0%	5	10	10	11
Zovirax (5% ACV)	25	10	0	6
Triapten (2% PFA-Na_3)	25	0	0	1
Control 0%	25	10	10	11

fection (CS = 11). PFA-Na_2 ointment 1.0% prevented the appearance of satellites (CS = 6). Treatment with 3.0% PFA-Na_2 completely prevented formation of cutaneous lesions (CS = 1). The results are presented in Table 1.

Treatment with TPFA-Na_3 ointment at 3.0%, 1.0%, or 0.5% completely inhibited the infection of the guinea pigs (CS = 1). 0.1% TPFA-Na_3 prevented the appearance of satellites (CS = 6). Concentrations above 1.0% led to skin irritations. The results are presented in Table 1.

Treatment with ACV and PFA-Na_3

In the 5-fold treatment scheme, neither 5.0% ACV in ointment base I nor 5.0% ACV in ointment base II (Zovirax cream) were able to prevent virus infection (CS = 11). Only the number of satellites was reduced (3 to 9 satellites) in comparison to the controls (> 15 satellites). In contrast, 2.0% PFA-Na_3 in ointment base I (Triapten ointment) completely inhibited virus infection (CS = 1).

A 25-fold treatment with Zovirax cream five times daily at four-hourly intervals for 5 days prevented the infection as long as treatment was continued. Two days after finishing the treatment (six days after virus inoculation) vesicles appeared at the inoculation sites. The skin of the animals was irritated (redness). Incrustation of the vesicles began 8 days after virus inoculation. Satellites did not appear (CS = 6). Triapten ointment completely inhibited the virus infection (CS = 1). In the control area with ointment base I without antiviral substances irritations of the skin were seen, which disappeared after cessation of treatment. In the area treated with Triapten ointment the skin was also irritated and a secondary infection occurred, which subsided within 4 to 5 days after finishing the treatment. The results are presented in Table 2.

Discussion

The effects of PFA-Na_2 and TPFA-Na_3 on the course of cutaneous herpes simplex virus type 1 infection of guinea pigs were investigated in comparison to the effects obtained with Triapten ointment and ACV ointment.

The cutaneous guinea pig model is convenient for the investigation of topically applicable antiviral substances (Hubler et al., 1974; Burkhardt and Wigand, 1983) because of the efficient scoring of the symptoms.

The efficacy of PFA-Na_3 in this animal model is well documented (Helgstrand et al., 1980; Wicht and Wiedemann, 1985; Helbig et al., 1987). PFA-Na_3 0.5% in ointment base inhibited infection of the guinea pig skin even when treatment began 8 h after virus inoculation. Therefore, a 2.0% PFA-Na_3 ointment was chosen as the control, as it would certainly inhibit the infection and is commercially available (Triapten ointment).

A similar excellent inhibition was obtained with TPFA-Na_3. Concentrations of 3.0, 1.0 and 0.5% TPFA-Na_3 prevented the infection and 0.1% TPFA-Na_3 completely inhibited the appearance of satellites. PFA-Na_2 was less effective than TPFA-Na_3. PFA-Na_2 at 3.0% prevented the infection; at 1.0% it inhibited the appearance of satellites; 0.5% and 0.1% PFA-Na_2 had no effect. These results are in agreement with the in vitro data of Thiel et al. (1986). Whether PFA-Na_2 and TPFA-Na_3 are deposited in the bone, as is PFA-Na_3 (Helgstrand et al., 1980), has to be clarified. Treatment with PFA-Na_2 at 3.0% and TPFA-Na_3 at 1.0% did not result in skin irritation, such skin irritation was seen with TPFA-Na_3 in concentrations above 1.0%.

The anti-HSV activity of ACV was originally described by Schaeffer et al. (1978). ACV has been investigated successively in vitro (Collins and Bauer, 1979), in vivo (Burkhardt and Wigand, 1983) and in clinical trials (Spruance et al., 1982; Corey et al., 1982). Zovirax cream (Wellcome, London, U.K.) is an ointment containing 5.0% ACV. Comparison of Triapten ointment and Zovirax cream in a five-fold treatment scheme pointed to the superiority of Triapten ointment (CS = 1). Zovirax cream had no effect on the cutaneous HSV infection in guinea pigs (CS = 11). The incorporation of 5.0% ACV in ointment base I did not improve its efficacy. According to Wellcome, Zovirax cream should be applied five times daily for 5 days. A 25-fold application of Zovirax cream prevented the infection as long as the treatment was continued, two days after termination of treatment (six days after virus inoculation) vesicles appeared at the inoculation sites (CS = 6). Spruance et al. (1986) found a similar slight effect of a 5.0% ACV ointment. In contrast, Triapten ointment completely inhibited the virus infection (CS = 1).

These results are in keeping with those of Corey et al. (1982) and Spruance et al. (1982) who did not observe differences in healing time and duration of pain between patients with genital or labial herpes treated with ACV and placebo. For genital herpes, oral ACV administration seems to be the treatment of choice, particularly if intended for prophylactic purposes (Reichman et al., 1983).

The relatively low efficacy of cutaneously administered ACV in the guinea pig model could be attributed to the high thymidine content of guinea pig skin cells

(Harmenberg et al., 1985). Thymidine competitively inhibits the phosphorylation of ACV by virus-specific thymidine kinase. According to Spruance et al. (1986) penetration of ACV into guinea pig skin would be much slower than that of PFA-Na_3.

References

Alenius, S. and Öberg, B. (1978) Comparison of the therapeutic effects of five antiviral agents on cutaneous herpes virus infection in guinea pigs. Arch. Virol. 58, 277–288.

Burkhardt, U. and Wigand, R. (1983) Combined chemotherapy of cutaneous herpes simplex infection of the guinea pig. J. Med. Virol. 12, 137–147.

Collins, P. and Bauer, D.J. (1979) The activity in vitro against herpes virus of 9-(2-hydroxyethoxymethyl)guanine (acycloguanosine), a new antiviral agent. J. Antimicrob. Chemother. 5, 431–436.

Corey, L., Nahmias, A.J., Guinan, M.E., Benedetti, J.K., Critchlow, C.W. and Holmes, K.K. (1982) A trial of topical acyclovir in genital herpes simplex virus infection. N. Engl. J. Med. 306, 1313–1319.

Eriksson, B., Larson, A., Helgstrand, S., Johansson, N.-G. and Öberg, B. (1980) Pyrophosphate analogues as inhibitors of herpes simplex virus type 1 DNA polymerase. Biochem. Biophys. Acta 607, 53–64.

Harmenberg, J., Malm, M. and Abele, G. (1985) Deoxythymidine pools of human skin and guinea pig organs. FEBS Lett. 188, 219–221.

Helbig, B., Sauerbrei, A., Klöcking, R., Wutzler, P., Wicht, N., Wiedemann, U. and Herrman, G. (1978) Therapeutic effect of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine, caffeic acid oxidation product, and trisodiumphosphonoformate on cutaneous herpes simplex virus type 1 infection in guinea pigs. J. Med. Virol. 23, 303–309.

Helgstrand, S., Flodh, H., Lernestedt, J.G., Lundström, J. and Öberg, B. (1980) Trisodium phosphonoformate: antiviral activities, safety evaluation and preliminary clinical results. In: L.H. Collier and J. Oxford (eds.), Developments in Antiviral Therapy, pp. 63–83. Academic Press, London.

Henkler, G., Klotzbach, M., Koch, H., Müller, W. and Richter, J. (1982) Fortschritte auf dem Gebiet der Arzneimittelentwicklung, Teil 15. Pharmazie 37, 753–765.

Hubler, W.R. Jr., Felber, D., Troll, D. and Jarratt, M. (1974) Guinea pig model for cutaneous herpes simplex virus infection. J. Invest. Dermatol. 62, 92–95.

Hutchinson, D.W., Cload, P.A. and Haugh, M. (1985) Organophosphorus compounds as antiviral agents. Phosphorus and Sulfur 14, 285–293.

Issleib, K., Balszuweit, A., Mögelin, W., Bertram, D., Wutzler, P. and Thiel, K.-D. (1985a) Verfahren zur Herstellung von Phosphonoformiaten. WP-DD 219 198 (Cl. C 07 F).

Issleib, K., Balszuweit, A., Mögelin, W., Lettau, H., Wutzler, P. and Thiel, K.-D. (1985b) Verfahren zur Herstellung von Phosphonigformiaten. WP-DD 219 199 (Cl. C 07 F).

Klöcking, R., Sauerbrei, A., Wicht, N. and Wiedemann, U. (1986) Inokulationsapparatur zur intradermalen Applikation von Virusantigenen. Versuchstierkunde 28, 65–68.

Öberg, B. (1983) Antiviral effects of phosphonoformate (PFA, foscarnet sodium). Pharmacol. Ther. 19, 387–415.

Reichmann, R.C., Badger, G.J., Mertz, G.J., Corey, L. and Richman, D. (1983) Patient-initiated therapy of recurrent herpes simplex genitalis with orally administered acyclovir. Clin. Res. 31 (2), 373A.

Schaeffer, H.J., Beauchamp, L., de Miranda, P. and Elion, G.B. (1978) 9-(2-hydroxyethoxymethyl)guanine activity against viruses of the herpes group. Nature 272, 583–585.

Spruance, S.L., Schnipper, L.E., Overall Jr., J.C., Kern, E.R., Wester, B., Modlin, J., Wenerstrom, G., Burton, C., Arndt, K.A., Chiu, G.L. and Crumpacker, C.S. (1982) Treatment of herpes simplex labialis with topical acyclovir in polyethylene glycol. J. Infect. Dis. 146, 85–90.

Spruance, S.L., Freeman, D.J. and Sheth, N.V. (1986) Comparison of Foscarnet cream, Acyclovir cream, and Acyclovir ointment in the topical treatment of experimental cutaneous herpes simplex virus type 1 infection. Antimicrob. Agents Chemother. 30, 196–198.

Thiel, K.-D., Wutzler, P., Isleib, K., Mögelin, W. and Balszuweit, A. (1986) Antivirale Aktivität einiger neuer pyrophosphatanaloger Verbindungen. Pharmazie 41, 295–296.
Wicht, N. and Wiedemann, U. (1985) Meerschweinchenmodell zur Testing lokal wirksamer antiviraler Substanzen gegen Herpesvirus homonis Typ. 1. Doctoral thesis, Medical Academy Erfurt.