ASSESSMENT OF PHOSPHONOFORMATE-TREATMENT OF PIGEON HERPESVIRUS INFECTION IN PIGEONS AND BUDGERIGARS, AND AUJESZKY'S DISEASE IN RABBITS

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INTRODUCTION

The trisodium salt of phosphonoformic acid (PFA) is a recently discovered antiherpetic compound which is slightly cytotoxic and exhibits an important antiviral effect due to a specific inhibition of the herpesvirus-induced DNA-polymerase (Helgstrand, Eriksson, Johansson, Lannerö, Larsson, Misiorny, Noren, Sjöberg, Stenberg, Stening, Stridh, Öberg, Alenius and Philipson, 1978).

PFA has been successfully used for the treatment of experimental infection of mice and guinea pigs with either herpes simplex type 1 or 2 (*Human herpesvirus 1, 2*, $HSV_{1,2}$), especially when used locally soon after infection (Alenius, Dinter, and Öberg, 1978; Alenius and Nordlinder, 1979; Kern, Glasgow, Overall, Reno and Boezi, 1978), whereas in mice inoculated intraperitoneally or intracerebrally with HSV_1 or intraperitoneally with murine cytomegalovirus (*Murid herpesvirus 1*), treatment had little effect (Kern *et al.*, 1978).

PFA was shown to inhibit the growth of pseudorabies virus (Aujeszky's disease virus, *Sus herpesvirus 1*, SHV_1) and pigeon herpesvirus (*Pigeon herpesvirus 1*, PHV_1) in tissue cultures (Schwers, Pastoret, Vindevogel, Leroy, Aguilar-Setién and Godart, 1980). Significant differences in the susceptibility of PFA were observed between several strains of PHV_1, but none of them was found to be naturally resistant (Schwers, Vindevogel, Leroy and Pastoret, 1981). PFA was therefore assayed for the treatment of PHV_1 infection in pigeons and budgerigars (Vindevogel and Duchatel, 1977) and Aujeszky's disease in rabbits. These models were chosen because of the different pathogenesis of the diseases (Kaplan, 1969; Vindevogel and Pastoret, 1981). As PHV_1 infection is mainly restricted to the anterior respiratory and digestive tracts of pigeons (Vindevogel and Pastoret, 1981), it is difficult to treat them locally, especially when numerous animals must be handled simultaneously and repeatedly. For that reason, treatment by a systemic route has been used.

We also wanted to test whether treatment of pigeons with PFA before experimental infection with PHV_1 could prevent the production of latent carriers (Vindevogel, Pastoret and Burtonboy, 1980).

0021-9975/82/020177+04 $03.00/0

MATERIALS AND METHODS

Animals. Fifteen 5- to 6-week-old pigeons with an average body weight of 400 g from parents free of PHV_1 infection, 20 3- to 5-month-old budgerigars with an average body weight of 40 g obtained from a flock devoid of anti-PHV_1 antibodies and 10 4-month-old rabbits weighing 1·5 to 1·6 kg were used.

Virus and inoculation. The PHV_1/Cz/236 69 strain isolated in Czechoslovakia (Krupička, Šmíd, Valíček and Pleva, 1970) was chosen for its high in vitro susceptibility to PFA (Schwers *et al.*, 1981). Pigeons were infected by pharyngeal painting with 10^5 pfu, and budgerigars were inoculated intranasally with the same dose. Rabbits were injected intramuscularly with 10 to 30 pfu of the SHV_1/B/Cu_1 strain isolated in Belgium (Schwers *et al.*, 1980).

PFA solutions. Sterile 1 and 2 per cent solutions of PFA in water were adjusted to pH 7. The susceptibility of PHV_1/Cz/236 69 and SHV_1/B/Cu_1 to these solutions was tested in vitro as previously described (Schwers *et al.*, 1980).

Drug treatments. Animals were injected intramuscularly 4 times a day, at 07·00, 12·00, 17·00 and 22·00 h. Pigeons were injected with 0·5 ml of the 2 per cent solution, and rabbits with 2 ml. Budgerigars were injected with 0·1 ml of the 1 per cent solution, so that each animal received 100 mg per kg per day, as previously described (Alenius *et al.*, 1978; Alenius and Nordlinder, 1979) and as recommended by Öberg (personal communication).

Treatment of PHV_1 infection in pigeons. Pigeons were divided into 3 equal groups; group A was untreated and there were 2 treated groups. PFA-treatment began in group B 2 days before inoculation (day 1), and in group C 2 days after; in each group the treatment continued for 8 days.

Three weeks after inoculation, antibodies in pooled sera of each group were titrated as previously described (Hoskins, 1967; Vindevogel and Duchatel, 1978). Forty days after the beginning of the experiment, pigeons were treated with cyclophosphamide (Cy) for 4 days as previously described (Coignoul and Vindevogel, 1980). The pharyngeal mucous membrane of each pigeon was swabbed daily on days 1 to 13 and 40 to 48 for titration of infectious viral particles and clinical signs were scored according to previously described methods (Vindevogel *et al.*, 1980; Vindevogel and Pastoret, 1981).

Treatment of PHV_1 infection in budgerigars. Budgerigars were divided into 2 equal groups: a control untreated group and a PFA-treated group. Treatment began 2 days before inoculation and lasted for 8 days. Deaths were recorded and infectious viral particles titrated in the livers of all dead budgerigars as previously described (Vindevogel and Duchatel, 1977).

Treatment of SHV_1 infection (Aujeszky's disease) in rabbits. Rabbits were divided into 2 equal groups: a control untreated group and a PFA-treated group. Treatment began 2 days before inoculation and deaths were recorded.

RESULTS

In vitro Efficacy of PFA Solutions

The PFA solutions used in the experiments inhibited the multiplication of strains PHV_1/Cz/236 69 and SHV_1/B/Cu_1 in chicken embryo fibroblast cultures as previously described (Schwers *et al.*, 1980, 1981).

Effect of PFA-Treatments

In all groups of pigeons, either treated or untreated, inoculation was followed by a similar pattern of simultaneous viral excretion and clinical disease,

both usually developing soon after inoculation and lasting for from 5 to 10 days. Three weeks after inoculation, titres in pooled sera were 64, 64, 128 for groups A, B and C, respectively. Forty days after inoculation, one pigeon of each group excreted virus spontaneously, and after Cy-treatment all the others re-excreted virus, with or without clinical signs, for an indefinite period.

With the budgerigars, no significant differences in death rates were seen between treated and untreated animals (8 of 10 and 10 of 10) and virus at similar titres was isolated from the liver of all dead animals.

All the treated and untreated rabbits died within 4 days after inoculation.

DISCUSSION

Intramuscular administration of PFA in pigeons did not prevent the occurrence of a classical clinical disease and the development of carriers was unmasked by Cy-treatment. The results may have been due to poor distribution of the drug in the target organ and to the low susceptibility of *Pigeon herpesvirus*$_1$ to weak concentrations of the compound (Schwers *et al.*, 1980, 1981). In budgerigars, where PHV$_1$ infection is always a systemic infection generalized by viraemia (Vindevogel and Duchatel, 1977; Vindevogel and Pastoret, 1981), fatal hepatitis was not prevented by PFA injections which began before inoculation.

This result may have been due to either a too weak drug concentration or to a rapid degradation of PFA in the liver. Unfortunately, no data are available on PFA bio-transformation in these species.

In Aujeszky's disease of the rabbit, virus infection is essentially spread by the nervous pathway (Kaplan, 1969) but fatal encephalitis was not prevented by PFA treatment even though rabbits were infected with a minimal dose of a strain which is very susceptible to the in vitro effect of PFA (Schwers *et al.*, 1980). The failure of the treatment seems unlikely to be due to resistance of the virus but was probably related to problems of drug distribution or bio-transformation.

Nevertheless, further experiments are needed to understand the discrepancy between the results obtained in vitro and in vivo.

SUMMARY

Trisodium phosphonoformate (PFA) was used to treat *Pigeon herpesvirus 1* (PHV$_1$) infection in pigeons and budgerigars and Aujeszky's disease in rabbits.

Intramuscular administration of PFA in pigeons, even when commenced before experimental inoculation of PHV$_1$, did not prevent the occurrence of clinical disease, or reduce the viral excretion or the serological response, and did not prevent the appearance of carriers.

In budgerigars infected with PHV$_1$, fatal hepatitis was not prevented by PFA-treatment begun before infection, and in rabbits the treatment did not prevent fatal encephalitis due to Aujeszky's disease virus.

ACKNOWLEDGMENTS

We wish to thank L. Dagenais, E. Thiry and J. Carleer for their help during the experiments, B. Öberg (Astra Häkemedel AB, Sweden) for providing us with phosphonoformate, and B. Šmíd (Veterinary Institute, Hudcova, Czechoslovakia) for the strain PHV_1/Cz/236 69.

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[*Received for publication, January 20th,* 1981]