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Anne Schwers, H. Vindevogel, P. Leroy & P.P. Pastoret

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SUSCEPTIBILITY OF DIFFERENT STRAINS OF PIGEON HERPESVIRUS TO TRISODIUM PHOSPHONOFORMATE

ANNE SCHWERS, H. VINDEVOGEL, P. LEROY and P.P. PASTORET

Faculty of Veterinary Medicine, University of Liège
45 rue des Vétérinaires, B-1070 Brussels, Belgium

SUMMARY

The susceptibility to trisodium phosphonoformate of five strains of pigeon herpesvirus (Pigeon Herpesvirus 1, PHV) was compared by measuring the mean plaque size variations in the presence of different concentrations of the compound.

Significant differences in their susceptibility to phosphonoformate were observed, but none of them was found to be naturally resistant. This finding may be of significance for a possible clinical application in the treatment of pigeon herpesvirus infection.

INTRODUCTION

Disodium phosphonoacetate and trisodium phosphonoformate are recently discovered antitherpetic compounds which are slightly cytotoxic and exhibit an important antiviral effect due to a specific inhibition of the herpesvirus-induced DNA-polymerase (Overby et al., 1974; Mao and Robishaw, 1975; Helgstrand et al., 1978). A possible mechanism of inhibition is an interference with a pyrophosphate binding site on the polymerase (Öberg and Helgstrand, 1978; Helgstrand and Öberg, 1978).

Susceptibility to these two compounds has been reported for all the herpesviruses so far studied, for instance turkey herpesvirus (Turkey Herpesvirus 1, HVT), Marek's disease virus (Phasianid Herpesvirus 2, MDV) (Lee et al., 1978; Reno et al., 1978) and pigeon herpesvirus (Pigeon Herpesvirus 1, PHV) (Schwers et al., 1980). Some strains of several herpesviruses were found to be resistant (Jofre et al., 1977; Duff et al. 1978; Reno et al., 1978).

For therapeutic purpose, the use of phosphonoacetate is limited because of its topical irritative potential (Kern et al., 1978), its toxicity for laboratory animals and its accumulation in bones (Bopp et al., 1977). Phosphonoformate does not irritate the skin or the mucous membranes, and has been successfully used for the treatment of experimental cutaneous and ocular herpes simplex type 1 (Human Herpesvirus 1, HSV1) infection (Alenius et al., 1978; Öberg et al., 1978) or genital infection of guinea pigs.
with herpes simplex type 2 (Human Herpesvirus 2, HSV2) (Alenius and Nordlinder, 1979). Furthermore, phosphonoformate has only minor effects on normal cellular metabolism (Stenberg and Larsson, 1978).

The present report deals with the comparison between the susceptibility of several strains of pigeon herpesvirus to phosphonoformate by measuring the effect of this compound on the mean size of plaques produced under agarose overlay. This investigation was performed with a view to a possible clinical application for the treatment of local lesions produced by PHV.

MATERIALS AND METHODS

Viruses
Two strains of PHV (PHV/B/Cu$_2$ and PHV/B/Cu$_3$) were isolated in our laboratory at Brussels (Cureghem) from young pigeons (Vindevogel and Duchatel, 1978), and a third one from psittacine birds (PHV/ Psi/B/Cu) (Vindevogel et al., 1978; Vindevogel, et al., 1980).

A strain of PHV isolated in Czechoslovakia (PHV/Cz/V 236/69) (Krupiška et al., 1970) was kindly provided by Dr. Šmid (Veterinary Research Institute, Hudcova), and another one isolated in Australia (PHV/A/B 50) (Boyle and Binnington, 1973) was obtained from Dr. Simmons (Animal Research Institute, Queensland).

Cell cultures
Chicken embryo fibroblasts (CEF) were cultured as previously described (Vindevogel et al., 1975).

Production of plaques
Confluent cell monolayers, grown in Petri dishes (plastic Falcon; 30 mm diam.) were inoculated with 0.2 ml of 10-fold viral dilutions. After 1 hour of adsorption, the infected cells were covered with Minimum Essential Medium (MEM) containing 1% agarose (indubiose) and 2% foetal bovine serum. This overlay medium was supplemented with trisodium phosphonoformate at the following final concentrations: 10 and 100 µM/ml. As controls, the same procedure was followed on uninfected cells and inoculated cells were incubated with maintenance medium devoid of phosphonoformate.

All the experiments were done on the same day, in the same CEF culture and with the same medium.

The cells were fixed with formaldehyde after 4 days of incubation, then stained with May-Grünwald Giemsa (Vindevogel et al., 1975).

The experiments were twice repeated.

Measurement of mean plaque size
Cell cultures inoculated with the optimal viral dilution, causing a sufficient number of isolated plaques, were examined according to a method previously described (Jetteur et al., 1979; Pastoret et al., 1979; Schwers et al., 1980).

Briefly, plaques were printed with an offset camera to obtain a final magnification of x 20. For each virus at each concentration of phosphonoformate, the areas of 50 plaques taken at random were measured with an OH Kempton planimeter.

Statistical tests
Normality test. The normality of the plaque size distribution was studied by skewness and kurtosis tests.
Susceptibility of PHV to phosphonoformate

Analysis of variance. The effect of the addition of trisodium phosphonoformate at increasing concentrations in the medium was studied by a one-way analysis of variance. Student’s t-test was used to determine which concentrations of phosphonoformate were producing a significant reduction of the plaque size.

Interaction test. To see if the susceptibility to phosphonoformate was the same for each strain, a two-way analysis of variance was performed and the hypothesis of absence of interaction was tested by the F-test.

RESULTS

The mean of the plaque size for PHV is given in Table 1.

Table 1. Mean plaque size of PHV strains.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Mean plaque size at different concentrations (μM/ml) of phosphonoformate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PHV/B/Cu₂</td>
<td>0.7627</td>
</tr>
<tr>
<td>PHV/B/Cu₃</td>
<td>0.4438</td>
</tr>
<tr>
<td>PHV/Psi/B/Cu</td>
<td>0.5644</td>
</tr>
<tr>
<td>PHV/Cz/V 236/69</td>
<td>0.5160</td>
</tr>
<tr>
<td>PHV/A/B 50</td>
<td>0.4800</td>
</tr>
</tbody>
</table>

Values are given in mm² and calculated from 50 plaques.

Normality test

The sizes of the plaque population produced by PHV strains were not quite normally distributed in the presence of phosphonoformate. The square root of the plaque size always followed a normal distribution, thus allowing variance analysis.

Analysis of variance

Addition of phosphonoformate induced a marked reduction of the mean plaque size ($P < 0.001$) for each of the five strains studied.

By use of the t-test, a significant reduction of the mean plaque size with each concentration of trisodium phosphonoformate (10, 100 μM/ml) ($P < 0.02$) was found, except for PHV/B/Cu₃ and PHV/Psi/B/Cu strains. Indeed, the addition of more than 10 μM/ml phosphonoformate in the presence of PHV/B/Cu₃ did not induce a further reduction in the mean size; for PHV/Psi/B/Cu we did not observe a significant reduction of the plaque size after addition of 10 μM/ml of phosphonoformate, but we did so after the addition of 100 μM/ml (Text-fig.1).

Comparison between the strains by their mean plaque size reduction in the presence of phosphonoformate

Significant differences were observed between the strains from the point of view of mean plaque size reduction in the presence of phosphonoformate ($P < 0.001$). All the strains studied differed from each other on the basis of the determination of their reduction rate in the presence of 10 or 100 μM/ml of phosphonoformate (Text-fig.1). The most susceptible strain was PHV/Cz/V 236/69, followed by PHV/B/Cu₂, PHV/A/B 50 and PHV/B/Cu₃; PHV/B/Cu₃ was less susceptible than the other strains at high concentrations, whereas PHV/Psi/B/Cu was the least susceptible. A second experiment gave similar results.
Text-fig. 1. Comparison between the mean plaque size reduction of PHV strains with increasing concentrations of trisodium phosphonoformate.
DISCUSSION

All the PHV strains studied were susceptible to trisodium phosphonoformate. In fact, the mean plaque size of the different PHV strains was reduced in the presence of phosphonoformate and, by comparing their reduction rate, it can be shown that each strain differs from any other according to its susceptibility to the compound. This agrees with the observations of Reno et al. (1978) who described variations between several strains of HVT in their susceptibility of phosphonoformate, some of them being resistant.

The strain isolated from psittacines was less susceptible than the other strains although it was a typical PHV strain (Vindevogel et al., 1980).

It remains to be studied whether these differences within the same viral species are as important as those between two different species (Schwers et al., 1980).

The finding that there are differences between several strains of PHV in the presence of phosphonoformate may be important from the point of view of a possible clinical application in the treatment of local lesions produced by PHV infection, notwithstanding the fact that none of the PHV strains studied was found to be naturally resistant to the compound.

Acknowledgements

We wish to thank Dr. B. Öberg (Astra Häkemedel AB, Sweden) for providing us with trisodium phosphonoformate, Dr. Šmid (Veterinary Research Institute, Hudcova, Czechoslovakia) and Dr. Simmons (Animal Research Institute, Queensland, Australia) for providing the pigeon herpesvirus strains V 236/69 and B 50.

We also wish to thank Dr. A. Aguilar-Setién, Annick Verheyden and Martine Godart for their help, and Lise Dagenais for revising the manuscript.

REFERENCES


RESUME

Etude la sensibilité de différentes souches de virus Herpès du pigeon au phosphonoformate de sodium

La sensibilité au phosphonoformate de sodium de cinq souches de virus herpes du pigeon (Pigeon Herpesvirus 1, PHV) a été étudiée en comparant les variations de la surface des plages liées aux différentes concentrations du produit.

Les cinq souches montrent des sensibilités différentes au phosphonoformate, mais aucune d’entre elles n’y est naturellement résistante. Ces résultats sont encourageants pour une utilisation clinique du phosphonoformate dans le traitement de l’infection herpétique du pigeon.
Die Empfindlichkeit verschiedener Taubenherpesvirusstämme gegen Trinatriumphosphonofomate

Die Empfindlichkeit von fünf Stämmen des Taubenherpesvirus (Taubenherpesvirus 1, PHV) gegen Trinatriumphosphonofomate wurde durch Messungen der Größenvariationen der Plaques in Gegenwart verschiedener Konzentrationen dieser Substanz vergleichend untersucht.

Es wurden signifikante Unterschiede in der Empfindlichkeit gegenüber Phosphonoformat beobachtet, aber keinerlei natürliche Resistenz. Dieses Ergebnis könnte bei einer evtl. klinischen Anwendung zur Behandlung der Taubenherpesvirus-Infektion von Bedeutung sein.