British Journal of Ophthalmology, 1990, 74, 532–535

Antiviral drug sensitivity in ocular herpes simplex virus infection

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Abstract

Thirty-nine herpes simplex virus (HSV) isolates were assayed for their sensitivity to 10 different antiviral agents. Of these 39 HSV isolates 10 were cultured from recipient buttons obtained at penetrating keratoplasty in patients with inactive stromal scarring due to recurrent herpetic keratitis, 25 were cultured from patients with conjunctival and ulcerative ocular infections, and the remaining four were laboratory strains with known drug sensitivity patterns, thus providing controls for the experiment. All but one of the 35 clinical isolates of HSV were type 1 and all were sensitive to the 10 antiviral agents. A single type 2 isolate from a young man with recurrent conjunctivitis proved to be resistant to a number of the antiviral agents. Since many of the clinical isolates had been exposed to multiple and protracted antiviral drug treatment, it is suggested that antiviral drug resistance in type 1 HSV ocular infection is not a significant problem.

Herpes simplex virus (HSV) resistance to antiviral drug treatment has long been suspected as a reason for therapeutic failure in herpetic keratitis. Failure to heal despite a protracted course of topical antiviral drug treatment has often been attributed to virus-drug resistance, but very few studies have looked at laboratory resistance of ocular herpetic infection. We have previously reported the isolation of HSV from 30% of corneal discs, removed during penetrating keratoplasty, of patients with scarring due to previous herpes simplex keratitis.[1-3] The demonstration of virus in apparently inactive stromal scars, despite previous long-term treatment with multiple antiviral drugs, suggests possible resistance of these isolates.

This study reports the antiviral drug sensitivity of 10 such recipient button isolates. In addition 25 isolates cultured from other conjunctival and ulcerative ocular HSV infections were included to determine the general level of antiviral drug sensitivity in our ophthalmic practice. Four laboratory strains with known drug sensitivity patterns provided controls for the experiments.

Patients and methods

Ten HSV type 1 isolates from corneal discs of patients with inactive scarring due to herpetic keratitis were collected in Bristol and Glasgow. Twenty-five HSV isolates were grown in cell culture at the Bristol Public Health Laboratory from swabs taken from patients presenting with ocular infections to the casualty department of the Bristol Eye Hospital. These were from a variety of clinical conditions (Table 1). Details of previous antiviral therapy were recorded, where possible, for both groups of isolates (Table 2). All but one of the viral isolates were shown to be type 1 by restriction endonuclease analysis using Bst I and Pvu II.[5] The only type 2 isolate was cultured from a young man with recurrent conjunctivitis and no history of genital infection.

Four laboratory strains were included as controls. The SC16[6] and KOS type 1 strains are known to be fully sensitive to all the antiviral agents. An in-vitro-derived acyclovir-resistant mutant of SC16 (kindly provided by Drs W A Blyth and T J Hill, Department of Microbiology, University of Bristol) and a multiply resistant type 2 strain, AR15, were also included. These provided both positive and negative controls for the experiment.

The 39 viral isolates were assayed for their sensitivity to ten antiviral agents (Table 3). These antiviral agents were selected on the basis of their known potential for the chemotherapy of HSV infections.[7-9]

The experimental method for assaying antiviral activity has previously been reported.[10] All assays were carried out in confluent embryonic skin-muscle fibroblast (E_6SM) cell cultures in

Table 1 Sources of viral isolates

Source	*No of cases*
Culture of recipient corneal buttons	10
Dendritic ulcer (first presentation)	11
Dendritic ulcer (recurrence)	6
Follicular conjunctivitis	7
Lid vesicles	1
Control strains	4
Total	39

Table 2 Previous treatment of patients with antiviral drugs

	Corneal discs (10)	*Casualty isolates (25)*
No previous treatment	0	17
Previous treatment	10	8
Antiviral drugs used		
ACV	5	3
IDU	5	2
TFT	4	0
Ara-A	5	0
Unknown	2	4

Table 3 Antiviral agents used in the drug sensitivity determinations[7]

1 IDU: idoxuridine, 5-iodo-2′-deoxyuridine
2 TFT: trifluridine, 5-trifluoro-2′-deoxythymidine
3 Ara-A: vidarabine, 9-beta-D-arabinofuranosyladenine
4 ACV: acyclovir, 9-(2-hydroxyethoxymethyl) guanine
5 BVDU: (E)-5-(2-bromivinyl)-2′-deoxyuridine
6 EDU: 5-ethyl-2′-deoxyuridine
7 DHPG: 9-(1, 3-dihydroxy-2-propoxymethyl) guanine
8 CEDU: 5-(2-chloroethyl)-2′-deoxyuridine
9 PFA: foscarnet, phosphnoformate
10 (S)-HPMPA: (S)-9-(3-hydroxy-2-phosphonyl (methyoxypropyl) adenine

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Accepted for publication 11 May 1990

Table 4 Mean ID_{50} concentrations of antiviral agent for the HSV isolates

Anti-viral agents	Recipient corneal buttons		Casualty infections (type 1)		Casualty infection (single type 2)	SC16 lab strain (type 1)	ACV resistant SC16 lab strain (type 1)	AR15 lab strain (type 2)	KOS lab strain (type1)
	Mean	Range	Mean	Range					
IDU	0·20	0·07–0·37	0·29	0·07–0·70	2·00	0·09	8·94	2·83	0·30
TFT	1·55	0·53–2·00	1·54	0·37–3·74	20·00	0·37	2·83	20·00	2·00
ARA-A	12·2	3·7–20·0	9·6	1·2–22·1	11·8	3·7	11·8	11·8	14·6
ACV	0·04	0·02–0·07	0·03	0·02–0·06	0·02	0·02	20·00	0·02	0·04
BVDU	0·03	0·01–0·04	0·02	0·01–0·04	167·33	0·01	141·42	>200·00	0·02
EDU	1·59	0·53–2·00	0·77	0·20–2·00	2·00	0·26	141·42	2·00	0·70
DHPG	0·002	0·002–0·003	0·002	0·002–0·007	0·002	0·002	1·180	0·002	0·002
CEDU	0·13	0·02–0·20	0·14	0·02–0·37	4·47	0·05	>200·00	3·74	0·20
PFA	73·8	28·3–122·5	50·6	20·0–122·5	70·0	70·0	70·0	70·0	46·1
HPMPA	0·09	0·01–0·26	0·06	0·01–0·20	0·07	0·01	0·07	0·08	0·12

microtitre trays. These were inoculated with 100 $CCID_{50}$ ($CCID_{50}$=50% cell culture infective dose) of each virus for 1 h at 37°C.

Sequentially decreasing concentrations of each test compound were then added to the virus infected cell cultures. Controls for each experiment consisted of cell cultures which had been inoculated only with the test virus. The viral cytopathic effect (CPE) in each culture was recorded microscopically. In the control the infected cell cultures' viral CPE was generally completed at three to four days after viral inoculation. All experiments were repeated twice.

Antiviral drug sensitivity is expressed as the ID_{50} (50% of inhibitory dose), that is, the concentration of drug required to reduce the viral CPE by 50% at the time when complete cell destruction was noted in the control infected cell cultures.

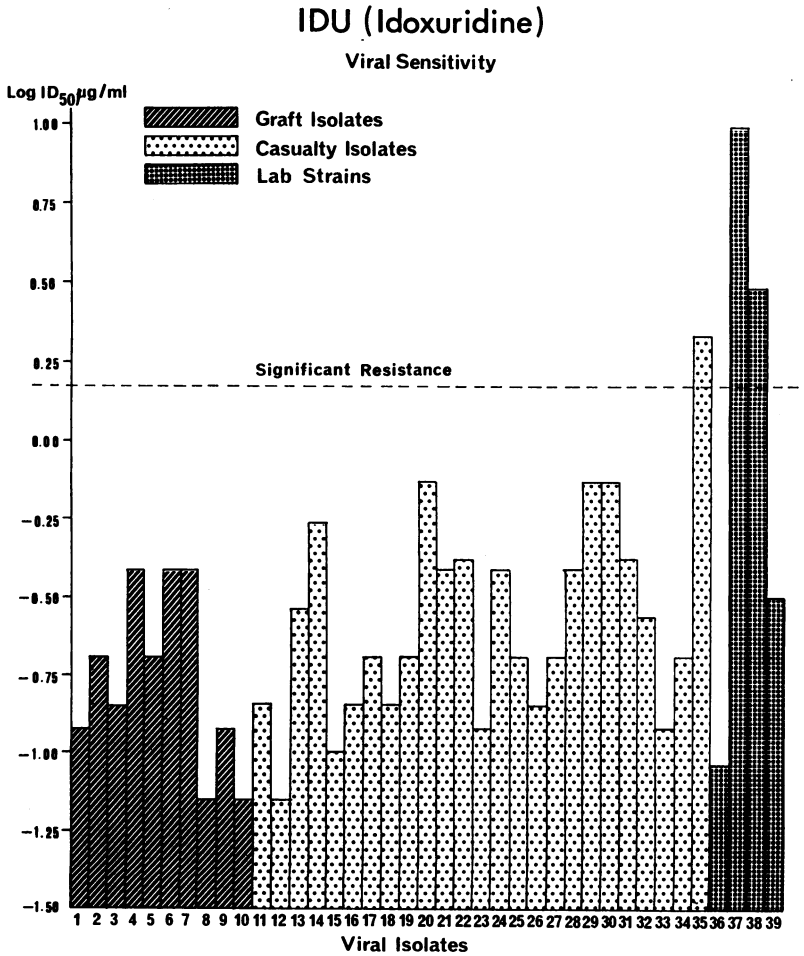

Figure 1

Figures 1–5 Sensitivity of the viral isolates to the five more commonly used antiviral drugs. Significant resistance is defined as a five-fold increase in the ID_{50} relative to the ID_{50} of the laboratory strain KOS (viral isolate no. 39). Viral isolate 35=type 2 isolate from recurrent conjunctivitis. Viral isolate 36=laboratory strain SC16. Viral isolate 37=acyclovir-resistant derivative of SC16. Viral isolate 38=type 2 laboratory strain AR15. Viral isolate 39=laboratory KOS.

Note that an arbitrary logarithmic scale is used; therefore sensitivity cannot be directly compared between separate graphs.

Results

The mean ID_{50} obtained for each compound with viral isolate was calculated for the two experiments. All 10 of the viral isolates from corneal discs of inactive stromal scarring and the 24 type 1 viral isolates from ulcerative and conjunctival infections showed no significant resistance to any of the 10 antiviral agents. We found all type 1 clinical isolates were fully sensitive to the 10 antiviral agents (Table 4).

Significant resistance is defined as a five-fold increase in the ID_{50} relative to that for the KOS strain, since this control virus was known to be sensitive to all the antiviral agents used. Other workers using a similar method have defined resistance as high as a 10-fold increase in the ID_{50} relative to that for the KOS strain.[11]

The single type 2 infection from a young man with recurrent conjunctivitis showed resistance to TFT, IDU, BVDU, and CEDU (see Table 3 for abbreviations), which was similar to the resistance pattern shown by the type 2 laboratory strain AR15.

Results for the four laboratory strains were as expected with the KOS and SC16 strains showing no evidence of resistance. The acyclovir-resistant derivative of SC16 were resistant to ACV and also showed cross resistance to IDU, BVDU, EDU, CEDU, and DHPG. The type 2 AR15 strain showed resistance to TFT, IDU, BVDU, and CEDU.

The results for the five more familiar antiviral drugs are presented in Figs 1–5.

Discussion

Considerable attention has focused on the development of HSV resistance to antiviral drugs, most recently with the introduction of acyclovir to clinical practice.[12,13] Reports of acyclovir resistant clinical strains have largely been confined to immunosuppressed patients receiving high dose intravenous treatment, and sensitivity has returned on withdrawal of the acyclovir.[14,16]

Report's of laboratory proved resistance to

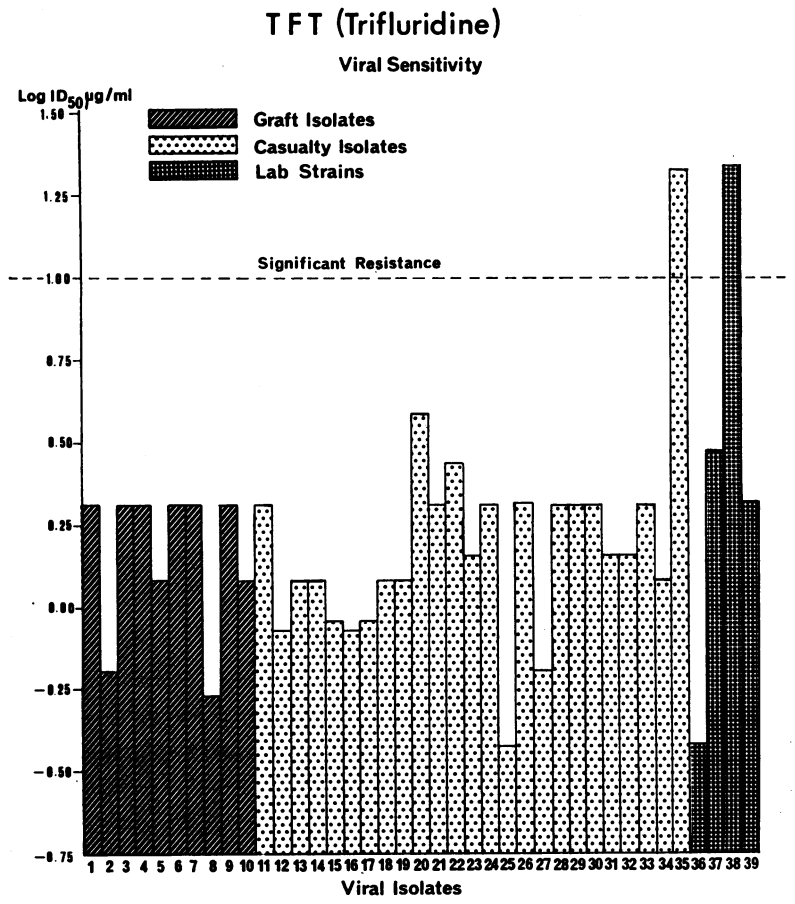

Figure 2

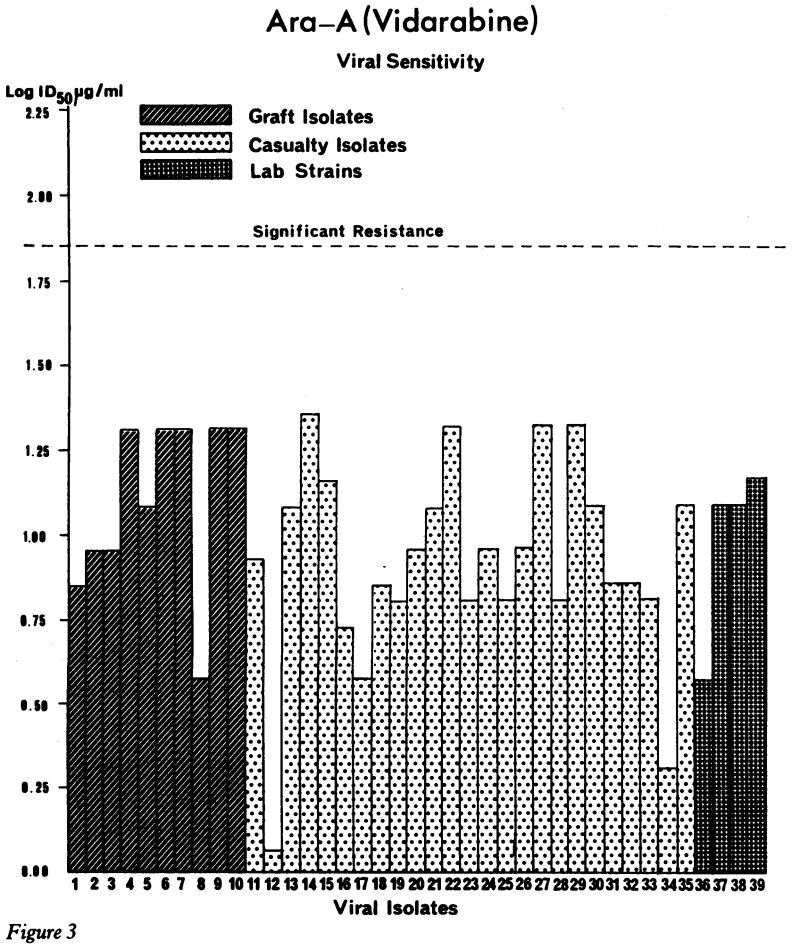

Figure 3

antivirals in ocular herpes simplex infection are very rare,[17] but failure to heal in clinical herpetic keratitis is a relatively common problem, with persistence of the infection despite apparently adequate treatment with a topical antiviral drug. A concept of 'treatment resistance' or 'clinical resistance' has been proposed in the situation where an ulcer fails to heal with apparently adequate treatment with one antiviral drug but responds well when another is used.[18,19] A complex definition of clinical resistance is necessary in order to try and exclude some of the many other causes of failure to respond to treatment.[18] These include poor stromal penetration, poor compliance, inadequate treatment, toxicity, coexisting epithelial disease, and inappropriate use of steroids. Clinical resistance rates of 37% with IDU and 11% with Ara-A have been reported.[17]

The results of our experiments indicate there is little if any evidence of resistance of type 1 ocular isolates to the commonly used antiviral agents to support the relatively common clinical experience of failure of antiviral drug treatment of herpetic keratitis. This only serves to emphasis the need for adequate delivery of sufficient antiviral agent to the virus and the other multiple causes of clinical resistance.

This study developed from the isolation of HSV from inactive scarring in patients with long histories of herpetic keratitis. The patients had been subjected to multiple courses of antiviral drugs over many years. The lack of resistance of the isolates indicates that repeated exposure to antiviral drugs does not easily generate virus drug resistance in ophthalmic infection.

Resistance of HSV to antiviral drugs[20] is mediated by two loci on the herpes virus genome, viral thymidine kinase and viral DNA polymerase. Thymidine kinase deficient mutants can easily be selected for in-cell culture and have occasionally been isolated from immunocompromised patients treated with acyclovir[21] but show markedly diminished pathogenicity in laboratory animals.[22] This is also the case in experimental ocular infections.[23,24] It is postulated that, if such drug-resistant HSV mutants were to emerge in the corneal stroma of patients with herpetic keratitis, their low pathogenicity would make them unlikely either to establish trigeminal ganglion infection or subsequently to reactivate from a latent stage.

The HSV isolates from conjunctival and ulcerative infections presenting to the casualty department showed no significant resistance to any of the antiviral agents. The lack of resistance to a large number of commonly used antiviral agents in all the clinical type 1 HSV infections would indicate a low incidence of virus-drug resistance in the vast majority of ocular HSV infections.

The only type 2 HSV clinical isolate in our study showed resistance to TFT, IDU, BVDU, and CEDU but was fully sensitive to the other antiviral agents, including ACV. Even the most resistant isolates tested showed sensitivity to some of the commonly used antiviral agents.

Ocular type 2 HSV infection is very rare, and no other similar isolates had been obtained at the time of the experiment. Some studies have

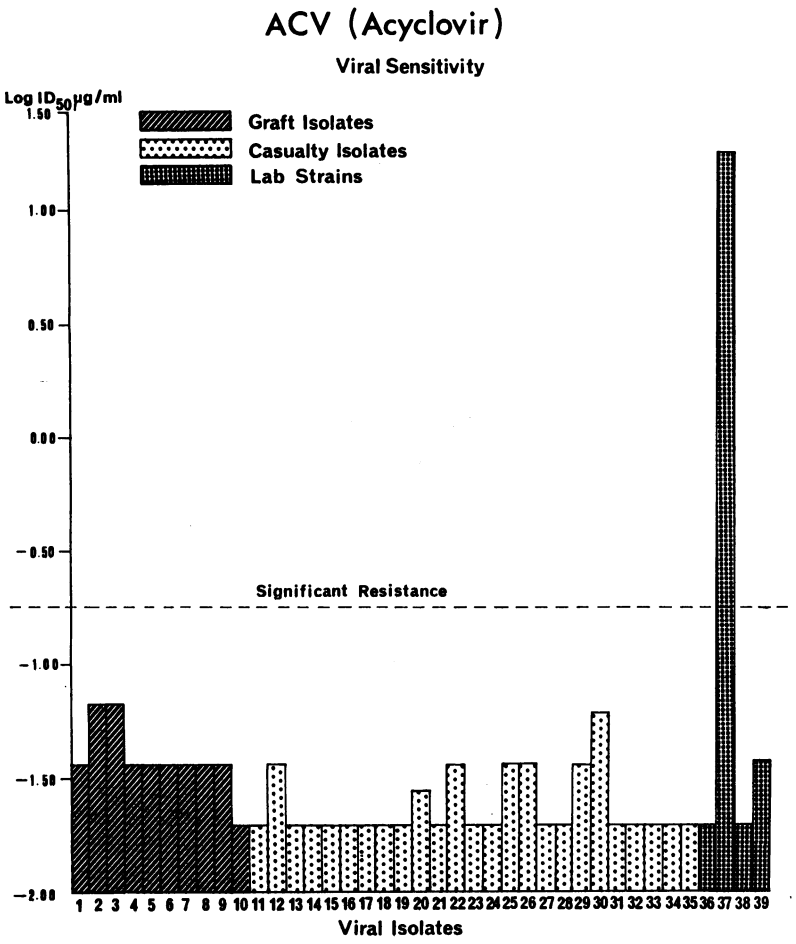

Figure 4

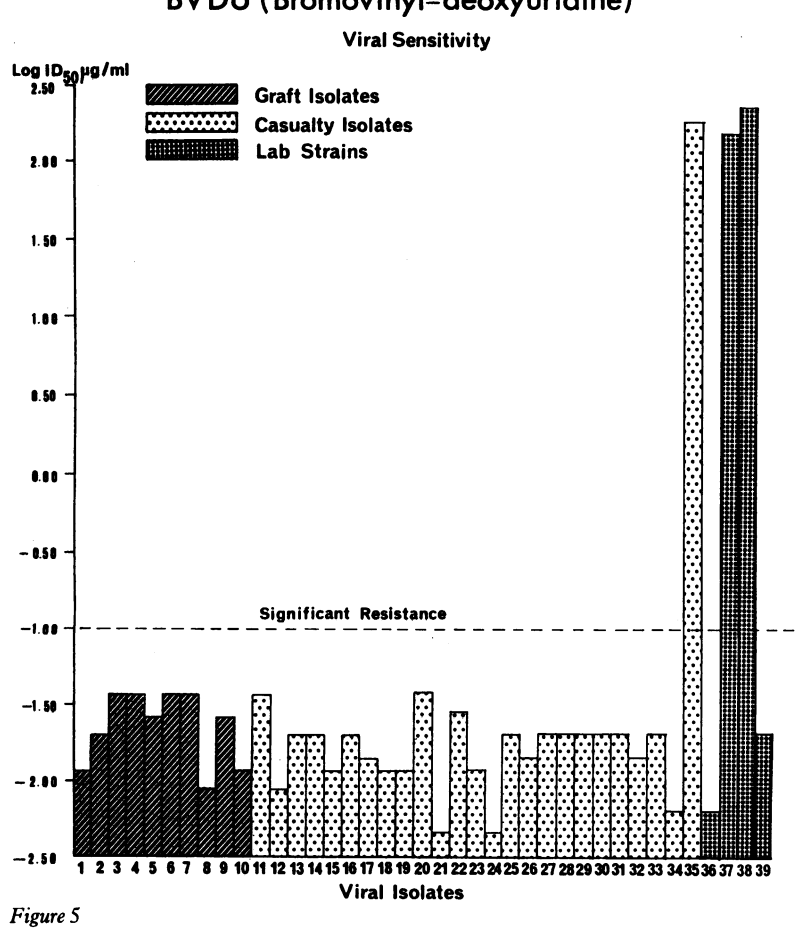

Figure 5

shown that type 2 HSV isolates have higher ID_{50} values than type 1 isolates when tested for acyclovir sensitivity,[14] and there is some evidence that breakthrough infections in patients on prophylactic acyclovir for genital type 2 HSV infections show increased resistance[16], but there is scant information on ocular infections.

It is unwise to draw any conclusions from a single result, but this obviously offers scope for further investigation into both the frequency of ocular type 2 herpetic infection and the level of resistance to antiviral drugs.

In conclusion, our results suggest that virus-drug resistance is not a significant problem in ocular type 1 HSV infections.

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