only upon a solid surface. Its physiology and chemical composition, and those of Gram-positive variants derived from it, suggest a relationship with both Streptococcus and Corynebacterium, especially the latter.

I wish to acknowledge the skilled and patient technical assistance of Mr Gary White.

REFERENCES


THE ANTIVIRAL ACTIVITY OF ISOQUINOLINE DRUGS FOR RHINOVIRUSES IN VITRO AND IN VIVO

Sylvia E. Reed and M. L. Bynoe*

Common Cold Research Unit, Salisbury, Wiltshire

Certain isoquinoline compounds have been found to have antiviral activity in vitro (Brammer, McDonald and Tute, 1968). Larin et al. (1968) showed that two such derivatives of dihydroxyisoquinoline, namely 1-(p-chlorophenoxymethyl)-3,4-dihydroisoquinoline hydrochloride (UK2054) and 1-(p-methoxyphenoxymethyl)-3,4-dihydroisoquinoline hydrochloride (UK2371) showed activity in vitro against a range of viruses, including myxoviruses and

Received 1 May 1969; accepted 20 Oct. 1969.

* Dr Bynoe died in June 1969.
some picornaviruses. UK2054, but not UK2371, was found to be active against some rhinoviruses. Whilst the effect of these compounds on myxoviruses appeared to involve direct inactivation of the infectious particle, other groups of viruses were evidently affected in the course of their reproductive cycle in tissue-culture cells. Beare, Bynoe and Tyrrell (1968) and Reed et al. (1969) reported tests of the antiviral effect of UK2371 in volunteers: this drug was weakly active in suppressing human experimental infection with an influenza-B virus and also a strain of influenza A2.

We report here some observations on the effect of UK2054 and UK2371 on the growth of rhinoviruses in tissue culture, and of oral treatment with UK2054 on experimental human infections with rhinovirus type 9.

**MATERIALS AND METHODS**

**Virus strains.** Rhinoviruses were standard laboratory strains. Type 1A (strain JH) and type 4 (strain 16/60) were adapted to passage in HeLa cells. Type 2 (strain HGP), type 9 (strain DC) and type 43 (strain GT) were normally maintained by man-to-man passage, but were used for tissue-culture experiments after three serial passages in HeLa cells. Rhinovirus type 9 (strain DC) used for experiments in volunteers was maintained by man-to-man passage.

**Tissue cultures.** A strain of HeLa cells sensitive to rhinoviruses was used. The methods and media used for its cultivation were as described by Stott and Tyrrell (1968). The semi-continuous line of human embryo lung fibroblasts, WI-38, and a similar line, HEL-218, were grown in Eagle's medium with 10 per cent. ox serum and maintained in the same medium with 2 per cent. ox serum.

**Virus titrations.** These were performed in tissue cultures of HeLa or HEL-218 cells. Serial 3.2-fold (0.5 log10) dilutions of virus were made, and three tubes were seeded with inocula of each virus dilution. Tubes were incubated at 33°C on a roller drum and the cytopathic effect was read after 6-7 days.

**Neutralisation tests.** Pairs of sera from volunteers were tested for neutralising antibody to rhinovirus type 9 by the micro-neutralisation colour test (Stott and Tyrrell). A four-fold rise in antibody titre was taken as evidence of infection.

**Drug toxicity tests in tissue cultures.** UK2054 or UK2371 at various concentrations were incorporated in the maintenance media used for HeLa and HEL-218 cells. The cells were incubated at 33°C; on each successive day the medium was changed and the monolayers were examined microscopically for evidence of drug toxicity.

**Tests with drugs in tissue cultures.** As a test for direct inactivation of virus by the drugs, a suspension of rhinovirus type 2, containing about 10^5.0 TCID50 per ml, was diluted with an equal volume of solution of either UK2054 or UK2371. The mixtures were incubated for 2 hr at 37°C, then diluted in maintenance medium and titrated in HeLa cells. A control virus suspension was incubated in the absence of drug and was similarly diluted and titrated.

In a second type of experiment rhinovirus type 2 was titrated in HeLa cells maintained in medium containing 15 μg per ml UK2054 or UK2371, or in a control medium without drug. The medium, with or without drug, was changed daily for 4 days; after 1 wk, the end-points of the titrations were compared. Similar comparisons were made in titrations in HEL-218 cells maintained in medium containing the drug at a concentration of 10 μg per ml.

In a third type of experiment the yields of rhinovirus obtained from cultures in treated and untreated cells were compared. Monolayers of HeLa cells or HEL-218 cells in tubes were maintained in medium with or without added drug. Tubes were given inocula of about 10^2.0 TCID50 of the test virus and were rolled at 33°C. The medium, with or without drug, was changed daily and the cultures were examined for cytopathic effect (CPE). On the 2nd, 3rd or 4th day of incubation, groups of three or four tubes from drug-treated and control cultures were harvested by freezing the monolayers in their supernatant medium. The infectivity of these harvests was subsequently titrated in HeLa cells, and the difference in titres of the harvests from drug-treated and control cultures was calculated.
Trials in volunteers. Volunteers were selected, isolated, inoculated and observed by methods standard for this Unit (Tyrrell, 1963). Rhinovirus type 9 (strain DC) was given in intranasal drops. UK2054 (1-5 g daily in four oral doses) was given to half the volunteers, while the remainder received placebo tablets. Drug treatment was started 24 hr before administration of the virus and was continued for 64 days. Volunteers were allocated randomly to the drug and placebo groups, and the administration of drug or placebo and the clinical observations were made "double blind". Clinical examinations were made daily to assess the degree of coryza, nasal obstruction, sore throat, malaise and other relevant symptoms. The reactions ultimately graded as severe, moderate or mild were scored as positive; doubtful reactions were included with those that were negative. Nasal washings were collected 2, 3 and 4 days after the administration of virus and were subsequently inoculated into WI-38 cells. Virus isolations were recorded as positive if a typical rhinovirus cytopathic effect was seen in the monolayers within 1 wk after they had been given inocula from one or more specimens from any volunteer. Serum samples were taken from all volunteers at the start of the trial and 2-3 wk later.

**TABLE I**

*Effect of incubation of a suspension of rhinovirus type 2 with UK2054 or UK2371 for 2 hr at 37°C on the titre of virus subsequently estimated in HeLa cells*

<table>
<thead>
<tr>
<th>Titre of virus (log_{10} TCID50 per ml) after suspension had been incubated</th>
<th>in the absence of drug (control)</th>
<th>in the presence of UK2054</th>
<th>in the presence of UK2371</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 µg per ml</td>
<td>100 µg per ml</td>
</tr>
<tr>
<td>4.6</td>
<td>4.75</td>
<td>4.6</td>
<td>4.75</td>
</tr>
</tbody>
</table>

Attempts to prepare drug-resistant virus. Virus was isolated from a volunteer who had been treated with UK2054 and who nevertheless developed a cold. Two further passages of this isolate were made in HeLa cells, and in its third passage the virus was tested for its sensitivity to UK2054.

In an attempt to prepare drug-resistant virus *in vitro*, rhinovirus type 9 was passaged three times serially in HeLa cells that were grown in medium containing 10 µg per ml of UK2054. In each passage the inoculum contained between 10 and 100 TCID50. This virus was then grown in cells cultured in medium without drug and its sensitivity to UK2054 was compared with that of a strain of rhinovirus type 9 that had undergone a parallel series of passages in medium without drug.

**RESULTS**

Toxicity in tissue cultures

When used at 20 µg per ml on HeLa cells, neither of the drugs UK2054 and UK2371 produced any observable toxic effect until the 6th day of observation, when the monolayers, though healthy, appeared somewhat thinner than control cultures. In cultures of HEL-218 cells this thinning was detectable on the 4th day. Thinning was not seen when the drugs were used at concentrations lower than 20 µg per ml, though occasional small, plaque-like foci of degeneration were produced in HeLa cell monolayers by either drug at concentrations down to 10 µg per ml. The latter effect, which was thought to be due to insoluble particles of drug, made rhinovirus plaque experiments somewhat unreliable.
Antiviral activity in vitro

The results of attempts to inactivate rhinovirus type 2 by incubation with UK2054 or UK2371 are shown in table I. No significant decrease of titre was found on comparison with the control titration.

Table II shows the results of experiments in which rhinovirus types 1A and 2 were titrated in HEL-218 or HeLa cells in media with or without an isoquinoline. The presence of UK2054 did not reduce the titre when the virus was titrated in HEL-218 cells. When, however, the titration was done in HeLa cells, the addition of either UK2054 or UK2371 to the medium at a concentration of 15 μg per ml led to a reduction in virus titre of 0.5 log}_{10} units. Although this reduction was apparently insignificant in amount, it was noticed that in the titration made in HeLa cells with added UK2054 the virus cytopathic effect progressed more slowly than in either the control titration or the titration made in the presence of UK2371.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Host cells</th>
<th>Virus titre (log}_{10} TCID50 per ml) estimated in cells maintained in medium containing drug*</th>
<th>control medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1A</td>
<td>HEL-218</td>
<td>4.25</td>
<td>4.25</td>
</tr>
<tr>
<td>Type 2</td>
<td>HeLa</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>Type 2</td>
<td>HEL-218</td>
<td>4.9</td>
<td>4.7</td>
</tr>
</tbody>
</table>

* Both drugs were used at 15 μg per ml in HeLa cells and at 10 μg per ml in HEL-218 cells.

The results of experiments in which the yields of virus obtained from cultures in drug-treated and control cells were measured are shown in table III. Significant changes were observed only in the experiments with UK2054 and HeLa cells. In this test system, UK2054 decreased or delayed the development of the virus cytopathic effect, and this inhibition of the CPE was evidently associated with a decrease in the yield of virus in the drug-treated cultures. No such inhibition occurred in the presence of UK2371, or when HEL-218 cells were used. When rhinovirus type 4 was grown in HeLa cell cultures in medium containing 5, 10 or 15 μg per ml of UK2054, the degree of inhibition of the yield of virus was found to be dependent on the dose of the drug.

Activity in experimental infections in man

The results of trials in human volunteers who were infected experimentally with rhinovirus type 9 and treated by oral administration of UK2054 are shown in table IV. Serological tests showed that comparable numbers of volunteers with antibody titres less than 16 in the pre-inoculation sample of serum had been assigned to the drug and placebo treatments. The differences found between the drug and placebo groups, whether assessed clinically, or by the rate of virus isolation, or by the serological response, were found not to be significant (P>0.05).

Attempts to isolate drug-resistant virus

Rhinovirus type 9 that had been passed three times in HeLa cells in the presence of UK2054 ("2054-treated virus") and control virus that had been passaged three times in
HeLa cells in the absence of drug were compared for their sensitivity to inhibition by UK2054. A similar dose of each virus was inoculated into HeLa cells treated with UK2054 15 μg per ml, or into control cells, and the yield of each virus was measured 2 days later. In the

**TABLE III**

*Effect of UK2054 and UK2371 on the yield of rhinoviruses obtained from virus-infected cell cultures maintained in the presence of the drug*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Host cells</th>
<th>Concentration of drug (μg per ml)</th>
<th>Changes in virus yield* produced by the presence of the drug, measured 3 or 4 days after inoculation of the virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>UK2054</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>Type 2</td>
<td>HEL-218</td>
<td>10</td>
<td>-0.15</td>
</tr>
<tr>
<td>Type 2</td>
<td>HeLa</td>
<td>15</td>
<td>-0.8</td>
</tr>
<tr>
<td>Type 4</td>
<td>HeLa</td>
<td>5</td>
<td>-0.65</td>
</tr>
<tr>
<td>Type 4</td>
<td>HeLa</td>
<td>10</td>
<td>-1.5</td>
</tr>
<tr>
<td>Type 4</td>
<td>HeLa</td>
<td>15</td>
<td>-1.8</td>
</tr>
<tr>
<td>Type 9</td>
<td>HeLa</td>
<td>15</td>
<td>-2.35</td>
</tr>
<tr>
<td>Type 43</td>
<td>HeLa</td>
<td>15</td>
<td>-0.9</td>
</tr>
</tbody>
</table>

* Changes are expressed as log10 (virus yield from drug-containing culture divided by virus yield from control, drug-free culture). The virus yields were estimated as number of TCID50 per ml by titration in HeLa cells maintained in drug-free medium.

In a test involving titrations of a suspension of virus in cell cultures maintained in the presence and absence of UK2054, the drug was found to decrease the virus yield by 0.7 log10 units on the 3rd day after inoculation and by 0.65 log10 units on the 4th day.

**TABLE IV**

*Effect of oral treatment with UK2054 on experimental infections with rhinovirus type 9 in human volunteers*

<table>
<thead>
<tr>
<th>Observation</th>
<th>Number of volunteers in whom observation was made/ number given virus inoculum and treated with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UK2054</td>
</tr>
<tr>
<td>Development of symptoms</td>
<td>12/31 (38.7 per cent.)</td>
</tr>
<tr>
<td>Virus isolation</td>
<td>13/31 (41.9 per cent.)</td>
</tr>
<tr>
<td>Four-fold rise in titre of antibody to virus</td>
<td>21/31 (67.7 per cent.)</td>
</tr>
<tr>
<td>Virus isolation or antibody rise or both</td>
<td>23/31 (74.1 per cent.)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Our experiments confirm that although the isoquinoline drug, UK2054, has no direct inactivating effect on rhinoviruses, it can inhibit the growth of these viruses in HeLa cells. In a test involving titrations of a suspension of virus in cell cultures maintained in the presence and absence of UK2054, the drug did not decrease the titre of virus to a significant extent.
ISOQUINOLINE DRUGS AND RHINOVIRUSES

It seems possible, however, that a more sensitive test based on plaque titration, which was found to be impracticable, might have revealed a minor degree of inhibition. When depression of the yield of virus in drug-treated cultures was observed as the measure of antiviral activity it was found that UK2054 inhibited the development of each of the four rhinovirus serotypes tested (table III). These effects were produced only in HeLa cells and not in HEL-218 cells. UK2371 was not inhibitory to production of virus in HeLa cells.

The concentrations of drug required to produce inhibition of the virus approached rather closely to the concentrations that produce detectable, though apparently mild, toxic damage to the cell cultures. It may therefore be questioned whether the inhibitory effect of the drug is indeed specifically antiviral, or is mediated by a more general toxic effect on the cells. However, if the suppression of virus yield found in the presence of the drug were related only to general cell damage, similar degrees of viral inhibition might be expected to occur in cells subjected to apparently comparable degrees of potential damage by the drug. The toxic effect of UK2054 on HEL-218 cells was apparently slightly greater than that on HeLa cells, yet 10 μg per ml of the drug did not inhibit the yield of rhinovirus type 2 from HEL-218 cells. The same concentration of drug in HeLa cells significantly depressed the yield of rhinovirus type 4 (table III).

The difficulty of predicting the effect of antiviral drugs in man from the results of laboratory tests is well known. There may be several explanations for the failure of UK2054 to reduce the incidence of infection in our trial of its prophylactic and therapeutic effectiveness in human volunteers. The effective concentration of the drug achieved at the actual site of viral multiplication is clearly important. UK2054 is found in higher concentration than UK2371 in human serum after comparable oral dosage, and the former compound also reaches higher concentration than UK2371 in the pharyngeal mucous membrane of dogs after administration by mouth (information obtained from the Therapeutics Research Division, Pfizer Ltd). It therefore seems likely that an adequate concentration of UK2054 is achieved in the human respiratory tract. If this is so, it may be assumed either that the antiviral activity of the drug is insufficient to suppress a clinical infection, or that the effect found in HeLa cells does not occur in human respiratory epithelium. The nature of the host cell is clearly important in determining whether a measurable antiviral action occurs in vitro, since UK2054 showed no activity when the virus was grown in HEL-218 cells. Human respiratory epithelium may be more closely analogous to these cells than to HeLa cells.

Another possible cause of failure of the drug to influence the course of infection in man is the emergence of drug-resistant virus. Attempts to demonstrate development of resistance in vitro were not successful. Virus isolated from a volunteer who had been treated with UK2054 was tested in vitro and was found to be inhibited by UK2054, although its sensitivity appeared somewhat less than that of the original strain. There is thus no clear indication that the development of drug resistance is a cause of failure of UK2054 to suppress human infections, but a more extensive comparison of the sensitivity of isolates from drug-treated and placebo-treated individuals would be necessary in order to reach a definite conclusion on this point.

SUMMARY

The isoquinoline drugs UK2054 and UK2371 were tested for activity against rhinoviruses. UK2054, but not UK2371, decreased the yield of rhinovirus types 2, 4, 9 and 43 from HeLa cells maintained in medium containing the drug. This activity was not demonstrable in a semi-continuous line of human embryo lung fibroblasts, HEL-218. In a double-blind, placebo-controlled trial, human volunteers were experimentally infected with rhinovirus type 9 and given prophylactic and therapeutic UK2054 by mouth. No significant antiviral activity was found in this trial.

We thank Messrs Pfizer Ltd for supplies of drug and placebo tablets, Miss M. D. Turnbull for help with clinical observations, and Mrs Susan Finniss for valuable technical assistance. We also thank Dr D. A. J. Tyrrell and Dr E. J. Stott for much helpful advice.
REFERENCES


Addendum

After the recent reports by Meenan and Hillary (1969a and b) that UK2054 given intranasally may have some effect on influenza infection, this route of administration was tried in volunteers infected with rhinovirus type 9. A suspension of UK2054 of either 15 mg per ml or 40 mg per ml was given as nasal drops, eight times daily, from 1 day before until 5 days after inoculation of virus. The total dose of drug per day was either 7.2 mg or 19.2 mg. Nine volunteers received the less concentrated drug suspension and 14 the more concentrated: 22 received placebo. Colds occurred in 7 of 23 drug-treated volunteers and 8 of 22 controls, and the severity of the symptoms was the same in the two groups. Laboratory evidence of infection, as revealed by virus isolations and specific serological responses, was shown in 15 of 23 volunteers who received active drug and 16 out of 22 controls. The higher dosage of drug showed no advantage in comparison with the lower.

REFERENCES


" " " . . 1969b. Ibid., 2, 641.

THE BACTERIAL FLORA OF TRICHOMYCOSIS AXILLARIS

J. A. SAVIN, DOROTHY A. SOMERVILLE AND W. C. NOBLE

St John's Hospital for Diseases of the Skin, London*

Trichomycosis axillaris is a condition in which nodules appear on the hair shaft in the axilla, particularly on the portion nearest the skin. The nodules appear to be composed of masses of bacteria, and the condition receives clinical attention when staining of the clothing occurs, presumably due to the growth of chromogenic bacteria. The subject has been reviewed by Lane (1919) and Crissey, Rebel1 and Laskas (1952).

Paxton (1869) first described trichomycosis axillaris and recorded the growth of a white mould from affected hairs. Subsequent investigations have not confirmed Paxton's findings and in recent studies only the diphtheroid flora has been considered. Trichomycosis has been attributed to one particular species of diphtheroid, Corynebacterium tenuis (sic) (Crissey et al.), but the observations of McBride, Freeman and Knox (1968) do not support this view. Shehadeh and Kligman (1963) had suggested that trichomycosis represents the overgrowth

Received 26 June 1969; accepted 22 Aug. 1969.