A NEW ANTIRHINOVIRUS COMPOUND, ICI 73602: STRUCTURE, PROPERTIES, AND SPECTRUM OF ACTIVITY

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Guanidine hydrochloride (Figure 1a) has long been known as a selective inhibitor of replication of small RNA viruses in tissue culture. The principal target for the antiviral activity of guanidine appears to be the virus genome coded viral RNA polymerase, hence its specificity for viral rather than cellular functions. The metabolic block caused by guanidine, however, is readily circumvented by picornaviruses, and after only a few passages in presence of the drug the viruses become completely resistant to its effect.

This defect of guanidine itself, coupled with its total lack of activity in vivo stimulated an intensive search for antiviral activity in derivatives of guanidine. It is probably still correct to say, as Loddo et al. did in 1964, that practically all guanidine derivatives are inactive against picornaviruses. There are, fortunately, some exceptions to the general rule.

Two years ago we reported the discovery of a substituted guanidine ICI 65709 (Figure 1b) that had high activity against a number of RNA viruses particularly rhinoviruses.

Subsequent development of this discovery led us to the structurally related compound ICI 73601 (Figure 2), which has a broader spectrum of activity against viruses causing "the common cold," i.e., rhinoviruses and coronaviruses.

The methods used for testing for in vitro antiviral activity have already been described in full. Briefly, monolayers of human embryonic lung (HEL) cells in 3 x 0.5 in. tubes are infected with 100 TCD₅₀ of test virus, and simultaneously test compound is added at a range of concentrations. After incubation at 33° for 2 days the cells are inspected under a low-power microscope and the concentration of compound that causes a 50% cytopathic effect and that causing 50% inhibition of viral cytopathic effect are determined.

Table 1 shows the activity of ICI 73602 against 15 strains of rhinovirus and one strain of coronavirus determined by the above method. It is evident that the compound has very considerable selectivity between effects on virus replication and effects on cellular function. This was confirmed by studies on the synthesis of cellular RNA, DNA, and protein in HEL cells. There was no effect on the synthesis of these components at concentrations of compound up to 50 times greater than the antiviral levels. The most stringent test of the effect of a drug on cellular function is to grow the cells in its presence. When HEL cells are grown in the presence of ICI 73602 for 15 days, the yield of viable cells compared with untreated controls is completely unaffected by 1.0 µg/ml but is reduced by about 50% at 2.5 µg/ml. Thus the antiviral activity we have found in the range 0.2–1.0 µg/ml is indeed absolutely selective.
Further testing of the compound has been carried out in transverse sections of human embryonic trachea. The cumulative virus yield over 5 days from a challenge of 100 TCD₅₀ of rhinovirus 2 is reduced 10-fold by 3 μg/ml and nearly 100 fold by 9 μg/ml of drug in the medium. The 50% toxic concentration as judged by cessation of ciliary activity is 25-30 μg/ml so that the antiviral activity is selective by factors of 10 and 3, respectively. In this type of experiment, the drug is present continuously in the culture medium which is renewed each day of the 4-5 day experiment. Intermittent dosing of drugs is characteristic of most schedules of chemotherapy and, from the point of view of the patient, the less frequently the better. We devised an intermittent dosing schedule for tracheal sections in which they were exposed to ICI 73602 for either 5 or 60 sec per day for 4 days. The results of several tests are shown in Table 2. Virus yields in TCD₅₀/ml culture fluid were measured daily from treated sections and from control sections that were treated daily with isotonic saline in exactly the same way as the drug-treated sections. Experiments on fragments from the same trachea are grouped together each with its own control. It can be seen that the compound will produce effectively complete inhibition of viral replication when dosed once per day for one min at a concentration of 0.35 mg/ml or for 5 sec per day at concentrations between 1.0 and 1.5 mg/ml.

Attempts to make rhinovirus cultures resistant to ICI 73602 by repeated passage in subinhibitory concentrations were not successful. Thus the compound differs significantly from guanidine, where resistance begins to develop after only one passage, and from some other antiviral guanidine derivatives, where resistance developed more slowly but involved cross-resistance to guanidine.

Preliminary mode-of-action studies by adding drug to tissue culture cells at in-
Inhibition of Growth of Rhinovirus and Coronavirus Strains in Human Embryonic Lung Cells by ICI 73602

| Virus       | Type          | 50% Inhibitory Concentration (µg/ml) | Therapeutic Ratio* |
|-------------|---------------|--------------------------------------|-------------------|
| Rhinovirus  | 9             | 0.2                                  | 150               |
|             | 1A, 2, 11, 19, 26, 36, 43 | 0.4                                  | 75                |
|             | 1B, 4, 16     | 0.8                                  | 38                |
|             | 5, 14, 17, 35 | 1.6                                  | 19                |
| Coronavirus | 229E          | 1.0                                  | 30                |

*Based on a 50% toxic concentration of 30 µg/ml.

Intervals 0-8 hr after infection and measuring virus yield at 9-16 hr after infection indicate that ICI 73602 affects a late stage in viral replication, possibly the assembly of viral RNA and coat protein. Some later biochemical studies indicated that only 4% of viral single-stranded RNA found in infected cells was actually encapsidated into mature virions when drug was present, confirming our original finding. However, these studies also showed the compound to have similarities to guanidine in that RNA-dependent RNA polymerase activity was inhibited by the drug, although synthesis of polymerase polypeptides appeared to be unaffected.

Toxicological examination of ICI 73602 has shown that it is a well-tolerated compound. No adverse effects were observed when it was administered to rats either by instillation into the nostrils six times per day for 7 days at concentrations up to 400 µg/ml, or by intraperitoneal injection at 100 mg/kg daily for 14 days. There is little or no systemic absorption of the compound from an oral or intranasal dose in rats.

An investigation of structure-activity relationships in this type of compound served only to demonstrate that the occurrence of activity was unpredictable as can be seen in Table 3, which contains a small selection of the compounds examined. Activity was present most frequently when R₂ is an isobutyl group (i-Bu), but even

### Table 2

**Effect on Rhinovirus Yield from Infected Human Embryonic Tracheal Fragments When Treated Once per Day with ICI 73602**

| Concentration of ICI 73602 at Time of Treatment | Period of Daily Exposure | Virus Yield TCD₅₀/ml on Day | Total TCD₅₀ |
|------------------------------------------------|--------------------------|----------------------------|-------------|
| 0.35 mg/ml                                     | 5 sec                    | 250 160 63 5              | 478         |
| 0.35 mg/ml                                     | 60 sec                   | 7 5 <2.5 <2.5 <2.5 <2.5 17 |           |
| Control                                        |                          | 63 100 63 5              | 231         |
| 0.4 mg/ml                                      | 5 sec                    | 160 100 4 5              | 305         |
| 1.36 mg/ml                                     | 5 sec                    | 100 63 5 <5 <5 <5 <168    |             |
| Control                                        |                          | 400 25 8 5              | 438         |
| 0.8 mg/ml                                      | 5 sec                    | 25 25 <5 <5 <5 <5 <60     |             |
| Control                                        |                          | 630 400 111 160 160 1501  |             |
| 1.49 mg/ml                                     | 5 sec                    | 40 10 5                  | 55          |
| Control                                        |                          | 2500 630 630             | 3760        |
TABLE 3

| R₁   | R₂   | Benzene Substitution | ICI No. | Activity* µg/ml | Toxicity µg/ml |
|------|------|----------------------|---------|----------------|----------------|
| p-Cl | H    | m                    | 94458   | NA             | 25             |
| Me   | m    |                      | 81011   | NA             | >45            |
| n-Pr | m    |                      | 81012   | NA             | 45             |
| i-Pr | m    |                      | 80656   | 1.8            | 9              |
| n-Bu | m    |                      | 81013   | NA             | 45             |
| i-Bu | m    |                      | 73602   | 0.1            | 30             |
| s-Bu | m    |                      | 81014   | NA             | 45             |
| i-Am | m    |                      | 81015   | NA             | 45             |
| Ph   | m    |                      | 77688   | 0.25           | 5              |
| i-Pr | p    |                      | 81262   | 1.8            | 45             |
| i-Bu | p    |                      | 81263   | 5              | 45             |
| p-Br | i-Bu | m                    | 80497   | 1.0            | 30             |
| i-Bu | p    |                      | 81679   | NA             | 45             |
| p-OMe| i-Bu | m                    | 80459   | NA             | 45             |
| p-OEt| i-Bu | m                    | 76961   | 1.5            | 30             |

*NA indicates a ratio between toxicity and activity of <5.

minor changes in \( R_1 \) when \( R_2 = i-Bu \) could result in loss of activity, e.g., the last pair of compounds in the Table. Activity was less frequently encountered when the central aromatic linkages were in the para position rather than meta, but all compounds in which they were ortho were inactive. Reversal of the relative positions of the guanidine and urea moieties in the molecule led to a series of compounds in which activity appeared even less frequently.

In ICI 73602 we have a guanidine derivative that differs significantly in its antiviral behavior from guanidine itself. It has very high activity against rhinovirus in two types of in vitro test systems, it is relatively nontoxic to laboratory animals, and studies in the only satisfactory in vivo system, man, are in progress.

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