Relationship Between the Chemical Structure of Thiazolidine Acetic Acid Derivatives and the Antiherpetic Activity in Human Embryonic Cell Cultures

Recently it was found that 3-(4'-bromophenyl)-4-oxo-5-thiazolidine acetic acid 2-benzylidenehydrazone (DFT)[1] prevents cytopathogenic changes in human embryonic kidney cell cultures infected with *Herpes simplex* and poliovirus type 1, if added to the cell cultures simultaneously with the virus, or even if added at different short-time intervals after infection[2].

A number of compounds chemically related to DFT were tested for their activity on *H. simplex* virus in order to determine if a relationship between their antiviral activity and chemical structure could be established.

For the experiments we used *H. simplex* virus strain Z obtained from Dr. G. W. A. DICK. This strain caused a total destruction of human embryonic kidney cell cultures in 48 h. Cell cultures were grown in 0.5% lactalbumin with 5% calf serum in Hanks' balanced salt solution.

The titration of virus controls in cell culture media containing derivatives of thiazolidine acetic acid was described previously[3]. The effect of compounds on non-infected cell cultures was determined in all experiments in which virus was grown in the presence of added compounds. After infection, cell cultures were examined for cytopathogenic changes from the 1st to the 5th day.

For the experiments all the compounds used have been dissolved in demineralized boiled water containing 5 mg/100 ml NaOH. Thus $2 \cdot 10^{-3} M$ solutions were obtained. The concentration of the compounds in the culture medium was $5 \cdot 10^{-5} M$. The compounds tested are presented in the Table.

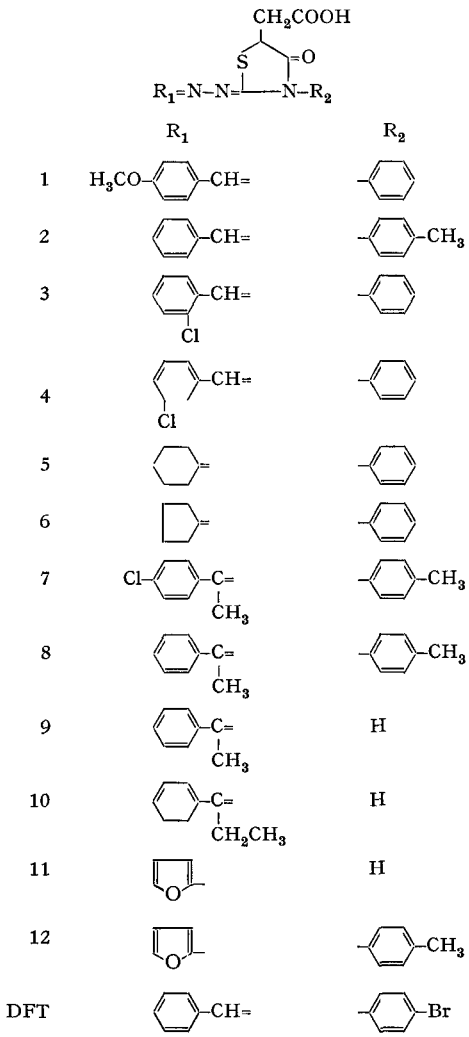
In virus controls, cytopathogenicity developed on the 1st day after infection. These changes progressed to the 5th day when the titre of virus was 6.5 $\log_{10}$.

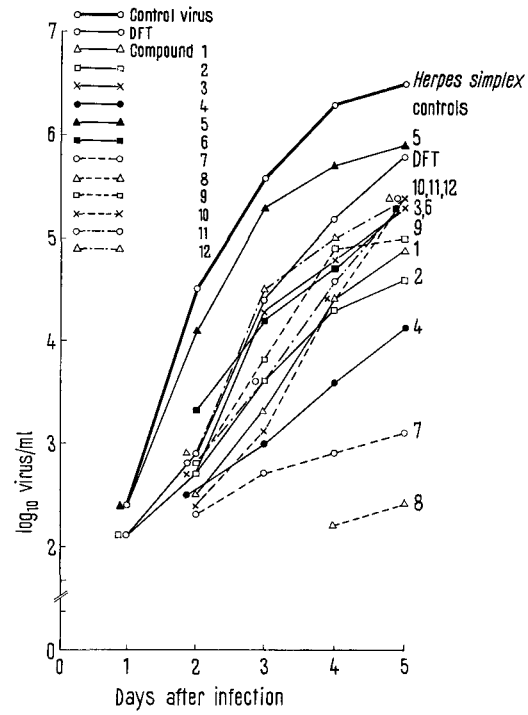
The derivatives of 4-oxo-5-thiazolidine acetic acid gave a whole spectrum of antiherpetic activity. They reduced the titre of *H. simplex* virus in cell cultures after 5 days of growth from 0.6 $\log_{10}$ (compound 5) to 4.1 $\log_{10}$ (compound 8).

Most of the substances tested reduced the titre of *H. simplex* virus between the 0.6 $\log_{10}$ and 1.6 $\log_{10}$ (Figure). The greatest effect on *H. simplex* virus in human embryonic kidney cells was exhibited by compounds 2, 4, 7 and 8. Virus titre in the culture fluids containing these compounds was lower on the 5th day after infection for 1.9 $\log_{10}$, 2.4 $\log_{10}$, 3.4 $\log_{10}$ and 4.1 $\log_{10}$ than in culture fluids of the virus controls.

Our experiments have shown that the changes of the substituents R_1 and R_2 of the 4-oxo-5-thiazolidine acetic acid molecule exhibited a pronounced effect on *H. simplex* virus. The greatest inhibition of viral multiplication

Chemical structure of 4-oxo-5-thiazolidine acetic acid derivatives





Inhibition with 4-oxo-5-thiazolidine acetic acid derivatives of the cytopathogenic changes produced by *Herpes simplex* in the tissue culture of human embryonic kidneys.

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showed the derivatives, which have in the molecule as R_2 a *p*-tolyl group and as R_1 an aromatic ring. For the antiherpetic activity the presence of a halogen atom is not necessary. But if it is, then its place in the molecule is of some importance. 1'-furylidene group as R_1 decreases the antiherpetic activity of the molecule.

It was established for the isatine β-thiosemicarbazone, a compound from the group of related substances-thiosemicarbazones, that the primary site of action lies in the late phases of virus replication[4–6]. About the intracellular site of action of our compounds, we can only suppose from experiments with one of the thiazolidine acetic acid derivatives – DFT, which indicate that the late phases of virus multiplication are affected[7].

Zusammenfassung. Durch Abwandlung der Struktur der 4-Oxo-5-thiazolidinessigsäure ergeben sich starke inhibitorische Effekte auf die cytopathogenen Wirkungen, die vom Virus *Herpes simplex* in Kulturen menschlicher Embryonalzellen ausgeübt werden.

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Histamine Release by Passive Sensitization with Murine Neoplastic Mast Cells in vitro[1]

Histamine release and mast cell damage have been reported recently by Humphrey and Austen[2] and by Keller[3] from the incubation of isolated rat peritoneal mast cells with rabbit antiserum against rat γ-globulin (passive reversed anaphylaxis). Histamine release from isolated rat peritoneal mast cells, exposed in vitro to the specific antigen, has been achieved by Perera and Mongar[4] and confirmed by Zilletti et al.[5]. So far, no information is available on the possibility of inducing an active or passive sensitization of isolated mast cells from mice. Information is also lacking on the possibility of sensitizing, actively or passively, murine neoplastic mast cells, maintained in tissue culture or in the inbred mice. The isolation of a clone (i.e. the progeny of a single cell) from a murine mastocytoma tumour possessing high histidine-decarboxylase activity, and fairly constant in endogenous amines (histamine and serotonin[6]), provided us with a system for investigating the problem of the histamine release by passive sensitization with murine neoplastic mast cells.

Methods. Furth mastocytoma cells, which were grown subcutaneously as a solid tumour in LAF_1 mice, were transplanted in tissue culture according to the method described by Fischer and Sartorelli[7]. From the original mixed population a clone of cells was obtained by means of the cloning technique by dilution[8]. This clone was maintained for one year, both in tissue culture and as an ascitic tumour in LAF_1 mice. The biochemical and biological characteristics (doubling time, 24 h; chromosome modal number, 45; histamine levels, 650 ± 54 ng/10^6 cells; serotonin levels, 750 ± 80 ng/10^6 cells) of the cells continuously grown either in culture or in the mice were the same. Since these neoplastic cells grown in mice afford higher cell population than when grown in culture, ascitic tumours were used as a source of cells throughout these experiments. The approach used to sensitize the cells passively was the production in the rabbit of an antiserum against mast cells from the same colonie. The procedure was carried out as follows: Neoplastic mast cells were obtained from the mice, washed and resuspended in ice-cold tyrode solution. Microscopic examination of small samples revealed a pure and uniform cell population. Two male albino rabbits (weighing 3 kg each) were injected endovenously with $2–3 \cdot 10^7$ cells, and subcutaneously with 2 ml of pertussis vaccine. Before the procedure for sensitization was begun, a sample of about 10 ml of blood was collected from each rabbit; the sera obtained were kept frozen until used. Booster injections of $5 \cdot 10^6–10^7$ cells were given subcutaneously every 10 days, and the schedule repeated four times. At the end of the sensitization procedure, blood was collected by cardiac puncture from each rabbit, and the sera obtained kept frozen.

Mast cells obtained from a single mouse were harvested, washed and resuspended in 6 ml of tyrode solution. A set of 5 small centrifuge tubes was prepared as follows: tube 1, 0.2 ml of tyrode + 0.8 ml of tyrode containing mast cells; tube 2, 0.2 ml of serum of rabbit No. 1 before sensitization + 0.8 ml of tyrode containing mast cells; tube 3, 0.2 ml of serum of rabbit No. 1 after sensitization + 0.8 ml of tyrode containing mast cells; tube 4, 0.2 ml of serum of rabbit No. 2 before sensitization + 0.8 ml of tyrode

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