Inhibitory Effect of a Cyclic Urea Derivative on Rubella Virus Replication

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Cyclic Ureas, 1-(4-Morpholinomethyl)-tetrahydro-2(1H)-pyrimidinone, Rubella Virus

1-(4-Morpholinomethyl)-tetrahydro-2(1H)-pyrimidinone (mopyridone) exhibited a marked activity against rubella virus (Judith and RA27/3 strains), a MIC_{50} value of 0.9 μM and selectivity ratio of 557.7 been found in the case of Judith strain. These data, in addition to the previous ones about its anti-alphavirus effects suggest the compound to be considered as a broad spectrum inhibitor of togavirus replication.

Introduction

1-(4-Morpholinomethyl)-tetrahydro-2(1H)-pyrimidinone (mopyridone, MCU) is an antiviral substance, synthesized by Sidzhakova *et al.* (1982), which manifested a marked activity against alpha- and orthomyxoviruses (Galabov *et al.*, 1984, 1994). Noteworthy the inhibitory effect of the compound on replication of alphaviruses Semliki forest (SFV), Sindbis (SV) and Western equine encephalomyelitis (WEEV) (Galabov *et al.*, 1984; Karparov *et al.*, 1986). Moreover, mopyridone was highly efficient *in vivo* when administered intravenously or subcutaneously in mice infected with SFV and SV, and a significant protection was recorded even at massive virus inocula (Karparov *et al.*, 1985). Recently, the compound given subcutaneously was found to be effective also in experimental Eastern equine encephalomyelitis virus (EEEV) infection in mice (A. A. Davydova, A. A. Lazarenko and I. F. Barinsky, unpublished data). By its main chemotherapeutic characteristics mopyridone could be considered as the most potential antiviral among known anti-alphavirus compounds.

It was of interest to study the mopyridone effect on replication of another representative of *Togaviridae* family, the rubella virus.

Materials and Methods

Compound tested

Mopyridone (MCU, mol. weight 199.25, white fine crystals, very soluble in water) was supplied by D. Sidzhakova (Faculty of Pharmacy, Higher Medical School, Sofia).

Virus strains and cells

Two rubella virus strains were used, Judith (supplied by the National Institute of Biological Standards and Control, London, U. K.) and RA27/3 (the Plotkin's vaccinal strain), cultivated in BHK_{21} (baby hamster kidney) cells from the collection of the National Institute of Biological Standards and Control, London (culture medium of Eagle's MEM Difco supplemented with 5% newborn calf serum).

Cytotoxicity test

The compound maximal tolerated (nontoxic, MTC) concentration for the cell culture was determined beforehand by tracing the effect of the compound on uninfected confluent cell monolayer and cellular morphology for overt signs of cytotoxicity during 4–5 days.

Antiviral tests

The compound effect was studied in a multicycle virus growth experimental setup. In this case the cell monolayers were inoculated at low values (0.005, 0.0005 and 0.00005) of multiplicity of infection (the number of viral infectious doses inoculated per cell). Virus inoculum was added directly to the cell suspension at the time of seeding in test tubes. Compound at nontoxic concentrations was applied immediately after virus inoculation. Then, the infected cell cultures were observed during 7 days. The cytopathic effect (CPE) was scored on a 0–4 basis with 4 representing total cell destruction. The infectious virus yields (in three times frozen and thawed cell culture samples) were as-

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sayed on the 7th day after infection by the end-point dilution method.

The CPE inhibition method using monolayer BHK_{21} cell cultures in 96-well plastic microplates (Flow, Glasgow) was used in some experiments with the Judith strain. The cells sheets were inoculated with serial 10-fold virus dilutions – 1–1000 $CCID_{50}$ (50% cell culture infectious doses), 0.02 ml per well, by 60 min adsorption at room temperature. Then the compound was added to the maintenance medium (0.2 ml per well of Dulbecco's MEM Sigma without serum) with 0.5 $\log_{10}$ increasing consecutive concentrations. The plates were incubated for 6 days and CPE was estimated as described above, the compound dose-response traced at 10–100 $CCID_{50}$ viral dose. From the graphs the minimal concentration was evaluated causing a 50% reduction of CPE as compared to the untreated controls (MIC_{50}).

Results and Discussion

Mopyridone, known as an alphavirus replication inhibitor, was tested vs. two rubella virus strains: the pathogenic Judith and the vaccinal RA27/3. No reference (control) substance was tested for comparison as, according to the literature data, a compound possessing a marked anti-rubella virus activity has not been described yet.

Initially the compound was studied in the multicycle virus growth setup applied at comparatively high concentrations, 200.7/401.4 μM, and the maximal tolerated concentration for BHK_{21} cells of 501.9 μM had been found.

As seen in Table I, mopyridone showed a marked inhibitory effect on replication of the rubella virus strains in BHK_{21} cells, expressed as suppression of CPE development and decrease of the infectious virus yield.

In further experiments the compound was tested against the Judith strain by the CPE inhibition method in 96-well microplates. The compound activity was confirmed. A MIC_{50} value of 0.9 μM, and a selectivity ratio (MTC/MIC_{50}) of 557.7, was established.

These data suggest mopyridone to be considered as a broad-spectrum inhibitor of togavirus replication. Obviously, the anti-rubella virus effect of this compound needs further elucidation.

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Table I. Effect of mopyridone on rubella virus replication in BHK_{21} cells (multicycle virus growth setup).

Virus strain	M. O. I.[a]	Mopyridone concentration (μM)					
		0		200.7		401.4	
		CPE[b]	$\log CCID_{50}$/ml[c]	CPE	$\log CCID_{50}$/ml	CPE	$\log CCID_{50}$/ml
Judith	0.005	4.0	3.7	3.0	2.5	2.75	2.3
	0.0005	3.5	3.7	2.0	2.5	1.75	2.3
	0.00005	1.75	3.3	0.5	1.5	0	1.3
RA27/3	0.005	4.0	4.0	4.0	3.3	4.0	3.0
	0.0005	3.25	3.7	1.5	2.0	1.25	2.0
	0.00005	1.0	2.0	0.12	1.0	0	0.7

Virus replication estimated on day 7 after infection:
[a] M. O. I., multiplicity of infection;
[b] CPE mean value (4 samples per test group; two experiments);
[c] $CCID_{50}$, infectious titer in 50% cell culture infectious doses.

Galabov A. S., Khristova M. L., Uzunov S., Wassilewa L., Bucher D. J. and Kharitonenkov I. G. (1994), Alteration in the antigenic structure of M1 protein of influenza A virus mutant resistant to a new antiviral compound mopyridone. Acta Virol. **38**, 5–10.

Galabov A. S., Velichkova E., Karparov A., Sidzhakova D., Danchev D. and Chakova N. (1984), Antiviral activity of tetrahydro-2(1H)-pyrimidinone and related compounds. Arzneim.-Forsch./Drug Res. **34**, 9–14.

Karparov A., Galabov A. S., Sidzhakova D., and Danchev D. (1985), Antiviral effect of 1-morpholinomethyl-tetrahydro-2(1H)pyrimidinone (DD-13) in experimental alphavirus infections in white mice. Arzneim.-Forsch./ Drug Res. **35**, 1269–1275.

Karparov A. A., Galabov A. S., Sidzhakova D. and Danchev D. (1986), Inhibitory effect of Mannich bases of tetrahydro-2(1H)-pyrimidinone and its analogues on the reproduction of some alphaviruses in cell cultures. C. r. Acad. bulg. Sci. **39**, 149–152.

Sidzhakova D., Danchev D., Galabov A. S., Velichkova E., Karparov A. and Chakova N. (1982), Structure and properties of cyclic polymethyl ureas. III. Synthesis and biological activity of some Mannich bases of tetrahydro-2(1H)-pyrimidinone. Arch. Pharm. (Weinheim) **315**, 509–514.