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ANTIVIRAL ACTIVITY OF ARYL-FURFURANE DERIVATIVES

V. I. VOTYAKOV, M. N. SHASHIKHINA, E. A. IZERGINA, S. V. ZHAVRID, N. P. STASHKEVICH, A. F. OLEINIK

Byelorussian Research Institute of Epidemiology and Microbiology, 220020 Minsk, U.S.S.R.

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Summary. — Of 27 aryl-furfurane derivatives (AFD) studied, 13 compounds were found to have antiviral activity against ECHO 6, herpes simplex, vaccinia, and influenza A/Hong Kong/1/68 (H3N2) viruses, the highest inhibition being observed with ECHO 6 virus. The maximum tolerated concentrations (MaTC) of AFD inhibited the macromolecular syntheses in ECHO 6 virus-infected cell cultures.

Key words: aryl-furfurane derivatives; antiviral activity; cell culture

Introduction

The activity of AFD, many of which had been previously described as tuberculostatics (Oleinik *et al.*, 1976*a*, *b*), was studied in cell culture against 7 viruses. Some indices characterizing the antiviral activity of the compounds were compared.

Materials and Methods

Chemical compounds. AFD were synthesized at the S. Ordzhonikidze All-Union Research Chemopharmaceutical Institute, Moscow.

Viruses. Influenza A/Hong Kong/1/68 (H3N2), parainfluenza 3, Venezuelan equine encephalomyelitis (VEL-230 strain), ECHO type 6, fixed rabies (Moscow strain), human adenovirus type 3, herpes simplex (L2 strain) obtained from D. I. Ivanovsky Institute of Virology, Moscow, and vaccinia virus (white clone) produced at the Byelorussian Research Institute of Epidemiology and Microbiology, Minsk, were used.

Cell culture. Influenza virus was grown in cultured pieces of surviving chick embryo chorioallantoic membranes (CAM), VEE, herpes simplex (HSV) and vaccinia viruses in primary chick embryo fibroblasts, ECHO 6 virus in human embryo diploid skin-muscle or lung cells, adenovirus and parainfluenza virus in primary human embryo kidney and diploid skin- muscle cells.

Medium. The growth medium for primary and diploid cell cultures consisted of 5% haemohydrolysate prepared at the Byelorussian Research Institute of Epidemiology and Microbiology containing 10% normal bovine serum; medium 199 was used as maintenance medium. For CAM pieces, the maintenance chloride medium supplemented with glucose and gelatine was used (Fazekas de St. Groth and White, 1958).

Laboratory animals. Random-bred white mice weighing 6—8 g were used.

Antiviral properties of AFD. Screening and assessment of antiviral activity were performed according to previously published methods (Boreko *et al.*, 1974; Denisova *et al.*, 1974; Votyakov, 1974; Galitskaya, 1977; Votyakov and Boreko, 1977).

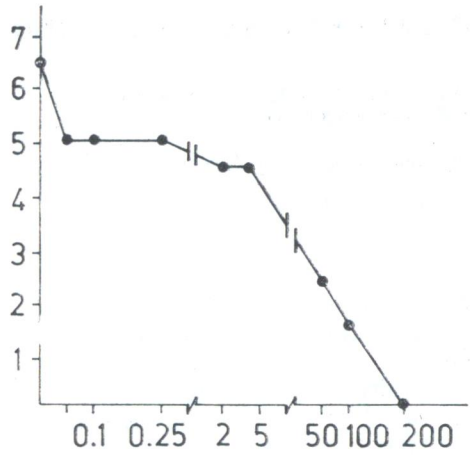


Fig. 1.
The effect of compound No. 9 on the infectivity of ECHO-6 virus
Abscissa: compound concentration (μg/ml); ordinate: virus infectivity (log $TCID_{50}$ values)

Assessment of the level of macromolecular syntheses in virus-infected cells in the presence of AFD. RNA and protein syntheses were determined by incorporation of ^{3}H-uridine and ^{14}C-chlorella hydrolysate, respectively, into the acid-insoluble fraction of the cells exposed to virus at the multiplicity of infection of 1 PFU/cell. Radioactivity was measured in a Packard-Tricarb counter. To test the effect of AFD on RNA synthesis of ECHO 6 virus-infected cells, AFD in the MaTC and actinomycin D (1 μg/ml) were added to the cells early after virus inoculation.

Statistical treatment of the data. The Spearman coefficient of rank correlation was determined by the formula:

$$\beta = 1 - \frac{6\Sigma(x - y)^2}{n(n^2 - 1)},$$

where $(x - y)^2$ is the sum of squares of rank differences, and n is the number of pairs compared. The significance of ranks was evaluated using a special Table (Merkov, 1965).

Results

Antiviral activity of AFD

Among 27 AFD studied, 7 were found to be highly active: 6 compounds (Nos 9, 11, 13, 15, 18 and 21) against ECHO 6 virus, and one (No. 8) against HSV; 3 compounds possessed moderate activity: 2 against vaccinia virus (Nos 4 and 27) and one (No. 14) against ECHO 6; five compounds showed weak activity against influenza virus. None of the compounds tested was active against VEE, rabies and adenovirus. Table 1 presents the maximum tolerated (MaTC) and minimal active (MiAC) concentrations. Further it shows the maximum antiviral effect (MAVE), the reduction of the infectious virus titres at MiAC, and the chemotherapeutic index (TI) of the compounds having a marked antiviral effect. The effect of the most active compound No. 9 on ECHO 6 virus infectivity is shown in Fig. 1.

Study of the effect of AFD on macromolecular syntheses

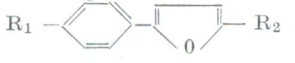
Because AFD were found most active against ECHO 6 virus, the effect of compounds Nos 9, 11, 13, 14 and 15 on ECHO 6 virus-induced RNA

Table 1. Influence of AFD on virus infectivity in cell culture

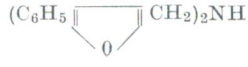
Compound No.	R_1	R_2	Tested virus	MaTC	MiAC	Virus titre reduction MAVE	Virus titre reduction MiAC	TI
9	Br	COC_6H_5	ECHO 6	400*	0.05*	6.5**	1.5**	8000
11	Cl	COC_6H_5	ECHO 6	400	0.5	2.5	1.5	800
18	Cl	$COC_6H_4OCH_3$	ECHO 6	12.5	0.5	not done	1.5	25
15	Br	COC_6H_4Cl	ECHO 6	400	25	2.5	2.0	16
21	Cl	CH_2OH	ECHO 6	100	10	not done	2.5	10
13	NO_2	COC_6H_4Cl	ECHO 6	400	50	4.0	1.83	8
14	NO_2	$COC_6H_4OCH_3$	ECHO 6	200	50	6.0	2.0	4
8	Cl	COOH	Herpes	800	100	2.0	1.5	8
4	Cl	$COCH_2SC$	Vaccinia	400	100	4.0	3.67	4
27			Vaccinia	800	200	2.33	1.84	4

*μg/ml **$\log TCID_{50}$

MaTC = maximum tolerated concentration; MiAC = minimum active concentration; MAVE = maximum antiviral effect; TI = = therapeutic index



formula of compounds No. 9, 11, 18, 15, 21, 13, 14, 8, 4

27 

formula of the compound No. 27

Table 2. Effect of AFD on RNA synthesis in ECHO 6 virus-infected cells

Compound No.	Incorporation of ^{3}H-uridine	Inhibition %
9	369*	83.7
11	870	61.5
13	646	71.6
14	711	68.6
15	1,233	45.5
Control cells	32,369	
Control cells + actinomycin D	337	
Infected cells + actinomycin D	2,260	

* c.p.m.

synthesis (after treatment with actinomycin D) was studied. As follows from Table 2, these compounds effectively inhibited viral RNA synthesis in the infected cells but exerted no inhibiting effect on cellular RNA synthesis. The assessment of the effect of AFD on total protein synthesis showed 3 compounds (Nos 9, 11 and 14) to be capable of complete restoring of protein synthesis in infected cells (Table 3).

While virus infection inhibited cellular protein synthesis by more than 85%, the synthesis was restored when virus reproduction took place in the presence of AFD compounds tested. The compound No. 13 inhibited the syntehsis of virus-specific RNA by 71.6% (Table 2) but was not conducive to complete recovery of protein synthesis in the infected cells (Table 3). Compound No. 15 inhibited RNA synthesis by 45.5% and slightly restored protein synthesis in the infected cells. Statistical treatment of the results using Spearman coefficient of rank correlation revealed no correlation between the TI value and the level of inhibition of viral RNA synthesis ($\varrho = 0.2$; $P < 0.05$), between TI and the level of stimulation of viral protein

Table 3. The effect of AFD on protein synthesis

Compound No.	Incorporation of ^{14}C-hydrolysate uninfected cells		infected cells	
	control	cells + compound	control	cells + compound
9	31,829*	30,249	3632	33,111
11	10,435	11,437	1191	11,029
13	8,724	8,876	1110	7,207
14	8,902	9,772	1337	10,360
15	8,724	8,366	1110	2,720

*c.p.m.

synthesis ($\varrho = 0.1$; $P < 0.05$), or between TI and the level of virus titre reduction ($\varrho = 0.1$; $P < 0.05$). At the same time a significant correlation was found between the levels of virus titre reduction and viral RNA synthesis inhibition ($\varrho = 0.9$; $P < 0.05$), between the levels of virus titre reduction and stimulation of viral protein synthesis ($\varrho = 1.0$, $P = 0.01$), as well as between the levels of viral RNA synthesis inhibition and stimulation of viral protein synthesis ($\varrho = 0.9$, $P = 0.05$).

Discussion

Screening of compounds with possible antiviral effect is based on two data: the virus titre reduction in the presence of a compound and TI. While TI reflects a range of possible application of a compound and depends on its toxicity for a biological system, MAVE at a MaTC indicates the antiviral activity of the compound. MaTC in cell culture is usually determined according to morphological signs of the viability of cells with regard to the cell damage in the presence of the compound under study. However, life activity of the cell is most completely reflected by their RNA synthesis. Our previous data showed the correlation of MaTC values as determined by morphological signs and by RNA synthesis. Based on this we concluded that the toxicity of antiviral substances, which affect virus replication, may be determined according to the level of RNA synthesis in their presence rather than according to morphological signs of cell damage. The determination of MaTC for antiviral substances of other mechanisms of action (at cellular level, at the cell population level) should be based on the sign of cell damage caused by the compound.

As follows from our results, the most marked antiviral activity of AFD was demonstrated against ECHO 6 virus. The intensity of antiviral effect was found to depend on different fragments of the molecule of the aryl--furfurane derivatives under study. The optimal combinations for the manifestation of antiviral activity were Br in R_1 and COC_6H in R_2 (compound No. 9) as well as NO_2 in R_1 and $COC_6H_4OCH_3$ in R_2 (compound No. 14); these compounds reduced the infectivity of ECHO 6 virus by 6.5—6.0 log TCD_{50}. Introduction of Cl in R_1 or R_2 resulted in a decrease of antiviral activity (compounds Nos. 13, 15, 11): compound No. 13 reduced virus titre by 4.0 log TCD_{50}, compounds Nos 15 and 11 by 2.5 log TCD_{50}. Because synthesis of nucleic acids and protein is so important for cell life, comparative evaluation of various antiviral substances should include tests of their effects on macromolecular syntheses of cells and virus. To characterize the substances inhibiting picornaviruses, we chose the tests of inhibition of cellular and viral RNA syntheses and recovery of protein synthesis in virus-infected cells.

Assessment of the effect of AFD on macromolecular syntheses of ECHO 6 virus-infected cell cultures confirmed the antiviral selectivity of the effect of the compounds. All the compounds under study except No. 15 which exerted a poor effect on macromolecular syntheses produced more marked recovery of protein synthesis than inhibition of viral RNA synthesis. The

value of MAVE was found to correlate strictly with the values characterizing the effect of a compound on the virus-induced synthesis. The processes of inhibition of ECHO 6 virus RNA synthesis correlated also with the recovery of protein synthesis in this virus-infected cell in the presence of AFD. At the same time, no correlation of the level of viral RNA synthesis inhibition, stimulation of viral protein synthesis or virus titre decrease with TI value was observed.

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