METHOTREXATE POTENTIATES ANTI-HERPES SIMPLEX VIRUS TYPE 1 ACTIVITY OF E-5-(2-BROMOVINYL)-2'-DEOXYURIDINE

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Summary. – The pharmacologic of induction of low level of deoxyribonucleoside triphosphates (dNTP) is a novel approach to combined inhibition of virus replication. In accordance with this concept the alteration of antiherpes activity of E-5-(2-bromovinyl-2'-deoxyuridine (BVDU) in combination with methotrexate (MTX), an inhibitor which lowers the intracellular pool of thymidine triphosphate (dTTP) was studied. MTX applied alone at non-toxic concentrations had a moderate effect on herpes simplex virus type 1 (HSV-1) replication in human embryonic skin-muscle fibroblasts. The combination of BVDU and MTX acts synergistically, as measured by virus yield assay in the above mentioned system. The potentiating effect of MTX on the anti-HSV-1 activity of BVDU was reversed by thymidine (dThd).

Key words: methotrexate; bromovinyldeoxyuridine; antivirals; synergism; herpes simplex virus type 1

It has been demonstrated recently that the reduction of dNTP pools size in virus-infected cells could result in an increased antiviral effectiveness of some selective antiviral nucleoside analogues (Spector *et al.*, 1985; Baba *et al.*, 1987; Balzarini *et al.*, 1989, 1990; Machida *et al.*, 1991). In our recent studies it was shown that ribavirin, which causes depletion of the intracelullar guanine nucleotides pool, potentiates the antiherpes activity of acyclovir (Pancheva, 1991). In this paper investigating the antiherpes activity of BVDU combined with MTX, an additional confirmation of this concept is presented. BVDU exhibits very strong and selective antiviral activity against HSV-1 (De Clercq *et al.*, 1976). BVDU triphosphate acts as a competitive inhibitor with respect to the natural substrate dTTP for initial binding of viral DNA polymerase (Allauden *et al.*, 1981). MTX blocks the *de novo* dThd synthesis through inhibition of dihydrofolate reductase (DHFR), and consequently lowers the intracellular pools of dTTP (Friedland, 1974). The decrease of the cellular pools of dTTP could give a better opportunity to its competitor to interact with the target enzyme HSV-1 DNA polymerase. It was demonstrated that MTX significantly enhanced the antiviral activity of BVDU against HSV-1 in cell culture of human embryonic skin-muscle fibroblasts. The increased inhibition of HSV-1 replication by the two combined substances was reversed when the infection was carried out in excess of dThd. In this case dTTP could be considered as a "key metabolite" responsible for the higher effectivity of the combination of drugs.

Abbreviations: BVDU = E-5-(2-bromovinyl)-2'-deoxyuridine; DHFR = dihydrofolate reductase; dNTP = deoxyribonucleoside triphosphate; dThd = thymidine; dTTP = thymidine triphosphate; HESMF = human embryonic skin-muscle fibroblast; HSV-1 = herpes simplex virus type 1; MTX = methotrexate

BVDU was gifted by courtesy of Dr. Reefschlager (Bayer A.G., Pharmaceutical Research Center, Germany), MTX (amethopterin) originated from Werfft-Chemie. HSV-1 strain DA was received from Dr. S. Dundarov, Institute of Infectious and Parasitic Diseases, Bulgarian Medical Academy, Sofia. The virus was cultivated in cultures of diploid human embryonic skin-muscle fibroblast (HESMF) cells. The activity of the combination of BVDU and MTX was evaluated against HSV-1 in HESMF cells using the yield reduction method. Investigations were carried on confluent cell monolayers in tubes infected with 100 $CCID_{50}$ in 0.1 ml. The drugs alone and in combination were added after 1 hr of virus adsorption and cells were then incubated for 48 hrs at 37 °C. Virus titers of the samples (pools from 4 test tubes) were determined by titration in microplates cultures, and expressed in $CCID_{50}$/0.1 ml. Cytotoxicity was monitored by the trypan blue dye exclusion method.

We had previously established that MTX was toxic for HESMF cells at concentration of 0.5 μmol/l. No cytotoxicity of combination of MTX and BVDU in the concentrations used in the antiviral assays was observed.

As it is demonstrated in Table 1 MTX showed a moderate antiviral activity against HSV-1 in HESMF cells at non-toxic concentrations, e.g. a 66-fold reduction of virus yield at 0.1 μmol/l.

Table 1. Effect of BVDU in combination with MTX on reduction of yield of HSV-1 in HESMF cells

BVDU (μmol/l)	MTX (μmol/l)	Virus yield ($CCID_{50}$/0.1 ml x 10^{-4})	N	Q
-	-	210	-	
0,15	-	4,7	45	
-	0,1	3,2	66	
-	0,05	4,7	45	
-	0,025	17,0	12	
-	0,0125	100,0	2,1	
0,15	0,1	0,00047	$4,5 \times 10^5$	151
0,15	0,05	0,001	$2,1 \times 10^5$	104
0,15	0,025	0,0047	$4,5 \times 10^4$	83
0,15	0,0125	0,032	$6,6 \times 10^3$	70

N - Virus yield reduction; ratio of virus yield in control to that in drug-treated cells.

$$Q = \frac{N_{A+B}}{N_A \times N_B}$$

N_{A+B} = N for the combination of drugs A and B; N_A = N for the drug A alone; N_B = N for the drug B alone.

If Q is higher, equal or lower than 1, the interaction is synergistic, additive or antagonistic, respectively (Smith *et al.*, 1982). The values represent the means of three independent determinations.

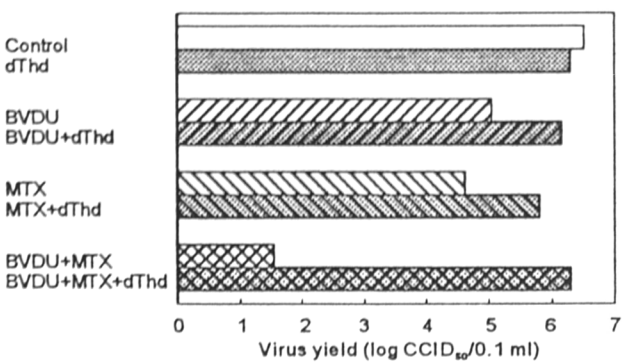

Fig. 1
Reversal of the combined inhibitory effect of BVDU and MTX on HSV-1 replication by dThd
Concentrations: 2 μmol/l dThd, 0,075 μml/l BVDU, 0.1 μmol/l MTX.

The combination of MTX with BVDU enhanced enormously their anti-HSV-1 activity. 0.15 μmol/l (0.05 μg/ml) BVDU and 0.1 μmol/l (0.05 μg/ml) MTX, which alone reduced the viruses yield 45 and 66 times, respectively, suppresed in combination the virus replication 450,000 times, indicating a high degree of synergism. By using a non-effective dose of MTX, 0.0125 μmol/l (0.0062 μg/ml), the virus yield was reduced more than 6000 times. Fig. 1 presents the reversal of the combined inhibitory effect by dThd, administered together with the drugs. A 97% recovery of the virus yield 48 hrs p.i. was observed in the presence of 2 μmol/l dThd (20-fold higher concentration than that of MTX). As dThd is converted within the cells to dTTP, our data suggest that it is the reduction in dTTP level which accounts for the potentiating effect of MTX on the antiviral activity of BVDU. It is well known that MTX is widely used in cancer chemotherapy as a cytostatic agent. The viral infections associated with administration of cytotoxic drugs are primarily due to herpesviruses. In this context our results could be important and inspirating for specialists who are interested in the therapy of associated herpesvirus infections.

The data shown in this paper confirm the validity of the above mentioned concept as an approach to the combined chemotherapy of viral infections of man and may gain also clinical merits in suppressing exogenous or endogenous DNA virus infections in patients treated by cytostatic therapy.

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