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N^2-SUBSTITUTED GUANINE DERIVATIVES ACT AS SELECTIVE NON SUBSTRATE INHIBITORS OF HSV I THYMIDINE KINASE

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INTRODUCTION

Herpes simplex virus type I (HSV I) induces a unique virus specific thymidine kinase (TK) in infected cells (1-3) that in contrast to the cellular enzyme, not only phosphorylates thymidine, but also a variety of pyrimidine and purine deoxyribonucleosides and nucleoside analogs such as acycloguanosine or bromovinyl-deoxyguanosine. The "plasticity" of the active site represents a vulnerable point for the viral enzyme and so far great efforts have been made to develop antiherpes compounds that would serve as selective substrate for the viral thymidine kinase in the virally infected cell. Since studies of HSV pathogenesis and replication in vivo have suggested that the viral TK is required for viral replication and reactivation of latent virus in non-dividing cells in which host-specific pathways to dTTP are depressed or absent such as trigeminal ganglion (4) or adult brain (5) we have sought to develop a specific inhibitor of the viral enzyme. To this purpose we have screened a variety of guanine, uracile and adenine analogs or their nucleoside derivatives for capacity to inhibit viral or cellular thymidine kinase.

MATERIALS AND METHODS

Cells, viruses and enzymes: The cells HeLa S3, its TK deficient derivative (TK^-) and the HeLa TK^-/HSV I TK^+, a derivative of HeLa TK^- stably transormed to the TK^+ phenotype with a functional copy of the HSV I TK gene were grown in monolayer culture in Dulbecco's modified Eagles medium. HSV I (17 sym^+)-infected HeLa TK^- served as the source of viral thymidine kinase.
Both the virus- and the host- specific thymidine kinases were purified approximately one hundred fold from cytoplasmic extracts of their respective sources through a DEAE-cellulose and phosphocellulose chromatography.

Thymidine Kinase assay: The reaction mixture (50 µl) 30 mM Hepes-K^+ pH 7.5, 6 mM $MgCl_2$, 6 mM ATP (Mg^{++} salt), 0.5 mM DTT, 1 mM (^{3}H)Thymidine (25 Ci/mmol), incubated with thymidine kinase at 37°C for 30 min, was terminated by spotting 45 µl on a 25 mm DE 81 paper disc. The disc was washed with an excess of 1 mM ammonium formate, H_2O and ethanol and counted in 5 ml omnifluor.

Inhibitors: The compounds listed in the table were synthesized as previously described (6,7).

RESULTS AND DISCUSSION

The table summarizes the results of examination of a variety of 6-aryl-uracil and of N^2-aryl-aminopurine and their nucleoside derivatives on the activity of HSV I specific and cellular

thymidine kinases. Several guanines and their nucleoside derivatives were found active against HSV I TK. The anti-HSV TK activity requires a purine nucleus of the guanine (6-oxo) type. The equivalent 6 $-NH_2$ adenine derivative is inactive. The potency of the 2'-deoxyribonucleoside derivative exceeds that of the base, the ribonucleoside and the more complex nucleotide form.
The most active derivative, phenyldeoxyguanosine, competes with both thymidine and cytidine kinase activity of HSV I TK and is not converted to its 5'-phosphate form. Most probably it binds to the enzyme in the area of its catalytic site sequestering it in a catalytically non-productive form.
At concentrations ten times higher than that causing 50% inhibition of TK the compound displays limited toxicity for the viral host HeLa cell line and it might thus be a starting structure for the synthesis of an ideal agent. It could also represent a novel tool with which to probe the structure and functions of the active site of HSV TK.

Derivative	concentration (μM) causing 50% inibition * of HSV I TK
(p-n-butylanilino)-deoxyadenosine	inactive
(")- " triphosphate	"
(anilino)-uracil	"
(p-methylanilino)-uracil	"
(p-methyl, m-ethylanilino)-uracil	"
(p-n-butylanilino)-3-methyluracil	86
(p-n-butylanilino)-uracil	35
(toluen)-guanine	50
(toluen)-guanosine	30
(p-n-butylphenyl)-guanine	50
(")-guanosine	75
(")-deoxyguanosine	25
(")-acycloguanosine	140
(")-deoxyguanosine monophosphate	400
(")- " triphosphate	800
(p-Br-phenyl)-guanine	1
(phenyl)-guanine	8
(p-methyl-m-ethylphenyl)-guanine	7
(phenyl)-deoxyguanosine	0.3
(")- " monophosphate	3
(m-ethylphenyl)-guanine	3
(m-Cl-phenyl)guanine	1.3

* All tested compounds were inactive against HeLa thymidine kinase.

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