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Effects of Inhibitors of *De Novo* Pyrimidine Biosynthesis on Human Cytomegalovirus DNA Replication. Fayez M. Hamzeh, Frances E., Lee, Nadia B. Assadi, and Paul S. Lietman. Division of Clinical Pharmacology of the Department of Medicine, The Johns Hopkins School of Medicine, Baltimore, MD 21287-5554 USA.

Our previous observation that brequinar, which inhibits *de novo* pyrimidine biosynthesis by inactivating dihydro-orotate dehydrogenase, selectively inhibits human cytomegalovirus (HCMV) DNA synthesis has led us to hypothesize that the inhibition of *de novo* pyrimidine biosynthesis leads to a preferential inhibition of HCMV DNA synthesis as compared to cellular DNA synthesis, possibly due to the depletion of the endogenous pyrimidine pools. Other inhibitors of *de novo* pyrimidine biosynthesis are invariably potent and selective inhibitors of HCMV DNA replication with lesser effects on host cell DNA synthesis. The compounds tested were acivicin (ACN), azaserine (AZS), and 6,diazo-5-oxo-1-norleucine (DON) which inhibit carbamyl-phosphate synthetase, dichloroallyllawson (DL) and brequinar which inhibit dihydro-orotate dehydrogenase, phosphoracetyl-L-aspartic acid (PALA) which inhibits aspartate transcarbmylase and pyrazofurin (PYRA) which inhibits orotidine-5'monophophate decarboxylase. The preferential inhibition of HCMV DNA synthesis as compared to host cell DNA synthesis, (i.e. greater therapeutic index, TI; TD50/ED50) may be related to an inhibition of the HCMV induced buildup of endogenous nucleotide pools in HCMV-infected cells.

Drug	GCV	ACN	AZS	BREQ	DL	DON	PALA	PYRA
$ED_{50}(\mu M)$	5	0.5	5	0.05	5	6	40	0.1
$TD_{50}(\mu M)$	>150	>500	>2000	>10	>64	>2000	>10000	>400
TI	>30	>100	>400	>200	>12	>333	>250	>4000

Similar to brequinar, these agents were generally more potent inhibitors of HCMV than HSV-1 DNA replication.

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Rapid Detection of Thymidine Kinase (TK)-Positive and Negative Varicella-Zoster Virus (VZV) Isolates Using 1-ß-D-arabinofuranosyl-*E*-5-(2-[^{125}I]-iodovinyl)uracil (^{125}IVaraU) Plaque Autoradiography (PA). E.M. Smyrnis, S.E. Straus, H. Dougan. and S.L. Sacks. University of BC and TRIUMF, Vancouver, BC, Canada and National Institutes of Health, Bethesda, MD, USA

PA has become an important tool in determining the heterogeneity of wild-type and resistant isolates for herpes simplex virus and VZV. Previously described PA employ [^{125}I]-iododeoxycytidine or [^{14}C]-thymidine and require trichoroacetic acid, formalin fixation and film exposures of 5-7d. To improve the quality and speed for obtaining a radioimage, we modified the current technique and utilized ^{125}IVaraU as the TK-dependent nucleoside probe. HFF subconfluent monolayers grown in 6-well plates were infected (60-90min) with 75–100 plaque forming cells (PFC's) of TK$^-$ VZV or 5-7 PFC's of TK$^+$ VZV strains. After formation of plaques (5-7d), 0.5 µCi of ^{125}IVaraU were added to each well for 6h. Monolayers were washed X4; air dried; well-bottoms excised and positioned against X-OMAT-AR® film X 4-48h. Samples were also immunocytologically (IP) examined and compared with PA film images using NIH-Image version 1.45 for optical densities (OD) on a 1–256 scale.

VZV strain	PFC [IP]	PFC [PA]	IP $Image_{max}$ OD (mean)	PA $Image_{max}$ OD (mean) 4 h	48h
CI1205 (TK$^+$)	7	7	117.7	113.1	244.9
CIwt-1 (TK$^+$)	5	5	130.6	nd	241.6
ppIIa (TK$^+$)	6	6	126.5	120.7	244.4
40a2 (TK$^-$)	100	0	150.0	68.3*	92.6*
101 (TK$^-$)	75	0	162.5	69.7*	88.9*

*background; nd=not done

TK$^+$ VZV strains were quickly and accurately detected using ^{125}IVaraU-PA. Uptake of ^{125}IVaraU was shown to correspond to only those regions in the infected cell monolayer which also expressed VZV-specific antigens, making this a sensitive and accurate method for detection of TK$^+$ VZV. Substantial reductions in the time required for producing an adequate film image was possible by avoiding chemical fixation and directly imaging air-dried monolayers. This technique may be applicable to a variety of radiolabeled nucleoside TK-dependent antiviral compounds.