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ANTI-HERPES ACTIVITY OF THE ADENINE ARABINOSIDE ANALOG, 2'-AZIDO-2'-DEOXY-β-D-ARABINOFURANOSYLADENINE

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Recently, 9-β-(D-Arabinofuranosyl)adenine (ara-A) has been shown to be clinically effective against herpes zoster in immune compromised patients[1]. Similar, encouraging results have been obtained with other, serious infections caused by viruses, such as herpes simplex, varicella zoster, and cytomegalo-virus[2]. By contrast, ara-A did not influence herpes genitalis when applied locally as an ointment[3,4].

Since appropriate sugar intermediates recently became available to us[5], we decided to synthesize 9-β-(2'-Azido-2'-deoxy-D-arabinofuranosyl)adenine (arazide), the 2'-azido analog of ara-A, with the purpose of obtaining access to ara-A derivatives with potentially improved therapeutic properties[6]. The present report describes the behaviour of arazide against herpes simplex virus *in vitro* and *in vivo*. After completion of this study, we learned of similar work being carried out at several other institutions[7-10]. The behaviour of arazide and its hydrogenation product, 9-β-(2'-Amino-2'-deoxy-D-arabinofuranosyl)adenine (aramine) toward L1210 leukemia cells and toward cell-free adenosine deaminase systems has been reported[11].

The anti-herpes *in vitro* activity of arazide (in comparison to ara-A and aramine) was determined from the reduction in the virus-induced cytopathogenic effect (CPE) on HF I, Vero, RK II, and Hep-2 monolayers. Arazide inhibited the CPE of herpes simplex 1 and 2 to an extent qualitatively similar to that of ara-A. Inhibition of the CPE by aramine was somewhat weaker

than that of ara-A or arazide (Table 1).

Table 1.- Reduction of the CPE of herpes simplex virus 1 and 2 by ara-A, arazide and aramine*

virus	host cell line	cell toxicity, µg/mL			inhibition of CPE µg/mL		
		ara-A	arazide	aramine	ara-A	arazide	aramine
HSV-1	HF I	-	10	1000	-	3.2	320
	Vero	32	10	1000	10	3.2	100
	Hep II	320	32	1000	3.2	0.32	10
	RK II	320	10	320	3.2	10	1
HSV-2	HF I	-	10	1000	-	0.32	-
	Vero	32	10	1000	10	10	-
	Hep II	320	32	1000	3.2	0.32	32
	RK II	320	10	320	3.2	3.2	32

* Washed monolayers of susceptible cells were incubated with appropriate dilutions of the test compounds in MEM (0.5 mL per well, two wells for each dilution step). Subsequently, medium containing 100 $TCID_{50}$ ($TCID_{50}$ = mean tissue culture infectious dose) of the appropriate virus was placed in each well. After an incubation time of 96 h at 37^{o} in a humidified carbon dioxide (3 %) atmosphere, the fluid overlays were removed. The monolayers were then stained with Ziehl-Neelson's carbolfuchsin solution (0.5 mL per well) and, after one minute, the staining overlays were removed and the wells rinsed with distilled water. The protective effect of a compound was evaluated by comparing the CPE on treated and untreated monolayers. The untreated monolayers were entirely destroyed at the virus inocula used. The effective dose of a compound was the dose which completely inhibited any CPE as determined by visual inspection of the stained host cell monolayers. Cell toxicity levels are those concentrations of compounds which caused slight but clearly visible changes in cell monolayers, as determined by microscopic examination prior to staining. ara-A and IUdR (active against herpes simplex virus 1 and 2) were used as antiviral

standards.

The antiviral in vivo activity of arazide in comparison to ara-A was tested in a mouse infection model of herpes simplex virus 2-induced encephalitis, and is expressed as percent survivors. It is seen that arazide showed only weak antiviral, chemotherapeutic activity in comparison to ara-A (Table 2).

Table 2.- Increase in survival of herpes simplex virus 2-infected mice upon treatment with ara-A or arazide, relative to untreated controls*

daily dose mg/kg	increase in survival, %		mean survival time, days§	
	ara-A	arazide	ara-A	arazide**
300	73	20	23.07 ± 3.52	13.87 ± 6.23
150	60	13	21.20 ± 5.25	13.33 ± 5.74
75	67	13	21.20 ± 5.82	12.93 ± 5.82

* Groups of fifteen mice (and one untreated control group of twenty mice) were injected intraperitoneally with 3 LD_{50} of herpes simplex virus 2, causing 90-100 % mortality of untreated mice. The first of these die between the 6^{th} and the 8^{th} day post infection. Test compounds (300, 150 and 75 mg/kg body weight) were given intraperitoneally, at first 24 h after infection, then once daily on five consecutive days. The chemotherapeutic activities of both the standard, ara-A, and arazide were statistically ascertained using the H-test followed by the U-test ($p < 0.001$ for all three dosages of ara-A; $p < 0.05$ for 75, < 0.02 for 150 and 300 mg/kg of arazide).

§ Mean survival time of untreated controls was 9.35 ± 2.03 d. Experiments were terminated after 21 days. Animals surviving at this time were considered, for statistical calculations, to have died on day 23. This assumption does not alter the results of the experiment, but influences the means and deviation values.

** The dose dependence of increased survival time with arazide treatment can not be statistically ascertained.

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