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Effect of Cytosine Arabinoside, Iododeoxyuridine, Ethyldeoxyuridine, Thiocyanatodeoxyuridine, and Ribavirin on Tail Lesion Formation in Mice Infected with Vaccinia Virus (39241)

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Mice infected intravenously with vaccinia virus readily develop dermal lesions over the tail surface (1). The formation of these lesions is suppressed if the mice are treated with methisazone (marboran, 1-methylisatin-3-thiosemicarbazone) after virus inoculation (1) or with interferon or interferon inducers before virus challenge (2, 3). The pox tail lesion system has also been employed to assess the *in vivo* antiviral activity of branched-chain sugar nucleosides (2′-C-methyladenosine, 3′-C-methyladenosine) (4). The system itself is advantageous in that its sensitivity and reliability compare favorably to more severe testing procedures based on mortality.

Two deoxynucleoside analogs, 5-ethyl-2′-deoxyuridine (EtUdR) and 5-thiocyanato-2′-deoxyuridine (NCSUdR), whose *in vitro* antiviral activity has only recently been described (5, 6), were evaluated for inhibition of vaccinia tail lesions in the mouse. Several additional compounds were tested as reference materials: thymidine (TdR), ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, previously referred to as virazole), cytosine arabinoside (ara-C, cytosar, 1-β-D-arabinofuranosylcytosine), and 5-iodo-2′-deoxyuridine (IUdR, IDU). Ribavirin has been found effective against a wide variety of RNA and DNA viruses, both *in vitro* and *in vivo* (7, 8, and references cited therein). Ara-C and IUdR are particularly active against DNA viruses and, although both compounds have been evaluated in numerous cell culture and experimental animal systems (9, 10), only an occasional report (1) has appeared on the effects of ara-C and IUdR on dermal pox lesions in mice infected intravenously with vaccinia virus.

Materials and methods. Mice. Female NMRI mice, weighing 10–11 g were used throughout all experiments.

Virus. Calf lymph vaccine of *vaccinia virus* was provided by the Rijksentstofinrichting of Brussels (lot no. 7003/4). It titered 1.9×10^8 PFU/ml and was stored at 4°. Before use, the virus stock was diluted $1/10^4$ in phosphate buffered saline (PBS). Mice were inoculated intravenously (in a tail vein) with 0.2 ml of this virus dilution and tail lesions were counted 7 days later.

Compounds. Ara-C (cytosar) and IUdR (IDU) were obtained from Upjohn (Puurs, Belgium) and Ludeco (Brussels), respectively. Ribavirin was generously supplied by Cyanamid International (Pearl River, New York) through Cyanamid Benelux (Lederle Laboratories Division, Brussels). TdR was purchased from BDH Chemicals Ltd. (Poole, England). EtUdR and NCSUdR were synthesized as described previously (11, 12). All compounds were dissolved in PBS at the appropriate concentrations. These solutions were stored at 4° until used. They were administered intraperitoneally in 0.2-ml volumes.

Statistical significance of the results was assessed by Student's *t* test.

Results and discussion. At the treatment regimens employed (repeated daily injections started immediately after virus inoculation), ara-C was slightly more effective than IUdR in inhibiting vaccinia tail lesion formation (Table I). Both compounds caused a significant reduction in the number of tail lesions when administered at daily doses of either 4, 20, or 100 mg/kg, and the inhibitory effects obtained with ara-C and IUdR were equally dose-dependent.

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The beneficial effects obtained with ara-C and IUdR in the vaccinia tail lesion system (Table I) contrast with the lack of activity reported for ara-C and IUdR in mice infected with vaccinia virus by the intracerebral route (13). The latter system, based on mortality, may present too severe a challenge to the test compounds, especially if these compounds do not, as suspected, cross the blood brain barrier.

EtUdR, NCSUdR, and ribavirin, all active against vaccinia virus in cell culture (5, 6, 8), were also active in inhibiting the formation of vaccinia tail lesion in mice, when administered at daily doses of 20 mg/kg (Table I). At 4 mg/kg neither compound offered significant protection. At 100 mg/kg, NCSUdR was lethal for 100% of the mice, when given as a single intraperitoneal injection immediately after virus infection. EtUdR and ribavirin were not tested at 100 mg/kg. NCSUdR was clearly more effective in suppressing pox tail lesion formation than EtUdR. In cell culture, however, EtUdR proved definitely more active than NCSUdR in inhibiting both vaccinia virus growth and vaccinia virus-induced cytopathogenicity (5, 6).

Ribavirin effectively suppressed vaccinia tail lesion formation. Yet, ribavirin has previously been shown ineffective in reducing the mortality rate of mice inoculated intracerebrally with vaccinia virus (14). The failure of parenterally administered ribavirin to inhibit vaccinia encephalitis may be attributed to the inability of the compound to cross the blood brain barrier (14).

Although EtUdR was less effective than NCSUdR in inhibiting the development of vaccinia tail lesions, it compared favorably to TdR. At a dosage level (20 mg/kg) at which EtUdR reduced the number of pox lesions by 30%, TdR remained inactive, and, even at a fivefold higher dose, TdR did not cause a significant reduction in tail lesion number (Table I).

Attempts to improve the therapeutic effects of our compounds through drug combinations invariably failed. Ara-C and IUdR acted antagonistic when administered together at 20 and 100 mg/kg, respectively (Table II). They were also antagonistic when given at 4 and 100 mg/kg, respectively, and even at the lowest dosage levels (0.8 and 4 mg/kg, respectively) drug combination did not prove more efficacious than

TABLE 1. EFFECT OF REPEATED DOSES OF ARA-C, IUdR, EtUdR, NCSUdR, RIBAVIRIN AND TdR ON THE FORMATION OF VACCINIA TAIL LESIONS IN MICE.[a]

Compounds	Daily dose (mg/kg)	Number of mice	Number of lesions per mouse: Mean ± Standard deviation	Probability (compared to control group)
Ara-C	100	17	2.1 ± 2.7	$P < 0.001$
	20	20	3.1 ± 2.8	$P < 0.001$
	4	20	4.1 ± 2.4	$P < 0.01$
	0.8	10	6.0 ± 3.8	Nonsignificant
IUdR	100	20	2.8 ± 3.3	$P < 0.001$
	20	20	3.7 ± 4.2	$P < 0.005$
	4	20	4.7 ± 3.6	$P < 0.025$
	0.8	10	6.0 ± 3.3	Nonsignificant
Control		39	8.2 ± 6.0	–
EtUdR	20	20	6.6 ± 3.3	$P < 0.05$
	4	10	9.6 ± 1.9	Nonsignificant
NCSUdR	20	20	4.5 ± 3.8	$P < 0.001$
	4	20	7.1 ± 4.0	Nonsignificant
Ribavirin	20	20	5.4 ± 3.9	$P < 0.01$
	4	20	7.8 ± 4.0	Nonsignificant
Control		20	9.4 ± 4.5	–
TdR	100	20	6.4 ± 2.9	Nonsignificant
	20	20	7.9 ± 2.6	Nonsignificant
Control		20	8.1 ± 3.8	–

[a] Vaccinia virus was injected intravenously. All compounds were administered intraperitoneally, starting immediately after virus inoculation, and continued daily for 7 consecutive days.

TABLE II. EFFECT OF COMBINATIONS OF REPEATED DOSES OF ARA-C, IUdR, NCSUdR AND/OR RIBAVIRIN ON THE FORMATION OF VACCINIA TAIL LESIONS IN MICE.[a]

Compounds	Daily dose (mg/kg)	Number of mice	Number of lesions per mouse: Mean ± Standard deviation	Probability (compared to control group)
Ara-C	20	10	3.4 ± 1.5	$P < 0.001$
IUdR	100	10	3.0 ± 2.4	$P < 0.001$
Ara-C + IUdR	20 + 100	10	4.4 ± 2.3	$P < 0.005$
Ara-C	4	20	4.6 ± 2.1	$P < 0.001$
IUdR	20	20	4.7 ± 3.2	$P < 0.001$
Ara-C + IUdR	4 + 20	20	6.6 ± 2.7	$P \sim 0.05$
Ara-C	0.8	30	8.0 ± 3.8	Nonsignificant
IUdR	4	30	6.5 ± 2.6	$P < 0.02$
Ara-C + IUdR	0.8 + 4	20	7.5 ± 4.0	Nonsignificant
Control		40	8.7 ± 4.3	–
NCSUdR	4	30	7.7 ± 4.1	Nonsignificant
Ara-C	0.8	30	8.0 ± 3.8	Nonsignificant
IUdR	4	30	6.5 ± 2.5	$P < 0.005$
Ribavirin	4	10	7.2 ± 3.3	Nonsignificant
NCSUdR + Ara-C	4 + 0.8	20	7.7 ± 4.1	Nonsignificant
NCSUdR + IUdR	4 + 4	10	7.9 ± 3.2	Nonsignificant
NCSUdR + Ribavirin	4 + 4	10	7.8 ± 5.3	Nonsignificant
Control		30	9.3 ± 4.3	–

[a] Vaccinia virus was injected intravenously. All compounds were administered intraperitoneally starting immediately after virus inoculation and continued daily for 7 consecutive days.

either compound alone.

Similarly NCSUdR, administered at a dosage level (4 mg/kg) which did not afford significant protection, did not become more efficacious when combined with either ara-C, IUdR, or ribavirin (Table II). Other drug combinations (ribavirin + ara-C, ribavirin + IUdR) also failed to confer greater protection than that obtained with either compound separately (data not shown).

The data presented herein extend our *in vitro* observations on the antivaccinia activity of ara-C, IUdR, EtUdR, NCSUdR, and ribavirin (5, 6, 8) to the *in vivo* situation. When administered at 20 mg/kg daily for 7 consecutive days starting immediately after virus infection, all compounds brought about a significant reduction in the number of vaccinia tail lesions: ara-C (62% reduction), IUdR (55%), NCSUdR (52%), ribavirin (43%), and EtUdR (30%). Quantitatively, the reductions in pox counts obtained with ara-C, IUdR, etc., may seem less pronounced than those recorded previously with either interferon or interferon inducers (polyacrylic acid, *Brucella abortus*) (2, 3). It should be recognized, however, that, unlike ara-C, IUdR, EtUdR, NCSUdR, and ribavirin, which were active when administered after virus infection, interferon and its inducers must be given before infection in order to achieve a substantial reduction of pox lesions.

Summary. Mice infected intravenously with vaccinia virus develop characteristic lesions over the entire tail surface. This experimental virus infection presents a highly sensitive and reliable model for evaluating the antivaccinia activity of antiviral compounds. Ara-C (1-β-D-arabinofuranosylcytosine), ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), IUdR (5-iodo-2′-deoxyuridine) as well as two novel analogs of IUdR, EtUdR (5-ethyl-2′-deoxyuridine), and NCSUdR (5-thiocyanato-2′-deoxyuridine), were found to inhibit the formation of vaccinia tail lesions, when administered intraperitoneally once daily for 7 days starting

immediately after virus infection. The order of (decreasing) activity was: ara-C > IUdR > NCSUdR > ribavirin > EtUdR. Various drug combinations, involving IUdR + ara-C, NCSUdR + ara-C, NCSUdR + IUdR, NCSUdR + ribavirin, etc., were evaluated, but none proved more efficacious than either compound administered alone.

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