Antiviral Activity of Adenine Arabinoside and Iododeoxyuridine in Human Fetal Intestinal and Tracheal Organ Cultures

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In vitro antiviral activities of two potentially clinically useful antiviral compounds, adenine arabinoside and iododeoxyuridine, were examined in human fetal intestinal and tracheal organ cultures infected with *Herpesvirus hominis* (types 1 and 2) or vaccinia virus. The two compounds were similarly active against the viruses in organ culture, and minimal inhibitory concentrations could be determined by titration of organ culture fluid harvests into tissue culture or directly in the organ cultures themselves. Minimal inhibitory concentrations were consistently lower in tracheal than in intestinal organ cultures and were consistently higher for *H. hominis* type 2 than for *H. hominis* type 1. Thus the organ cultures are promising systems in which to evaluate antiviral activity against those agents that replicate in vitro only in organ culture, and they may have particular application to the study of herpetic tracheitis and esophagitis.

Organ cultures appear to provide a more sensitive medium for the cultivation of fastidious viral agents than do conventional tissue culture systems. Certain human gastrointestinal pathogenic viruses, including the recently described reovirus-like agents [1] and possibly the Norwalk agent [2], appear to replicate in vitro only in human intestinal organ culture. Similarly, certain strains of coronavirus and rhinovirus replicate only in human tracheal organ culture [3, 4]. In contrast to monolayer tissue cultures, the organ cultures maintain intact tissue organization and contain a variety of differentiated cells that preserve many in vivo metabolic functions [5]. Thus the organ cultures more closely resemble in vivo target organs for these viruses and may therefore represent a superior in vitro model for the study of viral pathogenesis and for the possible evaluation of antiviral mechanisms.

However, in vitro evaluation of antiviral drugs has generally been restricted to tissue culture systems [6, 7]. Two drugs that have been demonstrated to have antiviral activity in tissue

culture and that are currently being considered for clinical use in man are adenine arabinoside (ara-A) [8] and iododeoxyuridine (IUDR) [9]. The purpose of these studies was to evaluate the effect of these compounds on morphology, function, and viral replication in organ cultures. The increasingly frequent reports of herpetic tracheitis [10] and esophagogastritis [11] in patients with compromised host defenses suggested that investigation of *Herpesvirus hominis* infections of trachea and intestine would be clinically relevant. Therefore, human fetal intestinal and tracheal organ cultures were established and infected with *H. hominis* types 1 and 2 and with vaccinia virus; the effects of various concentrations of ara-A and IUDR were then examined.

Materials and Methods

Establishment of organ cultures. Human fetal intestinal and tracheal organ cultures were established as previously described [5] from 10- to 14-week-old embryos obtained from therapeutic abortions performed via hysterectomy or hysterotomy. Briefly, the intestine and trachea were dissected in toto, cut into 1-mm² sections, and placed on 50- × 15-mm plastic petri dishes that had been scratched with a scalpel. Five pieces were placed at equidistant points on each

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dish, and each piece was oriented under the dissecting microscope with the mucosal side up. Leibowitz (L-15) medium (Flow Laboratories, Rockville, Md.) (1.25 ml) was added, and was supplemented with a final concentration of 0.2% bovine albumin, 2.0 mM glutamine, 250 μ g of streptomycin/ml, and 33 μ g of tetracycline/ml. The dishes were placed on a rocker platform, which completed a full cycle eight times per min, and were incubated at 34 C. Ara-A and IUDR were added to the medium in the concentrations indicated below. Fluid was harvested every 48 hr, frozen at -70 C, and replaced with 1.25 ml of identical medium containing an antiviral drug.

Viruses. Three strains of *H. hominis* type 1 were isolated from human oral lesions in 1973, and three strains of *H. hominis* type 2 from human genital lesions in 1973. All strains were passed once in human diploid fibroblasts (WI-38) and were typed by immunofluorescence [12] (kindly performed by Dr. A. Nahmias). Vaccinia virus, strain IC, was provided by the Bureau of Biologics (Bethesda, Md.) and was passed twice in WI-38 cells. All viruses were titrated in WI-38 cells, and virus (100–150 TCID₅₀) was inoculated into each dish in a volume of 0.5 ml.

Antiviral drugs. 9- β -D-Arabinofuranosyladenine (ara-A) and 5-iodo-2'-deoxyuridine (IUDR) were obtained from P-L Biochemical, Milwaukee, Wis. Dilutions were made in L-15 medium in two- to 10-fold increments, with final concentrations ranging from 0.1 to 300 μ g/ml. Original concentrations of drug were added to each change of fluid.

Assessment of morphology. The organ cultures were examined daily under the dissecting microscope for cytoarchitectural changes. Intestinal organ cultures were evaluated for smooth muscle contractions, and tracheal organ cultures for respiratory ciliary motion. Every two days organ culture explants were fixed in formalin, and sections stained with hematoxylin and eosin were examined under the light microscope.

Viral assays and determination of MIC. Fluid harvests were titrated for viral growth in WI-38 tissue culture tubes, and titers of infectious virus were calculated by the method of Reed and Muench [13]. Inhibition of viral growth was defined as a $\geq$ 100-fold reduction of virus yield in the bathing medium. The MIC of each drug was

defined as the smallest concentration of drug in the medium that resulted in viral inhibition.

Results

Toxicity. Intestinal organ culture morphology and smooth muscle contractions were unaffected by concentrations of up to 350 μ g of ara-A/ml and up to 300 μ g of IUDR/ml. At 400 μ g of ara-A/ml and at 350 μ g of IUDR/ml, contractions were depressed and cytoarchitectural degeneration was seen. Ciliary motion in tracheal organ cultures was depressed with concentrations of 400 μ g of ara-A/ml and 350 μ g of IUDR/ml. Cytoarchitectural degeneration of the trachea was observed after doses of 500 μ g of ara-A/ml and 400 μ g of IUDR/ml.

Viral growth and histopathology. *H. hominis*. *H. hominis* types 1 and 2 grew to titers of $10^{4.0}$ – $10^{5.5}$ /TCID₅₀ per 0.1 ml of medium four to six days after inoculation in both intestinal and tracheal organ cultures (figures 1 and 2). In the intestinal organ cultures, suppression of smooth muscle contraction was noted by days 6–8, along with histopathologic disruption in the explants. The histopathology was observable directly through the dissecting microscope and consisted first of swelling ("ballooning") of the villi (figure 3), followed progressively by flattening of the villi, loss of the epithelial border, and frank destruction of the explant within 72 hr after the onset of "ballooning." Similar changes were noted by light microscopy of fixed intestinal explants (figure 4). The earliest findings were blunting of the villi with superficial ulceration of the epithelial cells, progressing to denudation of the entire epithelial surface (figure 4B). Eosinophilic intranuclear inclusions were seen within epithelial cells (figure 4C). No histopathologic differences were detected between explants infected with *H. hominis* type 1 and those infected with *H. hominis* type 2.

In tracheal organ cultures infected with *H. hominis*, loss of ciliary motion was noted by days 5 and 6 after inoculation, and histopathology was detected by day 6 or 7. The pathology was not observed under the dissecting microscope but was seen only by light microscopy of fixed sections. Early histopathology consisted of focal loss of epithelial cells, with progression to ulcer-

Figure 1. Growth of *Herpesvirus hominis* types 1 and 2 in human fetal intestinal organ culture in the presence of high (closed circles) and low (open circles) concentrations of adenine arabinoside (ara-A) and iododeoxyuridine (IUDR). Fluid was harvested every two to three days and assayed in tissue cultures. Values represent mean titer $\pm$ SD for a single strain.

ation of the entire epithelial surface within 72–96 hr after the onset of changes (figure 5A and 5B). Intranuclear inclusions were also seen within epithelial cells (figure 5C).

Vaccinia virus. Vaccinia virus grew to peak titers of $10^{4.5}$ TCID₅₀/0.1 ml in intestinal and tracheal organ cultures within eight to 10 days after inoculation (figure 6). In intestinal organ cultures, loss of contractions and histopathology were noted by day 10–12 after inoculation. Loss of ciliary motion and histopathology were seen in tracheal organ cultures by day 8–10. Pathology in both intestinal and tracheal organ cultures consisted primarily of ulcerations of the epithelial surface that were generally less extensive than

the ones seen with *H. hominis* infections (figures 4D and 5D).

Inhibition of viral growth. Growth of *H. hominis* type 1 was inhibited by concentrations of ≥ 10 μg of ara-A/ml in intestinal organ culture (figure 1A) and by ≥ 1 $\mu\text{g/ml}$ in tracheal organ culture (figure 2A). Concentrations of 75 μg of ara-A/ml inhibited *H. hominis* type 2 in intestine (figure 1B), while 50 $\mu\text{g/ml}$ inhibited this virus in trachea (figure 2B). IUDR inhibited *H. hominis* type 1 in concentrations of 5–12.5 $\mu\text{g/ml}$ in intestine (figure 1C) and 1 $\mu\text{g/ml}$ in trachea (figure 2C). *H. hominis* type 2 was inhibited by 37.5 $\mu\text{g/ml}$ in intestine (figure 1D) and by 25–37.5 $\mu\text{g/ml}$ in trachea (figure 2D). By day 4 no virus was de-

Figure 2. Growth of *Herpesvirus hominis* types 1 and 2 in human fetal tracheal organ culture in the presence of high (closed circles) and low (open circles) concentrations of adenine arabinoside (ara-A) and iododeoxyuridine (IUdR). Fluid was harvested every two to three days and assayed in tissue culture. Values represent mean titer $\pm$ SD for a single strain.

tectable in the medium of either system when the concentrations mentioned above were employed. The development of histopathology and the loss of contraction or ciliary motion were prevented when the above inhibitory concentrations were present. Similar MICs could be derived when the histopathology or loss of ciliary and contractile motion mentioned above were employed as indicators of viral growth.

MICs of ara-A against vaccinia virus were 5–10 $\mu\text{g/ml}$ in intestine (figure 6A) and 1 $\mu\text{g/ml}$ in trachea (figure 6C). MICs of IUdR against vaccinia virus were 5–12.5 $\mu\text{g/ml}$ in intestine (figure 6B) and 1 $\mu\text{g/ml}$ in trachea (figure 6D).

Concentrations lower than those indicated above appeared to have no effect on viral growth patterns; the growth curves were identical to those seen in the absence of drug.

Discussion

Both ara-A and IUdR appear to be relatively nontoxic in organ culture, since no effect is noted on explant morphology, on intestinal contraction, or on respiratory ciliary motion until high levels of drug (five to 400 times greater than the antiviral MICs) are reached in the bathing medium.

Figure 3. Human fetal intestinal organ culture photographed in situ (unfixed) through dissecting microscope. A, uninfected; B, six days after infection with *Herpesvirus hominis* type 1, with "ballooning" of villus ($\times 27$).

Ara-A and IUDR have been reported to be active in tissue culture systems against *H. hominis* [8, 15] and vaccinia virus [16, 17] and are clearly active against these viruses in organ cultures as well. The activities of ara-A and IUDR on a weight basis appear to be similar against each virus, although IUDR appears to be somewhat more active against *H. hominis* type 2 (table 1). The MICs of both drugs against each virus appear to be consistently higher in intestinal than in tracheal organ cultures (table 1). This differential sensitivity to antiviral activity appears to be independent of the peak titers of viral growth in the absence of drug, since the titers in intestinal culture are similar to those in tracheal culture. This individual sensitivity of target organs may play a role in the in vivo response of viral infections to antiviral compounds.

Isolates of *H. hominis* type 2 appear to be more resistant than type 1 isolates to the action of both ara-A and IUDR in both types of organ culture (table 1). This relative resistance has

been observed in most, but not all, of the tissue culture systems studied [14, 15, 18, 19].

The precise relationship of this type of MIC, which is calculated on the basis of extracellular concentrations in either tissue or organ culture, to the in vivo antiviral activity of these compounds is not established, since the action of these nucleoside analogues is entirely intracellular. In addition, the tissue culture values vary with the strain of virus, the type of cell, and the assay system employed [14, 15, 18]. In this laboratory, MICs for ara-A and IUDR against the same strains of virus in a variety of tissue culture systems are two- to 50-fold less than the above organ culture values (R. Dolin and M. Coker, unpublished observations).

The ability to obtain MICs directly from inhibition of histopathology and preservation of ciliary motion and contractions in the organ cultures bypasses the need for use of an additional assay system (i.e., tissue culture). These findings suggest that organ culture will be a suitable in

Figure 4. Light micrographs of sections of human fetal intestinal organ culture eight days after explantation and viral inoculation. A, uninoculated; B and C, inoculated with *Herpesvirus hominis* type 1 (arrow points to intranuclear inclusion); D, inoculated with vaccinia virus (hematoxylin and eosin; A, B, and D, $\times 350$; C, $\times 880$).

Figure 5. Light micrographs of sections of human fetal tracheal organ culture eight days after explantation and viral inoculation. A, uninoculated; B and C, inoculated with *Herpesvirus hominis* type 1 (arrow points to intranuclear inclusion); D, inoculated with vaccinia virus (hematoxylin and eosin; A, B, and D, $\times 350$; C, $\times 880$).

Figure 6. Growth of vaccinia virus in human fetal intestinal and tracheal organ cultures in the presence of high (closed circles) and low (open circles) concentrations of adenine arabinoside (ara-A) and iododeoxyuridine (IUdR). Fluid was harvested every two to three days and assayed in tissue culture. Values represent mean titer $\pm$ SD for a single strain.

Table 1. MICs of antiviral drugs in organ cultures.

Culture system	Drug*	<i>Herpesvirus hominis</i>		Vaccinia virus
		Type 1	Type 2	
Human fetal intestinal organ culture	Ara-A	10	75	5-10
	IUDR	5-12.5	37.5	5-12.5
Human tracheal organ culture	Ara-A	1	50	1
	IUDR	1	25-37.5	1

NOTE. MICs are expressed as range of concentrations of drug ($\mu\text{g/ml}$ in medium) required to decrease virus yield 100-fold. Three strains of each type of *H. hominis* were used.

* Ara-A = adenine arabinoside; IUdR = iododeoxyuridine.

vitro system in which to assay antiviral activity against viruses that replicate only in organ cultures, provided that a detectable end point exists in those cultures and that appropriate antiviral substances become available.

The similarity of the histopathology of tracheal and intestinal organ cultures infected with *H. hominis* to that of herpetic tracheitis [10] and esophagogastroenteritis [20] in patients with compromised host defenses suggests that the organ cultures may represent a promising in vitro model in which to study these entities. Since both ara-A (A. J. Glazko, personal communica-

tion) and IUDR [21] are poorly absorbed after oral administration, it is possible that topical administration of these nucleoside analogues may provide a local antiviral effect while bypassing potential systemic toxicity. Experiments with in vivo models of herpetic infections are underway to examine this possibility.

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