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A new in vivo anti-viral assay using microencapsulated infected cell cultures

X.Q. Li[1], E. Gorelik[2,3], R.W. Atchison[1], A. Ovejera[3] and M. Ho[1,2]

[1]*Department of Infectious Diseases and Microbiology, Graduate School of Public Health,* [2]*Department of Pathology, School of Medicine, University of Pittsburgh and* [3]*Pittsburgh Cancer Institute, Pittsburgh, PA 15261, U.S.A.*

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Summary

Microencapsulation technology makes it possible to encapsulate virus infected human or animal cells in microcapsules with semipermeable membranes. These may be implanted intraperitoneally into mice which may then be treated with antiviral drugs. The implanted microcapsules may be recovered at various intervals following in vivo treatment and the effect of the drug is evaluated by assaying the virus titers inside the microcapsules.

In this paper, the feasibility of this model was tested using microencapsulated human or non-human cells infected with herpes simplex virus type 1. The microcapsules were implanted in the peritoneal cavity of mice, and the effect of systematically administered acyclovir on HSV-1 replication was ascertained. We found that (a) HSV-1 can replicate in both human (A549 and FEMx) and non-human (Vero) cells after they are infected and encapsulated. (b) HSV-1 replication was inhibited by 0.005 μg/ml to 0.08 mg/ml of acyclovir in the medium when virus producing A549 cells were encapsulated or when they were in monolayers. (c) Acyclovir (20–80 mg/kg), injected twice daily by intraperitoneal, subcutaneous or intravenous routes in mice, significantly inhibited HSV-1 production in encapsulated Vero cells implanted in the peritoneal cavity.

The major advantage of this in vivo model is that it can be used to study antivirals in experimental animals in which viruses do not replicate in non-permissive animals. Toxicity, pharmacokinetic and efficacy data may be obtained. It can also be used to test drugs which require activation in vivo to be effective.

Microencapsulated anti-viral assay in mice; Herpes simplex virus; Acyclovir

Correspondence to: R.W. Atchison, Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, 439 Parran Hall, 130 DeSoto Street, Pittsburgh, PA 15261, U.S.A.

Introduction

Animal models are essential for the proper evaluation of a putative antiviral agent. This is no problem if the virus in question is one such as herpes simplex virus type-1 (HSV-1) which can infect a number of available experimental animals. Models for HSV in mice, rats, guinea pigs, rabbits and dogs have been developed (Collins, 1983; Schaeffer et al., 1978). However, many human viruses infect only humans or primates. Lack of an appropriate experimental animal model may substantially affect efficient screening of antiviral drugs. A suitable animal model is particularly needed at present for studying acquired immunodeficiency syndrome (AIDS), a fatal human disease caused by the narrowly tropic human immunodeficiency virus (HIV) (Mitsuya and Broder, 1987; Devita, 1987). HIV infects only humans and higher primates such as chimpanzees (Gibbs et al., 1986), which cannot be used for large scale screening of anti-HIV drugs.

Recently, using a new encapsulation technology (Encapcel™, Damon Biotech, Inc., Boston, MA), Gorelik et al. (1987) developed a new in vivo short-term assay for testing drugs against human tumors. Cells from malignant lines were encapsulated in microcapsules with semipermeable membranes and implanted into the mouse peritoneal cavity. Human tumor cells grew in microcapsules; some better in the mouse peritoneal cavity than in vitro. Antitumor drugs administered to mice were able to enter these microcapsules, inhibit the proliferation of tumor cells or kill them. The sensitivity of drugs at levels achievable in vivo was assessed. Compounds requiring in vivo metabolic activation could be tested and the test was applicable to many types of human tumor cells (Gorelik et al., 1987).

We postulate that a similar approach could be used for in vivo evaluation of antiviral drugs. First human or non-human cells are infected by a virus to be tested. They are then encapsulated in about 0.8 mm microcapsules which are subsequently implanted in the peritoneal cavity of mice. The semipermeable membranes of the microcapsules protect the cells inside from destruction by the host's immune mechanisms, but permit the free flow of essential nutrients and compounds less than 150000 daltons (Gorelik et al., 1987). Secondly, antiviral drugs can then be administered to implanted animals by intraperitoneal (i.p.), intravenous (i.v.) and subcutaneous (s.c.) routes. Microcapsules can be recovered periodically and virus replication could be evaluated.

We chose herpes simplex type-1 (HSV-1) and a known effective anti-HSV-1 drug acyclovir, to test the feasibility of this new antiviral model. Acyclovir has been studied extensively in mice and used clinically (Collins, 1983; Corey et al., 1983; Schaeffer et al., 1978). The purpose of this study was to determine whether HSV-1 infection of the microencapsulated human or monkey cells could be treated with acyclovir. To do this, we needed to know whether cells which support the growth of HSV-1 would survive after being encapsulated and implanted into the mouse peritoneal cavity; whether HSV-1 will replicate under such conditions in microencapsulated infected cells; and whether acyclovir can penetrate the semipermeable membrane of the microcapsules and inhibit the replication of HSV-1 in the capsules.

Materials and Methods

Experimental animals

Female CD-1 mice, 4–8 weeks old, 25–28 g each, were obtained from Charles River Laboratory (Wilmington, MA). Mice were maintained in a filter barrier facility and received feed and water ad libitum.

Cells and media

Vero cells, a continuous cell line of African green monkey kidney cells was obtained from the American Type Culture Collection (Rockville, MD) and cultured in Eagle's minimum essential medium (MEM, Hanks' salts; Gibco Grand Island, NY) supplemented with 25 mM *N*-2-hydroxy-ethylpiperazine-*n'*-2-ethane sulfonic acid (Hepes; Calbiochem-Behring, La Jolla, CA), 100 units penicillin G 100 ugm streptomycin sulfate per ml, and 8% heat-inactivated (56°C, 30 min) newborn calf serum (Gibco). Cell culture monolayers were grown at 37°C in 175 cm^2 Nunclon flasks. A549, a cell line of non-small cell human lung carcinoma was obtained from Dr. Sidney E. Grossberg of the Medical College of Wisconsin and cultured in Dulbecco minimum essential medium (DMEM; Gibco) supplemented as above.

FEMx cells, a line of human melanoma cell were obtained from the same source as in the study of microencapsulated tumor assay (Gorelik et al., 1987). HR1K and CEM, a human B and a T lymphoma cell lines were grown in RPMI 1640 medium (Gibco) similarly supplemented except for substitution of 8% heat-inactivated fetal calf serum (Gibco).

All cells were grown and maintained at 37°C until they were encapsulated.

Virus

HSV-1, strain F was obtained from B. Roizman (Chicago, IL). The virus pool used herein had a titer of 2.68×10^7 plaque-forming units (PFU) per ml in Vero cells.

Virus infection

Cells taken from routinely maintained stock culture were counted and grown in 25 ml of fresh culture medium overnight at 37°C. Medium was withdrawn from flasks and cells were infected with HSV-1 (0.1 PFU/cell; 10% of the cell were infected as given by the Poisson distribution) for 1 h at 37°C. They were then washed with 5 ml of medium 4 times and detached using 0.25% trypsin (Difco Lab, Detroit, MI) and 0.02% ethylene diamine-tetracetic acid (EDTA Fisher Scientific Co., Pittsburgh, PA) by incubating for 20 min at 37°C. Infected cells were collected by centrifuging at $600 \times g$ for 10 min.

Cell encapsulation

Encapsulation procedures were based on the Encapcel™ technology developed by Damon Biotech Inc., Boston, MA (U.S. Patent Number 4352883), as described by Gorelik et al. (1987). Cell encapsulation consists of 3 steps: (a) formation of spherical gel beads entrapping viable cells; (b) construction of semi-

permeable membranes around the gel beads and (c) liquefying and removing the interior gel to create the final microcapsules with suspended cells. A virus-infected or uninfected cell suspension was first mixed with 1.6% sodium alginate in a 1:4 ratio (v/v) to reach the final cell concentration of 2×10^6 cells/ml. This mixture was then passed through a jet-head droplet forming device into an isotonic solution (1.2%) of $CaCl_2$. The spherical microdroplets were washed with poly-1-ornithine (30–70 kDa; Sigma, St. Louis, MO) to form a semipermeable membrane on their surface and then with 55mM sodium citrate (Sigma) for liquefying the interior gel to allow sodium alginate to diffuse out of the capsules. Microcapsules with a thin semipermeable membrane containing viable cells in liquid suspension were thus formed. The quantity of microcapsules was expressed by the volume they occupied measured in ml in a pipet or centrifuge tube.

Implantation of microcapsules

Microcapsules were incubated at 37°C in medium appropriate for the cell line and with 10% serum and antibiotics for 1 h before inoculation into mice. The medium was removed and Hanks' balanced salt solution (HBSS, Gibco), containing the antibiotics as in MEM, was substituted and a 25% (v/v) suspension of microcapsules prepared. Two ml of this suspension were routinely inoculated in the peritoneal cavity of a mouse through a 16 gauge needle.

Antiviral drug

Acyclovir (Zovirax sterile powder; 9-[(2-hydroxy ethoxyl)methyl] guanine sodium) (Burroughs Wellcome Co., Research Triangle Park, NC) was purchased. The powder was stored at 4°C until used. A stock solution of 50 mg/ml in distilled water was made and stored at −70°C until used. Further dilutions were made in physiologic saline as needed.

Recovery of microcapsules and encapsulated cells

At different time periods after treatment, mice were killed by cervical dislocation. The peritoneal cavity of each mouse was washed with HBSS and floating microcapsules were aspirated using a large bore plastic pipet. Microcapsules were transferred to 15 ml tubes and washed 3 times with 0.9% saline to remove host peritoneal cells. The washed microcapsules were taken up into 1 ml serological pipettes, allowed to sediment, and the volume of packed microcapsules recovered from each mouse was determined. Microcapsules were transferred to a glass dounce homogenizer (7 ml volume; Wheaton Scientific, New York, NY) and disrupted with 3 strokes of an A-tolerance plunger. Cell suspensions were then filtered through a 150 μm nylon mesh to remove the disrupted membranes. Cells were centrifuged and pellets were resuspended in 0.5 ml of 0.9% saline. After mixing equal volumes of cell suspension and trypan blue, total and viable cell counts were determined using a leukocyte counting chamber. The number of total cells per 1 ml of packed microcapsules (a) was determined as follows:

$$a = b/c$$

where b = total number of cells in the suspension, c = total volume of microcapsules used to prepare the suspension.

Virus titration

For virus titration of encapsulated infected cells homogenate samples were prepared by the same method as above except that the harvested microcapsules were diluted 1:10 in HBSS before being homogenized. Ten-fold serial dilutions of the above were titrated for infectious HSV-1 by methyl cellulose plaque assay method using duplicate Vero cell monolayers grown in 4.5 cm^2 diameter wells in 12-well plastic cell culture plates (Gibco). One-tenth ml of virus dilution was adsorbed to cells for 1 h, then overlaid with MEM complete medium containing 1% methyl cellulose (Fisher Scientific Co., Pittsburgh, PA). After 5 days of incubation in a CO_2 incubator at 37°C, plate-well contents were stained with crystal violet for 10 min, then washed and dried for plaque counts and calculation of virus PFU/ml. Total virus from harvested microcapsule contents was determined after sonication (Heat System Ultrasonics, Farmingoale, NY) of 3 ml of the mixture of infected cells and supernatant for 5 min at 4–6°C prior to making serial 10-fold dilutions for virus plaque assay. Intracellular virus was determined by assay of the sonicate of infected cells centrifuged (600 × *g*) out of the harvested microcapsular contents at experimental times tested. Extracellular virus was determined by testing the supernatant fluid after infected cells were removed from microcapsule contents by centrifugation.

Cell counts were also performed by mixing 1 part of trypan blue and 9 parts of this 1/10 dilutions of cell suspension and counting as above. The cell concentration was used to express virus titers as PFU/cell.

Results

Virus retention in microcapsules

In order to evaluate the permeability of the microcapsule membrane to HSV, an experiment was set up to test whether virus produced by encapsulated cells could be found in the medium outside the microcapsules. Vero cells were infected with HSV-1 (0.1 PFU/cell) for 1 h, washed 3 times and encapsulated as described in Materials and Methods. Two 0.5 ml aliquots of these microcapsules were harvested immediately after encapsulation for cell counts and virus titration. At this time, there were 4.0×10^5 cells/ml of capsules and no titrable virus inside the microcapsules; 2 ml of these microcapsules were placed in 2 separate tubes and incubated in 10 ml of medium at 37°C. After two and three days, 0.5 ml microcapsules were harvested from each tube for cell counts and virus titrations. We found on these two days 3.02×10^2 and 2.05×10^2 PFU/cell inside the microcapsules. No virus was detected in the medium outside the microcapsules in either case. This result showed that virus did not escape from the capsules.

In vivo survival of encapsulated Vero cells

The following human and animal cell lines were tested for their ability to support HSV-1 replication: Vero, A549, FEMx, CEM (human T lymphoma cell) and HR1-K (human B lymphoma cell) cell line. The last three are non-adherent tumor cell lines which grew well encapsulated, but were found not to be consistent and effective producer of HSV-1 (data not shown). A549 and FEMx are monolayer tumor cell lines which were previously shown to grow in microcapsules (Gorelik et al., 1987) and which we use in HSV experiments in subsequent sections. Vero cells are a non-malignant monolayer cell line known to be optimal in supporting proliferation of HSV (Rawls, 1979).

The survival and proliferation of Vero cells inside of microcapsules were tested under in vivo and in vitro conditions. Vero cells were counted and mixed with the sodium alginate to reach the concentration of 2×10^6 cells/ml in 1.6% sodium alginate, then encapsulated. At this time, 3.6×10^5 cells were counted per 1 ml of microcapsules and 98% were viable. Aliquots of 0.5 ml of microcapsules were inoculated into the peritoneal cavity of each of three mice, and another aliquot was cultured in a test flask in 10 ml complete medium at 37°C. Three, five, and seven days thereafter, 0.5 aliquots of microcapsules were collected from the in vitro culture and capsules were harvested from one mouse each time. The viable cell counts and percent viability of cells on day 3 from capsules obtained from the mouse was 9.68×10^5/ml and 92%, respectively. This represented an increase from the original 3.6×10^5 cells/ml capsules. Thus there was evidence of cell multiplication with maintenance of viability. In contrast there were only 4.37×10^5/ml total cells in the in vitro cultured capsules, and viability was 7%. On day 7 the corresponding figures for cells from the microcapsules implanted in mice were 1.08×10^6/ml and 97% viability. The microcapsules cultured in vitro yielded only 0.04×10^5/ml cells and 1% viability. We conclude that encapsulated Vero cells survive satisfactorily in the peritoneal cavity but not in vitro.

TABLE 1

HSV-1 production by microencapsulated Vero cells in the mouse peritoneal cavity

Hours A.I.	Total cell $\times 10^5$/ml of MC	% Viability of cell	Intracellular Virus log PFU/cell	Extracellular Virus log PFU/cell	Total Virus log PFU/cell
0	11.8	86	N.V.	N.V.	N.V.
4	11.7	85	N.V.	N.V.	N.V.
8	11.7	79	−3.32	−3.13	−1.16
16	11.6	79	0.43	0.92	1.44
24	11.6	78	0.95	2.01	2.13
36	11.8	76	1.00	1.87	2.05
48	11.7	75	1.10	1.77	1.92

The titer represents log PFU per total cells assayed at times after inoculation (A.I.) of microcapsules containing cells infected 4 h previously. N.V. = no virus detected at 1:10 dilution of microcapsules. MC = microcapsule.

HSV-1 replication in encapsulated HSV-1 infected cells in mice

Vero cells were infected with HSV-1 at an input multiplicity of 0.1 PFU/cell for 1 hour at 37°C, then encapsulated; 2 ml of 25% suspension of capsules were implanted into the peritoneal cavity of 6 separate mice. For each time period indicated on Table 1, one mouse was killed and microcapsules were harvested. The virus yields expressed as intracellular, extracellular and total virus for each of the assayed time periods are presented. No virus was detected in the cells or in extracellular fluids taken at 0 and 4 hours after inoculation of capsules. Virus was first detected 8 h after inoculation of encapsulated cells, and reached its maximum total virus titers 28 h after infection of capsules or 24 h after implantation of cells.

Inhibition of HSV by acyclovir in encapsulated cells

Acyclovir is a guanosine nucleoside analogue of 225 daltons. Theoretically, it should pass through the membrane of the microcapsule which is permeable to molecules less than 150000 d (Gorelik et al., 1987). The ability of different doses of acyclovir to inhibit HSV-1 replication in encapsulated and non-encapsulated human A549 cells was compared. We chose this lung carcinoma cell line because its cells proliferate both in vivo and in vitro in microcapsules, as opposed to Vero cells which do not survive in vitro in microcapsules. A549 cells (3×10^7) were first infected with HSV-1 at input multiplicity of 1:10 as described in Materials and Methods. Two thirds (about 2×10^7) of the cells were encapsulated in 12 ml microcapsules, which were used to set up 10 separate cultures containing 1 ml microcapsules each. The remainder of the cells was used to make 10 separate flask cultures of

TABLE 2

Comparison of inhibition of HSV-1 production from encapsulated and unencapsulated A549 cells by acyclovir in vitro[a]

	Acyclovir dose	HSV-1 production (Log PFU/cell)	
		encapsulated cells	unencapsulated cells
1.	0.08 mg/ml	N.V.[b]	N.V.
2.	0.04 mg/ml	N.V.	N.V.
3.	0.02 mg/ml	N.V.	N.V.
4.	0.01 mg/ml	N.V.	N.V.
5.	2.5 μg/ml	N.V.	N.V.
6.	0.1 μg/ml	N.V.	N.V.
7.	0.05 μg/ml	−1.36	−1.08
8.	0.02 μg/ml	−0.47	−0.10
9.	0.005 μg/ml	0.93	0.65
10.	No drug	2.47	2.89

[a] A549 cells were infected with input multiplicity of HSV-1 at 0.1 PFU/cell. Encapsulated and unencapsulated cultures were set up as described in text. The final cell content in each culture was calculated to be 2×10^6 cells. The two types of cultures were then overlaid with medium containing 9 different concentrations of acyclovir or control.

[b] N.V. = no virus detected. The least amount of virus detectable in log PFU/cell was −3 or 1 PFU per 1000 cells.

non-encapsulated cells. The final calculated number of cells in both types of cell cultures was 1×10^6 in one ml. To each culture containing capsulated or uncapsulated cells, 9 ml of medium containing sufficient acyclovir to give the final concentrations indicated in Table 2 were added. After 24 h of incubation, samples were collected for assay of total virus. The results, expressed in HSV plaques produced per cell (Table 2), show inhibition of virus by acyclovir in a dose dependent manner from 0.005 μg/ml to 0.08 mg/ml in both types of cell cultures. No significant difference was found in virus production in encapsulated and non-encapsulated A549 cell cultures exposed to any of the different drug concentrations. A small dose, 0.005 μg/ml was inhibitory in both cases. The results indicate that acyclovir probably freely penetrates the membrane of the microcapsules.

Inhibition of HSV-1 by acyclovir in encapsulated cells in mice

In the next series of experiments, the effect of acyclovir on the replication of HSV-1 inside encapsulated Vero cells was evaluated in mice. Vero cells were infected in vitro with HSV-1 (0.1 PFU/cell) and infected cells were encapsulated and implanted i.p. into mice 4 hours after HSV-1 infection as described in Materials and Methods. Acyclovir was administered i.p. into the mice twice daily starting 0.5 hour before implantation of microcapsules. Three different doses of acyclovir (80, 40 and 20 mg/kg) in 0.2–0.5 ml were given to 4–5 mice in each dose group and 0.5 ml saline was given to controls. Microcapsules were harvested from mice 24 h after HSV-1 infection and the total virus titers of the capsular homogenate were determined. Table 3 shows a dose dependent reduction of HSV-1 production in encapsulated Vero cells. The differences in virus production between each drug treated group and controls were significant ($P < 0.001$).

An experiment was next performed to assess the effect of duration of administration as well as dosage of acyclovir on virus replication in mice bearing microcapsules with HSV-1 infected Vero cells. Vero cells were infected with HSV-1, en-

TABLE 3

The relationship between HSV-1 production from implanted encapsulated Vero cells and doses of acyclovir administered to mice

Acyclovir mg/kg[c]	Number of mice in each group	Mean log PFU/cell[a]	S.D.	*P*-values[b]
80 mg/kg	5	−1.47	0.25	<0.001
40 mg/kg	4	0.22	0.13	<0.001
20 mg/kg	4	0.65	0.23	<0.001
None	5	2.14	0.05	

[a] HSV-1 production 24 h after infection. Vero cells were infected in vitro with HSV-1 (MOI=0.1 PFU/cell), encapsulated and implanted i.p. into CD-1 mice 4 h after HSV-1 infection.

[b] *P*-values are based on comparison between titers in controls which received no drug and each dose group by Student *t*-test.

[c] Acyclovir was administered i.p. to mice every 12 h starting on 0.5 h before implantation of microcapsules. Microcapsules were harvested from mice 24 hours after HSV-1 infection.

S.D. = standard deviation.

capsulated and implanted as above into 18 mice. The mice were treated with 5 different doses (2.5 to 80 mg/kg) of acyclovir, or saline controls twice a day for 1, 2 or 3 days i.p. One mouse was killed for each of the 18 different treatment time interval points shown in Fig. 1. The figure shows a dose dependent reduction of HSV-1 production by acyclovir. There was a similar degree of virus reduction irrespective of whether the microcapsules were collected on day 1, day 2 or day 3 of treatment using analysis of variance (ANOVAR) ($P > 0.05$). Hence virus titration after one day of acyclovir treatment was sufficient to assess its effect.

Effect of different routes of acyclovir administration

Intraperitoneal injections are not common practice and they may result in artifactually high local concentrations of drug in the peritoneal cavity where the microcapsules are implanted. Thus, the anti-HSV-1 effects of acyclovir administered to mice by the i.p., i.v. and s.c. routes were compared. Procedures for Vero cell infection with HSV-1, encapsulation and implantation were done as described. Two doses of acyclovir were given by i.p., i.v. and s.c. routes 12 h apart. Five different doses ranging from 80 to 2.5 mg/kg are shown in Fig. 2 starting 0.5 h before implantation of microcapsules into mice. One mouse was killed for each dose and each route for which acyclovir was given. Microcapsules were harvested after 1 day of treatment and titers of HSV-1 from encapsulated Vero cells were determined. As shown previously for i.p. administration, a dose dependent anti-HSV-1 effect of acyclovir also shown for s.c. and i.v. administration. No significant differences in the antiviral effects of acyclovir were found regardless of the routes of admin-

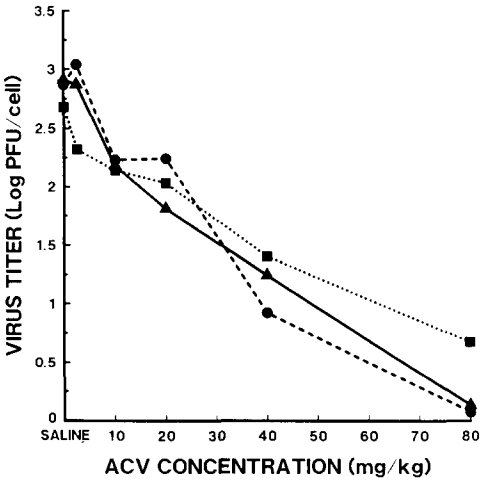

Fig. 1. Dose-dependent inhibition of HSV-1 production from infected Vero cells by acyclovir (ACV) 1, 2 and 3 days post infection. Vero cells were infected in vitro with HSV-1, encapsulated, then implanted i.p. into CD-1 mice. Acyclovir (2.5, 10, 20, 40 and 80 mg/kg) was given to mice i.p. every 12 h for 3 days starting at 0.5 hours before microcapsule implantation. Microcapsules were harvested from mice up to 3 days after inoculation of capsules into mice and cells were recovered and assayed for HSV-1 by plaque titration assay (▲, day 1; ●, day 2; ■, day 3).

istration by using analysis of variance (ANOVAR) ($P > 0.05$). It should be pointed out however that when different lots of infected cells were encapsulated and implanted, there may be quite a bit of variation between experimental groups even if one method of acyclovir administration was used. This may be seen by comparing the 80/μg/kg i.p. categories in Figs. 1 and 2.

Anti-viral effect of acyclovir using HSV-1 infected human cells

Although in vivo experiments were performed using HSV-1 infected monkey (Vero) cells, it was of interest to use human cells for in vivo evaluation of the antiviral effects of acyclovir. Two human cell lines of malignant origin (A549 and FEMx) previously shown to grow in implanted microcapsules in the peritoneal cavity (Gorelik et al., 1987) were tested. Monolayers of each cell line were first infected with HSV-1 at input multiplicities of 0.1, trypsinized and encapsulated as described above for Vero cells. Two mice were implanted with either infected A549 or FEMx cells for each of the dosages of acyclovir represented in Fig. 3 ranging from 2.5 to 80 mg/kg. They received four doses i.p. at 12 h intervals. Forty-eight hours after implantation, the mice were sacrificed and the capsules were harvested for virus titration. The mean total virus titers expressed as log PFU/cell were plotted in Fig. 3. In parallel with in vitro data virus production was less efficient and lower in these two cell lines than in encapsulated Vero cells (see Table 1), but of the two, A549 cells were better than FEMx cells. Acyclovir inhibited virus production in these cells in a dose-dependent manner, although the reduction is less apparent than in Vero cells.

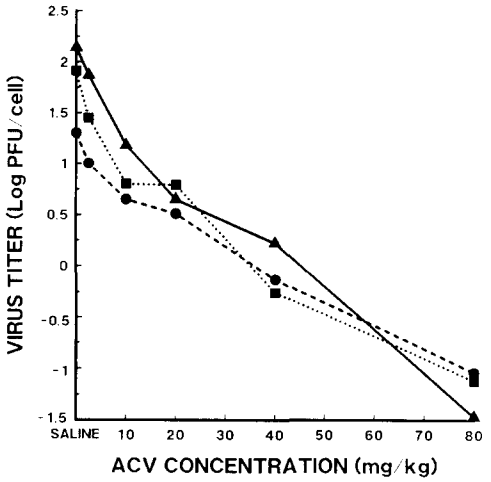

Fig. 2. Effect of routes of acyclovir administration or HSV-1 production from implanted encapsulated Vero cells. Cells were infected in vitro with HSV-1, encapsulated and implanted i.p. into CD-1 mice. Two doses of acyclovir (2.5, 10, 20, 40 and 80 mg/kg) were administered by 3 routes (▲, i.p.; ●, s.c.; ■, i.v.). Microcapsules were harvested from mice 24 h after implantation and cells were recovered and assayed for HSV-1 by plaque titration assay.

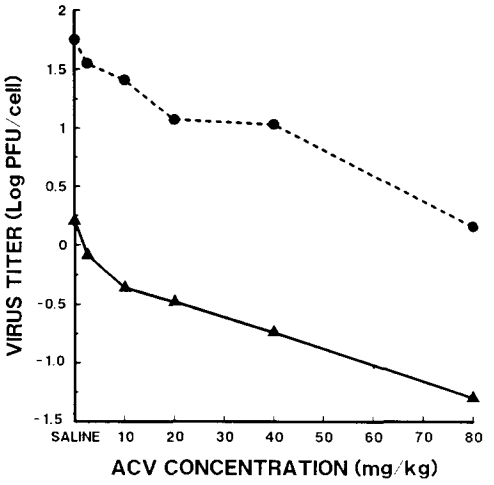

Fig. 3. Effect of acyclovir on HSV-1 production from implanted encapsulated A549 and FEMx cells. A549 (●) and FEMx cells (▲) were infected with HSV-1 in vitro with input multiplicity of 0.1 PFU/cell, encapsulated and implanted i.p. Acyclovir was administered i.p. and samples were collected for virus titration as in Figs. 1 and 2.

Discussion

By using the microencapsulation technique, we have developed an in vivo model for evaluation of the effect of the antiviral-drug, acyclovir, on the replication of HSV-1. We found that non-malignant monolayer cell cultures, such as Vero cells did not grow well in microencapsules in vitro. But malignant cell lines (A549 and FEMx) from a human carcinoma and melanoma proliferated well inside microcapsules in vivo and in vitro (Gorelik et al., 1987). As opposed to the latter lines, Vero cells are diploid and attachment dependent (American Type Culture Collection, 1981). However, microencapsulated Vero cells survived and proliferated in the peritoneal cavity. This is consistent with the observation by Gorelik et al. (1987) that encapsulated human tumor cells often grow better in the mouse peritoneal cavity than in vitro. It is possible that the peritoneal cavity is a better source of nutrients and growth factors or it permits removal of toxic metabolic products.

Implanted microencapsulated HSV-1 infected Vero cells provided excellent conditions for virus replication making the development of an anti-HSV model in vivo feasible. Cells from the human cell lines, A549 and FEMx, could also be infected with HSV-1, encapsulated and inoculated into the mice. But virus production was not optimal. The maximum growth of HSV-1 appeared 24 h after the microencapsulated virus infected Vero cells were implanted into mice or 28 h after Vero cells were infected with HSV-1. Titers of the virus did not change after an additional 48 h of culture.

We chose a known effective antiviral drug (acyclovir) to test the feasibility of the microencapsulation virus infected cells technique for the evaluation of antiviral drugs. Collins (1983) collected data from six different laboratories which showed

that acyclovir inhibited HSV-1 production in Vero cells in doses (ID_{50}) ranging from 0.02 to 1.8 μM (0.004 and 0.396 μg/ml). The mean ID_{50} was 0.18 μM (0.039 μg/ml). These results are consistent with our lowest inhibitory doses (Table 2). Our results also showed that the semipermeable membranes of microcapsules allowed small molecules such as acyclovir (225 d) to pass through but retained virus particles within the capsules. In culture flasks, acyclovir in medium outside the capsules inhibited HSV-1 production by encapsulated cells as well as by non-encapsulated A549 cells. In mice, HSV-1 production in encapsulated cells was inhibited by 20–80 mg/kg of acyclovir. This is consistent with the inhibition of HSV-1 titers in mouse brain by acyclovir (Park et al., 1979). Virus inhibition was about the same regardless of the routes of drug administration.

The effect of acyclovir on HSV-1 could be evaluated one day after implantation of the capsules and administration of the drug. The time required will obviously vary with the characteristics of growth of the virus and the properties of the drug to be tested.

As with any other experimental model, the microencapsulation antiviral assay has problems as well as limitations. We do not as yet have rigorous data on any diffusion gradient which the capsular membrane may create. This gradient may also vary with the molecular size of the drug tested, and possibly other properties. Separate studies should be done for each drug for their penetration of microcapsules. The effect of a putative antiviral on the viral disease process cannot be tested since the virus is restricted within the capsules. The level of drug attained inside the capsules may or may not reflect tissue levels. Nevertheless, the assay described herein should be useful for screening antiviral drugs for preclinical evaluation. The efficacy and toxicity of any antiviral drug may be obtained once the proper cell-virus system is encapsulated and implanted. A therapeutic index may be obtained. Drugs to be tested may act directly or may require metabolic modification or activation. Acyclovir is an example of such a drug since it must be phosphorylated in cells in order to be active (Schaeffer et al., 1978). The potential application of this method for screening anti-HIV drugs is under current investigation.

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