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Antiviral Activity of Carbobenzoxy Di- and Tripeptides on Measles Virus

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A series of simple carbobenzoxy peptides showed high and consistent antiviral chemotherapeutic activity in cell culture. In general, greatest activity was found against the measles-distemper or herpesvirus groups, or both, but various representatives of the series had quantitatively and qualitatively different antiviral activities. Several of the compounds, showing the highest antimeasles activity, were investigated extensively. In human cell culture plaque assays, these compounds were active against measles virus at levels of from 15 to 500 μg/ml. At single doses of about 250 to 500 mg/kg, orally in three animal species, significant serum levels of drugs were detected in virus cell culture assays. The mode of action appeared to be therapeutic, as an effect was seen in cell systems infected for at least 24 hr before treatment.

In recent years, antiviral chemotherapy has been given considerable impetus as a result of the demonstration of the chemotherapeutic effectiveness of at least two antiviral drugs; namely, 2'-deoxy-5-iodouridine (IDU) and 1-methylisatin 3-thiosemicarbazone (ITC). IDU has shown activity against both experimental and clinical herpes keratitis infections (5, 6), and ITC has shown statistically significant activity against poxvirus infections in experimental animal systems and in man (1, 19).

Many different compounds and antibiotic preparations have shown antiviral activity against different viruses both in cell culture and in in vivo systems. However, virtually none has been found with good activity against measles virus (rubeola). An antibiotic produced by *Alternaria tenuis* and by a species of *Aspergillus*, tenuazonic acid, inhibits measles virus growth in cell culture (8, 17), and three other compounds, gliotixin (7), guanidine (20), and phlorizin (12), have reportedly shown antimeasles activity in cell culture. Phlorizin is a glucoside which, in experimental animals, blocks transport of glucose across cell membranes (21). We were unable to demonstrate the antimeasles activity of either gliotoxin (15) or guanidine (14), with our standard screening procedures. Another agent which has been reported to have an inhibitory effect against measles virus is 6-mercaptopurine (18). However, all of the drugs mentioned above have a relatively low chemotherapeutic index and their clinical usefulness as antimeasles agents is improbable.

In the course of testing synthetic chemicals for antiviral activity, several carbobenzoxy-(di- or tri-)peptides showed significant chemotherapeutic effect against a number of viruses in in vitro mammalian cell culture systems. Certain myxoviruses (particularly members of the measles subgroup) or the herpesviruses, or both, were inhibited in varying degrees by members of the series. The most extensive biological work has been performed with measles virus and three members of this chemical series. Most of the antimeasles virus results reported in this paper concern carbobenzoxy-L-phenylalanyl-nitro-L-arginine (SV-1772), carbobenzoxy - D- phenylalanyl - D - phenylalanine (SV-3936), and carbobenzoxy-D-phenylalanyl-L-phenylalanyl-nitro-L-arginine (SV-4814). However this report also includes results on SV-4364, carbobenzoxy - D - phenylalanyl - D -methionine; SV-4337, carbobenzoxy-D-phenylalanyl-D-methionine; SV-4339, carbobenzoxy-D-phenylalanyl - L - alanine; SV-4892, carbobenzoxy- D - phenylalanyl-*S*-benzyl-L-cysteine.

The Chemical Abstracts nomenclature for the above-mentioned peptides is as follows: SV-1772, *N*-benzyl ester of N^2-(*N*-carboxy-3-phenyl-L-alanyl)-N^5-(nitroamidino)-L-ornithine; SV-3936, *N*-benzyl ester of *N*-(*N*-carboxy-3-phenyl-D-alanyl) 3 phenyl-D-alanine; SV-4814, *N*-benzyl

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ester of N^2-[*N*-(*N* carboxy-3-phenyl-D-alanyl-3-phenyl - L - alanyl] - N^5 - (nitroamindino) - L - ornithine; SV-4364, *N*-benzyl ester of *N*-(*N*-carboxy - 3 - phenyl - D - alanyl) - D - methionine; SV-4337, *N*-benzyl ester of *N*-(*N*-carboxy-3-phenyl - D - alanyl) - L - methione; SV-4339, *N*-benzyl ester of *N*-(*N*-carboxy-3-phenyl-D-alanyl)-L-alanine; SV-4892, *N*-benzyl ester of 3-(benzylthio) - *N* - (*N* - carboxy - 3 - phenyl - D - alanyl)-L-alanine.

Studies relating structure to activity showed that the carbobenzoxy group was essential as an integral part of the molecule. These relationships and the chemistry of these compounds are discussed in another paper (11).

MATERIALS AND METHODS

The cell culture methods used in testing these compounds have been reported previously (4, 9, 16). The HEp-2 cells (10) used for measles virus studies were obtained in 1955 from Alice Moore of the Sloan Kettering Institute for Cancer Research and have been maintained by passage and frozen storage. They were propagated in modified fermentors (New Brunswick Scientific Co., New Brunswick, N.J.) with Basal Medium Eagles (BME; 3) containing 15% tryptose phosphate broth (TPB) and 10% bovine serum.

The Edmonston strain of measles virus, obtained in 1955 from John Enders of Harvard Medical School, was grown in HEp-2 cells in a fermentor or in stationary bottle cultures in BME supplemented with 10% calf serum. These preparations gave titers of 10^4 50% cell culture infective dose ($CCID_{50}$)/ml [the concentration of virus showing evidence of cytopathology in 50% of the test units, as calculated by the method of Reed-Muench (13)] or higher in tubes or plastic panels read microscopically for development of a typical measles virus cytopathic effect (CPE) and 10^4 to 10^5 plaque-forming units (PFU)/ml when assayed on HEp-2 cell monolayers, following the technique described below.

Plaque reduction assays were carried out in 4-oz (113.4 g) stoppered prescription bottles. HEp-2 cells were planted in the bottles at a concentration of 5×10^6 cells in 20 ml of BME supplemented with 10% calf serum and 10% TPB or with 5% fetal bovine serum. These cells were allowed to grow for 48 hr at 37 C with one complete medium change at 24 hr. The virus suspension was placed on the confluent monolayers and was allowed to adsorb for 1 hr; with no washing to remove unadsorbed virus, the drug concentrations were added to the culture and 30 min later the monolayers were overlaid with nutrient agar. This overlay procedure was described previously (9); in certain of these assays, however, the vital stain, 2 - (*p* - iodophenyl) - 4 - (*p* - nitro - phenyl) - 5 - phenyl-2H-tetrazolium chloride (INT), was used, at a concentration of 400 μg/ml, in place of neutral red in a secondary overlay added after about 11 days of incubation. The results of the plaque reduction assays are reported as the per cent plaque reduction, based on plaque counts of virus seeded but not drug-treated control cultures, caused by a given amount of the drug expressed as the final concentration (μg/ml) after overlay. Duplicate or triplicate bottles were used for each variable, and most assays were performed two or more times. In tests comparing compounds, those reported are simultaneous evaluations using the same control.

Plastic panel viral inhibition tests were performed as reported previously (16) for initial activity screening test. Viral activity ratings (4) indicative of antiviral effect were calculated. The peptides were generally prepared at 10 mg/ml in Hanks' Balanced Salt Solution (HBSS) for these screening tests. The stock preparation was diluted and added to the panel cup in a volume of 0.2 ml, thus giving a final drug concentration of 2.0 mg/ml as the "undiluted" or most concentrated level. Delayed drug addition studies on SV-1772 were performed by incubating two levels of measles virus (10 and 100 $CCID_{50}$) with HEp-2 cells at 37 C for various lengths of time (0 to 24 hr) with gentle agitation. At the end of each time period, samples of the virus-cell suspension were pipetted into plastic panels and selected concentrations of test drug were added. The panels were sealed with cellophane tape, incubated for 6 days at 37 C, and examined microscopically for CPE.

In one series of assays performed with SV-1772, we used the disc-zone agar diffusion system, following the general method previously reported for poliovirus testing (9). The system was modified by adding the measles virus to the cell cultures 24 hr before the filter disc containing the drug was applied to the agar overlay.

For secondary and expanded testing, drug solubility was increased by forming the sodium salt with added NaOH immediately prior to testing. Propylene glycol or 0.4% carboxymethyl cellulose (CMC) in saline were used as suspending agents for some animal work. Dimethyl sulfoxide (DMSO) was found to be an excellent solvent for some of these drugs and was used as indicated in these studies.

A procedure has been reported (2) for evaluating the probable in vivo potential of antiviral compounds by determining the serum level of the drug after parenteral or oral dosing. Three species of animals (rats, dogs, and monkeys) were used to evaluate the peptides in this manner. SV-1772 and SV-3936 were administered as the sodium salt. SV-4814 was administered orally either in gelatin capsules or suspended in CMC. SV-1772, SV-3936, and SV-4814 were tested in dogs in cooperation with W. A. Knapp of Morris Research Laboratories, because these drugs gave some indication of activity against distemper virus in embryonated eggs as well as measles in cell culture, and it was hoped that subsequent challenge studies with distemper might be feasible. Normal serum was obtained, and after one drug treatment the animals were bled at various time intervals. The serum specimens were tested against measles virus in cell culture by the plastic panel or the plaque reduction method. Some assays were performed by adding serum dilutions to virus-infected monolayers. Others were carried out by adding a mixture of virus and serum after combining for 1 hr at 4 C.

TABLE 1. *Antiviral spectrum of selected peptides*[a]

Antiviral activity[b]			Virus	Host
SV-1772	SV-3936	SV-4814		
			RNA	
			Myxovirus	
+	+	+	Measles (Edmonston)	HEp-2 cells[c]
±	±	±	Distemper (Onderstepoort)	Embryonated egg[d]
0	0	—	Influenza type A (PR8)	Mouse[e]
0	0	0	Influenza type A (PR8)	Embryonated egg
0	0	0	Influenza type A_2 (Asian)	Embryonated egg
0	0	0	Influenza type B (Great Lakes)	Embryonated egg
0	0	0	Influenza type A_2 (Asian)	MK cells[f]
0	0	0	Newcastle disease (Cal)	Embryonated egg
0	—	—	Rabies (CVS)	Mouse
—	±	—	Parainfluenza 1 (Sendai)	MK cells
0	0	0	Parainfluenza 3 (HA-1)	HEp-2 cells
—	±	±	Respiratory syncytial (Parrot)	HEp-2 cells
0	0	0	Rous sarcoma (Bryan)	Chick embryo cells[g]
0	0	0	Avian leukosis (RPL 12)	Chick embryo cells
0	—	0	Rubella (M-33)	RK-13 cells[h]
			Picornavirus	
0	0	0	Poliovirus type 2 (MEF_1)	HEp-2 cells
0	0	0	Coxsackie B_1 (Conn 5)	HEp-2 cells
0	0	0	ECHO-9 (Hill)	MK cells
			DNA	
			Herpesvirus	
+	—	0	Herpes simplex (HF)	HEp-2 cells
0	—	—	Herpes simiae B virus (Yale)	HEp-2 cells
			Poxvirus	
0	0	0	Vaccinia (Lederle CA)	HEp-2 cells
			Adenovirus	
0	—	—	Adenovirus type 3 (GB)	MK cells

[a] Highest final drug concentration in systems, 500 μg/ml.
[b] Symbols: + = active; ± = possible activity; 0 = inactivity; — = not tested.
[c] Fermentor or bottle line.
[d] Eleven day.
[e] ICR Swiss, 18 to 20 g.
[f] Primary monkey kidney cell.
[g] Three day.
[h] Established rabbit kidney cell.

Attempts were made to reverse, prevent, or modify, with several metabolites, the antimeasles activity of SV-1772 using the plastic panel procedure. Reversal agents used included mixtures of purines, pyrimidines, amino acids, and vitamins. In these studies, the following control cultures were employed: (i) cell controls, (ii) virus controls, (iii) reversal agent cytotoxicity controls, (iv) SV-1772 cytotoxicity controls, (v) SV-1772 reversal agent controls.

RESULTS

Cell culture and animal studies. The spectrum of virus activity reported in Table 1 for the three peptides includes tests in cell culture and in animals. Measles was the only virus highly and consistently sensitive to the members of the series chosen for extensive work-up (Table 1). Marginal activity against certain other myxoviruses and paramyxoviruses was occasionally seen. Other members of the series had greater activity against other myxoviruses or paramyxoviruses and many had good but selective activity against various members of the herpesvirus group.

The first member of the dipeptide series found to be a markedly effective inhibitor of measles virus CPE in cell culture was SV-1772. The results of two SV-1772 assays are given in Table 2; these results demonstrate the consistency of the tests and the high viral activity ratings found repeatedly with this drug, indicating definite activity at concentrations well below the cell toxic level.

Many of the peptides which were tested as part

TABLE 2. *Antimeasles activity of SV-1772 in plastic panel[a] antiviral chemotherapy screening tests*

Test[b]	Virus level ($CCID_{50}$)	Microscopic readings on cells[c] at various drug levels (μg/ml)						
		2,000	625	200	62.5	20	6.2	0
1	10	T	T	0	0	1	3	3
	100	T	T	0	1	4	4	4
2	10	T	0	0	1	2	3	3
	100	T	0	0	0	4	4	4

[a] *See* reference 16.

[b] Viral rating [based on effective therapeutic index; i.e., reduction in viral effect (CPE reading rated 1 to 4) correlated with drug toxicity and infecting virus dose] for test 1 = 4.4, for test 2 = 6.6. *See* reference 4.

[c] Symbols: T = toxic; 0 = no CPE; 1 to 4, graded CPE; 4, complete cell destruction.

TABLE 3. *Virus plaque reduction assay[a] results of peptides*

Drug	Virus[b]	Per cent plaque reduction at various drug levels (μg/ml)				
		250	78	25	7.8	2.5
SV-1772	M	100	100	90	41	0
	H	0	0	0	0	0[c]
SV-3936	M	98	98	99	84	27
	H[d]					
SV-4814[e]	M	100	100	100	94	66
	H[d]					
SV-4364	M	100	100	79	42	0
	H[d]					
SV-4337	M	97	93	78	40	0
	H	68	52	14	0	0
SV-4339	M	100	78	81	79	53
	H	82	65	60	52	26
SV-4892	M[f]					
	H	94	80	55	47	24

[a] *See* reference 4.

[b] M = measles virus; H = herpes simplex virus.

[c] Active in plastic panel test. *See* reference 16.

[d] Not tested in plaque reduction system. Negative in plastic panel test.

[e] SV-4814 was not tested simultaneously, but is included for comparison. Virus control for SV-4814 was 110 (PFU) per bottle in untreated cultures. The virus control for the other compounds was 85 PFU per bottle in untreated cultures.

[f] Not tested in plaque reduction system. Active in plastic panel test. *See* reference 16.

of the evaluation of the series of compounds showed excellent measles chemotherapeutic activity (minimal active concentration, 7.8 and 2.5 μg/ml); but on the basis of solubility, relative activity, and cost of synthesis, SV-1772 and SV-3936 were selected for further study. It was not until later that the tripeptide, SV-4814, was synthesized, found to be extremely active, and added to the measles study group. Several peptides active against measles in the panel test were compared by plaque reduction assays in order to select candidates for further measles study. Table 3 shows the plaque assay results of the three peptides (SV-1772, SV-3936, and SV-4814) finally selected for extensive study along with their antiherpes activity where tested. In addition, certain results with other members of the series are given to illustrate comparative herpes and measles activity. Only SV-1772 was completely soluble under these test conditions. The other peptides were used, at the higher concentrations, as suspensions. This difference, however, was of minor importance since SV-3936 showed greater antimeasles activity than SV-1772, even though it was incompletely soluble. As indicated above, SV-4814 was not available when this study was made. The results shown for SV-4814 are from an entirely comparable trial done later and are included in the table to indicate the higher potency of the tripeptide.

Results of direct measles plaque reduction assay comparisons of the three selected peptides (SV-1772, SV-3936, and SV-4814) are given in Table 4. To avoid the possible effect of the differing solubilities on relative effectiveness, each peptide was tested dissolved in DMSO and also partially solubilized with added NaOH. Suprisingly, only the drug which was almost completely soluble in the alkalinized buffer (SV-1772) showed evidence of significantly higher activity in DMSO (50 as compared with 156 μg/ml). Anitviral activity of the other two peptides did not increase when dissolved in DMSO prior to addition to cell cultures. DMSO alone did not inhibit development of measles plaques.

It should be noted that a threefold higher virus challenge level was used in the study reported in

TABLE 4. *Effect of peptides on antimeasles activity in plaque assay[a]*

Drug	Per cent viral plaque reduction[b] at drug level indicated					
	Concn (μg/ml) in 50% DMSO[c]			Concn (μg/ml) as sodium salt[d]		
	156	50	15.6	156	50	15.6
SV-1772.....	100	51	0	94	0	0
SV-3936.....	100	100	86	98	100	63
SV-4814.....	100	100	100	100	100	100

[a] Tested in solution in 50% DMSO or as the sodium salt in HBSS. *See* reference 4.

[b] Virus control: average 248 PFU.

[c] DMSO control: no plaque reduction.

[d] Only SV-1772 completely in solution in HBSS.

TABLE 5. *Inverse variation of chemotherapeutic activity of SV-3936 with three concentrations of measles virus*

Virus level[a]	Per cent plaque reduction with SV-3936 at various concentrations (μg/ml)			
	500	156	50	15.6
PFU				
61	92	80	51	23
142	98	58	28	4
190	93	57	12	8

[a] Plaque count in monolayers containing no drug. The per cent plaque reduction is based on this count. Average of two tests.

TABLE 6. *Effect of delayed addition of SV-1772 on measles virus in plastic panel test*[a]

Time of drug addition after exposure to measles virus	Virus rating[b]
hr	
0	3.1
0.25	3.2
0.5	3.3
1.0	3.1
2.0	2.9
3.0	1.2
6	1.3
12	1.1
24	0.47

[a] Drug at levels of 2,000 to 2 μg/ml were tested against 10 and 100 $CCID_{50}$ of measles virus at each time period. *See* reference 16.

[b] *See* reference 4.

Table 4 than was used in the test described in Table 3. Evidence of decrease in per cent plaque reduction with increase in number of inoculated virus particles has been observed by us in antiviral activity assays using the plaque reduction technique. An experiment was performed with three levels of virus to determine antiviral activity of SV-3936 under these conditions. The results in Table 5 show that, at levels below the concentration giving almost complete suppression of viral activity (500 μg/ml for SV-3936), the per cent plaque reduction varied inversely with the number of PFU seeded. Based on these studies, 60 PFU provides a more sensitive test for this particular drug.

The results of the delayed treatment studies with SV-1772 are given in Table 6. When HEp 2 cells were in contact with measles virus for up to 2 hr prior to addition of the drug, there was essentially no difference in the protective effect of the drug. When the drug was added to the culture 3 hr after the cells had been exposed to the virus, the protective effect (or virus inhibitory activity) of the drug was still quite apparent and the same degree of cell protection was seen even after 12 hr.

TABLE 7. *Normal metabolites and vitamins used in attempting to reverse the antimeasles activity of peptides*

Compounds	Final concn in test (μg/ml)
Purine mixture	
Adenine	15
Guanine	20
Xanthine	20
Hypoxanthine	20
Pyrimidine mixture	
Uracil	25
Thymine	25
Cytosine	25
Amino acids mixture (A)	
Norleucine	200
Glycine	100
L-Serine	100
L-Histidine	100
L-Alanine	100
L-Threonine	100
L-Leucine	100
L-Isoleucine	100
L-Sarcosine	100
L-Valine	100
L-Norvaline	100
L-2-Aminobutyric acid	100
Amino acids mixture (B)	
Aspartic acid	50
L-Glutamic acid	50
L-Glutamine	150
L-Lysine	50
L-Arginine	50
L-Ornithine	25
L-Citrulline	25
L-Cystine	25
L-Cysteine	50
L-Methionine	12.5
L-Homocysteine	12.5
Betaine	12.5
Amino acids mixture (C)	
L-Phenylalanine	25
L-Tyrosine	25
L-Proline	25
L-Hydroxyproline	25
L-Tryptophan	12.5
Vitamin mixture	
Ascorbic acid	50
Citrovorum factor	10
B_{12}	0.5
Biotin	1
Choline	1
Folic acid	1
Nicotinamide	1
Pantothenic acid	1
Pyridoxal	1
Thiamine	1
Riboflavine	0.1
Inositol	4
L-Phenylalanine	50
L-Histidine	100
L-Phenylalanine and L-histidine combined	50 100
L-Ornithine	50
L-Phenylalanine and L-ornithine combined	50 50

TABLE 8. *Summary of animal tests with peptides versus measles virus to determine drug concentration in serum by plaque inhibition*

Compound	Animal	Route	Dose (mg/kg)	Results[a]
SV-1772 sodium salt	Rat[b]	Oral	2,000	Active at 2 and 18 hr
			1,000	Active at 2 and 18 hr
	Dog[c]	Oral	500	Inactive at 24, 72, and 168 hr
	Dog	Subcutaneous	250	Inactive at 1, 2, and 48 hr
SV-3936 sodium salt	Rat	Oral	1,000	Inactive at 0.25, 0.75, 1.5, and 3 hr
			500	Inactive at 0.25, 0.75, 1.5, and 3 hr
	Rat	Intraperitoneal	400	Active at 0.25 and 0.75 hr
			200	Active at 0.25 and 0.75 hr
	Dog	Oral	500	Active at 2–8 hr
	Monkey[d]	Oral	250	Active at 2–6 hr
		Intramuscular	125	Inactive at 2–6 hr
SV-4814	Rat	Oral (in CMC)[e]	1,000	Active at 0.5–3 hr
			250	Active at 0.5–3 hr
SV-4814 gelatin capsules (sodium salt)	Dog	Oral	500	Active at 2–8 hr

[a] Activity was based on per cent plaque reduction with serum from dosed animals compared with serum from nondosed animals.
[b] Holtzmann female, 200 g.
[c] Immature beagles, 7 to 9 kg.
[d] Rhesus, 2 to 4 kg.
[e] Carboxymethyl cellulose, 0.4%.

However, when the drug was added 24 hr after virus infection, the virus inhibitory effect was not significant by our arbitrary criterion for antiviral activity (4).

Paper-disc agar diffusion assay. With the disc zone test system on HEp #2 cell monolayers in Pyrex dishes, the peptide SV-1772 showed very good zones of virus inhibition. When 0.08 ml of the drug per disc was applied, zones of 40, 30, and 23 mm, in which the cells were protected from virus destruction, were observed for drug concentrations of 5, 1.5, and 0.5 mg/ml, respectively.

Attempts to reverse the antimeasles activity of SV-1772. The compounds used in attempting to reverse the antimeasles activity of SV-1772 are listed in Table 7 along with their respective concentrations (i.e., final concentrations in micrograms per milliliter in the test cultures). We used 55 compounds in this study. To minimize the work involved, the various purines, pyrimidines, amino acids, and vitamins were added as mixtures rather than individually. There was no apparent reversal or modification of antimeasles activity by any of the metabolites or combinations of the metabolites employed.

Animal serum level studies. At various times during our studies with the three peptides (SV-1772, SV-3936, and SV-4814), rats, dogs, and monkeys were used to determine whether effective drug serum levels for measles virus could be achieved in vivo with a single oral or intraperitoneal dose. Table 8 gives a summary of results of representative tests. Two of the three peptides (SV-3936 and SV-4814) showed measles virus inhibitory serum levels in one or more species after administration of doses ranging from 250 to 500 mg/kg. SV-1772 showed virus inhibitory levels only at 1,000 mg/kg when administered orally. Significant levels of drug activity against measles were not found in serum after subcutaneous or intramuscular administration of tolerated doses.

Tables 9 and 10 give detailed results of animal serum level tests of SV-3936 in rats (intraperitoneal) and in dogs (oral). All of these assays showed some degree of antimeasles virus activity by serum from drug-treated animals. The control tests of normal serum collected just prior to treatment with the test compound frequently showed some nonspecific plaque reduction at the very high serum concentrations required. Evidence of measles virus plaque reduction, over the base line levels, was generally apparent, particularly with undiluted serum, and there was less antimeasles activity at higher dilutions. Antimeasles activity 26 to 63% greater than that in control serum was seen in serum of rats treated intraperitoneally with 400 mg of SV-3936 per kg and bled at 15 min (Table 9). The results shown in Table 10 indicated that there was detectable antiviral

activity in serum taken from dogs 2 to 4 hr after an oral dose of 500 mg/kg. Antimeasles activity could not be determined with enough precision to calculate the drug concentration present in the serum by reference to the serum-drug mixture controls.

DISCUSSION

The consistent in vitro antimeasles activity and the high chemotherapeutic index of the peptides indicate that they are extremely interesting antiviral agents. They appear to possess true chemotherapeutic activity in cell culture, since they are quite effective in systems in which the cells have been infected up to 24 hr prior to treatment. One of the observations which makes this class of compounds unique is that certain members of this series are active against both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) viruses. The delayed treatment experiments suggest that the drugs interfere with the replicative process and probably do not inhibit penetration of the cell or exert direct virucidal action. We have determined that essentially all measles virus infective units are adsorbed to cells by 1 hr; therefore, from this period on, the drug is acting on virus replication within the infected cells or is blocking secondary or subsequent generation of cell infections. The latter possibility seems less likely since the degree of antiviral effect was constant whether the drug was added before or any time up to 2 hr after the viral infection was initiated (Table 6). Additional studies on the mode of action are needed, as no reversal activity was seen in the studies performed. Possibly, certain simple dipeptides or tripeptides would reverse the activity of these drugs, but only a few of these substances are commercially available and have not been studied.

It is quite encouraging that detectable antimeasles drug levels were present in the serum of several species which had been treated with a single oral or intraperitoneal dose of drug which was well below the maximal tolerated dose. Thus, chemotherapeutic trials in higher animals could be undertaken with some assurance that active drug

TABLE 9. *Effect of SV-3936 sodium salt rat serum levels on measles virus after a single intraperitoneal dose*[a]

Intraperitoneal injection (mg/kg)	Time bled (min)	Avg per cent plaque reduction		
		Serum undiluted	Diluted 1:2	Diluted 1:4
200	15	34	42	17
200	45	56	27	28
400	15	83	60	26
Control rat serum		20	19	0
SV-3936 in rat serum[b]		100	70	47

[a] Virus controls averaged 112 PFU per culture.

[b] Drug (3.2 mg/ml) was added to whole normal rat blood prior to clotting. The blood was allowed to clot and the serum was separated by centrifugation.

TABLE 10. *Blood levels of SV-3936*[a] *in dogs (single oral dose, 500 mg/kg)*[b]

Bleeding time after drug treatment (hr)	Per cent plaque reduction at serum various dilutions																	
	Animal 1713			Animal 1715			Animal 1716			Animal 1722			Animal 1723			Animal 1725		
	Undiluted	1:2	1:4	Undiluted	1:2	1:4	Undiluted	1:2	1:4	Undiluted	1:2	1:4	Undiluted	1:2	1:4	Undiluted	1:2	1:4
0 (base line)[c]	14	21	23	10	16	10	20	15	0	0	0	0	12	21	6	20	26	15
1	24	0	0	10	21	10				23	0	0				45	0	0
2				36	2	12	48	20	24	45	0	0	34	8	13			
4	33	0	0							53	35	23	48	28	3	35	40	24
8	5	0	0	30	12	8	20	0	0							19	28	0
24	16	0	0				20	0	0	7	0	0	13	0	3			
48				1	3	9	0	0	0				15	0	0	5	0	0

[a] SV-3936 prepared in HBSS (high levels of drug tested simultaneously as positive control): 10 mg/ml = 100% plaque reduction; 5 mg/ml = 96% plaque reduction; 2.5 mg/ml = 96% plaque reduction.

[b] Serum sample versus measles virus assayed by plaque reduction method (*see* reference 4). Per cent plaque reduction of serum samples from treated animals were calculated with the base line count. Blanks in the table indicate that the particular animal was not bled at that time interval. Virus control, 117 PFU.

[c] Base line plaque counts were carried out on serum collected from each animal prior to drug treatment.

is absorbed and circulated through the animal at effective antiviral levels and for an appreciable period of time.

In spite of these encouraging findings, we have decided not to pursue further evaluation or clinical studies at this time. With the wide acceptance and reduced reactions of the further attenuated measles virus vaccines, the need for a chemotherapeutic agent to treat the natural disease or to control vaccine reactions has diminished. Moreover, the antimeasles activity reported here was more striking than the antiviral activity of other dipeptides, studied to date, against the paramyxoviruses and the herpesvirus group.

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