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Effects of Amphotericin B and Its Methyl Ester on the Antiviral Activity of Polyinosinic:Polycytidylic Acid

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Since the macrolide polyene antibiotics, amphotericin B (AmB) and its methyl ester (AmBME), augment interferon production by polyriboinosinic:polyribocytidylic acid [poly(I):poly(C)] in vitro, experiments were undertaken to determine how AmB and AmBME affect the antiviral activity of poly(I):poly(C) and interferon. AmBME increased the direct antiviral activity of poly(I):poly(C) 10^2- to 10^4-fold in L929, Flow 6000, and T98G cells. Viral replication, measured by either direct plaque formation or virus yield, was markedly reduced. Serum interferon levels in mice induced by poly(I):poly(C) were enhanced by concomitant treatment with AmB. However, the therapeutic effects of poly(I):poly(C) in encephalomyocarditis and Semliki Forest virus infections were not augmented by combined treatment with poly(I):poly(C) and AmB. In vitro, the antiviral effects of exogenous interferon were markedly inhibited by AmB and AmBME. This inhibition may have contributed to the adverse effects of the macrolide polyenes in encephalomyocarditis and Semliki Forest virus infections in vivo. These findings further substantiate the effectiveness of macrolide polyenes in augmenting cellular penetration of macromolecules. However, therapeutic application may be limited by the complex interactions which occur between compounds administered in vivo.

Interferon production by polyriboinosinic:polyribocytidylic acid [poly(I):poly(C)] is enhanced up to 100-fold when L929 cells are treated with macrolide polyene antibiotics such as amphotericin B (AmB) and nystatin (5, 7). Polyene antibiotics bind to sterols in both fungal and animal cell membranes and cause substantial changes in membrane permeability (1, 11, 13). Membrane alterations induced by polyenes enhance cell penetration by anionic compounds and potentiate intracellular action of metabolic inhibitors (1, 9, 15-17, 19). Studies of the mechanism of enhancement of interferon production suggest that the polyenes increase cell penetration of poly(I):poly(C) (5). This observation is further substantiated by the finding that amphotericin B methyl ester (AmBME) augments the infectivity of encephalomyocarditis virus ribonucleic acid for L929 cells (6) and simian virus 40 deoxyribonucleic acid for CV-1 cells (manuscript in preparation).

The following experiments were conducted to determine how AmB and AmBME affect the antiviral activity of poly(I):poly(C) and interferon in vitro and in vivo.

MATERIALS AND METHODS

Cells. L929 mouse fibroblasts (CCL 1, ATCC) were grown as monolayers in Eagle minimal essential medium (MEM) (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 50 μg of gentamicin per ml and 10% fetal calf serum (FCS) (GIBCO) in a 36°C humidified incubator containing 5% CO_2 in air. WI38-VA13 cells (CCL 75.1, ATCC), Flow 6000 human fetal tonsil cells (Flow Laboratories, Rockville, Md.), and T98G human astrocytoma cells (a gift from Gretchen Stein, Boulder, Colo.) were grown under identical conditions.

Viruses. Vesicular stomatitis virus (VSV) (Indiana serotype) was propagated in L929 cells by infection at a multiplicity of 0.01 and harvested from the supernatant after 24 h. Stock virus had a titer of 3×10^9 plaque-forming units per ml in L929 cells.

Semliki Forest (SLF) virus was propagated in suckling mouse brains. Stock preparations titered $10^{7.3}$ 50% lethal dose (LD_{50}) units upon subcutaneous inoculation of 0.1 ml of virus into adult mice.

Encephalomyocarditis (EMC) virus was propagated by infecting L929 cells using a multiplicity of 2 and harvesting the intracellular virus after 7 to 8 h by repeated freeze-thaw cycles on cells scraped from the sides of roller bottles. Stocks, clarified by centrifuging at $800 \times g$ and 0.45 μM filtration, had titers of 3×10^9 to 5×10^9 plaque-forming units per ml. Virus stocks were stored at −80°C in MEM with 2% FCS.

Mice Five- to eight-week-old male Swiss mice (Sch: ARS[ICR]$_f$) were obtained from Sprague Dawley, Madison, Wis. and given water and commercial rodent chow ad libitum.

Drugs. Poly(I):poly(C) for in vitro experiments was obtained from Miles Laboratories, Elkhart, Ind. Lot

no. 12, with a sedimentation coefficient ($S_{20,w}$) of 14, was dissolved in Dulbecco phosphate-buffered saline without calcium or magnesium. Poly(I) (PL Biochemicals, Milwaukee, Wis.) had an $S_{20,w}$ of 9, and polyriboxanthylic acid [poly(X)] (Sigma Chemical Co., St. Louis, Mo.) had an $S_{20,w}$ of 8.2. Poly(I):poly(C) (PL Biochemicals, Milwaukee, Wis.) for animal experiments was prepared as described above. AmB (E. R. Squibb and Sons, Princeton, N.J.) was the parenteral form of Fungizone, USP. AmBME (a gift from E. R. Squibb and Sons) was prepared by two methods. The latter drug was either ground in Earle balanced salt solution (EBSS) or dissolved in a minimal volume of 10 mM ascorbic acid in 5% dextrose and further diluted with EBSS. Both methods resulted in a partially solubilized suspension of AmBME and provided comparable experimental results. Both AmB and AmBME were freshly prepared, protected from light, and only used in serum-free solutions to prevent biological inactivation by proteins. Diethylaminoethyl-dextran (Sigma Chemical Co., St. Louis, Mo.) was combined with poly(I):poly(C) at room temperature for in vitro experiments. Mouse fibroblast interferon was produced in L929 cells induced with ultraviolet-inactivated Newcastle disease virus and partially purified by perchloric acid precipitation. It had a titer of 3×10^4 international reference units.

In vitro antiviral assays. Confluent replicate monolayers in 35-mm dishes (Costar, Cambridge, Mass.) were washed twice with EBSS and overlaid with the appropriate dilutions of drugs in an EBSS–Dulbecco phosphate-buffered saline mixture at pH 7.3 and 1 mM Ca^{2+}. After a 60-min incubation in 3% CO_2 at 36°C, the monolayers were washed three times and overlaid with MEM containing 2% FCS before being reincubated. After 24 h, the medium was removed, and about 30 plaque-forming units of VSV in 0.1 ml of MEM with 2% FCS were inoculated. After a 60-min incubation, an overlay of 1% agarose in MEM with 2% FCS was applied, and the trays were returned to the incubator. Plaques were counted after 36 to 48 h by adding a neutral red liquid overlay (100-μg/ml solution in EBSS).

Cytotoxic effects of the polyene antibiotics or poly(I):poly(C) were determined by serial cell counts after treatment. Both adherent (after trypsinization) and nonadherent cell densities were determined with a Coulter Counter, model B. Viability was assessed in triplicate monolayers by trypan blue exclusion and hemacytometer count.

Survival studies. Doses of poly(I):poly(C) and/or either AmB or AmBME suspended in a balanced salt solution (milligrams per kilogram of body weight) administered intraperitoneally to groups of 10 to 15 mice. Two or four LD_{50} of SLF or EMC virus in MEM were given subcutaneously or intraperitoneally, respectively. The viruses were administered concomitantly or up to 6 h after the drug, usually 2 h after treatment. Survival was recorded daily for 3 weeks.

Serum interferon levels and assay. Drugs, prepared as in the survival experiments, were administered intraperitoneally to groups of six mice. At 2, 6, or 16 h after treatment, the mice were lightly anesthetized with ether and bled from the brachial artery. The serum was titrated for interferon by a modification (8) of a colorimetric assay (10). Briefly, twofold dilutions of each interferon sample in MEM with 0.5% FCS were added in triplicate to confluent L929 cells. After 24 h at 37°C, interferon was aspirated, and the monolayers were washed with EBSS and infected with VSV (multiplicity of infection = 0.001). After 48 h, the media were aspirated and the cells were incubated with neutral red (33 μg/ml) in EBSS for 75 min at 37°C in 5% CO_2. Monolayers were then washed with EBSS, and the dye was eluted into a 0.1 M NaH_2PO_4–50% ethanol mixture. Eluted dye was measured at 540 nm. The sample titer was the reciprocal of that interferon dilution at which uptake of dye was 50% of controls. Interferon titer was standardized and expressed as international reference units by incorporation of a mouse fibroblast interferon laboratory standard into each assay and by repeated comparison of that with the international mouse interferon reference standard.

All results presented are representative of findings in two to four experiments.

RESULTS

Antiviral effects in vitro. In prior studies, enhancement of interferon production by poly(I):poly(C) was observed with several polyene antibiotics, including both the pure and deoxycholate preparations of AmB, the aqueous suspension of AmBME, and preparations of nystatin and filipin (5). An aqueous suspension of AmBME was prepared which had minimal toxicity for L929 cells and significantly enhanced interferon production. To determine whether AmBME would increase the direct antiviral effects of poly(I):poly(C), L929 cells were treated with these compounds both alone and in combination. A 50-μg amount of AmBME per ml did not alone affect VSV yield or plaque formation in L929 cells (Table 1). Poly(I):poly(C) alone (100 μg/ml) modestly decreased VSV yield and plaque formation. These reductions were markedly augmented by AmBME (Table 1). At AmBME concentrations as low as 10 μg/ml, the effect of poly(I):poly(C) on VSV plaque formation was potentiated 3,000-fold; at 50 μg/ml, the effect of poly(I):poly(C) was potentiated approximately 60,000-fold. Increasing the AmBME concentration beyond 50 μg/ml did not further augment the antiviral effects of poly(I):poly(C). The increased antiviral activity of poly(I):poly(C) resulting from diethylaminoethyl-dextran was further augmented by AmBME (Table 1). Optimal concentrations of AmBME (50 μg/ml) and diethylaminoethyl-dextran (500 μg/ml) enhanced the reduction in VSV plaques by poly(I):poly(C) to a similar degree (data not shown).

To determine whether AmBME would induce antiviral activity by single-strand ribonucleic acid, L929 cells were treated with 1, 3, 10, 30, and 100 μg of poly(I) or poly(X) per ml in com-

TABLE 1. *Antiviral effect of poly(I):poly(C) and AmBME in L929 cells*

Additions			VSV (plaques/dish) by plaque reduction assay	Virus yield (PFU/ml) by yield reduction assay[a]
Poly(I):poly(C) (μg/ml)	AmBME (μg/ml)	Diethylaminoethyl-dextran (μg/ml)		
0	0	0	33	1.3×10^5
0	50	0	31	3.4×10^5
100	0	0	17	2.2×10^4
10	0	0	27	1.3×10^5
0.1	10	0	0	—[b]
0.03	10	0	18	—
0.1	50	0	0	$<10^1$
0.01	50	0	4	6.0×10^4
0.003	50	0	36	—
0.001	50	0	34	6.4×10^5
0.1	0	50	0	—
0.03	0	50	29	—
0.01	50	50	0	—
0.003	50	50	30	—

[a] Supernatants from infected cells were collected for virus titration 11 h after VSV infection, and virus was titrated on L929 cells by plaque assay. PFU, plaque-forming units.

[b] —, Not tested.

bination with AmBME (50 μg/ml). No inhibition in VSV plaque formation resulted.

To ascertain the degree of selectivity for viral replication, the effect of poly(I):poly(C) and AmBME on L929 cell viability and growth was examined. Monolayers were treated with 20 μg of poly(I):poly(C) per ml and 10, 50, and 100 μg of AmBME per ml, both separately and combined. The number of detached cells in treated monolayers did not increase when compared with untreated monolayers. More than 99% of attached cells were viable after this treatment, as determined by trypan blue exclusion. Cell growth in monolayers was examined by comparing cell counts after treatment with poly(I):poly(C) at 0.01, 0.1, or 1 μg/ml and with AmBME at 50 μg/ml (Fig. 1). Neither drug by itself affected cell growth. A slight inhibition in cell proliferation was apparent with 0.01 μg of poly(I):poly(C) per ml and 50 μg of AmBME per ml. Increased concentrations of poly(I):poly(C) in the presence of AmBME resulted in up to fourfold inhibition of cell proliferation (Fig. 1). Thus, neither AmBME nor poly(I):poly(C) alone or in combination inhibited cell viability, but the combination resulted in inhibitory effects on cell proliferation.

The effect of AmBME in cells which are more sensitive to the antiviral effects of poly(I):poly(C) was then determined. T98G and Flow 6000 cells were treated with AmBME and the antiviral effects of poly(I):poly(C) were examined (Table 2). The antiviral activity of poly(I):poly(C) in both cell lines was augmented. In Flow 6000 cells, the enhancements by AmBME was up to 1,000-fold; in T98G cells, it was up to 100-fold. WI38VA13, a human cell line with a high membrane sterol content (12), was also tested. AmBME did not augment the antiviral effects of poly(I):poly(C) for VSV in this cell.

Interferon production in vivo. To further evaluate the enhancement in antiviral effects of poly(I):poly(C) by macrolide polyenes, interferon induction in mice was examined. Mice were injected with 0.25 or 2.5 mg of poly(I):poly(C) per kg and 0, 4, or 8 mg of AmB per kg. Serum was collected for interferon determinations at 2, 6, and 16 h after drug administration. Interferon production was enhanced up to 12-fold when the drugs were injected concomitantly. The degree of enhancement was dependent upon both poly(I):poly(C) and AmB dose; at the lower poly(I):poly(C) dose, interferon production was augmented by AmB to a greater degree (Fig. 2A and 2B). In the presence of AmB, interferon levels tended to be slightly less at 2 h after drug administration, and slightly greater at 16 h than the corresponding levels in mice that did not receive AmB (Fig. 2A). When AmB was given 24, 6, or 2 h before poly(I):poly(C), no enhancement in interferon production occurred (data not shown).

Antiviral effects in vivo. AmB or AmBME enhanced the direct antiviral effects of poly(I):

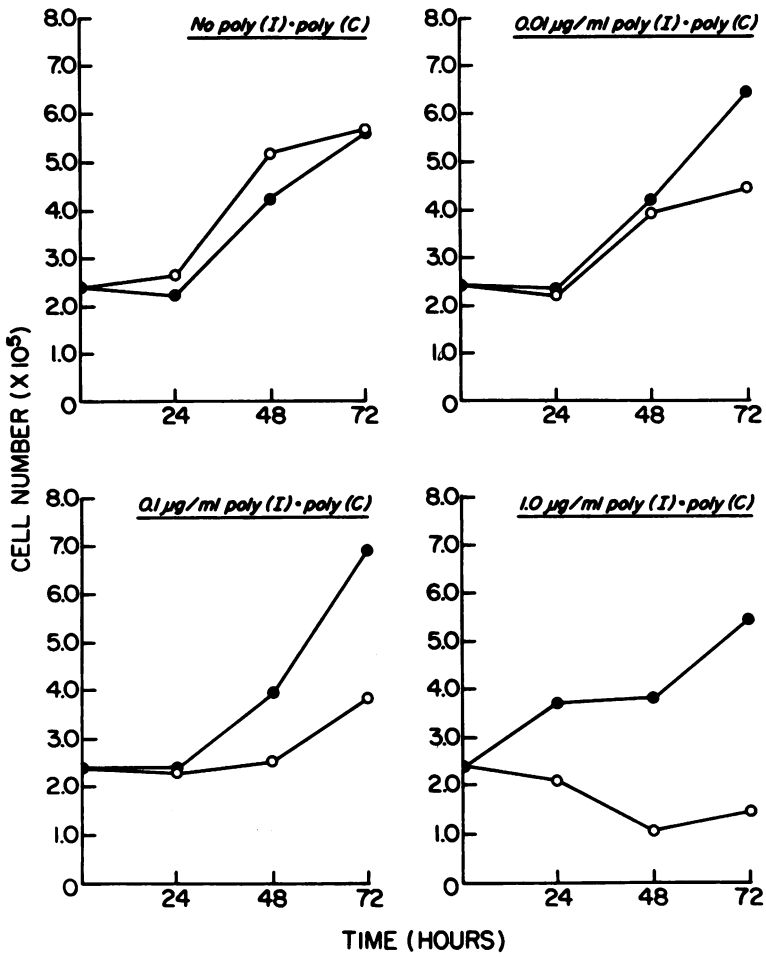

FIG. 1. *Effect of poly(I):poly(C) and AmBME on L929 cell growth. Replicate L929 monolayers were treated at time zero with poly(I):poly(C), or AmBME, or both. After 1 h, cells were washed twice and overlaid with MEM with 2% FCS. Individual cell suspensions from triplicate wells were prepared by trypsinization; each suspension was counted three times. Each graph depicts mean cell count at 24-h intervals at given poly(I):poly(C) concentrations and with no* (●) *and 50 μg* (○) *of AmBME per ml.*

poly(C) in L929 mouse fibroblasts and also enhanced interferon production by poly(I):poly(C) in L929 cells and in mice. Therefore, experiments were undertaken to evaluate the effect of these macrolide polyenes on the antiviral activity of poly(I):poly(C) for SLF and EMC virus infections in mice.

Initial experiments were conducted with poly(I):poly(C) and the macrolide polyenes, each given separately at various doses and schedules. Poly(I):poly(C) was given intraperitoneally in the range of 0.01 to 4.5 mg/kg at 1 or 2 h before small infecting doses of the viruses (2 to 6 LD_{50}). At the higher doses of poly(I):poly(C), all EMC virus-infected mice and up to 75% of SLF virus-infected mice survived. Later administration resulted in decreased effectiveness of poly(I):poly(C). For example, untreated mice infected with SLF virus had a median survival of 5.7 days, whereas 0.5 mg of poly(I):poly(C) per kg administered 1 h before infection with SLF virus resulted in a median survival of 15 days; the same dose given 2 h after infection resulted in a median survival of 8.5 days.

The influence of either AmB or AmBME alone on survival rates of infected mice was examined by using a range of doses and different time intervals of administration. AmB (4, 8, or 12 mg/kg), given 2 h before 2 LD_{50} of subcutaneous SLF virus, resulted in a modest, although not significant, decrease of survival (Fig. 3A). In other experiments, neither lower doses of AmB (0.5, 1, and 2 mg/kg) nor a range of doses of AmBME (10, 30, 60, and 90 mg/kg) ameliorated

TABLE 2. *Antiviral effect of poly(I):poly(C) and AmBME in F6000 and T98G cells*

Additions		VSV[a] (plaques/dish)	
Poly(I):poly(C) (μg/ml)	AmBME (μg/ml)	F6000 cells	T98G cells
0	0	103	26
0.03	0	—[b]	25
0.1	0	100	8
1	0	50	7
10	0	0	0
0	5	102	—
0.001	5	99	—
0.003	5	85	—
0.01	5	4	—
0.1	5	0	—
0	10	—	40
0.001	10	—	30
0.003	10	—	17
0.01	10	—	19
0.03	10	—	0
0.1	10	—	0
0	50	107	44
0.001	50	94	53
0.003	50	105	42
0.01	50	8	16
0.03	50	—	6
0.1	50	0	0

[a] Plaque reduction assay.
[b] —, Not tested.

the detrimental effects of the macrolide polyenes. Mean body weights of the untreated mice remained stable for the first several days after infection, whereas those receiving macrolide polyenes lost from 5 to 15% of their weight. Altering the dose schedule did not decrease the detrimental effects of AmB on survival rates of mice infected with EMC or SLF viruses. Mice treated with 4 mg of AmB per kg at various times before EMC virus survived significantly less time ($P < 0.01$) than untreated mice (Fig. 3B). Although survival rates of mice infected with EMC virus were more markedly affected by polyenes than those mice infected with SLF virus, altering the dose schedule of AmB did not decrease the adverse effects on SLF virus infection.

Combined treatment with AmB and poly(I):poly(C) did not augment the therapeutic effects of poly(I):poly(C) alone. When 4 mg of AmB per kg was given 2 h before SLF or EMC virus infection, the drug only modestly enhanced ($P < 0.1$) (Fig. 4A) or did not alter (Fig. 4B) the effects of suboptimal doses of poly(I):poly(C). No increase in systemic toxicity resulted from combined treatment. Similar results were obtained when the same doses of both drugs were

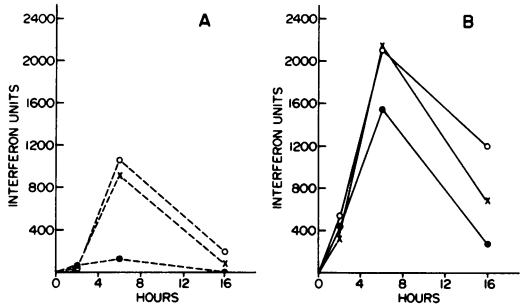

FIG. 2. *Serum interferon production in mice after poly(I):poly(C) and AmB. Groups of six mice were injected intraperitoneally with concomitant AmB and poly(I):poly(C). Serum was collected for interferon assay at indicated times thereafter. (A) poly (I): poly(C), 0.25 mg/kg (---); no AmB (●); AmB, 4 mg/kg (×); and AmB, 8 mg/kg (○). (B) Poly(I):poly(C), 2.5 mg/kg (——); no AmB (●); AmB, 4 mg/kg (×); and AmB, 8 mg/kg (○).*

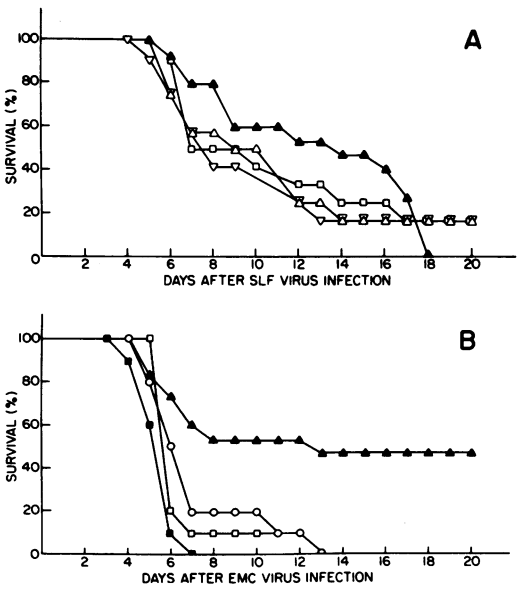

FIG. 3. *Survival periods of virus-infected mice treated with AmB. (A) Groups of 12 mice were treated with AmB intraperitoneally and infected 2 h later with SLF virus (2 50% lethal doses) subcutaneously. No AmB (▲); AmB, 4 mg/kg (△); AmB, 8 mg/kg (□); and AmB, 12 mg/kg (▽). (B) Groups of 10 mice were treated with AmB, 4 mg/kg, and infected at various times thereafter with EMC virus (2 LD_{50}) intraperitoneally. No AmB (▲); AmB concomitant with EMC virus (■); AmB 2 h before EMC virus (□); and AmB 6 h before EMC virus (○).*

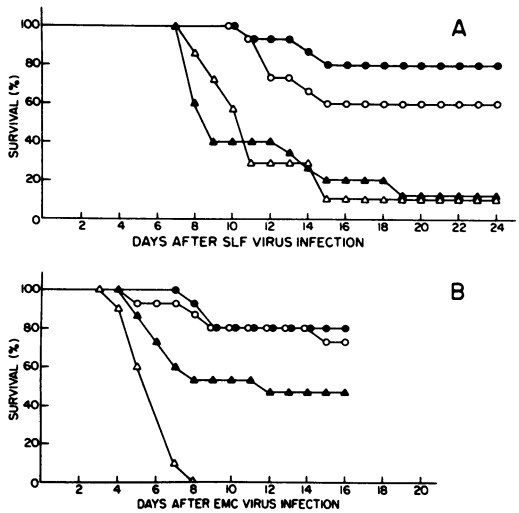

FIG. 4. *Survival periods of virus-infected mice treated with poly(I):poly(C) and AmB. (A) Groups of 15 mice were treated with AmB (4 mg/kg) and poly(I):poly(C) (0.4 mg/kg) intraperitoneally and infected 2 h later with SLF virus subcutaneously (2 LD_{50}). No treatment (▲); AmB only (△); poly(I):poly(C) only (○); and poly(I):poly(C) and AmB (●). (B) Groups of 15 mice were treated with AmB (4 mg/kg) and poly(I):poly(C) (0.04 mg/kg) intraperitoneally and infected 2 h later with EMC virus intraperitoneally (2 LD_{50}). No treatment (▲); AmB only (△); poly(I):poly(C) only (○); and poly(I):poly(C) and AmB (●).*

given concomitant with or 6 h before SLF and EMC viruses. Altering the dose of AmB (0.5, 1, 2, 8, or 12 mg/kg) or using AmBME (10, 30, 60, and 90 mg/kg) also failed to enhance the therapeutic effects of poly(I):poly(C).

Interferon action. Any alteration in the antiviral activity of poly(I):poly(C) by AmB and AmBME in vivo would probably be influenced by effects of these polyene antibiotics on interferon action. To determine these effects, L929 cells were treated with a range of concentrations of AmB 24 h before addition of twofold dilutions of a mouse fibroblast interferon preparation. Interferon was then titrated at each AmB concentration. At 1 and 2 μg of AmB per ml, a slight enhancement in antiviral activity of interferon was observed (Table 3). However, at higher AmB concentrations, the antiviral effects of interferon were markedly inhibited (Table 3). Similar inhibition was observed when AmBME at 20, 40, 80, and 160 μg/ml was used.

DISCUSSION

After treatment with AmBME at concentrations as low as 10 μg/ml, the antiviral effects of poly(I):poly(C) in L929, T98G, and Flow 6000 cells were augmented. These effects may have been mediated by increased interferon production or by augmentation in the direct effects of poly(I):poly(C) on viral macromolecular synthesis. However, effects of poly(I):poly(C) and AmBME in L929 cells were not completely selective for viral replication. Therefore, the antiviral effects may also have been partially mediated via secondary effects on cellular macromolecular synthesis. The enhancement by polyene antibiotics in the effects of biologically active, single- and double-stranded polyribonucleotides probably results from increased cell penetration (5, 6). The lack of development of antiviral activity by poly(I) and poly(X) in L929 cells treated with AmBME suggests that the absence of antiviral activity of these compounds does not result from failure of cell penetration.

Studies of membranes have demonstrated that fundamental structural alterations result from treatment with polyene antibiotics (1, 9, 11). On the basis of interferon binding to membrane gangliosides (2, 21), and the inhibition of interferon action by treatment of cells with anti-interferon globulin (22), it has been postulated that interferon action may be a membrane-mediated event. Inhibition of interferon action by membrane-active macrolide polyenes is consistent with this hypothesis.

Much of our work on the enhancing effects of AmB and its methyl ester on the biological activity of exogenous nucleic acids has focused on L929 cells (4–7). However, the antiviral activity of poly(I):poly(C) in Flow 6000 and T98G cells was augmented by AmBME, interferon induction by poly(I):poly(C) was increased by treatment with AmB in vivo, and infectivity of simian virus 40 virus deoxyribonucleic acid for CV-1 cells was enhanced by AmBME (manuscript in preparation). These findings demonstrate that augmentation of nucleic acid activity by macrolide polyenes is not unique to L929 cells. Furthermore, the biological effects of tetracycline, actinomycin D, and rifampin in 3T3, KB, and HeLa, in addition to L929 cells, was potentiated by AmB (15–17). Failure of AmBME to augment the antiviral activity of

TABLE 3. *AmB effect on interferon action*

AmB[a] (μg/ml)	Interferon titer[b] (IU)
0	26,000
1	39,000
2	37,000
5	10,000
10	9,500
25	2,800

[a] Added to L929 cells 25 h before interferon.

[b] Titer of a single interferon preparation in the presence of indicated AmB concentration.

poly(I):poly(C) for a cell with high membrane sterols (WI38-VA13) suggests that not all cells are sensitive to the synergistic interactions of polyribonucleotides and macrolide polyenes.

Survival of leukemic AKR mice treated with a combination of 1,3-bis(2-chloroethyl)-1-nitrosourea and AmB was augmented as compared to treatment with 1,3-bis(2-chloroethyl)-1-nitrosourea alone (18). Possible enhancement of the antitumor effects of adriamycin by AmB was recently reported in humans (20). These observations, together with the in vitro enhancement in direct antiviral activity of poly(I):poly(C) and the in vitro and in vivo enhancement in interferon production by AmB and AmBME, suggested that the macrolide polyenes might augment the antiviral activity of poly(I):poly(C) in vivo. The failure of these compounds to do this may have resulted, at least in part, from the increased pathogenicity of the infecting viruses after treatment with AmB and AmBME alone. The increased viral pathogenicity, which was particularly evident in EMC virus infection, may have been caused by systemic toxicity, inhibition of interferon action, or other undefined effects. The decreased survival rate was particularly surprising in view of the immunostimulatory effects of AmB (3, 14).

The observations reported herein expand the range of biological effects of exogenous nucleic acids which are enhanced by treatment of eucaryotic cells with AmB or AmBME. The macrolide polyenes may prove useful as experimental tools for augmenting cellular penetration of compounds or macromolecules (5, 6, 15–18) and for probing biological mechanisms (5, 19). However, therapeutic application may be limited by the complex effects which occur when biologically potent compounds interact in vivo.

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LITERATURE CITED

1. **Andreoli, T. C.** 1973. On the anatomy of amphotericin B-cholesterol pores in lipid bilayer membranes. Kidney Int. **4**:337–345.
2. **Besancon, F., H. Ankel, and S. Basu.** 1976. Specificity and reversibility of interferon ganglioside interaction. Nature (London) **259**:576–578.
3. **Blanke, T. J., J. R. Little, S. F. Shirley, and R. G. Lynch.** 1977. Augmentation of murine immune responses by amphotericin B. Cell. Immunol. **33**:180–190.
4. **Borden, E. C.** 1978. Synergistic cytotoxicity for L929 cells of polyriboinosinic-polyribocytidylic acid and amphotericin B methyl ester. J. Natl. Cancer Inst. **60**:1309–1311.
5. **Borden, E. C., B. W. Booth, and P. H. Leonhardt.** 1978. Mechanistic studies of polyene enhancement of interferon production by polyriboinosinic-polyribocytidylic acid. Antimicrob. Agents Chemother. **13**:159–164.
6. **Borden, C. C., B. W. Booth, and J. A. McBain.** 1979. Enhancement of infectivity of encephalomyocarditis virus RNA by amphotericin B-methyl ester. J. Gen. Virol. **42**:297–303.
7. **Borden, E. C., and P. H. Leonhardt.** 1976. Enhancement of rI_n:rC_n-induced interferon production by amphotericin B. Antimicrob. Agents Chemother. **91**:551–553.
8. **Borden, E. C., and P. H. Leonhardt.** 1977. A quantitative semi-micro, semi-automated colorimetric assay for interferon. J. Lab. Clin. Med. **89**:1036–1042.
9. **Finkelstein, A., and R. Holz.** 1973. Aqueous pores created in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. Membranes **2**:377–408.
10. **Finter, N. B.** 1969. Dye uptake methods for assessing viral cytopathogenicity and their application to interferon assays. J. Gen.O Virol. **5**:419–427.
11. **Hamilton-Miller, J. M. T.** 1974. Fungal sterols and the mode of action of the polyene antibiotics. Adv. Appl. Microbiol. **17**:109–134.
12. **Howard, B. V., and D. Kritchevsky.** 1969. The lipids of normal diploid (WI-38) and SV-40-transformed human cells. Int. J. Cancer **4**:393–402.
13. **Kinsky, S. C.** 1970. Antibiotic interaction with model membranes. Annu. Rev. Pharmacol. **10**:119–142.
14. **Lin, H. S., G. Medoff, and G. S. Kobayashi.** 1977. Effects of amphotericin B on macrophages and precursor cells. Antimicrob. Agents Chemother. **11**:154–160.
15. **Medoff, G., C. N. Kwan, D. Schlessinger, and G. S. Kobayashi.** 1973. Permeability control in animal cells by polyenes: a possibility. Antimicrob. Agents Chemother. **3**:441–443.
16. **Medoff, G., C. N. Kwan, D. Schlessinger, and G. S. Kobayashi.** 1973. Potentiation of rifampicin, rifampicin analogs, and tetracycline against animal cells by amphotericin B and polymyxin B. Cancer Res. **33**:1146–1149.
17. **Medoff, G., D. Schlessinger, and G. S. Kobayashi.** 1974. Sensitivity of transformed and nontransformed cells to amphotericin B and several rifamycin derivatives. Cancer Res. **34**:823–826.
18. **Medoff, G, F. Valerioti, R. G. Lynch, D. Schlessinger, and G. S. Kobayashi.** 1974. Synergistic effect of amphotericin B and 1,3-bis(2-chloroethyl)-1-nitrosourea against a transplantable AKR leukemia. Cancer Res. **34**:974–978.
19. **Morton, B. E., and H. A. Lardy.** 1967. Cellular oxidative phosphorylation. III. Measurement in chemically modified cells. Biochemistry **6**:57–61.
20. **Presant, C. A., C. Klahr, and R. Santala.** 1977. Amphotericin B induction of sensitivity to adriamycin, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) plus cyclophosphamide in human neoplasia. Ann. Intern. Med. **86**:47–51.
21. **Vengris, V. E., F. H. Reynolds, Jr., M. D. Hollenberg, and P. M. Pitha.** 1976. Interferon action: role of membrane gangliosides. Virology **72**:486–493.
22. **Vengris, V. E., B. D. Stollar, and P. M. Pitha.** 1975. Interferon externalization by producing cell before induction of antiviral state. Virology **65**:410–417.