ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1976, p. 551-553
Vol. 9, No. 3

Printed in U.S.A.

Enhancement of rI_n:rC_n-Induced Interferon Production by Amphotericin B

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Received for publication 30 September 1975

When mouse L929 cells are treated with amphotericin B before exposure to polyriboinosinic-polyribocytidylic acid or polyriboinosinic-polyribocytidylic acid-dextran, they make significantly more interferon than do cells not receiving amphotericin. This effect may be due to enhanced cell membrane penetration by the polynucleotide.

Polyene antibiotics, such as nystatin and amphotericin B, bind to sterols in the cell membrane and abolish the membrane's ability to act as a selective permeability barrier (2, 8, 10, 12, 14). Amphotericin B increased penetration of eukaryotic cells by antibiotics, nucleases, latex particles, and *Escherichia coli* deoxyribonucleic acid (13, 16, 17). Both enhanced uptake of the radiolabeled compounds and enhanced biological effects of the antibiotics have been demonstrated. These permeability changes may result from the development of functional pores in membranes treated with polyenes (2, 10, 12). These pores selectively favored penetration of anionic compounds (2, 8).

Whether polyriboinosinic-polyribocytidylic acid (rI_n:rC_n) must enter cells or need only contact a membrane-associated trigger site to effect antiviral resistance and interferon production remains an unresolved question. In an effort to partially delineate the site of interferon induction by rI_n:rC_n, the effect of amphotericin B on interferon production and action in L929 cells was studied.

Amphotericin B treatment of L929 cells enhanced interferon production by both rI_n:rC_n and rI_n:rC_n-dextran (Table 1). Interferon titers were enhanced significantly by amphotericin B at 5 μg/ml and increased almost 10-fold at 25 μg/ml. No interferon production by amphotericin B alone (25 μg/ml) was observed. Amphotericin B alone at 5 μg/ml and 10 μg/ml produced no decrease in cell viability or cell number, but at 25 μg/ml cell number was halved, although viability was unaffected. Higher concentrations of amphotericin B alone (50 μg/ml) reduced cell number 80%, and 100 μg/ml killed the cell inoculum. Consistent with the decrease in cell viability at the higher amphotericin B concentrations was an inhibition of interferon production (Table 1). Absence of cytotoxicity in L929 and other cells at the lower concentrations of amphotericin B has been previously reported (1, 16). Although combined treatment with amphotericin B and rI_n:rC_n decreased cell number, enhanced interferon production was observed (Table 2).

Interferon production by rI_n:rC_n in L929 cells (a continuous mouse fibroblast line) is generally minimal or absent; only after formation of a complex between rI_n:rC_n and a polycation, such as dextran, has interferon been induced in these cells. However, after treatment of cells with 10 μg of amphotericin B per ml, interferon production occurred even at relatively low (25 μg/ml) rI_n:rC_n concentrations (Fig. 1).

Whether rI_n:rC_n per se must penetrate the cell membrane to induce interferon remains unresolved. Direct studies of the cellular distribution of radiolabeled rI_n:rC_n by radioautography or cell fractionation have proven difficult (3, 24). No correlation between the amount of cell-bound rI_n:rC_n and interferon produced has been observed, and both active and inactive polynucleotides became cell associated at equivalent rates (5, 7, 19). Treatment of cells with agents such as neuraminadase or phospholipase C, which alter membrane topological orientation, resulted in inhibition of interferon production without effect on rI_n:rC_n binding (18). These findings suggested that interferon induction may result from an intracellular event after membrane binding. However, rI_n:rC_n, covalently linked to particulate carriers and thus unable to enter cells, induced interferon (20, 23, 25). Furthermore, induction of antiviral activity required persistence of intact polymer at a superficial site (6), and intracellular concentrations of rI_n:rC_n, capable of inducing interferon in other cells, were not identified

after $rI_n:rC_n$ treatment (11). These findings suggested that $rI_n:rC_n$ either acted on the membrane and did not enter the cell intact or was partially broken down at the cell surface with only pieces entering (22).

The membrane alterations induced by amphotericin B resulted in enhanced uptake by eukaryotic cells of *E. coli* deoxyribonucleic acid (13) and enhanced inhibition of ribonucleic acid and protein synthesis by the antibiotics rifampin and tetracycline (16, 17). Thus, although alteration of a putative membrane receptor by amphotericin B cannot be eliminated, the observed enhancement in interferon production caused by amphotericin B in L929 cells probably resulted from increased cell penetration of the anionic polymer, $rI_n:rC_n$. If enhancement of interferon production by polyenes also occurs in other cell lines, entry into cells of $rI_n:rC_n$ may prove one of the critical negative controls for interferon induction. Cell lines, resistant to the cytotoxic effects of actinomycin D, as a result of decreased permeability, can be rendered specifically sensitive by amphotericin B (15) or the

TABLE 1. *Enhancement of interferon production by amphotericin in L929 cells*[a]

Amphotericin (μg/ml)	Interferon (IU) produced by[b]	
	$rI_n:rC_n$[c]	$rI_n:rC_n$-dx[d]
0	0	140
5	50	730
10	40	830
25	100	1000
50	–	105
100	–	30

[a] Amphotericin B (Fungizone, GIBCO, Grand Island, N.Y., was added to suspensions of 2.5×10^5 L929 cells (ATCC, CCL 1) to the final concentrations indicated. The L929 cells were then grown as monolayers for 24 h in minimal essential medium (Eagle base) with added 2 mM glutamine, 50 μg of gentamicin per ml (MEM), and 10% fetal calf serum (FCS). After 24 h, medium and amphotericin were removed, and 50 μg of $rI_n:rC_n$ per μl (Miles Laboratories, Kankakee, Ill.) or 50 μg of $rI_n:rC_n$ per μl annealed to 100 μg of dextran (diethylaminoethyl-dextran, 100 μg/ml, Sigma Chemical Co., St. Louis, Mo.) at 22 C in phosphate-buffered saline, 0.85%, pH 7.3, was mixed with the indicated concentrations of amphotericin B and added to the cells. After 60 min at 37 C, cells were washed twice with Earle balanced salt solution, pH 7.3, and MEM with 2% FCS was added. After incubation at 37 C for 24 h, cell supernatants were collected for interferon assay. Interferon, characterized as previously described (4), was assayed in triplicate on L929 cells by a modification of the Finter colorimetric technique (4, 9). Results presented in this and subsequent tables are representative of two or three separate experiments.

[b] At 24 h (international units).

[c] $rI_n:rC_n$, 50 μg/ml.

[d] $rI_n:rC_n$, 50 μg/ml-dextran, 100 μg/ml.

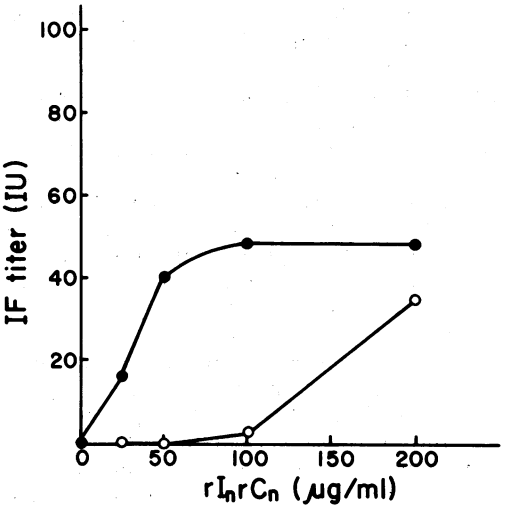

FIG. 1. *Interferon production by $rI_n:rC_n$ in absence (○) and presence (●) of amphotericin B (10 μg/ml). Interferon production and assay as described in Table 1.*

TABLE 2. *Effect of amphotericin on cell number, viability, and interferon production*[a]

Treatment[b]	L929 cells[c]		Interferon (IU)[d]
	Viability (%)	No. (% of control)	
0	100	100	0
Amphotericin (20 μg/ml)	99	59	0
$rI_n:rC_n$ (100 μg/ml)	100	44	<8
Amphotericin (20 μg/ml), $rI_n:rC_n$ (100 μg/ml)	98	8	55

[a] Cells were counted and viability was determined by staining with trypan blue and performing counts in triplicate in a hemocytometer chamber. Interferon production and interferon assay were as described in Table 1.

[b] Cells were plated in 20 μg of amphotericin per ml, and $rI_n:rC_n$ was added at 24 h in 20 μg of amphotericin per ml, adsorbed 60 min, and washed.

[c] Determined after 24 h of indicated treatment.

[d] Produced at 24 h (international units).

detergent dimethylsulfoxide (21). The effectiveness of $rI_n:rC_n$ as an interferon inducer in L929 cells, otherwise relatively resistant to interferon induction by $rI_n:rC_n$, may result from a similar mechanism.

This work was supported by institutional research funds from the American Cancer Society and Surgical Associates. Ernest C. Borden is a Junior Faculty Fellow of the American Cancer Society.

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