[CANCER RESEARCH 33, 1146–1149, June 1973]

Potentiation of Rifampicin, Rifampicin Analogs, and Tetracycline against Animal Cells by Amphotericin B and Polymyxin B[1]

G. Medoff, C. N. Kwan, D. Schlessinger, and G. S. Kobayashi

Divisions of Infectious Diseases [G. M.] and Dermatology [G. S. K.], Department of Medicine, and Department of Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110

SUMMARY

The membrane-active antibiotics amphotericin B and polymyxin B enhanced the action of rifampicin, rifampicin analogs, and tetracycline against macromolecular synthesis and growth of mouse L-cells, human HeLa cells, and KB cells in tissue culture. The specificities of the second agents were maintained in that rifampicin inhibited RNA synthesis and tetracycline inhibited protein synthesis.

INTRODUCTION

A number of antibiotics alter the permeability of the membranes of eukaryotic cells (1). Previously, we have exploited this property of amphotericin B to increase the uptake of 2nd drugs into fungi and animal cells, thereby enhancing the effects of the 2nd agents (5–7). Recently our studies have been confirmed by Kuwano *et al.* (4).

This report is an extension of our previous work and represents a 1st attempt to screen membrane-active antibiotics as well as inhibitors of macromolecular synthesis against a number of different mammalian cells in tissue culture. In these experiments, amphotericin B and polymyxin B were used to enhance the effects of rifampicin, rifampicin analogs, and tetracycline against mouse L-cells and human KB and HeLa cells.

MATERIALS AND METHODS

Materials. Amphotericin B (Fungizone) was a gift of E. R. Squibb & Sons, Inc., New Brunswick, N. J. Rifampicin and the rifampicin analogs used were obtained from Dr. Pierro Sensi, Gruppo Lepetit, Milan, Italy. Polymyxin B (Aerosporin) was donated by Burroughs Wellcome Co., Research Triangle Park, N. C. and tetracycline was donated by Lederle Laboratories, Pearl River, N. Y.

Methods. Mouse L-cells were grown in spinner cultures with α-MEM[2] (Flow Laboratories, Rockville, Md.) or leucine-deficient MEM plus 5% fetal calf serum. HeLa cells were grown in Joklik-modified MEM plus 5% horse serum or in leucine-deficient medium, and KB cells were grown in MEM plus 5% horse serum or leucine-deficient medium. At the start of the experiment, the cells were diluted to 1 to 2 $\times$ 10^5 cells/ml and were divided into separate cultures and incubated with the antibiotics being tested and with leucine-^{3}H, 0.5 μCi/ml (specific activity, 51 Ci/mM), or uridine-^{3}H, 0.5 μCi/ml (specific activity, 25 Ci/mM), for 3 hr. One-ml samples were then removed in triplicate and centrifuged. The cell pellets were precipitated with 2 ml of 5% trichloroacetic acid, and the chilled precipitates were filtered onto glass filters and counted.

For viable cell counts after antibiotic treatment, the cells were grown in spinner cultures as described above. They were diluted to 1 to 2 $\times$ 10^5 cells/ml at the beginning of the experiment and placed in Falcon Petri dishes (3.5 cm in diameter) with the antibiotics being tested. After 18 hr, the cells were scraped from the Petri dishes and counted under the microscope. Cell viability was determined by trypan blue dye exclusion.

Each point in the experiments measuring RNA and protein synthesis and cell viability is the average of 3 replicate determinations in each experiment, and each experiment was repeated at least 3 times with similar results.

RESULTS

Chart 1 shows that amphotericin B, in combination with rifampicin, had a marked effect on RNA synthesis in mouse L-cells and human HeLa and KB cells, whereas the same concentration of each agent tested alone against the cells had no effect. Treatment with amphotericin B, at the concentrations used, did not alter the specificity of rifampicin because, in our experiments, rifampicin preferentially inhibited RNA synthesis without markedly affecting protein synthesis. Similar results were obtained when several rifampicin analogs were used in combination with amphotericin B (Table 1). The potency of each of the analogs was different and conformed to the general pattern described for them in the past (9).

The cultured cells were also tested for their response to polymyxin B and rifampicin. Polymyxin B enhanced the effect of rifampicin on RNA synthesis in L-cells with a sparing of protein synthesis (Chart 2).

Chart 3 shows that cell viability data were consistent with our results describing inhibitions of RNA and protein synthesis. The viability of the cells exposed to amphotericin B or polymyxin B and maximum concentrations of rifampicin was 10 to 50% of the values obtained by exposing the cells to no drug or to each drug alone at the concentrations shown.

[1] Supported by USPHS Research Grants 1 RO1 AI 10622, AI 06213, and CA-125602-1 and by Training Grant AM 05611 and a grant from the John A. Hartford Foundation, Inc.

[2] The abbreviation used is: MEM, minimum essential medium.

Received December 18, 1972; accepted February 19, 1973.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/33/6/1146/2391855/cr0330061146.pdf by guest on 19 August 2022

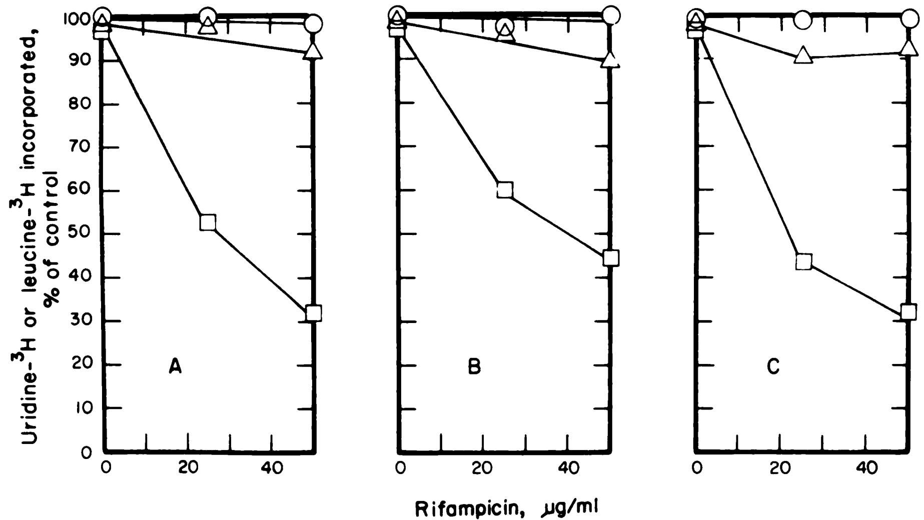

Chart 1. Dose response to rifampicin of uridine-^{3}H or leucine-^{3}H incorporation into mouse L-cells (*A*), human HeLa cells (*B*), and human KB cells (*C*) incubated in the presence or absence of amphotericin B. ○, no amphotericin (uridine-^{3}H or leucine-^{3}H incorporation); □, 25 µg amphotericin B per ml (uridine-^{3}H incorporation); △, 25 µg amphotericin B per ml (leucine-^{3}H incorporation).

Table 1

Concentrations of rifampicin derivatives[a] which resulted in 50% inhibition of RNA synthesis in mouse L-cells

Rifampicin derivative[b]	Concentration with amphotericin B (µg/ml)	Concentration alone (µg/ml)
M/14	25	100
AF/AP	25	100
SV	15	50
AF/ABDP	5	25
AF/013	1	10

[a] At each concentration, there was little or no inhibition of protein synthesis.

[b] Symbols designated by Lepetit. See Ref. 8 for structure.

The results illustrated show the effects of the drugs on L-cells. Similar results were obtained with HeLa and KB cells.

When tetracycline was used in combination with polymyxin B, protein synthesis was preferentially inhibited, whereas net RNA synthesis continued to be stable (Chart 4). Therefore, as with the rifampicin potentiation, the selective effect of tetracycline on protein synthesis was maintained.

Both gramicidin and valinomycin are membrane-active antibiotics (3). Neither was able to potentiate the effects of the 2nd agents used in the experiments already described when tested against mouse L-cells and human KB and HeLa cells.

DISCUSSION

Amphotericin B and polymyxin B both potentiate the effects of 2nd agents against mammalian cells. As with yeast, the argument that the amphotericin B and polymyxin B enhance the effects of the 2nd agents rather than the reverse is supported by the results which show that the specificity of the 2nd agent is maintained in the combination. That is, rifampicin selectively inhibited RNA synthesis, whereas tetracycline affected net protein synthesis. The effects of amphotericin B were not selective, because high concentrations inhibited DNA, RNA, and protein synthesis concomitantly with cell death (data not shown). Not only was the selectivity

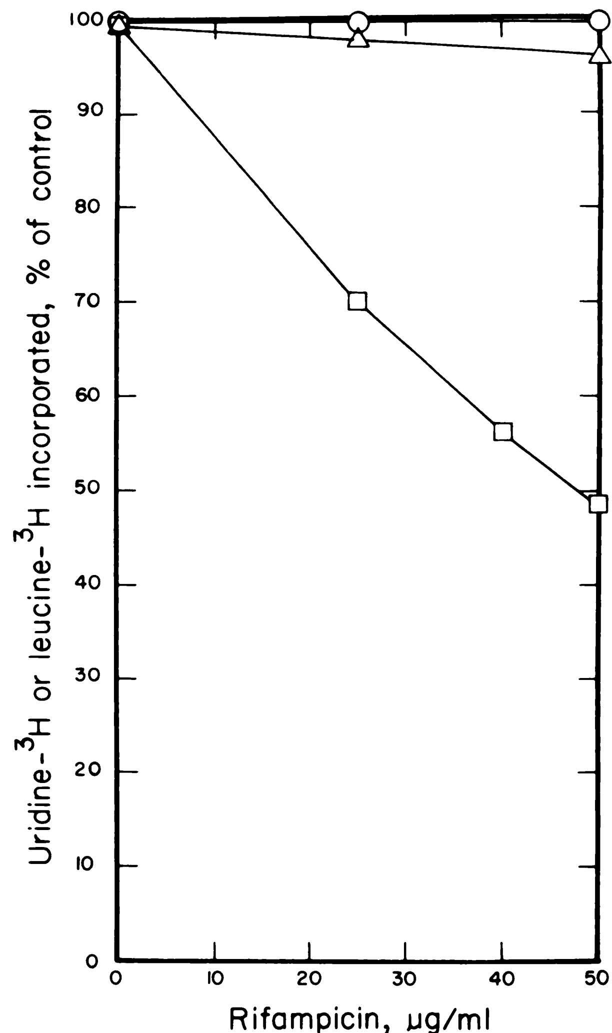

Chart 2. Dose response to rifampicin of uridine-^{3}H or leucine-^{3}H incorporation into mouse L-cells incubated in the presence or absence of polymyxin B. ○, no polymyxin B (uridine-^{3}H or leucine-^{3}H incorporation); □, 50 µg polymyxin B per ml (uridine-^{3}H incorporation); △, 50 µg polymyxin B per ml (leucine-^{3}H incorporation).

Downloaded from http://aacrjournals.org/cancerres/article-pdf/33/6/1146/2391855/cr0330061146.pdf by guest on 19 August 2022

of rifampicin for RNA synthesis maintained by the amphotericin B potentiation, but the relative potency of the rifampicin analogs in combination with amphotericin B was in agreement with those data previously reported by Smith *et al.* (9) when they screened the derivatives against human leukemic cells. In our experiments, we measured the effects of the rifampicin analogs on RNA rather than DNA synthesis so that their potency could be compared with that of rifampicin. We do not mean to imply by this that we think that the effect on RNA synthesis may be the major mechanism of action of the analogs.

In other experiments, we found that the different rifampicin derivatives also had the same relative degree of potency against the fungus *Histoplasma capsulatum* as they had against animal cells (2). Although mammalian cells were less sensitive to the membrane effects of amphotericin B and polymyxin B, the excellent agreement between our studies on fungi and animal cells suggests that yeast may represent a usable model for study of many features of animal cell biology.

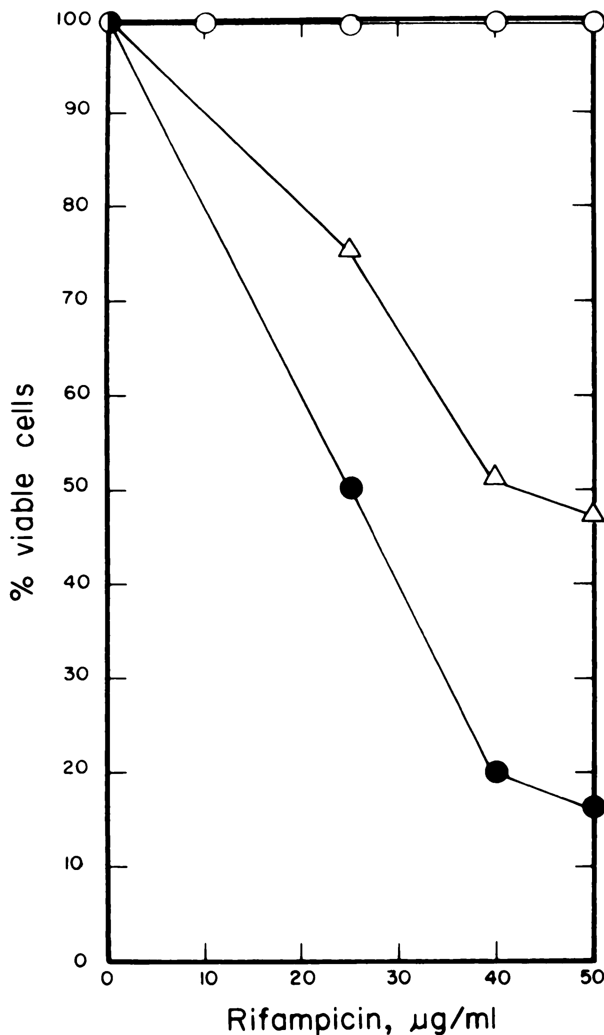

Chart 3. Dose response to rifampicin of the viable cell count of mouse L-cells in the presence or absence of amphotericin B or polymyxin B. ○, no amphotericin B or polymyxin B; ●, 25 µg amphotericin B per ml; △, 25 µg polymyxin B per ml.

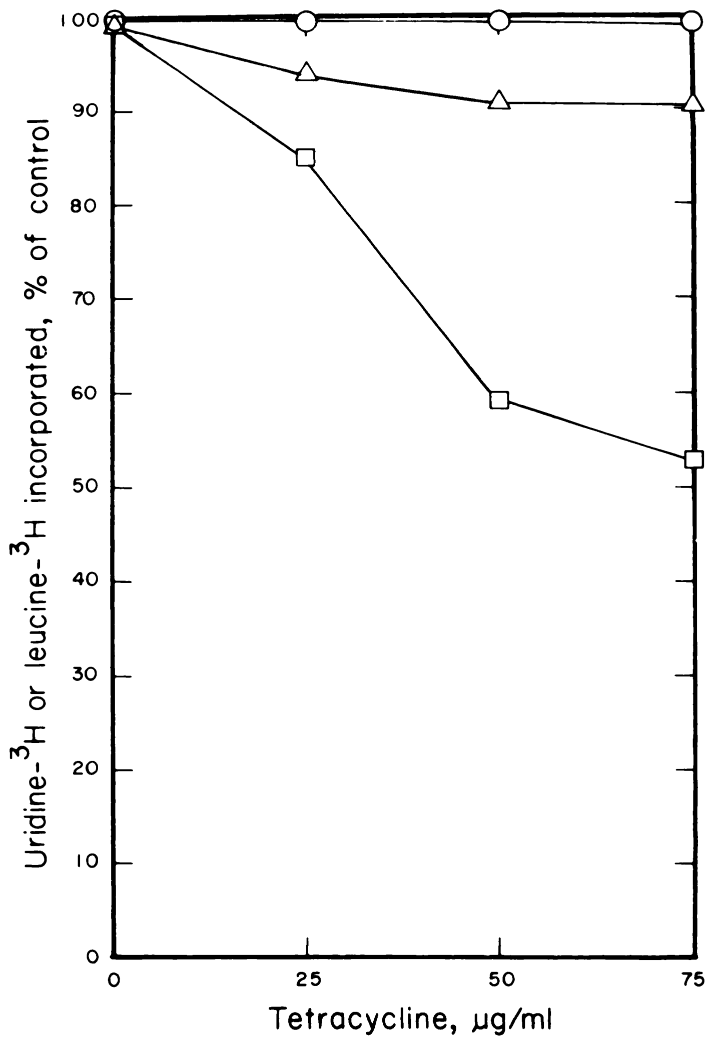

Chart 4. Dose response to tetracycline of uridine-^{3}H or leucine-^{3}H incorporation into mouse L-cells incubated in the presence or absence of polymyxin B. ○, no polymyxin B (uridine-^{3}H or leucine-^{3}H incorporation); □, 50 µg polymyxin B per ml (leucine-^{3}H incorporation); △, 50 µg polymyxin B per ml (uridine-^{3}H incorporation).

The concept that cellular integrity can be altered by a variety of drugs affecting the cell membrane is an important one. Its clinical usefulness will depend on the degree of selectivity that can be achieved so that only specified cell populations will be affected (malignant cells). There is evidence that malignant cells are more sensitive than normal cells to the action of polyenes (8), and preliminary animal experiments in our laboratory suggest that there is selectivity of the polyenes for populations of malignant cells (unpublished results). If this is the case, the synergistic combinations demonstrated in our *in vitro* experiments will prove to have important therapeutic implications.

ACKNOWLEDGMENTS

We acknowledge the excellent technical assistance of Mary Blum in this work.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/33/6/1146/2391855/cr0330061146.pdf by guest on 19 August 2022

REFERENCES

1. Kinsky, S. C. Antibiotic Interactions With Model Membrane. Ann. Rev. Pharmacol., *10:* 119–142, 1970.
2. Kobayashi, G. S., Medoff, G., Schlessinger, D., Kwan, C. N., and Musser, W. E. Amphotericin B Potentiation of Rifampicin as an Antifungal Agent against the Yeast Phase of *Histoplasma capsulatum.* Science, *177:* 709–710, 1972.
3. Krasne, S., Eisenman, G., and Szabo, G. Freezing and Melting of Lipid Bilayers and the Mode of Action of Nonactin, Valinomycin and Gramicidin. Science, *174:* 412–415, 1971.
4. Kuwano, M., Akiyama, S., Endo, H., and Kohga, M. Potentiation of Fusidic Acid and Lentinan Effects Upon Normal and Transformed Fibroblastic Cells by Amphotericin B. Biochem. Biophys. Res. Commun., 49: 1241–1247, 1972.
5. Kwan, C. N., Medoff, G., Kobayashi, G. S., Schlessinger, D., and Raskas, H. J. Potentiation of the Antifungal Effects of Antibiotics by Amphotericin B. Antimicrobiol. Agents Chemotherapy, *2:* 61–65, 1972.
6. Medoff, G., Kobayashi, G. S., Kwan, C. N., Schlessinger, D., and Venkov, P. Potentiation of Rifampicin and 5-Fluorocytosine as Antifungal Antibiotics by Amphotericin B. Proc. Natl. Acad. Sci. U. S., *69:* 196–199, 1972.
7. Medoff, G., Kwan, C. N., Kobayashi, G. S., and Schlessinger, D. Enhancement by Membrane Active Antibiotics of Inhibitors of RNA and Protein Synthesis in Animal Cells. J. Cell Biol., *55:* 1739, 1972.
8. Mondovi, B., Strom, R., Argo, A. F., Caiafa, P., DeSole, P., Bozzi, A., Rotilio, G., and Fanelli, A. R. Effect of Polyenic Antibiotics in Ehrlich Ascites and Novikoff Hepatoma Cells. Cancer Res., *31:* 505–509, 1971.
9. Smith, R. G., Whang-Peng, J., Gallo, R. C., Levine, P., and Ting, R. C. Selective Toxicity of Rifampicin Derivatives for Leukaemic Human Leucocytes. Nature New Biol., *236:* 166–171, 1972.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/33/6/1146/2391855/cr0330061146.pdf by guest on 19 August 2022