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DISCLAIMER

SYNERGISTIC EFFECT OF RIFAMYCIN DERIVATIVES AND AMPHOTERICIN B ON VIRAL TRANSFORMATION OF A MURINE CELL LINE

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ABSTRACT

One of the most potent inhibitors of RNA-dependent DNA polymerase activity so far described (rifazacyclo-16) was found not to be as correspondingly active in focus inhibition. This discrepancy was thought to be due to the inability of the drug to penetrate the cell membrane. It has been found that a very low level of amphotericin B allows this drug, as well as the previously described 2',6'-dimethyl-N(4')benzyl-N(4')-[desmethyl]rifampicin (DMB), to exhibit a very high capability to inhibit focus formation. Since these two drugs are highly lipophilic, their activity may be expected to be dependent upon any lipophilic components in the medium such as serum or detergents. The use of amphotericin B as well as serum in tissue cultures is common and could account for some of the variability in focus inhibition reported in the literature.

Running title: Rifamycin derivatives and amphotericin

One of the rifampicin derivatives, 2',6'-dimethyl-N(4')benzyl-N(4')[desmethyl]rifampicin (DMB), inhibited focus formation and infectious virus production in BALB/3T3 cells by Moloney Sarcoma Virus (1,2). It also inhibited Moloney leukemia virus induced focus formation in the UC1-B cell line derived from BALB/3T3 cells (3a,3b).

Three recently synthesized derivatives of rifampicin (rifazacyclo-16, dirifampin, and rifamazine) have been described (4). Rifazacyclo-16 was the most effective inhibitor of the RNA-instructed DNA polymerase (RIDP) yet tested (5,6), while the others were less active. However, these drugs were all found to be ineffective against viral transformation of mouse cells, presumably because they were unable to penetrate the cell membrane.

It was shown that amphotericin B, an antibiotic commonly used against fungal infection in tissue cultures, has the property of increasing the membrane permeability of susceptible fungi (7,8,9). Recently, it was shown that low levels of the polyene antibiotic potentiate the effects of rifampicin on the yeast phase of Histoplasma capsulatum (10) and on Saccharomyces cerevisiae (11). We have found that the inhibition of viral transformation of mouse cells by rifampicin derivatives is enhanced by low levels of amphotericin B.

Toxic effects of the drugs may alter the cellular growth rate resulting in reduction of focus formation in virus-infected cells (12). Efficiency of plating (EOP) of UC1-B cells in the presence of increasing concentration of both drugs was used to measure these effects. Representative data are presented in Table 1. AT 5 μg/ml of amphotericin (with

6 μg/ml rifazacyclo-16) the EOP was reduced by 92%, while no effect was detectable at the lower dose levels. All subsequent experiments were done at 1 μg/ml of amphotericin B.

The effect of increasing levels of rifazacyclo-16 (with 1 μg/ml amphotericin B) on the EOP of UC1-B cells is also shown in Table 1. No significant reduction could be demonstrated up to 12 μg/ml. The toxicity for cells of the other derivatives used in these experiments was tested previously (1,2) and 6 μg/ml of each drug was used in the focus inhibition tests.

Four rifampicin derivatives are compared for their effects on focus formation in UC1-B cells with and without amphotericin B (Table 2). A significant increase in the effects of all of the rifampicin derivatives was found in the presence of amphotericin B. Dirifampin is a much less effective inhibitor of leukemia virus induced focus formation than rifamazine, and the latter is less inhibitory than either rifazacyclo-16 or DMB.

Rifazacyclo-16 alone had very little effect on leukemia virus induced focus formation. In the presence of 1 μg/ml amphotericin B and increasing concentrations of rifazacyclo-16, focus formation was reduced by 90 to 100% at both 6 and 12 μg/ml. The effect of DMB is also potentiated by the presence of amphotericin B, reducing the number of foci to 14% of the controls at 6 μg/ml, which concentration without amphotericin B only reduced the number of foci to 54% (Table 3).

Variation in the effects of these drugs (as much as 30 to 40%) has been encountered in these experiments. These fluctuations are partially due to the (sampling) errors inherent in the procedures of the assay, and

to the pH variation of the culture medium. Replicate cultures in which the pH was adjusted to low (pH 6.0), intermediate (pH 7.0) and high (pH 7.5) were infected with virus and the average number of foci counted after five days incubation. Foci formed at all pH levels: at pH 6.0, 16% and pH 7.5, 70% of the number formed at pH 7.0. These results showing pH sensitivity are consistent with observations made with this assay system during the past year.

The protein content of the fetal calf serum used in the growth medium may nonspecifically adsorb some of the rifampicin derivatives, and may also contribute to the variability of the focus inhibition test (13). Another source of variation is the apparent temperature sensitivity of the transformation of UCl-B cells by murine leukemia virus. Fluctuation in incubator temperature above 37.5° reduces focus formation significantly (14).

An alteration of the permeability barrier of the cytoplasmic membrane, resulting in increased penetration of the rifampicin derivatives, could account for the enhanced reduction in focus formation observed. Direct tests of this are underway using labeled drugs.

The results of this work suggest that studies on the biodynamics of mammalian cell membranes should be interpreted with caution when these antibiotics are in the milieu, as amphotericin frequently is.

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Table 1

Effect of Rifazacyclo-16 in the Presence of Amphotericin B on the Plating Efficiency of UCl-B Cells

Amphotericin B µg/ml (with 6 µg rifazacyclo-16)	# Colonies Produced	% Reduction
0	25	0
0.01	33	0
0.1	21	0
1	25	0
5	2	92
Rifazacyclo-16 µg/ml (with 1 µg/ml amphotericin B		
0	25	0
1.5	23	8
3	29	0
6	21	6
12	22	5

Cells were suspended with trypsin-versene, counted, and distributed into 50 mm petri dishes at levels of 10,000, 1,000 and 100 cells/dish. The cells were allowed to become attached to the substrate (2 hr at 36°C) and the medium was then changed to contain the appropriate drug level. All cell cultures were grown without antibiotics, except, as indicated, where amphotericin B was added. Growth medium consisted of Dulbeco's MEM with 10% fetal calf serum.

Rifazacyclo-16 and all other rifampicins were dissolved just before use in dimethylsulfoxide as a ten-fold concentrate and diluted therefrom in growth medium.

Table 2

Effect of Rifampicin Derivatives on Induction of Focus Formation by Moloney Leukemia Virus in UCl-B Cells

Rifampicin Derivative	Average # Foci Formed	
	Without Amphotericin B	With Amphotericin B
None	110	110
Dimethylbenzylrifampicin	45	2
Rifazacyclo-16	42	0
Rifamazine	100	29
Dirifampin	135	52

Subconfluent monolayers were inoculated with an estimated 300 plaque-forming units of leukemia virus in 0.5 ml growth medium with 2 µg/ml polybrene (15). Cultures were fluid changed at day 3 without added polybrene or drugs. Foci of transformed cells were counted 5 to 6 days post infection, unstained.

Table 3

Effects of Amphotericin B and Rifampicin Derivatives on Moloney Leukemia Virus Transformation of UCl-B Cells

Rifampicin Derivative	Average # Foci Formed		
	g/ml	With Amphotericin B (1 μg/ml)	Without Amphotericin B
Dimethylbenzyldesmethyl-rifampicin	0	298	287
	3	180 (60)*	234 (80)
	6	42 (14)	157 (54)
	12	0 (0)	0 (0)
Rifazacyclo-16	0	298	287
	3	284 (94)	291 (91)
	6	30 (10)	251 (86)
	12	0 (0)	0 (0(

* Figure in parenthesis: percent of control.

Focus inhibition assay was done as described in Table 2.

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