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AMPHOTERICIN B RENDERS STATIONARY PHASE HEPATOMA CELLS SENSITIVE TO DIPYRIDAMOLE

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This study provides evidence that the dipyridamole inhibitory effect on nucleoside incorporation changed with culture time. Lag and log phase hepatoma 3924A cells were highly sensitive to dipyridamole with IC_{50} values for thymidine incorporation of 0.20 and 0.31 μM, respectively. In contrast, stationary phase cells were comparatively insensitive to dipyridamole with an IC_{50} of 38.9 μM. Amphotericin B (10 μM) restored the sensitivity of stationary phase cells to dipyridamole, lowering the IC_{50} value for thymidine incorporation to 0.25 μM. Amphotericin B also enhanced the cytotoxicity of dipyridamole to hepatoma 3924A cells. The combination of amphotericin B and dipyridamole may be useful in cancer chemotherapy.

Dipyridamole is a nucleoside transport inhibitor which blocks the utilization of exogenous nucleosides by the cell. Our previous study demonstrated that dipyridamole effectively blocked incorporation of nucleosides in hepatoma 3924A cells and prevented the rescue effect of nucleosides against acivicin, a glutamine antagonist. These results suggested that dipyridamole may be useful in combination with acivicin or other antimetabolites in cancer chemotherapy (1,2). It has been reported that dipyridamole enhanced the cytotoxicity of acivicin against human colon cancer cells (3). In the present study we found that hepatoma 3924A cells in stationary phase were insensitive to dipyridamole inhibitory action and the sensitivity was restored in cells treated with amphotericin B.

MATERIALS AND METHODS

Cell Culture: Rat hepatoma 3924A cells were grown in McCoy's 5A medium (Grand Island Biological Co., Grand Island, NY), supplemented with 10% fetal bovine serum; penicillin, 100 unit/ml; and streptomycin, 100 μg/ml. Cells were incubated at 37 °C in a 5% CO_2-95% air humidified atmosphere.

ABBREVIATIONS: IC_{50}, 50% inhibitory concentration; TCA, trichloroacetic acid.

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Incorporation Assay: The procedure used was as described (2), except that 24-well plates instead of culture flasks were utilized. Samples were incubated in serum-free medium and analyzed for the incorporation of ^{14}C-labeled nucleosides into TCA-insoluble material.

Growth Inhibition: Hepatoma 3924A cells were seeded in 24-well plates with a density of 10^4 cells/cm^2 (2 x 10^4 cells/well). After 48 h, the spent medium was removed and fresh medium containing 10% dialyzed fetal bovine serum was added, followed by dipyridamole and amphotericin B (Sigma Co., St. Louis, MO) after which the cells were incubated for another 48 h. The cells were then harvested by trypsinization and counted by a Coulter counter. Based on the difference in cell number, the percent inhibition was calculated.

RESULTS AND DISCUSSION

1. The sensitivity to dipyridamole changed with growth phase of the cultured cells. The utilization of exogenous, preformed nucleosides in stationary phase hepatoma 3924A cells was insensitive to the inhibitory action of dipyridamole. The growth curve for hepatoma 3924A cells is shown in Figure 1. The lag, log and stationary phases occur at 0-24, 24-72 and 72-96 h, respectively. When seeded at 10^4 cells/cm^2, the cell densities at mid-log phase (48 h) and stationary phase (96 h) were 5 x 10^4 and 2.5 x 10^5 cells/cm^2. Cells in lag and log phase were highly sensitive to dipyridamole (10 μM) where 54 to 99% of cytidine, guanosine, thymidine and uridine incorporation was inhibited (Table 1). In contrast, late stationary phase cells were insensitive to dipyridamole (10 μM) action displaying lower or no inhibition of nucleoside incorporation. Dipyridamole in this concentration and up to 75 μM did not affect protein or nucleic acid biosynthesis (4). The IC_{50} values of dipyridamole for thymidine incorporation were 0.20 and 0.31 μM in lag (2) and log phase cells whereas the IC_{50} in stationary phase cells was 38.9 μM (Table 2).

2. Amphotericin B restored the sensitivity to dipyridamole in stationary phase cells. As shown in Table 2, the dipyridamole IC_{50} for thymidine incorporation was 38.9 μM in stationary phase cells but in combination with amphotericin B (10 μM) the IC_{50} was restored to 0.25 μM. It is evident that the addition of amphotericin B rendered stationary phase cells as sensitive as log phase cells to dipyridamole inhibition. The effect of dipyridamole on thymidine incorporation was related to amphotericin B

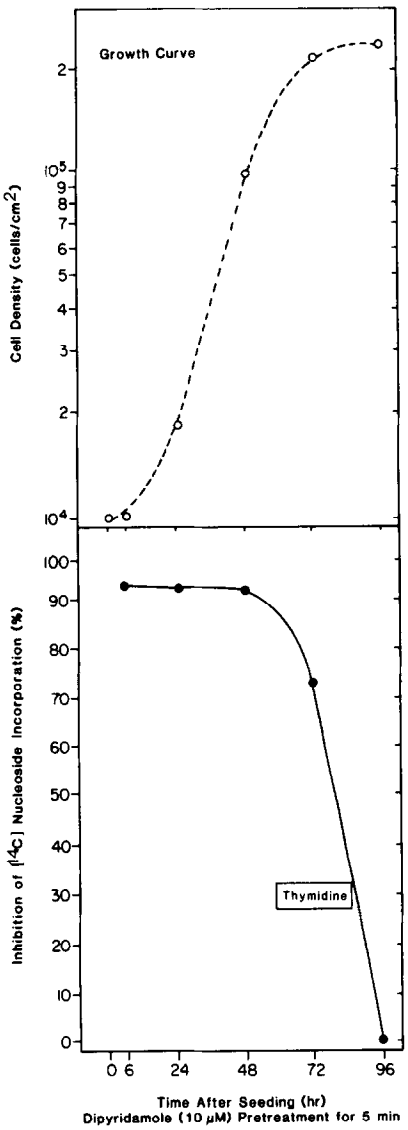

Figure 1. (Upper box) Growth curve for hepatoma 3924A cells. (Lower box) Inhibition of thymidine incorporation by dipyridamole (10 μM). Cells at 6, 24, 48, 72 and 96 h after seeding were used for incorporation assay. Dipyridamole pretreatment was for 5 min and incubation with ^{14}C-labeled thymidine was for 20 min. Points are means of duplicate determinations.

concentration. With 2.5 μM of amphotericin B, the IC_{50} was 0.72 μM. However, amphotericin B (10 μM) alone showed no inhibition on thymidine incorporation.

Various compounds such as cycloheximide, 2-deoxy-D-glucose, neuraminidase, tunicamycin and valinomycin were also tested for their ability to render

Table 1: Effect of growth phase on inhibition of dipyridamole of nucleoside incorporation in hepatoma 3924A cells

Precursors	Time: phases and h									
	Lag		Log				Stationary			
	6		24		48		72		96	
	Control	+DP	Control	+DP	Control	+DP	Control	+DP	Control	+DP
Cytidine	1350	95	3083	99	1439	96	1467	89	2100	44
Guanosine	432	68	1072	54	539	83	823	62	596	0
Thymidine	1588	92	2336	92	1983	93	1347	71	1296	0
Uridine	1069	94	2757	76	1026	94	1391	45	1339	0

Cells at 6, 24, 48, 72 and 96 h after seeding were used for incorporation assay. Dipyridamole (10 μM) pretreatment was for 5 min and incubation with ^{14}C-labeled nucleosides was for 20 min.

Control values are cpm/10^6 cells; the effects of dipyridamole (+DP) are expressed as % inhibition.

stationary phase hepatoma cells sensitive to dipyridamole action but all were found ineffective.

3. Amphotericin B enhanced cytotoxicity of dipyridamole to hepatoma cells. At a concentration of 50 μM, dipyridamole showed a 50% growth

Table 2: Effect of amphotericin B on the inhibitory activity of dipyridamole on thymidine incorporation in log and stationary phase hepatoma 3924A cells

Cell phase	Treatment	Dipyridamole IC_{50} (μM)
Log	None	0.31
Stationary	None	38.90
Stationary	In combination with amphotericin B (10 μM)	0.25
Stationary	In combination with amphotericin B (2.5 μM)	0.72

Log phase cells, 48 h after seeding; stationary phase cells, 96 h after seeding. Stationary cells were treated with amphotericin B for 5 min before dipyridamole was added and then incubated in the presence of both drugs.

After the addition of graded concentrations of dipyridamole (5 min), ^{14}C-labeled thymidine was added and cells were incubated at 37 °C for 60 min. Radioactivity in TCA-insoluble fractions was measured. The IC_{50} values were calculated with the linear regression equation. Thymidine incorporation in control, untreated log and stationary phase cells were 16,953 and 2,315 cpm/10^6 cells, respectively.

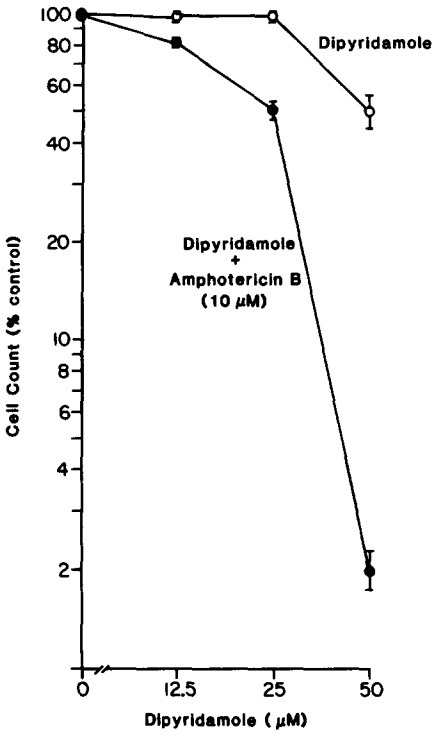

Figure 2. Amphotericin B enhanced dipyridamole growth inhibition on hepatoma cells. Procedure used was as described in Materials and Methods. Points are means ± S.E. of triplicate determinations.

inhibition of hepatoma 3924A cells and amphotericin B (10 μM) alone did not inhibit cell growth. However, the combination of the two drugs inhibited cell growth by 98%, indicating synergism (Figure 2). Our study also showed that log phase human colon cancer HT-29 cells were very sensitive to dipyridamole inhibition of thymidine incorporation (IC_{50} = 0.07 μM) and this sensitivity was markedly reduced in stationary phase cells (IC_{50} = 46 μM). The use of amphotericin B also successfully restored the sensitivity and enhanced the cytotoxicity of dipyridamole in stationary phase human colon cancer HT-29 cells (data not shown).

Amphotericin B is an antifungal drug without established antitumor activity; however, it has been shown to potentiate the effect of some cytotoxic agents (5,6,7). To our knowledge, no report on restoring sensitivity of stationary phase cells to dipyridamole by amphotericin B treatment has been published.

In some respects, monolayer cancer cells in stationary phase are more similar to solid tumors than cells in log phase; for example, cells are highly compacted and there exists a smaller growth fraction. Moreover, stationary phase cells are less sensitive to some anticancer drugs, especially antimetabolites (8). Our finding of the enhancement of dipyridamole inhibitory activity by amphotericin B in stationary phase cells may have interesting implications for the treatment of solid tumors where growth fractions are low. Both amphotericin B and dipyridamole are licensed drugs; therefore, this combination may be potentially useful in cancer chemotherapy.

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