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Amphotericin B (Fungizone®) Enhancement of Nitrogen Mustard Uptake by Human Tumor Cells

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SUMMARY

In HT29 human colon carcinoma cells, amphotericin B at doses above 120μg/ml increased nitrogen mustard uptake, and this was due to an increase in the apparent V_{max} without a change in the apparent K_m. Longer incubations (24 to 48 hr) of ascites fluid human ovarian carcinoma cells or SKMES-1 human epidermoid carcinoma cells with amphotericin B 4μg/ml enhanced the uptake of nitrogen mustard to a greater degree than that observed when cells were incubated for only 30 min. Therefore, amphotericin B can enhance nitrogen mustard by human tumor cell lines and by fresh human tumor cells.

INTRODUCTION

Amphotericin B has been found to increase the cellular uptake of ions, antibiotics and DNA by long-term mammalian cell lines and fungi (1,2). Moreover, in actinomycin D-resistant HeLa cells, amphotericin B (Fungizone®) increased the uptake of actinomycin D and increased cytotoxicity of the drug (3).

Therefore, we studied the effect of amphotericin B on the uptake of nitrogen mustard, an active alkylating agent that does not require in vivo

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metabolism to an active form, and for which the transport mechanism is known to be by the choline carrier (4,5). Our results indicate a dose-and time-dependent amphotericin B enhancement of nitrogen mustard uptake into fresh ovarian carcinoma cells as well as into cultured human colon and epidermoid carcinoma cells.

METHODS

Human ovarian carcinoma cells (obtained from paracentesis of patients with ascites), HT29 colon carcinoma cells (obtained from Dr. G. Philpott, Washington University), or SKMES-1 epidermoid carcinoma cells (obtained from Dr. J. Fogh, Sloan Kettering Institute for Cancer Research) were suspended at concentrations of 5×10^4 to 5×10^5 cells per 0.5 ml 0.9% NaCl final volume (the number of cells is given with each of the experiments listed below). The total volume of 0.5 ml contained {^{14}C}-nitrogen mustard (4.3 Ci/mole, California Bionuclear Corp., Sun Valley, CA) at final concentrations of 0.5 to 5.0µg/ml, plus amphotericin B (Fungizone®, Grand Island Biological Co., Grand Island, NY) at final concentrations of 0 to 1000µg/ml. In all experiments, solutions or suspensions were adjusted to pH 7.4 prior to addition to cell cultures.

After incubation for 30 min at 37°, the cell suspension was centrifuged at 900 x G for 10 min. The cell pellet was washed twice with 0.9% NaCl and was then dissolved in 0.5 ml of NCS tissue solubilizer. After addition of 10 mg of Bray's solution, radioactivity was determined in a Packard scintillation spectrometer. Triplicate cultures were performed under each condition.

Incubations with {^{14}C}-nitrogen mustard were limited to 30 min because the half-life of nitrogen mustard in tissue culture medium at 37° has been shown to be 75 min (5). Furthermore, uptake in certain cell lines remains linear for the first 90 min (4) so that 30 min incubations study only the initial uptake velocities. In experiments associated with longer incubations, {^{14}C}-nitrogen mustard was added only for the final 30 min.

RESULTS:

Nitrogen mustard uptake studies were performed using human HT29 colon carcinoma cells maintained in cell culture. A short incubation (30 min) with amphotericin B produced a dose-dependent increase in nitrogen mustard uptake into HT29 cells (Fig. 1). Only concentrations of amphotericin B 300µg/ml or greater produced an increase in uptake of the lowest concentration of nitrogen mustard (0.5µg/ml). At a higher concentration of nitrogen mustard, 0.5µg/ml, amphotericin B concentration above 60µg/ml increased nitrogen mustard uptake. At the highest concentration of nitrogen mustard, 2.0µg/ml, all concentrations of amphotericin B (20µg/ml to 1000µg/ml) enhanced nitrogen mustard uptake. Uptake appeared maximal with amphotericin B 600µg/ml, and at 1000 µg/ml, uptake of nitrogen mustard appeared to decline slightly. Deoxycholate at 2 concentrations, 96µg/ml or 480 µg/ml, failed to increase nitrogen mustard uptake.

To determine the mechanism of enhancement of drug uptake by amphotericin B, we calculated the apparent V_{max} and K_m for nitrogen mustard uptake at each concentration of amphotericin B using linear regression analysis of data in Fig. 1 when plotted by the Lineweaver-Burke method. Plots for each concentration were linear, with regression coefficients greater than 0.92 for each set of data. Nitrogen mustard uptake kinetics in cells cultured in the absense of amphotericin B was characterized by a V_{max} of 6.31×10^{-17} mole per min per cell, and a K_m of 1.54×10^{-6}M This calculated V_{max} is twice that described for hydrolyzed nitrogen mustard in L5178Y lymphoblasts (4), but the K_m is 30-fold less. Additions of amphotericin B at any concentration resulted in an apparent K_m 70% to 144% of that calculated for incubation without amphotericin B. The apparent V_{max} was not changed by

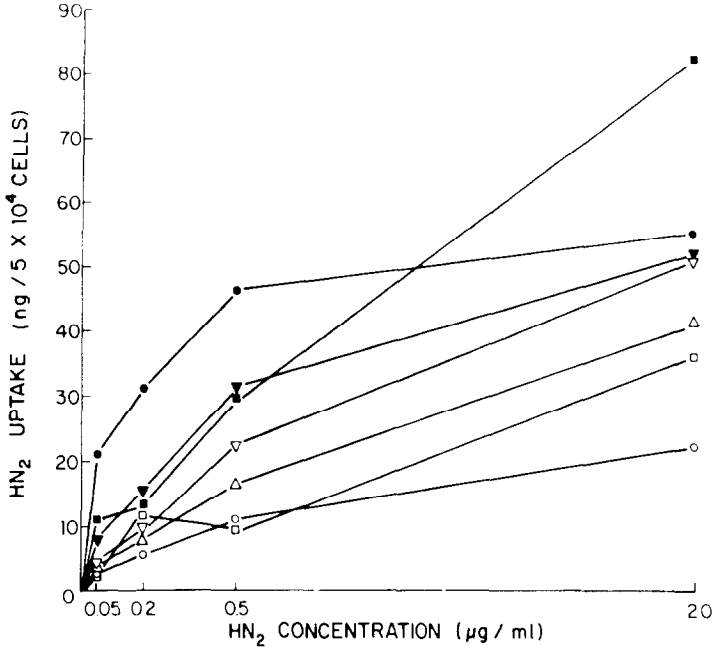

Fig. 1 Nitrogen mustard (HN_2) uptake by HT29 colon carcinoma cells. Cells were incubated at a final concentration of 5×10^4 cells per 0.5 ml 0.9% NaCl containing {^{14}C}-HN_2 0.05 to 1.8μg/ml and amphotericin B at a final concentration of 0 (○), 20 (□), 60 (△), 120 (▽), 300 (■), 600 (●), or 1000 (▼) μg/ml. Nitrogen mustard uptake was measured after 30 min. Points represent means of duplicate determinations.

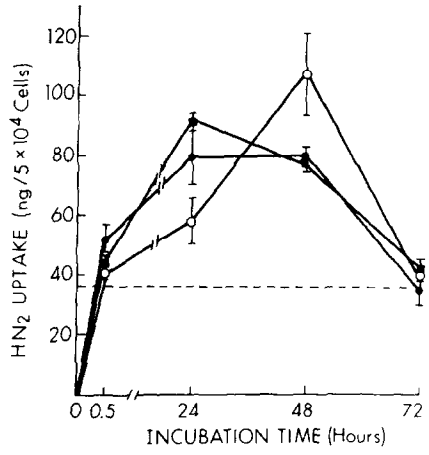

Fig. 2 Uptake of nitrogen mustard (HN_2) by SKMES-1 human epidermoid carcinoma cells. Cells were incubated at a concentration of 5 x 10^4 cells per 0.5 ml with amphotericin B at 4μg/ml (- O -), 40 μg/ml (- ● -), or 240 μg/ml (- ■ -). Nitrogen mustard 1 μg/ml was added to the culture for the final 30 min, and uptake was determined. Each point represents the mean ± S.E.M. Uptake of nitrogen mustard in the absence of amphotericin B was 35.9 ± 0.7 ng per 5 x 10^4 cells (indicated by horizontal line ---------).

addition of amphotericin B at concentrations of 120μg/ml or less, but was increased 3.1-fold at 300μg/ml, 4-fold at 600μg/ml, and 2.3-fold at 1000μg/ml.

Since amphotericin B as used in the clinical setting produces serum amphotericin B concentrations of 2 to 3μg/ml for 2 to 3 days, we next asked whether longer incubations with amphotericin B produced enhanced nitrogen mustard uptake at pharmacological amphotericin B concentrations. To determine this, SKMES-1 human epidermoid carcinoma cells were incubated for 30 min or 24, 48, or 72 hr with amphotericin B. Nitrogen mustard was added for the final 30 min only, and uptake was measured (Fig. 2). The uptake of nitrogen mustard in control cells incubated without amphotericin B was 35.9 ± 0.72 ng nitrogen mustard per 5 x 10^4 cells. Although amphotericin B at a concentration of 4μg/ml did not significantly increase nitrogen mustard uptake after a 30-min incubation, uptake was significantly increased after 24 or 48 hr incubations. In comparison, amphotericin B at concentrations of 40 or 240μg/ml increased nitrogen mustard uptake after 30 min, 24 hr, or 48 hr incubations. The

Table 1
Nitrogen Mustard Uptake by
Ovarian Carcinoma Cells

Amphotericin B Concentration (μg/ml)	{^{14}C}-Nitrogen Mustard Uptake (ng/30 min/5x 10^4 cells)
0	37.7
4	53.6
40	74.0
240	72.2

Human ovarian carcinoma cells from neoplastic ascites (patient MN) were incubated at a concentration of 5 x 10^4 cells per 0.5 ml with amphotericin B as described above for 24 hr. {^{14}C}-nitrogen mustard was added for the final 30 min and uptake was measured during the final 30 min.

increase in nitrogen mustard uptake induced by amphotericin B at the lower concentration (4μg/ml) when incubated for 48 hr was greater than the enhancement produced by higher concentrations of amphotericin B incubated for 30 min or 24 hr. Very prolonged incubations with amphotericin B for 72 hr interfered with accumulation of nitrogen mustard since no concentration of amphotericin B enhanced nitrogen mustard uptake above that of control cells incubated without amphotericin B. This may have been due to cytotoxicity at 72 hr, but no cytotoxic effects were evident at 24 or 48 hr.

To confirm that human carcinoma cells obtained directly from a patient also demonstrated enhancement of nitrogen mustard uptake at low concentrations of amphotericin B, human ascites ovarian carcinoma cells from a patient were incubated with amphotericin B for 24 hr (Table 1). Nitrogen mustard was added for the final 30 min and uptake was determined. All concentrations of amphotericin B enhanced nitrogen mustard uptake in such cells, although higher concentrations were more effective at the 24 hr time point, a result consistent with effects seen in SKMES-1 cells.

DISCUSSION:

Amphotericin B produced a dose- and time-dependent increase in nitrogen mustard uptake in all human tumor cells studied, including both tissue culture human tumor cells and ascites tumor cells from patients with ovarian carcinoma. Kinetically, this was due in cultured colon carcinoma cells to an increase in the apparent V_{max} of transport with no appreciable change in the apparent K_m. This could represent an increased number of transport sites, or an increased capacity of each site to transport nitrogen mustard.

When cells were incubated with amphotericin B for only 30 minutes, the amphotericin B concentration required to increase nitrogen mustard uptake was markedly higher than the concentration which can be achieved in man with amphotericin B infusion (approximately 3μg/ml). However, in clinical trials in man (7,8), amphotericin B was given for several days prior to other drugs. In the present study, longer incubations with amphotericin B at low concentrations produced enhanced nitrogen mustard uptake. Indeed, enhancement by a low concentration of amphotericin B after a 48 hr incubation was greater than or equal to the enhancement by higher concentrations after 30 min or 24 hr incubations.

Resistance of long-term cultured cell lines to nitrogen mustard is characterized in part by decreased nitrogen mustard uptake (5,9,10). It is theoretically possible, considering our findings, that amphotericin B could reverse the resistance of these cell lines to nitrogen mustard. Amphotericin B has recently been shown to increase the antileukemia effects of nitrogen mustard and other alkylating agents (F. Valeriote, T. Vietti, and D. Coulter, personal communication).

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