Eur J Cancer Clin Oncol, Vol. 24, No. 2, pp. 117–121, 1988.
Printed in Great Britain

0277-5379/88$3.00+0.00
© 1988 Pergamon Journals Ltd.

Perspectives and Commentaries

Amphotericin B as a Potentiation Agent to Cytotoxic Chemotherapy

A. COUNE

Service de Médecine Interne et Laboratoire d'Investigation Clinique H.J. Tagnon, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, 1 rue Héger-Bordet, 1000 Bruxelles, Belgium

(A COMMENT ON: Presant CA, Multhaus P, Metter G. Reversal of cancer chemotherapeutic resistance by amphotericin B. Broad phase I–II pilot study. *Eur J Cancer Clin Oncol* 1987, **23**, 683–686.)

DEVELOPMENT of tumor cells resistant to chemotherapeutic drugs prevents the successful eradication of most human disseminated malignancies. Known mechanisms of drug resistance are many: defective drug transport, defective drug metabolism to active species, altered intracellular nucleotide pools, increased drug inactivation, altered DNA repair, gene amplification and altered target protein. Resistance to a given drug can result from several unrelated mechanisms. Moreover, resistance to a specific agent belonging to the groups of antitumor antibiotics or plant alkaloids can confer cross-resistance to structurally dissimilar drugs with different mechanisms of action. The pathway by which this pleiotropic resistance is acquired by tumor cells appears to be at the level of membrane transport. In membranes of hamster, mouse and human tumor cell lines that display multiple resistance to drugs, increased expression of a 170,000 dalton surface antigen has been shown to be correlated with resistance. A P-glycoprotein of identical molecular size, sharing some immunogenic homology with this membrane component, has been demonstrated in colchicine-resistant Chinese hamster ovarian cells. In these cells the colchicine resistance, the pleiotropic resistance and a reduced drug accumulation in the cells have been shown to be due to the same genetic alteration. The P-glycoprotein marker for drug resistance has also been demonstrated in the membrane of cells directly derived from ovarian cancer in two patients [1]. Pleiotropic resistance has been shown to be reversible in some conditions by compounds such as calcium channel blockers or calmodulin inhibitors. Therefore it is not surprising that amphotericin B, an antifungal antibiotic known for its interaction with cell membrane components, was investigated for reversal of cancer chemotherapeutic resistance as reported in the paper by Presant *et al.* [2].

Amphotericin B is a macrolide polyene antibiotic used for the treatment of disseminated fungal diseases. Its action on cells is characterized by a decrease in intracellular potassium ions resulting from the modification of the membrane permeability. As bacteria do not contain sterol and are insensitive to amphotericin B, it was considered that the mechanism of action of the antibiotic was an interaction with the fungal membrane sterol. This hypothesis was supported by the demonstration of an amphotericin B–sterol interaction in water or in water–methanol mixtures as well as by the sensitivity of natural and artificial membranes to amphotericin B. However, experiments conducted in recent years have demonstrated that amphotericin B was able to bind to membranes containing no sterol and to induce cell permeability in sterol-free model membranes. In fungi no exact correlation was found between sterol level and sensitivity to amphotericin B. These results indicate that the natural membrane sensitivity to amphotericin B is not determined by the level of a single membrane component, but by overall membrane organization including the level of sterols. Morphological alterations induced in membranes by

Accepted 13 July 1987.

amphotericin B have been shown by freeze-etch electron microscopy, but there is no definite proof that these changes are responsible for the enhanced permeability of the membrane. In fact, it is generally considered that aqueous channels formed by the antibiotic and the sterol molecules are the cause of the increased permeability of the membrane [3].

Amphotericin B has a relative specificity for fungi due perhaps to its greater affinity for ergosterol, the sterol of fungi membranes, than for cholesterol, the sterol found in animal cell membranes. Although amphotericin B is less toxic for animal cells, it does interact with their membranes. Therefore, despite the lack of an established antitumor activity, amphotericin B has been investigated *in vitro* and *in vivo* as a possible potentiation agent of chemotherapeutic drugs, because it may increase their intracellular levels.

RNA synthesis measured by [^{3}H]uridine incorporation in mouse L cells incubated *in vitro* with a combination of BCNU and amphotericin B was markedly inhibited whereas each compound tested alone at the same concentration had no effect. The enhanced efficacy of the combination was also demonstrated by viability studies of mouse L cell cultured *in vitro* [4].

HeLa cells, selected for their resistance to actinomycin D, became sensitive to the cytotoxic effects of the drug after *in vitro* incubation with amphotericin B, as shown by loss of viability, typical morphological changes and inhibition of RNA synthesis. It was demonstrated that treatment of these HeLa cells with amphotericin B resulted in an increased cellular uptake of labelled [^{3}H]actinomycin D [5].

Using laser flow cytometry it has been shown that coincubation of murine leukemia P388 cells with amphotericin B resulted in an increase in doxorubicin retention. This increase was larger in cells sensitive to doxorubicin than in cells resistant. Such an enhanced drug retention was also demonstrated in splenocytes and in some bone marrow subpopulations [6]. Potentiation by amphotericin B of dipyramidole, a drug used in the treatment of some vascular diseases, has also been shown in malignant cells. Stationary phase rat hepatoma 3924A cells, contrary to the lag and log phase cells, are insensitive to dipyridamole that has an inhibitory effect on nucleoside incorporation in sensitive cells. Amphotericin B was able to restore the sensitivity in stationary phase cells and to increase the cytotoxicity of this drug to hepatoma cells. Identical results were obtained on human colon cancer HT-29 cells [7]. In an established human ovarian cancer cell line, amphotericin B did not potentiate the cytotoxicity of melphalan and doxorubicin. In a soft agar cloning assay using fresh human ovarian cancer cells, only a minor potentiation effect of these drugs by amphotericin B was observed in nine of 15 specimens [8]. Amphotericin B was able to increase cellular uptake of [^{14}C]nitrogen mustard in HT-29 human colon carcinoma cells, human ovarian carcinoma cells and SKMES-1 human epidermoid carcinoma cells [9]. Recently interesting results on the synergism between amphotericin B and two different cytotoxic drugs—actinomycin D and CCNU—on the human promyelocytic leukemia cell line HL-60 have been reported [10]. Both combinations induced toxicity to HL-60 cells as measured by their effect on [^{3}H]thymidine incorporation and on total cell number. The potentiation of actinomycin D by amphotericin B was correlated with an increase in the uptake of [^{3}H]actinomycin D into the cells whereas an increase of [^{14}C]CCNU could not be demonstrated. Further experiments suggested that CCNU or products of its decomposition made cells more vulnerable to amphotericin B oxidative injury. These *in vitro* data indicate that a synergistic action of combinations of amphotericin B and different cytotoxic drugs does not result from a unique mechanism of action.

In the last 15 years experiments conducted in animals on potentiation of cytotoxic drugs by amphotericin B have pointed out the complex basis of this effect. Amphotericin B has been shown to potentiate the activity of cytotoxic drugs from different classes in AKR mice bearing a transplantable, widely disseminating lymphocyte leukemia [11]. Quantitative assessment of the potentiation index by a spleen colony assay showed a large range of values from 3 for BCNU to over 1000 for CCNU. A dose–response effect was observed for BCNU and doxorubicin. Most phase-specific drugs showed a low level of potentiation by amphotericin B. An increased life-span of animals treated with combinations of amphotericin B and cytotoxic drugs was observed and correlated with the potentiation index measured by the spleen colony assay, with the exception of vincristine and BCNU. Despite a low potentiation index the amphotericin B and BCNU regimen produced a cure rate at 28 days ranging from 0 to 80% according to dose levels and treatment schedules. Potentiation by amphotericin B was also investigated in CD2F1 mice bearing L1210 leukemia. This leukemia was somewhat less sensitive to cytotoxic drug potentiation [12]. Results obtained on the potentiation by amphotericin B of melphalan in L1210 leukemia were not confirmed by another group of investigators [8], but the schedules of administration of amphotericin B were different in the two studies. In BALB/c mice having received MOPC-315 cells i.v., a transplantable myeloma model, potentiation of CCNU and melphalan by amphotericin B was demonstrated using a spleen colony assay but no

potentiation of BCNU could be shown [13]. Potentiation is not limited to animal hematological malignancies but has also been demonstrated in three animal solid tumor systems, a situation much closer to that encountered in daily clinical oncology. In Lewis lung carcinoma growing in BDF or C57B1/6 mice, a marked potentiation of the cytotoxicity of CCNU by amphotericin B was demonstrated by tumor growth curves and calculation of cell survival [13]. In C3HeB/FeJ mice bearing a transplantable murine ovarian cancer simultaneous administration of amphotericin B and doxorubicin resulted in long-term survival in 83% of mice compared to 65% of animals treated with doxorubicin alone ($P = 0.06$). Potentiation of melphalan was also shown in this model, the combination treatment producing long-term survival in 44% of mice compared to 7.5% of mice treated with melphalan alone ($P < 0.05$) [8]. In this latter case amphotericin B administration had to precede the cytotoxic treatment in order to induce potentiation. In C51BL mice bearing a subcutaneous transplanted murine ependymoblastoma O1B111, administration of amphotericin B 10 h before im injection of CCNU resulted in an increase of the 2-month cure rate from 15 to 58% ($P < 0.01$), although no increased cellular uptake of [^{14}C]CCNU was measured [14], a situation similar to that encountered in *in vitro* experiments with HL-60 cells. Experiments conducted in AKR mice bearing a transplantable lymphocytic leukemia have disclosed that, at least in some conditions, the mechanisms of potentiation of the activity of a cytotoxic drug by amphotericin B may be multiple and involve modifications of the host response [15]. Survivors at the end of a 28-day follow up period were resistant to further challenge with 10^6 leukemic cells, a dose that resulted in a fatal leukemia by day 6 in all untreated control animals. The resistance to AKR leukemia could be transferred to untreated mice by spleen cells but not by bone marrow cells or by serum of mice cured with the amphotericin B-BCNU regimen. Moreover, potentiation could be prevented by induced immunosuppression by preirradiation or cyclophosphamide. These data support an immune basis of the antitumor activity of the amphotericin B-BCNU regimen in the AKR leukemic mice.

Modification of the host response by amphotericin B suggested by potentiation experiments in animal tumor systems should not have been a great surprise because of the different actions of the antibiotic according to its concentration on fungi and animal cells [3]. These actions range from a stimulatory effect to a lytic or killing effect. Amphotericin B can stimulate the function of specific cell types such as polyclonal B lymphocytes, can activate macrophages to kill bacteria and parasites and can modulate the macrophage tumoricidal capability. In experiments carried out in inbred strains of mice, amphotericin B has been shown to increase the immune response to several different antigens by probably enhancing the immune reactivity of lymphoid cells and having a selectively toxic activity for suppressor T cells. However, the use of different experimental conditions may result in imnunosuppressive effects.

Amphotericin B is an amphiphilic compound that is not soluble in water. Because experiments reported previously were performed using Fungizone®, a deoxycholate complex of amphotericin B that forms a colloidal dispersion when hydrated, one might wonder whether the bile salt did not play a role in the potentiation of cytotoxicity by amphotericin B. In fact, it was demonstrated that deoxycholate alone was devoid of potentiation effect and that amphotericin B methylester, a water-soluble derivative of the antibiotic, and other polyene antibiotics could potentiate the activity of cytotoxic drugs [12].

Whatever mechanisms might be operating in animal tumor systems to induce potentiation of cytotoxic activity by amphotericin B, the significant results obtained in AKR mice several years ago incited clinical investigators to start therapeutic trials in patients with disseminated cancer. Contrary to what could be expected from animal experiments, no potentiation of CCNU by amphotericin B was observed in 13 patients with colon carcinoma and five with renal carcinoma [16]. In 37 patients with a lung non small cell carcinoma, randomized between a combination of doxorubicin, CCNU, hexamethyl melamine and methotrexate and the same combination with amphotericin B, a higher objective response rate was obtained with the latter regimen (39% vs. 23%), but the difference was not statistically significant; moreover, the median response duration and the survival of the group receiving amphotericin B were much shorter, respectively, 3 vs. 7 months and 4 vs. 8 months [17]. These data may suggest a possible toxic effect of amphotericin B. In fact, in the group receiving this compound, a greater hematological toxicity was observed. In another randomized trial, 94 patients with a bone or a soft tissue sarcoma were randomized between doxorubicin, cyclophosphamide and methotrexate (ACM) and the same combination with amphotericin B [18]. A significant difference ($P < 0.05$) was observed in the response rates between the two arms: 4% complete response and 34% partial response in the ACM group and only 5% partial response in the arm receiving ACM and amphotericin B. Despite this unexpected difference in efficiency, no differences in median time to progression and in survival were reported. Seven

patients not responding to ACM were crossed over to the ACM plus amphotericin B regimen but remained resistant.

In the paper by Presant *et al.* [2], the possible reversal of resistance to cytotoxic therapy is addressed, a possibility also investigated by Krutchik *et al.* who had shown one antitumor response induced by the addition of amphotericin B to chemotherapy in 14 patients suffering from refractory disseminated breast carcinoma or sarcoma [19]. Presant *et al.* show that in evaluable patients amphotericin B was able to reverse resistance in one patient not responding to a single drug and in five patients not responding to drug combinations. The overall response rate of 12% was rather low and was probably not due to the great array of different malignant tumors included in this trial, as suggested by the results obtained in the other clinical studies. How unsatisfactory this low rate of potentiation might be, the results should not be dismissed without further consideration, because the advent of resistance to combination chemotherapy is associated with a bad prognosis in patients with a disseminated solid tumor.

As stressed previously, mechanisms of resistance are multiple and only a few of them can probably be modified by amphotericin B. Data from animal tumor systems indicate that potentiation by amphotericin B is not consistent in all tumor lines, that innate sensitivity to a given cytotoxic drug is not predictive of potentiation by amphotericin B and that dose and time schedules of administration are main factors for inducing chemotherapy potentiation. In some *in vitro* systems the concentration of amphotericin B needed to increase the cell membrane permeability is of about 20 μg/ml or even higher, values never reached in the serum of patients treated with Fungizone®. Conflicting results observed in different animal tumor systems might possibly be due at least in some cases to the immunosuppressive properties of amphotericin B that might counteract its potentiation effects. Finally synergistic effects of one cytotoxic drug and amphotericin B were due to an unexpected mechanism, i.e. potentiation by the drug of the oxidative injury induced in the malignant cells by amphotericin B. From all these points it appears that adequate design of further clinical trials will require more information on the potentiation mechanisms of amphotericin B at the cell level and of its modulating properties on the immune system. However, at the present time use of new delivery systems for the administration of amphotericin B should be considered as a possible answer to some of the challenges we are faced with. As intravenous infusion in man of amphotericin B entrapped in liposomes has resulted in unexpectedly high serum concentrations and as these lipid vesicles are well known for their interaction with cells of the monocyte macrophage system, the use of this carrier should be tested in further investigation of the potentiation properties of amphotericin B to cytotoxic chemotherapy [20].

REFERENCES

1. Bell DR, Gerlach JH, Kartner N *et al.* Detection of P-glycoprotein in ovarian cancer: a molecular marker associated with multidrug resistance. *J Clin Oncol* 1984, **3**, 311–315.
2. Presant CA, Multhaus P, Metter G. Reversal of cancer chemotherapeutic resistance by amphotericin B. Broad phase I–II pilot study. *Eur J Cancer Clin Oncol* 1987, **23**, 683–688.
3. Medoff G, Brajtburg J, Kobayashi GS. Antifungal agents useful in therapy of systemic fungal infections. *Ann Rev Pharmacol Toxicol* 1983, **23**, 303–330.
4. Medoff G, Schlessinger D, Kobayashi GS. Polyene potentiation of antitumoral agents. *J Natl Cancer Inst* 1977, **50**, 1047–1050.
5. Medoff J, Medoff G *et al.* Amphotericin B-induced sensitivity to actinomycin D in drug resistant HeLa cells. *Cancer Res* 1975, **35**, 2548–2552.
6. Krishan A, Saverteig A, Gordon K. Effect of amphotericin B on adriamycin transport in P388 cells. *Cancer Res* 1985, **45**, 4097–4102.
7. Zhen YS, Reardon MA, Weber G. Amphotericin B renders stationary phase hepatoma cells sensitive to dipyridamole. *Biochem Biophys Res Comm* 1986, **140**, 434–439.
8. Ozols RF, Hogan WM, Grotzinger KR *et al.* Effects of amphotericin B on adriamycin and melphalan cytotoxicity in human and murine ovarian carcinoma and in L1210 leukemia. *Cancer Res* 1983, **43**, 959–964.
9. Presant CA, Carr D. Amphotericin B (Fungizone) enhancement of nitrogen mustard uptake by human tumor cells. *Biochem Biophys Res Comm* 1980, **93**, 1067–1073.
10. Vertut-Croquin A, Brajtburg J, Medoff G. Two mechanisms of synergism when amphotericin B is used in combination with actinomycin D or 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea against the human promyelocytic leukemia cell line HL-60. *Cancer Res* 1986, **46**, 6054–6058.
11. Medoff G, Valeriote F, Dieckman J. Potentiation of anticancer agents by amphotericin B. *J Natl Cancer Inst* 1981, **67**, 131–135.
12. Valeriote F, Medoff G, Tolens S, Dieckman J. Amphotericin B potentiation of the cytotoxicity of anticancer agents against both normal hematopoietic and leukemia cells in mice. *J Natl Cancer Inst* 1984, **73**, 475–482.

13. Valeriote F, Dieckman J, Flentje D *et al.* Potentiation by amphotericin B of the cytotoxicity of anticancer agents against MOPC-315 plasmocytoma and Lewis lung carcinoma. *Cancer Chemother Pharmacol* 1984, **13**, 126–130.
14. Laurent G, Atassi G, Hildebrand J. Potentiation of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea by amphotericin B in murine ependymoblastoma. *Cancer Res* 1976, **36**, 4069–4073.
15. Medoff G, Valeriote F, Ryan J, Tolen S. Response of transplanted AKR leukemia to combination therapy with amphotericin B and 1,3-bis(2-chloroethyl)-1-nitrosourea: dose and schedule dependency. *J Natl Cancer Inst* 1977, **58**, 949–953.
16. Presant CA, Farbstein M, Multhaus P *et al.* Lack of clinical synergism with amphotericin B and CCNU in renal and colorectal carcinomas. *Eur J Cancer Clin Oncol* 1986, **22**, 329–330.
17. Presant CA, Metter GE, Multhaus P *et al.* Discordant effects of amphotericin B and combination chemotherapy on response rates and on survival in non-small cell carcinoma of the lung. *Cancer Treat Rep* 1984, **68**, 651–654.
18. Presant CA, Lowenbraun S, Bartolucci AA. Effect of amphotericin B on chemotherapy of metastatic sarcomas. *Cancer* 1984, **53**, 214–218.
19. Krutchik AN, Buzdar AU, Blumenschein GR *et al.* Amphotericin B and combination chemotherapy in the treatment of refractory metastatic breast carcinoma and sarcoma. *Cancer Treat Rep* 1978, **62**, 1565–1567.
20. Sculier JP, Coune A, Meunier F *et al.* Pilot study of amphotericin B entrapped into sonicated liposomes in cancer patients with mycotic complications. *Proc ASCO*, Los Angeles, 1986, No. 964.