INABILITY OF AMPHOTERICIN B(FUNGIZONE) OR OF SODIUM DEOXYCHOLATE TO ALTER NATURAL DRUG RESISTANCE OR SENSITIVITY OF TRANSPLANTED MURINE LEUKEMIAS

U. K. GANU, D. D. KHANDALEKAR AND S. V. GOKHALE*

Chemotherapy Division,
Cancer Research Institute,
Tata Memorial Centre, Parel, Bombay - 400 012

(Received on January 11, 1984)

Summary : The ability of Amphotericin B ('Fungizone') to alter the natural resistance of leukemia L1210 to vincristine was studied. in BDF_1 mice Neither Fungizone nor the "solubilizing agent" sodium deoxycholate, when used in combination with vincristine potentiated the activity of the drug against L1210. There was no change in the activity pattern of 5-fluorouracil against L1210 or vincristine against P388 lymphocytic leukemia respectively, which are sensitive to these drugs. Thus, both Fungizone and sodium deoxycholate failed to improve the activity of the drugs in either a naturally resistant or sensitive murine leukemia *in vivo*.

Key words : vincristine, amphotericin B (Fungizone), L1210 leukemia, 5-fluorouracil, P388 leukemia, sodiumdeoxycholate, natural resistance

INTRODUCTION

Amphotericin B (Fungizone) is a polyene antifungal antibiotic derived from *Streptomyces nodosus*. Although it does not possess anticancer activity, it has been shown to potentiate the anticancer activity of a number of cytotoxic agents, both in tissue culture systems (16,28) and in mice bearing AKR leukemia (17, 19). This synergistic effect has been attributed to the immune adjuvant properties of Fungizone (17,27). It has also been shown to induce sensitivity in actinomycin-D resistant HeLa cells (18). This transformation of resistant into sensitive cell lines is supposedly brought about by an increased cell permeability to the drugs due to Fungizone resulting into increased intracellular levels of the cytotoxic agents (11, 15).

On the basis of these observations, Phase I and Phase II clinical trials were conducted by several workers with doxorubicin, carmustine and cyclophosphamide in combination

*Requests for reprints

with Fungizone on breast carcinoma and sarcomas (20, 23, 24, 25, 29). However, the results have not proved unequivocally the efficacy of Fungizone in rendering the drug-resistant cells sensitive to the treatment (14, 20).

Although potentiation of the effect of doxorubicin and Melphalan by Fungizone was observed in murine ovarian cancer, similer results were not observed in human ovarian cell lines (20). Further, Fungizone was unable to potentiate the activity of Melphalan against L1210 leukemia in either the drug-sensitive or the drug-resistant cell line (20).

Medoff *et al.* (19) had observed cures in mice bearing AKR leukemia on a dose of vincristine (VCR) equivalent to human dose, when combined with Fungizone. The results were similar to those with doxorubicin. VCR is a leading agent in the curative treatment of acute lymphoblastic leukemia in humans, but is ineffective against L1210 leukemia in mice (6). Hence, the capacity of the combination of Fungizone and VCR to reverse this natural resistance was studied and has been presented in this communication. The possibility that natural sensitivity may be enhanced was also tested using the Fungizone-VCR combination against P388 leukemia and using Fungizone-5-fluorouracil (5-FU) combination against L1210 leukemia since these leukemias are naturally sensitive to VCR and 5-FU, respectively.

MATERIAL AND METHOD

Drugs : The drugs used were Amphotericin B (Fungizone, intravenous, Sarabhai Chemicals, Baroda, India), sodium deoxycholate (SDC; L. Light & Co. Ltd., Colnbrook, England), 5-fluorouracil (Biochem Laboratories, Bombay) and vincristine sulphate ('Cansovin', Immu-Kimia Laboratories, Bombay).

Tumours : Leukemia L1210 and P388 lymphocytic leukemia were used. The tumours were maintained in DBA/2 mice. For the purpose of testing BDF_1 mice (DBA/2 ♂♂ x C57BL/6 ♀♀ F_1 generation) were used.

The inoculum in case of L1210 was 10^5 cells/mouse while in case of P388, each mouse received 10^6 cells in 0.1 *ml* of the inoculum under aseptic conditions. Each group comprised of 6 animals unless stated otherwise. Treatment was started after 24 hr of tumour implantation. *Mean survival time* (days) was calculated for L1210 while *median survival time* (days) was calculated for P388. For calculation of activity of a treatment, these results were also expressed as percent T/C (treatment/control) and the values compared as described by Geran *et al.* (7).

Two different treatment schedules, viz., (a) single injection daily (1–9 days) and (b) injections on day 1,5 and 9 were followed using different doses. In one experiment, a gap of 24 hr was kept between the Fungizone and VCR treatment, as the combination is reported to be schedule-dependant for optimal results (17). Drugs were administered ip. In case of VCR, on two occasions, either Fungizone or VCR was given iv to check the effect of route of administration on the activity.

RESULTS

Table I shows that on day 1–9 schedule, the VCR Fungizone combination showed a marginal potentiation of VCR activity against L1210 leukemia once, which was statistically not significant. The activity could not be observed on day 1,5,9 treatment schedule. Further, there was no effect on the activity pattern when Fungizone was used by ip route and VCR by iv route, or vice versa. Even a gap of 24 hr between the two drugs (Fungizone followed by VCR) failed to show VCR activity-potentiation against the tumour.

Table II shows the effect of SDC and fungizone on the activity of 5-FU or VCR against L1210 or P388 leukemia. There was no increase in the survival rates.

TABLE I : Effect of Fungizone VCR combination against L1210 leukemia in BDF_1 mice.

Treatment schedule (days)	*Dose (mg/kg)*		*T/C % and Mean survival time (in days)*			
	Fungizone	*Vincristine*	*Control*	*Fungizone*	*Vincristine*	*Vincristine+ Fungizone*
1 to 9	1.25 (ip)	0.5 (ip)	—	105	141	151
			(7.6)	(8.0)	(10.7)	(11.5)
1,5 and 9	2.5 (iv)	1.0 (ip)	—	93	135	105
			(9.8)	(9.1)	(13.2)	(10.3)
-do-[a]	2.5 (iv)	1.0 (ip)	—	107	145	148
			(8.4)	(9.0)	(12.2)	(12.5)
-dp-	2.5 (ip)	1.0 (iv)	—	98	107	106
			(9.0)	(8.83)	(9.67)	(9.5)
-do-[b]	10 (ip)	1.0 (ip)	—	97	136	130
			(7.33)	(7.1)	(10.0)	(9.5)

a = There were 10 animals per group in this set. In all other sets 6 animals per group were taken.

b = A gap of 24 hr was kept between Fungizone and Vincristine, i.e. Fungizone was given on day 1,5, 9 and Vincristine on day 2,6 and 10. In all other cases the drugs injected simultaneously.

TABLE II : Effect of Fungizone and SDC on 5-FU activity against L1210[a] leukemia. and on VCR activity against L1210 and P388[b] leukemias.

Tumour	*Drug*	*T/C % and median survival time (days)*			
		Control	*Only drug*	*SDC+Drug*	*Fungizone +Drug*
L1210	5-FU	— (8.77)	166 (14.6)	165 (14.5)	176 (15.4)
L1210	VCR	— (9.1)	126 (11.5)	117 (10.6)	121 (11.0)
P388	VCR	— (11.0)	195 (21.5)	215 (23.5)	182 (20.0)

a – There were 10 animals per group
b – There were 6 animals per group
VCR – Vincristine
SDC – Sodium deoxycholate (solvent in Fungizone)
5-FU – 5-Flurouracil

DISCUSSION

Extensive work has been carried out on the Fungizone combination *in vitro* and *in vivo* for potentiation of cytotoxic drugs in sensitive as well as resistant cell lines (16, 17, 18, 19, 20, 28).

There are a number of pathways by which a cell can become resistant to anti-cancer agents (4). Inability to cross plasma membrane makes many agents ineffective in different tumour systems e.g. alkylating agents (8, 31), antitumour antibiotics (9), vinca alkaloids (5) and even antimetabolites such as methotrexate (10). Modification of tumour resistance by using surface active agents has been reported in the past. Non-ionic detergents Tween-80 (3, 26) and Triton derivatives (1) and lipid vesicles containing Actinomycin-D (21, 22) have been used to advantage.

Fungizone aided enhancement of nitrogen mustard uptake by human tumour cells *in vitro* and subsequent resensitization of these tumours to the combination chemotherapy regimen to which it had developed resistance has been shown in the past (24, 25).

Although Valeriote *et al.* did not find a manifold increase in the activity of VCR on combining with Fungizone *in vitro* against AKR leukemia cells (unlike doxorubicin) (28), the *in vivo* studies showed cures, similar to those due to doxorubicin (19).

Very little information is available about the transport of VCR in the tumour cells. Bleyer *et al.* (2) carried out studies using ^{3}H labelled VCR in sensitivea and resistant murine leukemias. They have shown that the influx of VCR was, P388 cells $\geqslant\geqslant$ L1210 cells $\geqslant$ P388/VCR resistant cells. The lack of proper transport system for VCR makes

L1210 leukemia naturally resistant to the drug. This provides an excellent system for studying the ability of Fungizone to assist the transport of VCR across the cell membrane.

Kessel (11) had shown that Fungizone and SDC but not amphotericin B alone caused an enhancement of actinomycin-D uptake in murine leukemia cells *in vitro*. This was most marked in L1210 which is relatively insensitive to the drug. Whether Fungizone or SDC alters the permeability of VCR into the L1210 cells could not be ascertained in the present studies due to the nonavailability of the labelles drug.

Our studies, however, do not show any increase In the efficacy of VCR when used in combination with Fungizone over the positive control group. Fungizone contains SDC as a solubilizing agent. SDC itself is a surface active agent and hence, was used in combination with VCR as a proper control for Fungizone solution. The potentiation of anticancer activity by Fungizone has been described to be dose – and schedule-dependent (17). However, in our studies, both Fungizone and SDC failed to improve the anticancer activity of VCR even with different schedules, varied doses or different routes of administration. Fungizone can be said to have failed to alter the natural resistance of L1210 to VCR.

Another drug used in the studies was 5-FU, L1210 leukemia is sensitive to this drug. Also used was P338 lymphocytic leukemia, which is sensitive to VCR. In both cases, however, there was no potentiaion of the activity of the drugs on combination with either Fungizone or SDC.

These results raise a few questions. Polyene antibiotics exert their effect on specific micro-organisms by interacting with their specific lipid content, sterol, of the membranes (12, 13, 30). Such molecules which preferentially interact with Fungizone may be lacking in these tumours.

It may also be possible that the mechanism of natural resistance may not be dependent upon the transport across the cell membrane, but a on difference in binding site or on a rapid egress of the drug molecule out of the cell. The upper limit to sensitivity also does not depend upon transport across the cell membrane.

REFERENCES

1. Allison, A. The role of lysosomes in the action of drugs and hormones In : "Advances in Chemotherapy by Goldin, A., F. Hawking and R.J. Schnitzer. New York & London Academic Press p. 281, 1968.
2. Bleyer, W.A., S.A. Frisby and V.T. Oliverio. Uptake and binding of vincristine by murine leukemia cells. *Biochem. Pharmacol.*, **24** : 633–639, 1975.
3. Bowen, D. and I.D. Goldman. The relationship among transport, intracellular binding and inhibition of RNA synthesis by actinomycin D in Ehrlich ascites cells *in vitro*. *Cancer Res.*, **35** : 3054–3060, 1975.
4. Broackman, R.W. Mechanisms of resistance, In : Sartorelli, A.C. and D.G. Johns (eds.), Antineoplastic Immunosuppressive Agents Vol. 1, pp. 352–410, Berlin: Springer-Verlag, 1974.
5. Creasey, W.A. Modification in biochemical pathways produced by the vinca alkalcids. *Cancer Chemother. Rep.*, **52** : 501–507, 1968.
6. Crowther, D. Cancer Chemotherapy. In "Treatment of Cancer" by Halnan, K.E., London, Chapman and Hall, p. 74, 1982.

7. Geran, R.I., N.H. Greenberg, M.M. MacDonald, A.M. Schumacher and B.J. Abbott. Protocols for screening chemical agents and natural products against animal tumours and other biological systems. *Caner Chemother. Rep.*, Part (3) **3** : 3–103, 1972.

8. Goldenberg, G.J. Properties of L5178 Y lymphoblasts highly resistant to nitrogen mustard. *Ann. N.Y. Acad. Sci.*, **163** : 936–953, 1969.

9. Goldstein, M.N., K. Hamm and E. Amrod. Incorporation of tritiated actinomycin D into drug-sensitive and drug-resistant HeLa cells. *Science*, **151** : 1555–1556, 1966.

10. Johns, D.G. and J.R. Bertino, The Chemotherapeutic Agents, Folate Antagonists: In "Cancer Medicine" Holland J.F. & E. Frei III Phiadelphia, Lea & Febiger, p. 739–1973.

11. Kessel, D. Alteration of cell permeability barriers by amphotericin B–deoxycholate (fungizone) *in vitro*. *Biochem. Pharmacol.*, **25** : 483–485, 1976.

12. Kinsky, S.C. Comparative responses of mammalian erythrocytes and microbial protoplasts to polyene antibiotics and vitamin. *Arch. Biochem. Biophys.*, **102** : 180–188, 1963.

13. Kinsky, S.C., J. Avruch, M. Permutt, H.B. Rogers and A.A. Schonder. The lytic effect of polyene antifungal antibiotics on mammalian erythrocytes. *Biochem. Biophys. Res. Commun.* **9** : 503–507, 1962.

14. Krutchik, A.N., A.U. Buzdar, G.R. Blumenschein, C.K. Tashima, P.Y. Holoye, G. Bodey, and J.G. Sinkovics. Amphotericin B and combination chemotherapy in the treatment of metastatic breast carcinoma and sarcoma. *Proc. Am. Assoc. Cancer Res. and ASCO*, **19** : 14, 1978.

15. Kuwano, M., S. Akiyama, H. Endo and M. Kobyn. Potentiation of fusidic acid and Letinan effects upon normal and transformed fibroblastic cells by amphotericin B. *Biochem. Biophys. Res. Commun.*, **49** : 1241–1248, 1972.

16. Medoff, G., D. Schelessinger and G.S. Kobayashi: Potentiation of Antitumour Agents. *J. Natl. Cancer Inst.*, **50** : 1047–1050, 1973.

17. Medoff, G., F. Valeriote, R.G. Lynch, D. Schlessinger and G.S. Kobayashi. Synergistic effect of Amphotericin-B and 1,3-bis (2-chloroethyl)-1-nitrosourea against a transplantable AKR-leukemia. *Cancer Res.*, **34** : 974–978, 1974.

18. Medoff, J., G. Medoff, M.N. Goldstein, G. Schlessinger and G.S. Kobayashi. Amphotericin B induced sensitivity to Actinomycin D in drug-resistant HeLa cells. *Cancer Res.*, **35** : 2548–2552, 1975.

19. Medoff, G., F. Valeriote and J. Dieckman. Potentiation of anticancer agents by amphotericin *B. J. Natl. Cancer Inst.*, **67 (1)** : 131–135, 1981.

20. Ozols, R.F., W. Micheal Hogan, K.R. Grotzinger, W. McCoy and R.C. Young. Effects of Amphotericin B on adriamycin and melphalan cytotoxicity in human and murine ovarian carcinoma and in L1210 Leukemia. *Cancer Res.*, **43** : 959–964, 1983.

21. Papahjadjopoulos, D., G. Poste, W.J. Vail and J.L. Biedler. Use of lipid vesicles as carriers to introduce actinomycin D into resistant tumour cells. *Cancer Res.*, **36** : 2988–2994, 1976.

22. Peterson, R.H.F., J.A. O'Neil and J.L. Biedler. Some biochemical properties of Chinese hamster cells sensitive and resistant to actinomycin D. *J. Cell. Biol.*, **63** : 733–779, 1974.

23. Presant, C.A., C. Klahr, J. Olander and D. Gatewood. Amphotericin B plus, 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) in advanced cancer. *Cancer*, **38** : 1917–1921, 1976.

24. Presant, C.A., C. Klahr and R. Santala. Amphotericin B induction of sensitivity to adriamycin, 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) plus cyclophosphamide in human neoplasia. *Ann. Intern. Med.*, **86** : 47–51, 1977.

25. Presant, C.A., S. Hillinger and C. Klahr. Phase II Study of 1,3-bis (2-chloroethyl)–1-nitrosourea (BCNU), NSC 409962) with amphotericin B in Bronchogenic carcinoma. *Cancer*, **45** : 6–10, 1980.

26. Riehm, H. and J.L. Biedler. Potentiation of drug effect by Tween 80 in Chinese hamster cells resistant to actinomycin D and daunomycin. *Cancer Res.*, **32** : 1195–1200, 1972.

27. Stein, S.H., E.T. Plut, T.E. Shine and J.R. Little. The importance of different murine cell types in the immunopotentiation produced by amphotericin methyl ester. *Cellular immunol.*, **40** : 211–221, 1978.

28. Valeriote, F., G. Medoff and J. Dieckman. Potentiation of anticancer agent cytotoxicity against sensitive and resistant AKR leukemia by Amphotericin B. *Cancer Res.*, **39** : 2041–2045 1979.

29. Vogl. S.E Amphotericin B and CCNU in advanced lung cancer. *Proc. Am. Assoc. Cancer Res. and ASCO*, **18** : 169, 1977.

30. Weissmann G. and Grazia Sessa. The Action of Polyene Antibfotics on Phospholipid cholesterol structures. *J. Biol. Chem.*, **242 (4)** : 616–625, 1967.

31. Wolpert, M.K. and R.W.A. Ruddon. A study on the mechanism of resistance to nitrogen mustard (HN_2) in Ehrlich ascites tumour cells: Comparison of uptake of HN_2–^{14}C into sensitive and resistant cells. *Cancer Res.*, **29** : 873–879, 1969.