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

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Summary: The ability of Amphotericin B ('Fungizone') to alter the natural resistance of leukemia L1210 to vincristine was studied in BDF1 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 sodium deoxycholate 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...
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, similar 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 BDF₁ mice (DBA/2 ♂ ♂ x C57BL/6 ♀ ♀ F₁ generation) were used.

The inoculum in case of L1210 was 10⁳ cells/mouse while in case of P388, each mouse received 10⁴ cells in 0.1 ml of the inoculum under aseptic conditions. Each group comprised of 8 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>
<thead>
<tr>
<th>Treatment schedule (days)</th>
<th>Dose (mg/kg)</th>
<th>TIC % and Mean survival time ('n days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fungizone</td>
<td>Vincristine</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Fungizone</td>
</tr>
<tr>
<td>1 to 9</td>
<td>1.25 (ip)</td>
<td>0.5 (ip)</td>
</tr>
<tr>
<td></td>
<td>(7.6)</td>
<td>(8.0)</td>
</tr>
<tr>
<td>1.5 and 9</td>
<td>2.5 (iv)</td>
<td>1.0 (ip)</td>
</tr>
<tr>
<td></td>
<td>(9.8)</td>
<td>(9.1)</td>
</tr>
<tr>
<td>-do-</td>
<td>2.5 (iv)</td>
<td>1.0 (ip)</td>
</tr>
<tr>
<td></td>
<td>(8.4)</td>
<td>(9.0)</td>
</tr>
<tr>
<td>-dp-</td>
<td>2.5 (ip)</td>
<td>1.0 (iv)</td>
</tr>
<tr>
<td></td>
<td>(9.0)</td>
<td>(8.83)</td>
</tr>
<tr>
<td>-do-</td>
<td>10 (ip)</td>
<td>1.0 (ip)</td>
</tr>
<tr>
<td></td>
<td>(7.33)</td>
<td>(7.1)</td>
</tr>
</tbody>
</table>

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 leukemia and on VCR activity against L1210 and P388b leukemias.

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Drug</th>
<th>Control</th>
<th>Only drug</th>
<th>SDC+Drug</th>
<th>Fungizone+Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1210</td>
<td>5-FU</td>
<td>—</td>
<td>(8.77)</td>
<td>166</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td></td>
<td>—</td>
<td>(14.6)</td>
<td>(14.5)</td>
<td>(15.4)</td>
</tr>
<tr>
<td>L1210</td>
<td>VCR</td>
<td>—</td>
<td>(9.1)</td>
<td>126</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td></td>
<td>—</td>
<td>(11.5)</td>
<td>(10.6)</td>
<td>(11.0)</td>
</tr>
<tr>
<td>P388</td>
<td>VCR</td>
<td>—</td>
<td>(11.0)</td>
<td>195</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td></td>
<td>—</td>
<td>(21.5)</td>
<td>(23.5)</td>
<td>(20.0)</td>
</tr>
</tbody>
</table>

- There were 10 animals per group
- There were 6 animals per group
VCR = Vincristine
SDC = Sodium deoxycholate (solvent in Fungizone)
5-FU = 5-Fluouracil

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 anticancer 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, 28) 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 3H labelled VCR in sensitive and resistant murine leukemias. They have shown that the influx of VCR was, P388 cells > L1210 cells > 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 labelled 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 P388 lymphocytic leukemia, which is sensitive to VCR. In both cases, however, there was no potentiation 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


