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# EFFECTS OF CIMETIDINE COMBINATION WITH CYCLOPHOSPHAMIDE IN TRANSPLANTED MURINE TUMORS

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**Abstract** : Studies were carried out on the combination of Cimetidine (CMTD) with Cytoxan (CTX) in three murine tumors. While the combination significantly potentiated the anticancer effect of CTX in L1210 leukemia, the results with P388 leukemia were not significantly different. The results with Lewis Lung Carcinoma showed a consistent reduction in the number of metastases. However, there was no consistent concomitant prolongation in survival. The host strain, biology of the tumour and the drug used in combination with CMTD might be some of the factors responsible for the varied response.

**Key words** : cimetidine, cyclophosphamide, combination therapy, L1210, P388, Lewis lung carcinoma

## INTRODUCTION

Cimetidine (CMTD) is an $H_2$-receptor blocker used in the treatment of duodenal ulcer. It has been shown to cause tumour regression in two patients with metastatic carcinoma of bronchial origin (1). A direct anti-tumour effect has also been observed by several workers with regression of tumour and an increase in survival (2, 3). An immunoregulatory mechanism has been proposed by several workers (4, 5) for the inactivation of suppressor cells, thereby slowing down the development of lung metastases in CMTD treated Lewis Lung Carcinoma (3). CMTD enchancement of T-cell mediated function, thereby immunologically thwarting tumour cell growth has also been indicated (3). However, some investigators have not only ruled out the immunoregulatory properties associated with CMTD, but have observed an enhancement of tumour growth and an increased trapping of tumour cells in the lung (6).

CMTD has been shown to depress the clearance of microsomally metabolised drugs in rodents (7, 8, 9) and man (10, 11, 12, 13, 14). It has also been shown to cause a number of uncommon suppressive haematological reactions, including agranulocytosis (15, 16, 13), pancytopenia (17) and elimination of pruritus in polycythemia vera (18). An accentuation of haematological toxicity in patients treated with Lomustine for brain tumours has also been reported (19). Enhancement of anticancer activity of razoxan, an anticancer drugs, on combination with CMTD in Walker 256 carcinoma has been reported. It has been suggested that there is possibly an effect

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on the tumour blood vessels after the finding of a reduced liver blood flow by Feely *et al* (20). Dorr and Alberts have shown potentiation of cytoxan antitumour activity in combination with CMTD in DBA mice bearing P388 lymphocytic leukemia (21). However, Collins and Hellman (22) did not observe the efficacy of the combination against the solid tumours such as S180, Lewis Lung Carcinoma and L1210 (S.C.).

We have worked on the combination of cytoxan with CMTD against ascitic and solid tumours at our laboratories. The effect of this combination has been presented in this communication, on P388 and L1210 leukemia in $BDF_1$ ($C_{57}BL/6♀ \times DBA/2♂$) strain of mice, and on Lewis Lung Carcinoma in $C_{57}BL/6$ mice.

## METHODS

*Tumours :* Leukemia L1210 and P388, which were serially maintained in DBA/2 mice were used. $BDF_1$ ($C_{57}BL/6♀ \times DBA/2♂$) mice were used for experimental studies. Each mouse received $10^5$ cells in the case of L1210 and $10^6$ cells in the case of P388 in 0.1 ml of the medium by intraperitoneal route on day 0. For Lewis Lung Carcinoma (LL) $C_{57}BL/6$ mice were used for both, maintainance as well as experimental studies. The tumours were transplanted as per the method of Geran *et al* (23). Animals of the same sex were used in a particular set of experiment.

Pure Cimetidine was obtained from Cadilla Laboratories (Ahmedabad, India). This was ground well and used as a fine suspension in 0.5% CMC in normal saline. Cytoxan (Cycloxan, injectable) was purchased from Biochem Pharmaceutical Industries, Bombay.

Animals were randomized after tumour transplantation and kept in four different cages with minimum six animals per cage, The four groups were as follows : (i) Control, (ii) Cimetidine (200 mg/kg), (iii) Cytoxan (50 mg/kg or 30 mg/kg), (iv) Cimetidine (200 mg/kg) and Cytoxan (50 mg/kg).

The schedules of drug administration in mice bearing various tumours are as shown in Table I (Leukemia, P388), Table II (Leukemia L1210) and Table III (Lewis Lung Carcinoma, LL).

TABLE I : Effect of combination of cyclophosphamide (CTX) and cimetidine (CMTD) on Leukemia P388

| *Treatment* | *Dose (mg/kg)* | *Injection 1 schedule (day)* | *MST T/C* | *% T/C* | |
|---|---|---|---|---|---|
| Control | — | — | 10.0 | — | |
| CMTD | 200 | 1—9 | 12.0/10.0 | 120 | |
| CTX | 100 | 1, 5 | (5)[2] | <300 | |
| CTX | 100 | 1, 5 | | | |
| + | + | | 26.5/10.0 | 265 | |
| CMTD | 200 | 1—9 | (2)[2] | | |
| Control | — | — | —/10.0 | — | |
| CMTD | 200 | 1—9 | 10.5/10.0 | 105 | |
| CTX | 50 | 1, 5, 9 | 26.5/10.0 | 265) | |
| CTX | 50 | 1, 5, 9 | | ) | |
| + | + | | 22.5/10.0 | ) | N.S. |
| CMTD | 200 | 1—9 | | 225) | |
| CTX | 30 | 1, 5, 9 | 22.0/10.0 | 220) | |
| CTX | 30 | 1, 5, 9 | | ) | N.S. |
| + | + | | 26.0/10.0 | ) | |
| CMTD | 200 | 1—9 | | 260) | |

(1) One injection of drug (ip) adminstered as indicated each day.

(2) Long term servivore beyond 30 days.

## RESULTS

Table I shows the effect of the combination of CMTD and CTX on P388 leukemia. While there were 5 long (30 day) survivors with a percent T/C of <300 for CTX alone, there were only 2 long (30 day) survivors with a percent T/C of 265 for the combination at a CTX does of 100 mg/kg (d–1 and 5) and CMTD 200 mg/kg (d–1–9) showing a reduction in the activity for the combination. However, at lower doses of CTX (50 mg/kg and 30 mg/kg on day 1, 5, 9), the differences in the activity pattern of CTX alone, and the combination, were statistically not significant.

The effect of the combination of L1210 leukemia has been shown in Table II. The percent T/C for the combination was greater than 300 (4 long term survivors) as against 224 (2 long term survivors) for CTX alone at 100 mg/kg (day 1, 5). Even at a lower dose of CTX (50 mg/kg on day 1, 5, 9) the enhancement in the activity could not be observed.

TABLE II : Effect of combination of cyclophosphamide (CTX) and cimetidine (CMTD) on Leukemia L1210.

| *Treatment* | *Dose (mg/kg)* | *Injection 1 schedule (days)* | *MST T/C* | *% T/C* | |
|---|---|---|---|---|---|
| Control | — | — | 0/8.7 | — | |
| CMTD | 200 | 1—9 | 8.0/8.7 | 92 | |
| CTX | 100 | 1, 5 | 19.5/8.7 (2)[2] | 224) | |
| CTX | 100 | 1, 5 | | ) 3 | |
| + | + | | | 300) | |
| CMTD | 200 | 1—9 | (4)[2] | ) | |
| Control | — | — | 0/9.3 | — | |
| CMTD | 200 | 1—9 | 9.3/9.3 | 100 | |
| CTX | 50 | 1, 5, 9 | 14.4/9.3 | 156) | |
| CTX | 50 | 1, 5, 9 | | ) | |
| + | + | | 19.8/9.3 | ) | p 0.001 |
| CMTD | 200 | 1—9 | | 213) | |
| Control | 200 | 1—9 | 9.3/9.3 | 100 | |
| CTX | 30 | 1, 5, 9 | 12.3/9.3 | 135) | |
| CTX | 30 | 1, 5, 9 | | ) | N. S. |
| + | + | | 13/9.3 | ) | |
| CMTD | 200 | 1—9 | | 143) | |

1. One injection of drug (ip) administratered as indicated each day.
2. Long term survivors beyond 30 days
3. Exact P value cannot be calculated; but it is highly significant.

Table III shows the results on the effect of the combination on the formation of pulmonary metastases in Lewis Lung Carcinoma. A reduction in the number of pulmonary metastases in the combination treated group as compared to the cytoxan treated group shows the efficacy of the combination. However, no consistent enhancement in the life-span of CTX–CMTD combination treated Lewis Lung Carcinoma bearing animals over CTX treated animals could be observed (data not shown). CMTD alone at a dose of 200 mg/kg for days 1–9 did not possess any antitumour activity against all the three tumours tested.

TABLE III : Effect of combination of cimetidine and cyclophosphamide (CTX) on lung metastasis in Lewis Lung carcinoma (LL).

| *Drugs* | *No. of lung metastatic nodules*, c* | *Nodule size* | *% Regression* |
|---|---|---|---|
| Control | 14 | 3 mm | — |
| CMTD | 13 | 3 mm | 7 |
| CTX | 12 | 2-3 mm | 14 |
| CTX[a] + CMTD[b] | 2.3 | 1-2 mm | 83 |

*Average of two sets of experiments

a) Cyclophosphamide 50 mg/kg injected ip on days 1, 5, 9 and 13

b) Cimetidine 200 mg/kg injected ip from days 1 to 9.

c) Animals were sacrificed on day 17.

## DISCUSSION

Inconsistent results ranging from anticancer activity (1, 2, 3) to tumour growth potentiation (2, 24) have been reported for CMTD, in the literature. A link between the $H_2$–receptor blockade and potentiation of anticancer activity has been suggested. Collin and Hellman reported the potentiation of anticancer activity of Razoxane, an anticancer drug by CMTD (25, 26), metiamide and ranitidine (22). However, they say that histamine antagonism is necessary for the compound and structural analogs devoid of antagonism do not show potentiation of anticancer activity. The $H_2$–antagonists given alone, did not possess anticancer activity (22). Our results with CMTD concur with the above findings. However, clonidine, a central α–adrenergic st mulant and $H_2$–receptor antagonist neither showed anticancer activity by itself, nor potentiated that of CTX in L1210 or P318 leukemia (unpublished data).

The potentiation of anticancer activity by CMTD of CTX against L1210 leukemia is in accordance with the results of Dorr and Alberts (21). However, our results in P388 leukemia are somewhat inconsistent. These observations are difficult to explain only on the basis of Histamine antagonism

of CMTD. May be, in our case, we have used $BDF_1$ mice in our studies instead of DBA/2 mice as used by Dorr and Albert (21). The strain of the mice used (hybrid against pure) might have some relevance as far as the effect of CMTD on CTX activity potentiation is concerned.

Further, the results on LL are quite inconsistent. While there was reduction in the number of pulmonary metastases, no consistent prolongation in the life-span of LL bearing animals for CTX-CMTD combination over only CTX treated animals was observed. Only CMTD had very little influence upon the metastases to the lungs.

The inconsistent results suggest a mechanism of potentiation which is dependent upon the host strain, the biology of the transplanted tumour and the drug with which Cimetidine in combined. Further studies would be necessary to arrive at firm conclusions.

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