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Effect on natural killer and antibody-dependent cellular cytotoxicity of adjuvant cytotoxic chemotherapy including melphalan in breast cancer

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Summary. *Natural killer (NK) cell activity and antibody-dependent (K) cell activity were studied sequentially in 30 patients with early node-positive breast cancer entered into an adjuvant chemotherapy trial. The drugs used were melphalan, and melphalan with methotrexate, given for 12 months. Estimations were made 3-monthly during chemotherapy, and then at 15 and 24 months to assess recovery. Mean values for NK-cell activity during chemotherapy were significantly lower than the mean pre-chemotherapy baseline value at all time-points from 3 to 15 months, but there was recovery by 24 months. Mean values for K-cell activity during chemotherapy did not appear to differ from the mean pre-chemotherapy value, but variability in individual values was high. Over a 4-year follow-up period, a comparison of 16 patients who did not develop recurrent breast cancer with 14 who did showed that NK-cell activity was significantly lower in the latter group 12 months after the start of chemotherapy.*

Introduction

Adjuvant chemotherapy with cytotoxic drugs given after radical mastectomy prolongs the disease-free interval and survival in early node-positive breast cancer. The initial good results with melphalan [4] were later confirmed with various combinations of drugs [1] and, in the dosages used, the short-term adverse effects of adjuvant chemotherapeutic drugs have proved to be mostly mild and impermanent, but there are few detailed studies of late immunological effects of adjuvant chemotherapy [15]. We have examined short- and long-term effects of adjuvant chemotherapy with melphalan and methotrexate on indices of haematological and immunological function, with attention to the degree of recovery after withdrawal of treatment. We have detected residual long-term deficits, with melphalan held to be responsible, in numbers of circulating blood cells [6], and in T- and B-lymphocytes in blood, but without long-term deficits in immune functional assays [9]. In view of the postulated role of natural cell-mediated immunity in defence against tumours [7, 8], we examined the effects in patients with early breast cancer of adjuvant chemotherapy including melphalan on the natural killer (NK) and antibody-dependent (K) cell activity in vitro of peripheral blood leucocytes.

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Methods

Patients. Thirty patients with breast cancer and histological evidence of axillary node metastases were entered into a randomized adjuvant chemotherapy trial to compare melphalan with melphalan and methotrexate; 14 received melphalan at each cycle in a dose of 0.15 mg/kg for days 1–6 of each treatment cycle, increasing to 0.2 mg/kg per day for days 1–5 if toxicity permitted, so that the total dose per course was 0.9–1.0 mg/kg; 16 received melphalan as above, together with 0.2 mg/kg methotrexate on day 8 of each treatment cycle [6, 9]. Cycles were repeated 6-weekly for 1 year, and doses of drugs were adjusted according to leucocyte counts in peripheral blood. When a patient showed evidence of recurrence of cancer, monitoring measurements were discontinued, so that mean values derived represent only those for patients with no clinically detectable cancer at the time of testing. Blood samples for K- and NK-cell activity were taken immediately prior to the upcoming cycle of chemotherapy.

NK-cell and K-cell activity. The manner of referral of cases to this study precluded estimations before surgery. Thus, the baseline estimations were made after mastectomy and before chemotherapy. The interval between surgery and initiation of chemotherapy was usually 2–4 weeks and was never greater than 6 weeks. According to published reports [10, 16], there should be no residual effects of surgery on K- or NK-cell activity after this interval. Thereafter estimations were made at 3-monthly intervals over 15 months, and finally at 24 months, this being 12 months after cessation of treatment. NK-cell and K-cell activity was measured as previously described [5]. At the time when this study was developed, Chang cells were used as NK-target cells rather than the currently preferred cells of the K562 line. Additional target cell lines were not used in view of the belief that substantial advantages do not accrue by including a range of targets in studies on NK- and K-cell activity [13]. Effector (E) cells were derived as peripheral blood mononuclear cells (PBMN) using Isopaque Ficoll centrifugation of heparinized blood, and target (T) cells from the Chang cell line (Commonwealth Serum Laboratories, Melbourne) using subconfluent cultures and labelling with ^{51}chromium; E : T ratios studied were 100 : 1 and 50 : 1, and the duration of exposure was 18 h. NK-cell activity was estimated with no anti-Chang cell immunoglobulin (IgG) in the assay. K-cell activity was estimated using anti-Chang cell antibody raised by multiple immunization of rabbits with Chang cells in complete Freund's adjuvant (CFA); IgG was

obtained using a column of protein-A Sepharose, and the dilution of immune rabbit IgG in phosphate-buffered saline was chosen, which provided optimal antibody-dependent cytotoxicity. $F(ab')_2$ fragments prepared by pepsin digestion from the immune rabbit IgG [5] did not augment ^{51}Cr release. Spontaneous release (range 23%–30%) and maximum release of ^{51}Cr were ascertained and these data, together with release in the presence of effector cells, were used to measure the percentage cytotoxicity, according to the formula: (E-S ÷ M-S) × 100, where E = experimental, S = spontaneous and M = maximal release respectively.

Presentation of results and statistics. Mean values for NK-cell and K-cell activity were calculated for successive 3-monthly time points from 3 to 15 months after starting chemotherapy, and after 24 months, and were compared with means before treatment. The significance of differences was tested by the Wilcoxon rank test.

When 4 years had elapsed after the start of chemotherapy, each patient was allocated to the group NER, comprising cases with no evidence of recurrence of breast cancer, or the group Rec in which recurrence had occurred; for the Rec group, only data before recurrence was evident were used. Mean values for NK- and K-cell activity from months 0 to 24 were compared in order to ascertain whether the Rec group had lower values in these assays at any time over the 2-year period.

Results

Results for patients treated with melphalan, and patients treated with melphalan and methotrexate, were found not to differ significantly for any time point and hence data for the two groups were combined. A panel of 24 healthy female subjects provided blood for use as a "test control" on each occasion when a study was done. The mean specific lysis ± SD for 20 NK assays on these subjects done over the first 15 months of the study was 20.3 ± 15.1, and for 23 assays done over the second 15 months of the study 27.5 ± 13.0; this higher mean for the normal subjects in the latter half of the study, although not significantly different, may have reflected technical changes with the assay over time. Normal values for females in a previous study in this laboratory were 20% specific lysis for NK-cell cytotoxicity and 12% specific lysis for K-cell cytotoxicity [12]. The fact that mean values for NK- and K-cell activity for the patients before entry for chemotherapy were comparable with those of healthy female subjects indicates that any residual post-surgical depression of NK- or K-cell activity was unlikely.

Successive mean values for NK-cell activity (percentage cytotoxicity) at E : T ratios of 50 : 1 (values for ratios of 100 : 1 were comparable, data not shown) from pretreatment through 3, 6, 9, 12, 15, and 24 months after starting treatment (Table 1) show that means from 3 to 15 months were significantly lower than the mean before treatment and the mean for healthy subjects, whereas at 24 months (12 months after stopping chemotherapy) full recovery had occurred.

Successive mean values for K-cell activity (percentage cytotoxicity) at E : T ratios of 50 : 1 (values for ratios of 100 : 1 were comparable, data not shown) from pretreatment until 24 months after starting treatment (Table 1) show that means after chemotherapy did not differ significantly from the mean before treatment, but when the degree of variability of individual values for K-cell activity was allowed for, it was estimated that the sample size was not sufficiently large to detect an effect of chemotherapy on K-cell activity.

At the time when the last-entered patient had been under observation for 4 years, sequential mean levels of NK- and K-cell activity for the two groups, NER and Rec, over 24 months were compared. The results (Table 1) show that the mean NK activity for the NER group was higher than that of the Rec group at 12 months (15.7 ± 9.9 vs 7.2 ± 6.4, $P < 0.02$) and was higher (non-significantly) at 24 months. There were no clear differences in K-cell activity for the two groups at any time-point (data not shown).

Discussion

The postulated interrelationship between immune capacity and either the genesis or growth-potential of cancer raises the interesting question of effects of adjuvant cytotoxic chemotherapy on indices of immune responsiveness, and whether drug-induced depression can be related to recurrence of cancer after surgical excision. The present study examines these questions in relation to NK-cell and K-cell activity. Before chemotherapy, the patients studied with early breast cancer had no measurable impairment in any of several immunological indices studied [9], in line with our present finding that mean NK- and K-cell activity was comparable with that of

Table 1. Natural (NK cell) cytotoxicity and antibody-dependent (K cell) cytotoxicity (% specific cytotoxicity ± SD) in patients with early breast cancer treated by adjuvant chemotherapy for 12 months and grouped according to whether, after 4 years, there had been no evidence of recurrence of cancer (NER) recurrence (Rec)

Time (months)	NK-cell cytotoxicity			K-cell cytotoxicity (all cases)
	All cases	NER	Rec	
Pre-treatment	24.4 ± 12.3 (19)	23.7 ± 10.5 (8)	25.4 ± 14.3 (11)	10.6 ± 9.2 (20)
3	16.1 ± 11.4 (24)**	17.8 ± 12.9 (13)	14.2 ± 9.6 (11)	17.1 ± 17.2 (24)
6	15.7 ± 7.2 (26)**	15.4 ± 5.9 (13)	15.6 ± 8.5 (13)	17.7 ± 15.3 (25)
9	17.5 ± 11.5 (24)*	18.4 ± 8.4 (11)	16.6 ± 18.5 (13)	14.2 ± 15.5 (22)
12	12.1 ± 9.5 (25)***	15.7 ± 9.9 (13)[a]	7.2 ± 6.4 (12)[a]	19.6 ± 13.3 (23)
15	17.0 ± 9.0 (22)**	17.3 ± 9.5 (15)	17.8 ± 8.8 (7)	34.2 ± 11.6 (21)
24	29.0 ± 18.7 (23)	33.2 ± 20.4 (15)	21.0 ± 12.1 (8)	13.9 ± 16.1 (22)

Brackets indicate number of cases tested; numbers decrease with time in Rec group because of withdrawal of cases upon evidence of recurrent disease

*–*** Significance of difference from pretreatment mean: *, $P < 0.05$; **, $P = 0.01$; ***, $P = 0.001$

[a] Mean for Rec group significantly lower than mean for NER group at 12 months $P < 0.02$

healthy sex-matched controls; this is in accord with other reported studies [3, 10, 16], although evidence is cited for depressed NK activity in early cancer, including lung cancer and melanoma [5], and breast cancer [2].

Cytotoxic drugs would be expected to depress immune responsiveness in the short-term, and this was found to be the case for NK-cell activity in the present study, there being highly significant differences from pre-chemotherapy values. However, full recovery occurred within 3–12 months after treatment was stopped. When patients were grouped according to being free of recurrence for 4 years, the level of NK activity after two years was higher (non-significantly) than the baseline before chemotherapy; this change, if meaningful, is not readily explainable. Substantial depressions of both NK- and K-cell activity were reported be Saijo et al. [14] to occur within weeks after cytotoxic chemotherapy and in their study, there was recovery shortly thereafter. We cannot state, from the present study, whether the observed decrease in NK-cell activity during chemotherapy is attributable to a relative decrease in numbers of NK cells among the population of blood mononuclear cells, or defective activity of these cells, or both. There was a pronounced fall during chemotherapy in numbers in blood of all nucleated cells, including lymphocytes and T- and B-cells [6, 9]; the degree to which NK cells were specifically depleted was not measured, since antisera enabling quantitation of NK cells were not available.

McCredie and MacDonald [10] found that levels of NK- or K-cell activity before, during or 1 year after chemotherapy did not differ significantly between women who did, or did not, have recurrences of breast cancer. In our study, the decrease in NK-cell activity during chemotherapy affected all patients; a comparison of patients who remained free of recurrence with those who did experience recurrence in a 4-year follow-up showed, for one time-point at least (12 months), that NK-cell activity was significantly lower in the latter group. However, this difference does not seem decisive enough to implicate drug-induced depression of NK-cell activity as a risk factor for recurrence of breast cancer, or for the possible occurrence subsequently of secondary malignancy, which is a known risk after treatment with immunosuppressive or cytotoxic drugs [11].

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