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Clinical and Laboratory Evaluation of the Myeloprotective Effect of Medroxyprogesterone Acetate in Head and Neck Cancer

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The action of high-dose medroxyprogesterone acetate (MPA) was studied by analysing the behaviour of colony-forming-unit granulocyte–macrophage (CFU-GM) during chemotherapy. 21 non-pretreated men with locally advanced carcinoma of the head and neck were randomised into two arms: A (11 patients) received three alternating cycles of cisplatin, 5-fluorouracil (CF)/cisplatin, methotrexate, bleomycin, vincristine and then CF every 4 weeks and B (10 patients) were treated with the same schedule plus 1000 mg per day of MPA. MPA was administered 14 days before the start of chemotherapy (day 0) and continued daily up to the 90th day. Bone marrow was harvested in arm A on days 0, +14 and +90, and in B, also on day −14. There was diverse CFU-GM behaviour in the two arms on the 14th day. These data support the hypothesis that the myeloprotective effect of MPA is due to induction of a mitotic rest in the stem cells, which protects them from drug action.
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INTRODUCTION

SINCE THE first report by Pannuti *et al.* [1], it has been observed in clinical trials, for the most part in hormone-related tumours [2–6], that when medroxyprogesterone acetate (MPA) was used in combination with chemotherapy it enhanced marrow cellularity by preventing drug-related haematological toxicity, even though the peripheral bloo count was scarcely modified in some cases. In contrast, Umbach *et al.* [7] demonstrated that MPA does not protect human bone marrow progenitor cells exposed to doxorubicin *in vitro*. Wils [8] studied the use of MPA in combination with chemotherapy in breast cancer patients, both in the adjuvant and advanced settings, noting significant differences in the low haematological toxicities (WHO grade I and II) induced by different chemotherapy treatments [9].

The conflicting results obtained thus far in the limited number of clinical and experimental trials justify further investigations, mainly in non-endocrine-related tumours [10].

Myelotoxicity is one of the major dose-limiting side effects of the cytotoxic drugs normally employed in the treatment of non-hormone-related solid tumours. We, therefore, selected patients with non-hormone-related carcinoma of the head and neck and designed this study with the aim of verifying: (1) the action of MPA on marrow stem cells in the absence of antiblastic treatment by ascertaining the variation in the number of CFU-GM in bone marrow samples; (2) the myeloprotective effect of MPA by comparing those patients receiving chemotherapy alone with the ones receiving chemotherapy in association with MPA.

PATIENTS AND METHODS

From 1 September 1987 to 31 March 1989, 21 male patients with carcinoma of the head and neck were randomised into two treatment arms. Inclusion criteria were: Karnofsky performance status ≥ 60, white blood cells (WBC) $\geq 3500/mm^3$, platelets $\geq 120{,}000/mm^3$, age ≤ 75 years, good hepatic, renal and cardiac function. All patients gave written consent prior to enrolment. Exclusion criteria were: metastatic bone marrow involvement, brain metastases, heart failure, previous myocardial infarction, diabetes, inadequate renal or hepatic function, coagulation disorders, and uncontrolled hypertension.

Arm A received 3 cycles of chemotherapy alone every 4 weeks: cisplatin + 5-fluorouracil (CF), followed by cisplatin + methotrexate + bleomycin + vincristine (CABO), then CF once again. CF comprised cisplatin 20 mg/m^2 and 5-FU 800 mg/m^2 (8h infusion) intravenously on days 1–5. CABO comprised cisplatin 50 mg/m^2 on day 4, methotrexate 40 mg/m^2 on days 1 and 15, and bleomycin 10 U and vincristine 2 mg on days 1, 8 and 15 (all intravenously). Arm B received the same treatment regimen in combination with high-dose MPA (Upjohn) (1000 mg/day) administered orally 14 days before the start of chemotherapy and continuing up to day 90.

Bone marrow samples were taken in the two groups following the experimental scheme shown in Fig. 1. Patients were considered evaluable only if marrow aspirate had been performed on day 14.

Before starting treatment and prior to each chemotherapy cycle a clinical evaluation of the lesions was performed as well as a complete blood count, hepatic and renal function tests, and ECG. X-rays (chest and skeleton) and abdominal ultrasound were done prior to and at the end of treatment. Bone scans were performed only in those cases of doubtful skeletal findings. A computed tomography (CT) scan was performed both at the beginning and end of treatment in cases where it was the only means of diagnosis and evaluation.

The grade of haematological toxicity was classified and response to treatment defined according to standard WHO criteria as suggested by Miller *et al.* [9].

In vitro cloning of committed haematopoietic progenitor cells, being a simple and repeatable test, was used as an index of stem

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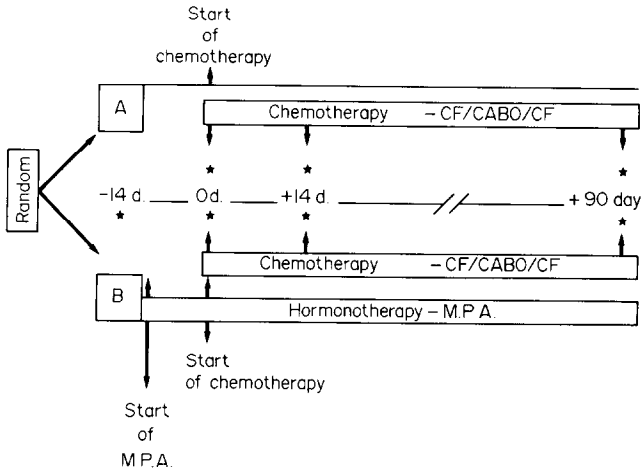

Fig. 1. Design of the study. Group A: chemotherapy CF/CABO/CF without hormonotherapy (not MPA). Group B: chemotherapy CF/CABO/CF with hormonotherapy (MPA). * Bone marrow aspiration.

cell activity [11–16]. 5 ml of bone marrow from the posterior iliac crest were aspirated into heparinised syringes. The specimens were then diluted 1:1 with Iscove's modified Dulbecco's medium (IMDM: Gibco), stratified over Ficoll–Hypaque (FH, 1.077 g/ml Gibco) and centrifuged (1000 *g* for 20 min). Adherent cells were removed by overnight incubation of the cell suspension in IMDM supplemented with 10% fetal calf serum at 37°C and 7% CO_2 in air. Non-adherent cells were cultured in IMDM supplemented with 0.3% agar (Difco) and 10% partially purified human placental granulocyte–macrophage colony-stimulating factor (GM-CSF). The number of cells cultured was 2×10^5/ml. The cultures were incubated at 37°C in a humidified atmosphere of 7% CO_2 in air. After 10 days of incubation, the plates were examined using an inverted microscope. Groups of 40 or more cells were considered a colony.

Statistical analysis

The data were analysed using the Student's *t*-test for matched data. The behaviour of the CFU-GM in the two arms over a period of time was tested by the repeated measures analysis of variance [17].

RESULTS

The two arms were homogeneous as to age, performance status and number of chemotherapy cycles received.

All 11 patients in arm A had advanced disease at diagnosis and 5 out of 10 patients in arm B had relapsed after surgery. The trend for the number of marrow CFU-GM is shown in Table 1 and Fig. 2. In arm A (chemotherapy alone), a total of 29 marrow aspirations was performed. A decrease in the number of CFU-GM colonies was observed on day 14 of chemotherapy in all but one case, statistically significant according to the Student's *t*-test for matched data ($t = -5.27$, 10 DF, $P < 0.001$). On day 90, 3 out of 7 patients had a CFU-GM value higher than the initial one, but this difference was not statistically significant ($t = 1.46$, 6 DF, P = n.s.).

In arm B (chemotherapy + HD-MPA) (Table 1 and Fig. 3), 37 bone marrow samples were taken; in this group, an additional bone marrow aspiration was performed 14 days before the start of chemotherapy, i.e. on the first day of HD-MPA. After 14 days of MPA alone, the number of CFU-GM colonies decreased

Table 1. Mean number of CFU-GM/2 × 10^5 plated cells (S.E.)

	Timing of bone marrow aspirations (days)			
Patient	Day −14	Day 0	Day +14	Day +90
Arm A				
1	—	116.0 (4.2)	68.3 (8.7)	127.7 (14.5)
2	—	80.0 (9.5)	83.0 (2.5)	133.0 (20.7)
3	—	208.0 (4.2)	88.0 (17.0)	18.7 (2.9)
4	—	216.0 (9.9)	178.5 (10.6)	
5	—	176.5 (3.5)	126.5 (21.9)	83.0 (7.0)
6	—	157.7 (8.5)	122.5 (3.5)	
7	—	176.3 (2.5)	104.6 (14.2)	
8	—	158.3 (5.8)	87.7 (3.5)	176.6 (12.4)
9	—	74.0 (2.8)	53.3 (3.5)	25.3 (3.5)
10	—	142.0 (4.2)	36.7 (4.7)	73.3 (5.7)
11	—	183.0 (11.4)	96.0 (5.3)	
Arm B				
1	192.7 (9.2)	110.0 (2.8)	187.2 (27.8)	207.0 (13.1)
2	112.7 (14.7)	4.0 (2.0)	54.6 (1.5)	
3	152.7 (20.2)	38.0 (4.2)	194.5 (17.7)	
4	77.5 (7.8)	88.0 (2.0)	135.6 (8.1)	124.0 (7.1)
5	109.7 (7.1)	35.3 (12.9)	51.3 (6.1)	125.0 (23.6)
6	32.5 (4.9)	113.3 (5.9)	6.0 (3.6)	36.5 (3.5)
7	67.3 (5.5)	172.0 (11.5)	43.5 (2.1)	103.6 (15.3)
8	109.3 (8.3)	82.0 (6.2)	123.3 (5.5)	126.5 (3.5)
9	102.6 (7.1)	71.7 (3.5)	120.0 (5.5)	
10	162.3 (10.2)	27.0 (4.4)	97.3 (7.0)	166.0 (5.3)

in 7 out of 10 patients and increased in 3 ($t = -1.45$, 9 DF, P = n.s.). An increase in the number of CFU-GM in 8 out of 10 patients was noted after 14 days of chemotherapy whereas a decrease was observed in 2 cases ($t = 1.01$, 9 DF, P = n.s.). The CFU-GM values were higher than the original ones on day 90 in all 7 patients.

A total of 7 patients (4 in arm A and 3 in arm B) did not have a bone marrow aspiration on day 90, either because of refusal or progression of the disease. The repeated measures analysis of variance of bone marrow recovery on day +14 showed a significant difference in favour of arm B ($F = 6.74$, $P = 0.02$) (Table 2), however, no significant difference between the two arms was observed at marrow evaluation on day 90. The lack of

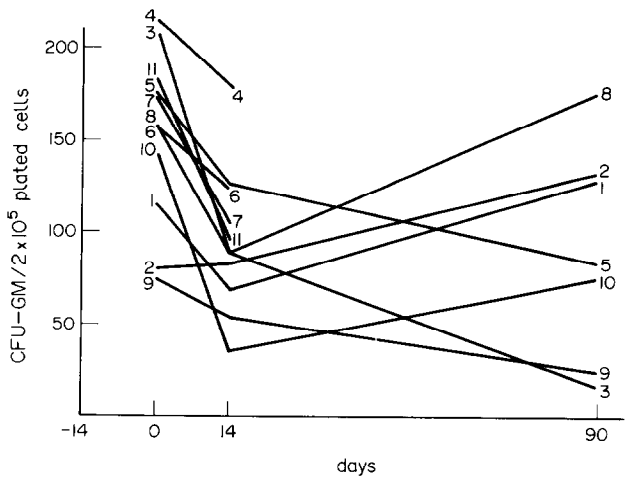

Fig. 2. Effect of cytotoxic chemotherapy on committed granulopoietic stem cells (group A).

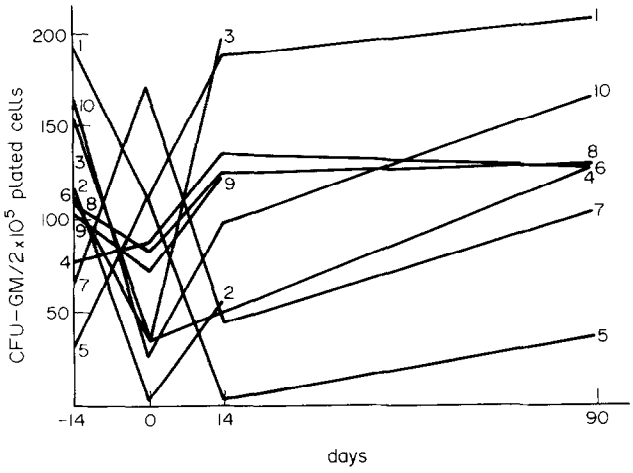

Fig. 3. Effect of medroxyprogesterone acetate on committed granulopoietic stem cells under cytotoxic chemotherapy (group B).

a significant difference between the two arms could be due to the relatively small number of patients undergoing final bone marrow evaluation on day 90, i.e. 7 out of 11 in arm A and 7 out of 10 in arm B.

DISCUSSION

According to Goldie and Coldman's hypothesis [18] the lack of response to chemotherapy could be due to drug resistance induced by mutation of the neoplastic cells. One of the mechanisms to overcome resistance is the use of high doses of drug combinations in alternating regimens of chemotherapy.

Myelosuppression represents one of the dose-limiting factors of cancer chemotherapy. Hence, numerous trials have been carried out with chemotherapy regimens designed to maximise the therapeutic effect with minimal bone marrow toxicity. In recent years colony stimulating factors (CSF) have been employed to selectively stimulate bone marrow stem cells; this new approach seems to be of great interest and is under intensive investigation [19–28].

Medroxyprogesterone acetate (MPA) is widely used at high doses (1–2 g/day) in the treatment of hormone-dependent tumours, and some authors have found it to have a positive antileukopenic effect [1–5, 10]. The antileukopenic mechanism of MPA is still not well understood, and various hypotheses have been proposed to explain its action: (a) the recruitment of multipotent stem cells from the GO phase into the cell cycle; (b) the induction of differentiation of committed progenitor cells into proliferating precursors of granulocytes and megakaryocytes; (c) shifting of leucocytes from reserve compartments to the blood pool; (d) capability to protect committed progenitor stem cells from the cytotoxic activity of antiblastic drugs.

Table 2. CFU-GM plated cells at T.0 and T.14 in the two arms with and without MPA

	Repeated measures analysis of variance				
Source	DF	Sum of squares	Mean square	*F* value	*P*
Between arms	1	13951	13951	6.74	0.02
Among patients in each arm	19	5550	2921	1.41	0.23
Between time	1	3270	3270	1.23	0.28
Interaction arm × time	1	19209	19209	9.28	0.007
Error	19	39325	2069		
Total	41	131259			
Test of hypothesis using mean square for patients in each arm as an error term					
Between arms	1	13951	13951	4.78	0.004

Our results support the hypothesis that the myeloprotective effect of MPA is due to its capability to induce a mitotic rest in stem cells which are thus protected from the action of chemotherapeutic drugs. In arm B, in fact, after 14 days of MPA administration the CFU-GM value on day 0 was nearly always lower than its initial value on day −14. Only 3 cases showed an opposite trend with an increase in the number of CFU-GM.

On day 14, after the first cycle of chemotherapy, the bone marrow harvested from all but 1 patient in arm A (chemotherapy alone) presented a definite decrease in the CFU-GM (no change in 1 case), whereas in arm B (chemotherapy plus MPA) there was a net recovery of the CFU-GM in all but 2 cases. As the 2 patients in question regularly took the required dose of MPA, this leads us to suspect that there were some problems as to absorption or metabolic action of the drug on the stem cells.

As regards our second objective, verification of the myeloprotective effect of MPA during chemotherapy, we can confirm that MPA administration permits a more rapid recovery of the CFU-GM in the stem cell compartment. Even considering the limited number of patients and the not particularly aggressive chemotherapy regimen used, we observed lower haematological toxicity in the peripheral bloodstream in arm B: only 3 cases of grade 1 leukopenia and thrombocytopenia in comparison with 2 cases of grade 2 thrombocytopenia, 1 case of grade 3, and 4 cases of grade 1 leukopenia in arm A.

In conclusion, our results indicate a need for further studies on larger populations in order to assess the relationship between dose intensity and therapeutic efficacy in the treatment of solid tumours.

1. Pannuti F, Martoni A, Pilutri E, Camera P, Losinno F, Giusti H. Massive-dose progestational therapy in oncology (Medroxyprogesterone): Preliminary results. *Panminerva Med* 1976, **18**, 129–136.
2. Robustelli della Cuna G, Bernardo-Strada MR. High dose medroxyprogesterone acetate (HD-MPA) combined with chemotherapy for metastatic breast carcinoma. In: Iacobelli S, DiMarco A, eds. *Role of Medroxyprogesterone in Endocrine-Related Tumors*. New York, Raven Press, 1980, 152 (*Prog Cancer Res Ther* **15**).
3. Gercovich FG, Drewinko B, Morgenfeld E, *et al.* High-dose parenteral MPA prevents granulocytopenia from FAC chemotherapy in advanced breast cancer patients. *Proc Ann Meet ASCO* 1984, 25–196.
4. Lopez M, Di Lauro L, Perno CF, Papaldo P, Barduagni M, Barduagni A. 5-Fluorouracil, adriamycin and cyclophosphamide combined with high-dose medroxyprogesterone acetate in advanced breast cancer. *Tumori* 1983, **69**, 545–551.
5. Pannuti F, Martoni A, Cricca A. Pilot study of the treatment of renal clear-cell carcinoma by high doses of medroxyprogesterone acetate (MPA). IRCS Med Sci. *Cancer* 1978, **6**, 177.
6. Roncoli B, Simoncini E, Marpicati P, *et al.* Decrease of granulocyte-marcophage colony-forming units during high dose medroxyprogesterone acetate treatment in advanced breast cancer. *Chemioterapia* 1985, **4**, 475–477.
7. Umbach GE, Spitzer G, Drewinko B, Gercovich G, Hortobagyi G. Medroxyprogesterone acetate does not protect human bone marrow progenitor cells exposed to adriamycin *in vitro*. *Breast Cancer Res Treat* 1985, 5, 87–89.

8. Wils JA. Myeloprotective effect of high dose medroxyprogesterone acetate (MPA). *Chemioterapia* 1988, **7**, 60–62.
9. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981, **47**, 207–214.
10. MPA-Hematology Italian Cooperative Group. Protective effect of high-dose medroxyprogesterone acetate (HD-MPA) on hematological toxicity induced by chemotherapy for advanced solid tumors: a multi-centric controlled clinical trial. *Chemioterapia* 1986, **5**, 134–139.
11. Metcalf D. *In vitro* cloning of normal and leukemic cells. In *Hemopoietic Colonies. Recent Advances in Cancer Research*. Berlin, Springer, 1977.
12. Burgess AW, Wilson VC, Metcalf D. Stimulation by human placental conditioned medium of hemopoietic colony formation by human marrow cells. *Blood* 1977, **49**, 573–583.
13. Metcalf D. The granulocyte–macrophage colony stimulating factors. *Cell* 1985, **43**, 5–6.
14. PluznikDH, Sachs L. The cloning of normal mast cells in tissue culture. *J Cell Physiol* 1965, **66**, 319–324.
15. Bradley TR, Metcalf D. The growth of mouse bone marrow cells *in vitro*. *Aust J Exp Biol Med Sci* 1966, **44**, 287–299.
16. Pike BL, Robinson WA. Human bone marrow colony growth in agar-gel. *J Cell Physiol* 1970, **76**, 77–84.
17. Winer BJ. *Statistical Principles in Experimental Design*, 2nd edition. New York, McGraw-Hill, 1971.
18. Goldie JH, Coldman AJ. The genetic origin of drug resistance in neoplasms: implications for systemic therapy. *Cancer Res* 1984, **44**, 3643–3653.
19. Vadhan-Raj S, Beuscher S, LeMaistre A, *et al.* Stimulation of hematopoiesis in patients with bone marrow failure and in patients with malignancy by recombinant human granulocyte–macrophage colony-stimulating factor. *Blood* 1988, **72**, 134–141.
20. Antman KS, Griffin JD, Elias A, *et al.* Effect of recombinant human granulocyte-macrophage colony-stimulating factor on chemotherapy-induced myelosuppression. *N Engl J Med* 1988, **319**, 593–598.
21. Nemunaitis J, Singer JW, Buckner CD, *et al.* Use of recombinant human granulocyte-macrophage colony stimulating factor in autologous marrow transplantation for lymphoid malignancies. *Blood* 1988, **72**, 834–836.
22. Devereux S, Linch DC. Clinical significance of the haemopoietic growth factors. *Br J Cancer* 1989, **59**, 2–5.
23. Metcalf D. The colony stimulating factors: Discovery, development, and clinical applications. *Cancer* 1990, **65**, 2185–2195.
24. Groopman JE, Molina J-M, Scadden DT. Hematopoietic growth factors: biology and clinical applications. *N Engl J Med* 1989, **321**, 1449–1459.
25. Laver J, Moore MAS. Clinical use of recombinant human hematopoietic growth factors. *J Natl Cancer Inst* 1989, **81**, 1370–1382.
26. Morstyn G. The impact of colony stimulating factors on cancer chemotherapy. *Br J Haematol* 1990, **75**, 303–307.
27. Theriault R, Frye D, Fraschini G, *et al.* Vinblastine (VLB) and Vinblastine with granulocyte–macrophage colony stimulating factor (GM-CSF) in the treatment of patients (pts) with metastatic breast cancer (MBC). *Proc Ann Meet ASCO* 1990, **9**, A159.
28. Spitzer G, Deisseroth A, Ventura G, *et al.* Use of recombinant human haematopoietic growth factors and autologous transplantation to attenuate the neutropenic trough of high-dose therapy. *Int J Cell Cloning* 1990, **8** (suppl. 1), 249–259.

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Psychological Effects of Participation in a Prevention Programme for Individuals with Increased Risk for Malignant Melanoma

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The Swedish Melanoma Study Group runs a programme aimed at prevention and early detection of premalignant and malignant melanoma in families with two or more members having malignant melanoma. Psychological consequences of participation in this programme were studied. A questionnaire containing items concerning cognitive and emotional responses to the programme was completed by 115 consecutive individuals at their first visit to the clinic. The same questionnaire was administered by mail 7 months later. The levels of psychological and psychosomatic problems were relatively low at both points of assessment. No negative psychological effects were found, neither in the group with dysplastic naevus syndrome (DNS) with increased risk for malignant melanoma, nor in the group without dysplastic naevi. Only one variable, "emotional responses to the visit" differentiated between the groups, with higher scores in the group without DNS. A majority of the individuals expressed positive attitudes to the clinic.
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INTRODUCTION

PREVENTION AND early detection of malignant diseases is a new and growing field in oncology. The aim is to identify individuals having an increased risk of developing a malignant disease in order to allow primary prevention and treatment of the disease at an early stage [1]. Healthy persons will be informed of their increased risk of developing a life-threatening disease, and medical procedures will be recommended for some. This information and suggestions of preventive medical