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Letters

Recombinant Human GM-CSF Enhances the Anti-proliferative Activity of Vitamin D in MCF-7 Human Breast Cancer Clonogenic Cells

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EPIDEMIOLOGICAL AND laboratory evidence suggests that vitamin D may play a role in reducing risk of breast cancer [1]. Also, the presence of 1,25-dihydroxyvitamin D3 (calcitriol, vitamin D) receptors is associated with longer remission duration and disease-free survival in patients with breast cancer [2]. Moreover, topical vitamin D is effective in the treatment of locally advanced or cutaneous metastatic breast cancer [3]. Furthermore, vitamin D inhibits the proliferation of several human breast cancer cell lines, including MCF-7 in which vitamin D receptors are present [2].

Recombinant human granulocyte–macrophage colony-stimulating factor (GM–CSF) stimulates the recovery of granulocytes and reduces the duration of neutropenia following intensive chemotherapy in patients with advanced breast cancer [4], showing that GM–CSF can allow a safe escalation of dose intensity of cytotoxic chemotherapy which may translate into improved therapeutic response in breast cancer patients [5]. Therefore, we investigated the effect of GM–CSF on the antiproliferative activity of vitamin D in MCF-7 cells, in an effort to explore their interaction which could provide the basis for the clinical use of their combination in breast cancer patients.

MCF-7 cells were cultured at 10^4/ml in glass capillaries containing RPMI-1640 medium supplemented with l-glutamine, 15% fetal calf serum and 0.18% agar in a final volume of 30 μl for 9 days at 37°C in a humidified incubator in 5% CO_2. GM–CSF was added at 1–1000 U/ml either alone or with vitamin D at 0.1, 1, 10 and 100 nmol/l. Also, cultures containing vitamin D alone and control cultures containing neither GM–CSF nor vitamin D were set up in conditions otherwise identical. After 9 days, colonies (aggregates of at least 20 cells) were counted in each capillary using a dissecting microscope. IC_{50} was the concentration of vitamin D inducing 50% inhibition of MCF-7 colony formation compared with control cultures, calculated from dose–response curves as described before [6]. The dose reduction index indicates how many fold of dose reduction is needed to achieve a given effect in combination compared with the agent alone [7] and was calculated by dividing the IC_{50} for vitamin D alone by the IC_{50} for vitamin D required in combination with GM–CSF.

Table 1. Effect of GM–CSF on the antiproliferative activity of vitamin D in MCF-7 human breast cancer clonogenic cells

Vitamin D	IC_{50} (pmol/l) Mean*	Median	Dose reduction index(fold)
Alone	1069	1028	—
+ GM–CSF (U/ml)			
1	465	596	2.3
10	234	227	4.6
100	184	190	5.8
1000	68	51	15.7

* Mean values of nine determinations from three separate experiments with S.D. for each value ≤9% of mean.

GM–CSF alone slightly stimulated the proliferation of MCF-7 clonogenic cells; MCF-7 colony numbers increased from [46.1 (3.7) mean (S.D.)] in control cultures to 50.9 (4.1) in GM–CSF-treated cultures, in line with previous findings [8]. GM–CSF enhanced the antiproliferative activity of vitamin D in MCF-7 clonogenic cells (Table 1). GM–CSF plus vitamin D significantly reduced the concentrations of vitamin D alone required to induce the same antiproliferation effect in MCF-7 clonogenic cells by up to 15-fold (Table 1). Similarly, GM–CSF and several other recombinant human cytokines enhanced the antiproliferative and differentiation inducing effects of vitamin D in human myeloid leukaemia cells [7].

The combination of vitamin D with GM–CSF could clinically not only increase the effective drug levels *in vivo* but also limit the toxicity by providing shorter and less toxic courses of treatment in breast cancer patients. Moreover, since both GM–CSF [9] and vitamin D [10] stimulate normal human myelopoiesis, their combination could provide a more effective therapy in stimulating the recovery of myelopoiesis after intensive chemotherapy in breast cancer patients, which may allow a safer dose escalation of cytotoxic drugs and consequently an improved therapeutic response in these patients.

In conclusion, a clinical trial of the combination of GM–CSF and vitamin D is warranted in breast cancer patients.

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Late Onset of Gallbladder Carcinoma with Meningeal Carcinomatosis

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GALLBLADDER CARCINOMA is rare, chiefly a disease of older people and associated in nearly all cases with cholelithiasis. The most common findings at presentation are pain, nausea and vomiting, weight loss and jaundice [1]. We report a case with onset of meningeal carcinomatosis.

A 61-year-old woman presented with pain in the muscles of the left thigh and heaviness of the limb. The symptoms worsened despite treatment with diclofenac. During the third week, pain spread over both legs, with mild paresthesias and dysesthesias, and weakness and progressive difficulty in standing and walking. Computed tomography (CT) showed a degenerate L5-S1 without any alteration of intradural content.

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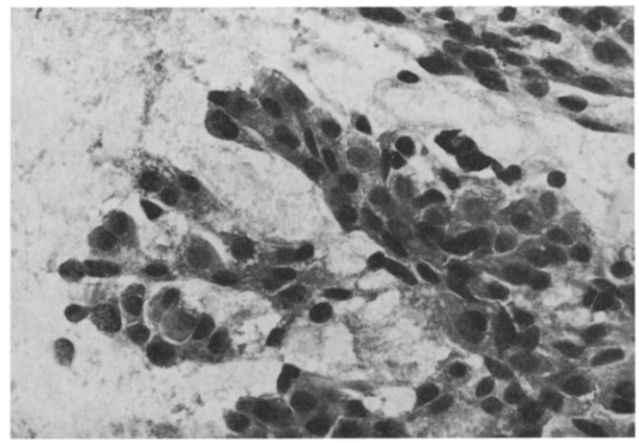

Fig. 1. Polimorphous malignant epithelial cells in clusters with pseudoglandular or papillary configuration.

One month after the onset of symptoms she was admitted. Neurological examination revealed upper limb involvement (slight weakness, hypotonia and mild atrophy) and brick tendon reflexes. The lower limbs were very weak with muscular hypotonia and bilateral atrophy. She could not walk or stand. Abdominal and plantar reflexes were absent. Magnetic resonance imaging of the spinal cord demonstrated thickening of the cauda with roundish nodules on the roots' walls. Administration of contrast agent mildly enhanced the nodules and revealed an irregular enhancement at the origin of the cauda and of the distal portion of the dural sac. These findings were either meningeal carcinomatosis or sarcoidosis.

The cerebrospinal fluid contained malignant cells in clusters or isolated with a pleomorphic appearance. The cytoplasm was basophilic and often vacuolated, suggesting epithelial origin. Meningeal carcinomatosis was diagnosed and the primary tumour was looked for. Serum levels of carcinoembryonic antigen and CA199 were greatly increased, which suggested a primary gastrointestinal tract neoplasm. CT revealed thickened gallbladder wall with a mass protruding into the lumen, invasion of the adjacent quadrate lobe of the liver and metastatic nodes in the region posterior to the pancreatic head. Cholelithiasis was present. A biopsy specimen of the hepatic lesion revealed neoplastic epithelial cells with cytological features related to primary biliary tract adenocarcinoma (Fig. 1). Cachexia rapidly ensued and the patient died 33 days after admission. No chemotherapy had been attempted.

Involvement of leptomeninges in solid tumours is rare and may be the first evidence of disease. In a Mayo Clinic series [2] the most frequent primary sites of malignancy were the breast and the lung. In 2 out of 29 cases of the same series, diagnosis of primary tumour was not established. In our patient, neurological symptoms were the first evidence of disease, while presentation of gallbladder tumour is usually related to local spreading (e.g. biliary tract obstruction, pain). Gallbladder carcinoma should be taken into account while looking for a primary tumour in patients with meningeal carcinomatosis.

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