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Immunoreactive calcitonin in leukaemia

CARMEL J HILLYARD, D G OSCIER, R FOA, D CATOVSKY, J M GOLDMAN

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Summary and conclusions

A radioimmunoassay was used to measure concentrations of immunoreactive human calcitonin (HCT) in plasma and leucocytes from patients with various leukaemic and myeloproliferative disorders. Plasma immunoreactive HCT concentrations were increased in 32 out of 33 patients with chronic granulocytic leukaemia (CGL) and in all eight patients with acute myeloid leukaemia (AML) at presentation or in relapse. Out of 11 patients with other myeloproliferative disorders, eight had increased plasma immunoreactive HCT concentrations. Buffy-coat-cell extracts and culture media from peripheral leucocytes of patients with CGL also contained increased immunoreactive HCT concentrations. In contrast, plasma from patients with chronic lymphocytic leukaemia, acute lymphoblastic leukaemia, and AML in remission had low or undetectable immunoreactive HCT concentrations.

Increased plasma and cellular concentrations of immunoreactive HCT may be a consequence of abnormal proliferation of myeloid cells and might prove to be valuable in predicting relapse in patients with myeloid leukaemias.

Introduction

Increased hormone concentrations may be associated with various non-endocrine tumours,¹ and immunoreactive human calcitonin (HCT) has been found in several different non-thyroid tumours.² In some cases immunoreactive HCT may be shown to be of ectopic origin by in-vitro techniques.^{3,4}

We have measured calcitonin concentrations in the plasma and cells of patients with different types of leukaemia and myeloproliferative disorders. The finding of increased concentrations in several of these conditions may help in assessing such patients, and changes in immunoreactive HCT concentrations during the course of the disease could lead to changes in therapeutic strategy.

Subjects and methods

The subjects studied were divided into seven groups: (1) 33 patients with chronic granulocytic leukaemia (CGL), 27 in chronic phase and six in blast-cell transformation before undergoing intensive treatment; (2) six patients with acute myeloid leukaemia (AML) at presentation and two in relapse before reinduction treatment; (3) five patients with AML in remission; (4) 10 patients with chronic lymphocytic leukaemia (CLL); (5) two patients with acute lymphoblastic leukaemia (ALL); (6) 11 patients with chronic myeloproliferative disorders (eight with myeloid sclerosis, one with polycythaemia rubra vera, and two with essential thrombocythaemia); and (7) 113 normal volunteers.

Plasma calcitonin assay—Peripheral blood samples from all the patients and normal controls were collected into cold heparinised tubes, centrifuged, and separated immediately. Plasma samples were frozen and stored at -20°C until assayed. Calcitonin concentrations were measured by an overnight radioimmunoassay similar to that described.⁵ The antiserum used is directed against the central and C-terminal parts of the calcitonin molecule. Normal calcitonin concentrations ($<0.08\text{ }\mu\text{g/l}$) are undetectable with this assay.

Cell extraction procedure—In ten cases CGL cryopreserved buffy-coat cells were used as these were readily available in adequate quantities.⁶ Fresh cells were obtained from two patients with CGL and two controls. The frozen cells were thawed in a water bath at 37°C and washed in TC-199 and saline to remove residual plasma. Fresh cells were washed in saline. The cells were weighed and extracted as described.⁶ After centrifuging at $10\,000\text{ g}$ the supernatants were decanted and applied to ODS-porasil columns twice.⁷ The columns were then eluted with 80% methanol and 1% trifluoroacetic acid, the eluate being vacuum-dried and reconstituted in phosphate buffer before calcitonin assay.

Leucocyte cultures—Heparinised peripheral blood samples from patients with CGL and controls were collected and allowed to stand at room temperature for one to two hours. The leucocyte-rich plasma was collected and the leucocytes washed three times and resuspended in McCoy's 5A modified medium supplemented with 20% horse serum. A total of 12×10^6 leucocytes in 6 ml McCoy's 5A modified medium was placed in a 90 mm sterile plastic Petri dish and incubated in a 5% CO_2 incubator in air. After seven days' incubation the culture medium was collected, centrifuged to remove all cells, and the supernatant tested for calcitonin.

Results

With one exception all patients with CGL whether in chronic phase or in blast-cell transformation had increased plasma immunoreactive HCT concentrations (mean $0.78 \pm \text{SE of mean } 0.35\text{ }\mu\text{g/l}$) (figure). Patients in blast-cell transformation tended to have higher concentrations (mean $3.14 \pm 2.21\text{ }\mu\text{g/l}$) than those in chronic phase ($0.36 \pm 0.04\text{ }\mu\text{g/l}$). There was no correlation between immunoreactive HCT concentrations and the patients' leucocyte counts, though all counts were high (range 86×10^9 – $572 \times 10^9/\text{l}$).

The patients with AML studied at diagnosis and in relapse had increased plasma immunoreactive HCT concentrations (mean $0.97 \pm 0.45\text{ }\mu\text{g/l}$). In contrast, four of the five patients with AML

Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0HS

CARMEL J HILLYARD, BSC, PHD, senior research officer, endocrine unit
D G OSCIER, MRCP, registrar in haematology
R FOA, MD, research fellow, MRC Leukaemia Unit
D CATOVSKY, MD, MRCPATH, honorary consultant physician
J M GOLDMAN, BM, FRCP, honorary consultant physician

Plasma immunoreactive calcitonin concentrations in patients with leukaemia and other myeloproliferative disorders. CGL = Chronic granulocytic leukaemia. AML = Acute myeloid leukaemia. CLL = Chronic lymphocytic leukaemia. ALL = Acute lymphoblastic leukaemia.

* ● = Studied in chronic phase. ○ = Studied in transformation.

studied in complete remission had undetectable plasma immunoreactive HCT concentrations, whereas the fifth had a slightly increased concentration. Eight of the 11 patients with myeloproliferative disorders had increased circulating calcitonin concentrations (mean 0.27 ± 0.07 µg/l). Nine of the 10 patients with CLL and both patients with ALL had undetectable plasma immunoreactive HCT concentrations.

Immunoreactive HCT concentrations in extracts from CGL buffy-coat cells were much higher than those found in normal leucocytes (table I). Fresh cells from two patients with CGL contained the same amounts of immunoreactive HCT as their frozen cells. Culture media obtained after incubation of chronic-phase CGL peripheral leucocytes showed increased immunoreactive HCT concentrations (table II), whereas concentrations in normal peripheral blood leucocytes from controls were undetectable.

also had increased immunoreactive HCT concentrations. Though all patients with CGL studied had greatly increased total leucocyte counts, there was no direct correlation between plasma immunoreactive HCT concentrations and leucocyte numbers. Generally, however, patients in blast-cell transformation had higher plasma immunoreactive HCT concentrations than those in the chronic phase. Increased immunoreactive HCT concentrations are probably associated with proliferation of leukaemic myeloid cells but not with disordered proliferation in the lymphoid series.

If our findings are confirmed measurement of immunoreactive HCT concentrations might be useful in assessing the response to treatment and in monitoring the subsequent clinical progress of patients with AML, CGL, and other myeloproliferative disorders. Thus rising concentrations in patients thought to be in complete remission of AML or in chronic phase of CGL might lead to more intensive chemotherapeutic regimens. Rising immunoreactive HCT concentrations may also be useful for diagnostic differentiation between myeloid and lymphoid leukaemias.

Characterisation of the immunoreactive material is still incomplete. Nevertheless, it resembles human calcitonin in two of its properties: it has similar immunological reactivity, and it may be extracted from plasma with Spherosil, which adsorbs calcitonin.²

The source of immunoreactive HCT is still unknown. Increased immunoreactive HCT concentrations in extracts of CGL cells as well as in plasma from patients with CGL, however, suggest that it may be of leucocyte origin. The results of our cell culture studies support this contention. Circulating calcitonin is probably not merely attached to the surface membrane of the CGL leucocyte because in both our extraction and cell-culture studies the leucocytes were washed repeatedly. Furthermore, leucocytes are already known to produce other proteins—for example, the increased serum values of transcobalamin I observed in CGL are due to increased synthesis by the leukaemic leucocytes.⁸ Whatever the source of immunoreactive HCT the clinical implications are clear: using only this relatively simple technique it may be possible to monitor the course of myeloid leukaemias and predict relapse or transformation.

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TABLE I—Concentrations of immunoreactive calcitonin in plasma and cryopreserved thawed buffy-coat cells from patients with chronic-phase CGL

	Case No										Normal value
	1	2	3	4	5	6	7	8	9	10	
Cell concentration (ng/g)	3.40	2.79	4.48	3.00	1.93	0.48	1.85	0.88	1.04	0.53	<0.04
Plasma concentration (µg/l)	0.76	0.63	0.09	0.20	0.09	0.11	0.18	0.54	0.12	0.21	<0.08

TABLE II—Concentrations of immunoreactive calcitonin in supernatant from incubated peripheral leucocytes from patients with chronic-phase CGL

	Case No				Normal value
	8	10	11	12	
Immunoreactive HCT (µg/l culture medium)	0.80	0.66	1.48	1.68	<0.08

Discussion

Our studies show that plasma concentrations of immunoreactive HCT are usually increased in CGL, AML in relapse, and various myeloproliferative disorders. In contrast, essentially normal values were found in patients with AML in remission, CLL, and ALL. Extracts from leucocytes of patients with CGL

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