Rosenberg in patients with lymphomas in the United States may have been partly due to an effect of vitamin D, as the normal range for vitamin D is known to be higher in the United States than Great Britain and ingestion of multivitamin preparations is fairly common in the general population in the United States¹⁹ and may be more common among patients with cancer.

We cannot make any useful comment on the effect that alfalcidol might have on the survival of patients from this study. Clearly this would require longer follow up and more patients in a randomised study. Nevertheless, if alfalcidol could be substituted for cytotoxic treatment as a means of controlling disease without necessarily affecting survival this would represent a major advance in the treatment of low grade lymphomas: the replacement of a toxic treatment with a fairly non-toxic treatment is obviously beneficial, and more effective treatment of transformed low grade lymphomas might be achieved, as it has been suggested that these tumours respond so poorly to chemotherapy partly because they acquire resistant cell populations during exposure to chemotherapy in the low grade state.²⁰ Furthermore, the substitution of chemotherapy with alfalcidol might reduce the risk of secondary leukaemia in patients with lymphoma because this risk is related to the duration of treatment with alkylating agents.²¹

These preliminary data suggest that the antitumour effect of alfalcidol is not limited to in vitro systems or animal tumour models and that the drug may have an important role as a biological modifier in the management of non-Hodgkin's lymphomas.

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References

- 1 Non-Hodgkin's Lymphoma Pathological Classification Project. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. *Cancer* 1982;49:2112-35.

- 2 Skarin AT, Canellos GP. Chemotherapy of advanced non-Hodgkin's lymphoma. In: Wolvsey W, ed. *Clinics in haematology*. Philadelphia: W B Saunders, 1979:667-84.
- 3 Anderson T, Bender RA, Fisher RI, et al. Combination chemotherapy in non-Hodgkin's lymphoma: results of long-term follow up. *Cancer Treat Rep* 1977;61:1057-66.
- 4 Schein PS, Chabner BA, Canellos GP, Young RC, DeVita VT. Non-Hodgkin's lymphoma: patterns of relapse from complete remission after combination chemotherapy. *Cancer* 1975;35:354-7.
- 5 Horning SJ, Rosenberg SA. The natural history of initially untreated low-grade non-Hodgkin's lymphomas. *N Engl J Med* 1984;311:1471-5.
- 6 Anonymous. Vitamin D and the lymphohematopoietic system [Editorial]. *Lancet* 1984;ii:1105-6.
- 7 Abe E, Miyaura C, Sakagami H, et al. Differentiation of mouse myeloid leukaemia cells induced by 1 α ,25-dihydroxyvitamin D₃. *Proc Natl Acad Sci USA* 1981;78:4990-4.
- 8 McCarthy DM, Miguel SFS, Freake HC, et al. 1,25-dihydroxyvitamin D₃ inhibits proliferation of human promyelocytic leukaemia (HL60) cells and induces monocyte-macrophage differentiation in HL60 and normal human bone marrow cells. *Leuk Res* 1983;7:51-5.
- 9 Reitsma PH, Rothberg PG, Astrin SM, et al. Regulation of myc gene expression in HL-60 leukaemia cells by a vitamin D metabolite. *Nature* 1983;306:492-5.
- 9a Sato T, Takusagawa K, Asou N, Konno K. Antitumour effect of 1-hydroxyvitamin D₃. *Tohoku J Exp Med* 1982;138:445-6.
- 9b Honma Y, Hozumi M, Abe E, et al. 1,25-Dihydroxyvitamin D₃ and 1 hydroxyvitamin D₃ prolong survival time of mice inoculated with myeloid leukaemia cells. *Proc Natl Acad Sci USA* 1983;80:210-4.
- 10 Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1,25-Dihydroxyvitamin D₃ receptors in human leukocytes. *Science* 1983;221:1181-2.
- 11 Rheinhardt TA, Horst RL, Orf JW, Hollis BW. Microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. *J Clin Endocrinol Metab* 1984;58:91-8.
- 12 Preece MA, O'Riordan JLH, Lawson DEM, Kodicek E. A competitive protein binding assay for 25-hydroxycholecalciferol and 25-hydroxy ergocalciferol in serum. *Clin Chim Acta* 1974;54:235-42.
- 13 Channess GC, McGuire WL. Scatchard plots: common errors in correction and interpretation. *Steroids* 1975;26:538-42.
- 14 Lowry QH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265-75.
- 15 Miller AB, Hoogstraten B, Stequet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981;47:207-14.
- 16 Tsoukas CD, Provvedini DM, Manolagas SC. 1,25-Dihydroxyvitamin D₃: a novel immunoregulatory hormone. *Science* 1984;224:1438-9.
- 17 Lemire JM, Adams JS, Sakai R, Jordan SC. 1,25 Dihydroxyvitamin D₃ suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. *J Clin Invest* 1984;74:657-61.
- 18 Wiernik PH. Spontaneous regression of hematologic cancers. *Natl Cancer Inst Monogr* 1976;44:35-8.
- 19 Smith PG, Jick H. Cancers among users of preparations containing vitamin A. *Cancer* 1978;42:808-11.
- 20 Armitage JO, Dick FR, Corder MP. Diffuse histiocytic lymphoma after histologic conversion: a poor prognostic variant. *Cancer Treat Rep* 1981;65:413-8.
- 21 Greene MH, Young RC, Merrill JM, DeVita VT. Evidence of a treatment dose response in acute nonlymphocytic leukemias which occur after therapy of non-Hodgkin's lymphoma. *Cancer Res* 1983;43:1891-8.

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Treatment with danazol and plasma glucagon concentration

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Abstract

The association between treatment with danazol and hyperglucagonaemia was studied. Plasma glucagon concentrations were measured during an oral glucose tolerance test in seven women taking danazol and six healthy controls not taking danazol. Results showed that treatment with danazol is associated with severe hyperglucagonaemia, and in three patients glucagon concentrations reached the range suggestive of glucagonoma.

It is important to recognise that this increasingly used drug may cause severe hyperglucagonaemia to prevent patients treated with danazol undergoing unnecessary investigations to localise glucagonoma.

Introduction

Grossly increased plasma glucagon concentrations (>50 pmol/l (>17.4 ng/ml)) were found fortuitously in a woman receiving danazol for premenstrual exacerbations of lupus erythematosus. Known causes of hyperglucagonaemia were excluded. Glucagon concentrations were found to parallel the dosage of danazol, suggesting that hyperglucagonaemia was due to the drug. To explore this possibility we measured plasma glucagon concentrations during an oral glucose tolerance test in seven women taking danazol and six healthy controls.

Patients, methods, and results

Seven women aged 20-45 (mean 33.9) years treated with danazol (100-600 mg/day for four to 24 weeks) for endometriosis (five patients) or mastalgia (two patients) were randomly selected from the gynaecological outpatient clinic. One patient was taking phenobarbitone and lorazepam for epilepsy and another norethisterone 20 mg/day. Body weight ranged from 45.5 to 82.7 kg (mean 62.7 kg). The six control women did not differ noticeably from the group treated with danazol in either age (range 23-40, mean 29.8 years) or body weight (range 50 to 68 kg, mean 58.3 kg); one was taking an oral contraceptive.

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A 50 g oral glucose tolerance test was performed after an overnight fast. Venous blood was taken immediately before ingestion of glucose and at 30, 60, 90, and 120 minutes thereafter into lithium-heparin tubes containing aprotinin (4000 kIU/10 ml blood) and immediately centrifuged at room temperature. Plasma glucose concentration was measured by an autoanalyser (glucose oxidase method) and insulin and glucagon concentrations by radioimmunoassay. One patient treated with danazol was re-examined eight weeks after stopping treatment.

Statistical analyses were performed by the paired or unpaired Wilcoxon test.

The figure shows the mean (SEM) changes in plasma concentrations of glucagon, insulin, and glucose in the two groups. In the patients treated with

(3096 (SEM) 1487) v 112 (30) pmol/l $\times$ 120 min (1078 (518) v 39 (10.5) ng/ml $\times$ 120 min); $p < 0.01$). Plasma insulin concentrations, initially similar in the two groups, were significantly higher in the danazol group after 30 minutes, and the area under the plasma insulin curve was significantly increased (39.8 (8.9) v 15.6 (2.5) nmol/l $\times$ 120 min (231 (52) v 90.5 (14) μ g/l $\times$ 120 min); $p < 0.01$). Glucose tolerance was significantly worse in the danazol group than in the control group with significant hyperglycaemia at 60 and 90 minutes and a greater area under the glycaemic curve (910 (71) v 645 (51) mmol/l $\times$ 120 min (16 396 (1369) v 11622 (919) mg/100 ml $\times$ 120 min); $p = 0.05$).

In the patient re-examined after withdrawal of danazol fasting plasma glucagon concentration had fallen from 58 to 2 pmol/l (20.2 to 0.7 ng/ml), and all glucagon, insulin, and glucose data were within one SD of the control mean.

Mean (SEM) changes in plasma concentrations of glucagon, insulin, and glucose in seven women treated with danazol (●) and six control women (○). Significance of differences between danazol and control groups (Wilcoxon's test): * $p < 0.05$; ** $p < 0.02$; and *** $p < 0.01$.

Conversion: SI to traditional units—Plasma glucagon: 1 pmol/l $\approx$ 348 pg/ml. Plasma insulin: 1 pmol/l $\approx$ 0.006 μ g/l. Plasma glucose: 1 mmol/l $\approx$ 18 mg/100 ml.

danazol plasma glucagon concentrations were significantly higher (fasting range 7-193 pmol/l (2.4-67.4 ng/ml); > 50 pmol/l (> 17.4 ng/ml) in three cases) than in the control group (fasting range 2-5 pmol/l (0.7-1.8 ng/ml)), and the total area under the plasma glucagon curve was significantly greater

Discussion

The recognised causes of hyperglucagonaemia include glucagonoma, renal and hepatic disease, diabetes mellitus, and pancreatitis.¹ Except for B-piridylcarbinol (a nicotinic acid derivative) drugs are not known to cause hyperglucagonaemia.¹ Our results show that treatment with danazol is associated with severe hyperglucagonaemia, which in three of our patients reached the range suggestive of glucagonoma (> 50 pmol/l (> 17.4 ng/ml)). Glucagon concentrations returned to normal in one patient after withdrawal of danazol, further suggesting a causative role of the drug.

Danazol, a synthetic derivative of ethisterone which suppresses the release of gonadotrophin, is widely used to treat endometriosis and mastalgia.² It causes hyperinsulinaemia and hyperglycaemia,³ perhaps by its mild androgenic activity which could produce post-receptor resistance to insulin.⁴ Hyperglucagonaemia could also cause resistance to insulin.¹ The mechanism of hyperglucagonaemia, not previously recognised as a side effect of treatment with danazol, is unknown.

Gross hyperglucagonaemia was not accompanied by features of the glucagonoma syndrome (necrolytic rash, stomatitis, and loss in weight⁵) and was reversible when treatment with danazol was withdrawn. It is important, however, to recognise that this increasingly used drug may cause severe hyperglucagonaemia as patients taking danazol may otherwise undergo unnecessary and often highly invasive investigations designed to localise a glucagonoma.

We thank Professor Hawkins for permission to report on his patients.

References

- 1 Lefebvre PJ. *Glucagon. Handbook of experimental pharmacology*. Vol 66. Berlin: Springer, 1983.
- 2 Ward GD. Dosage aspects of danazol therapy in the treatment of endometriosis. *Postgrad Med J* 1979;55 (suppl 5):7-9.
- 3 Wynn V. Metabolic effects of danazol. *J Int Med Res* 1977;5 (suppl 3):25-35.
- 4 Barbieri RL, Ryan KJ. Hyperandrogenism, insulin resistance and acanthosis nigricans syndromes: a common endocrinopathy with distinct pathophysiological features. *Am J Obstet Gynecol* 1983;147:90-101.
- 5 Mallinson CN, Bloom SR, Warin AP, Salmon PR, Cox B. A glucagonoma syndrome. *Lancet* 1974;iii:1-5.

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100 YEARS AGO

There appears to be especially good reason why the Police-Commissioners should exercise to the full extent their powers for the control, and, if necessary, the destruction, of stray dogs. A fatal case of hydrophobia was the subject of an inquest in Holloway last week. The deceased had been bitten a month ago by a mongrel cur, whose owner could not be found. At Dunstable, another animal has lately been shot, after making havoc among sheep and dogs, and biting a boy. In a former article, we have shown why the stray dog, as being hungrier and more weak than another, is, therefore, more likely to be attacked by the germ of rabies. Experience also shows that the dog that is mad wanders as if by a restless instinct; the more need, therefore, for the control to which we have referred. Some would go further, and advise that the mere fact of a bite inflicted on the person should seal the doom of the biting animal. This is going too far. It should be remembered

that, with the death of the dog, all proof of madness or the contrary is obliterated, and thus the best means of reassuring the person attacked of his safety in a case where rabies does not exist is removed. This loss of evidence is of some consequence to nervous people. Of course, where madness, that is, hydrophobia, is certain or where dogs have been bitten by one of their kind known to be mad, there is no alternative but destruction. Death is then a wise and provident measure, but the need for its infliction should in any such case be fairly apparent. A frothy or watery mouth; a wild, bright, bloodshot eye, and, in a late stage, more or less paralysis of the lower jaw and hinder extremities, are among the most reliable signs of hydrophobia in dogs. Avoidance of water is not in them so reliable a symptom as in man; on the contrary, they often make for water, and swallow it freely. (*British Medical Journal* 1885;ii:402.)