Development and significance of antibodies to salmon calcitonin in patients with Paget’s disease on long-term treatment

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Summary
Sixteen consecutive patients in one unit were studied during long-term treatment of Paget’s disease of bone with salmon calcitonin. Eleven patients developed detectable antibody titres at some time during treatment. In one patient with a high antibody titre evidence of resistance to treatment emerged two years after the development of antibodies, but no other patient showed evidence of resistance. The clinical and biochemical response could be maintained in the absence of an acute calcium-lowering effect of calcitonin.

Although antibodies often develop during treatment with heterologous calcitonin, they are only rarely the cause of clinical resistance.

Introduction
Calcitonin has been used for some years for Paget’s disease of bone. It extends the disease and induces radiological healing. Long-term or even lifelong treatment may be necessary in patients whose disease is potentially crippling, or in those with spinal cord compression or high-output cardiac failure. The only hormones available commercially so far, porcine and salmon calcitonin, differ appreciably from human calcitonin in their amino-acid sequence. They are antigenic in man, and resistance developing during treatment has been ascribed to these antibodies. On the other hand, in 38 patients treated for up to four years with porcine calcitonin no patient developed clinical resistance, although there was a 40% incidence of detectable antibody titres. Furthermore, relapse may also occur during treatment with human calcitonin, which is not antigenic. The results reported here provide further evidence that the development of antibodies to calcitonin, even in high titre, need not necessarily be accompanied by clinical resistance.

Patients and methods
Informed consent was obtained from 16 consecutive patients with symptomatic Paget’s disease who were referred to a single unit for management. Urine collections were made while patients were taking a self-selected low-gelatin diet. Blood samples for serum alkaline phosphatase and antibody measurements were taken before or at least three hours after the first injection of calcitonin, which was given on the ward. Sera were stored at -20°C until assayed. At least four daily urine collections were obtained every three to six months during treatment and assayed for total hydroxyproline. Salmon calcitonin (Calsynar, Armour) was given subcutaneously, 100 units/day as a single injection or in two doses of 50 units.

The acute response to salmon and to human calcitonin was tested by taking blood for serum calcium as follows. With the patients fasting, samples were taken from an indwelling arm catheter without stasis at 0800, 0830, and 0900. The hormone injection was given at 0900, and samples were taken at 1000, 1100, 1200, and 1500. The response to human calcitonin (Giba Geigy Ltd) was tested three days after that to salmon calcitonin.

Serum calcium was measured by atomic absorption spectrophotometry, alkaline phosphatase on the SMA12 autoanalyser, and urinary total hydroxyproline by the method of Kivirikko et al. Calcitonin antibody assays were carried out by incubating dilutions of serum with 125I-labelled synthetic salmon calcitonin for 48 hours at 4°C, with separation of bound from free hormone by dextran-coated charcoal. The bound to free ratio was calculated and results expressed as a titre, which was taken as the last dilution of plasma which gave a bound/free ratio greater than 0.10. Sera were tested at dilutions of 1:50, or greater. A standard guinea-pig anti-salmon calcitonin serum was included in every assay to check the reproducibility of binding of labelled hormone.

Results
In the first few months of treatment urinary hydroxyproline concentrations fell in all patients. They reached a plateau, as indicated by the grouped data in fig 1. Fig 2 shows details of three patients, in one of whom urinary hydroxyproline returned to pretreatment concentrations despite continued calcitonin treatment and a high antibody titre. A small rebound increase in hydroxyproline occurred in the other two patients.

During treatment (mean duration 29 months) antibodies were detected in 11 of the 16 patients (69%). In a larger series of assays carried out on patients from several centres (to be published), the overall incidence was about 45% after six months’ treatment. Antibodies developed within 3 to 18 months and increased rapidly in titre in four patients (fig 3). Antibody titres fell in nine patients during treatment, and levels in three of them eventually fell below a serum dilution of 1:50.

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The acute calcium-lowering effects of salmon and human calcitonin were tested in eight patients, six of whom had antibodies to salmon calcitonin. In two patients a calcium-lowering response was obtained with human but not with salmon calcitonin (fig 4). Both of these patients had high anti-salmon calcitonin titres, but only one (case 12) had evidence of resistance to long-term treatment with salmon calcitonin (fig 2). The other patient (case 16) maintained low serum alkaline phosphatase concentration and urinary hydroxyproline excretion 24 months after first developing an antibody titre of 1/3200.

**Discussion**

These findings draw attention to the high incidence of antibody formation during treatment with salmon calcitonin. Clinical and biochemical improvement occurred and was maintained in spite of these antibodies, which were observed in variable titres at some stage during treatment in over half the patients. In some cases the antibodies appeared transiently and in others their titres fell appreciably during treatment.

In most patients hydroxyproline excretion was maintained at a plateau level during treatment. One patient (case 12) showed a biochemical relapse, and her urinary hydroxyproline excretion returned to pretreatment levels. This woman had gross skull disease, worsening neurological damage, and high antibody titres. Furthermore, she responded to an acute injection of human, but not salmon calcitonin, with a prompt fall in serum calcium concentration. Although this might suggest that her biochemical relapse had occurred as a result of neutralisation of hormone activity by antibody, an almost identical relapse has been observed in a similarly affected patient treated with human calcitonin, and furthermore, the biochemical relapse in case 12 did not occur until almost two years after development of an antibody titre of 1/6400 (figs 2 and 3). The other patient with an identical acute calcium-lowering response and high antibody titres (case 16) did not relapse biochemically or clinically. This observation is significant in that it draws attention to the fact that the failure of calcitonin to lower serum calcium acutely need not indicate that the hormone is unable to exert a long-term effect on bone. Calcitonin lowers serum calcium by inhibiting the activity of osteoclasts and strongly that in-vivo evidence. The calcium-lowering effect of the hormone probably requires rapid availability of the injected dose to the active osteoclasts, and this may be prevented by binding to antibody. Nevertheless, the binding may not prevent the long-term effect of calcitonin on bone cells: in other words, bound calcitonin may be available for action, albeit at a slower rate. This concept was advanced by Berson et al when antibodies to insulin were first reported, and it finds support from the work of Kurtz et al who showed that antibody-bound insulin is not unavailable to cells.

Rojanasathit et al have reported a higher incidence of biochemical resistance to salmon calcitonin when antibodies are present. This occurred in five out of 27 patients and within a year of starting treatment in four of them. Two of their patients had previously received porcine calcitonin and three had been given much larger doses of salmon calcitonin than those used in our study.

Our findings and those of others indicate that antibody development occurs relatively often in the course of treatment of Paget’s disease with non-human calcitonin. Resistance to treatment occurs from the outset or may develop after some months of treatment, and it may also develop during treatment with human calcitonin. It is not possible to conclude that antibodies are causally related to resistance. The acute calcium-
lowering effect of calcitonin need not reflect the ability of the hormone to affect Paget’s disease of bone chronically, and thus the only acceptable evidence in support of antibody-mediated relapse during treatment would be obtained by demonstrating a restored response to porcine or human calcitonin in the face of high antibody titres to salmon calcitonin. Such evidence is available for a total of five patients.11

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Plasma creatinine and urea: creatinine ratio in patients with raised plasma urea

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Summary
We examined the plasma urea and creatinine concentrations and the ratio between them according to diagnosis in 100 unselected and 31 selected adult hospital patients with a plasma urea concentration > 10 mmol/l (60 mg/100 ml). We also examined plasma urea and creatinine concentrations in 350 unselected consecutive patients, but found no useful relation between the two values.

Congestive heart failure was the most common identifiable cause of a raised plasma urea concentration in the 100 unselected patients (38%). Among these 100 patients the plasma creatinine concentration was a more useful discriminant between prerenal uremia and intrinsic renal failure than was the urea:creatinine ratio or the plasma urea concentration.

A plasma creatinine concentration > 250 μmol/l (2.8 mg/100 ml) indicated intrinsic renal failure with a 90% probability.

Introduction
Measurement of the plasma urea concentration is still the usual screening test for renal glomerular failure, but it is generally recognised that the failure must be gross before the plasma urea concentration is clearly raised, and that it may be raised for reasons other than glomerular failure. It has been stated that the causes of a raised plasma urea concentration can be distinguished by measuring the plasma creatinine concentration and calculating the ratio of the two values.1-4

Two reasons prompted us to re-examine the diagnostic role of measuring plasma creatinine concentrations and the urea:creatinine ratios. Firstly, the introduction of multichannel analysers has made measurements of plasma creatinine concentrations more generally available, and they are often made when they are not requested. Hence the urea:creatinine ratio is probably being increasingly used as an aid to interpret a raised plasma urea concentration. Furthermore, we were surprised to find that the publications which support the use of the urea:creatinine ratio contain little detailed information, and the conclusions seem to be based more on physiological principles than on a study of the usefulness of the ratio in clinical practice.

As a first step in the re-examination of this problem we studied the relation between plasma urea and plasma creatinine values in 350 consecutive patients in whom the two measurements had been made on the same blood sample. Secondly, we studied the case histories of 100 consecutive patients with a plasma urea concentration > 10 mmol/l (60 mg/100 ml) who had creatinine measured on the same plasma sample. We also examined the records of an additional 17 consecutive patients with acute intrinsic renal failure and 14 consecutive patients with chronic renal failure to increase the size of the groups with these disorders. We examined the plasma urea and creatinine values and the ratio between them according to the cause of the raised plasma urea concentration. In the larger