SHORT COMMUNICATION

THE CALCIUM LOWERING EFFECT OF SYNTHETIC HUMAN, PORCINE, AND SALMON CALCITONIN IN PATIENTS WITH PAGET'S DISEASE

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SUMMARY

1. Four patients with active Paget's disease were given acute injections of a synthetic calcitonin. The calcium lowering effect of human and porcine calcitonin was compared in two patients, and the human and salmon hormone in the other two patients.

2. All three hormones were effective in lowering the plasma calcium, but weight for weight salmon calcitonin was the most potent. Thus salmon calcitonin produced approximately the same effect as a ten times greater dose of human calcitonin. However, porcine calcitonin was the least potent, and compared with human calcitonin, required an approximately tenfold dose for a similar response.

Keys words: synthetic calcitonin, human calcitonin, porcine calcitonin, salmon calcitonin, Paget's disease.

Calcitonin has a hypocalcaemic and hypophosphataemic action in experimental animals (Kumar, Foster & MacIntyre, 1963; Hirsch, Voelkel & Munson, 1964). These effects are due to inhibition of bone resorption (Milhaud, Perault & Moukhtar, 1965; Raisz, 1965; Martin, Robinson & MacIntyre, 1966; Reynolds, 1968). In normal adult man injection of large doses of porcine or human calcitonin produces little or no change in plasma calcium (Woodhouse, Reiner, Kalu, Galante, Leese, Foster, Joplin & MacIntyre, 1970). However, in Paget's disease and in other conditions where there is increased bone turnover, injection of either hormone inhibits bone resorption, with a marked fall in serum calcium and urine hydroxyproline excretion (Bijvoet, Sluys Veer & Jansen, 1968; Singer, Neer, Parsons, Krane & Potts, 1970; Woodhouse, Reiner, Bordier, Kalu, Fisher, Foster, Joplin & MacIntyre, 1971). As synthetic porcine, human and salmon calcitonins are currently available for clinical trials their calcium lowering effects after single intravenous injections were compared in patients with active Paget's disease.

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PATIENTS

Informed consent was obtained from four patients with active and symptomatic Paget's disease who were considered suitable for long-term outpatient calcitonin treatment. There were three male and one female subject aged between 60 and 66, with plasma calcium between 2.4 and 2.7 mol/l, plasma alkaline phosphatase between 40 and 330 K.A. units per 100 ml and urinary hydroxyproline excretion between 120 and 475 mg/24 h.

Throughout the study the patients received a constant diet containing less than 5 mmol of calcium per day. In all cases, at least 3 days separated each of the calcitonin injections. The patients fasted overnight and until 13.00 hours on the day of injection. An indwelling catheter was inserted into an arm vein to minimize discomfort during venous sampling. On the morning of treatment five basal samples were taken without stasis at half-hourly intervals from 07.30 to 10.00 hours and then calcitonin was injected. Thereafter blood samples were taken at half-hourly intervals until 13.00 hours; and then at hourly intervals until 15.00 or 16.00 hours; and finally at 09.00 hours on the next day. Two days before the first calcitonin injection each patient received a control injection containing the buffer solution alone at 10.00 hours, and similar blood samples were taken.

MATERIALS AND METHODS

Calcitonins

Synthetic human calcitonin Ciba 47 175-Ba (Sieber, Riniker, Brugger, Kamber & Rittel, 1970) and synthetic porcine calcitonin were supplied by Ciba-Geigy and synthetic salmon calcitonin by Sandoz Limited.

The calcitonin was dissolved in sterile 0.2 M-sodium acetate buffer at pH 4.6, containing 0.1% human plasma albumin, added to prevent adsorption of the hormone onto the glass, and administered intravenously as a single injection. Plasma albumin was obtained from Blood Products Laboratory, Lister Institute, Elstree, Herts.

Assessment of the potency of the preparations used

The activity of each batch of ampoules used in the study was tested in the standard rat four-point assay (Moseley, Matthews, Breed, Galante, Tse & MacIntyre, 1968).

The potency of the preparations was measured by the rat assay using the MRC Research Standard B which is a porcine standard. The potencies were: synthetic porcine calcitonin 100 MRC Units/mg, synthetic human calcitonin 93 MRC Units/mg; synthetic salmon calcitonin 3833 MRC Units/mg.

Plasma calcium and magnesium

These were determined by emission flame photometry, calcium by the method of MacIntyre (1961) and magnesium by the method of Alcock, MacIntyre & Radde (1960).

RESULTS

The change in plasma calcium before and after injection of the control buffer solution is indicated by the dotted lines in Fig. 1(a) and (c). The zero value for the change is the last value, obtained before the injection at 10.00 hours.
Calcitonin in Paget's disease

Fig. 1. Comparison of porcine and human calcitonin (a and b) and human and salmon calcitonin (c and d). For each patient the plasma calcium on a control day is shown by a dotted line on one graph. H—synthetic human, P—synthetic porcine and S—synthetic salmon calcitonin.
Comparison of human and porcine synthetic calcitonins

Fig. 1(a) shows a comparison in patient W.W. of the effects of 3 μg of porcine and 3 μg of human calcitonin. Both hormones produced hypocalcaemia, the lowest calciums being recorded 1.5–2 h after the injection; human calcitonin produced a greater fall in plasma calcium than an equal weight of the porcine hormone.

In Fig. 1(b) (patient W.W.), 0.3 and 3.0 μg of human calcitonin was compared with 30.0 μg of porcine hormone. The 3.0 μg dose of the human hormone produced a greater fall in plasma calcium than did a ten times higher dose of the porcine material; 0.3 μg of the human hormone produced little or no effect in this patient.

In a second patient, A.S., 3 μg of human and porcine calcitonin were compared; similar results to those shown in Fig. 1(a) were obtained. In this patient the effect of 30.0 μg of human and porcine calcitonin was also compared; there were no differences in the responses which might indicate maximum fall had been achieved by both preparations (not illustrated).

Comparison of human and salmon synthetic calcitonins

Fig. 1(c) shows a comparison of the effects of 3.0 and 30.0 μg of human and 3.0 μg of salmon calcitonin in patient C.P.; 3.0 μg of salmon calcitonin produced more marked hypocalcaemia than 3.0 μg of the human hormone, and 30.0 μg of the human hormone produced a similar effect to 3.0 μg of the salmon material. In this patient (Fig. 1d) even a 0.3 μg dose of the salmon material produced a greater fall in plasma calcium than did 3.0 μg of the human hormone. In another patient, E.B., similar results were obtained, but in this patient comparison of 0.3 μg of salmon and 3 μg of human calcitonin produced an equal response.

On several occasions the plasma calcium levels were found to be higher 23 h after the calcitonin injection, compared with previous control values. This effect was transient and the plasma calcium had returned to normal in 48 h. This rise could possibly be explained by increased secretion of parathyroid hormone during the hypocalcaemic phase.

In patient W.W. plasma magnesiums were determined on all samples following injection of the calcitonin preparations; neither of the two hormones tested produced a fall in magnesium.

DISCUSSION

Though the results presented here do not constitute a formal biological assay, these studies demonstrate a clear-cut difference in the calcium lowering activity in man of the three available synthetic calcitonins. Thus, when equal weights of these hormones are given as acute intravenous injections, salmon calcitonin is approximately ten times more effective than human, which in turn is approximately ten times more effective than the porcine hormone. This applies whether the maximum fall in plasma calcium or the area under the curve is considered.

Although each hormone contains thirty-two amino acid residues there are only nine conserved residues in the amino acid sequence of the three calcitonins. It is therefore not surprising that variations in the calcium lowering ability of these hormones occur in different species. For example, when intravenous injections of all three hormones are compared in the rat, one-fortieth of the weight of salmon calcitonin will produce a similar fall in plasma calcium to that produced by the human or porcine material (Galante, 1972). This contrasts with our findings in man where one-tenth of the weight of the salmon hormone has the same calcium
lowering effect as the human hormone. There are similar differences in urine sodium excretion (Ardaillou, Milhaud, Rousselet, Vuagrat & Richet, 1967; Singer, Woodhouse, Parkinson & Joplin, 1969), and in the rat salmon calcitonin weight for weight produces a much greater natriuresis than the other calcitonins (Williams, Mathews, Moseley & MacIntyre, 1972). The reason for these striking differences is unknown but may be related to the apparent resistance of the salmon calcitonin to enzymic degradation (De Luise, Martin & Melick, 1970). In addition to the interspecies variation in response to calcitonin there is also variation within the same species which depends on the route of administration of the hormone (Maier, Neher, Rittel & Staehelin, 1970; Galante et al., 1971). As we have only tested the intravenous route in man we cannot be certain that the calcium lowering effect would be the same if intramuscular or subcutaneous injections had been given, nor whether this effect is related to the long-term therapeutic potential of the hormone.

Comparison of these hormones in man is of considerable clinical importance since long-term administration of the human synthetic hormone has produced a complete clinical and biochemical remission in Paget’s disease (Woodhouse et al., 1971) without the formation of antibodies (Woodhouse, 1972). Somewhat similar observations have been made using porcine (Haddad, Birge & Avioli, 1970) and salmon calcitonin (Singer, Keutmann, Neer, Potts & Krane, 1972; Shai, Baker & Wallach, 1971), but in a proportion of patients antibody titres may be sufficiently high to limit their clinical usefulness (Singer, Aldred, Neer, Krane, Potts & Bloch, 1972).

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REFERENCES


