SHORT COMMUNICATION

Bone-resorbing activity in the sera of patients with rheumatoid arthritis

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Summary

1. Foetal rat hemi-calvaria were incubated in organ culture with sera from patients with active rheumatoid arthritis.
2. Increased $^{45}$Ca resorption was produced by sera from patients who were hypercalcaemic.
3. This bone-resorbing effect could be inhibited by calcitonin.

Key words: bone, calcitonin, calcium, hypercalcaemia, rheumatoid arthritis.

Introduction

Hypercalcaemia and other biochemical features suggestive of hyperparathyroidism have been demonstrated in patients with rheumatoid arthritis (Kennedy, Allam, Boyle, Nuki, Rooney & Buchanan, 1975). The hypercalcaemia was subsequently confirmed in a further study of twenty-three patients with rheumatoid arthritis, of whom eight had an elevated ionized fraction of serum calcium (A. C. Kennedy, B. F. Allam, P. J. Rooney, M. Watson, A. Fairney, K. Bowling, K. D. Buchanan, C. J. Hillyard, P. Finch, J. A. Anderson, W. W. Buchanan & G. Morgan, unpublished work). In this same study, a biochemical pattern compatible with 'hyperparathyroidism' was demonstrated. However, parathyroid hormone levels measured by immun assay (Fairney, Jackson & Clayton, 1973) were markedly reduced. In addition, calcitonin levels were increased in two of the patients, both of whom had elevated serum gastrin. Twenty-five hydroxycholecalciferol concentrations were found to be essentially normal in the work by A. C. Kennedy et al. referred to above.

These findings seemed to indicate that 'hyperparathyroid-like' activity was present in the serum of some patients with rheumatoid arthritis but that this was not due to the action of parathyroid hormone itself, since the immunoassay results of this hormone were so low. The possibility of a bone-resorbing substance being present in the serum of patients with rheumatoid arthritis, possibly accounting for the above findings, prompted us to carry out the following study.

Materials and methods

Sera were obtained at the same time as all other blood samples during a previous study and stored at $-20^\circ$C. As explained previously (Kennedy et al., 1975), 65% of patients with rheumatoid arthritis have a low serum albumin, and commonly a normal total serum calcium. In some of these patients, serum ionized calcium has been found to be elevated. It was decided, therefore, to categorize the results from our experiments with foetal rat calvariae purely on the concentration of serum ionized calcium. Fourteen patients were divided into two groups, hypercalcaemic and normocalcaemic, on the basis of the serum ionized calcium concentration (normal range 1.10±0.07 SD mmol/l). On this criterion, there were eight samples from patients in the normocalcaemic range and six samples in the hypercalcaemic range. Serum ionized calcium was measured with a flow-through calcium electrode
Female Sprague-Dawley rats were injected with 100 μCi of $^{45}$CaCl$_2$ on the seventeenth day of pregnancy. Not less than 24 h and not more than 48 h later the rats were killed and paired half-calvariae dissected, washed in medium no. 199 on plastic dishes and maintained at 37°C for 48 h. One of each pair of half-calvariae served as a control, with pooled human serum added (0.1 ml), and the serum to be tested was added to the other half (0.1 ml). Serum from each patient was always tested in triplicate.

Resorption was measured in terms of the percentage of $^{45}$Ca released into the medium, determined by a liquid-scintillation well counter at 48 h after test sera were added.

Results were expressed as the ratio of $^{45}$Ca released by treated and control members of the bone pair (test/control $^{45}$Ca ratio). Cell-mediated bone resorption was calculated by subtracting the $^{45}$Ca released from a dead explant (frozen and subsequently thawed three times), from that of the living paired bone (Reynolds & Minkin, 1970; Raisz & Niemann, 1969).

Results
The results (Table 1) show the $^{45}$Ca released from the bones cultured with sera from rheumatoid patients in each group, compared with responses to two levels of parathyroid hormone in the same system. In each case, the results have been calculated as the ratio of the $^{45}$Ca released from the bone treated with rheumatoid sera to that released from its control.

It can be seen that the hypercalcaemic group of patients had a significantly higher ratio than those patient groups who were normocalcaemic ($t$ = 4.29; $P$ < 0.001 or $P$ < 0.01, by Wilcoxon rank test). The bone-resorption activity of the hypercalcaemic serum is seen to be equivalent to 0.5 unit/ml of parathyroid hormone and the highest individual value in the group was equivalent to approximately 0.75 unit/ml.

Experiments with dead bone, by the technique of Reynolds & Minkin (1970), confirmed that the increased Ca resorption was cell-mediated. When salmon calcitonin (final concentration 4 munits/ml) was added to the bone culture at the same time as sera from all patients with high concentrations of serum ionized calcium, the resorbing effect of these sera was inhibited (Table 1).

Discussion
The process of bone loss occurring in rheumatoid arthritis has long been an area of contention. The term osteoporosis is generally applied to describe the thinning of bone occurring in this disease and erosions are attributed to local factors in the diseased joint such as granulation tissue. However, from X-ray appearances, Bywaters (1959) commented that the rheumatoid erosion bore a definite similarity to that occurring in hyperparathyroidism. Muirden (1975) examined the area of bone adjacent to articular cartilage from biopsies taken at the time of synovectomies in fifteen patients with rheumatoid arthritis. He found that resorption of bone was active around numerous osteoclasts along the periosteal surface of the bone and that these areas

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<th>Table 1. Bone-resorbing activity of rheumatoid sera</th>
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<td>Results are expressed as the ratio of $^{45}$Ca resorbed from hemi-calvaria treated with rheumatoid sera to the $^{45}$Ca resorbed from its pair incubated with control pooled human serum D ($\pm$ SEM). Calcitonin inhibits the resorbing power of the sera with high calcium concentrations. Responses to two concentrations of parathyroid hormone are shown for comparison. Significance values were obtained by Wilcoxon's rank test.</td>
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<tr>
<td><strong>Rheumatoid sera</strong></td>
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<td>Normal Ca</td>
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<td>(n = 8)</td>
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<td>Test/control $^{45}$Ca ratio</td>
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<td>Significance</td>
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(Orion 99/20) at 37°C with correction for pH and direct sodium interference.
were quite detached from synovial and sub-synovial granulation tissue. In addition, osteoclastic and osteoblastic reactions were seen on the marrow surface of the trabeculae. Thus biopsy evidence appeared to conflict with the generally held belief that rheumatoid granulation tissue was the primary stimulus of bone erosion.

Independently of this study Kennedy et al. (1975), in an extensive study of calcium metabolism in patients with rheumatoid arthritis, showed that there was a high incidence of hypercalcaemia and other biochemical features suggestive of hyperparathyroidism. In a further study these features were confirmed, but shown not to be due to parathyroid hormone (as measured by two separate immunoassays). Other possible agents, such as prostaglandin E\(_2\), were considered, but prostaglandin E\(_2\), because of degradation, is unlikely to be a causative factor (J. Morley, personal communication).

The question then arose as to whether the serum of the rheumatoid patients did indeed contain bone-resorbing activity. From our current study, it would appear that just such activity has been demonstrated in the sera of those patients who were hypercalcaemic. The hypercalcaemia itself could not account for the increased resorption of \(^{45}\)Ca observed (Raisz & Nieman, 1969). The fact that calcitonin inhibited this bone resorption is of considerable interest when it is recalled that calcitonin considerably reduced bone resorption in rats with adjuvant arthritis and, indeed, when given in conjunction with indomethacin and phenylbutazone the adjuvant effect was still less evident (Bobalik, Aldred, Kleszynski, Stubbs, Zeedyk & Bastian, 1974).

Thus it would appear that the serum of some patients with rheumatoid arthritis contains a bone-resorbing substance, unlikely to be parathyroid hormone, yet giving rise to a biochemical picture strongly suggestive of excess parathyroid activity.

It seems reasonable to postulate, especially when also considering Muirden's (1975) evidence, that the presence of this substance in sera indicates a possible explanation for the aetiology of erosion and 'osteoporosis' occurring in rheumatoid arthritis. Perhaps the potential role which a substance like 'osteoclast-activating factor' (Raisz, Trummel, Mundy & Luben, 1975) may play in such clinical situations is worthy of further consideration and study.

The fact that calcitonin inhibits the bone resorption in vitro, suggests a possible therapeutic role of this drug in rheumatoid arthritis, and this aspect is currently under study.

Acknowledgment

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References


