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An investigation into the progress of calcification in simple calcergy

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The injection of a small quantity of certain chemical salts (calcergens) into the subcutis of animals has been found to induce a localised inflammatory response followed by the deposition of calcium phosphate salts in the affected area.[1 2] This phenomenon is termed simple calcergy and is being extensively studied as a model for the pathogenesis of calcification in osteoarthritic tissue[3] and the effect of diphosphonates and anti-inflammatory drugs on mineralisation and dissolution of calcium phosphates in bone.[4 5]

Extensions to this simple model of calcergy[6] have shown mast cells to be possible mediators of the inflammatory response and subsequent calcification.[6] We have therefore investigated the morphological aspects of simple calcergy by examining the progress of induced calcification in young male rats over two weeks. The chemical nature of the deposits has also been analysed to find evidence for calcification occurring via one or more intermediate calcium phosphate crystal phases to stable hydroxyapatite.

Male Lester hooded rats weighing 200–250 g were used. Six dorsal sites, three either side of the mid-lumbar region, were shaved; those on the right side were injected subcutaneously with 0·2 ml of 1 in 40 dilution in saline of a saturated solution of potassium permanganate; those on the left side with 0·2 ml of neutral saline.

At three, five, seven, and 14 days after injection, four animals were sacrificed for each time point using ether anaesthesia and divided into two groups. In the first group the animals were examined for any evidence of subdermal calcific deposits. These deposits were usually seen as circular white discs, as found by other workers,[2] and were measured across their diameter in two places and their average area determined. The plaques were then removed and their wet and dry weights recorded. The samples were characterised using powder *x*-ray diffraction, *x*-ray energy spectroscopy, and scanning electron microscopy.

Plaques were measured in the same way for the second group, but in addition a histological analysis was performed on the lesions using both optical light and electron microscopy.

Samples were taken from both sides in each animal and also from sites that had not received any treatment (controls).

All the samples for morphological examination were prepared by immersion fixation using Bouin's solution with ester wax embedding for light microscopy, and 3% glutaraldehyde followed by 1% osmium tetroxide, both in cacodylate buffer at pH 7·2 with araldite embedding for electron microscopy. Sections were stained with Alcian blue and haematoxylin eosin for light microscopy and with lead acetate, with or without uranyl acetate, for electron microscopy.

RESULTS

Plaques were observed at sites injected with $KMnO_4$ after five days, increasing in size and weight up to seven days. No further change in the plaques was observed up to 14 days (Table 1).

X-ray diffraction revealed hydroxyapatite and showed a general increase in apatite content with time. No other crystalline phase was identified. Scanning electron microscopy showed collagen fibrils aligned in parallel bundles throughout the plaques. These fibrils had a petrified appearance, as if coated by some material. Within the interstices of the fibrils 'chalky' deposits were observed which were not bound to the collagen matrix. *X*-ray energy spectroscopy gave variable ratios of calcium to phosphoros in the range 2·1 to 2·6; hydroxyapatite having a ratio of 2·17.

Histological analysis of the lesions showed that three days after injection of $KMnO_4$, oedema was evident in association with mast cells, macrophages, and other connective tissue cells. Large numbers of circulating monocytes and lymphocytes were also seen migrating to the affected area. After five days the cellular populations were mainly confined to mast cells, macrophages, and fibroblasts; the latter two cell types exhibiting a degree of motility. Seven days after injection, fibroblasts were seen to be actively producing collagen, and after 14 days were almost the only cell type present, with the collagen in close packed arrays of fibrils extending throughout the area—as confirmed by scanning electron microscopy.

Isotopic scanning with ^{99m}Tc labelled methylene diphosphonate (MDP) was conducted on a third group of male rats after three, six, nine, and 15 days. The animals were prepared as before with subcutaneous injections of $KMnO_4$ and saline. At each time interval four rats were selected and intravenous 50 μCi ($\approx$0·2 ml) ^{99m}Tc labelled MDP administered. Four hours after injection each animal

Table 1 *Mean (SD) variation in size and weight of plaques excised from young male hooded rats 5, 7, and 14 days after subcutaneous injections of 0·2 ml $KMnO_4$*

Day	*5*	*7*	*14*
Average size of plaque (mm)	103 (11)	125 (29)	122 (22)
Wet weight of plaque (g)	126	246	300
Dry weight of plaque (g)	—	41	94

was scanned using a General Electric maxicamera II scanner and 400T formatter. ^{99m}Tc labelled diphosphonate was found to be concentrated within the plaques; activity being apparent by the sixth day.

To estimate the mean turnover rate of mineral in the plaques we are attempting to quantify this technique.

CONCLUSIONS

Simple calcergy is an easy, reproducible model of pathological calcification. Hydroxyapatite is found to deposit without forming intermediary crystalline phases and may nucleate on young collagen fibrils laid down during the inflammatory reaction. Mast cells are observed in large numbers during the initial phase of the inflammatory response, along with macrophages and fibroblasts. After 14 days fibroblasts are the predominant cell type, exhibiting electron microscopical profusions, and are actively laying down new collagen to encapsulate the affected area.

Isotope scanning may be a tool for assessing mineral turnover and for monitoring the progress of calcification.

The calcergy model is now being applied to investigate potential therapeutic inhibitors of hydroxyapatite deposition.

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Periarticular calcification of the shoulder in articular chondrocalcinosis

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In articular chondrocalcinosis calcium deposits commonly occur within the articular cartilages and menisci; extra-articular linear deposits have, however, been described in the Achilles, quadriceps, triceps, and supraspinatus tendons.[1-3]

We report on the prevalence and morphology of periarticular calcification of the shoulder in patients suffering from articular chondrocalcinosis compared with a control group.

Two groups of patients were studied. The first comprised 30 consecutive patients (22 women, 8 men; mean age 73 years, range 40–89) with definite articular chondrocalcinosis diagnosed radiologically in at least two joints. The disease was idiopathic in 28 cases; one was associated with hypothyroidism, and another with gout. A comparable control group (22 women, 8 men; mean age 72·5 years, range 41–90) with no radiological evidence of articular chondrocalcinosis was taken at random and matched for sex and age. Patients with diabetes mellitus or severe renal insufficiency were not included in view of the increased prevalence of calcifying tendinitis among them.[4]

In all cases anteroposterior views of both shoulders were obtained with conventional radiological methods. When periarticular calcification was found xeroradiographic films were made to accentuate their density.

Periarticular calcification was found in nine patients with chondrocalcinosis; all had idiopathic disease; the calcium deposits were bilateral in three cases and unilateral in six. Calcification was also found in one control patient; the deposits were bilateral. The difference between the groups in those with calcification was significant ($p<0·006$) by the exact Fisher test.

Morphologically, two types of calcification were encountered: linear punctate and dense homogeneous. Linear punctate calcification was found in eight patients in the study group; in two cases punctate calcium deposits were also observed at the insertion of the long head of the biceps. Dense homogeneous calcification was found in one control and four of the study group, in three of whom they were associated with linear calcification. The difference between the groups in prevalence of dense calcification is, however, not significant ($p = 0·18$).

Of the 10 cases with periarticular calcification, three (all in the study group) had chronic shoulder pain, one suffering from a rupture of the rotator cuff; the remainder were asymptomatic.