

The inhibitory effects of colchicine on cell proliferation and mineralisation in culture

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Colchicine is often used in the treatment of diseases such as familial Mediterranean fever (FMF) and gout. We have previously reported that patients with FMF who had colchicine on a daily basis and who had a total hip arthroplasty showed no heterotopic ossification after surgery. The mechanism by which colchicine causes this clinical phenomenon has never been elucidated. We therefore evaluated the effect of various concentrations of colchicine on cell proliferation and mineralisation in tissue culture, using rat and human cells with and without osteogenic potential. Cell proliferation was assessed by direct cell counts and uptake of (³H)thymidine, and mineralisation by measuring the amount of staining by Alizarin Red.

Our findings indicate that concentrations of colchicine of up to 3 ng/ml did not affect cell proliferation but inhibition was observed at 10 to 30 ng/ml. Mineralisation decreased to almost 50%, which was the maximum inhibition observed, at concentrations of colchicine of 2.5 ng/ml. These results indicate that colchicine at low concentrations, of up to 3 ng/ml, has the capacity to inhibit selectively bone-like cell mineralisation in culture, without affecting cell proliferation. Further clinical and laboratory studies are necessary to evaluate the effects of colchicine on biological processes involving the proliferation of osteoblasts and tissue mineralisation in

vivo, such as the healing of fractures, the formation of heterotopic bone and neoplastic bone growth.

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We have previously reported 18 patients with familial Mediterranean fever (FMF) who had had a total hip arthroplasty and had been followed up for a mean of seven years (14 months to 14 years). They had negligible heterotopic ossification (HO) after the operation compared with the reported incidence of 20% to 70% in patients who did not have FMF.¹⁻² All had received daily prophylactic treatment with colchicine to prevent the periodic attacks of FMF.

This effect on HO is thought to occur as a result of inhibitory effects the proliferation of bone-forming cells or by interference with mineralisation of bone tissue.

We have therefore investigated the effect of various concentrations of colchicine on osteoblast-like cells (ost-cl), and skin fibroblasts (RSF) in rats, and on human stromal bone-marrow cells (HSBMC). Assessment of cell proliferation was done by direct cell counts and incorporation of (³H)thymidine. The effect on mineralisation of bone tissue was assessed by staining with Alizarin Red.^{3,4}

Our aim was to determine a possible preferential inhibition by colchicine of tissue mineralisation rather than of cell proliferation.

Materials and Methods

We obtained alpha-modified minimum essential medium (α MEM), fetal calf serum (FCS), penicillin, streptomycin, fungizone, glutamine, and trypsin versene solution from Biological Industries (Beth Haemek, Israel). The FCS was heat-inactivated at 56°C for 30 minutes before use. Tissue-culture dishes and flasks were purchased from Nunc (Roskilde, Denmark). Basic FGF (bFGF) was a generous gift from Amgen (Boulder, Colorado) and was dissolved in α MEM supplemented with 0.5% tissue-culture-grade bovine serum albumin (BSA) obtained from Sigma (St Louis, Missouri). Alizarin Red S was obtained from BDH Chemicals Ltd (Poole, UK), (³H)thymidine (2 Ci/mmol) from Amersham Radiochemicals (Buckinghamshire, UK) and dexamethasone (DEX) from Sigma.

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We performed the studies in a sequential manner. First, the effect of colchicine on cell proliferation, as determined by actual cell counting or by assay of (^3H)thymidine, was tested on ost-cl, RSF, HSBMC, and human gingival fibroblasts (HGF). Then its effect on mineralisation was tested in tissue cultures using ost-cl as previously described by Pri-Chen et al.³ and Pitaru et al.⁴

Cell-culture conditions

Cell types. We obtained rat osteoblast-like cells from the long-term culture of a clonal population of rat bone-marrow-derived, bone-like cells prepared and cultured as previously described.⁴

Human stromal bone-marrow cells were obtained from young female patients who had undergone removal of iliac bone for grafts during surgery for scoliosis. Approximately 1 ml of bone marrow was cultured in a 75 cm² tissue-culture flask. The cultures were then grown in 15 ml of complete medium containing 10⁻⁸M DEX as previously described.³ Cultures were maintained in a humidified incubator in 10% CO₂ at 37°C for 14 days. During the first week of culture, the unattached cells were washed out extensively and only colonies of attached cells developed in the tissue-culture flasks. On day 14, the primary HSBMC cultures were trypsinised, counted, and subcultured for the experiments. Human gingival fibroblasts were prepared and cultured as previously described⁵ and RSF were prepared from rat skin explants and cultured in a similar manner.

Cell counting. The cells were cultured in α -modified essential medium (α MEM) supplemented with 15% FCS, 2 mM glutamine, 10 mM β -glycerolphosphate (β -GP), a mixture of three types of antibiotic, namely penicillin (100 U/ml), streptomycin (0.1 mg/ml) and nystatin (12.5 U/ml), and 50 g/ml of vitamin C. Induction of differentiation towards the osteogenic phenotype and proliferation were achieved by adding both DEX 10⁻⁷M and 3ng/ml of bFGF to the medium (complete medium).

For the experiment, the cells were seeded at a density of 1 \times 10⁵ cells/cm². After 24 hours the cultures were treated without or with increasing concentrations of colchicine (each point in tetraplicate) for six days and bFGF was added again on day 3. On day 7, all plates were harvested by trypsin digestion and counted under the microscope.

Uptake of (^3H)thymidine. The basic method for determination of (^3H) uptake with the various tested cell types was the same with minor variation. The cells were seeded at a density of 1 \times 10⁵ cells/well in 24 (12 mm)-well plates. After incubation for 24 hours in complete medium, various concentrations of colchicine were added.

After 48 hours, (^3H)thymidine, 1 Ci per well, was added, for a further 24-hour period of incubation. The cells were then harvested by washing (10) with phosphate-buffered saline, lysed by 0.2 N NaOH solution, and 200 μ l were taken from each well and scintillation fluid added. The radioactivity was counted by a scintillation counter (Packard 1600, Downers Grove, Illinois).

Mineralisation of bone-like tissue. Cultures of ost-cl cells

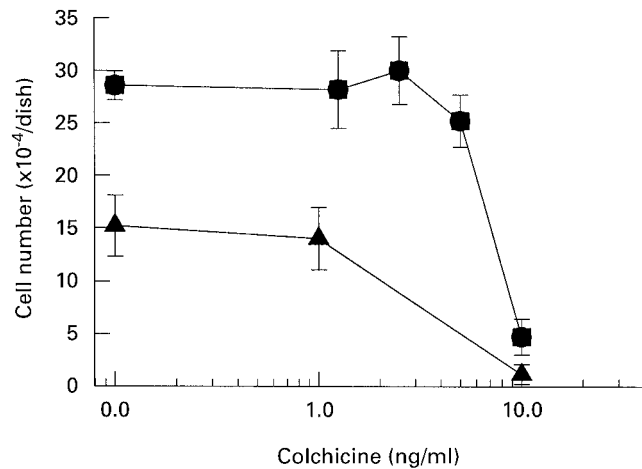


Fig. 1

Effect of colchicine on the proliferation of RSF (■) and ost-cl cells (▲).

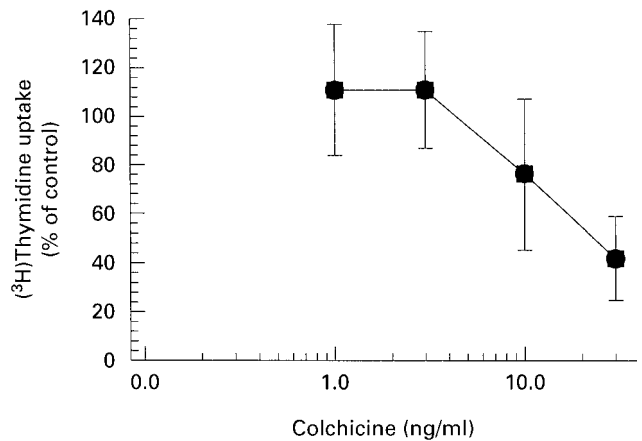


Fig. 2

Inhibitory effect of colchicine on uptake of (^3H)thymidine by ost-cl cells. Control cultures were considered as 100%.

were grown in complete medium for 21 days in 35 mm dishes and then fixed for 24 hours in a 1:1:1.5 solution of formalin:methanol:water. The dishes were then stained for five minutes with a saturated solution of Alizarin Red S (at pH 4.0) and thereafter washed with water and dried. The area of the dish covered by a red stain indicating mineralised bone tissue was measured by an image-analysis system (Supercue-3; Galai, Migdal Haemek, Israel).

Statistical analysis. The results are expressed as the mean \pm SD. We used Student's *t*-test to evaluate the degree of significance and $p < 0.05$ was considered as significant.

Results

Effect of colchicine concentration on cell proliferation. Cultures of ost-cl cells and RSF were grown in the presence of colchicine at different concentrations, and the rate of cell proliferation was evaluated (Figs 1 and 2). Our studies

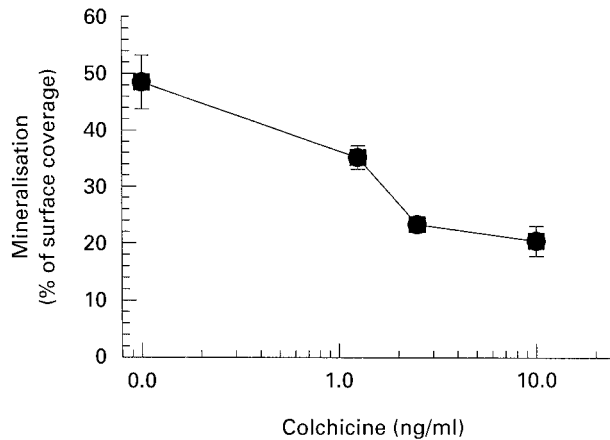


Fig. 3

Mineralisation of ost-cl cultures in the presence of colchicine.

show that at a concentration of up to 3 ng/ml there was no inhibition and only at concentrations of 10 to 30 ng/ml was significant inhibition observed. Similar results were seen also with cultures of HBM and HGF, at which only a minimal and not significant inhibition of cell proliferation of 15% was observed at a concentration of colchicine of 3 ng/ml (data not shown).

Effect of colchicine on mineralisation. To quantitate the effect of colchicine on mineralisation, ost-cl cells were cultured for 21 days, fixed, stained and the percentage of the area of the dish stained by Alizarin Red was determined by an image analyser. Our results showed a decrease of 50% in mineralisation at a concentration of 2 ng/ml of colchicine (Fig. 3).

Discussion

Colchicine is the alkaloid of the plant *Colchicum autumnale* which has been known to medicine for centuries. Today it is used mostly in the treatment of gout, FMF and other arthritides,^{6,7} as well as in Paget's disease of bone.⁸ Our aim in this study was to assess the inhibition of the formation of HO by colchicine at a cellular and tissue-culture level.⁹⁻¹³

We investigated sequentially whether this phenomenon was cell-specific affecting only osteoblasts and fibroblasts, and whether colchicine also inhibited the development of mineralised bone tissue in culture.

Our findings indicated that colchicine was a non-specific inhibitor of the proliferation of all the cell lines which we had studied. This inhibitory effect was demonstrated both by the direct cell counts and by the uptake of (³H)thymidine. It was most prominent at concentrations of 10 to 30 ng/ml. Mineralisation seemed to be more sensitive to this inhibitory effect than proliferation, being affected at a concentration of 1 to 2 ng/ml.

The microcellular mechanisms by which colchicine exerts these effects and the reasons for the 'mineralisation

hypersensitivity' remain to be determined. Several studies have investigated its exact mode of action.¹⁴⁻¹⁸ It seems that colchicine modulates the activity of both osteoblasts and osteoclasts, probably by direct antimicrotubular activity and/or by expression of mRNA.

We suggest that since the bone-marrow cells are heterogeneous, colchicine inhibits non-selectively the osteogenic lineage, and presumably inhibits selectively the differentiation of osteoblasts.

We have shown that it has the capacity to inhibit both the proliferation and mineralisation of bone-marrow cells in vitro, which is the basic process of the formation of HO in vivo. Clinical studies have shown that it is a safe drug with few adverse reactions. The well-tolerated dose is usually up to 2 mg/day often on a regular basis, as in patients with FMF.¹⁹

Our findings may be of value in conditions of pathological formation of bone such as in pelvic and acetabular fractures, the formation of HO associated with brain injury, in total hip arthroplasty and in other osteoblastic bone processes.

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