Effects of agents interacting with microtubules on collagen synthesis and breakdown

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Any drug found to inhibit net collagen synthesis selectively might be clinically useful in pneumoconiosis, cirrhosis, schistosomiasis, scleroderma, and certain operations such as tendon transplantation where adhesions are a problem. One group of agents which has attracted attention interacts with tubulin, the protein constituent of microtubules. These include colchicine and vinblastine, which bind to different sites on the tubulin molecule, preventing their assembly and breaking down labile microtubules (Borisy and Taylor, 1967). Since the mitotic spindle is based on microtubules it is not surprising that colchicine is a classical spindle poison, arresting mitosis in metaphase.

Colchicine has been widely used to study the role of microtubules in cell functions. Microtubules and the contractile system made up of actin, myosin, and regulatory proteins interact to produce cell motility, the uptake of materials from the medium by endocytosis, and the release of packaged secretions (Allison, 1973). In several cell types there is well-defined polarity. This is true, for example, of macrophages and fibroblasts in culture. The cells are elongated and thin veils of cytoplasm are observed at the poles. These are the ruffles where pinocytosis takes place and where new sites of adhesion of the plasma membrane to the substratum form. Consequently the cells show directional movement towards the ruffles.

In electron micrographs many microtubules, arranged more or less in parallel along the long axis of the cell, are seen below the plasma membrane. The ruffles have many microfilaments but no microtubules. Microtubules interact with membrane constituents and restrict their mobility (Ukena and Berlin, 1972). When the fibroblasts are treated with low concentrations of colchicine which prevent the assembly of cytoplasmic microtubules ruffling is seen all over the surface of the cell and the cells show random amoeboid rather than directional gliding movements (Goldman, 1971; Bhishey and Freed, 1971). If cells are treated with cytochalasin B, which interacts with the microfilament system, ruffling and motility cease (Allison, 1973). Thus movements of the plasma membrane, including phagocytosis and pinocytosis visible by light microscopy, are mediated

by microfilaments, whereas microtubules are required for cell polarity and for the translocations of certain secretions within cells.

Among the secretory processes inhibited by colchicine are the release of iodoprotein from stimulated thyroid (Wolff and Williams, 1974), the release of newly synthesized lipoprotein and protein from liver cells (Stein *et al.*, 1974), and release of insulin from pancreatic islet cells (Lacy *et al.*, 1972).

Effects of colchicine on collagen synthesis

Ehrlich and Bornstein (1972) and Ehrlich *et al.* (1974) found that in cultured embryonic chick or rat cranial bones colchicine (10^{-4} M) inhibited procollagen secretion and conversion to collagen and specifically inhibited collagen synthesis. Cells exposed to collagen showed an increased number of dilated Golgi-associated vacuoles and vesicles, some of which contained parallel arrays of filamentous structures. The authors suggest that disruption of cytoplasmic microtubules may interfere with the movement of Golgi-derived vesicles, and the resulting accumulation of collagen precursors in the Golgi complex may lead secondarily to an inhibition of synthesis. Vinblastine also inhibited procollagen secretion and conversion to collagen but it produced marked changes in the overall structure of the endoplasmic reticulum and inhibited protein synthesis, so that there was some doubt about the specificity of the effect.

Similarly, Diegelmann and Peterkofsky (1972) found that colchicine ($10\ \mu\text{M}$) and vinblastine block collagen release from chick embryo frontal bone and 3T3 fibroblasts into the culture medium. Dehm and Prockop (1972) and Gordon and Werb (1976) studied the effects of colchicine and related compounds on secretion of enzymes by thioglycollate-elicited mouse peritoneal macrophages in culture. Colchicine stimulated secretion of inducible proteinases, including collagenase, elastase, gelatinase and azocaseinase, two- to sixfold over a period of several days. Similar effects were obtained with colcemid and vinblastine but not with lumicolchicine, a structurally related compound which does not interfere with microtubule assembly.

Treatment of scleroderma and liver cirrhosis

Alarcón-Segovia and his colleagues (1974) presented a preliminary report of a randomized double-blind crossover trial of colchicine (1 mg/day 6 days/week) and placebo (3 months each) in 14 patients with scleroderma. After completing the 6 months' trial the patients were placed in an open study of colchicine at tolerance doses with a 6 months' end point. Before the trial and at the end of each period patients were assessed by photographs, measuring grip strength, buccal opening and first dimensions, biopsy of skin, determination of serum proline and hydroxyproline, and, in 11 patients, by studies of ^{14}C -proline incorporation into skin collagen.

Nine patients improved on colchicine in the crossover trial and all these improved further in the higher-dose open study. Histological examination showed that 6 patients receiving colchicine had decreased dermal collagen. Furthermore, colchicine reduced incorporation of ^{14}C -proline into skin collagen in 6 out of 11 patients. In 8 out of 14 patients serum proline fell and serum hydroxyproline rose during colchicine treatment, suggesting increased collagen degradation. The authors concluded that colchicine might be useful in the management of scleroderma.

Rojkind *et al.* (1973) reported preliminary findings on the administration of colchicine 1–2 mg/day to 7 patients with liver cirrhosis. All were kept under observation for 3 weeks before starting colchicine. Neither their clinical condition nor liver function tests improved. After 2 weeks of treatment liver function tests of all patients showed improvement.

Discussion

Observations from several different groups of experts in the field show that colchicine in micromolar concentrations inhibits collagen secretion by fibroblasts and stimulates release of collagenase by fibroblasts and macrophages (Harris and Krane, 1971; Harris *et al.*, 1975b). Preliminary results of clinical trials suggest that well-tolerated doses of colchicine (1 mg/day) may be effective in some cases of scleroderma.

Since colchicine has proved to be a safe and effective drug for the long-term prevention of gout (Seegmiller, 1974), its efficacy might be tried in scleroderma and other diseases in which collagen synthesis is increased. An example is pneumoconiosis, where the fibrogenesis continues long after exposure to the offending dust has ceased and there is no known way of preventing progressive impairment of lung function. However, in view of the marked individual variation in fibrogenesis in pneumoconiosis, scleroderma, and other conditions the effect of colchicine will be difficult to prove conclusively.

Since fibroblasts treated with colchicine show accumulation of collagen-containing vacuoles (Scharft and Heersche, 1975), the question arises whether long-term colchicine treatment would produce a situation analogous to a storage disease. This seems unlikely, since procollagen can be degraded by cellular enzymes and colchicine can promote autophagocytosis (Hirsimäki *et al.*, 1975). No evidence for any such effect has been obtained in gouty patients treated with colchicine. Nevertheless, careful morphological observations of fibroblasts in patients on long-term colchicine treatment would be of interest.